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Environmental activity of bacteria degrading aromatic pollutants

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Faculté de biologie
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Environmental activity of bacteria degrading aromatic pollutants

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Environmental activity of bacteria degrading aromatic pollutants.

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P. Goloubinoff

pour le Doyen
de la Faculté de biologie et de médecine

Prof. Pierre Goloubinoff

What am I, Life? A thing of watery salt
Held in cohesion by unresting cells
Which work they know not why, which never halt,
Myself unwitting where their master dwells.
I do not bid them, yet they toil, they spin;
A world which uses me as I use them,
Nor do I know which end or which begin,
Nor which to praise, which pamper, which condemn.
So, like a marvel in a marvel set,
I answer to the vast, as wave by wave
The sea of air goes over, dry or wet,
Or the full moon comes swimming from her cave,
Or the great sun comes north, this myriad I
Tingles, not knowing how, yet wondering why.

(Poem II of Seven Poems from 'Lollingdon Downs' by John Masefield, 1917)

We are drowning in information but starved for knowledge –John Naisbitt

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SUMMARY

Successful bioremediation and specially bioaugmentation have been considered as one of the best techniques to clean up the environment from toxic man-made compounds (like dibenzofurans, DBF, and 4-chlorophenols, 4CP), at low cost and without major perturbations for the environment. Unfortunately, even though several microorganisms have demonstrated their efficacy to degrade toxic compounds under laboratory conditions, several attempts to apply them into the environment did not give the expected success. These failures may be the result of a poor knowledge about the reactions of such microorganisms in the environment. The purpose of my work was to better understand the genome-wide response of bacteria after inoculation or growth in conditions more close to reality but still enough controlled to elucidate their behaviour.

Resistance to dry conditions has been considered as a key factor in the survival of strains meant to be used for bioremediation; this implies a series of mechanisms used by cells to deal with water stress. A general introduction to the state of the art in bioremediation and the species intended for this study is presented in chapter I. In chapter II, through a genome-wide responses approach, I compared the reactions of three promising strains for bioremediation purposes (*Arthrobacter chlorophenolicus* A6, *Sphingomonas wittichii* RW1 and *Pseudomonas veronii* 1YdBTEX2) to standard laboratory induced water stress. The objective here was to discover and describe the common and specific stress-resistance strategies deployed by the bacteria. My results showed that the three strains had different sensitivities to water stress. The common trait among them included diminished expression of flagellar motility and increased expression of compatible solutes, the choice of those solutes were strain-specific.

I studied more in detail the genome-wide response of RW1 to inoculations into and growth within non-sterile contaminated sand (chapter III) compared to regular batch liquid cultures. My results indicated that RW1 can efficiently persist and grow under dry conditions and degrade the contaminant (here DBF) if the conditions of preculturing were made in the target contaminant. In contrast to our hypotheses from Chapter II, the behaviour of RW1 in sand was very different from that to water stress induced by addition of salt or PEG. More interestingly, the responses of RW1 are very different from liquid cultures, meaning that the strain has a way to recognize its growth environment.

The same types of experiments in contaminated sand (this time with 4CP) were performed for A6 (chapter IV) in an attempt to complete the comparison of induced water stress in liquid versus soil adaptation. Unfortunately it was impossible to obtain good quality hybridization samples for the study of transcriptome responses during the different phases of growth in (contaminated) sand. Nevertheless I learned that *Arthrobacter* cannot grow in highly contaminated soil if the conditions are very dry; they need much more water to degrade high amounts of 4CP.

These observations warn that studies focusing on inoculation strain efficacy should be tested under conditions as close as possible to the intended environmental objective, as well as to the optimum cell concentration for the inoculum.

Finally, we studied the behaviour of A6 in a phyllosphere habitat under two levels of humidity (chapter V). A6 did not show any particular reaction to changes in humidity, but again the phyllosphere response could not be related to the types of gene expression changes measured under induced water stress in liquid. This study also allowed the demostration of the presence of phenolic compounds on leaves, which

can potentially enhance the degradation properties or faster reaction to contaminants in a process of phylloremediation by *A. chlorophenolicus*.

RESUME

Une des meilleures techniques pour décontaminer l'environnement d'éléments toxiques (comme par exemple le dibenzofuan, DBF et le 4-chlorophenol, 4CP) déposés par l'homme, à bas coûts et sans le perturber considérablement, est sans doute la biorémédiation, et particulièrement la bioaugmentation. Malheureusement, si plusieurs microorganismes ont démontré leur efficacité à dégrader les composés toxiques en conditions de laboratoire, plusieurs tentatives afin de les utiliser dans l'environnement n'ont pas abouti. Ces échecs sont probablement le résultat des pauvres connaissances des réactions de ces mêmes microorganismes dans l'environnement. L'objectif de mon travail a été de mieux comprendre les réponses de ces bactéries au niveau de leurs gènes lorsqu'elles sont introduites ou prospèrent dans des conditions plus proches de la réalité, mais encore suffisamment contrôlées pour pouvoir élucider leur comportement.

Le fait de résister à des conditions de sécheresse a été considéré en tant que facteur clé dans la survie des bactéries amenées à être utilisées pour la biorémédiation; cela implique une série de mécanismes utilisés par la cellule pour faire face au stress hydrique. Le chapitre II, par une approche métagénomique, compare les réactions de trois souches prometteuses pour la biorémédiation (*Arthrobacter chlorophenolicus* A6, *Sphingomonas wittichii* RW1 and *Pseudomonas veronii* 1YdBTEX2) vis-à-vis du stress hydrique simulé en conditions de laboratoire. L'objectif ici est de découvrir et de décrire les stratégies de résistance au stress,

communes ou spécifiques, employées par les bactéries. Mes résultats montrent que les trois souches ont des sensibilités différentes au stress hydrique. Entre les traits communs trouvés, il y a une diminution de l'expression des gènes flagellaires ainsi qu'une augmentation de l'expression de solutes compatibles, mais qui sont souche-spécifiques.

J'ai étudié plus en détail la réponse génomique de RW1 par rapport aux inoculations ainsi que sa croissance dans le sable contaminé et non-stérile (chapitre III), et je les ai comparé à des cultures en milieu liquide. Mes résultats indiquent que RW1 peut résister efficacement et peut croître dans des conditions presque sèches et peut également dégrader le contaminant (DBF, dans le cas présent) si les pré-cultures sont réalisées dans le même type de contaminant. Par contre, notre hypothèse du chapitre II se révèle fausse car le comportement de RW1 est très diffèrent de celui observé dans des conditions avec stress hydrique induit par l'addition de sel ou de PEG. Plus intéressant, les réponses de RW1 en milieu liquide sont très différentes de celles observées dans le sable, révélant ainsi que cette souche peut reconnaître le milieu dans lequel elle se trouve.

Les mêmes expériences en sable contaminé, cette fois–ci avec 4CP, ont été réalisées pour A6 (chapitre IV) dans l'espoir de compléter la comparaison entre le stress hydrique et l'adaptation dans le sol. Malheureusement, il n'a pas été possible d'obtenir d'échantillons de bonne qualité pour les hybridations des micropuces afin d'étudier la réponse transcriptionnelle dans les différentes phases de croissance dans le sable (contaminé ou non). Toutefois, j'ai appris qu'*Arthrobacter* ne peut pas croitre dans les sols hautement contaminés si les conditions du sol sont très sèches, elles ont en effet besoin de suffisamment d'eau pour dégrader des quantités importantes de 4CP.

Ces observations dirigent l'attention sur le fait que les études sur l'efficacité de l'inoculation de bactéries doivent être testées dans des conditions le plus proche possible de l'environnement ciblé, tout comme les concentrations optimales pour l'inoculum.

Finalement, nous avons étudié le comportement de A6 dans la phytosphère avec deux dégrés d'humidité (chapitre V). A6 ne montre pas de réaction particulière face aux changements d'humidité, et à nouveau, ces réponses ne peuvent être liées aux changements d'expression des gènes observées dans les conditions de stress hydrique simulées. Cette étude a permis d'identifier la présence de composés phénoliques dans les feuilles qui peuvent potentiellement améliorer les propriétés de dégradation ou qui permettent d'effectuer de façon plus rapide la réaction de dégradation des contaminants dans un processus de phytoremédiation par *A. chlorophenolicus*.

CHAPTER I

General Introduction

CHAPTER I. General Introduction

In the world of microbiology there is still a huge knowledge gap in several aspects of microbial life. Microorganisms show an extraordinary capability to cope with a broad kinds of environments, they are capable to metabolize a large variety of compounds that are toxic to humans and they have been reported to spontaneously remove pollutants directly in the environment (Camilli et al 2010, Hazen et al 2010, Medina-Bellver et al 2005, Mrozika and Piotrowska-Segetb 2010). For decades, efforts have been made to exploit at best these properties to clean up the environment, but the processes are still misunderstood

(Mrozika and Piotrowska-Segetb 2010, Tyagi et al 2011, Vila et al 2015).

Better understanding of the functioning of microorganisms degrading toxic compounds would give us a key to solve one of the major current societal problems: environmental pollution. Environmental pollution has an important negative impact on natural biodiversity and human health (Camilli et al 2010, Hazen et al 2010, Kulkarni et al 2008, Megharaj et al 2011). In the last decade there has been an increasing interest in understanding the specific roles of bacteria in the process of degradation of pollutants (Andreoni and Gianfreda 2007, de Lorenzo 2001, de Lorenzo 2009, de Lorenzo et al 2013, Haritash and Kaushik 2009, Jeon and Madsen 2013).

A wide variety of bacterial strains have been isolated from contaminated sites, and their catabolic properties have been studied, but mostly under laboratory conditions. It has been proposed that some of those strains may be beneficial to enhance the degradation of toxic compounds in contaminated sites where no “natural degrading organisms” are present (de Lorenzo 2001, de Lorenzo 2009, de Lorenzo et al 2013, Tyagi et al 2011). Of these, we chose to study three strains on the basis of their

capabilities to degrade particularly toxic compounds: *Sphingomonas wittichii* RW1, *Arthrobacter chlorophenolicus* A6 and *Pseudomonas veronii* 1YdBTEX2.

Sphingomonas wittichii RW1

Strain RW1 was isolated from enrichment of water samples taken from the Elbe River (Germany) on dibenzo-p-dioxin (DBD) and dibenzofurans (DBF) (Wittich et al 1992). Since then several studies have tried to characterize the genes implicated in the pathway of degradation of these compounds. An overview of these genes was compiled by Coronado et al (2012), who used a combination of transcriptomics and transposon library screening to unravel the metabolic pathway exhibited by *S. wittichii* during salicylate and dibenzofuran mineralization.

Other studies have looked at other compounds degraded by RW1: mono and dichlorinated dibenzofurans but not highly chlorinated derivates (Wilkes et al 1996), 2,7-dichloro- and 1,2,3,4-tetrachlorodibenzo-p-dioxin (Hong et al 2002) and carbazole (Nam et al 2012). A complete taxonomic characterization and description of strain RW1 is found in Yabuuchi et al (2001) and its genome sequence was published in 2010 by Miller and collaborators.

In 1997 a study on the survival and degradation properties of RW1 in soil slurries (80% of maximal water capacity) showed up to 90% of degradation of DBF within 12 days, maintaining the high cell density that was inoculated (Megharaj et al 1997). Later Halden et al (1999) followed the degradation of DBD, DBF and 2-chlorodibenzo-*p*-dioxin (2-CDD) in soil slurries (60% of field capacity) with different amounts of organic matter. They showed that high densities of cells were necessary for the total degradation of compounds and that higher levels of organic matter resulted in an increase in the half-life of the strain during degradation of 2-CDD. A

genome-wide transposon screening was performed to identify genes important for growth in DBF and survival under sand conditions (Roggo et al 2013). Coronado et al (2015) studied the *in situ* DBF degradation activity of *S. wittichii* through fluorescent gene reporters in soil microcosms. Their results indicated a competition for DBF metabolites between RW1 and native aromatic-compound degrading bacteria present in the contaminated soil. As a consequence, RW1 was not able to grow on DBF as well as in a soil without such competing aromatic-compound degraders. This study further pointed out the importance for survival studies to use low-density inoculation and ensure availability of the target carbon source.

Two studies have specifically addressed the proteome of RW1 during growth on a variety of carbon sources, one using mass spectrometry as a tool to identify and monitor microorganisms (Halden et al (2005) and the other to identify the proteomic changes between the carbon sources (Colquhoun et al (2012). Finally, Johnson et al (2011) described the genome-wide behaviour of *S. wittichii* in conditions of water stress after short and acute exposition to NaCl and PEG8000 and found that cells react differently to these two types of hydric stress.

***Arthrobacter chlorophenolicus* A6**

A.chlorophenolicus is a particularly interesting gram-positive strain that was isolated from enrichments of contaminated soil suspensions with high concentrations of 4-chlorophenol (4CP) (Westerberg et al 2000). A6 can mineralize chloro and nitrophenols (Elvang et al 2001, Westerberg et al 2000), para-nitrophenol (Sahoo et al 2011) and bromophenols (Sahoo et al 2014).

The pathway of 4CP degradation through hydroxyquinol was described by Nordin et al (2005). They further described the complete cluster of genes responsible for the

4CP degradation and suggested that strain A6 had acquired those genes by horizontal gene transfer.

The genus *Arthrobacter* is especially abundant in soil environments and being an isolate from soil, strain A6 would seem the perfect candidate for bioremediation in soil. Jernberg and Jansson (2002) describe the changes in soil communities after contamination with 4CP and/or inoculation of A6 chromosomally tagged with the *luc* luciferase gene. They found fluctuations of the communities in response to the changes imposed. Their results also indicated that the tagged strain was stimulated by the presence of 4CP. Backman and Jansson (2004) studied the effects of soil temperature on the degradative responses of A6. They found lower rates of 4CP degradation and growth at 5°C. At 28° the degradation rates were higher during the first week, but after 7 days the rates decreased to those at 5°C. Finally, the strain managed to degrade the same amount of 4CP after 17 days, both at 5°C and 28°C. The study further found that at 28°C most of the cells lost their membrane integrity while they remained intact and metabolically more active at 5°C, making A6 a suitable strain for chlorophenol-contaminated soils in cold climates (Backman et al 2004). In 2007 Unell and others described the adaptations of the A6 cell membrane fatty acids to different concentrations of phenolic compounds and temperatures. This study showed that a more rigid membrane counterbalances the increase in fluidity resulting from exposure to organic solvents or higher temperatures.

Unell and collaborators (2008) demonstrated the capability of A6 to degrade high concentrations of nitrophenols, chlorophenols and phenols in liquid and soil slurries. Experiments with mutants only able to grow on phenol suggested a different catabolic pathway for phenol than for 4CP degradation. A characterization of the proteome during growth on 4CP, 4-nitrophenol and phenol at two different

temperatures (5°C and 28°C) for the wild type and the mutant disabled for growth on substituted phenols confirmed the 4CP degradation pathway (Nordin et al 2005, Unell et al 2009).

***Pseudomonas veronii* 1YdBTEX2**

The genus *Pseudomonas* contains a variety of strains capable to cope with environmental contaminants Examples of these can be found in Andreoni et al (2004), Andreoni and Gianfreda (2007), Cebron et al (2014), Chikere et al (2011), Desai et al (2010), Haferburg and Kothe (2010), Haritash and Kaushik (2009), Head et al (2006), Heuer and Smalla (2012), Megharaj et al (2011), Mrozika and Piotrowska-Segetb (2010). *P. veronii* strains 1YdB and 1YdBTEX2 were isolated from the former army airforce base Hradçany (Czech Republic), a well-known site contaminated with petroleum hydrocarbons (Brennerova et al 2009). Strain 1YdBTEX2 is capable to degrade both benzene, toluene, *m*- and *p*-xylene and ethylbenzene (BTEX). It carries a unique catabolic pathway for the degradation of benzene and toluene (de Lima-Morales et al 2013, Junca and Pieper 2004, Witzig et al 2006).

Bioremediation

Two approaches have been used to enhance bioremediation of organic compounds in contaminated environments. These are biostimulation and bioaugmentation (El Fantroussi and Agathos 2005, Mrozika and Piotrowska-Segetb 2010, Singer et al 2005, Tyagi et al 2011). Biostimulation is a technique that increases the amount of nutrients to stimulate the growth and activity of microbes already present at a site. The idea here is that biodegradation activity can become limited for certain nutrients

(N, P) when a large excess of carbon in form of organic material is present at the site. In contrast, bioaugmentation consists of inoculation of strains with well-known biodegradative capacities into a contaminated environment (Mrozika and Piotrowska-Segetb 2010, Singer et al 2005). The objective of bioaugmentation is to eliminate the contaminant with bacteria that have demonstrated their efficacy in laboratory, with the idea that the number or characteristics of such bacteria are limiting in the site. Biostimulation has had considerable success, but bioaugmentation efforts have been frequently frustrated by poor survival of the inoculants, which might be due to a poor understanding of the behaviour of such strains in real environments (Andreoni and Gianfreda 2007, de Lorenzo 2009, El Fantroussi and Agathos 2005, Tyagi et al 2011).

The “Achilles heel” for bioaugmentation in contaminated soil seems to be the survival of the inoculum (Singer et al 2005). For good survival and growth of inoculated microorganisms in "real" environments there are several important factors to consider. These include accessibility of water, availability of nutrients, accessibility of the target pollutant, oxygen, organic matter, pH, temperature, light from sun, atmospheric pressure, redox potential, presence of other metabolizable substrates, or interactions with plants and animals (Backman and Jansson 2004, Halden et al 1999, Megharaj et al 1997, Standing and Killham 2007). Auto-ecological properties such as adaptation to carbon sources and different environments, motility, capability to form biofilms or biosurfactant production (Cunliffe and Kertesz 2006), and genome structure have been implicated as well (Mongodin et al. 2006). But the general molecular and physiological responses of strains to a given environment are still poorly understood (Desai et al 2010, Puglisi et al 2010, Wang et al 2011). The behaviour of pure cultures introduced in a contaminated soil is still not understood

sufficiently well with respect to their interactions with the environment and with the native microbial community (Jeon and Madsen 2013, Megharaj et al 2011, Mrozika and Piotrowska-Segetb 2010, Tchelet et al 1999).

Water stress

It has been considered that the key factor for survival of bacteria inoculated in contaminated soils is the availability of water, even more than other factors such as competition with native microorganisms for nutrients or predation (van Elsas et al 2007). The capability of bacteria to cope with water stress has frequently been decomposed into either solute stress or matric stress. Cells experience solute stress when the concentration of solutes is higher outside the cell than inside, which requires the cell to compensate this osmotic difference by different means (see further below) (Csonka 1989, Potts 1994, Sleator and Hill 2002). Matric stress on the other hand is the result of increased binding of water with surfaces or pores. As a result, the cell will experience damage to proteins and nucleic acids, changes in growth rate and even death (Gulez et al 2014, Johnson et al 2011, Potts 1994).

Methods that mimic water stress in the laboratory generally use the addition to the culture media of increasing amounts of salts like NaCl for solute stress, and addition of polyethylene glycol with high molecular weight to achieve matric stress (Chang et al 2007, Coronado et al 2015, Halverson and Firestone 2000, Johnson et al 2011, van de Mortel and Halverson 2004). Finally, some authors have used gravimetric methods to achieve controlled levels of water stress (Gulez et al 2014, van de Mortel and Halverson 2004).

Different strategies adopted by microorganisms when exposed to water stress have been described (Booth et al 2015, Csonka 1989, Feehily and Karatzas 2013, Paul

2013, Poolman and Glaasker 1998, Sleator and Hill 2002). Stimulation of the uptake of potassium ions (Dominguez-Ferreras et al 2006, Feehily and Karatzas 2013, Fu et al 2014, Hahne et al 2010) and glutamate biosynthesis (Feehily and Karatzas 2013, Fu et al 2014, (He et al 2010) have been described as a first response of bacteria to water stress. This is followed by production of compatibles solutes (Hernández Garcia 2011, Poolman and Glaasker 1998, Sleator and Hill 2002) like proline (Brill et al 2011, Hahne et al 2010, Hernández Garcia 2011), glycine/betaine/choline (Hoffmann et al 2013, Niewerth et al 2012, Wargo 2013, Zhou et al 2011) and trehalose (Dominguez-Ferreras et al 2006, Fida et al 2012, Freeman et al 2013, Iturriaga et al 2009, Johnson et al 2011, Singh et al 2005). Compatible solutes can accumulate to high concentrations in the cell without affecting general cellular process (Brill et al 2011, Feehily and Karatzas 2013, Poolman and Glaasker 1998, Sleator and Hill 2002). Changes in fatty acid composition of membranes (Chang et al 2007, Halverson and Firestone 2000, Johnson et al 2011) as well as production of exopolysaccharides have also been mentioned as adaptation to water stress (Chang et al 2007, Gulez et al 2014).

Transcriptomic approach

The development of techniques to analyse genome-wide transcriptional responses has led several groups to study the bacterial stress responses to water stress. For example, osmoadaptation of *Sinorhizobium meliloti* was studied by DNA microarrays of cultures exposed to changes in osmolarity caused by NaCl or sucrose (Dominguez-Ferreras et al 2006). Osmotolerance of two strains of *P. syringae* was examined by microarrays, showing the superior epiphytic competence of one of them due a their higher tolerance and proactive response to osmotic shock (Freeman et al

2013). Fu et al (2014) studied the responses of several strains of *Vibrio cholerae* facing salt stress by using qRT-PCR of 53 specific salt stress-response genes. Results reveal that the use of common mechanisms and sigma factors activates their salt concentration-dependent response. Gulez and others (2014) studied genome-wide transcription in *P. putida* strain KT2440 and mutants deficient in the production of alginate or other exopolysaccharide proteins, under conditions of matric stress using a Pressurized Porous Surface Model. While their results show the importance of alginate, in its absence, other mechanisms are activated. Two other groups had studied the responses of *Bacillus subtilis* to exposition to salt stress, whereas Steil and collaborators (2003) used microarrays to report the responses of a mutant which is more sensitive to prolonged growth in high salinity (1.2M NaCl) as well as the sudden solute increase in the growth media revealing distinctively different physiological adaptations. Hahne and collaborators (2010) analysed the global transcriptome and membrane proteomics of the response of *B. subtilis* to severe and sudden solute osmotic change. The response of *Desulfovibrio vulgaris* Hildenborough to long term salt exposition was studied by He et al (2010) showing that the addition of aminoacids or yeast extract to the culture medium mitigates the inhibition of growth in the presence of salts. As mentioned above, Johnson and collegues (2011) using custom microarrays studied the genome-wide responses of *S. wittichii* to chronic and shock matric and solute stress. Finally, Singh et al (2005) examined the transcriptional response of *Saccharomyces cerevisiae* to desication by air-drying and later rehydration and their results suggest that the general dessication response is independent of the conditions of water removal, whereas *Shewanella oneidensis* MR-1 responses to high levels of salt in growth media were examined by microarray analysis by Liu et al (2005) where they showed a reduction in the

expression of motility/chemotaxis genes and an increase in expression of genes related to Na^+ extrusion and glutamate biosynthesis.

Gene expression was recorded directly in the soil environment for *Rhodococcus jostii* RHA1 in sterilized soil (Iino et al 2012); identifying soil-specific genes mainly associated with metabolism, another *Rhodococcus* strain was studied in minimal medium and soil slurries contaminated with polychlorinated biphenyls (PCBs) and the results show the transcriptomic response is fundamentally directed to reduce oxidative stress (Puglisi et al 2010). Wang et collaborators (2011) further studied the behaviour of *P. putida* KT2440 in sterilized soils contaminated or not with 3-chlorobenzoic acid (3CBC) at the beginning of the stationary phase of growth, their results show the activation of several genes implicated in the transport of 3CBC, which is useful in understanding the degradation pathway. Finally an catabolic array of the key alkane degradation and aromatic catabolic gene families were designed as tool to evaluate the general catabolic activity of an environment (Vilchez-Vargas et al 2012).

Aim

The purpose of this thesis work was to improve the understanding of the strategies adopted by bacteria useful for biodegradation under conditions as expected for contaminated sites. I focused mainly on studying genome-wide responses with help of micro-array analysis under carefully replicated experimental conditions. I used three selected strains (*S. wittichii* RW1, *A. chlorophenolicus* A6 and *P. veronii* 1YdBTEX2) as examples of potentially useful strains for bioaugmentation. In addition, these strains belong to different taxonomic groups, which might show differences in strategies to cope with environmental conditions. As main

environmental conditions I focused on calibrated water stress through solute and matric potential changes, as well as on sandy soil with added contaminants as methods to induce transcriptomic changes representative for what may prevail in contaminated sites.

My specific objectives were:

1- Analyse general and common properties in cellular adaptation programs to simulated drought conditions across a three strains with different catabolic properties

2- Study survival and genome-wide gene expression of bacteria upon introduction into sand with or without contamination for short and longer periods after inoculation and in comparison to liquid cultures.

3- Analyse the cellular responses during transition from laboratory growth conditions in agar surfaces, with or without contamination, to plant leaves in dry or humid conditions.

Outline of the thesis

The thesis is organized as follows:

Chapter I. General Introduction

Chapter II. Comparison of genome-wide responses to water stress

This chapter describes a "metagenomic" analysis of water stress response by custom-made microarray hybridisations of RNA from three strains: *Sphingomonas* wittichii RW1, *Arthrobacter* chlorophenolicus A6 and *Pseudomonas veronii* 1YdBTEX2. I used the same imposed water stress with the two main components of the osmotic pressure, solute stress and matric stress.

Chapter III. Genome-wide analysis of *Sphingomonas wittichii* RW1 behaviour during inoculation and growth in contaminated sand

This chapter was previously published in ISME J. 2015 Jan;9(1):150-165 and describes a comprehensive analysis of the transcriptomic behaviour of RW1 during inoculation and growth in contaminated sand compared with liquid cultures.

Chapter IV. Behaviour of *Arthrobacter chlorophenolicus* A6 in liquid cultures and sand inoculations

Here I present the results obtained in an attempt to describe the genome-wide expression of strain A6 in similar conditions of inoculation in contaminated sand as for RW1 in Chapter III.

Chapter V. Transcriptional profiling of Gram-positive *Arthrobacter* in the phyllosphere: Induction of pollutant degradation genes by natural plant phenolic compounds

This work was previously published in Environmental Microbiology. 2014 Jul;16(7):2212-2225. This chapter includes the results obtained in a collaboration with Tanja Scheublin and others (Scheublin et al 2014) from the Netherlands Institute

of Ecology (NIOO-KNAW), Department of Microbial Ecology, on the transcriptome response of *A.chlorophenolicus* A6 to the phyllosphere environment, subjected to high or low humidity and a comparison with the behaviour on agar plates. My role was to perform the transcriptome analysis. In annex I also include a description of the comparison of phyllosphere-induced genes with previously described water stress genes.

Chapter VI. General discussion

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CHAPTER II

Comparison of genome-wide responses to water stress

CHAPTER II. Comparison of genome-wide responses to water stress

Abstract

Resistance to semi-dry state and water stress has been considered a crucial trait for superior growth and survival of strains used for bioaugmentation in contaminated soils. Here we compare three strains with promising characteristics for bioremediation for their reaction to standard laboratory induced water stress: *Arthrobacter chlorophenolicus* A6, *Sphingomonas wittichii* RW1 and *Pseudomonas veronii* 1YdBTEX. First we compared growth rates of the three strains in liquid medium with gradually diminishing water potential, induced either by addition of solutes (NaCl, solute stress) or by addition of polyethylene glycol (matric stress). The genome-wide gene expression of the three strains was then compared to conditions of sudden but subinhibitory water stress induced by solute or polyethylene glycol. Growth of *P. veronii* 1YdBTEX2 was the most sensitive to water potential decrease, followed by *S. wittichii* RW1 and *A. chlorophenolicus* A6. The number of genes differentially expressed under decreasing water potential was lowest for A6, followed by *S. wittichii* and *P. veronii*. Gene inspection and gene ontology analysis indicated that common reactions among the three strains included diminished expression of flagellar motility and increased expression of compatible solutes (which were strain-specific). Furthermore, a set of common genes with ill-defined function was found between all strains, including ABC transporters and aldehyde dehydrogenases, suggesting a core conserved response to water stress.

Introduction

Improving bioremediation of contaminated soils by the introduction of specific microorganisms has frequently been proposed (Andreoni and Gianfreda 2007, de Lorenzo 2009, de Lorenzo et al 2013, El Fantroussi and Agathos 2005, Haritash and Kaushik 2009, Mrozika and Piotrowska-Segetb 2010) but poses a variety of practical problems. Most importantly, the bacterial strains raised under laboratory conditions that degrade contaminants very efficiently seem to have large difficulties to adapt to a "real" contaminated soil environment (de Lorenzo et al 2013, Jeon and Madsen 2013, Mrozika and Piotrowska-Segetb 2010).

One evident problem in the application of strains for bioremediation is the passage from liquid state (i.e., their preculturing) to a rather dry environment (e.g., the contaminated site). The question as to how bacteria react to changes in water availability or water potential, and whether this influences their capacity to degrade the contamination under in situ conditions has therefore attracted attention (van de Mortel and Halverson 2004). Cells can experience stress when they are confronted to changes in the water potential of their environment, which can be the result of changes in solute potential and producing osmotic differences across the cell envelope. Secondly, water stress can result from changes in matric potential, for example, when water in the environment is retained by small pores (Potts 1994). The two types of water stress have been mimicked in laboratory experiments by adding salts (NaCl) to culture media to induce solute stress and polyethylene glycol (PEG8000) for matric stress (Johnson et al 2011a, van de Mortel and Halverson 2004).

The purpose of the underlying study was to better understand and compare strain-specific as well as common responses of bacteria useful for bioremediation to

induced water stress as a function of their water stress sensitivity. We chose three bacteria, which previously have been proposed as suitable candidates for bioaugmentation: *Sphingomonas wittichii* RW1, *Arthrobacter chlorophenolicus* A6 and *Pseudomonas veronii* 1YdBTEX2. RW1 is an alphaproteobacterium capable to degrade dibenzo-p-dioxins and dibenzofurans, and some of their chlorinated substitutes (Wittich et al 1992, Yabuuchi et al 2001). Strain A6 is considered a typical gram-positive soil bacterium, which is capable to use a wide number of carbon sources and resist extreme environments (Mongodin et al 2006, Westerberg et al 2000). The interest in the strain lays in its efficient degradation of high concentrations of chloro- and nitrophenols (Elvang et al 2001, Westerberg et al 2000). Strain 1YdBTEX2 is a gammaproteobacterium, which was isolated from the former army airforce base Hradçany (Czech Republic), a well-known site contaminated with petroleum hydrocarbons (Brennerova et al 2009). The strain can efficiently degrade benzene, toluene, ethylbenzene and xylenes (de Lima-Morales et al 2013).

The sensitivity of each strain to water stress was tested by measuring maximum specific growth rates at gradually decreasing water potential of culture media, by addition of NaCl or PEG. The genome-wide transcriptional response of the strains was then determined by micro-array hybridizations of reverse-transcribed fluorescently labelled cDNA isolated from cultures at a water stress level at which growth rates were not more than 20% decreased compared to non-stressed growth conditions. Data on *S. wittichii* RW1 water stress responses were included from previous published work (Johnson *et al.*, 2011). Bioinformatic tools were used to infer common and/or strain-specific adaptations to water stress among the three strains.

Materials and Methods

Bacterial strains and growth media

A. chlorophenolicus (A6) was isolated from soil supensions with increasing amounts of 4-chlorophenol (4CP). It can degrade 4CP at concentrations of maximal 350 ppm (2.7 mM) (Westerberg et al 2000). *P. veronii* strain 1YdBTEX2 was isolated from an aquifer contaminated with jet fuel in Czech Republic. Strain 1YdBTEX2 grows on benzene, toluene, ethylbenzene, *m*- and *p*-xylene (BTEX) as sole carbon and energy sources (Junca and Pieper 2004). *Pseudomonas putida* KT2440 is a plasmid-free derivative of the toluene-degrader strain mt2, and has been used widely for a variety of studies (Dominguez-Cuevas et al 2006, Halverson and Firestone 2000, Martinez-Garcia et al 2014a, Martinez-Garcia et al 2014b, Roberson and Firestone 1992, Wang et al 2011). It was included here to compare growth rate effects to *P. veronii*. *S. wittichii* strain RW1 is capable of using dibenzofuran (DBF) as sole carbon and energy source (Wittich et al 1992, Yabuuchi et al 2001).

The growth medium for *A. chlorophenolicus* (GM) consisted of (in $g \cdot l^{-1}$): K_2HPO_4, 2.10; KH_2PO_4, 0.40; NH_4NO_3, 0.50; $MgSO_4 \cdot 7H_2O$, 0.20; $CaCl_2 \cdot 2H_2O$, 0.023; and $FeCl_3 \cdot 6H_2O$, 0.002 (Alexander and Lustigman 1966), supplemented with 1 $g \cdot l^{-1}$ yeast extract (GM+YE) as carbon source. Cultures of *A. chlorophenolicus* were incubated at 28°C on a rotary shaker at 180 rpm. Growth medium for *P. veronii* was based on 21C minimal medium (Gerhardt et al 1981), which was supplemented with 5 mM succinate (21CS) as carbon source. Cultures of *P. veronii* were incubated at 30°C on a rotary shaker at 180 rpm. *P. putida* was grown in minimal medium (MM) with 5 mM benzoate as carbon source, the culture was incubated at 30°C and 180 rpm in a rotary shaker. Growth media and conditions for *S. wittichii* have been described

previously (Johnson et al 2011a). All chemicals were of the highest grade purity and were obtained from Sigma Aldrich (Steinhelm, Germany).

Growth rate reduction as function of water availability

The effect of decreasing water potential in the medium on the specific growth rate (μ) was tested by increasing the amount of NaCl (salt stress) or polyethylene glycol (PEG8000, matric stress). Growth media were produced in which the water potential was decreased by 0.25, 0.5, 1.0, 1.5 and 2.5 MPa of the initial water potential. Following the calculations made by Johnson et al (2011) for matric stress, this translated into PEG8000 additions of 139, 203, 295, 366 and 477 g l^{-1} to the growth medium, respectively. For solute stress, this was equivalent to adding 2.9, 5.8, 11.6, 17.4, 29 and 58 g l^{-1} NaCl to the growth medium, respectively. Culture turbidity as the absorbance at 600 nm (OD600) was followed in quadruplate biological replicates over time, from which the maximum specific growth rate (μ_{max}) was calculated by linear regression from ln-transformed absorbance values as a function of time of at least 3 points. The water potential decrease at which the μ_{max} decreased by no more than 20% (i.e., subinhibitory) was chosen as the condition to measure genome-wide gene expression under drought stress compared to control growth media conditions (i.e., without additional PEG8000 or NaCl).

Genome-wide expression analysis of induced drought stress

To measure the effects of drought stress on genome-wide gene expression, we used a 30 min incubation of exponentially growing cultures to subinhibitory concentrations of NaCl or PEG8000 (as defined above), compared to non-supplemented cultures (Table 1). For *A. chlorophenolicus* precultures of 20 ml in 100 ml Erlenmeyer flasks

were grown on GM+YE starting from a single isolated colony that had been freshly grown on an LB plate. For *P. veronii* 1YdBTEX2 precultures we used 21CS medium that had been inoculated with a single colony freshly grown on 21C-agar with toluene supplied through the gas phase. Stationary phase precultures were diluted in quadruplate in 100 ml flasks with the same growth medium to obtain a starting OD600 of 0.02, and growth was followed until cultures reached an OD600 of 0.2. Ten ml of this starter culture was then either twofold diluted into a new 100 ml Erlenmeyer flask containing 10 ml of pre-warmed (28°C for *A.chlorophenolicus* or 30°C for *P. veronii* and *P. putida*) standard medium (GM+YE, 21CS or MM), to 10 ml pre-warmed medium with decreased solute potential by addition of NaCl, or to 10 ml medium with decreased matric potential by addition of PEG8000. Table 1 summarises the final water potential decreases used for each strain. After 30 min further incubation at the same growth temperature and rotary shaking conditions, the cells were collected by vacuum filtration, frozen on filter in liquid nitrogen, which was crushed in an eppendorf tube and stored at -80°C until RNA extraction, as described previously (Johnson et al 2011a, Moreno-Forero and van der Meer 2015).

RNA isolation (hot phenol protocol)

The acid hot phenol method was used to extract RNA from the cells collected on the filter, as described in Johnson et al (2011). The RNA quality was verified using a Nanodrop spectrophotometer (ThermoFisher Scientific) by quantification of the A260/A280 and A260/A230 ratios and by electrophoresis on an Agilent Bioanalyser to detect intact 16S- and 23S-rRNA. RNA was stored at −80°C prior to cDNA labelling.

Microarray hybridisations

Reverse complementary oligonucleotides for *A. chlorophenolicus* A6 and for *P. veronii* 1YdBTEX2 were designed with the software YODA (Nordberg 2005). The parameters for the designed 50-mer probes that target all genes in chromosome and plasmids of A6 and 1YdBTEX2 are summarized in table S1. Every gene was represented by a minimum of three probes, if possible. Oligonucleotides were printed on a 8x15K custom gene microarray (Agilent-024142, NCBI platform GPL 17332) for *A. chlorophenolicus and* on a 8X60K custom gene microarray (Agilent-038776, NCBI platform GPL 20216) for *P. veronii*, using the Agilent custom e-array service (Agilent technologies, Santa Clara, CA).

The procedure for the production, purification and verification of labelled cDNAs, and the hybridization of arrays was performed as described previously (Johnson et al 2011a). Samples were adjusted to have at least 2 pmol of labelled cDNA per array. The AGILENT FEATURE EXTRACTION SOFTWARE (version 10.7.1.1; Agilent technologies, Santa Clara, CA) was used to extract the signal intensities of the probes from the scanned images. The subsequent text data file was then used as input in GeneSpring GX (version 12; Agilent technologies, Santa Clara, CA). Data were quantile normalized by GeneSpring and baseline transformed.

Data analysis

Genome-wide expression data were first analysed independently for each bacterial strain in order to infer genes with statistically significantly different expression under conditions of subinhibitory water stress. All replicas were hierarchically clustered, and variation among replicates and between different conditions was tested by Principal

Component Analysis. Multiple probes were grouped per gene and the mean expression values per gene were compared between control and stress conditions in a t-test with unequal variance (Welch's t-test) to calculate p-values. p-Values were then corrected into false discovery rates (FDRs) using the Benjamini and Hochberg procedure for multiple hypotheses testing. Genes were considered statistically significantly different expressed between the control and stress conditions if the FDR was less than 0.05 and the fold-difference in normalized hybridization signal intensities was higher than 2.

The list of genes differentially expressed under matric and solute stress compared to control conditions for each bacterial species was subsequently analysed by Gene Ontology (GO) terminology (GOConsortium et al 2000). Gene Ontology (GO) terms for genes from strains RW1 and A6 were retrieved using DAVID (Huang da et al 2009), whereas for *P. veronii* we used Blast2go v.2.5 (Conesa et al 2005) using the published assemblage accession number GenBank: AOUH00000000.1 (de Lima-Morales et al 2013). The web-based tool GOEAST (Zheng and Wang 2008) under implementation of the Alexa's algorithm (Alexa et al 2006) was then used to further compare the statistical relevance of the identified groups of differentially expressed genes under either matric or solute stress.

For three-strain visual comparison, the complete list of GO terms associated to *Biological process* was used to construct a simplified network in the open source software Cytoscape (version 3.1), on which the enriched terms identified for each of the three tested strains and conditions were mapped.

Lists with genes statistically significantly different expressed between stress and control conditions were further compared among the three strains by pair-wise BlastP of the encoded proteins. Proteins with reciprocal scores of lower than 10^{-8}, with

similar functional prediction and with extended amino acid overlap were considered orthologues with similar function. Gene order and orientation on the genome of each of the three strains were inspected to infer possible cotranscribed regions that would support coordinated stress induction.

Results and discussion

Comparative growth under induced water stress

The reduction in maximum specific growth rate of the three strains *A. chlorophenolicus* A6, *P. veronii* 1YdBTEX2 and *S. wittichii* RW1 was tested in standard medium by the addition of either salt (NaCl, for solute stress) or PEG8000 (for matric stress). For further comparison, we also included *P. putida* KT2440. Figure 1 shows the normalized maximum specific growth rate as function of decreasing water potential.

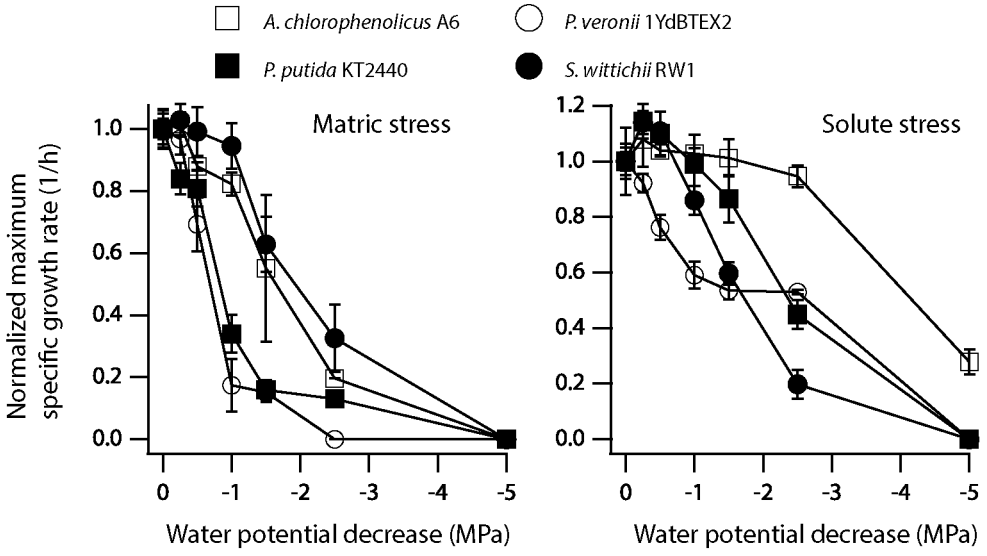

Figure 1. Effect of decreasing water potential by addition of PEG8000 (matric stress) or NaCl (solute stress) on the maximum specific growth rate of *A. chlorophenolicus* A6, *P. putida* KT2440, *P. veronii* 1YdBTEX2 and *S. wittichii* RW1. Maximum specific growth rates normalized compared to control medium (i.e., no additional water potential decrease).

Comparatively speaking, matric stress produced a stronger effect on growth rates of *P. veronii* and *P. putida* than on *S. wittichii* RW1 or *A. chlorophenolicus.* A decrease of -1 MPa in the medium by PEG8000 addition caused a reduction in the growth rate by 80% (Fig. 1A). By contrast, S. *wittichii* RW1 and *A. chlorophenolicus* displayed 20% growth rate reduction at a decrease of -1 MPa through matric potential, whereas only at -2.5 MPa growth rates decreased by 80%. We noted that the turbidity of cultures of *A. chlorophenolicus* A6 exposed to PEG8000 but not to NaCl almost instantly decreased by tenfold (Fig. 2). Upon microscopic inspection it appeared that cells exposed to PEG8000 dramatically reduced their cell volume by an estimated tenfold (Fig. 2), which caused the reduction in culture turbidity. The mechanism for this cell volume reduction is not known but may involve instant water loss from the cell. This can include modifications in the cell wall (Cava and de Pedro 2014), or activation of mechanosensitive channels (Booth et al 2015).

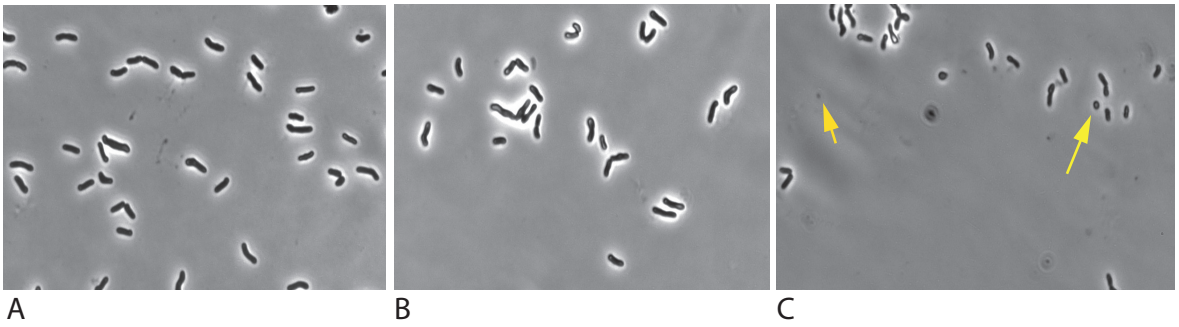

Figure 2. Cell volume changes of *A. chlorophenolicus* A6 upon addition of PEG. A: cells growing in control medium. B: cells after addition of NaCl to -1.5 MPa. C: cells after exposure to PEG8000 to -0.25 MPa. Arrows indicate the smaller cells formed after PEG contact.

Judging from growth rate decrease, *P. veronii* was also the most sensitive strain to decreasing medium water potential by addition of salt, followed by RW1, *P. putida* and *A. chlorophenolicus* (Fig. 1B). For example, at -1 MPa water potential decrease

the growth rate of *P. veronii* reduced by 40%. In contrast, that of RW1 reduced by 20%, whereas strain A6 maintained almost the same growth rate until beyond -2.5 MPa water potential decrease. This showed that strains can react quite differently to water potential decrease induced by either matric or solute stress, suggesting possibly different defence mechanisms against such stresses and different sensitivities of the individually tested strains.

For subsequent study of genome-wide expression, we chose for each strain a decrease in water potential in which the growth rate was only subinhibitorily affected, in order to avoid that the transcriptomes would reflect more the effects of reduced growth rates rather than adaptations to the applied stress *per se*. The choice was somewhat arbritarily placed at approximately 20% growth rate reduction (Table 1). Furthermore, because of the cell volume changes observed in *A. chlorophenolicus* exposed to matric stress, we tested two different matric stress levels (Table 1). In contrast to the growth experiments under decreasing water potential used to calculate μ_{max} (Fig. 1), for the genome-wide analysis we opted for transition exposure of cells (30 min) to the stress condition, which would maximize the detection of differentially expressed genes (Johnson et al 2011a).

Table 1. Imposed conditions for the analysis of matric or solute induced water stress.

Strain	Matric stress (MPa)	Solute stress (MPa)
Arthrobacter chlorophenolicus A6	-0.25 / -1.0	-1.5
Pseudomonas veronii 1YdBTEX2	-0.5	-0.5
Sphingomonas wittichii RW1	-0.25	-0.25

Transcriptional responses to water stress

Figure 3 summarizes the numbers of statistically significantly different expressed genes for the three species under each of the induced stress conditions compared to control incubations. The matric stress level of -0.25 MPa did not produce any significant difference in genome-wide gene expression in *A. chlorophenolicus* and will not be further described in the following text. *S. wittichii* RW1 displayed the highest number of genes whose expression was affected under water stress (equivalent to around 8% of all genes in the genome). Slightly higher numbers (305) were measured upon reduction of water potential by solute than by matric stress (239). A core of 88 genes responded both to matric and solute stress and in the same direction (e.g., higher under solute and higher under matric stress). Changes in water potential also caused an estimated 5% of genes in the *P. veronii* genome to change expression levels (Fig. 3), with slightly lower numbers for solute (148) than for matric-induced stress (187), and with a common core of 66 genes. Clearly lower numbers of genes of *A. chlorophenolicus* exhibited expression differences under matric (37 genes) or solute stress (88 genes), with a common core of only 15 genes. There was, therefore, not a direct correlation between the number of genes with statistically significant expression changes and the deduced sensitivity of strains to water stress from the calculated growth rate reduction (Fig. 1), which might have been expected given that we attempted to impose the same stress level to the cells. In addition, all strains generally resisted the same level of water potential decrease by solute stress better than by matric stress (Fig. 1), but the number of differentially expressed genes under solute stress was higher for *S. wittichii* and *A. chlorophenolicus* A6 than under matric stress at approximately the same growth rate reduction (Fig. 3).

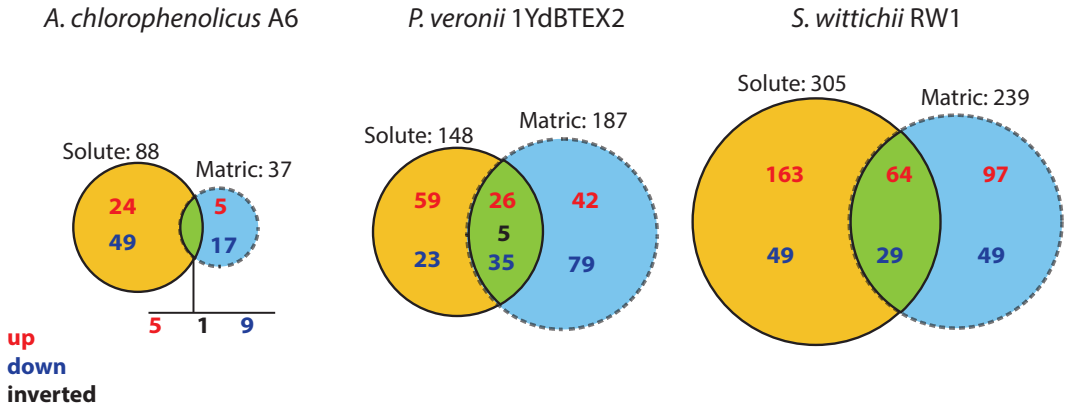

Figure 3. Numbers of statistically significantly different expressed genes under conditions of matric or solute stress for *A. chlorophenolicus* A6, *P. veronii* 1YdBTEX2 or *S. wittichii* RW1 (Venn diagram). Circle area is proportional to the number of genes in category. Numbers inside intersections refer to those genes whose expression changes both under matric or solute stress. Blue numbers represent decreased expression under imposed stress compared to the control; red numbers represent increased expression; black numbers represent different directions of expression under solute versus matric stress.

At the gene level the responses of the three strains seem very diverse and with only few immediate apparent similarities (Table 2, Tables S2-S10). Between the highly expressed genes in solute stress we found that all the strains produce osmoprotectants, all of them as part of several co-localized genes in the same direction that suggest operon-type regulation. This was the case for, e.g., trehalose synthesis in RW1 (Swit_3608-3610, Table S4), choline synthesis for A6 (Achl_3494-3496 and Achl_3686-3689, Table S5 and S7), and transporters of L-proline, glycine and betaine in 1YdBTEX2 (YdB-peg6296-6299, Table S10). It is well known that the production of osmoprotectants is key for osmotic stress control caused by alteration in the water potential (Iturriaga et al 2009, Potts 1994, Roberson and Firestone 1992, Sleator and Hill 2002, Wargo 2013). Fida and collaborators (Fida et al 2012) also found increased expression of genes related to trehalose production in *Sphingomonas* strain LH128 biofilms exposed to conditions of chronic and acute salt stress.

Table 2. Typical examples of protein functions expressed during solute or matric stress in either of the three bacteria.

Condition	Expression	*S. wittichii* RW1	*A. chlorophenolicus* A6	*P. veronii* 1YdBTEX2
Solute stress	up	Putative extracytoplasmic function (ECF) sigma 24 factor, outer membrane proteins, catalase, regulators.	General transporters. Synthesis and transport of osmoprotectant choline.	Osmotically inducible proteins, modulation of ribosomes, cytochromes.
	down	Amino acid metabolism (glycine, glutamate and methionine), lipid metabolism.	Periplasmic receptors, cell wall peptidase, transporters, chaperones.	Metabolism of glycine, porins.
Matric stress	up	RNA polymerase sigma 32 factor, chaperones, cell wall biosynthesis.	Periplasmic binding protein, NADH oxidase.	Lactate dehydrogenases, biosynthesis of alginates, permeases, Fe transport.
	down	Aromatic compound metabolism	Transmembrane transporters, cytochrome, several transcriptional regulators.	Glutamate precursor, chemotaxis response, transporters.
Common to both matric and solute stress	up	Trehalose biosynthesis, cold-shock protein, exopolysaccaride biosynthesis, cytoskeleton proteins, chaperones.	Na^+/H^+ antiporter, Mn^{2+}/Fe^{2+} transporter, glucose-methanol-choline oxidoreductase, catalase.	Outer membrane proteins, Fe transport, L-proline, glycine, betaine transport, catalase
	down	Flagella biosynthesis, TonB receptors.	Flagella synthesis and motility, chaperone DnaJ, GrpE protein, ABC transporter.	Flagella synthesis and motility, chemotaxis, cytochrome.

We also found expression of catalases in all three strains, highly expressed in RW1 and 1YdBTEX2 under solute stress, and expressed both under solute and matric stress in case of strain A6 (Table 2). Catalases protect cells from the damage of reactive oxygen species (ROS) (Kim and Park 2014, Mishra and Imlay 2012), which can be produced in response to water limitation, as shown for e.g., *P. putida* (Gulez et al 2014, Kim and Park 2014). Catalases were also higher expressed in solute-stressed cells of root associated bacteria (Dominguez-Ferreras et al 2006).

Among the genes whose expression was lower under both solute and matric stress in all three strains were almost complete operons for flagella biosynthesis (Table 2, Tables S4, S7, S10, S16). Repression of flagella synthesis has frequently been detected in other bacteria under water stress (Dominguez-Ferreras et al 2006, Fida et al 2012, Freeman et al 2013, Liu et al 2005, Mukhopadhyay et al 2006, Steil et al 2003). It has been proposed that the reason for this is a divergence of cellular energy for stress defence rather than for flagellar production and maintenance (Martinez-Garcia et al 2014b).

Further striking strain-specific gene functions whose expression diminished under water stress included several TonB receptors for strain RW1 (Table S2, S3 and S4), possibly implicated in nutrient scavenging (Lim 2010); or chaperones (Achl_3619-Achl_3620, Table S5, S6, S7) and ABC transporters for *A. chlorophenolicus* (Tables S5, S6, S7). Several genes in an operon for alginate biosynthesis were induced in *P. veronii* under matric stress (YdB-peg2245-2248, Table S9). Alginate is an extracellular polymer that has been implicated in osmotic and matric stress in other *Pseudomonas* species (Chang et al 2007, Freeman et al 2013, Gulez et al 2014, Hay et al 2014).

Gene Ontology Interpretation of changes observed under solute stress

In order to better describe and understand the general behaviour of the three strains under the same stress condition we used GO analysis of the differentially expressed genes. GO analysis provides a verbal account of the functions implicated in processes rather than precise gene names, and can link various individual gene names to biologically relevant processes.

Table 3 summarizes the number of GO terms related to the number of differentially expressed genes under the stress conditions. For RW1 and A6 the GO terms cover at least 54% but in most cases between 60 to 74% of the genes, presenting a reasonable landscape of involved functions. In the case of 1YdBTEX2 between 35 and 55% of differentially expressed genes are covered by GO terms, which is mainly due to the fragmented state of the draft genome. A detailed list of the enriched terms for each strain under either matric or solute stress is shown in Tables S11-S16. Figure 4 shows a simplified comparative tree of the enriched GO terms at the level of *Biological process* under either water stress condition.

Twelve GO terms in the category *Biological process* were enriched among the RW1 genes with lowered expression under solute stress (Fig. 4A). Notably shared among those with the other two species were *flagellar motility* (GO:0001539) and *chemotaxis* (GO:0006935), and two closely related GO terms *flagellar organization* (GO:0044781, for RW1 and 1YdBTEX) and *flagellar assembly* (GO:0044780, for A6). This confirmed the preliminary observation on the individual gene level that flagella biosynthesis and motility functions are highly repressed during water stress (solute and matric stress) in all strains.

Table 3. Numerical account of GO interpreted differentially expressed genes under solute or matric stress.

Strain	**Genome**		**Solute stress**						**Matric stress**					
			Lower expressed genes			**Higher expressed genes**			**Lower expressed genes**			**Higher expressed genes**		
	Estima-ted no. genes	No. genes with GO	No. genes	No. genes with valid GO	No. of GO terms	No. genes	No. genes with valid GO	No. of GO terms	No. genes	No. genes with valid GO	No. of GO terms	No. genes	No. genes with valid GO	No. of GO terms
S. wittichii RW1	5400	3459	73	55	407	227	124	579	73	57	295	161	96	526
A. chlorophenolicus A6	4632	2536	59	44	325	29	20	160	26	17	183	11	8	102
P. veronii 1YdBTEX2	6500	3072	61	31	311	87	30	273	116	49	307	71	28	247

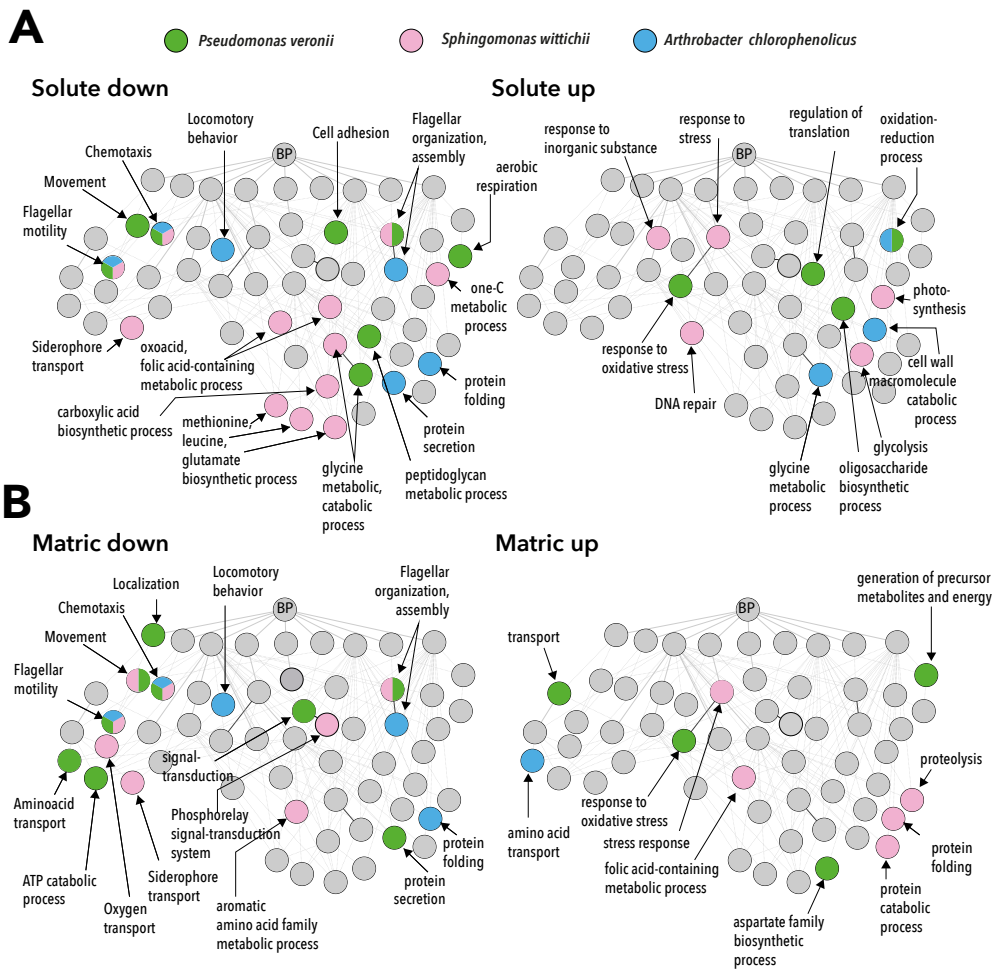

Figure 4. Simplified network of common and strain-specific enriched GO terms (circles) under the hierarchy *Biological process* (BP), derived from the list of differentially expressed genes under conditions of imposed solute (**A**) or matric stress (**B**) for the three used bacteria. Single colors, GO terms specifically enriched for one of the three bacteria only. Mixed colors, shared enriched GO terms.

Other diminished responses to solute stress in RW1 include *methionine* (GO:0009086), *glutamate* (GO:0006537), and *glycine biosynthesis* (GO:0006544). Glycine and glutamate are precursors for the biosynthesis of methionine, which has been indirectly implicated in cellular motility and chemotaxis (Springer et al 1975). Glutamate is also used for osmoprotection by cells (Feehily and Karatzas 2013).

In contrast to RW1, *A. chlorophenolicus* A6 reacted to solute stress by decreasing expression of genes related to *protein folding* (GO:0006457, chaperones Achl_3619-3620, Table S5) and *secretion* (GO:0009306, in flagella operon Achl_2972, 2988, Table S5) but this term seems, from inspection of the genes implicated, more related to flagella biosynthesis. In *P. veronii* the term *peptidoglycan catabolic process* (GO:0009253) was enriched, but the main gene involved (i.e., YdB-peg4827) is located within an operon with other flagellar proteins (Table S8 and S16). Finally, GO term analysis in *1YdBTEX2* pointed to genes involved in *respiration* (Fig. 4A, GO:0009060, YdB0976-0977, Table S8 and S16).

In contrast to gene functions with diminished expression under solute stress there were few common functions with increased expression among the strains. The only shared GO term was *oxidation-reduction process* (GO:0055114) between *A. chlorophenolicus* and *P. veronii.* For A6 the genes in this category may be related to synthesis of osmoprotectant choline, including several operons Achl_3494-3496, Achl_3673-3674 and Achl_3686-3689. In the case of 1YdBTEX2 the genes in this GO category seem more implicated in generation of energy, but this is hard to interpret at this point.

Terms with increased expression under solute stress for RW1 included *glycolysis* (GO:0006096), which may point to the increasing need for biosynthesis precursors. Also the GO term *photosynthesis* (GO:0015979) was enriched, but this term contains

genes with encoded PRC-barrel domains, an Mg-chelatase implied in RNA processing (Lovgren et al 2004), and encoding electron transport (Brusslan and Peterson 2002). Other GO terms for RW1 suggest general stress functions such as, *DNA repair* (GO:0006281), *response to inorganic substance* (GO:0010035) and *response to stress* (GO:0006950, Fig. 4A).

In *P. veronii* the term *response to oxidative stress* (GO:0006979), a child GO term of *response to stress*, was enriched in both solute and matric stress conditions. This term is mainly represented by a catalase that is important in defense against ROS (Kim and Park 2014, Mishra and Imlay 2012). Genes related to trehalose biosynthesis in *P. veronii* are enriched under the GO term *Oligosaccharide biosynthetic process* (GO:0009312, Table S8), which indicates that in addition to transporters of other osmoprotectants, 1YdBTEX2 is probably able to produce trehalose for protection against solute stress.

GO terms enriched under matric stress

Among the genes with diminished expression under matric stress (Fig 4B) we found a series of GO terms related to flagella functions in all three species (i.e., *Flagellar dependent motility, Chemotaxis, Movement, Locomotory behaviour, Localization, Flagellar organization and assembly)*. Similar terms were found in experiments of inoculation in sand with RW1 (Moreno-Forero and van der Meer 2015).

Several TonB receptors and associated GO terms (TonB-dependent sideropore receptor, GO:0015891, Table S11) were enriched in RW1 under matric stress. In addition, several response regulator receivers (Swit_0067, Swit_3186, Swit_3187 and Swit_5296) were enriched under the GO term *Two-component signal transduction system (phosphorelay)* (GO:0000160), indicating specific regulatory

systems to be involved in reaction to matric stress. Further specific GO terms for RW1 included *Oxygen transport* (GO:0015671) and *Metabolism of aromatic amino acids* (GO:0009072), both of which covered genes whose expression was lower upon matric stress.

GO terms of genes with lowered expression under matric stress in strains A6 and 1YdBTEX2 covered *Protein folding* (GO: 0006457, Table S13) and *Protein secretion* (GO:0009306, Table S15), but inspection pointed again to genes within these categories being related to flagella synthesis and export.

Not a single GO term was shared between the strains that covered genes whose expression was increased upon matric stress (Fig. 4). Individual reactions included, for example for RW1, GO terms related to *Response to stress* (GO:0006950, Table S11), which was represented by heat-shock proteins, known to act as chaperones under stress for protein refolding (Sabate et al 2010, Schlesinger 1990). Further terms associated with this idea of the need for protein refolding were *Protein catabolic process* (GO:0030163)*, Protein folding* (GO:0006457) and *Proteolysis* (GO:0006508, Table S11). Individual genes within these GO terms related to energy-dependent proteases, which are known to remove aggregated or misfolded proteins (Koodathingal et al 2009).

Very different GO terms describe increased gene expression of *P. veronii* to matric stress (Table S15). Notably, this included *Responses to oxidative stress* (GO:0006979), a term which was also enriched under solute stress conditions, and others like *Generation of energy* (GO:0006091), *Transport* (GO:0006810) and *Aspartate biosynthetic process* (GO: 0009067). This indicates a variety of different gene functions being activated, suggesting that *P. veronii* needs a considerable amount of energy to cope with matric stress. Quite the contrary, only a single GO

term was enriched among the genes with increased expression of *A. chlorophenolicus* upon matric stress. This term *Amino acid transport* (GO:0006865) may cover exchange of compatible solutes. The fact that matric stress of -0.25 MPa did not elicit any change in gene expresion suggests that *A. chlorophenolicus* can adapt much quicker than the other two strains to changes in water stress caused by matric potential changes.

Stress-induced protein orthologs among the three strains

In order to detect whether direct protein orthologues would exist among the three used strains, which are differentially expressed under either solute or matric induced water stress, we used pair-wise BlastP comparisons between the proteins encoded from the lists of stress-induced genes. At an arbitrary cut-off level of 10^{-8}, similar functional annotation and extended amino acid overlap between pair-wise compared proteins, a number of obvious but also a number of surprising orthologues were detected. As expected from the GO terminology interpretation described above, it was not surprising to find direct orthologous proteins implicated in motility or flagella biosynthesis (Table 4). More intriguing was a set of putative ABC transporters, of which each of the strains possesses multiple copies but which differed between solute and matric imposed water stress (Table 4). Of note is that the *A. chlorophenolicus* orthologous ABC transporters are twice the size of those in *P. veronii* or *S. wittichii*, with both halves showing high homology (Table S17, S18). Along similar lines are a number of ortholog proteins annotated as aldehyde dehydrogenase, of which four paralogs exist in *A. chlorophenolicus* and two in *S. wittichii* RW1 that are all higher expressed under solute imposed water stress.

Table 4. Conserved proteins expressed during solute or matric stress

Annotation	Locus_Pv[1]	Locus-AchI	Locus_Swit
SOLUTE			
ABC transporter related	YdB-peg2154[2] YdB-peg5565 YdB-peg5567	AchI_1181 AchI_4608	Swit_0257 Swit_2743
Aldehyde dehydrogenase	YdB-peg2758	AchI_3686 AchI_1277 AchI_3495 AchI_2799	Swit_0703 Swit_2698
OmpA/MotB domain-containing protein	YdB-peg1841	AchI_2980	Swit_1172 Swit_2278 Swit_2132
Flagellar basal-body rod protein FlgF	YdB-peg1455 YdB-peg4830 YdB-peg1452	AchI_2994	Swit_1267
Flagellar motor switch protein FliM	YdB-peg4804	AchI_2979	Swit_1458
Flagellin-specific chaperone FliS	YdB-peg4820	AchI_2996	Swit_0212
Transglycosylase domain protein	YdB-peg1667	AchI_0996	Swit_2353
MATRIC			
ABC transporter related	YdB-peg2233	AchI_3732 AchI_4608	Swit_0125 Swit_2917
Glucose-methanol-choline oxidoreductase	YdB-peg1057	AchI_3687	Swit_0379
Major facilitator transporter	YdB-peg1473	AchI_0186	Swit_0553
Aldehyde dehydrogenase	YdB-peg1186	AchI_3686	Swit_1880
GntR family transcriptional regulator	YdB-peg777	AchI_3609	Swit_3081
Flagellar basal-body rod modification protein	YdB-peg1453	AchI_2984	Swit_3128

1) Pv, Locus numbering for *P. veronii* 1YdBTEX2; AchI, for *A. chlorophenolicus* A6; Swit, for *S. wittichii* RW1.
2) For full overview of pair-wise conserved proteins, overlaps and e-values, see Table S17,S18.

Interestingly, another pair of orthologous aldehyde dehydrogenases is differentially expressed under matric-imposed water stress. Further orthologous proteins differentially expressed among the three strains include an outer membrane protein (OmpA/MotB, Table 4) and a transglycosylase, which may be implicated in modifying the peptidoglycan in the cell wall (Cava and de Pedro 2014). Under matric stress we further found an orthologous GntR-type regulator protein and glucose-methanol-

choline oxidoreductase (Table 4), which may be implicated in biosynthesis of the compatible solute choline. Further pair-wise but not triple orthologs included a variety of membrane receptors, glycosyltransferases, response regulators or catalase (*S. wittichii* versus *P. veronii*), ROK family protein (*S. wittichii* and *A. chlorophenolicus*), or permease and aminotransferase (*P. veronii* and *A. chlorophenolicus*, Table S17, S18).

Concluding remarks

In conclusion, we found that the three strains had different sensitivities to diminishing water potential as a result of NaCl or PEG8000 addition. *P. veronii* 1YdBTEX2 was the most sensitive bacterium, *S. wittichii* RW1 showed better tolerance and *A. chlorophenolicus* A6 was the strain that maintained the most stable growth rate across a range of decreasing water potentials. The macroscopic change in growth rate correlated only to a certain extent with the numbers of genes with statistically significantly altered expression under subinhibitory water stress. For example, *A. chlorophenolicus* displayed the lowest number of genes with altered expression to water stress (Fig. 3) and was the most resistant of the three to maintaining growth rate at decreasing water potential (Fig. 1). However, *S. wittichii* was relatively speaking more resistant than *P. veronii* to decreasing water potential in terms of growth rate changes, but displayed similar numbers of genes altering expression under subinhibitory water stress exposure. One could argue that expression modulation of fewer genes is advantageous for rapid adaptation to a stress and will require less energy from the cell, which explains the behavior seen for *A. chlorophenolicus*. On the other hand, having more genes at hand to modulate behavior could be advantageous (but more costly) and could explain the behaviour of

S. wittichii and *P. veronii*. It also needs to be said that induced water stress by addition of solutes or PEG8000 is not the same as the conditions experienced in e.g., dry sand or soil (Moreno-Forero and van der Meer 2015), and therefore, the adaptations displayed by the strains may not be quite the same as their expected behaviour in soil (as a previous study on *S. wittichii* demonstrated).

An initial goal of this study was to discern strain-specific as well as common reactions of cells to induced water stress, with the idea that this might help to understand and predict their behaviour in a contaminated soil environment. *A. chlorophenolicus* has been considered a typical soil bacterium (Westerberg et al 2000), in contrast to *S. wittichii*, even though the latter strain can grow easily on contamination with DBF in sand (Coronado et al 2015, Moreno-Forero and van der Meer 2015). Interestingly, the only common reaction to all strains was an instant decrease of expression of genes implicated in flagellar motility, which was apparent both from individual gene inspection as well as GO terms. It has been suggested that the absence of flagella contributes to increase the energy available for the cell to cope with environmental stresses (Martinez-Garcia et al 2014b). Controlled experimental inoculations with *S. wittichii* revealed that expression of genes implicated in motility is decreased in sand with DBF, indicating that control of motility is an important behavioral trait for survival in semi-dry conditions (Moreno-Forero and van der Meer 2015). All strains also responded to some extent by increasing expression of genes for synthesis or transport of osmoprotectants and compatible solutes, but which were different among the strains. Finally, all three strains similarly expressed a set of conserved genes to either solute or matric stress (Table 4), the most striking of which are annotated as ABC transporters and aldehyde dehydrogenases. These may constitute an important conserved core of the reactions to decreasing water potential, but their precise

functions are currently ill-defined. Previous mutation analysis of *S. wittichii* (Roggo et al 2013) showed that at least some of those are important for growth and survival in sand, such as Swit_0379, Swit_0703, Swit_1172, Swit_1458, Swit_2353, Swit_2917 and Swit_3081. In contrast, inactivation of Swit_0553 actually increased survival of *S. wittichii* in sand (Roggo et al 2013).

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Table S1. Parameters applied to the program YODA to obtain probes for microarray

	A6	1YdBTEX2
% probes with stringent parameters	99.50%	96%
Number of non overlaping probes by gen.	1-3	1-30
Maximun % of identity to non-target sequences	70%	80%
Maximun consecutives matches to non-target sequences	15	15
Range of melting temperature	8°C	10°C
Range of GC content	12%	15%
remaining probes with less stringent parameters	0.30%	2.86%
Maximun % of identity to non-target sequences	80%	80%
Maximun consecutives matches to non-target sequences	15	19
Range of melting temperature	15°C	15°C
Range of GC content	30%	30%
% remaining genes with no probes	0.20%	1.14%
Total probes designed	13589	40757
aditional positive control probes	7	10
Format microarray Agilent	8 X 15000	8 X 60000

Table S2. **Complete list of *Sphingomonas wittichii* RW1 genes whose expression levels responded to water stress with sodium chloride but not PEG8000 (FDR<0.05, fold difference>2.0)**

yellow/beige: possibly same operon

Gene ID	Sodium chloride expression fold-change	Regulation type	Gene product
Swit_0067	3.1	down	response regulator receiver protein
Swit_0212	2.3	down	flagellin-specific chaperone FliS-like protein
Swit_0224	2.4	down	hypothetical protein
Swit_0226	2.4	down	hypothetical protein
Swit_0228	2.1	down	acetolactate synthase
Swit_0231	2.2	down	putative adenylate/guanylate cyclase
Swit_0393	2.1	down	hypothetical protein
Swit_0428	2.0	down	hypothetical protein
Swit_0429	2.6	down	periplasmic-like protein
Swit_0430	2.2	down	PepSY-associated TM helix domain-containing protein
Swit_0535	2.1	down	TonB-dependent receptor
Swit_0588	2.2	down	hypothetical protein
Swit_0589	2.3	down	hypothetical protein
Swit_0628	2.5	down	type II secretion system protein E
Swit_0657	2.6	down	glutamate synthase (NADPH) large subunit
Swit_0658	2.4	down	hypothetical protein
Swit_0958	2.2	down	butyryl-CoA:acetate CoA transferase
Swit_0959	2.1	down	3-oxoacid CoA-transferase, A subunit
Swit_1141	3.0	down	acyltransferase 3
Swit_1217	2.6	down	hypothetical protein
Swit_1218	2.2	down	TPR repeat-containing protein
Swit_1219	3.8	down	hypothetical protein
Swit_1264	2.2	down	flagellar basal body P-ring protein
Swit_1266	2.1	down	FlgG flagellar basal body rod protein FlgG
Swit_1267	2.2	down	flagellar basal-body rod protein FlgF
Swit_1268	2.4	down	flagellar basal body FlaE domain-containing protein
Swit_1270	2.5	down	flagellar basal-body rod protein FlgC
Swit_1286	2.3	down	flagellar hook-basal body complex subunit FliE
Swit_1293	2.3	down	flagellar basal body-associated protein FliL
Swit_1309	2.3	down	nucleotidyl transferase
Swit_1458	2.8	down	flagellar motor switch protein FliM
Swit_2365	2.9	down	SCP-like extracellular
Swit_2399	2.8	down	methionine synthase
Swit_2400	3.0	down	methionine synthase (B12-dependent)
Swit_2401	2.8	down	methylenetetrahydrofolate reductase
Swit_2402	2.4	down	ArsR family transcription regulator
Swit_2475	8.5	down	electron transport protein SCO1/SenC
Swit_2476	2.9	down	hypothetical protein
Swit_2477	8.7	down	TonB-dependent receptor
Swit_2559	7.7	down	acyl-CoA synthetase
Swit_2674	2.8	down	S-adenosyl-L-homocysteine hydrolase
Swit_2694	2.0	down	gcvT glycine cleavage system aminomethyltransferase T
Swit_2696	2.2	down	glycine dehydrogenase subunit 1
Swit_2697	2.0	down	glycine dehydrogenase subunit 2
Swit_2698	2.2	down	hypothetical protein
Swit_2703	2.2	down	TetR family transcriptional regulator
Swit_2880	2.0	down	hypothetical protein
Swit_3008	4.2	down	hypothetical protein
Swit_3127	2.1	down	hypothetical protein
Swit_3176	2.3	down	hypothetical protein
Swit_3187	2.8	down	response regulator receiver protein
Swit_3190	2.1	down	polypeptide-transport-associated domain-containing protein
Swit_3191	2.0	down	filamentous haemagglutinin outer membrane protein
Swit_3192	2.3	down	TPR repeat-containing protein
Swit_3373	2.4	down	S-adenosylmethionine synthetase
Swit_3380	2.0	down	glycosyl transferase family protein
Swit_3748	5.3	down	TonB family protein
Swit_3750	6.3	down	TonB-dependent receptor
Swit_3778	2.3	down	hypothetical protein
Swit_3862	2.0	down	radical SAM domain-containing protein
Swit_3903	5.4	down	diacylglycerol kinase, catalytic region
Swit_3904	4.8	down	hypothetical protein
Swit_3907	3.4	down	fatty acid hydroxylase
Swit_3908	2.3	down	hypothetical protein
Swit_3986	2.1	down	Glu/Leu/Phe/Val dehydrogenase, dimerisation region
Swit_4025	3.1	down	TonB-dependent siderophore receptor
Swit_4088	2.6	down	TonB-dependent receptor
Swit_4375	2.2	down	hypothetical protein
Swit_4433	2.0	down	hydrolase
Swit_4494	2.0	down	3-isopropylmalate dehydrogenase
Swit_4696	14.2	down	TonB-dependent receptor
Swit_4750	2.6	down	hypothetical protein
Swit_4784	2.3	down	glutamate synthase (NADPH)
Swit_4785	3.7	down	dihydroneopterin aldolase
Swit_4786	3.4	down	5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase
Swit_4788	2.2	down	methylitaconate delta2-delta3-isomerase
Swit_5151	2.1	down	dihydrolipoamide dehydrogenase
Swit_5152	2.2	down	pyruvate dehydrogenase complex dihydrolipoamide acetyltransferase

Gene ID	Sodium chloride expression fold-change	Regulation type	Gene product
Swit_0072	7.3	up	hypothetical protein
Swit_0097	12.0	up	MarR family transcriptional regulator
Swit_0129	4.4	up	hypothetical protein
Swit_0142	3.7	up	phospholipase D
Swit_0150	4.4	up	hypothetical protein
Swit_0183	2.1	up	hypothetical protein
Swit_0239	12.6	up	hypothetical protein
Swit_0257	2.2	up	ABC transporter related
Swit_0442	2.6	up	toluene tolerance family protein
Swit_0443	4.0	up	hypothetical protein
Swit_0444	2.4	up	hypothetical protein
Swit_0490	6.6	up	hypothetical protein
Swit_0524	5.8	up	hypothetical protein
Swit_0545	16.1	up	hypothetical protein
Swit_0565	2.3	up	type IV pilus assembly PilZ
Swit_0617	2.7	up	peptidase M48, Ste24p
Swit_0619	3.2	up	heat shock protein Hsp20
Swit_0636	3.9	up	hypothetical protein
Swit_0654	10.2	up	CRP/FNR family transcription regulator
Swit_0655	7.3	up	hypothetical protein
Swit_0656	2.7	up	hypothetical protein
Swit_0689	2.6	up	hypothetical protein
Swit_0692	2.8	up	extracellular solute-binding protein
Swit_0693	2.4	up	pyrrolo-quinoline quinone
Swit_0696	6.8	up	hypothetical protein
Swit_0703	2.8	up	aldehyde dehydrogenase
Swit_0773	4.0	up	hypothetical protein
Swit_0784	6.3	up	hypothetical protein
Swit_0785	10.9	up	hypothetical protein
Swit_0858	13.3	up	hemerythrin HHE cation binding domain-containing protein
Swit_0862	2.3	up	phasin family protein
Swit_0873	4.2	up	PBP family phospholipid-binding protein
Swit_0878	16.7	up	hypothetical protein
Swit_0944	2.2	up	sarcosine oxidase, delta subunit, heterotetrameric
Swit_0953	6.0	up	CRP/FNR family transcription regulator
Swit_0954	6.6	up	hypothetical protein
Swit_0975	2.1	up	muconate cycloisomerase
Swit_0995	6.4	up	PRC-barrel domain-containing protein
Swit_1090	10.8	up	diacylglycerol kinase, catalytic region
Swit_1146	3.8	up	ATP-dependent protease La
Swit_1147	5.0	up	molecular chaperone (small heat shock protein)-like protein
Swit_1151	7.9	up	ROK family protein
Swit_1172	5.2	up	OmpA/MotB domain-containing protein
Swit_1247	26.8	up	hypothetical protein
Swit_1263	3.1	up	rod binding-like protein
Swit_1294	2.2	up	hypothetical protein
Swit_1295	9.2	up	hypothetical protein
Swit_1320	3.6	up	DGPFAETKE family protein
Swit_1359	2.0	up	CutA1 divalent ion tolerance protein
Swit_1361	7.2	up	hypothetical protein
Swit_1363	2.1	up	Serine-type D-Ala-D-Ala carboxypeptidase
Swit_1388	2.6	up	hypothetical protein
Swit_1412	4.9	up	glutathione-dependent formaldehyde-activating, GFA
Swit_1507	17.4	up	17 kDa surface antigen
Swit_1509	9.3	up	17 kDa surface antigen
Swit_1625	2.1	up	glucokinase
Swit_1688	2.1	up	TonB-dependent receptor
Swit_1816	2.8	up	protein of unknown function DUF306, Meta and HsIJ
Swit_1881	2.1	up	cyclase family protein
Swit_1916	2.9	up	endonuclease/exonuclease/phosphatase
Swit_1919	2.1	up	hypothetical protein
Swit_1920	2.4	up	patatin
Swit_1937	2.7	up	uroporphyrinogen III synthase HEM4
Swit_2027	3.4	up	hypothetical protein
Swit_2076	2.9	up	dihydrodipicolinate reductase
Swit_2132	2.0	up	peptidoglycan-associated lipoprotein
Swit_2218	2.2	up	hypothetical protein
Swit_2245	3.7	up	glutathione S-transferase domain-containing protein
Swit_2278	3.6	up	OmpA/MotB domain-containing protein
Swit_2294	2.3	up	alcohol dehydrogenase
Swit_2322	10.3	up	OmpA/MotB domain-containing protein
Swit_2324	13.2	up	hypothetical protein
Swit_2325	19.3	up	hypothetical protein
Swit_2334	16.0	up	hypothetical protein
Swit_2335	3.9	up	hypothetical protein
Swit_2353	2.2	up	hypothetical protein
Swit_2360	2.4	up	inositol monophosphate
Swit_2422	13.2	up	transglycosylase-associated protein
Swit_2423	2.9	up	hypothetical protein
Swit_2433	3.7	up	AsmA family protein
Swit_2540	8.5	up	response regulator receiver protein
Swit_2551	2.2	up	hypothetical protein

Gene ID	Sodium chloride expression fold-change	Regulation type	Gene product
Swit_2576	4.2	up	glycosidase, PH1107-related
Swit_2577	6.3	up	glycosyl transferase, group 1
Swit_2714	2.0	up	gmk guanylate kinase
Swit_2734	2.0	up	pseudo
Swit_2742	2.6	up	hypothetical protein
Swit_2743	3.0	up	ABC transporter related
Swit_2744	3.4	up	hypothetical protein
Swit_2745	3.8	up	ABC-type uncharacterized transport system auxillary component-like protein
Swit_2750	15.6	up	hypothetical protein
Swit_2778	2.5	up	hypothetical protein
Swit_2779	4.6	up	ferritin, Dps family protein
Swit_2867	2.3	up	extradiol ring-cleavage dioxygenase III subunit B
Swit_2868	3.6	up	hypothetical protein
Swit_2933	2.3	up	superoxide dismutase
Swit_2951	2.1	up	acyltransferase 3
Swit_2974	3.1	up	LytTR family two component transcriptional regulator
Swit_3015	4.8	up	pyrophosphate-dependent phosphofructokinase
Swit_3114	2.4	up	hypothetical protein
Swit_3193	16.8	up	hypothetical protein
Swit_3279	4.6	up	short-chain dehydrogenase/reductase SDR
Swit_3430	8.6	up	hypothetical protein
Swit_3455	3.6	up	large conductance mechanosensitive channel protein
Swit_3475	8.0	up	citrate transporter
Swit_3488	3.1	up	glucan biosynthesis protein G
Swit_3489	3.4	up	glucosyltransferase MdoH
Swit_3529	2.3	up	methyltransferase type 12
Swit_3568	5.4	up	hypothetical protein
Swit_3596	11.2	up	hypothetical protein
Swit_3608	3.4	up	HAD family hydrolase
Swit_3609	8.3	up	glycoside hydrolase 15-related
Swit_3610	3.9	up	alpha,alpha-trehalose-phosphate synthase (UDP-forming)
Swit_3612	3.1	up	Mg chelatase, subunit ChlI
Swit_3613	14.0	up	hypothetical protein
Swit_3687	2.2	up	lytic transglycosylase, catalytic
Swit_3690	2.9	up	hypothetical protein
Swit_3739	2.1	up	chloride channel, core
Swit_3740	2.6	up	ArsR family transcriptional regulator
Swit_3741	2.6	up	1-Cys peroxiredoxin
Swit_3793	3.5	up	membrane protein involved in aromatic hydrocarbon degradation
Swit_3794	4.0	up	hypothetical protein
Swit_3803	4.7	up	ErfK/YbiS/YcfS/YnhG family protein
Swit_3804	2.3	up	peptidase M23B
Swit_3813	2.5	up	hypothetical protein
Swit_3835	2.0	up	peptidase S8 and S53, subtilisin, kexin, sedolisin
Swit_3836	2.7	up	ECF subfamily RNA polymerase sigma-24 factor
Swit_3837	2.5	up	putative transmembrane anti-sigma factor
Swit_3839	2.4	up	hypothetical protein
Swit_3851	4.8	up	hypothetical protein
Swit_3855	2.2	up	hypothetical protein
Swit_3863	2.1	up	fumarylacetoacetate hydrolase
Swit_3864	2.4	up	homogentisate 1,2-dioxygenase
Swit_3865	2.5	up	4-hydroxyphenylpyruvate dioxygenase
Swit_3867	2.0	up	hypothetical protein
Swit_3893	3.5	up	GCN5-related N-acetyltransferase
Swit_3911	3.0	up	exodeoxyribonuclease III
Swit_3923	11.4	up	hypothetical protein
Swit_3924	7.2	up	ECF subfamily RNA polymerase sigma-24 factor
Swit_3925	3.5	up	two-component response regulator
Swit_3926	3.0	up	signal transduction histidine kinase
Swit_3927	17.6	up	entericidin EcnAB
Swit_3978	2.1	up	exonuclease of the beta-lactamase fold involved in RNA processing-like protein
Swit_3979	2.4	up	ATP-dependent DNA ligase
Swit_3981	8.8	up	Ku family containing protein
Swit_3982	4.5	up	DNA ligase D
Swit_3983	2.9	up	antibiotic biosynthesis monooxygenase
Swit_4023	2.3	up	rod shape-determining protein MreB
Swit_4044	2.4	up	hypothetical protein
Swit_4096	13.3	up	hemerythrin HHE cation binding domain-containing protein
Swit_4121	2.7	up	AMP-dependent synthetase and ligase
Swit_4122	3.1	up	hypothetical protein
Swit_4125	2.1	up	TonB-dependent receptor
Swit_4209	8.0	up	glutathione-dependent formaldehyde-activating, GFA
Swit_4391	4.8	up	transglutaminase domain-containing protein
Swit_4399	2.3	up	hypothetical protein
Swit_4400	2.3	up	hypothetical protein
Swit_4401	2.1	up	hypothetical protein
Swit_4432	10.3	up	PAS/PAC sensor hybrid histidine kinase
Swit_4475	14.8	up	hypothetical protein
Swit_4497	3.7	up	hypothetical protein
Swit_4499	2.0	up	phosphomannose isomerase-like protein
Swit_4523	4.1	up	glycosyl transferase family protein
Swit_4524	3.3	up	hypothetical protein
Swit_4526	2.3	up	glycosyl transferase family protein

Gene ID	Sodium chloride expression fold-change	Regulation type	Gene product
Swit_4527	3.7	up	polysaccharide biosynthesis protein
Swit_4528	3.5	up	non-specific protein-tyrosine kinase
Swit_4529	2.4	up	hypothetical protein Swit_4529
Swit_4530	3.4	up	O-antigen polymerase
Swit_4531	4.6	up	polysaccharide export protein
Swit_4532	16.2	up	sugar transferase
Swit_4533	4.3	up	glycoside hydrolase family protein
Swit_4541	2.8	up	SAF domain-containing protein
Swit_4545	2.9	up	phosphoribosyltransferase
Swit_4546	4.0	up	hypothetical protein
Swit_4547	5.1	up	hypothetical protein
Swit_4548	2.5	up	asparagine synthesis
Swit_4564	5.3	up	hypothetical protein
Swit_4573	4.2	up	hypothetical protein
Swit_4575	4.5	up	hypothetical protein
Swit_4582	5.0	up	hypothetical protein
Swit_4590	6.6	up	hypothetical protein
Swit_4591	8.3	up	hypothetical protein
Swit_4637	2.2	up	protein of unknown function, zinc metallopeptidase putative
Swit_4647	3.6	up	hypothetical protein
Swit_4648	10.1	up	hypothetical protein
Swit_4671	3.3	up	hypothetical protein
Swit_4743	2.3	up	hypothetical protein
Swit_4746	2.9	up	hypothetical protein
Swit_4747	3.3	up	hypothetical protein
Swit_4749	7.9	up	transglycosylase-associated protein
Swit_4764	5.3	up	hypothetical protein
Swit_4765	6.7	up	hypothetical protein
Swit_4863	15.1	up	hypothetical protein
Swit_5007	2.1	up	type IV secretion/conjugal transfer ATPase
Swit_5011	2.0	up	pseudogene
Swit_5012	5.4	up	regulatory protein, LuxR
Swit_5045	2.1	up	TonB-dependent receptor
Swit_5237	5.6	up	hypothetical protein
Swit_5248	10.5	up	catalase
Swit_5249	6.8	up	ankyrin
Swit_5250	2.8	up	histidine kinase
Swit_5270	7.5	up	signal transduction histidine kinase
Swit_5274	2.7	up	protease Do
Swit_5275	3.0	up	hypothetical protein
Swit_5282	3.5	up	DNA ligase D
Swit_5283	5.3	up	exodeoxyribonuclease III Xth
Swit_5284	3.8	up	hypothetical protein
Swit_5285	8.7	up	putative DNA topoisomerase I
Swit_5286	6.7	up	alpha/beta hydrolase domain-containing protein
Swit_5287	7.6	up	hypothetical protein
Swit_5290	13.6	up	MscS mechanosensitive ion channel
Swit_5291	7.9	up	short-chain dehydrogenase/reductase SDR
Swit_5292	6.9	up	hypothetical protein
Swit_5310	5.8	up	hypothetical protein
Swit_5311	5.2	up	catalase
Swit_5312	3.2	up	short-chain dehydrogenase/reductase SDR
Swit_5313	9.0	up	2Fe-2S iron-sulfur cluster binding domain-containing protein
Swit_5314	2.9	up	molybdopterin dehydrogenase, FAD-binding
Swit_5315	6.9	up	xanthine dehydrogenase, molybdenum binding subunit apoprotein
Swit_5339	2.0	up	cold-shock DNA-binding protein family protein
Swit_5343	3.9	up	PRC-barrel
Swit_5344	13.2	up	cyclase/dehydrase
Swit_5345	17.2	up	alcohol dehydrogenase
Swit_5347	6.6	up	hypothetical protein
Swit_5348	6.3	up	hypothetical protein
Swit_5396	4.1	up	response regulator receiver protein

Table S3. **Complete list of *Sphingomonas wittichii* RW1 genes whose expression levels responded to water stress with PEG8000 but not sodium chloride (FDR<0.05, fold difference>2.0)**

yellow/beige: possibly same operon

Gene ID	PEG expression fold-change	Regulation type	Gene product
Swit_0067	4.4	down	response regulator receiver protein
Swit_0211	2.1	down	hypothetical protein
Swit_0212	2.8	down	flagellin-specific chaperone FliS-like protein
Swit_0213	2.3	down	flagellar hook-associated 2 domain-containing protein
Swit_0224	2.4	down	hypothetical protein
Swit_0226	2.7	down	hypothetical protein
Swit_0393	2.0	down	hypothetical protein
Swit_0418	2.0	down	glutamate dehydrogenase
Swit_0429	2.3	down	periplasmic-like protein
Swit_0535	2.6	down	TonB-dependent receptor
Swit_0587	2.6	down	metallophosphoesterase
Swit_0588	2.1	down	hypothetical protein
Swit_0604	2.1	down	sporulation domain-containing protein
Swit_0628	2.8	down	type II secretion system protein E
Swit_0914	3.3	down	TonB-dependent receptor
Swit_0993	2.2	down	hypothetical protein
Swit_1141	2.3	down	acyltransferase 3
Swit_1143	2.4	down	RND efflux system outer membrane lipoprotein
Swit_1145	2.8	down	EmrB/QacA family drug resistance transporter
Swit_1219	3.4	down	hypothetical protein
Swit_1264	2.3	down	flagellar basal body P-ring protein
Swit_1267	2.2	down	flagellar basal-body rod protein FlgF
Swit_1268	2.3	down	flagellar basal body FlaE domain-containing protein
Swit_1270	2.7	down	flagellar basal-body rod protein FlgC
Swit_1286	2.5	down	flagellar hook-basal body complex subunit FliE
Swit_1293	2.7	down	flagellar basal body-associated protein FliL
Swit_1380	2.0	down	tetratricopeptide TPR_4
Swit_1879	5.7	down	putative indolepyruvate oxidoreductase subunit B
Swit_1880	3.9	down	indolepyruvate ferredoxin oxidoreductase
Swit_1881	3.9	down	cyclase family protein
Swit_1904	2.3	down	malic enzyme
Swit_1918	2.2	down	nitrogen regulatory protein P-II
Swit_2365	3.1	down	SCP-like extracellular
Swit_2634	3.2	down	benzoate 1,2-dioxygenase, alpha subunit
Swit_2703	2.1	down	TetR family transcriptional regulator
Swit_2765	3.7	down	hypothetical protein
Swit_3008	6.4	down	hypothetical protein
Swit_3081	2.3	down	GntR family transcriptional regulator
Swit_3083	2.4	down	ornithine cyclodeaminase
Swit_3084	3.0	down	5-oxopent-3-ene-1,2,5-tricarboxylate decarboxylase
Swit_3086	3.3	down	gentisate 1 2-dioxygenase-like protein
Swit_3087	3.9	down	2,4-dihydroxyhept-2-ene-1,7-dioic acid aldolase
Swit_3088	3.9	down	2-oxo-hepta-3-ene-1,7-dioic acid hydratase
Swit_3089	2.7	down	hypothetical protein
Swit_3090	2.7	down	acyl-CoA dehydrogenase type 2
Swit_3091	3.7	down	TonB-dependent receptor
Swit_3092	4.3	down	hypothetical protein
Swit_3093	4.3	down	hypothetical protein
Swit_3094	2.8	down	glyoxalase/bleomycin resistance protein/dioxygenase
Swit_3095	3.6	down	hypothetical protein
Swit_3127	2.5	down	hypothetical protein
Swit_3144	3.7	down	TonB-dependent receptor
Swit_3186	3.2	down	response regulator receiver modulated CheB methylesterase
Swit_3187	3.9	down	response regulator receiver protein
Swit_3190	2.2	down	polypeptide-transport-associated domain-containing protein
Swit_3192	2.3	down	TPR repeat-containing protein
Swit_3392	2.2	down	MarR family transcriptional regulator
Swit_3607	2.4	down	hypothetical protein
Swit_3778	2.5	down	hypothetical protein
Swit_3862	2.2	down	radical SAM domain-containing protein
Swit_3863	4.8	down	fumarylacetoacetate hydrolase
Swit_3864	3.6	down	homogentisate 1,2-dioxygenase
Swit_3865	3.4	down	4-hydroxyphenylpyruvate dioxygenase
Swit_3866	4.1	down	MarR family transcriptional regulator
Swit_4025	2.8	down	TonB-dependent siderophore receptor
Swit_4121	2.5	down	AMP-dependent synthetase and ligase
Swit_4122	3.1	down	hypothetical protein
Swit_4263	2.1	down	gentisate 1 2-dioxygenase-like protein
Swit_4274	2.0	down	TonB-dependent receptor
Swit_4412	2.5	down	type IV pilus assembly PilZ
Swit_4433	2.3	down	hydrolase
Swit_4576	2.3	down	DNA methylase N-4/N-6 domain-containing protein
Swit_4750	4.3	down	hypothetical protein
Swit_4779	3.0	down	hypothetical protein
Swit_4781	2.5	down	TonB-dependent receptor
Swit_5296	2.3	down	response regulator receiver protein
Swit_5297	3.5	down	sec-independent protein translocase protein TatC
Swit_5298	2.3	down	hypothetical protein

Gene ID	PEG expression fold-change	Regulation type	Gene product
Swit_0060	3.7	up	RNA polymerase factor sigma-32
Swit_0061	3.9	up	ribosomal large subunit pseudouridine synthase D
Swit_0074	2.3	up	peptide methionine sulfoxide reductase
Swit_0097	3.6	up	MarR family transcriptional regulator
Swit_0103	2.1	up	hypothetical protein
Swit_0124	2.6	up	hypothetical protein
Swit_0125	3.3	up	ABC transporter related
Swit_0126	2.8	up	ATP-dependent Clp protease, ATP-binding subunit clpA
Swit_0129	2.3	up	hypothetical protein
Swit_0263	4.1	up	hypothetical protein
Swit_0312	2.2	up	amidohydrolase 3
Swit_0379	2.1	up	glucose-methanol-choline oxidoreductase
Swit_0390	2.4	up	ATP-dependent protease La
Swit_0442	2.4	up	toluene tolerance family protein
Swit_0490	5.9	up	hypothetical protein
Swit_0524	2.7	up	hypothetical protein
Swit_0540	2.1	up	TonB-dependent receptor
Swit_0545	5.2	up	hypothetical protein
Swit_0553	2.8	up	major facilitator transporter
Swit_0586	2.0	up	homoserine O-succinyltransferase
Swit_0619	6.2	up	heat shock protein Hsp20
Swit_0636	3.7	up	hypothetical protein
Swit_0773	4.9	up	hypothetical protein
Swit_0780	2.1	up	branched-chain alpha-keto acid dehydrogenase E2 component
Swit_0782	3.0	up	3-methyl-2-oxobutanoate dehydrogenase (2-methylpropanoyl-transferring)
Swit_0785	3.0	up	hypothetical protein
Swit_0944	2.2	up	sarcosine oxidase, delta subunit, heterotetrameric
Swit_1090	3.2	up	diacylglycerol kinase, catalytic region
Swit_1107	8.8	up	hypothetical protein
Swit_1146	4.8	up	ATP-dependent protease La
Swit_1147	3.0	up	molecular chaperone (small heat shock protein)-like protein
Swit_1151	4.8	up	ROK family protein
Swit_1152	7.1	up	RND family efflux transporter MFP subunit
Swit_1153	15.6	up	hydrophobe/amphiphile efflux-1 (HAE1) family protein
Swit_1154	2.4	up	RND efflux system outer membrane lipoprotein
Swit_1172	5.7	up	OmpA/MotB domain-containing protein
Swit_1177	3.1	up	S-formylglutathione hydrolase
Swit_1178	4.8	up	glyoxalase/bleomycin resistance protein/dioxygenase
Swit_1179	3.3	up	S-(hydroxymethyl)glutathione dehydrogenase
Swit_1246	2.0	up	hypothetical protein
Swit_1247	8.0	up	hypothetical protein
Swit_1248	2.5	up	methylated-DNA--protein-cysteine methyltransferase
Swit_1250	3.6	up	chaperone protein DnaK
Swit_1263	3.5	up	rod binding-like protein
Swit_1363	2.7	up	Serine-type D-Ala-D-Ala carboxypeptidase
Swit_1412	2.1	up	glutathione-dependent formaldehyde-activating, GFA
Swit_1471	3.2	up	formate dehydrogenase family accessory protein FdhD
Swit_1472	2.5	up	formate dehydrogenase delta subunit
Swit_1473	3.6	up	formate dehydrogenase, alpha subunit
Swit_1939	3.4	up	peptidase M48, Ste24p
Swit_2026	2.3	up	isochorismatase hydrolase
Swit_2076	2.4	up	dihydrodipicolinate reductase
Swit_2144	2.6	up	branched-chain alpha-keto acid dehydrogenase E1 component
Swit_2145	2.2	up	branched-chain alpha-keto acid dehydrogenase E1 component
Swit_2156	3.3	up	luciferase family protein
Swit_2281	2.8	up	HxlR family transcriptional regulator
Swit_2315	2.2	up	3-isopropylmalate dehydratase, small subunit
Swit_2318	2.8	up	L-carnitine dehydratase/bile acid-inducible protein F
Swit_2355	2.1	up	band 7 protein
Swit_2423	4.2	up	hypothetical protein
Swit_2424	2.2	up	plasmid maintenance system killer
Swit_2425	2.3	up	XRE family plasmid maintenance system antidote protein
Swit_2447	4.0	up	NADH:flavin oxidoreductase/NADH oxidase
Swit_2465	2.8	up	hypothetical protein
Swit_2490	2.0	up	GreA/GreB family elongation factor
Swit_2527	2.1	up	hypothetical protein
Swit_2551	2.0	up	hypothetical protein
Swit_2682	2.6	up	thioredoxin
Swit_2816	2.5	up	methionine-R-sulfoxide reductase
Swit_2867	5.8	up	extradiol ring-cleavage dioxygenase III subunit B
Swit_2868	2.5	up	hypothetical protein
Swit_2902	2.1	up	integral membrane protein TerC
Swit_2914	2.0	up	FeS assembly SUF system protein
Swit_2917	2.1	up	FeS assembly ATPase SufC
Swit_2918	2.1	up	cysteine desulfurase activator complex subunit SufB
Swit_2951	2.2	up	acyltransferase 3
Swit_2974	2.9	up	LytTR family two component transcriptional regulator
Swit_3107	2.1	up	excinuclease ABC subunit A
Swit_3128	3.0	up	ATPase
Swit_3223	2.7	up	hypothetical protein

Gene ID	PEG expression fold-change	Regulation type	Gene product
Swit_3279	2.3	up	short-chain dehydrogenase/reductase SDR
Swit_3375	9.5	up	chaperonin Cpn10
Swit_3376	9.7	up	chaperonin GroEL
Swit_3457	4.0	up	glutathione S-transferase domain-containing protein
Swit_3463	3.6	up	cell wall hydrolase, SleB
Swit_3475	2.8	up	citrate transporter
Swit_3489	2.4	up	glucosyltransferase MdoH
Swit_3568	3.0	up	hypothetical protein
Swit_3608	2.2	up	HAD family hydrolase
Swit_3609	3.9	up	glycoside hydrolase 15-related
Swit_3610	2.5	up	alpha,alpha-trehalose-phosphate synthase (UDP-forming)
Swit_3667	2.8	up	thioredoxin domain-containing protein
Swit_3813	2.1	up	hypothetical protein
Swit_3855	2.1	up	hypothetical protein
Swit_3890	2.2	up	hemimethylated DNA binding protein
Swit_3913	2.1	up	peptidase M23B
Swit_3923	2.8	up	hypothetical protein
Swit_3986	2.2	up	Glu/Leu/Phe/Val dehydrogenase, dimerisation region
Swit_4023	4.1	up	rod shape-determining protein MreB
Swit_4056	2.2	up	phosphoadenosine phosphosulfate reductase
Swit_4072	2.3	up	hypothetical protein
Swit_4073	2.2	up	hypothetical protein
Swit_4075	2.2	up	transglutaminase domain-containing protein
Swit_4076	3.0	up	hypothetical protein
Swit_4096	4.0	up	hemerythrin HHE cation binding domain-containing protein
Swit_4098	2.4	up	lytic transglycosylase, catalytic
Swit_4099	2.8	up	uracil-DNA glycosylase superfamily protein
Swit_4209	2.8	up	glutathione-dependent formaldehyde-activating, GFA
Swit_4376	3.3	up	ATP-dependent protease peptidase subunit
Swit_4377	4.1	up	ATP-dependent protease ATP-binding subunit
Swit_4378	3.4	up	arsenate reductase and related
Swit_4470	2.8	up	hypothetical protein
Swit_4509	2.4	up	membrane protease FtsH catalytic subunit
Swit_4523	3.8	up	glycosyl transferase family protein
Swit_4524	2.7	up	hypothetical protein
Swit_4525	2.3	up	glycosyl transferase family protein
Swit_4526	2.8	up	glycosyl transferase family protein
Swit_4527	3.9	up	polysaccharide biosynthesis protein
Swit_4528	3.9	up	non-specific protein-tyrosine kinase
Swit_4529	2.4	up	hypothetical protein Swit_4529
Swit_4530	2.9	up	O-antigen polymerase
Swit_4531	3.1	up	polysaccharide export protein
Swit_4532	11.9	up	sugar transferase
Swit_4533	3.2	up	glycoside hydrolase family protein
Swit_4537	2.4	up	metallophosphoesterase
Swit_4541	4.3	up	SAF domain-containing protein
Swit_4545	4.1	up	phosphoribosyltransferase
Swit_4546	7.4	up	hypothetical protein
Swit_4547	5.6	up	hypothetical protein
Swit_4582	2.8	up	hypothetical protein
Swit_4590	3.5	up	hypothetical protein
Swit_4591	4.6	up	hypothetical protein
Swit_4619	2.0	up	hypothetical protein
Swit_4645	4.9	up	translation initiation factor 1
Swit_4646	6.1	up	hypothetical protein
Swit_4671	4.0	up	hypothetical protein
Swit_4721	2.1	up	response regulator receiver protein
Swit_4724	2.1	up	DEAD/DEAH box helicase domain-containing protein
Swit_4747	2.7	up	hypothetical protein
Swit_4763	5.1	up	hypothetical protein
Swit_4785	2.0	up	dihydroneopterin aldolase
Swit_4788	2.1	up	methylitaconate delta2-delta3-isomerase
Swit_4790	2.0	up	methylcitrate synthase
Swit_4791	2.7	up	2,3-dimethylmalate lyase
Swit_4856	2.0	up	GumN family protein
Swit_4937	2.8	up	cobyrinic acid a,c-diamide synthase
Swit_5003	2.1	up	P-type conjugative transfer protein VirB9
Swit_5005	2.3	up	TrbL/VirB6 plasmid conjugal transfer protein
Swit_5007	3.8	up	type IV secretion/conjugal transfer ATPase
Swit_5008	2.3	up	type IV secretory pathway, VirB3 family protein
Swit_5009	2.1	up	VIRB2 type IV secretion fmaily protein
Swit_5010	2.6	up	lytic transglycosylase, catalytic
Swit_5011	3.6	up	pseudogene
Swit_5012	8.1	up	regulatory protein, LuxR
Swit_5282	2.0	up	DNA ligase D
Swit_5287	2.1	up	hypothetical protein
Swit_5306	2.2	up	heat shock protein DnaJ domain-containing protein
Swit_5307	3.0	up	hypothetical protein Swit_5307
Swit_5339	13.6	up	cold-shock DNA-binding protein family protein
Swit_5351	4.0	up	heat shock protein 90
Swit_5397	4.0	up	2-octaprenylphenol hydroxylase

Table S4. Complete list of *Sphingomonas wittichii* RW1 genes whose expression levels responded to water stress with sodium chloride or PEG8000 (FDR<0.05, fold difference>2.0)

yellow/beige: possibly same operon

Gene ID	Sodium chloride expression fold-change	PEG8000 expression fold-change	Regulation type	Gene product
Swit_0067	3.1	4.4	down	response regulator receiver protein
Swit_0212	2.3	2.8	down	flagellin-specific chaperone FliS-like protein
Swit_0224	2.4	2.4	down	hypothetical protein
Swit_0226	2.4	2.7	down	hypothetical protein
Swit_0393	2.1	2.0	down	hypothetical protein
Swit_0429	2.6	2.3	down	periplasmic-like protein
Swit_0535	2.1	2.6	down	TonB-dependent receptor
Swit_0588	2.2	2.1	down	hypothetical protein
Swit_0628	2.5	2.8	down	type II secretion system protein E
Swit_1141	3.0	2.3	down	acyltransferase 3
Swit_1219	3.8	3.4	down	hypothetical protein
Swit_1264	2.2	2.3	down	flagellar basal body P-ring protein
Swit_1267	2.2	2.2	down	flagellar basal-body rod protein FlgF
Swit_1268	2.4	2.3	down	flagellar basal body FlaE domain-containing protein
Swit_1270	2.5	2.7	down	flagellar basal-body rod protein FlgC
Swit_1286	2.3	2.5	down	flagellar hook-basal body complex subunit FliE
Swit_1293	2.3	2.7	down	flagellar basal body-associated protein FliL
Swit_2365	2.9	3.1	down	SCP-like extracellular
Swit_2703	2.2	2.1	down	TetR family transcriptional regulator
Swit_3008	4.2	6.4	down	hypothetical protein
Swit_3127	2.1	2.5	down	hypothetical protein
Swit_3187	2.8	3.9	down	response regulator receiver protein
Swit_3190	2.1	2.2	down	polypeptide-transport-associated domain-containing protein
Swit_3192	2.3	2.3	down	TPR repeat-containing protein
Swit_3778	2.3	2.5	down	hypothetical protein
Swit_3862	2.0	2.2	down	radical SAM domain-containing protein
Swit_4025	3.1	2.8	down	TonB-dependent siderophore receptor
Swit_4433	2.0	2.3	down	hydrolase
Swit_4750	2.6	4.3	down	hypothetical protein

Gene ID	Sodium chloride expression fold-change	PEG8000 expression fold-change	Regulation type	Gene product
Swit_0097	12.0	3.6	up	MarR family transcriptional regulator
Swit_0129	4.4	2.3	up	hypothetical protein
Swit_0442	2.6	2.4	up	toluene tolerance family protein
Swit_0490	6.6	5.9	up	hypothetical protein
Swit_0524	5.8	2.7	up	hypothetical protein
Swit_0545	16.1	5.2	up	hypothetical protein
Swit_0619	3.2	6.2	up	heat shock protein Hsp20
Swit_0636	3.9	3.7	up	hypothetical protein
Swit_0773	4.0	4.9	up	hypothetical protein
Swit_0785	10.9	3.0	up	hypothetical protein
Swit_0944	2.2	2.2	up	sarcosine oxidase, delta subunit, heterotetrameric
Swit_1090	10.8	3.2	up	diacylglycerol kinase, catalytic region
Swit_1146	3.8	4.8	up	ATP-dependent protease La
Swit_1147	5.0	3.0	up	molecular chaperone (small heat shock protein)-like protein
Swit_1151	7.9	4.8	up	ROK family protein
Swit_1172	5.2	5.7	up	OmpA/MotB domain-containing protein
Swit_1247	26.8	8.0	up	hypothetical protein
Swit_1263	3.1	3.5	up	rod binding-like protein
Swit_1363	2.1	2.7	up	Serine-type D-Ala-D-Ala carboxypeptidase
Swit_1412	4.9	2.1	up	glutathione-dependent formaldehyde-activating, GFA
Swit_2076	2.9	2.4	up	dihydrodipicolinate reductase
Swit_2551	2.2	2.0	up	hypothetical protein
Swit_2867	2.3	5.8	up	extradiol ring-cleavage dioxygenase III subunit B
Swit_2868	3.6	2.5	up	hypothetical protein
Swit_2951	2.1	2.2	up	acyltransferase 3
Swit_2974	3.1	2.9	up	LytTR family two component transcriptional regulator
Swit_3279	4.6	2.3	up	short-chain dehydrogenase/reductase SDR
Swit_3475	8.0	2.8	up	citrate transporter
Swit_3489	3.4	2.4	up	glucosyltransferase MdoH
Swit_3568	5.4	3.0	up	hypothetical protein
Swit_3608	3.4	2.2	up	HAD family hydrolase
Swit_3609	8.3	3.9	up	glycoside hydrolase 15-related
Swit_3610	3.9	2.5	up	alpha,alpha-trehalose-phosphate synthase (UDP-forming)

Gene ID	Sodium chloride expression fold-change	PEG8000 expression fold-change	Regulation type	Gene product
Swit_3813	2.5	2.1	up	hypothetical protein
Swit_3855	2.2	2.1	up	hypothetical protein
Swit_3923	11.4	2.8	up	hypothetical protein
Swit_4023	2.3	4.1	up	rod shape-determining protein MreB
Swit_4096	13.3	4.0	up	hemerythrin HHE cation binding domain-containing protein
Swit_4209	8.0	2.8	up	glutathione-dependent formaldehyde-activating, GFA
Swit_4523	4.1	3.8	up	glycosyl transferase family protein
Swit_4524	3.3	2.7	up	hypothetical protein
Swit_4526	2.3	2.8	up	glycosyl transferase family protein
Swit_4527	3.7	3.9	up	polysaccharide biosynthesis protein
Swit_4528	3.5	3.9	up	non-specific protein-tyrosine kinase
Swit_4529	2.4	2.4	up	hypothetical protein Swit_4529
Swit_4530	3.4	2.9	up	O-antigen polymerase
Swit_4531	4.6	3.1	up	polysaccharide export protein
Swit_4532	16.2	11.9	up	sugar transferase
Swit_4533	4.3	3.2	up	glycoside hydrolase family protein
Swit_4541	2.8	4.3	up	SAF domain-containing protein
Swit_4545	2.9	4.1	up	phosphoribosyltransferase
Swit_4546	4.0	7.4	up	hypothetical protein
Swit_4547	5.1	5.6	up	hypothetical protein
Swit_4582	5.0	2.8	up	hypothetical protein
Swit_4590	6.6	3.5	up	hypothetical protein
Swit_4591	8.3	4.6	up	hypothetical protein
Swit_4671	3.3	4.0	up	hypothetical protein
Swit_4747	3.3	2.7	up	hypothetical protein
Swit_5007	2.1	3.8	up	type IV secretion/conjugal transfer ATPase
Swit_5011	2.0	3.6	up	pseudogene
Swit_5012	5.4	8.1	up	regulatory protein, LuxR
Swit_5282	3.5	2.0	up	DNA ligase D
Swit_5287	7.6	2.1	up	hypothetical protein
Swit_5339	2.0	13.6	up	cold-shock DNA-binding protein family protein

Table S5. Complete list of *Arthrobacter chlorophenolicus* A6 genes whose expression levels responded to water stress with sodium chloride but not PEG8000 (FDR<0.05, fold difference>2.0)

yellow/beige: possibly same operon

Gene ID	Sodium chloride expression fold-change	Regulation type	Gene product
Achl_0040	2.9	down	amine oxidase
Achl_0111	2.7	down	hypothetical protein Achl_0111
Achl_0493	2.1	down	hypothetical protein Achl_0493
Achl_0964	2.7	down	Na+/solute symporter
Achl_1074	2.4	down	hypothetical protein Achl_1074
Achl_1075	2.1	down	thiamine pyrophosphate protein central region
Achl_1179	7.9	down	multiple sugar-binding periplasmic receptor
Achl_1180	7.1	down	Monosaccharide-transporting ATPase
Achl_1181	3.9	down	ABC transporter related
Achl_1182	2.8	down	ROK family protein
Achl_1183	2.3	down	Nitrilase/cyanide hydratase and apolipoprotein N-acyltransferase
Achl_1186	2.5	down	amine oxidase
Achl_1210	2.4	down	basic membrane lipoprotein
Achl_1277	2.7	down	Aldehyde Dehydrogenase
Achl_1530	2.5	down	sodium:dicarboxylate symporter
Achl_1759	2.1	down	Pseudogene
Achl_1985	2.6	down	glycerol kinase
Achl_2187	2.0	down	hypothetical protein Achl_2187
Achl_2302	2.2	down	General substrate transporter
Achl_2333	2.4	down	cold-shock DNA-binding domain protein
Achl_2798	2.7	down	4-aminobutyrate aminotransferase
Achl_2799	2.4	down	Aldehyde Dehydrogenase
Achl_2904	2.3	down	Transketolase central region
Achl_2968	2.1	down	RNA polymerase, sigma 28 subunit, FliA/WhiG subfamily
Achl_2972	2.5	down	flagellar biosynthesis protein FlhA
Achl_2979	3.0	down	surface presentation of antigens (SPOA) protein
Achl_2980	2.3	down	OmpA/MotB domain protein
Achl_2981	3.0	down	MotA/TolQ/ExbB proton channel
Achl_2982	3.0	down	flagellar FlbD family protein
Achl_2983	4.6	down	protein of unknown function DUF1078 domain protein
Achl_2984	4.2	down	flagellar hook capping protein
Achl_2986	4.2	down	NLP/P60 protein
Achl_2988	2.4	down	ATPase, FliI/YscN family
Achl_2989	2.6	down	hypothetical protein Achl_2989
Achl_2990	2.1	down	flagellar motor switch protein FliG
Achl_2991	5.9	down	flagellar M-ring protein FliF
Achl_2992	2.2	down	flagellar hook-basal body complex subunit FliE
Achl_2993	5.6	down	flagellar basal-body rod protein FlgC
Achl_2994	9.0	down	flagellar basal-body rod protein FlgB
Achl_2995	2.6	down	hypothetical protein Achl_2995
Achl_2996	2.2	down	flagellar protein FliS
Achl_2998	2.3	down	flagellin domain protein
Achl_2999	3.5	down	FlgN family protein
Achl_3000	2.8	down	flagellar hook-associated protein FlgK
Achl_3001	3.2	down	flagellar hook-associated protein 3
Achl_3100	2.2	down	phospho-2-dehydro-3-deoxyheptonate aldolase
Achl_3256	2.6	down	hypothetical protein Achl_3256
Achl_3612	2.2	down	2-oxo-hepta-3-ene-1,7-dioic acid hydratase
Achl_3618	2.9	down	transcriptional regulator, MerR family
Achl_3619	3.1	down	chaperone DnaJ domain protein
Achl_3620	2.9	down	GrpE protein
Achl_3621	2.0	down	chaperone protein DnaK
Achl_3812	2.0	down	putative transcriptional regulator, GntR family
Achl_3893	2.1	down	amino acid permease-associated region
Achl_3982	2.0	down	hypothetical protein Achl_3982
Achl_3988	2.3	down	thioredoxin reductase
Achl_4384	2.1	down	beta-lactamase domain protein
Achl_4385	2.6	down	hypothetical protein Achl_4385
Achl_4608	2.2	down	ABC transporter related

Gene ID	Sodium chloride expression fold-change	Regulation type	Gene product
Achl_0070	5.6	up	hypothetical protein Achl_0070
Achl_0071	3.5	up	putative lipoprotein
Achl_0082	2.0	up	protein of unknown function DUF1185
Achl_0105	2.7	up	hypothetical protein Achl_0105
Achl_0485	2.3	up	hypothetical protein Achl_0485
Achl_0518	7.4	up	General substrate transporter
Achl_0666	3.8	up	peptidase M14 carboxypeptidase A
Achl_0996	5.8	up	Transglycosylase domain protein
Achl_1348	2.9	up	citrate/H+ symporter, CitMHS family
Achl_1840	5.0	up	sulphate transporter
Achl_2561	2.5	up	major facilitator superfamily MFS_1
Achl_3027	4.7	up	Na+/H+ antiporter NhaA
Achl_3045	2.1	up	hypothetical protein Achl_3045
Achl_3070	4.5	up	Mn2+/Fe2+ transporter, NRAMP family
Achl_3148	2.1	up	hypothetical protein Achl_3148
Achl_3226	2.0	up	hypothetical protein Achl_3226
Achl_3365	2.5	up	catalase/peroxidase HPI
Achl_3469	2.1	up	FAD dependent oxidoreductase
Achl_3480	2.5	up	peptidase S1 and S6 chymotrypsin/Hap
Achl_3494	5.5	up	glucose-methanol-choline oxidoreductase
Achl_3495	6.2	up	Aldehyde Dehydrogenase
Achl_3496	6.5	up	choline/carnitine/betaine transporter
Achl_3514	2.2	up	protein tyrosine phosphatase, receptor type, F (predicted)-like protein
Achl_3673	2.5	up	Aldehyde Dehydrogenase
Achl_3674	2.2	up	amino acid permease-associated region
Achl_3686	2.7	up	Aldehyde Dehydrogenase
Achl_3687	2.0	up	glucose-methanol-choline oxidoreductase
Achl_3689	2.6	up	Rieske (2Fe-2S) domain protein
Achl_4576	2.2	up	Beta-ketoacyl synthase

Table S6. **Complete list of *Arthrobacter chlorophenolicus* A6 genes whose expression levels responded to water stress with PEG8000 but not sodium chloride (FDR<0.05, fold difference>2.0)**

yellow/beige: possibly same operon

Gene ID	PEG expression fold-change	Regulation type	Gene product
Achl_0186	2.5	down	major facilitator superfamily MFS_1
Achl_0211	2.6	down	protein of unknown function DUF125 transmembrane
Achl_0589	2.4	down	histidine ammonia-lyase
Achl_0753	7.2	down	putative secreted lipoprotein
Achl_0754	11.1	down	nuclear export factor GLE1
Achl_0755	9.0	down	hypothetical protein Achl_0755
Achl_1616	2.2	down	cytochrome bd ubiquinol oxidase subunit I
Achl_1986	4.6	down	major intrinsic protein
Achl_1988	2.4	down	transcriptional regulator, DeoR family
Achl_2536	2.0	down	lipolytic enzyme, G-D-S-L family
Achl_2982	2.9	down	flagellar FlbD family protein
Achl_2984	3.7	down	flagellar hook capping protein
Achl_2990	2.0	down	flagellar motor switch protein FliG
Achl_2995	2.7	down	hypothetical protein Achl_2995
Achl_2996	2.6	down	flagellar protein FliS
Achl_2998	4.6	down	flagellin domain protein
Achl_3609	2.9	down	transcriptional regulator, GntR family
Achl_3619	3.0	down	chaperone DnaJ domain protein
Achl_3620	2.8	down	GrpE protein
Achl_3666	2.5	down	hypothetical protein Achl_3666
Achl_4607	2.8	down	Monosaccharide-transporting ATPase
Achl_4608	4.4	down	ABC transporter related
Achl_4609	3.3	down	periplasmic binding protein/LacI transcriptional regulator
Achl_4610	3.6	down	transcriptional regulator, LacI family
Achl_4619	2.1	down	hypothetical protein Achl_4619
Achl_4621	2.0	down	extracellular solute-binding protein family 1

Gene ID	PEG expression fold-change	Regulation type	Gene product
Achl_0913	2.2	up	NLP/P60 protein
Achl_0988	2.2	up	NADH:flavin oxidoreductase/NADH oxidase
Achl_3027	2.5	up	Na+/H+ antiporter NhaA
Achl_3070	2.3	up	Mn2+/Fe2+ transporter, NRAMP family
Achl_3365	2.0	up	catalase/peroxidase HPI
Achl_3686	4.0	up	Aldehyde Dehydrogenase
Achl_3687	2.0	up	glucose-methanol-choline oxidoreductase
Achl_3688	2.4	up	amino acid permease-associated region
Achl_3732	3.0	up	ABC transporter related
Achl_3735	3.6	up	periplasmic binding protein
Achl_4385	2.2	up	hypothetical protein Achl_4385

Table S7. Complete list of *Arthrobacter chlorophenolicus* A6 genes whose expression levels responded to water stress with PEG8000 and sodium chloride (FDR<0.05, fold difference>2.0)

yellow/beige: possibly same operon

Gene ID	Sodium chloride expression fold-change	Regulation type	PEG8000 expression fold-change	Regulation type	Gene product
Achl_2982	2.99	down	2.86	down	flagellar FlbD family protein
Achl_2984	4.22	down	3.68	down	flagellar hook capping protein
Achl_2990	2.15	down	2.02	down	flagellar motor switch protein FliG
Achl_2995	2.55	down	2.69	down	hypothetical protein Achl_2995
Achl_2996	2.24	down	2.64	down	flagellar protein FliS
Achl_2998	2.25	down	4.56	down	flagellin domain protein
Achl_3619	3.10	down	3.00	down	chaperone DnaJ domain protein
Achl_3620	2.88	down	2.82	down	GrpE protein
Achl_4608	2.21	down	4.37	down	ABC transporter related
Achl_3027	4.68	up	2.48	up	Na+/H+ antiporter NhaA
Achl_3070	4.49	up	2.31	up	Mn2+/Fe2+ transporter, NRAMP family
Achl_3365	2.54	up	2.03	up	catalase/peroxidase HPI
Achl_3686	2.67	up	4.01	up	Aldehyde Dehydrogenase
Achl_3687	2.02	up	2.03	up	glucose-methanol-choline oxidoreductase
Achl_4385	2.61	down	2.19	up	hypothetical protein Achl_4385

Table S8. **Complete list of *Pseudomonas veronii* 1YdBTEX2 genes whose expression levels responded to water stress with sodium chloride but not PEG8000 (FDR<0.05, fold difference>2.0)**

yellow/beige: possibly same operon

Gene ID	Sodium chloride expression fold-change	Regulation type	Gene product
YdB-peg215	2.6	down	Outer membrane porin, OprD family
YdB-peg976	2.2	down	Cytochrome O ubiquinol oxidase subunit III (EC 1.10.3.-)
YdB-peg977	2.3	down	Cytochrome O ubiquinol oxidase subunit I (EC 1.10.3.-)
YdB-peg978	2.2	down	Cytochrome O ubiquinol oxidase subunit II (EC 1.10.3.-)
YdB-peg1452	2.3	down	Flagellar hook protein FlgE
YdB-peg1453	2.3	down	Flagellar basal-body rod modification protein FlgD
YdB-peg1454	2.7	down	Flagellar basal-body rod protein FlgC
YdB-peg1455	3.4	down	Flagellar basal-body rod protein FlgB
YdB-peg1766	2.6	down	hypothetical protein
YdB-peg1811	2.1	down	Ribosomal protein S12p Asp88 (E. coli) methylthiotransferase
YdB-peg2054	2.0	down	Tricarboxylate porin OpdH
YdB-peg2145	2.2	down	hypothetical protein
YdB-peg2179	2.5	down	C4-type zinc finger protein, DksA/TraR family
YdB-peg2417	2.3	down	Methyl-accepting chemotaxis protein
YdB-peg2972	2.0	down	YgjD/Kae1/Qri7 family, required for threonylcarbamoyladenosine (t(6)A) formation in tRNA
YdB-peg3015	2.3	down	RNA polymerase sigma factor for flagellar operon
YdB-peg3016	2.3	down	Chemotaxis regulator - transmits chemoreceptor signals to flagelllar motor components CheY
YdB-peg3076	2.2	down	Cobalt-zinc-cadmium resistance protein
YdB-peg3121	2.3	down	FIG00953950: hypothetical protein
YdB-peg3140	2.3	down	FIG00958406: hypothetical protein
YdB-peg3294	2.1	down	FIG00956593: hypothetical protein
YdB-peg3295	2.1	down	Cell division protein FtsH (EC 3.4.24.-)
YdB-peg3297	2.1	down	hypothetical protein
YdB-peg3453	2.0	down	Positive regulator of CheA protein activity (CheW)
YdB-peg3457	2.1	down	Flagellar motor rotation protein MotA
YdB-peg3611	2.7	down	Nucleoside-binding outer membrane protein
YdB-peg3937	2.1	down	FIG00954271: hypothetical protein
YdB-peg3995	6.2	down	Glycine cleavage system H protein
YdB-peg3996	3.2	down	Glycine dehydrogenase [decarboxylating] (glycine cleavage system P protein) (EC 1.4.4.2)
YdB-peg3997	2.8	down	L-serine dehydratase (EC 4.3.1.17)
YdB-peg3998	2.6	down	Aminomethyltransferase (glycine cleavage system T protein) (EC 2.1.2.10)
YdB-peg4055	2.3	down	Acyl-CoA dehydrogenase, type 2, C-terminal domain
YdB-peg4157	2.5	down	Omega-amino acid--pyruvate aminotransferase (EC 2.6.1.18)
YdB-peg4190	2.1	down	Translation initiation factor SUI1-related protein
YdB-peg4803	2.0	down	Flagellar motor switch protein FliN
YdB-peg4804	2.4	down	Flagellar motor switch protein FliM
YdB-peg4805	2.4	down	Flagellar biosynthesis protein FliL
YdB-peg4811	2.1	down	Flagellum-specific ATP synthase FliI
YdB-peg4815	2.4	down	Flagellar hook-basal body complex protein FliE
YdB-peg4820	2.4	down	Flagellar biosynthesis protein FliS
YdB-peg4821	2.1	down	Flagellar hook-associated protein FliD
YdB-peg4822	2.3	down	Flagellin protein FlaG
YdB-peg4823	2.1	down	Flagellin protein FlaB
YdB-peg4825	2.4	down	Flagellar hook-associated protein FlgL
YdB-peg4826	2.4	down	Flagellar hook-associated protein FlgK
YdB-peg4827	2.6	down	Flagellar protein FlgJ [peptidoglycan hydrolase] (EC 3.2.1.-)
YdB-peg4828	2.2	down	Flagellar P-ring protein FlgI
YdB-peg4829	2.2	down	Flagellar L-ring protein FlgH
YdB-peg4830	2.7	down	Flagellar basal-body rod protein FlgG
YdB-peg4831	2.3	down	Flagellar basal-body rod protein FlgF
YdB-peg5136	2.3	down	hypothetical protein
YdB-peg5139	2.3	down	FIG00965007: hypothetical protein
YdB-peg5506	2.1	down	Flagellar basal-body P-ring formation protein FlgA
YdB-peg5710	2.2	down	FIG00963370: hypothetical protein
YdB-peg6082	2.0	down	FIG00955680: hypothetical protein
YdB-peg6145	2.0	down	FIG00638366: hypothetical protein
YdB-peg6223	2.1	down	hypothetical protein
YdB-peg6295	2.7	down	Ornithine decarboxylase (EC 4.1.1.17) / Arginine decarboxylase (EC 4.1.1.19)
YdB-peg6307	2.3	down	Outer membrane porin, OprD family
YdB-peg6427	3.4	down	FIG00954582: hypothetical protein
YdB-peg6428	2.9	down	hypothetical protein

Gene ID	Sodium chloride expression fold-change	Regulation type	Gene product
YdB-peg556	2.6	up	hypothetical protein
YdB-peg794	2.1	up	Polymyxin resistance protein ArnT, undecaprenyl phosphate-alpha-L-Ara4N transferase; Melittin resistance protein PqaB
YdB-peg1195	2.2	up	MaoC-like domain protein
YdB-peg1359	2.1	up	Glutathione S-transferase, unnamed subgroup 2 (EC 2.5.1.18)
YdB-peg1410	2.0	up	hypothetical protein
YdB-peg1444	2.7	up	Transcriptional regulatory protein PhoP
YdB-peg1445	3.8	up	outer membrane protein H1
YdB-peg1482	2.6	up	NAD-dependent glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12)
YdB-peg1483	2.0	up	RNA polymerase sigma-70 factor, ECF subfamily
YdB-peg1484	2.3	up	hypothetical protein
YdB-peg1550	2.1	up	Ribosome hibernation protein YhbH
YdB-peg1667	3.5	up	FIG00954962: hypothetical protein
YdB-peg1698	2.0	up	Glucose dehydrogenase, PQQ-dependent (EC 1.1.5.2)
YdB-peg1813	2.0	up	NADH:flavin oxidoreductases, Old Yellow Enzyme family
YdB-peg1841	14.9	up	Outer membrane lipoprotein omp16 precursor
YdB-peg1842	7.2	up	FIG00953405: hypothetical protein
YdB-peg1843	2.1	up	FIG00958830: hypothetical protein
YdB-peg2176	2.7	up	FIG00955717: hypothetical protein
YdB-peg2255	2.1	up	alginate o-acetyltransferase AlgF
YdB-peg2260	2.2	up	2-methylaconitate isomerase
YdB-peg2496	2.1	up	Ferrous iron transport periplasmic protein EfeO, contains peptidase-M75 domain and (frequently) cupredoxin-like domain
YdB-peg2497	3.0	up	Ferrous iron transport peroxidase EfeB
YdB-peg2498	2.5	up	Ferrous iron transport periplasmic protein EfeO, contains peptidase-M75 domain and (frequently) cupredoxin-like domain
YdB-peg2758	2.1	up	Aldehyde dehydrogenase (EC 1.2.1.3)
YdB-peg2850	2.3	up	Probable transmembrane protein
YdB-peg2900	4.4	up	hypothetical protein
YdB-peg2902	2.6	up	Alpha-amylase (EC 3.2.1.1)
YdB-peg2948	2.7	up	ABC transporter, periplasmic spermidine putrescine-binding protein PotD (TC 3.A.1.11.1)
YdB-peg2965	3.1	up	Serine protein kinase (prkA protein), P-loop containing
YdB-peg2966	3.3	up	UPF0229 protein YeaH
YdB-peg2967	2.9	up	Stage V sporulation protein involved in spore cortex synthesis (SpoVR)
YdB-peg2988	3.1	up	Stress induced hydrophobic peptide
YdB-peg3206	2.4	up	RNA polymerase sigma factor
YdB-peg3255	19.8	up	Osmotically inducible protein Y precursor
YdB-peg3361	3.1	up	FIG00953225: hypothetical protein
YdB-peg3472	2.0	up	Butyryl-CoA dehydrogenase (EC 1.3.99.2)
YdB-peg3645	15.7	up	FIG00958237: hypothetical protein
YdB-peg3652	2.3	up	FIG00956492: hypothetical protein
YdB-peg3656	10.9	up	Deblocking aminopeptidase (EC 3.4.11.-) @ Cyanophycinase 2 (EC 3.4.15.6)
YdB-peg3657	17.8	up	Cyanophycin synthase (EC 6.3.2.29)(EC 6.3.2.30)
YdB-peg3658	12.6	up	Asparagine synthetase [glutamine-hydrolyzing] (EC 6.3.5.4)
YdB-peg3663	2.6	up	Fumarate hydratase class II (EC 4.2.1.2)
YdB-peg3936	4.2	up	FIG00954474: hypothetical protein
YdB-peg3942	5.9	up	FIG00959696: hypothetical protein
YdB-peg4082	2.1	up	hypothetical protein
YdB-peg4178	2.1	up	3-ketoacyl-CoA thiolase (EC 2.3.1.16) @ Acetyl-CoA acetyltransferase (EC 2.3.1.9)
YdB-peg4359	2.5	up	Pyruvate oxidase [ubiquinone, cytochrome] (EC 1.2.2.2)
YdB-peg4588	2.9	up	FIG00963541: hypothetical protein
YdB-peg4719	7.1	up	FIG00953718: hypothetical protein
YdB-peg4720	3.2	up	FIG00956804: hypothetical protein
YdB-peg4723	4.5	up	Osmotically inducible protein C
YdB-peg4736	2.2	up	Carbonic anhydrase (EC 4.2.1.1)
YdB-peg4740	2.2	up	Cytochrome c oxidase polypeptide I (EC 1.9.3.1)
YdB-peg4741	2.0	up	Cytochrome oxidase biogenesis protein Cox11-CtaG, copper delivery to Cox1
YdB-peg4745	2.1	up	hypothetical protein in Cytochrome oxidase biogenesis cluster
YdB-peg4752	6.5	up	Catalase (EC 1.11.1.6)
YdB-peg4770	8.6	up	FIG00956005: hypothetical protein
YdB-peg4771	5.7	up	probable exported protein YPO0432
YdB-peg4772	4.0	up	Inhibitor of vertebrate lysozyme precursor
YdB-peg4898	2.0	up	FIG00959721: hypothetical protein
YdB-peg4974	2.7	up	Ribosome modulation factor
YdB-peg4996	2.0	up	Permease of the drug/metabolite transporter (DMT) superfamily

Gene ID	Sodium chloride expression fold-change	Regulation type	Gene product
YdB-peg5143	2.3	up	Methyltransferase type 12
YdB-peg5144	2.4	up	LmbE-like protein
YdB-peg5145	2.5	up	Acyl-CoA dehydrogenase/oxidase domain protein
YdB-peg5146	4.4	up	Glycogen debranching enzyme (EC 3.2.1.-)
YdB-peg5148	3.5	up	FIG00953356: hypothetical protein
YdB-peg5150	2.2	up	4-alpha-glucanotransferase (amylomaltase) (EC 2.4.1.25)
YdB-peg5151	2.1	up	Malto-oligosyltrehalose trehalohydrolase (EC 3.2.1.141)
YdB-peg5296	2.6	up	FIG00953680: hypothetical protein
YdB-peg5365	2.5	up	hypothetical protein
YdB-peg5396	2.9	up	Lipoprotein, putative
YdB-peg5410	2.4	up	Acetate permease ActP (cation/acetate symporter)
YdB-peg5411	2.1	up	Putative membrane protein, clustering with ActP
YdB-peg5485	3.2	up	FIG00953288: hypothetical protein
YdB-peg5486	3.5	up	hypothetical protein
YdB-peg5512	2.1	up	hypothetical protein
YdB-peg5573	2.8	up	Aconitate hydratase (EC 4.2.1.3)
YdB-peg5669	2.2	up	FIG024006: iron uptake protein
YdB-peg5755	2.3	up	FIG00955916: hypothetical protein
YdB-peg5765	2.6	up	Transposase
YdB-peg5867	4.7	up	Bacterioferritin
YdB-peg5908	2.0	up	Ferric iron ABC transporter, iron-binding protein
YdB-peg6296	9.2	up	L-proline glycine betaine ABC transport system permease protein ProV (TC 3.A.1.12.1)
YdB-peg6297	7.3	up	L-proline glycine betaine ABC transport system permease protein ProW (TC 3.A.1.12.1)
YdB-peg6298	9.9	up	L-proline glycine betaine binding ABC transporter protein ProX (TC 3.A.1.12.1) / Osmotic adaptation
YdB-peg6299	7.9	up	L-proline glycine betaine ABC transport system permease protein ProW (TC 3.A.1.12.1)

Table S9. Complete list of *Pseudomonas veronii* 1YdBTEX2 genes whose expression levels responded to water stress with PEG8000 but not sodium chloride (FDR<0.05, fold difference>2.0)

yellow/beige: possibly same operon

Gene ID	PEG expression fold-change	Regulation type	Gene product
YdB-peg173	3.8	down	DNA-binding protein HU-alpha
YdB-peg213	7.5	down	Quino(hemo)protein alcohol dehydrogenase, PQQ-dependent (EC 1.1.99.8)
YdB-peg214	5.9	down	pentapeptide repeat family protein
YdB-peg348	2.1	down	hypothetical protein
YdB-peg777	2.8	down	Transcriptional regulator, GntR family
YdB-peg778	2.2	down	Leucine-, isoleucine-, valine-, threonine-, and alanine-binding protein
YdB-peg779	2.1	down	High-affinity branched-chain amino acid transport system permease protein LivH (TC 3.A.1.4.1)
YdB-peg989	2.2	down	benABC operon transcriptional activator BenR
YdB-peg1057	2.1	down	FIG022869: Oxidoreductase, GMC family
YdB-peg1154	2.1	down	RhlA, 3-(3-hydroxyalkanoyloxy)alkanoic acids (HAAs) synthase
YdB-peg1167	3.0	down	Transcriptional regulator containing an amidase domain and an AraC-type DNA-binding HTH domain
YdB-peg1184	2.2	down	Ethanolamine permease
YdB-peg1186	2.3	down	Aldehyde dehydrogenase (EC 1.2.1.3)
YdB-peg1452	4.0	down	Flagellar hook protein FlgE
YdB-peg1453	2.9	down	Flagellar basal-body rod modification protein FlgD
YdB-peg1454	4.1	down	Flagellar basal-body rod protein FlgC
YdB-peg1455	4.3	down	Flagellar basal-body rod protein FlgB
YdB-peg1466	2.4	down	lipoprotein, putative
YdB-peg1473	2.2	down	Dicarboxylate MFS transporter
YdB-peg1650	2.4	down	hypothetical protein
YdB-peg1651	2.1	down	putative Zn dependent protease(EC:3.4.24.-)
YdB-peg1654	2.5	down	hypothetical protein
YdB-peg1766	2.2	down	hypothetical protein
YdB-peg1850	2.9	down	hypothetical protein
YdB-peg1918	4.3	down	Lysine-arginine-ornithine-binding periplasmic protein precursor (TC 3.A.1.3.1)
YdB-peg1949	3.3	down	High-affinity leucine-specific transport system, periplasmic binding protein LivK (TC 3.A.1.4.1)
YdB-peg1951	3.0	down	FIG00953621: hypothetical protein
YdB-peg2233	3.2	down	ABC-type polar amino acid transport system, ATPase component
YdB-peg2234	3.3	down	Glutamate Aspartate transport system permease protein GltK (TC 3.A.1.3.4)
YdB-peg2235	2.9	down	Glutamate Aspartate transport system permease protein GltJ (TC 3.A.1.3.4)
YdB-peg2236	5.9	down	Glutamate Aspartate periplasmic binding protein precursor GltI (TC 3.A.1.3.4)
YdB-peg2417	2.5	down	Methyl-accepting chemotaxis protein
YdB-peg2472	2.6	down	Gamma-glutamyltranspeptidase (EC 2.3.2.2)
YdB-peg2475	2.7	down	Methyl-accepting chemotaxis protein I (serine chemoreceptor protein)
YdB-peg2614	4.2	down	Methyl-accepting chemotaxis protein I (serine chemoreceptor protein)
YdB-peg2615	3.4	down	Positive regulator of CheA protein activity (CheW)
YdB-peg2616	2.5	down	Chemotaxis protein methyltransferase CheR (EC 2.1.1.80)
YdB-peg2626	2.1	down	Transcriptional regulator, AraC family
YdB-peg2952	2.3	down	hypothetical protein
YdB-peg3012	2.8	down	Flagellar biosynthesis protein FlhA
YdB-peg3013	2.2	down	Flagellar biosynthesis protein FlhF
YdB-peg3014	2.2	down	Flagellar synthesis regulator FleN
YdB-peg3016	2.9	down	Chemotaxis regulator - transmits chemoreceptor signals to flagelllar motor components CheY
YdB-peg3017	2.6	down	Chemotaxis response - phosphatase CheZ
YdB-peg3294	2.4	down	FIG00956593: hypothetical protein
YdB-peg3295	2.3	down	Cell division protein FtsH (EC 3.4.24.-)
YdB-peg3296	2.2	down	hypothetical protein
YdB-peg3297	2.2	down	hypothetical protein
YdB-peg3452	2.1	down	FIG00953562: hypothetical protein
YdB-peg3453	3.3	down	Positive regulator of CheA protein activity (CheW)
YdB-peg3456	2.0	down	Flagellar motor rotation protein MotB
YdB-peg3458	2.3	down	Chemotaxis response regulator protein-glutamate methylesterase CheB (EC 3.1.1.61)
YdB-peg3459	2.3	down	Signal transduction histidine kinase CheA (EC 2.7.3.-)
YdB-peg3529	3.0	down	hypothetical protein
YdB-peg3530	2.3	down	hypothetical protein
YdB-peg3531	2.3	down	hypothetical protein
YdB-peg3586	3.2	down	Alcohol dehydrogenase (EC 1.1.1.1); Acetaldehyde dehydrogenase (EC 1.2.1.10)
YdB-peg3879	2.5	down	TRAP transporter solute receptor, unknown substrate 1
YdB-peg3931	2.2	down	FIG00964490: hypothetical protein
YdB-peg3998	2.1	down	Aminomethyltransferase (glycine cleavage system T protein) (EC 2.1.2.10)
YdB-peg4024	3.6	down	Outer membrane porin, OprD family
YdB-peg4054	3.2	down	Chemotactic transducer
YdB-peg4055	2.3	down	Acyl-CoA dehydrogenase, type 2, C-terminal domain
YdB-peg4520	2.9	down	hypothetical protein
YdB-peg4529	2.6	down	Outer membrane protein
YdB-peg4531	2.2	down	FIG00957387: hypothetical protein
YdB-peg4611	2.1	down	hypothetical protein

Gene ID	PEG expression fold-change	Regulation type	Gene product
YdB-peg4801	3.0	down	Flagellar biosynthesis protein FliP
YdB-peg4803	3.1	down	Flagellar motor switch protein FliN
YdB-peg4804	3.6	down	Flagellar motor switch protein FliM
YdB-peg4805	4.3	down	Flagellar biosynthesis protein FliL
YdB-peg4811	3.2	down	Flagellum-specific ATP synthase FliI
YdB-peg4812	2.8	down	Flagellar assembly protein FliH
YdB-peg4813	2.9	down	Flagellar motor switch protein FliG
YdB-peg4814	2.7	down	Flagellar M-ring protein FliF
YdB-peg4815	4.3	down	Flagellar hook-basal body complex protein FliE
YdB-peg4819	2.8	down	FIG00953167: hypothetical protein
YdB-peg4820	4.3	down	Flagellar biosynthesis protein FliS
YdB-peg4821	4.5	down	Flagellar hook-associated protein FliD
YdB-peg4822	3.6	down	Flagellin protein FlaG
YdB-peg4823	5.6	down	Flagellin protein FlaB
YdB-peg4825	3.8	down	Flagellar hook-associated protein FlgL
YdB-peg4826	3.3	down	Flagellar hook-associated protein FlgK
YdB-peg4827	3.9	down	Flagellar protein FlgJ [peptidoglycan hydrolase] (EC 3.2.1.-)
YdB-peg4828	3.0	down	Flagellar P-ring protein FlgI
YdB-peg4829	3.6	down	Flagellar L-ring protein FlgH
YdB-peg4830	5.7	down	Flagellar basal-body rod protein FlgG
YdB-peg4831	3.9	down	Flagellar basal-body rod protein FlgF
YdB-peg4945	2.1	down	FIG00953282: hypothetical protein
YdB-peg5047	2.0	down	FIG002465: BNR repeat protein
YdB-peg5137	2.7	down	FIG00956593: hypothetical protein
YdB-peg5139	3.4	down	FIG00965007: hypothetical protein
YdB-peg5199	2.3	down	hypothetical protein
YdB-peg5410	2.2	down	Acetate permease ActP (cation/acetate symporter)
YdB-peg5411	2.6	down	Putative membrane protein, clustering with ActP
YdB-peg5463	2.2	down	Acetyl-coenzyme A synthetase (EC 6.2.1.1)
YdB-peg5505	2.2	down	Negative regulator of flagellin synthesis FlgM
YdB-peg5506	3.6	down	Flagellar basal-body P-ring formation protein FlgA
YdB-peg5649	2.1	down	Methyl-accepting chemotaxis protein
YdB-peg5741	2.8	down	FIG002188: hypothetical protein
YdB-peg5742	3.2	down	FIG067310: hypothetical protein
YdB-peg5879	2.5	down	Branched-chain amino acid transport ATP-binding protein LivF (TC 3.A.1.4.1)
YdB-peg5880	2.2	down	Branched-chain amino acid transport ATP-binding protein LivG (TC 3.A.1.4.1)
YdB-peg5881	2.5	down	Branched-chain amino acid transport system permease protein LivM (TC 3.A.1.4.1)
YdB-peg5882	3.9	down	High-affinity branched-chain amino acid transport system permease protein LivH (TC 3.A.1.4.1)
YdB-peg5883	2.2	down	hypothetical protein
YdB-peg5884	3.8	down	Branched-chain amino acid ABC transporter, amino acid-binding protein (TC 3.A.1.4.1)
YdB-peg6053	2.9	down	Methyl-accepting chemotaxis protein I (serine chemoreceptor protein)
YdB-peg6071	2.0	down	Urocanate hydratase (EC 4.2.1.49)
YdB-peg6223	2.7	down	hypothetical protein
YdB-peg6306	3.0	down	Dipeptide-binding ABC transporter, periplasmic substrate-binding component (TC 3.A.1.5.2)
YdB-peg6307	5.1	down	Outer membrane porin, OprD family
YdB-peg6308	3.3	down	Dipeptide-binding ABC transporter, periplasmic substrate-binding component (TC 3.A.1.5.2)
YdB-peg6309	4.2	down	Dipeptide-binding ABC transporter, periplasmic substrate-binding component (TC 3.A.1.5.2)
YdB-peg6427	3.4	down	FIG00954582: hypothetical protein
YdB-peg6428	3.3	down	hypothetical protein

Gene ID	PEG expression fold-change	Regulation type	Gene product
YdB-peg432	2.8	up	lipoprotein, putative
YdB-peg976	2.3	up	Cytochrome O ubiquinol oxidase subunit III (EC 1.10.3.-)
YdB-peg977	2.3	up	Cytochrome O ubiquinol oxidase subunit I (EC 1.10.3.-)
YdB-peg978	2.1	up	Cytochrome O ubiquinol oxidase subunit II (EC 1.10.3.-)
YdB-peg1445	4.3	up	outer membrane protein H1
YdB-peg1667	2.4	up	FIG00954962: hypothetical protein
YdB-peg1733	2.2	up	Arginase (EC 3.5.3.1)
YdB-peg1734	2.6	up	Permeases of the drug/metabolite transporter (DMT) superfamily
YdB-peg1735	2.5	up	Permeases of the drug/metabolite transporter (DMT) superfamily
YdB-peg1737	2.9	up	2-hydroxy-3-keto-5-methylthiopentenyl-1-phosphate phosphatase related protein
YdB-peg1738	2.9	up	Aminotransferase, class III
YdB-peg1739	2.7	up	Probable esterase
YdB-peg1740	2.8	up	FIG00959827: hypothetical protein
YdB-peg1753	2.0	up	Glycerol uptake facilitator protein
YdB-peg1841	4.3	up	Outer membrane lipoprotein omp16 precursor
YdB-peg1842	2.8	up	FIG00953405: hypothetical protein
YdB-peg1902	3.1	up	S-(hydroxymethyl)glutathione dehydrogenase (EC 1.1.1.284)
YdB-peg1903	3.5	up	S-formylglutathione hydrolase (EC 3.1.2.12)
YdB-peg2151	2.3	up	5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase (EC 2.1.1.14)
YdB-peg2192	2.1	up	DNA-binding response regulator
YdB-peg2193	2.1	up	sensor histidine kinase
YdB-peg2245	3.5	up	GDP-mannose 6-dehydrogenase (EC 1.1.1.132)
YdB-peg2246	2.8	up	Alginate biosynthesis protein Alg8
YdB-peg2247	2.0	up	Alginate biosynthesis protein alg44
YdB-peg2248	2.1	up	Alginate biosynthesis protein AlgK precursor

Gene ID	PEG expression fold-change	Regulation type	Gene product
YdB-peg2255	2.3	up	alginate o-acetyltransferase AlgF
YdB-peg2256	2.1	up	Mannose-6-phosphate isomerase (EC 5.3.1.8) / Mannose-1-phosphate guanylyltransferase (GDP) (EC 2.7.7.22)
YdB-peg2265	2.2	up	Ni,Fe-hydrogenase I cytochrome b subunit
YdB-peg2496	2.7	up	Ferrous iron transport periplasmic protein EfeO, contains peptidase-M75 domain and (frequently) cupredoxin-like domain
YdB-peg2498	2.8	up	Ferrous iron transport periplasmic protein EfeO, contains peptidase-M75 domain and (frequently) cupredoxin-like domain
YdB-peg2499	2.0	up	Ferrous iron transport permease EfeU
YdB-peg2621	2.3	up	Ferrichrome-iron receptor
YdB-peg2811	2.3	up	Biofilm PGA synthesis auxiliary protein PgaD
YdB-peg2812	2.5	up	Biofilm PGA synthesis N-glycosyltransferase PgaC (EC 2.4.-.-)
YdB-peg2813	2.4	up	Biofilm PGA synthesis deacetylase PgaB (EC 3.-)
YdB-peg2814	2.5	up	Biofilm PGA outer membrane secretin PgaA
YdB-peg2900	2.1	up	hypothetical protein
YdB-peg2988	2.4	up	Stress induced hydrophobic peptide
YdB-peg3025	2.3	up	Biopolymer transport protein ExbD/TolR
YdB-peg3026	2.0	up	MotA/TolQ/ExbB proton channel family protein
YdB-peg3205	2.1	up	FIG00955037: hypothetical protein
YdB-peg3255	2.9	up	Osmotically inducible protein Y precursor
YdB-peg3287	22.6	up	L-lactate permease
YdB-peg3288	32.6	up	Predicted L-lactate dehydrogenase, Fe-S oxidoreductase subunit YkgE
YdB-peg3289	26.2	up	Predicted L-lactate dehydrogenase, Iron-sulfur cluster-binding subunit YkgF
YdB-peg3290	25.1	up	Predicted L-lactate dehydrogenase, hypothetical protein subunit YkgG
YdB-peg3291	28.2	up	Predicted D-lactate dehydrogenase, Fe-S protein, FAD/FMN-containing
YdB-peg3334	2.5	up	FIG00965356: hypothetical protein
YdB-peg3645	2.8	up	FIG00958237: hypothetical protein
YdB-peg3656	4.6	up	Deblocking aminopeptidase (EC 3.4.11.-) @ Cyanophycinase 2 (EC 3.4.15.6)
YdB-peg3657	8.2	up	Cyanophycin synthase (EC 6.3.2.29)(EC 6.3.2.30)
YdB-peg3658	6.6	up	Asparagine synthetase [glutamine-hydrolyzing] (EC 6.3.5.4)
YdB-peg3663	2.4	up	Fumarate hydratase class II (EC 4.2.1.2)
YdB-peg3674	79.2	up	L-lactate dehydrogenase (EC 1.1.2.3)
YdB-peg3716	2.3	up	RND efflux system, outer membrane lipoprotein CmeC
YdB-peg3717	2.1	up	RND efflux system, inner membrane transporter CmeB
YdB-peg3718	2.3	up	Membrane fusion protein of RND family multidrug efflux pump
YdB-peg4124	2.2	up	CidA-associated membrane protein CidB
YdB-peg4719	2.5	up	FIG00953718: hypothetical protein
YdB-peg4752	2.5	up	Catalase (EC 1.11.1.6)
YdB-peg4770	2.4	up	FIG00956005: hypothetical protein
YdB-peg4898	2.1	up	FIG00959721: hypothetical protein
YdB-peg5146	2.2	up	Glycogen debranching enzyme (EC 3.2.1.-)
YdB-peg5280	2.4	up	hypothetical protein
YdB-peg5396	2.0	up	Lipoprotein, putative
YdB-peg5669	3.2	up	FIG024006: iron uptake protein
YdB-peg5909	2.1	up	Ferric iron ABC transporter, permease protein
YdB-peg6296	5.0	up	L-proline glycine betaine ABC transport system permease protein ProV (TC 3.A.1.12.1)
YdB-peg6297	4.1	up	L-proline glycine betaine ABC transport system permease protein ProW (TC 3.A.1.12.1)
YdB-peg6298	6.2	up	L-proline glycine betaine binding ABC transporter protein ProX (TC 3.A.1.12.1) / Osmotic adaptation
YdB-peg6299	4.7	up	L-proline glycine betaine ABC transport system permease protein ProW (TC 3.A.1.12.1)

Table S10. **Complete list of *Pseudomonas veronii* 1YdBTEX2 genes whose expression levels responded to water stress with PEG8000 and sodium chloride (FDR<0.05, fold difference>2.0)**

yellow/beige: possibly same operon

Gene ID	Sodium chloride expression fold-change	Regulation type	PEG8000 expression fold-change	Regulation type	Gene product
YdB-peg976	2.2	down	2.3	up	Cytochrome O ubiquinol oxidase subunit III (EC 1.10.3.-)
YdB-peg977	2.3	down	2.3	up	Cytochrome O ubiquinol oxidase subunit I (EC 1.10.3.-)
YdB-peg978	2.2	down	2.1	up	Cytochrome O ubiquinol oxidase subunit II (EC 1.10.3.-)
YdB-peg1452	2.3	down	4.0	down	Flagellar hook protein FlgE
YdB-peg1453	2.3	down	2.9	down	Flagellar basal-body rod modification protein FlgD
YdB-peg1454	2.7	down	4.1	down	Flagellar basal-body rod protein FlgC
YdB-peg1455	3.4	down	4.3	down	Flagellar basal-body rod protein FlgB
YdB-peg1766	2.6	down	2.2	down	hypothetical protein
YdB-peg2417	2.3	down	2.5	down	Methyl-accepting chemotaxis protein
YdB-peg3016	2.3	down	2.9	down	Chemotaxis regulator - transmits chemoreceptor signals to flagelllar motor components CheY
YdB-peg3294	2.1	down	2.4	down	FIG00956593: hypothetical protein
YdB-peg3295	2.1	down	2.3	down	Cell division protein FtsH (EC 3.4.24.-)
YdB-peg3297	2.1	down	2.2	down	hypothetical protein
YdB-peg3453	2.0	down	3.3	down	Positive regulator of CheA protein activity (CheW)
YdB-peg3998	2.6	down	2.1	down	Aminomethyltransferase (glycine cleavage system T protein) (EC 2.1.2.10)
YdB-peg4055	2.3	down	2.3	down	Acyl-CoA dehydrogenase, type 2, C-terminal domain
YdB-peg4803	2.0	down	3.1	down	Flagellar motor switch protein FliN
YdB-peg4804	2.4	down	3.6	down	Flagellar motor switch protein FliM
YdB-peg4805	2.4	down	4.3	down	Flagellar biosynthesis protein FliL
YdB-peg4811	2.1	down	3.2	down	Flagellum-specific ATP synthase FliI
YdB-peg4815	2.4	down	4.3	down	Flagellar hook-basal body complex protein FliE
YdB-peg4820	2.4	down	4.3	down	Flagellar biosynthesis protein FliS
YdB-peg4821	2.1	down	4.5	down	Flagellar hook-associated protein FliD
YdB-peg4822	2.3	down	3.6	down	Flagellin protein FlaG
YdB-peg4823	2.1	down	5.6	down	Flagellin protein FlaB
YdB-peg4825	2.4	down	3.8	down	Flagellar hook-associated protein FlgL
YdB-peg4826	2.4	down	3.3	down	Flagellar hook-associated protein FlgK
YdB-peg4827	2.6	down	3.9	down	Flagellar protein FlgJ [peptidoglycan hydrolase] (EC 3.2.1.-)
YdB-peg4828	2.2	down	3.0	down	Flagellar P-ring protein FlgI
YdB-peg4829	2.2	down	3.6	down	Flagellar L-ring protein FlgH
YdB-peg4830	2.7	down	5.7	down	Flagellar basal-body rod protein FlgG
YdB-peg4831	2.3	down	3.9	down	Flagellar basal-body rod protein FlgF
YdB-peg5139	2.3	down	3.4	down	FIG00965007: hypothetical protein
YdB-peg5506	2.1	down	3.6	down	Flagellar basal-body P-ring formation protein FlgA
YdB-peg6223	2.1	down	2.7	down	hypothetical protein
YdB-peg6307	2.3	down	5.1	down	Outer membrane porin, OprD family
YdB-peg6427	3.4	down	3.4	down	FIG00954582: hypothetical protein
YdB-peg6428	2.9	down	3.3	down	hypothetical protein
YdB-peg1445	3.8	up	4.3	up	outer membrane protein H1
YdB-peg1667	3.5	up	2.4	up	FIG00954962: hypothetical protein
YdB-peg1841	14.9	up	4.3	up	Outer membrane lipoprotein omp16 precursor
YdB-peg1842	7.2	up	2.8	up	FIG00953405: hypothetical protein
YdB-peg2255	2.1	up	2.3	up	alginate o-acetyltransferase AlgF
YdB-peg2496	2.1	up	2.7	up	Ferrous iron transport periplasmic protein EfeO, contains peptidase-M75 domain and (frequently) cupredoxin-like domain
YdB-peg2498	2.5	up	2.8	up	Ferrous iron transport periplasmic protein EfeO, contains peptidase-M75 domain and (frequently) cupredoxin-like domain
YdB-peg2900	4.4	up	2.1	up	hypothetical protein
YdB-peg2988	3.1	up	2.4	up	Stress induced hydrophobic peptide
YdB-peg3255	19.8	up	2.9	up	Osmotically inducible protein Y precursor
YdB-peg3645	15.7	up	2.8	up	FIG00958237: hypothetical protein
YdB-peg3656	10.9	up	4.6	up	Deblocking aminopeptidase (EC 3.4.11.-) @ Cyanophycinase 2 (EC 3.4.15.6)
YdB-peg3657	17.8	up	8.2	up	Cyanophycin synthase (EC 6.3.2.29)(EC 6.3.2.30)
YdB-peg3658	12.6	up	6.6	up	Asparagine synthetase [glutamine-hydrolyzing] (EC 6.3.5.4)
YdB-peg3663	2.6	up	2.4	up	Fumarate hydratase class II (EC 4.2.1.2)
YdB-peg4719	7.1	up	2.5	up	FIG00953718: hypothetical protein
YdB-peg4752	6.5	up	2.5	up	Catalase (EC 1.11.1.6)
YdB-peg4770	8.6	up	2.4	up	FIG00956005: hypothetical protein
YdB-peg4898	2.0	up	2.1	up	FIG00959721: hypothetical protein
YdB-peg5146	4.4	up	2.2	up	Glycogen debranching enzyme (EC 3.2.1.-)
YdB-peg5396	2.9	up	2.0	up	Lipoprotein, putative
YdB-peg5410	2.4	up	2.2	down	Acetate permease ActP (cation/acetate symporter)
YdB-peg5411	2.1	up	2.6	down	Putative membrane protein, clustering with ActP
YdB-peg5669	2.2	up	3.2	up	FIG024006: iron uptake protein
YdB-peg6296	9.2	up	5.0	up	L-proline glycine betaine ABC transport system permease protein ProV (TC 3.A.1.12.1)
YdB-peg6297	7.3	up	4.1	up	L-proline glycine betaine ABC transport system permease protein ProW (TC 3.A.1.12.1)
YdB-peg6298	9.9	up	6.2	up	L-proline glycine betaine binding ABC transporter protein ProX (TC 3.A.1.12.1) / Osmotic adaptation
YdB-peg6299	7.9	up	4.7	up	L-proline glycine betaine ABC transport system permease protein ProW (TC 3.A.1.12.1)

Table S11. **Enriched GO terms among the significantly differentially expressed genes in the comparison between *Sphingomonas wittichii* RW1 cells after 30min exposure to matric stress with PEG8000 versus cells in control conditions**

Matric-RW1- up

GOID	Biological Process	No probes in class	Total No probes in class	Total No probes on array	No probes in comparison	log_odds_ratio	p-value	Genes
GO:0030163	protein catabolic process	2	5	3458	96	3.85	5.46E-02	Swit_4376, Swit_4509
GO:0006457	protein folding	3	17	3458	96	2.67	1.08E-02	Swit_3375, Swit_5306, Swit_5351
GO:0006760	folic acid-containing compound metabolic process	2	15	3458	96	2.26	6.42E-02	Swit_0944, Swit_4785
GO:0006950	response to stress	8	79	3458	96	1.87	4.31E-04	Swit_0060, Swit_0619, Swit_1147, Swit_1248, Swit_3128, Swit_5282, Swit_5306, Swit_5351
GO:0006508	proteolysis	7	96	3458	96	1.39	1.65E-02	Swit_0390, Swit_1146, Swit_1363, Swit_1939, Swit_3913, Swit_4376, Swit_4509
GOID	**Molecular Function**							
GO:0016671	oxidoreductase activity, acting on a sulfur group of donors, disulfide as acceptor	3	5	3458	96	4.43	1.86E-04	Swit_0074, Swit_2816, Swit_4056
GO:0003863	3-methyl-2-oxobutanoate dehydrogenase (2-methylpropanoyl-transferring) activity	3	5	3458	96	4.43	1.86E-04	Swit_0782, Swit_2144, Swit_2145
GO:0004175	endopeptidase activity	6	42	3458	96	2.36	8.11E-04	Swit_0390, Swit_1146, Swit_1939, Swit_3913, Swit_4376, Swit_4509
GO:0016846	carbon-sulfur lyase activity	2	14	3458	96	2.36	5.37E-02	Swit_1412, Swit_4209
GO:0005515	protein binding	6	67	3458	96	1.69	8.96E-03	Swit_0126, Swit_0780, Swit_2918, Swit_3128, Swit_5306, Swit_5351
GO:0008270	zinc ion binding	6	84	3458	96	1.36	2.53E-02	Swit_0060, Swit_0312, Swit_1179, Swit_1939, Swit_2867, Swit_4509
GO:0005524	ATP binding	13	228	3458	96	1.04	7.73E-03	Swit_0125, Swit_0126, Swit_0390, Swit_1146, Swit_2917, Swit_3128, Swit_3375, Swit_4023, Swit_4509, Swit_4724, Swit_5007, Swit_5282, Swit_5351
GO:0001883	purine nucleoside binding	14	326	3458	96	0.63	5.43E-02	Swit_0125, Swit_0126, Swit_0379, Swit_0390, Swit_1146, Swit_2917, Swit_3128, Swit_3375, Swit_4023, Swit_4509, Swit_4724, Swit_5007, Swit_5282, Swit_5351

Matric-RW1- down

GOID	Biological Process	No probes in class	Total No probes in class	Total No probes on array	No probes in comparison	log_odds_ratio	p-value	Genes
GO:0043064	flagellum organization	3	6	3458	57	4.92	9.16E-05	Swit_0212, Swit_0213, Swit_1267
GO:0006928	cellular component movement	6	18	3458	57	4.34	1.54E-02	Swit_0213, Swit_1267, Swit_1268, Swit_1270, Swit_1286, Swit_1293
GO:0001539	ciliary or flagellar motility	5	17	3458	57	4.16	6.36E-06	Swit_1267, Swit_1268, Swit_1270, Swit_1286, Swit_1293
GO:0015891	siderophore transport	1	6	3458	57	3.34	9.88E-02	Swit_4025
GO:0015671	oxygen transport	1	6	3458	57	3.34	9.88E-02	Swit_5297
GO:0009072	aromatic amino acid family metabolic process	3	22	3458	57	3.05	4.34E-02	Swit_3863, Swit_3864, Swit_3865
GO:0006935	chemotaxis	2	17	3458	57	2.84	3.33E-02	Swit_1293, Swit_3186
GO:0000160	two-component signal transduction system (phosphorelay)	5	127	3458	57	1.26	6.41E-02	Swit_0067, Swit_3127, Swit_3186, Swit_3187, Swit_5296
GOID	**Cellular Component**							
GO:0009288	bacterial-type flagellum	7	19	3458	57	4.48	6.11E-08	Swit_0212, Swit_0213, Swit_1267, Swit_1268, Swit_1270, Swit_1286, Swit_1293
GO:0043232	intracellular non-membrane-bounded organelle	7	40	3458	57	3.41	1.66E-05	Swit_0212, Swit_0213, Swit_1267, Swit_1268, Swit_1270, Swit_1286, Swit_1293
GO:0019867	outer membrane	8	177	3458	57	1.46	3.98E-02	Swit_0535, Swit_0914, Swit_3091, Swit_3144, Swit_3190, Swit_4025, Swit_4274, Swit_4781

Matric-RW1- down

GOID	Molecular Function	No probes in class	Total No probes in class	Total No probes on array	No probes in comparison	log_odds_ratio	p-value	Genes
GO:0003774	motor activity	4	14	3458	57	4.12	4.65E-05	Swit_1267, Swit_1268, Swit_1270, Swit_1286
GO:0005198	structural molecule activity	4	19	3458	57	3.67	1.70E-04	Swit_1267, Swit_1268, Swit_1270, Swit_1286
GO:0030234	enzyme regulator activity	1	6	3458	57	3.34	8.99E-02	Swit_1918
GO:0015343	siderophore transmembrane transporter activity	1	6	3458	57	3.34	8.99E-02	Swit_4025
GO:0019825	oxygen binding	1	6	3458	57	3.34	8.99E-02	Swit_5297
GO:0016831	carboxy-lyase activity	3	34	3458	57	2.42	1.51E-02	Swit_3084, Swit_3087, Swit_3088
GO:0016702	oxidoreductase activity, acting on single donors with incorporation of molecular oxygen, incorporation of two atoms of oxygen	6	97	3458	57	1.91	3.54E-03	Swit_2634, Swit_3086, Swit_3094, Swit_3864, Swit_3865, Swit_4263
GO:0004871	signal transducer activity	12	304	3458	57	1.26	1.88E-03	Swit_0067, Swit_0535, Swit_0914, Swit_3091, Swit_3127, Swit_3144, Swit_3186, Swit_3187, Swit_4025, Swit_4274, Swit_4781, Swit_5296

Table S12. **Enriched GO terms among the significantly differentially expressed genes in the comparison between *Sphingomonas wittichii* RW1 cells after 30min exposure to solute stress with NaCl versus cells in control conditions.**

Solute-RW1- up

GOID	Biological Process	No probes in class	Total No probes in class	Total No probes on array	No probes in comparison	log_odds_ratio	p-value	Genes
GO:0015979	photosynthesis	3	5	3458	124	4.06	3.86E-04	Swit_0995, Swit_3612, Swit_5343
GO:0010035	response to inorganic substance	2	5	3458	124	3.48	1.11E-02	Swit_1359, Swit_5248
GO:0006096	glycolysis	2	9	3458	124	2.63	3.66E-02	Swit_1625, Swit_3015
GO:0006281	DNA repair	6	43	3458	124	1.96	1.34E-02	Swit_3911, Swit_3979, Swit_3981, Swit_3982, Swit_5282, Swit_5283
GO:0006950	response to stress	11	79	3458	124	1.96	2.11E-02	Swit_0619, Swit_1147, Swit_2779, Swit_3911, Swit_3979, Swit_3981, Swit_3982, Swit_5248, Swit_5282, Swit_5283, Swit_5311
GOID	**Cellular Component**							
GO:0016021	integral to membrane	13	208	3458	124	0.80	8.65E-02	Swit_1172, Swit_2132, Swit_2278, Swit_2322, Swit_2324, Swit_2334, Swit_2422, Swit_3455, Swit_3475, Swit_3837, Swit_3926, Swit_4648, Swit_4749
GOID	**Molecular Function**							
GO:0005216	ion channel activity	2	5	3458	124	3.48	6.14E-02	Swit_3455, Swit_3739
GO:0016628	oxidoreductase activity, acting on the CH-CH group of donors, NAD or NADP as acceptor	2	7	3458	124	2.99	1.85E-02	Swit_2076, Swit_5274
GO:0004252	serine-type endopeptidase activity	3	12	3458	124	2.80	5.40E-03	Swit_1146, Swit_3835, Swit_5274
GO:0004527	exonuclease activity	3	15	3458	124	2.48	6.00E-02	Swit_1916, Swit_3911, Swit_3978
GO:0005509	calcium ion binding	3	16	3458	124	2.39	1.25E-02	Swit_0693, Swit_3839, Swit_4475
GO:0016798	hydrolase activity, acting on glycosyl bonds	3	17	3458	124	2.30	8.68E-02	Swit_2576, Swit_3609, Swit_4533
GO:0004519	endonuclease activity	3	19	3458	124	2.14	2.33E-02	Swit_1916, Swit_3911, Swit_5283
GO:0016846	carbon-sulfur lyase activity	2	14	3458	124	1.99	6.97E-02	Swit_1412, Swit_4209
GO:0000156	two-component response regulator activity	7	99	3458	124	0.98	3.46E-02	Swit_2540, Swit_2974, Swit_3925, Swit_4432, Swit_5012, Swit_5270, Swit_5396
GO:0005524	ATP binding	12	228	3458	124	0.55	5.17E-02	Swit_0257, Swit_1146, Swit_1625, Swit_2743, Swit_3015, Swit_3612, Swit_3979, Swit_3982, Swit_4023, Swit_4432, Swit_5007, Swit_5282

Solute-RW1-down

GOID	Biological Process	No probes in class	Total No probes in class	Total No probes on array	No probes in comparison	log_odds_ratio	p-value	Genes
GO:0001539	ciliary or flagellar motility	6	17	3458	55	4.47	1.50E-07	Swit_1267, Swit_1268, Swit_1270, Swit_1286, Swit_1293, Swit_1458
GO:0044781	flagellum organization	2	6	3458	55	4.39	3.80E-03	Swit_0212, Swit_1267
GO:0006537	glutamate biosynthetic process	2	6	3458	55	4.39	3.80E-03	Swit_0657, Swit_4784
GO:0009086	methionine biosynthetic process	3	9	3458	55	4.39	3.25E-04	Swit_2399, Swit_2401, Swit_4786
GO:0006544	glycine metabolic process	2	7	3458	55	4.17	5.26E-03	Swit_2696, Swit_2697
GO:0015891	siderophore transport	1	6	3458	55	3.39	9.47E-02	Swit_4025
GO:0009098	leucine biosynthetic process	1	6	3458	55	3.39	9.47E-02	Swit_4494
GO:0006760	folic acid-containing compound metabolic process	2	15	3458	55	3.07	2.42E-02	Swit_2399, Swit_4785
GO:0006935	chemotaxis	2	17	3458	55	2.89	3.07E-02	Swit_1293, Swit_1458
GO:0006730	one-carbon metabolic process	2	20	3458	55	2.65	4.55E-02	Swit_2674, Swit_3373
GO:0046394	carboxylic acid biosynthetic process	7	105	3458	55	2.07	7.89E-04	Swit_0657, Swit_2399, Swit_2401, Swit_3907, Swit_4494, Swit_4784, Swit_4786
GO:0043436	oxoacid metabolic process	12	225	3458	55	1.75	1.01E-04	Swit_0657, Swit_2399, Swit_2401, Swit_2696, Swit_2697, Swit_3907, Swit_3986, Swit_4494, Swit_4784, Swit_4785, Swit_4786, Swit_5152

Solute-RW1-down

GOID	Cellular Component	No probes in class	Total No probes in class	Total No probes on array	No probes in comparison	log_odds_ratio	p-value	Genes
GO:0009288	bacterial-type flagellum	7	19	3458	55	4.53	8.40E-08	Swit_0212, Swit_1267, Swit_1268, Swit_1270, Swit_1286, Swit_1293, Swit_1458
GO:0043232	intracellular non-membrane-bounded organell	7	40	3458	55	3.46	2.23E-05	Swit_0212, Swit_1267, Swit_1268, Swit_1270, Swit_1286, Swit_1293, Swit_1458
GOID	**Molecular Function**							
GO:0008172	S-methyltransferase activity	3	7	3458	55	4.75	1.19E-04	Swit_2399, Swit_2400, Swit_4786
GO:0003774	motor activity	5	14	3458	55	4.49	1.35E-06	Swit_1267, Swit_1268, Swit_1270, Swit_1286, Swit_1458
GO:0050897	cobalt ion binding	2	6	3458	55	4.39	3.43E-03	Swit_2399, Swit_3373
GO:0005198	structural molecule activity	4	19	3458	55	3.73	1.70E-04	Swit_1267, Swit_1268, Swit_1270, Swit_1286
GO:0015343	siderophore transmembrane transporter activity	1	6	3458	55	3.39	8.99E-02	Swit_4025
GO:0008410	CoA-transferase activity	2	14	3458	55	3.17	1.92E-02	Swit_0958, Swit_0959
GO:0000287	magnesium ion binding	3	28	3458	55	2.75	8.85E-03	Swit_0228, Swit_3373, Swit_4494
GO:0004872	receptor activity	6	159	3458	55	1.25	3.51E-02	Swit_0535, Swit_2477, Swit_3750, Swit_4025, Swit_4088, Swit_4696

Table S13. **Enriched GO terms among the significantly differentially expressed genes in the comparison between *Arthrobacter chlorophenolicus* A6 cells after 30min exposure to matric stress with PEG8000 versus cells in control conditions**

Matric-A6-Up

GOID	Biological Process	No probes in class	Total No probes in class	Total No probes on array	No probes in comparison	log_odds_ratio	p-value	Genes
GO:0006865	amino acid transport	1	21	2535	8	3.92	7.18E-02	Achl_3688
GOID	**Molecular Function**							
GO:0020037	heme binding	1	12	2535	8	4.72	3.99E-02	Achl_3365
GO:0015171	amino acid transmembrane transporter activity	1	18	2535	8	4.14	5.93E-02	Achl_3688
GO:0000166	nucleotide binding	3	404	2535	8	1.23	6.03E-02	Achl_3687, Achl_0988, Achl_3732

Matric-A6-Down

GOID	Biological Process	No probes in class	Total No probes in class	Total No probes on array	No probes in comparison	log_odds_ratio	p-value	Genes
GO:0006457	protein folding	2	9	2535	17	5.05	1.39E-03	Achl_3620, Achl_3619
GO:0001539	ciliary or flagellar motility	2	10	2535	17	4.90	1.73E-03	Achl_2998, Achl_2990
GO:0006935	chemotaxis	1	5	2535	17	4.90	3.23E-02	Achl_2990
GO:0007626	locomotory behavior	1	5	2535	17	4.90	3.23E-02	Achl_2990
GO:0009296	flagellum assembly	1	6	2535	17	4.64	3.87E-02	Achl_2996
GOID	**Cellular Component**							
GO:0042995	cell projection	5	24	2535	17	4.96	3.49E-05	Achl_2996, Achl_2984, Achl_2998, Achl_2982, Achl_2990
GOID	**Molecular Function**							
GO:0003774	motor activity	1	9	2535	17	4.05	5.20E-02	Achl_2990
GO:0030246	carbohydrate binding	1	11	2535	17	3.76	6.32E-02	Achl_1988
GO:0005351	sugar:hydrogen symporter activity	1	15	2535	17	3.31	8.53E-02	Achl_4608
GO:0005198	structural molecule activity	1	15	2535	17	3.31	8.53E-02	Achl_2998

Table S14. **Enriched GO terms among the significantly differentially expressed genes in the comparison between *Arthrobacter chlorophenolicus* A6 cells after 30min exposure to solute stress with NaCl versus cells in control conditions.**

Solute A6 up

GOID	Biological Process	No probes in class	Total No probes in class	Total No probes on array	No probes in comparison	log_odds_ratio	p-value	Genes
GO:0006546	glycine catabolic process	1	6	2535	20	4.40	5.32E-02	Achl_3469
GO:0016998	cell wall macromolecule catabolic process	1	7	2535	20	4.18	6.18E-02	Achl_0996
GO:0055114	oxidation-reduction process	5	257	2535	20	1.30	7.22E-02	Achl_3673, Achl_3365, Achl_3689, Achl_3495, Achl_3686
	Cellular Component							
GO:0005576	extracellular region	1	5	2535	20	4.66	4.95E-02	Achl_0996
	Molecular Function							
GO:0051537	2 iron, 2 sulfur cluster binding	1	9	2535	20	3.82	7.00E-02	Achl_3689
GO:0004252	serine-type endopeptidase activity	1	12	2535	20	3.40	9.23E-02	Achl_3480
GO:0050660	flavin adenine dinucleotide binding	2	43	2535	20	2.56	4.52E-02	Achl_3687, Achl_3494
GO:0004872	receptor activity	1	30	2535	20	2.08	4.72E-02	Achl_3514
GO:0046914	transition metal ion binding	4	136	2535	20	1.90	2.05E-02	Achl_0666, Achl_1840, Achl_3365, Achl_3689
GO:0016491	oxidoreductase activity	8	422	2535	20	1.26	6.26E-02	Achl_3673, Achl_3687, Achl_3365, Achl_3494, Achl_3689, Achl_3495, Achl_3469, Achl_3686
GO:0005215	transporter activity	6	331	2535	20	1.20	7.20E-02	Achl_1840, Achl_3496, Achl_3070, Achl_3674, Achl_1348, Achl_0518

Solute A6 down

GOID	Biological Process	No probes in class	Total No probes in class	Total No probes on array	No probes in comparison	log_odds_ratio	p-value	Genes
GO:0001539	ciliary or flagellar motility	9	10	2535	44	5.70	2.93E-16	Achl_2991, Achl_2998, Achl_2981, Achl_2983, Achl_2994, Achl_2990, Achl_3001, Achl_2979, Achl_2992
GO:0006935	chemotaxis	3	5	2535	44	5.11	4.09E-05	Achl_2981, Achl_2990, Achl_2979
GO:0007626	locomotory behavior	3	5	2535	44	5.11	4.09E-05	Achl_2981, Achl_2990, Achl_2979
GO:0044780	flagellum assembly	3	6	2535	44	4.85	8.09E-05	Achl_2996, Achl_2999, Achl_3000
GO:0009306	protein secretion	2	9	2535	44	3.68	8.96E-03	Achl_2988, Achl_2972
GO:0006457	protein folding	2	9	2535	44	3.68	8.96E-03	Achl_3620, Achl_3619
	Cellular Component							
GO:0009288	bacterial-type flagellum	10	15	2535	44	5.26	3.47E-13	Achl_2996, Achl_2991, Achl_2998, Achl_2983, Achl_2994, Achl_2990, Achl_3001, Achl_3000, Achl_2979, Achl_2992
GO:0019861	flagellum	15	24	2535	44	5.17	1.54E-07	Achl_2996, Achl_2991, Achl_2999, Achl_2984, Achl_2998, Achl_2983, Achl_2982, Achl_2994, Achl_2990, Achl_3001, Achl_3000, Achl_2979, Achl_2972, Achl_2993, Achl_2992
	Molecular Function							
GO:0003774	motor activity	7	9	2535	44	5.49	4.39E-12	Achl_2991, Achl_2981, Achl_2983, Achl_2994, Achl_2990, Achl_2979, Achl_2992
GO:0005198	structural molecule activity	6	15	2535	44	4.53	4.30E-08	Achl_2998, Achl_2983, Achl_2994, Achl_3001, Achl_3000, Achl_2992
GO:0004872	receptor activity	1	30	2535	44	0.94	9.02E-02	Achl_1179

Table S15. **Enriched GO terms among the significantly differentially expressed genes in the comparison between *Pseudomonas veronii* 1YdBTEX2 cells after 30min exposure to matric stress with PEG8000 versus cells in control conditions.**

Matric 1YdBTEX2 Up

GOID	Biological Process	No probes in class	Total No probes in class	Total No probes on array	No probes in comparison	log_odds_ratio	p-value	Genes
GO:0009067	aspartate family amino acid biosynthetic process	2	12	3071	28	4.19	5.88E-03	YdB-peg3658, YdB-peg2151
GO:0006979	response to oxidative stress	1	7	3071	28	3.97	6.75E-02	YdB-peg4752
GO:0006091	generation of precursor metabolites and energy	5	42	3071	28	3.71	5.09E-04	YdB-peg976, YdB-peg977, YdB-peg5146, YdB-peg2265, YdB-peg978
GO:0006810	transport	11	585	3071	28	1.04	3.56E-02	YdB-peg3287, YdB-peg6299, YdB-peg6297, YdB-peg2621, YdB-peg3716, YdB-peg3718, YdB-peg2265, YdB-peg3717, YdB-peg5909, YdB-peg1753, YdB-peg2499
	Cellular Component							
GO:0005887	integral to plasma membrane	1	7	3071	28	3.97	9.22E-02	YdB-peg3287
GO:0016020	membrane	15	786	3071	28	1.07	7.96E-03	YdB-peg3287, YdB-peg6299, YdB-peg6297, YdB-peg1734, YdB-peg2621, YdB-peg3716, YdB-peg976, YdB-peg977, YdB-peg3718, YdB-peg2265, YdB-peg3717, YdB-peg978, YdB-peg5909, YdB-peg1753, YdB-peg2499
	Molecular Function							
GO:0004129	cytochrome-c oxidase activity	3	9	3071	28	5.19	5.71E-05	YdB-peg976, YdB-peg977, YdB-peg978
GO:0008762	UDP-N-acetylmuramate dehydrogenase activity	1	6	3071	28	4.19	5.44E-02	YdB-peg3291
GO:0020037	heme binding	2	37	3071	28	2.57	4.51E-02	YdB-peg4752, YdB-peg977
GO:0046914	transition metal ion binding	5	139	3071	28	1.98	7.80E-03	YdB-peg2151, YdB-peg2621, YdB-peg977, YdB-peg2265, YdB-peg978
GO:0005215	transporter activity	12	417	3071	28	1.66	1.15E-02	YdB-peg3287, YdB-peg6299, YdB-peg6297, YdB-peg2814, YdB-peg2621, YdB-peg3716, YdB-peg976, YdB-peg977, YdB-peg3717, YdB-peg978, YdB-peg5909, YdB-peg1753

Matric 1YdBTEX2 down

GOID	Biological Process	No probes in class	Total No probes in class	Total No probes on array	No probes in comparison	log_odds_ratio	p-value	Genes
GO:0006928	cellular component movement	13	17	3071	49	5.58	1.28E-02	YdB-peg4830, YdB-peg4823, YdB-peg4821, YdB-peg4805, YdB-peg1455, YdB-peg1454, YdB-peg4827, YdB-peg4825, YdB-peg4829, YdB-peg4826, YdB-peg4811, YdB-peg4813, YdB-peg4814
GO:0001539	ciliary or flagellar motility	12	16	3071	49	5.55	2.77E-19	YdB-peg4830, YdB-peg4823, YdB-peg4805, YdB-peg1455, YdB-peg1454, YdB-peg4827, YdB-peg4825, YdB-peg4829, YdB-peg4826, YdB-peg4811, YdB-peg4813, YdB-peg4814
GO:0044781	bacterial-type flagellum organization	7	10	3071	49	5.46	3.50E-11	YdB-peg4830, YdB-peg4823, YdB-peg4821, YdB-peg4827, YdB-peg4826, YdB-peg4811, YdB-peg3012
GO:0006935	chemotaxis	8	43	3071	49	3.54	2.92E-08	YdB-peg4805, YdB-peg2614, YdB-peg2615, YdB-peg3453, YdB-peg4054, YdB-peg6053, YdB-peg4813, YdB-peg2475
GO:0006200	ATP catabolic process	2	18	3071	49	2.80	3.82E-02	YdB-peg2233, YdB-peg4811
GO:0009306	protein secretion	3	42	3071	49	2.16	3.56E-02	YdB-peg4811, YdB-peg4801, YdB-peg3012
GO:0006865	amino acid transport	2	30	3071	49	2.06	9.54E-02	YdB-peg778, YdB-peg1184
GO:0051179	localization	25	600	3071	49	1.38	6.92E-02	YdB-peg4830, YdB-peg4823, YdB-peg6307, YdB-peg4805, YdB-peg1455, YdB-peg1918, YdB-peg6309, YdB-peg1454, YdB-peg4827, YdB-peg5882, YdB-peg4825, YdB-peg4829, YdB-peg4024, YdB-peg6308, YdB-peg4826, YdB-peg4811, YdB-peg4801, YdB-peg6306, YdB-peg4813, YdB-peg3012, YdB-peg4814, YdB-peg4529, YdB-peg778, YdB-peg1184, YdB-peg5410
GO:0007165	signal transduction	8	228	3071	49	1.14	4.05E-02	YdB-peg2614, YdB-peg2615, YdB-peg3453, YdB-peg4054, YdB-peg3016, YdB-peg6053, YdB-peg2475, YdB-peg2417

Matric 1YdBTEX2 down

	Cellular Component	No probes in class	Total No probes in class	Total No probes on array	No probes in comparison	log_odds_ratio	p-value	Genes
GO:0009288	bacterial-type flagellum	14	18	3071	49	5.61	6.16E-20	YdB-peg4830, YdB-peg4823, YdB-peg4821, YdB-peg4805, YdB-peg1455, YdB-peg4820, YdB-peg1454, YdB-peg4827, YdB-peg4825, YdB-peg4829, YdB-peg4826, YdB-peg4813, YdB-peg4814, YdB-peg3017
GO:0044462	external encapsulating structure part	4	37	3071	49	2.76	2.23E-02	YdB-peg213, YdB-peg1918, YdB-peg4829, YdB-peg778
	Molecular Function							
GO:0003774	motor activity	7	11	3071	49	5.32	4.98E-11	YdB-peg4830, YdB-peg1455, YdB-peg1454, YdB-peg4829, YdB-peg4826, YdB-peg4813, YdB-peg4814
GO:0005198	structural molecule activity	6	44	3071	49	3.10	5.17E-05	YdB-peg4830, YdB-peg4823, YdB-peg1455, YdB-peg1454, YdB-peg4825, YdB-peg4826
GO:0015288	porin activity	2	20	3071	49	2.65	3.94E-02	YdB-peg6307, YdB-peg4024
GO:0004871	signal transducer activity	8	213	3071	49	1.24	1.40E-02	YdB-peg2614, YdB-peg2615, YdB-peg3453, YdB-peg4054, YdB-peg3016, YdB-peg6053, YdB-peg2475, YdB-peg2417

Table S16. **Enriched GO terms among the significantly differentially expressed genes in the comparison between *Pseudomonas veronii* 1YdBTEX2 cells after 30min exposure to solute stress with NaCl versus cells in control conditions.**

Solute 1YdBTEX2 Up

GOID	Biological Process	No probes in class	Total No probes in class	Total No probes on array	No probes in comparison	log_odds_ratio	p-value	Genes
GO:0009312	oligosaccharide biosynthetic process	2	11	3071	30	4.22	8.79E-02	YdB-peg5143, YdB-peg5151
GO:0006417	regulation of translation	1	6	3071	30	4.09	5.59E-02	YdB-peg4974
GO:0006979	response to oxidative stress	1	7	3071	30	3.87	6.49E-02	YdB-peg4752
GO:0055114	oxidation-reduction process	10	353	3071	30	1.54	1.43E-02	YdB-peg4752, YdB-peg5867, YdB-peg5146, YdB-peg2497, YdB-peg5145, YdB-peg4740, YdB-peg2758, YdB-peg3472, YdB-peg1698, YdB-peg1813
	Cellular Component							
GO:0005886	plasma membrane	2	42	3071	30	2.29	5.51E-02	YdB-peg3206, YdB-peg4740
	Molecular Function							
GO:0004601	peroxidase activity	2	6	3071	30	5.09	1.31E-03	YdB-peg4752, YdB-peg2497
GO:0005507	copper ion binding	2	11	3071	30	4.22	4.66E-03	YdB-peg4740, YdB-peg4741
GO:0004553	hydrolase activity, hydrolyzing O-glycosyl compounds	2	12	3071	30	4.09	5.56E-03	YdB-peg5146, YdB-peg5151
GO:0030976	thiamine pyrophosphate binding	1	9	3071	30	3.51	8.36E-02	YdB-peg4359
GO:0004129	cytochrome-c oxidase activity	1	9	3071	30	3.51	8.36E-02	YdB-peg4740
GO:0048038	quinone binding	1	9	3071	30	3.51	8.36E-02	YdB-peg1698
GO:0003995	acyl-CoA dehydrogenase activity	2	19	3071	30	3.43	1.38E-02	YdB-peg5145, YdB-peg3472
GO:0020037	heme binding	3	37	3071	30	3.05	4.98E-03	YdB-peg4752, YdB-peg2497, YdB-peg4740
GO:0005506	iron ion binding	3	61	3071	30	2.33	1.98E-02	YdB-peg5867, YdB-peg2497, YdB-peg4740
GO:0043169	cation binding	9	251	3071	30	1.88	8.19E-02	YdB-peg3657, YdB-peg5867, YdB-peg5146, YdB-peg2497, YdB-peg4359, YdB-peg4740, YdB-peg4736, YdB-peg5151, YdB-peg4741

Solute 1YdBTEX2 down

GOID	Biological Process	No probes in class	Total No probes in class	Total No probes on array	No probes in comparison	log_odds_ratio	p-value	Genes
GO:0006928	cellular component movement	11	17	3071	32	5.96	6.79E-03	YdB-peg1455, YdB-peg4830, YdB-peg1454, YdB-peg4827, YdB-peg4805, YdB-peg4826, YdB-peg4825, YdB-peg4829, YdB-peg4823, YdB-peg4821, YdB-peg4811
GO:0001539	ciliary or flagellar motility	10	16	3071	32	5.91	2.35E-17	YdB-peg1455, YdB-peg4830, YdB-peg1454, YdB-peg4827, YdB-peg4805, YdB-peg4826, YdB-peg4825, YdB-peg4829, YdB-peg4823, YdB-peg4811
GO:0044781	bacterial-type flagellum organization	6	10	3071	32	5.85	1.72E-10	YdB-peg4830, YdB-peg4827, YdB-peg4826, YdB-peg4823, YdB-peg4821, YdB-peg4811
GO:0006546	glycine catabolic process	2	7	3071	32	4.78	2.25E-03	YdB-peg3995, YdB-peg3996
GO:0009253	peptidoglycan catabolic process	1	7	3071	32	3.78	7.28E-02	YdB-peg4827
GO:0007155	cell adhesion	1	9	3071	32	3.41	9.26E-02	YdB-peg4821
GO:0009060	aerobic respiration	2	19	3071	32	3.34	1.69E-02	YdB-peg977, YdB-peg976
GO:0006935	chemotaxis	2	43	3071	32	2.16	7.95E-02	YdB-peg4805, YdB-peg3453

Solute 1YdBTEX2 down

	Cellular Component	No probes in class	Total No probes in class	Total No probes on array	No probes in comparison	log_odds_ratio	p-value	Genes
GO:0009288	bacterial-type flagellum	11	18	3071	32	5.87	1.13E-15	YdB-peg1455, YdB-peg4830, YdB-peg1454, YdB-peg4827, YdB-peg4805, YdB-peg4820, YdB-peg4826, YdB-peg4825, YdB-peg4829, YdB-peg4823, YdB-peg4821
GO:0009279	cell outer membrane	2	16	3071	32	3.58	4.58E-02	YdB-peg3611, YdB-peg4829
	Molecular Function							
GO:0003774	motor activity	5	11	3071	32	5.45	3.03E-08	YdB-peg1455, YdB-peg4830, YdB-peg1454, YdB-peg4826, YdB-peg4829
GO:0004129	cytochrome-c oxidase activity	3	9	3071	32	5.00	7.23E-05	YdB-peg977, YdB-peg976, YdB-peg978
GO:0015288	porin activity	3	20	3071	32	3.85	9.12E-04	YdB-peg215, YdB-peg6307, YdB-peg2054
GO:0005198	structural molecule activity	6	44	3071	32	3.71	3.05E-06	YdB-peg1455, YdB-peg4830, YdB-peg1454, YdB-peg4826, YdB-peg4825, YdB-peg4823

Table S17 **Pair-wise BlastP between the genes founded differentially expressed under conditions of Matric stress**

Swit_GI	Achl_GI	Pv	identities %	positives %	overlap	expect	Swit_Locus	Swit_annotation	Swit_Length	Achl	Annotation ACHL	Length ACHL	Annotation Pv
148498186	219861279		35.71	57.14	28	6.00E-04	Swit_0067	response regulator receiver protein	123	Achl_3666	conserved hypothetical p	154	
148498186		6666666.8973.YdB-peg3016	32.17	55.65	115	1.00E-19	Swit_0067	response regulator receiver protein	123				Chemotaxis regulator - transmits chemoreceptor signals to flagelllar motor components CheY
148498186		6666666.8973.YdB-peg3458	35.45	56.36	110	2.00E-12	Swit_0067	response regulator receiver protein	123				Chemotaxis response regulator protein-glutamate methylesterase CheB (EC 3.1.1.61)
148498186		6666666.8973.YdB-peg2192	27.2	48.8	125	1.00E-10	Swit_0067	response regulator receiver protein	123				DNA-binding response regulator
148498186		6666666.8973.YdB-peg4324	25.22	47.83	115	1.00E-09	Swit_0067	response regulator receiver protein	123				Histidine kinase/response regulator hybrid protein
148498243	219861344		39.29	58.04	112	1.00E-17	Swit_0125	ABC transporter related	229	Achl_3732	ABC transporter related	271	
148498243	219862219		32.54	47.85	209	3.00E-20	Swit_0125	ABC transporter related	229	Achl_4608	ABC transporter related	520	
148498243	219862219		28.57	44.76	210	4.00E-12	Swit_0125	ABC transporter related	229	Achl_4608	ABC transporter related	520	
148498243		6666666.8973.YdB-peg2233	35.19	53.7	216	5.00E-38	Swit_0125	ABC transporter related	229				ABC-type polar amino acid transport system, ATPase component
148498329	219860615		31.51	43.84	73	0.001	Swit_0212	flagellin-specific chaperone FliS-like protein	134	Achl_2996	flagellar protein FliS	173	
148498495	219861300		35.19	50.63	557	7.00E-79	Swit_0379	glucose-methanol-choline oxidoreductase	562	Achl_3687	glucose-methanol-cholir	547	
148498495		6666666.8973.YdB-peg1057	22.14	39.85	271	9.00E-06	Swit_0379	glucose-methanol-choline oxidoreductase	562				FIG022869: Oxidoreductase, GMC family
148498604		6666666.8973.YdB-peg2142	32.39	47.37	247	1.00E-28	Swit_0490	hypothetical protein Swit_0490	573				FIG00962473: hypothetical protein
148498604		6666666.8973.YdB-peg2142	36.96	54.35	46	3.00E-04	Swit_0490	hypothetical protein Swit_0490	573				FIG00962473: hypothetical protein
148498649		6666666.8973.YdB-peg2621	30.65	49.19	124	4.00E-09	Swit_0535	TonB-dependent receptor	809				Ferrichrome-iron receptor
148498649		6666666.8973.YdB-peg2621	38.71	54.84	31	2.00E-04	Swit_0535	TonB-dependent receptor	809				Ferrichrome-iron receptor
148498654		6666666.8973.YdB-peg2621	42.59	55.56	54	6.00E-07	Swit_0540	TonB-dependent receptor	700				Ferrichrome-iron receptor
148498667	219857846		25.49	48.04	102	0.004	Swit_0553	major facilitator transporter	391	Achl_0186	major facilitator superfa	476	
	219857846	6666666.8973.YdB-peg1473	37.25	56.86	51	8.00E-05	Swit_0553			Achl_0186	major facilitator superfa	476	Dicarboxylate MFS transporter
148498667		6666666.8973.YdB-peg1473	25.12	42.86	203	7.00E-09	Swit_0553	major facilitator transporter	391				Dicarboxylate MFS transporter
148499027		6666666.8973.YdB-peg2621	28.17	43.66	213	2.00E-12	Swit_0914	TonB-dependent receptor	803				Ferrichrome-iron receptor
148499027		6666666.8973.YdB-peg2621	29.77	43.51	131	2.00E-08	Swit_0914	TonB-dependent receptor	803				Ferrichrome-iron receptor
148499106		6666666.8973.YdB-peg3288	26.01	43.5	223	3.00E-18	Swit_0993	hypothetical protein Swit_0993	432				Predicted L-lactate dehydrogenase, Fe-S oxidoreductase subunit YkgE
148499106		6666666.8973.YdB-peg3291	25.59	36.03	383	3.00E-14	Swit_0993	hypothetical protein Swit_0993	432				Predicted D-lactate dehydrogenase, Fe-S protein, FAD/FMN-containing
148499257	219857846		26.34	46.43	224	2.00E-10	Swit_1145	EmrB/QacA family drug resistance transporter	508	Achl_0186	major facilitator superfa	476	
148499284		6666666.8973.YdB-peg1841	33.13	48.8	166	2.00E-16	Swit_1172	OmpA/MotB domain-containing protein	296				Outer membrane lipoprotein omp16 precursor
148499284		6666666.8973.YdB-peg3456	39.02	53.66	82	2.00E-09	Swit_1172	OmpA/MotB domain-containing protein	296				Flagellar motor rotation protein MotB
148499289		6666666.8973.YdB-peg1903	56.38	68.09	282	1.00E-110	Swit_1177	S-formylglutathione hydrolase	293				S-formylglutathione hydrolase (EC 3.1.2.12)
148499291		6666666.8973.YdB-peg1902	67.2	81.18	372	0	Swit_1179	S-(hydroxymethyl)glutathione dehydrogenase	371				S-(hydroxymethyl)glutathione dehydrogenase (EC 1.1.1.284)
148499374		6666666.8973.YdB-peg4827	38.37	60.47	86	8.00E-14	Swit_1263	rod binding-like protein	123				Flagellar protein FlgJ [peptidoglycan hydrolase] (EC 3.2.1.-)
148499378		6666666.8973.YdB-peg1452	31.03	45.69	116	2.00E-08	Swit_1267	flagellar basal-body rod protein FlgF	248				Flagellar hook protein FlgE
148499378		6666666.8973.YdB-peg1452	32.81	54.69	64	9.00E-07	Swit_1267	flagellar basal-body rod protein FlgF	248				Flagellar hook protein FlgE
148499379		6666666.8973.YdB-peg1452	36.58	53.03	462	3.00E-71	Swit_1268	flagellar basal body FlaE domain-containing protein	436				Flagellar hook protein FlgE
148499381		6666666.8973.YdB-peg1454	41.1	58.22	146	8.00E-31	Swit_1270	flagellar basal-body rod protein FlgC	135				Flagellar basal-body rod protein FlgC
148499397		6666666.8973.YdB-peg4815	43.06	65.28	72	3.00E-17	Swit_1286	flagellar hook-basal body complex subunit FliE	122				Flagellar hook-basal body complex protein FliE
148499986	219861299		24.57	40	175	3.00E-04	Swit_1880	indolepyruvate ferredoxin oxidoreductase	709	Achl_3686	Aldehyde Dehydrogenas	499	
	219861299	6666666.8973.YdB-peg1186	36.53	52.86	490	4.00E-91	Swit_1880			Achl_3686	Aldehyde Dehydrogenas	499	Aldehyde dehydrogenase (EC 1.2.1.3)
148500552	219858641		45.22	60.67	356	6.00E-95	Swit_2447	NADH:flavin oxidoreductase/NADH oxidase	352	Achl_0988	NADH:flavin oxidoreduct	360	
148500595		6666666.8973.YdB-peg4531	25.27	46.15	91	9.00E-04	Swit_2490	GreA/GreB family elongation factor	167				FIG00957387: hypothetical protein
148501015	219861344		25.35	44.13	213	1.00E-07	Swit_2917	FeS assembly ATPase SufC	248	Achl_3732	ABC transporter related	271	
	219861344	6666666.8973.YdB-peg2233	25.42	44.58	240	8.00E-23	Swit_2917			Achl_3732	ABC transporter related	271	ABC-type polar amino acid transport system, ATPase component
148501015	219862219		27.1	46.26	214	7.00E-09	Swit_2917	FeS assembly ATPase SufC	248	Achl_4608	ABC transporter related	520	
	219862219	6666666.8973.YdB-peg2233	28.9	48.17	218	1.00E-25	Swit_2917			Achl_4608	ABC transporter related	520	ABC-type polar amino acid transport system, ATPase component
	219862219	6666666.8973.YdB-peg2233	25.12	48.31	207	2.00E-18	Swit_2917			Achl_4608	ABC transporter related	520	ABC-type polar amino acid transport system, ATPase component
148501015		6666666.8973.YdB-peg2233	21.39	41.04	173	2.00E-08	Swit_2917	FeS assembly ATPase SufC	248				ABC-type polar amino acid transport system, ATPase component
148501177	219861223		30.57	45.22	157	7.00E-12	Swit_3081	GntR family transcriptional regulator	241	Achl_3609	transcriptional regulator	220	
	219861223	6666666.8973.YdB-peg777	26.92	42.95	156	1.00E-05	Swit_3081			Achl_3609	transcriptional regulator	220	Transcriptional regulator, GntR family
148501177		6666666.8973.YdB-peg777	25.79	40.27	221	5.00E-08	Swit_3081	GntR family transcriptional regulator	241				Transcriptional regulator, GntR family
148501223	219860603		24.64	44.93	69	0.005	Swit_3128	ATPase	860	Achl_2984	flagellar hook capping pr	139	
	219860603	6666666.8973.YdB-peg1453	46.81	63.83	47	1.00E-11	Swit_3128			Achl_2984	flagellar hook capping pr	139	Flagellar basal-body rod modification protein FlgD
148501279		6666666.8973.YdB-peg3458	30.29	43.97	373	2.00E-42	Swit_3186	response regulator receiver modulated CheB methylesterase	359				Chemotaxis response regulator protein-glutamate methylesterase CheB (EC 3.1.1.61)
148501279		6666666.8973.YdB-peg3016	26.61	45.16	124	2.00E-07	Swit_3186	response regulator receiver modulated CheB methylesterase	359				Chemotaxis regulator - transmits chemoreceptor signals to flagelllar motor components CheY
148501280		6666666.8973.YdB-peg3016	34.19	58.12	117	8.00E-21	Swit_3187	response regulator receiver protein	137				Chemotaxis regulator - transmits chemoreceptor signals to flagelllar motor components CheY
148501280		6666666.8973.YdB-peg2192	31.58	48.25	114	7.00E-11	Swit_3187	response regulator receiver protein	137				DNA-binding response regulator
148501280		6666666.8973.YdB-peg3458	31.19	50.46	109	1.00E-08	Swit_3187	response regulator receiver protein	137				Chemotaxis response regulator protein-glutamate methylesterase CheB (EC 3.1.1.61)
148501371		6666666.8973.YdB-peg2135	26.34	42.93	205	6.00E-12	Swit_3279	short-chain dehydrogenase/reductase SDR	256				Dehydrogenases with different specificities (related to short-chain alcohol dehydrogenases)
148501549		6666666.8973.YdB-peg2148	28.57	42.86	161	1.00E-11	Swit_3457	glutathione S-transferase domain-containing protein	233				Probable glutathione S-transferase (EC 2.5.1.18), YfcF homolog
148502114		6666666.8973.YdB-peg3480	30.4	46.26	227	3.00E-14	Swit_4023	rod shape-determining protein MreB	351				Chaperone protein HscA
148502116		6666666.8973.YdB-peg2621	25.67	39.39	787	1.00E-39	Swit_4025	TonB-dependent siderophore receptor	827				Ferrichrome-iron receptor
148502360		6666666.8973.YdB-peg2621	22.61	36.7	575	4.00E-12	Swit_4274	TonB-dependent receptor	759				Ferrichrome-iron receptor
148502554		6666666.8973.YdB-peg2146	28.99	42.03	69	4.00E-04	Swit_4470	hypothetical protein Swit_4470	87				Chromosome segregation ATPases
148502554		6666666.8973.YdB-peg3487	31.67	46.67	60	6.00E-04	Swit_4470	hypothetical protein Swit_4470	87				1-hydroxy-2-methyl-2-(E)-butenyl 4-diphosphate synthase (EC 1.17.7.1)
148502607		6666666.8973.YdB-peg2812	23.64	38.37	258	2.00E-08	Swit_4523	glycosyl transferase family protein	337				Biofilm PGA synthesis N-glycosyltransferase PgaC (EC 2.4.-.-)
148502609		6666666.8973.YdB-peg2812	26.09	45.65	92	2.00E-06	Swit_4525	glycosyl transferase family protein	338				Biofilm PGA synthesis N-glycosyltransferase PgaC (EC 2.4.-.-)
148502610		6666666.8973.YdB-peg2812	31.25	45.31	128	2.00E-11	Swit_4526	glycosyl transferase family protein	420				Biofilm PGA synthesis N-glycosyltransferase PgaC (EC 2.4.-.-)
148502612		6666666.8973.YdB-peg3014	26.39	44.44	144	3.00E-06	Swit_4528	non-specific protein-tyrosine kinase	711				Flagellar synthesis regulator FleN
148502804		6666666.8973.YdB-peg4324	36.71	54.43	79	2.00E-08	Swit_4721	response regulator receiver protein	136				Histidine kinase/response regulator hybrid protein
148502804		6666666.8973.YdB-peg2192	28.57	43.65	126	2.00E-07	Swit_4721	response regulator receiver protein	136				DNA-binding response regulator
148502804		6666666.8973.YdB-peg3016	26.4	42.4	125	8.00E-06	Swit_4721	response regulator receiver protein	136				Chemotaxis regulator - transmits chemoreceptor signals to flagelllar motor components CheY
148502864		6666666.8973.YdB-peg2621	22.31	37.95	780	2.00E-15	Swit_4781	TonB-dependent receptor	754				Ferrichrome-iron receptor
148503161	219861233		36.86	54.17	312	1.00E-48	Swit_5306	heat shock protein DnaJ domain-containing protein	298	Achl_3619	chaperone DnaJ domain	327	
	219859618	6666666.8973.YdB-peg1753	33.08	48.5	266	5.00E-29	#N/A			Achl_1986	major intrinsic protein	250	Glycerol uptake facilitator protein
	219861300	6666666.8973.YdB-peg1057	23.63	37.97	474	2.00E-09	#N/A			Achl_3687	glucose-methanol-cholir	547	FIG022869: Oxidoreductase, GMC family
	219861301	6666666.8973.YdB-peg1184	25.93	46.67	135	0.002	#N/A			Achl_3688	amino acid permease-as	539	Ethanolamine permease

Table S18 Pair-wise BlastP between the genes founded differentially expressed under conditions of Solute stress

RW1 hit GI nr	Pv hits	identities %	positives %	overlap	expect	score	Locus_tag	Annotation Swit	Swit_length	Locus-ACHL		ACHL_length	Locus_PV	annotation Pv
219862219 ACL42559.1	6666666.8973.YdB-peg2154	26.12	44.9	245	6.00E-18	71.2				Achl_4608	ABC transporter r	520	YdB-peg2154	Maltose maltodextrin transport ATP-binding protein
219858830 ACL39172.1	6666666.8973.YdB-peg2154	30.95	54.76	84	2.00E-09	44.7				Achl_1181	ABC transporter r	521	YdB-peg2154	Maltose maltodextrin transport ATP-binding protein
219860613 ACL40955.1	6666666.8973.YdB-peg1444	28.3	49.06	53	6.00E-04	23.9				Achl_2994	flagellar basal-boc	114	YdB-peg1444	Transcriptional regulatory protein PhoP
219860613 ACL40955.1	6666666.8973.YdB-peg2260	38.1	57.14	21	7.00E-04	20.4				Achl_2994	flagellar basal-boc	114	YdB-peg2260	2-methylaconitate isomerase
219860612 ACL40954.1	6666666.8973.YdB-peg1454	28.37	49.65	141	1.00E-20	70.1				Achl_2993	flagellar basal-boc	131	YdB-peg1454	Flagellar basal-body rod protein FlgC
219860612 ACL40954.1	6666666.8973.YdB-peg4830	30	60	30	2.00E-04	25.8				Achl_2993	flagellar basal-boc	131	YdB-peg4830	Flagellar basal-body rod protein FlgG
219858617 ACL38959.1	6666666.8973.YdB-peg5410	21.65	42.64	462	3.00E-10	48.5				Achl_0964	Na+/solute sympc	509	YdB-peg5410	Acetate permease ActP (cation/acetate symporter)
219860600 ACL40942.1	6666666.8973.YdB-peg3457	36.32	56.84	234	6.00E-47	147				Achl_2981	MotA/TolQ/ExbB	277	YdB-peg3457	Flagellar motor rotation protein MotA
219860617 ACL40959.1	6666666.8973.YdB-peg4823	41.18	64.71	170	3.00E-36	126				Achl_2998	flagellin domain p	392	YdB-peg4823	Flagellin protein FlaB
219860421 ACL40763.1	6666666.8973.YdB-peg4157	25.96	43.17	366	4.00E-22	85.1				Achl_2798	4-aminobutyrate a	457	YdB-peg4157	Omega-amino acid--pyruvate aminotransferase (EC 2
219860617 ACL40959.1	6666666.8973.YdB-peg4823	35.38	51.54	130	4.00E-17	69.7				Achl_2998	flagellin domain p	392	YdB-peg4823	Flagellin protein FlaB
219860620 ACL40962.1	6666666.8973.YdB-peg4825	26.28	45.26	137	2.00E-09	44.3				Achl_3001	flagellar hook-ass	295	YdB-peg4825	Flagellar hook-associated protein FlgL
219860620 ACL40962.1	6666666.8973.YdB-peg4823	27.82	47.37	133	5.00E-08	40.4				Achl_3001	flagellar hook-ass	295	YdB-peg4823	Flagellin protein FlaB
219860598 ACL40940.1	6666666.8973.YdB-peg4803	31.37	52.94	51	7.00E-07	33.9				Achl_2979	surface presentati	299	YdB-peg4803	Flagellar motor switch protein FliN
219860617 ACL40959.1	6666666.8973.YdB-peg4825	23.81	44.9	147	7.00E-05	30.8				Achl_2998	flagellin domain p	392	YdB-peg4825	Flagellar hook-associated protein FlgL
148498186 ABQ66440.1	6666666.8973.YdB-peg3016	32.17	55.65	115	1.00E-19	66.6	Swit_0067	response regulator receiver protein	123				YdB-peg3016	Chemotaxis regulator - transmits chemoreceptor sign
148498186 ABQ66440.1	6666666.8973.YdB-peg1444	28.7	49.57	115	6.00E-12	47	Swit_0067	response regulator receiver protein	123				YdB-peg1444	Transcriptional regulatory protein PhoP
148498329 ABQ66583.1	6666666.8973.YdB-peg4820	29.85	44.03	134	2.00E-08	35.8	Swit_0212	flagellin-specific chaperone FliS-like prot	134				YdB-peg4820	Flagellar biosynthesis protein FliS
148498329 gi|220913737|	219860615	31.51	43.84	73	0.001	22.7	Swit_0212	flagellin-specific chaperone FliS-like prot	134	Achl_2996	flagellar protein Fl	173		
148498374 ABQ66628.1	6666666.8973.YdB-peg6296	27.7	50.23	213	2.00E-21	79.3	Swit_0257	ABC transporter related	267				YdB-peg6296	L-proline glycine betaine ABC transport system perm
148498374 ABQ66628.1	6666666.8973.YdB-peg2154	29	47.62	231	5.00E-21	77.8	Swit_0257	ABC transporter related	267				YdB-peg2154	Maltose maltodextrin transport ATP-binding protein
148498374 ABQ66628.1	6666666.8973.YdB-peg5565	30.2	45.31	245	7.00E-16	63.9	Swit_0257	ABC transporter related	267				YdB-peg5565	ABC transporter, ATP-binding protein
148498374 ABQ66628.1	6666666.8973.YdB-peg5567	29.72	47.17	212	9.00E-16	63.5	Swit_0257	ABC transporter related	267				YdB-peg5567	ABC transporter, ATP-binding/permease protein
148498374 ABQ66628.1	6666666.8973.YdB-peg5565	29.49	48.08	156	5.00E-10	46.2	Swit_0257	ABC transporter related	267				YdB-peg5565	ABC transporter, ATP-binding protein
148498374 gi|219883374|	219862219	28.63	45.97	248	7.00E-22	81.6	Swit_0257	ABC transporter related	267	Achl_4608	ABC transporter r	520		
148498374 gi|219883374|	219862219	31.71	52.68	205	9.00E-22	81.3	Swit_0257	ABC transporter related	267	Achl_4608	ABC transporter r	520		
148498374 gi|220911952|	219858830	24.35	45.65	230	4.00E-16	64.3	Swit_0257	ABC transporter related	267	Achl_1181	ABC transporter r	521		
148498374 gi|220911952|	219858830	26.37	45.27	201	3.00E-12	52.8	Swit_0257	ABC transporter related	267	Achl_1181	ABC transporter r	521		
148498659 ABQ66913.1	6666666.8973.YdB-peg3936	43.1	53.45	58	4.00E-12	42	Swit_0545	hypothetical protein Swit_0545	60				YdB-peg3936	FIG00954474: hypothetical protein
148498806 ABQ67060.1	6666666.8973.YdB-peg1698	25.75	38.48	699	6.00E-38	136	Swit_0693	Pyrrolo-quinoline quinone	580				YdB-peg1698	Glucose dehydrogenase, PQQ-dependent (EC 1.1.5.2)
219861109 ACL41451.1	6666666.8973.YdB-peg2758	39.62	58.7	477	8.00E-107	319	Swit_0703	4E-102		Achl_3495	Aldehyde Dehydrc	500	YdB-peg2758	Aldehyde dehydrogenase (EC 1.2.1.3)
219861299 ACL41641.1	6666666.8973.YdB-peg2758	39.12	57.32	478	2.00E-106	318	Swit_0703	3E-101		Achl_3686	Aldehyde Dehydrc	499	YdB-peg2758	Aldehyde dehydrogenase (EC 1.2.1.3)
219858926 ACL39268.1	6666666.8973.YdB-peg2758	37.26	55.03	467	3.00E-89	273	Swit_0703	1E-65		Achl_1277	Aldehyde Dehydrc	500	YdB-peg2758	Aldehyde dehydrogenase (EC 1.2.1.3)
148498816 ABQ67070.1	6666666.8973.YdB-peg2758	38.87	56.51	476	5.00E-113	336	Swit_0703	aldehyde dehydrogenase	507				YdB-peg2758	Aldehyde dehydrogenase (EC 1.2.1.3)
148498816 gi|220914231|	219861109	37.55	56.53	490	4.00E-102	307	Swit_0703	aldehyde dehydrogenase	507	Achl_3495	Aldehyde Dehydrc	500		
148498816 gi|220914421|	219861299	38.57	55.92	490	3.00E-101	305	Swit_0703	aldehyde dehydrogenase	507	Achl_3686	Aldehyde Dehydrc	499		
148498816 gi|220913544|	219860422	34.17	51.15	477	2.00E-80	250	Swit_0703	aldehyde dehydrogenase	507	Achl_2799	Aldehyde Dehydrc	477		
148498816 gi|220912048|	219858926	34.09	51.86	484	1.00E-65	211	Swit_0703	aldehyde dehydrogenase	507	Achl_1277	Aldehyde Dehydrc	500		
148499263 gi|220911953|	219858831	27.12	44.92	118	1.00E-11	50.8	Swit_1151	ROK family protein	300	Achl_1182	ROK family protei	397		
148499284 ABQ67538.1	6666666.8973.YdB-peg1841	33.13	48.8	166	2.00E-16	63.5	Swit_1172	OmpA/MotB domain-containing protein	296				YdB-peg1841	Outer membrane lipoprotein omp16 precursor
148499284 gi|220913721|	219860599	32.79	42.62	61	0.001	25.4	Swit_1172	OmpA/MotB domain-containing protein	296	Achl_2980	OmpA/MotB dom	267		
148499374 ABQ67628.1	6666666.8973.YdB-peg4827	38.37	60.47	86	8.00E-14	54.3	Swit_1263	rod binding-like protein	123				YdB-peg4827	Flagellar protein FlgJ [peptidoglycan hydrolase] (EC 3.
148499375 ABQ67629.1	6666666.8973.YdB-peg4828	53.3	73.35	349	7.00E-132	374	Swit_1264	flagellar basal body P-ring protein	368				YdB-peg4828	Flagellar P-ring protein FlgI
219860613 ACL40955.1	6666666.8973.YdB-peg1455	28.33	45.83	120	1.00E-09	38.5	Swit_1267	0.0006		Achl_2994	flagellar basal-boc	114	YdB-peg1455	Flagellar basal-body rod protein FlgB
219860613 ACL40955.1	6666666.8973.YdB-peg4830	40.54	62.16	37	3.00E-06	30.8	Swit_1267	0.0006		Achl_2994	flagellar basal-boc	114	YdB-peg4830	Flagellar basal-body rod protein FlgG
219860613 ACL40955.1	6666666.8973.YdB-peg1452	38.89	46.3	54	1.00E-05	30	Swit_1267	0.0006		Achl_2994	flagellar basal-boc	114	YdB-peg1452	Flagellar hook protein FlgE
148499378 ABQ67632.1	6666666.8973.YdB-peg4831	37.66	58.58	239	7.00E-55	167	Swit_1267	flagellar basal-body rod protein FlgF	248				YdB-peg4831	Flagellar basal-body rod protein FlgF
148499378 ABQ67632.1	6666666.8973.YdB-peg4830	26.14	44.4	241	1.00E-14	58.2	Swit_1267	flagellar basal-body rod protein FlgF	248				YdB-peg4830	Flagellar basal-body rod protein FlgG
148499378 ABQ67632.1	6666666.8973.YdB-peg1452	31.03	45.69	116	2.00E-08	40.4	Swit_1267	flagellar basal-body rod protein FlgF	248				YdB-peg1452	Flagellar hook protein FlgE
148499378 ABQ67632.1	6666666.8973.YdB-peg1454	38.1	59.52	42	8.00E-07	33.1	Swit_1267	flagellar basal-body rod protein FlgF	248				YdB-peg1454	Flagellar basal-body rod protein FlgC
148499378 ABQ67632.1	6666666.8973.YdB-peg1452	32.81	54.69	64	9.00E-07	35.4	Swit_1267	flagellar basal-body rod protein FlgF	248				YdB-peg1452	Flagellar hook protein FlgE
148499378 gi|220913735|	219860613	30.12	43.37	83	6.00E-04	23.9	Swit_1267	flagellar basal-body rod protein FlgF	248	Achl_2994	flagellar basal-boc	114		
148499404 ABQ67658.1	6666666.8973.YdB-peg4805	23.7	45.93	135	1.00E-08	38.5	Swit_1293	flagellar basal body-associated protein F	200				YdB-peg4805	Flagellar biosynthesis protein FliL
219860598 ACL40940.1	6666666.8973.YdB-peg4804	22.9	39.31	262	3.00E-16	64.7	Swit_1458	0.000000001		Achl_2979	surface presentati	299	YdB-peg4804	Flagellar motor switch protein FliM
148499568 ABQ67822.1	6666666.8973.YdB-peg4804	27.64	48.45	322	7.00E-37	124	Swit_1458	flagellar motor switch protein FliM	332				YdB-peg4804	Flagellar motor switch protein FliM
148499568 gi|220913720|	219860598	23	39.02	287	1.00E-09	44.3	Swit_1458	flagellar motor switch protein FliM	332	Achl_2979	surface presentati	299		
148499568 gi|220913723|	219860601	28.89	46.67	45	0.004	21.2	Swit_1458	flagellar motor switch protein FliM	332	Achl_2982	flagellar FlbD fami	82		
148500237 ABQ68491.1	6666666.8973.YdB-peg1841	36.19	51.43	105	3.00E-19	69.3	Swit_2132	peptidoglycan-associated lipoprotein	176				YdB-peg1841	Outer membrane lipoprotein omp16 precursor

RW1 hit GI nr	Pv hits	identities %	positives %	overlap	expect	score	Locus_tag	Annotation Swit	Swit_length	Locus-ACHL		ACHL_length	Locus_PV	annotation Pv
148500237 gi|220913721|	219860599	30	44	50	3.00E-05	29.3	Swit_2132	peptidoglycan-associated lipoprotein	176	Achl_2980	OmpA/MotB dom	267		
219860599 ACL40941.1	6666666.8973.YdB-peg1841	24.11	39.92	253	2.00E-05	30.4	Swit_2278	0.0005		Achl_2980	OmpA/MotB dom	267	YdB-peg1841	Outer membrane lipoprotein omp16 precursor
148500383 ABQ68637.1	6666666.8973.YdB-peg1841	34.29	50.48	105	2.00E-14	58.5	Swit_2278	OmpA/MotB domain-containing protein	374				YdB-peg1841	Outer membrane lipoprotein omp16 precursor
148500383 gi|220913721|	219860599	29.13	47.57	103	5.00E-04	26.9	Swit_2278	OmpA/MotB domain-containing protein	374	Achl_2980	OmpA/MotB dom	267		
148500427 ABQ68681.1	6666666.8973.YdB-peg1841	42.72	58.25	103	4.00E-22	78.6	Swit_2322	OmpA/MotB domain-containing protein	223				YdB-peg1841	Outer membrane lipoprotein omp16 precursor
148500427 ABQ68681.1	6666666.8973.YdB-peg6223	30.3	48.48	33	8.00E-04	22.7	Swit_2322	OmpA/MotB domain-containing protein	223				YdB-peg6223	hypothetical protein
148500427 gi|220913721|	219860599	25.91	41.36	220	1.00E-08	40	Swit_2322	OmpA/MotB domain-containing protein	223	Achl_2980	OmpA/MotB dom	267		
148500429 ABQ68683.1	6666666.8973.YdB-peg2988	51.28	74.36	39	3.00E-13	45.1	Swit_2324	hypothetical protein Swit_2324	71				YdB-peg2988	Stress induced hydrophobic peptide
148500430 ABQ68684.1	6666666.8973.YdB-peg4974	53.85	76.92	13	8.00E-04	21.9	Swit_2325	hypothetical protein Swit_2325	153				YdB-peg4974	Ribosome modulation factor
148500439 ABQ68693.1	6666666.8973.YdB-peg4771	51.16	69.77	43	2.00E-12	42.4	Swit_2334	hypothetical protein Swit_2334	61				YdB-peg4771	probable exported protein YPO0432
148500440 ABQ68694.1	6666666.8973.YdB-peg2255	25.35	43.66	71	6.00E-04	23.9	Swit_2335	hypothetical protein Swit_2335	117				YdB-peg2255	alginate o-acetyltransferase AlgF
219858648 ACL38990.1	6666666.8973.YdB-peg1667	30	58	50	1.00E-05	29.6	Swit_2353	0.001		Achl_0996	Transglycosylase	213	YdB-peg1667	FIG00954962: hypothetical protein
148500458 gi|220911770|	219858648	48.15	77.78	27	0.001	24.3	Swit_2353	hypothetical protein Swit_2353	274	Achl_0996	Transglycosylase	213		
148500645 ABQ68899.1	6666666.8973.YdB-peg1444	30.25	45.38	119	6.00E-09	38.1	Swit_2540	response regulator receiver protein	119				YdB-peg1444	Transcriptional regulatory protein PhoP
148500645 ABQ68899.1	6666666.8973.YdB-peg3016	27.27	47.11	121	4.00E-08	34.7	Swit_2540	response regulator receiver protein	119				YdB-peg3016	Chemotaxis regulator - transmits chemoreceptor sign
148500799 ABQ69053.1	6666666.8973.YdB-peg3996	38.57	52.66	433	5.00E-72	237	Swit_2697	glycine dehydrogenase subunit 2	525				YdB-peg3996	Glycine dehydrogenase [decarboxylating] (glycine cle
148500799 ABQ69053.1	6666666.8973.YdB-peg1843	26.61	41.28	109	5.00E-04	25.4	Swit_2697	glycine dehydrogenase subunit 2	525				YdB-peg1843	FIG00958830: hypothetical protein
219860422 ACL40764.1	6666666.8973.YdB-peg2758	33.9	54.03	472	8.00E-82	253	Swit_2698	0.003		Achl_2799	Aldehyde Dehydr	477	YdB-peg2758	Aldehyde dehydrogenase (EC 1.2.1.3)
148500800 ABQ69054.1	6666666.8973.YdB-peg4822	33.33	45.1	51	9.00E-04	23.5	Swit_2698	hypothetical protein Swit_2698	262				YdB-peg4822	Flagellin protein FlaG
219862219 ACL42559.1	6666666.8973.YdB-peg2154	30.87	48.7	230	8.00E-23	85.9	Swit_2743	6E-20		Achl_4608	ABC transporter r	520	YdB-peg2154	Maltose maltodextrin transport ATP-binding protein
219858830 ACL39172.1	6666666.8973.YdB-peg2154	30.77	52.04	221	6.00E-22	83.6	Swit_2743	3E-13		Achl_1181	ABC transporter r	521	YdB-peg2154	Maltose maltodextrin transport ATP-binding protein
148500844 ABQ69098.1	6666666.8973.YdB-peg2154	39.19	52.7	222	1.00E-33	114	Swit_2743	ABC transporter related	273				YdB-peg2154	Maltose maltodextrin transport ATP-binding protein
148500844 ABQ69098.1	6666666.8973.YdB-peg5567	32.27	51.36	220	3.00E-19	74.3	Swit_2743	ABC transporter related	273				YdB-peg5567	ABC transporter, ATP-binding/permease protein
148500844 ABQ69098.1	6666666.8973.YdB-peg5565	29.65	46.46	226	9.00E-17	67	Swit_2743	ABC transporter related	273				YdB-peg5565	ABC transporter, ATP-binding protein
148500844 ABQ69098.1	6666666.8973.YdB-peg5565	27.27	46.02	176	5.00E-07	37	Swit_2743	ABC transporter related	273				YdB-peg5565	ABC transporter, ATP-binding protein
148500844 gi|219883374|	219862219	28.76	49.56	226	6.00E-20	75.9	Swit_2743	ABC transporter related	273	Achl_4608	ABC transporter r	520		
148500844 gi|219883374|	219862219	28.85	46.63	208	2.00E-14	59.3	Swit_2743	ABC transporter related	273	Achl_4608	ABC transporter r	520		
148500844 gi|220911952|	219858830	26.09	47.34	207	3.00E-13	55.8	Swit_2743	ABC transporter related	273	Achl_1181	ABC transporter r	521		
148500844 gi|220911952|	219858830	27.75	44.98	209	1.00E-11	51.2	Swit_2743	ABC transporter related	273	Achl_1181	ABC transporter r	521		
148501280 ABQ69534.1	6666666.8973.YdB-peg3016	34.19	58.12	117	8.00E-21	70.5	Swit_3187	response regulator receiver protein	137				YdB-peg3016	Chemotaxis regulator - transmits chemoreceptor sign
148501280 ABQ69534.1	6666666.8973.YdB-peg1444	31.19	50.46	109	1.00E-13	52	Swit_3187	response regulator receiver protein	137				YdB-peg1444	Transcriptional regulatory protein PhoP
148501660 ABQ69914.1	6666666.8973.YdB-peg2151	24.69	35.19	162	2.00E-04	27.7	Swit_3568	hypothetical protein Swit_3568	159				YdB-peg2151	5-methyltetrahydropteroyltriglutamate--homocystein
148501779 ABQ70033.1	6666666.8973.YdB-peg5566	39.23	49.23	130	3.00E-15	63.2	Swit_3687	lytic transglycosylase, catalytic	301				YdB-peg5566	Soluble lytic murein transglycosylase precursor (EC 3.
148501869 ABQ70123.1	6666666.8973.YdB-peg4898	19.1	41.01	178	7.00E-04	25.4	Swit_3778	hypothetical protein Swit_3778	250				YdB-peg4898	FIG00959721: hypothetical protein
148502016 ABQ70270.1	6666666.8973.YdB-peg1444	26.72	49.14	116	9.00E-07	34.3	Swit_3925	two-component response regulator	265				YdB-peg1444	Transcriptional regulatory protein PhoP
148502016 ABQ70270.1	6666666.8973.YdB-peg3016	21.55	48.28	116	7.00E-04	24.3	Swit_3925	two-component response regulator	265				YdB-peg3016	Chemotaxis regulator - transmits chemoreceptor sign
148502612 gi|220911957|	219858835	34.62	47.44	78	0.003	26.6	Swit_4528	non-specific protein-tyrosine kinase	711	Achl_1186	amine oxidase	579		
148502612 gi|219883342|	219862187	35.71	44.64	56	0.003	26.2	Swit_4528	non-specific protein-tyrosine kinase	711	Achl_4576	Beta-ketoacyl syn	408		
148502614 gi|220914354|	219861232	37.5	47.5	40	3.00E-04	26.6	Swit_4530	O-antigen polymerase	445	Achl_3618	transcriptional re	142		
148502632 ABQ70886.1	6666666.8973.YdB-peg3658	24.06	36.35	586	3.00E-19	78.2	Swit_4548	asparagine synthase	752				YdB-peg3658	Asparagine synthetase [glutamine-hydrolyzing] (EC 6.
148503166 ABQ71419.1	6666666.8973.YdB-peg4752	25	40.18	112	3.00E-04	28.9	Swit_5311	catalase	382				YdB-peg4752	Catalase (EC 1.11.1.6)
148503251 ABQ71504.1	6666666.8973.YdB-peg3016	36.49	55.41	74	2.00E-10	41.2	Swit_5396	response regulator receiver protein	133				YdB-peg3016	Chemotaxis regulator - transmits chemoreceptor sign
148503251 ABQ71504.1	6666666.8973.YdB-peg1444	32.41	49.07	108	6.00E-09	38.5	Swit_5396	response regulator receiver protein	133				YdB-peg1444	Transcriptional regulatory protein PhoP

CHAPTER III

Genome-wide analysis of *Sphingomonas wittichii* RW1 behaviour during inoculation and growth in contaminated sand

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ORIGINAL ARTICLE

Genome-wide analysis of *Sphingomonas wittichii* RW1 behaviour during inoculation and growth in contaminated sand

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The efficacy of inoculation of single pure bacterial cultures into complex microbiomes, for example, in order to achieve increased pollutant degradation rates in contaminated material (that is, bioaugmentation), has been frustrated by insufficient knowledge on the behaviour of the inoculated bacteria under the specific abiotic and biotic boundary conditions. Here we present a comprehensive analysis of genome-wide gene expression of the bacterium *Sphingomonas wittichii* RW1 in contaminated non-sterile sand, compared with regular suspended batch growth in liquid culture. RW1 is a well-known bacterium capable of mineralizing dibenzodioxins and dibenzofurans. We tested the reactions of the cells both during the immediate transition phase from liquid culture to sand with or without dibenzofuran, as well as during growth and stationary phase in sand. Cells during transition show stationary phase characteristics, evidence for stress and for nutrient scavenging, and adjust their primary metabolism if they were not precultured on the same contaminant as found in the soil. Cells growing and surviving in sand degrade dibenzofuran but display a very different transcriptome signature as in liquid or in liquid culture exposed to chemicals inducing drought stress, and we obtain evidence for numerous 'soil-specific' expressed genes. Studies focusing on inoculation efficacy should test behaviour under conditions as closely as possible mimicking the intended microbiome conditions.
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Introduction

Environmental pollution is one of the most relevant challenges of our time in terms of potential harmful effects on biodiversity and human health (Kulkarni *et al.*, 2008; Camilli *et al.*, 2010; Hazen *et al.*, 2010; Megharaj *et al.*, 2011). Many pollutants are spontaneously transformed and removed from the environment by microbial activity (Medina-Bellver *et al.*, 2005; Camilli *et al.*, 2010; Hazen *et al.*, 2010; Mrozika and Piotrowska-Segetb, 2010). As a consequence, there has been considerable interest in understanding the capacity and the roles of bacteria for degradation of pollutants (de Lorenzo, 2001; Andreoni and Gianfreda, 2007; de Lorenzo, 2009; Haritash and Kaushik, 2009; de Lorenzo *et al.*, 2013). This has resulted in isolation of a wide variety of specific strains capable to degrade particular contaminants and in characterization of their catabolic activity under laboratory conditions.

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It has been proposed that inoculation of preenriched strains or pure culture isolates with biodegradative properties may be beneficial for enhancing the degradation rates of organic pollutants at contaminated sites or for achieving degradation of one or more specific organic pollutants for which no 'inherent' capacity exists at a site (de Lorenzo, 2001; de Lorenzo, 2009; Tyagi *et al.*, 2011; de Lorenzo *et al.*, 2013). The success of such bioaugmentation, however, is mostly anecdotic and the activity of inoculated pure culture isolates to degrade pollutants in the environment is still relatively unpredictable (Tchelet *et al.*, 1999; Mrozika and Piotrowska-Segetb, 2010; Megharaj *et al.*, 2011; Jeon and Madsen, 2013). It is clear that we do not understand sufficiently well how introduced pure culture isolates behave under the environmental conditions and within a native microbiome. Strain behaviour in a complex system in first instance will depend on its ability to survive and/or grow to a sizeable population. Several factors have been implicated in survival of introduced bacterial strains in the environment, such as water availability, pH or temperature (Megharaj *et al.*, 1997; Halden *et al.*, 1999; Backman and Jansson, 2004). In second instance, even when surviving and growing, the activity of introduced bacteria for

degradation depends on the accessibility of the target pollutant to the cells, the presence of other metabolizable substrates and, more generally, available nutrients. Autoecological properties such as adaptation to a broad range of environments and carbon sources, biofilm formation, motility, biosurfactant production (Cunliffe and Kertesz, 2006) and genome structure have been implicated as well (Mongodin *et al.*, 2006), but the general molecular and functional response of cells to a given environment has been poorly explored (Desai *et al.*, 2010; Puglisi *et al.*, 2010; Wang *et al.*, 2011).

The overarching goal of this work is thus to improve our understanding of the environmental behaviour of bacterial strains degrading aromatic hydrocarbons and of the factors that determine their activity. In particular, we were interested to study the global reactions of bacteria with biodegradative properties under near-environmental conditions as compared with laboratory culture conditions. Global reactions can be deduced from analysing recorded genome-wide expression profiles under different growth conditions, which we hypothesize will highlight factors and pathways specifically needed under those conditions.

The microorganism we chose to work with is *Sphingomonas wittichii* RW1, which is capable to degrade dibenzo-*p*-dioxins and dibenzofurans (DBF), and to cometabolize some of their chlorinated substitutes (Wittich *et al.*, 1992; Wilkes *et al.*, 1996; Yabuuchi *et al.*, 2001; Hong *et al.*, 2002; Nam *et al.*, 2012). *S. wittichii* RW1 has been proposed as a candidate pure culture isolate to achieve targeted degradation of dioxins and DBFs and has been used in several studies. Megharaj *et al.* (1997) showed that preadaptation of RW1 to soil before inoculation enhanced its survival and increased biodegradation rates of DBF and dibenzodioxins. Halden *et al.* (1999) revealed the negative influence of organic matter on the kinetics of biodegradation of 2-chlorodibenzo-*p*-dioxin, whereas Nam *et al.* (2005) showed survival of RW1 in minimal medium with fly ash from solid waste incinerators and demonstrated that RW1 can act as a sorbent for dioxins. The complete genome of RW1 has been sequenced and was recently published (Miller *et al.*, 2010). Following up on this, we analysed gene expression in RW1 upon growth on salicylate, DBF and phenylalanine and showed how likely several parallel 'lower' pathways operate in DBF metabolism after the initial unique angular dioxygenase attack (Coronado *et al.*, 2012). We further examined genome-wide expression of RW1 in response to laboratory condition-induced water stress (Johnson *et al.*, 2011). Finally, we performed a genome-wide transposon scanning of RW1 to identify putative functions important for survival under drought stress and in soil (Roggo *et al.*, 2013).

In an attempt to better understand the strategies that RW1 displays once it is introduced into a (non-lab) environment, we compared here the genome-wide responses of RW1 between regular laboratory batch growth on the aromatic substrates DBF and salicylate with growth in sand with or without the same aromatic compounds. We analysed the cellular reactions immediately after introduction into the sand, during early and late growth phases, all in carefully controlled and replicated experimental conditions. DBF degradation by the inoculated RW1 population in the sand was measured. Genome-wide transcriptome changes were recorded by micro-array hybridizations of purified and reverse-transcribed labelled RW1 cDNA, as previously described (Johnson *et al.*, 2011). We find that global reactions of RW1 are extremely different to liquid batch cultures and soil batch incubations with the same major carbon substrate, even though the specific growth rates are not very different in the two situations.

Materials and methods

Culture conditions

S. wittichii RW1 was cultured in phosphate-buffered mineral medium (medium DSM457 from the Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany) with salicylate (5 mM) or DBF as the sole carbon source. Liquid batch cultures were incubated at 30 °C and with 180 r.p.m. rotary shaking. DBF was dosed as crystals (1.6 g l^{-1}) to sterilized medium.

Preparation of sandy soil

Sand was collected in Spring 2011 on a beach of Lausanne near Lake Leman (46.5079741 N, 6.545103 E). The sand was sieved through a 2-mm mesh, dried at 80 °C for 16 h and stored at room temperature (18–22 °C). pH-H_2O of the sand was 7.14 ± 0.02, its $CaCO_3$ content is between 25–55%, whereas the percentage of organic matter content is 0.028 ± 0.005. Dry sand was contaminated with DBF by spraying 10 ml of a dichloromethane/DBF solution (100 mg DBF per ml dichloromethane) on 500 g of sand on aluminium foil. Dichloromethane was allowed to evaporate for 16 h under a chemical hood after which aliquots of 2 g were placed in a 15-ml Greiner tube and homogenized by rotary mixing. Volumes of 25, 50, 250 and 500 µl of minimal medium were added per 1 g of sand to produce microcosms with gravimetric water content (GWC) of 2.4, 4.8, 20 and 33% (visible saturation), respectively.

Survival in sand

To measure the survival of RW1 in sand after inoculation and as a function of GWC and physiological state, we used cells from salicylate-grown cultures. For exponentially growing cells, we added 1 ml, 0.5 ml, 100 µl or 50 µl of culture with turbidity (optical density (OD), at 600 nm) of 0.3 into 2 g dry

sand in 15-ml tubes to produce 33, 20, 4.8 and 2.4% GWC, respectively. For stationary phase, the same volumes were added but sampled at a culture turbidity of 0.9. Cells were maintained in the sandy microcosm for 1 h at 24–26 °C, after which they were extracted by adding 5 ml of saline solution (0.9%) to the tube and vortexing for 30 s. Larger particles were allowed briefly to settle, after which the supernatant was 10-fold serially diluted in sterile saline solution. Fifty microlitres of aliquots were plated on DSM457 agar medium with 5 mM salicylate to calculate the number of colony-forming units (CFU) per g of soil. The inoculum was also serially diluted to count the number of CFU per ml. Survival was calculated as the percentage of total CFU recovered after 1 h from the soil compared with the inoculated CFU.

The recovered cell suspension from sand was also stained using Live/Dead staining (Life Technologies, Carlsbad, CA, USA). Volumes of 5 µl of SYBR Green and 0.1 µl of propidium iodide were added per aliquot of 500 µl of suspension, mixed by vortexing and incubated for 10 min in the dark at room temperature. After that, the sample was filtered over a black 0.2-µm isopore membrane filter (Merck Millipore AG, Zug, Switzerland). The proportion of red and green fluorescent cells was counted by epifluorescence microscopy (Zeiss Axioplan II imaging microscope, Carl Zeiss, Jena, Germany).

Growth in sand

To follow growth of RW1 in the sand contaminated or not with DBF, we inoculated $\sim 2.5 \times 10^5$ cells per g soil in triplicate microcosm series (50 ml Greiner tube with 10 g soil). RW1 cells in this case were precultured on DBF as the sole carbon and energy source in liquid medium, harvested at a culture turbidity of 0.3 and diluted to 5×10^6 per ml in sterile minimal medium without added carbon source. A volume of 500 µl of this diluted cell suspension was added to 10 g sand to produce 4.8% GWC at the start of incubation. At regular time intervals, three replicate microcosms were killed to extract and dilute cells from the sand as described above. To count the number of CFU per g soil, serial dilutions were plated on selective plates containing minimal media agar with salicylate as carbon source, streptomycin (50 µg ml^{-1}) to inhibit growth of other bacteria (RW1 is spontaneously resistant to streptomycin) and cyclohexamide (100 µg ml^{-1}, Chemie Brunschwig, Basel, Switzerland) to limit fungal growth. The growth rate in soil was calculated from the increase of the log CFU over time in the triplicate assays, which was compared with that measured in liquid batch culture on DBF.

DBF degradation by RW1 in sand

The DBF content in the sand was measured over time in parallel incubated triplicate sand microcosms, inoculated or not with RW1 as described above. For every time point, three microcosms were killed and extracted by adding 5 ml of a 80:20 (*v/v*) mixture of hexane:acetone per g sand. Extraction was allowed to proceed for 5 min in an ultrasound bath (35 kHz), after which the organic phase was recovered by decanting. This was followed by twice an extraction with 5 ml dichloromethane per g sand and ultrasound treatment (5 min, 35 kHz). The organic phases were pooled into a single 20-ml amber glass vial (Infochroma AG, Zug, Switzerland; G075B-27/057). For analysis, 0.2 ml of the organic phase was diluted in 20 ml isooctane, of which 0.2 ml was mixed in 1 ml of isooctane containing 200 mg ml^{-1} ^{13}C-DBF (Cambridge Isotope Laboratories, Inc., Tewksbury, MA, USA; 50 µg ml^{-1}, CLM-1561-1.2). Volumes of 2 µl were injected on a Thermo Scientific GC Trace 1310-ISQ gas chromatograph with mass spectrometer (Thermo Fisher Scientific, Inc., Waltham, MA, USA) (GC–MS) equipped with a 60 m × 0.25 mm × 0.25 µm ZB-5MS column. The GC–MS was operated under constant flow (1.5 ml min^{-1}) of He gas with a temperature programme of 80 °C for 0.5 min, an increase of 50 °C min^{-1} until 150 °C, and then 10.0 °C min^{-1} until 250 °C, followed by 3 min at 250 °C. Mass analysis was carried out at 250 °C, and DBF in sand was identified by comparison of the parent and fragment masses to an authentic DBF standard (Sigma-Aldrich Chemie GmbH, Buchs, Switzerland; 23,637-3).

Genome-wide responses of RW1 to inoculation in sand

To measure the immediate genome-wide reaction of RW1 to sand with or without DBF, we inoculated $\sim 10^8$ RW1 cells taken from exponentially growing liquid batch culture per g sand at 4.8% GWC, which had resulted in 80% survival after 1-h incubation. Cells for inoculation were harvested from a 500-ml culture in exponential phase either on salicylate or on DBF by centrifugation for 5 min at 4000 r.p.m. Cells were then resuspended in 1.5 ml leftover medium to obtain a minimum of 12 replicas of 100 µl, of which four replicas each were used per treatment. The following treatments were produced: (i) cells harvested from salicylate-growing cultures inoculated to sand without (*SAL-NOTH*) or (ii) with DBF (*SAL-DBF*); (iii) cells harvested from DBF-growing cultures inoculated to sand with DBF (*DBF-DBF*); (iv) as (i) but not inoculated (control *SAL*); (v) as (iii) but not inoculated (control *DBF*).

Aliquots of 100-µl cell suspension were added to 2 g dry sand in a 15-ml Greiner tube to start the incubation and produce 4.8% GWC. All tubes were incubated on a tube roller at 50 r.p.m. min^{-1} and 24–26 °C. After 1 h, the cells were extracted from the sand by adding 5 ml of sterile saline solution (0.9%) to the tube and vortexing for 30 s, after which the suspension was filtered over a cell strainer of 70-µm pore size to remove sand grains. The filtrate was then immediately filtered over a 0.22-µm Durapore

membrane filter (Merck Millipore AG) by vacuum suction. The filter with the cells was removed, placed in a 2-ml tube containing 0.5 g of acid-washed glass beads (Sigma-Aldrich Chemie GmbH), frozen in liquid nitrogen and stored at −80 °C for RNA isolation. For the control treatments, the cells were maintained in the resuspension solution (100 µl) for 1 h at 24–26 °C. Subsequently, each of the 100-µl control cell suspensions was added to 2 g of dry sand, after which immediately 5 ml saline solution was added to the mixture and vortexed. Cells were re-extracted immediately from the sand as described above and stored for RNA isolation.

Genome-wide response of RW1 during growth in sand
To measure genome-wide gene expression of RW1 during growth in sand, we inoculated microcosms contaminated with DBF (see above) with $\sim 2.5 \times 10^5$ CFU of cells pregrown on DBF (see above) per g sand. In order to obtain enough cells to extract RNA, we started with 80 microcosms, each with 10 g sand. After 16 h, 64 microcosms were killed, cells were extracted by adding 15 ml of saline solution, vortexing and filtering as described above. Cells from 16 microcosms were pooled to obtain four replicates for the 'exponential phase' in sand (*SAND-DBF-EXPO*). Forty hours after inoculation, we killed the remaining 16 microcosms, extracted cells as before and pooled cells from four microcosms together to produce four replicates of 'stationary phase' in sand (*SAND-DBF-STAT*). As controls for growth in sand, we inoculated fourfold replicate batch cultures with RW in liquid medium with DBF crystals. Cells were recovered by filtration at an OD of 0.2–0.3 (*LIQ-DBF-EXPO*) and at an OD of 0.9–1 (*LIQ-DBF-STAT*).

RNA isolation
For extraction of RNA from cells recovered after 1 h (that is, treatments *SAL-NOTH*, *SAL-DBF*, *DBF-DBF*, control *SAL* and control *DBF*), we used a modified acid-phenol method (Johnson *et al.*, 2008); (Supplementary Methods). For extraction of RNA from growing cells in sand, it was not possible to use the hot phenol procedure because of the large quantity of soil. Therefore, for the treatments *SAND-DBF-EXPO*, *SAND-DBF-STAT*, *LIQ-DBF-EXPO* and *LIQ-DBF-STAT*, we used the RNA PowerSoil Total RNA Isolation Kit (Mobio Laboratories, Carlsbad, CA, USA). The replicates were maintained at −80 °C until extraction, and filters still frozen were broken inside the tube with RNAse-free tweezers to reduce the size of the filters, before following the protocol indicated by the manufacturer. The RNA pellet was resuspended in a final volume of 20 µl to obtain concentrations $>500\ \mathrm{ng\,\mu l^{-1}}$ necessary for subsequent labelling.

RNA quality in the purified solutions was verified by quantification of the A260/A280 and A260/A230 ratios using a NanoDrop spectrophotometer (ThermoFisher Scientific, Waltham, MA, USA) and by electrophoresis on an Agilent Bioanalyser (Agilent Technologies, Santa Clara, CA, USA) to detect intact 16S and 23S rRNA. If ratio values in NanoDrop were below 1.9 or if rRNA bands were visually degraded on the Bioanalyser diagram, the RNA was discarded, and the experiment was repeated. RNA was stored at −80 °C before cDNA labelling.

Labelling and microarray hybridization
cDNAs were produced by reverse transcription using cyanine-3-labelled (Cy-3)-dCTP as described previously (Johnson *et al.*, 2011). The labelled cDNA was purified with a MinElute PCR Purification Kit (Qiagen, Hilden, Germany), and the quantity of Cy-3-dCTP was calculated by the MICROARRAY function of the NanoDrop spectrophotometer. The criteria for sufficient labelling were a ratio of absorbances at 260/280 nm >1.6 and an absorbance at 553 nm >0.01. If these criteria were not met, the labelling was repeated.

The volume of the different samples was adjusted for the hybridization in order to have at least 2 pmol of labelled Cy-3-cDNA per array on the slide. Slides consisted of a custom 8 × 15 K array format (Agilent Technologies), which have 8 replicate 15 000 feature arrays per slide, including on average 3–4 probes per gene (Johnson *et al.*, 2011). Slides were hybridized at 65 °C for 17 h, after which they were washed according to the procedures by Agilent and scanned. The AGILENT FEATURE EXTRACTION SOFTWARE (vs 10.7.1.1, Agilent Technologies) was used to extract the signal intensities of the probes from the scanned images. The text data file obtained was used as input in GeneSpring GX (vs 12, Agilent Technologies). Data were ^{2}log transformed, normalized by quantile and scaled with the baseline to the median of all samples. Genes with a signal intensity above the 20th percentile in at least one of the samples were retained for analysis and comparisons between treatments. The RW1 transcriptomes described here were deposited in the GEO database under accession numbers GSE54814-54816.

Data analysis
A Welch's *T*-test with unequal variances was used to calculate *P*-values on triplicate or quadruplate probe signal intensity comparisons. *P*-values were converted into false-discovery rates with the Benjamini and Hochberg procedure for multiple hypotheses testing. Genes were considered statistically significantly differentially expressed between two conditions at a false-discovery rate of <0.05 and a fold-difference between the average grouped probe-per-gene hybridization intensity of >2. Reproducibility of samples (genes and conditions) was examined in GeneSpring by hierarchical clustering of normalized data, which were filtered on expression levels, after which the distance and similarity were calculated by using Euclidean distance and linkage rule average. Data were further examined by Principal

Component Analysis (GeneSpring). Transcriptome data from the inoculation experiments in soil vs suspended batch liquid culture were further analysed using a two-way analysis of variance (ANOVA) with interpretation groups 'environment' and 'growth phase' (GeneSpring, Table 1).

Genes statistically different between two or more different treatments were subsequently interpreted by using Gene Ontology (GO) terminology (GOConsortium *et al.*, 2000). GO terms of all RW1 genes or a series of differentially expressed genes between comparisons were extracted using the program DAVID (Huang da *et al.*, 2009). The web-based bioinformatics tool GOEAST (Zheng and Wang, 2008) was then used to analyse GO data sets of statistically significantly differentially expressed genes in each pair-wise treatment comparison, under implementation of the Alexa's algorithm (Alexa *et al.*, 2006).

Heat maps of normalized expression of genes common to specific comparison sets were produced by using the web-based program Matrix2png (Pavlidis and Noble, 2003). Thresholded and ^{2}log transformed expression data from GeneSpring were used as input in Matrix2png under the parameters: *normalize rows* and *preset map 17*. The average of the expression data per gene (in ^{10}log) was used further as input in Matrix2png to produce an expression scale (that is, log AVG, with the minimum value displayed in white and the maximum value in black).

A network for RW1 metabolism of DBF and other aromatic compounds as predicted in earlier work (Coronado *et al.*, 2012) was manually created in Cytoscape (version 2.8.3), with nodes representing metabolites and edges gene expression data. Thickness of the edge line width (linear scale) was used to represent expression values of the relevant RW1 genes under the different experimental conditions, normalized per gene to the highest expression (100%, line width = 15).

Results

Survival and growth of S. wittichii RW1 in sand contaminated with DBF

In order to compare the global behaviour of *S. wittichii* RW1 between contaminated sand and liquid batch culture, we first ensured that cells were surviving after transfer from liquid batch and were growing in the sand (that is, detectable exponential growth and stationary phase).

First, we established the conditions under which at least 70% of RW1 cells survived a transition from culture flask to sand (equivalent to a lag phase). These conditions would then allow us to examine the immediate RW1 sand-exposed transcriptome compared with cells remaining in liquid suspension after batch growth. We inoculated approximately 10^8 RW1 cells per gram of sand taken from exponential or stationary phase in liquid culture and measured survival (as CFU) and percentage of live cells (by staining with SYBR Green) after 1 h in sand with different GWC. The best survival was found for cells taken from exponential phase and in sand with 20% GWC (Figure 1a). A threshold of 70% survival was also attained for exponential phase cells inoculated in sand with 4.8% GWC. Concomitant SYBR Green-staining of recovered cells revealed 80–90% live cells. In contrast, inoculation of exponential phase cells into sand with 2.4% GWC diminished the surviving fraction to below 70% (Figure 1a). Finally, not more than 30–40% of RW1 cells inoculated from stationary phase survived in the sand, irrespective of the water content. Because of the necessity to have sufficient live cells, but considering that soils may often be drier than 20% GWC, we decided to carry out transcriptome analysis in sand at 4.8% GWC and using exponential phase cells for inoculation.

Next, in order to measure their capacity to actually grow in the sand, we inoculated a low number of RW1 cells ($\sim 2.5 \times 10^5$ cells g^{-1}) in sand at 4.8% GWC contaminated with or without 2 mg DBF g^{-1}. RW1 growth in sand without added DBF reached a population size of 2.5×10^7 CFU g^{-1} (Figure 1b). Trace amounts of DBF (0.8–1.6 μg g^{-1}) were present in non-amended sand. The RW1 population in sand with added DBF increased after 2 days to up to 1.5×10^8 CFU g^{-1} sand, which is evidence for active growth on DBF in the sand (Figure 1b). RW1 cells used on average 0.2 mg DBF g^{-1} sand during the first 40 h of incubation (Figure 1c), which is sufficient to sustain the net development of an RW1 population of 10^8 cells (assuming 0.4 pg C per cell and a yield on DBF of 20%). Population development of RW1 during the early phase in sand with DBF (12–28 h) was best represented by exponential growth (Supplementary Figure S1), with a calculated growth rate of $0.24 \pm 0.06\ h^{-1}$. This is similar to the growth rate observed in liquid cultures with DBF crystals ($0.23 \pm 0.01\ h^{-1}$). Although population growth on poorly water-soluble substances like DBF is often controlled by their dissolution rates and is therefore better described by pseudolinear rather than exponential growth (Wick *et al.*, 2001), for simplicity we refer to the early phase (12–28 h) in sand as 'exponential growth phase' and the later phase (28–48 h) as 'stationary phase'. We concluded that

Table 1 Two-way ANOVA comparison groups of transcriptome data

Transcriptome	*Comparison group*	
	Environment	*Growth phase*
CTRL DBF	Liquid with DBF	LAG
WS-DBF	Liquid with DBF	EXPO
LIQ-DBF-STAT	Liquid with DBF	STAT
DBF-DBF	Sand with DBF	LAG
SAND-DBF-EXPO	Sand with DBF	EXPO
SAND-DBF-STAT	Sand with DBF	STAT

Abbreviations: ANOVA, analysis of variance; DBF, dibenzofuran.

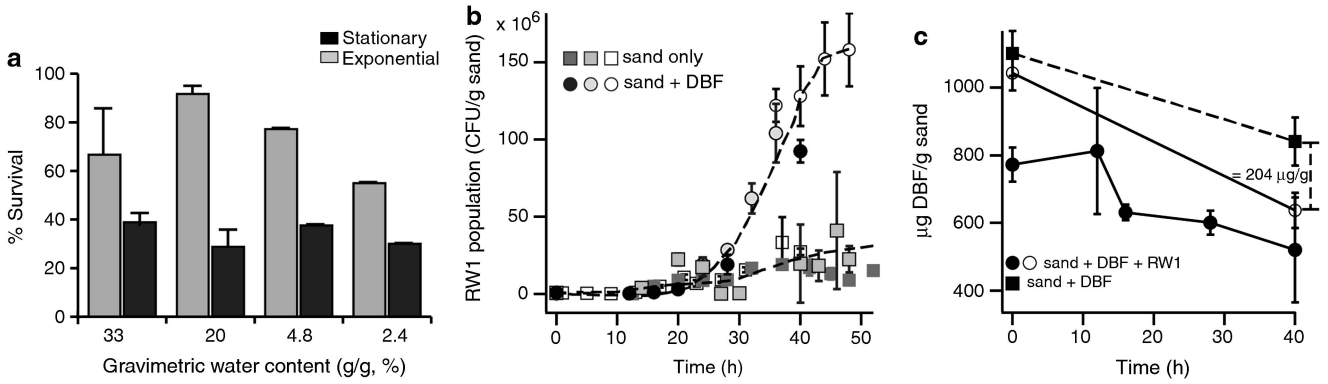

Figure 1 Survival (**a**), growth of (**b**) and degradation of DBF by (**c**) *Sphingomonas wittichii* RW1 in sand supplemented or not with DBF. (**a**) Percentage of survival of RW1 cells taken from exponential and stationary phase cultures on salicylate, inoculated in sand at different GWCs during 1 h. (**b**) Sampling points represent the average number of CFU per g sand from four independent microcosm replicates. Data are combined from three independent inoculation series (open, grey and black symbols) in sand with (circles) or without added DBF (squares). (**c**) DBF degradation was measured in two independent triplicate microcosm series inoculated with RW1 (circles) or not (squares).

the RW1 transcriptomes in exponential and stationary phases under both conditions could be compared without being compromised by growth rate effects alone.

Analysis of the immediate response of RW1 after inoculation into sand

Next we asked the question whether and how RW1 cells would respond to the transition into sand after having been cultured in liquid batch with a specific aromatic carbon source (similar to the 'inoculation' phase in a bioaugmentation process). In order to study this, we examined genome-wide gene expression of RW1 cells among five experimental conditions: (i) cells pregrown in liquid culture on DBF and inoculated for 1 h in sand (at 4.8% GWC) with DBF (*DBF-DBF*); (ii) cells grown on salicylate but introduced for 1 h in sand with DBF (*SAL-DBF*), or (iii) in sand without any addition of carbon substrate (*SAL-NOTH*); (iv) cells grown on DBF or (v) on salicylate but not incubated in sand (yet otherwise treated similarly—see Materials and methods section; named: control *DBF* and control *SAL*). Microarray analysis showed good replica clustering (Figure 2a), and replicas grouped closely together in the Principal Component Analysis (not shown). What becomes evident from the clustering analysis in Figure 2a is that cells pregrown on DBF or with salicylate display globally very different transcriptomes. In contrast, a transition from cells grown in DBF to sand with DBF is globally speaking less of a change than the difference between growth on DBF and salicylate. Cells grown on salicylate but introduced to sand with DBF or without any C-source addition maintain a global 'salicylate' signature (Figure 2a).

The expression of between 4% and 13% of all genes in the RW1 genome was affected by a 1 h presence in sand (Figure 3a). The largest number of (statistically significantly) differentially expressed genes was found in cells taken from salicylate culture and inoculated in sand without any further carbon source (*SAL-NOTH* vs control *SAL*: 707 genes). In all, 11% of all genes (632 genes) were differentially expressed in cells passed from salicylate culture to sand with DBF (*SAL-DBF* vs control *SAL*). The lowest number of differentially expressed genes (228) occurred among cells taken from DBF culture and introduced in sand contaminated with DBF (*DBF-DBF* vs control *DBF*). A common group of 45 genes were similarly differentially expressed (24 higher and 21 lower than in the controls) upon inoculation in sand with DBF, irrespective of preculturing of the cells with salicylate or DBF. A total of 40 differentially expressed genes were common to all sand transitions (Figures 2b and 3a). Of these, 19 were always lower expressed than in the controls, 6 were always higher expressed and 15 were either lower or higher expressed than in the controls (Table 2). These genes might thus represent the core reaction of RW1 to a transition in sand (under the used aromatic growth substrates).

Because a Venn representation is only informative for the identification of genes that are statistically significantly differentially expressed between treatments, but not on their expression levels, we examined more precisely the normalized and averaged expression levels of those 40 genes shared between all sand transitions and their controls. Figure 2b clearly shows how two opposing (control *SAL* and control *DBF*, vs *SAL-DBF* and *DBF-DBF*) and one intermediate expression pattern arise (*SAL-NOTH*) among the 40 shared differentially expressed genes. Functions that tend to be lower expressed after transition to sand with DBF (both *SAL-DBF* and *DBF-DBF*) include a group of genes (Swit_0975 to Swit_0978) previously implicated in degradation of salicylate (Coronado *et al.*, 2012), three genes (Swit_3144, Swit_3256 and Swit_3044) for TonB-dependent receptors, which may be associated with uptake of scarce resources such as vitamins, trace metals or heme (Lim, 2010), a small gene cluster encoding a cbb3-type cytochrome

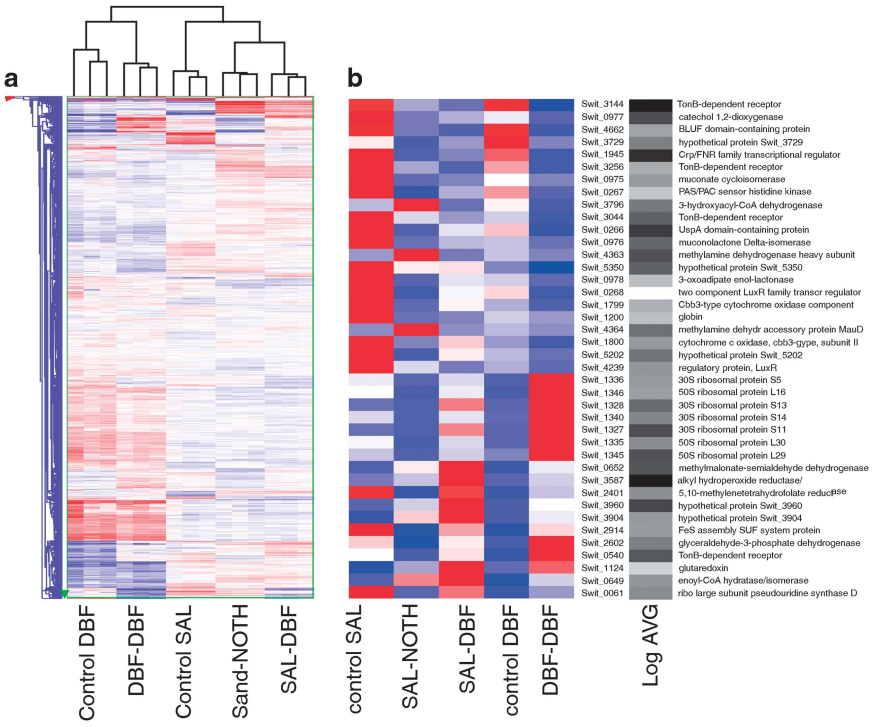

Figure 2 Hierarchical clustering of expression from (**a**) all RW1 genes (single blue or red lines) in microarray analysis or (**b**) of a set of 40 commonly differentially expressed genes. (**a**) Transcriptomes of RW1 cells introduced for 1 h in sand without or with DBF, compared with controls. Gene groups cluster in Y-direction; samples in X-direction. Colours represent genes exhibiting high (red), global average (white) or low (blue) intensity values of normalized signals in the comparison group. Sample designations, see main text. (**b**) Normalized gene expression of 40 RW1 genes commonly differentially expressed in all pair-wise comparisons of 1-h inoculation in sand with DBF (see Figure 3a). Colours indicate high (red), median (white) and low relative (blue) signal intensities. Log AVG, ^{10}log of the average absolute hybridization intensity per indicated gene, on a scale from low (white) to high (black). Heat map generated by Matrix2png (Pavlidis and Noble 2003).

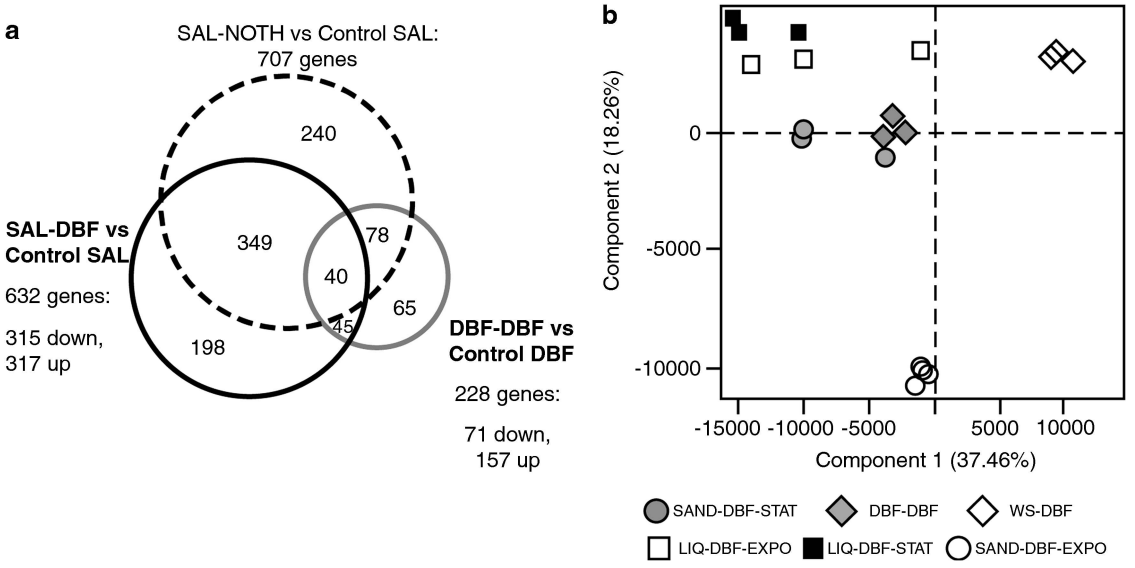

Figure 3 Group clustering of transcriptome data. (**a**) Venn diagram of the numbers of differentially expressed RW1 genes in pair-wise comparisons between 1-h treatments. *SAL-NOTH*, cells inoculated in bare sand from salicylate cultures; *SAL-DBF*, cells from salicylate cultures inoculated in sand contaminated with DBF; *DBF-DBF*, cells from DBF liquid cultures inoculated in sand contaminated with DBF. (**b**) Two-dimensional Principal Component Analysis of *Sphingomonas wittichii* transcriptomes under different growth conditions. Global patterns of RW1 cells growing in sand in exponential (SAND-DBF-EXPO) or stationary phase (SAND-DBF-STAT) vs liquid cultures with crystals of dibenzofuran in exponential (LIQ-DBF-EXPO) or stationary phase (LIQ-DBF-STAT). DBF-DBF, transcriptome from cells grown on DBF inoculated during 1 h in sand with DBF. WS-DBF exponential growth in liquid with saturated amounts of DBF (WS-DBF).

Table 2 Differentially expressed genes of *S. wittichii* RW1 common to pair-wise comparisons in short contact sand experiments

Gene	*Annotation*	*Fold change in comparison*[a]					
		SAL-NOTH[b] *vs ctrl SAL*	*SAL-DBF vs ctrl SAL*	*DBF-DBF vs ctrl DBF*	*Two-way ANOVA*[c]		
					1 h	*EXPO*	*STAT*
Swit_0061	Ribosomal large subunit pseudouridine synthase D	−5.3	−3.3	2	2.6	−1.9	1.0
Swit_0266	UspA domain-containing protein	−4.7	−5.7	−4.6	−1.7	−3.2	−1.1
Swit_0540	TonB-dependent receptor	−2.6	−2.3	3.5	2.6	−1.4	1.1
Swit_0652	Methylmalonate-semialdehyde dehydrogenase	4.2	4.5	6.5	1.1	6.2	1.9
Swit_0975	Muconate cycloisomerase	−21.7	−21.1	−6.3	1.5	−1.9	−1.1
Swit_0976	Muconolactone Delta-isomerase	−12.9	−11.9	−4.5	1.7	−5.4	1.2
Swit_0977	Catechol 1,2-dioxygenase	−11.4	−12.9	−9.8	−1.1	−2.6	1.6
Swit_1124	Glutaredoxin	3.7	4.3	2.7	1.8	2.2	33.9
Swit_1200	Globin	−21.8	−22.1	−3.3	−4.6	−1.3	−1.3
Swit_1327	30S ribosomal protein S11	−2.3	2.5	10.5	2.2	1.4	2.6
Swit_1328	30S ribosomal protein S13	−2.1	2.8	14.1	4.9	1.5	2.4
Swit_1336	30S ribosomal protein S5	−6.3	−2.3	35.2	7.7	−1.4	1.2
Swit_1346	50S ribosomal protein L16	−5.6	−2.2	22.2	4.6	−1.3	−1.0
Swit_1799	Cbb3-type cytochrome oxidase component	−4.4	−4.5	−3.5	−1.5	−2.8	−1.7
Swit_1800	Cytochrome *c* oxidase, cbb3-type, subunit II	−3.3	−3.9	−2.8	1.2	−2.4	−1.4
Swit_2401	5,10-Methylenetetrahydrofolate reductase	−14.3	−2.4	4.6	3.0	−3.1	−1.5
Swit_2914	FeS assembly SUF system protein	−6.2	−3.1	3.7	2.3	−1.1	1.4
Swit_3044	TonB-dependent receptor	−3.1	−8.8	−4.6	2.7	−4.0	−18.7
Swit_3144	TonB-dependent receptor	−3.1	−8.2	−17.8	−1.0	−1.4	3.4
Swit_3256	TonB-dependent receptor	−2.5	−8.2	−6.5	3.7	−1.6	1.6
Swit_3587	Alkyl hydroperoxide reductase	2.5	6.3	5.4	49.3	−1.1	7.4
Swit_3729	Hypothetical protein Swit_3729	−3	−3.9	−8.2	−1.2	−1.8	5.3
Swit_3904	Hypothetical protein Swit_3904	11.7	9.8	3.8	51.7	2.4	31.3
Swit_3960	Hypothetical protein Swit_3960	2.5	4.1	4.4	1.7	1.6	5.0
Swit_4364	Methylamine dehydrogenase accessory protein MauD	9	−2.2	−3.1	1.6	−1.1	−2.5
Swit_4662	BLUF domain-containing protein	−2.8	−7.9	−9.2	1.0	−3.1	1.8
Swit_5202	Hypothetical protein Swit_5202	−8.7	−5.6	−2.6	1.2	1.4	−2.5

Abbreviation: ANOVA, analysis of variance.
[a]Fold change (absolute) in condition compared with control, negative values indicate decreased expression in soil.
[b]Sample indications: Bare sand vs control cells growing in salicylate (SAL-NOTH vs ctrl SAL), sand contaminated with DBF vs control cells growing in salicylate (SAL-DBF vs ctrl SAL), and sand contaminated with DBF vs control cells growing in dibenzofuran (DBF-DBF vs ctrl DBF).
[c]Two-way ANOVA in the condition 'growth phase'; 1 h: *DBF-DBF* vs *ctrl DBF*, EXPO: *SAND-DBF-EXPO* vs *wsDBF*, and STAT: *SAND-DBF-STAT* vs *LIQ-DBF-STAT.*

oxidase (Swit_1799 and Swit_1800) and an UspA stress domain-containing protein (Swit_0266). Furthermore, a number of potential regulatory proteins are consistently lower expressed in sand with DBF, namely, a Crp/FNR family transcriptional regulator (Swit_1945), a LuxR-type two component system (Swit_0267 and Swit_0268) and another LuxR-type regulator (Swit_4239). In contrast, there is a very clear increase of expression of two gene clusters for ribosomal proteins (Swit_1327-1328, and Swit_1335-1346) in sand with DBF (Figure 2b). Furthermore, genes for another putative TonB-dependent receptor (Swit_0540) and for a putative glutaredoxin (Swit_1124) are higher expressed in sand with DBF. Genes specifically higher expressed in sand without extra added carbon include Swit_4363/_4364, part of a cluster putatively encoding methylamine degradation, and a 3-hydroxyacyl-CoA dehydrogenase (Swit_3796).

GO interpretation of the changes upon inoculation in sand

In order to further interpret the transcriptome changes and cellular reactions during the different transitions, we used GO terminology analysis (http://omicslab.genetics.ac.cn/GOEAST/). A complete detailed list of enriched GO terms for each comparison is presented in Supplementary Information (Supplementary Tables S1–S3), but reactions can be summarized as follows. From the GO category 'Biological processes', it became evident that cells taken from exponential phase on salicylate and introduced into sand without DBF completely interrupt their metabolism (deficient GO terms: respiration, oxidative phosphorylation, glycolysis, methionine biosynthetic process; Supplementary Table S1), try to scavenge nutrients and maintain cell survival (enriched GO terms: regulation of nitrogen compound metabolic process, methylamine and valine metabolic process, DNA repair, cellular homeostasis). This response is dramatically changed in cells introduced into sand supplemented with DBF and different for cells coming from cultures grown on salicylate or DBF. Cells grown on salicylate change their metabolism in order to adapt to the new carbon source (enriched GO terms: aromatic compound and catechol-containing compound catabolic process, Supplementary Table S2),

and adapt to life in sand (GO terms: polysaccharide, lipopolysaccharide and glutamate biosynthetic process). When the cells are already adapted to the carbon source present in the sand (that is, DBF), they continue without major changes in their metabolism. In the *DBF-DBF* vs control *DBF* comparison, enriched GO terms relate to oxidative phosphorylation, respiration and translation, indicative for growing and active cells (Supplementary Table S3). Interestingly, in all cases the transition to sand resulted in a decrease of expression of functions implicated in oxygen transport and binding, which may be due to the higher provision rate of oxygen in sand at 4.8% GWC (for example, thin water films) than in liquid culture.

Of further interest are a number of discernable stress functions that RW1 displays under different transition conditions (Supplementary Table S4). RW1 growing on DBF transited to sand with DBF turned off the expression of various two-component response regulators. A total of 21 genes distributed across 6 different GO terms related to stress is higher expressed in sand without DBF in comparison to the control on salicylate (Supplementary Table S4). Interesting among those is Swit_3927, predicted to code for an EcnAB entercidin (58.6-fold increase), which is part of a family of proteins called the entericidin antidote/toxin peptides. These proteins are activated in *Escherichia coli* in stationary phase under high osmolarity (Bishop *et al*., 1998).

Genome-wide expression differences between RW1 cells growing in sand contaminated with DBF vs liquid culture

After having examined the specific expression differences during a transition from liquid culture to sand at 4.8% GWC, we analysed the RW1 genome-wide gene expression under four new experimental conditions: (i) RW1 growing in sand on DBF, sampled in early phase (16 h, *SAND-DBF-EXPO*) and (ii) in late phase (40 h, *SAND-DBF-STAT*); (iii) cells growing in liquid batch culture on DBF crystals, sampled in early phase (*LIQ-DBF-EXPO*), and (iv) sampled during late phase (*LIQ-DBF-STAT*). In addition, we included previously established transcriptomes of RW1 during early exponential phase growth with water-saturated amounts of DBF (*WS-DBF*, Table 1) (Coronado *et al*., 2012).

Principal Component Analysis indicated that RW1 genome-wide gene expression during early growth in sand with DBF (open circles, Figure 3b) is very different from late growth phase in sand (filled circles), which on its turn resembles more the growth in liquid culture with DBF (open squares; both components explaining 59% of the variation). Genome-wide expression during early (OD = 0.3) and late phase (OD = 0.9) in liquid culture with DBF crystals varied quite a bit between replicates but was not statistically significantly different between them (Figure 3b). The reason for this may be that cells growing with DBF crystals in liquid culture actually display pseudolinear growth, in which case the growth rate is governed by the crystal dissolution rate (Wick *et al*., 2001). For this reason, we used the transcriptome data set *WS-DBF* instead of *LIQ-DBF-EXPO* in the two-way ANOVA presented below, which better represents RW1 exponential growth on DBF in liquid culture (Coronado *et al*., 2012). The genome-wide expression of RW1 cells 1 h after transition to sand with DBF (*DBF-DBF*, see above) was very different from both the liquid and the sand exponential growth phases (Figure 3b, filled diamonds) but more similar as in late growth phase in sand plus DBF (*SAND-DBF-STAT*, two components explaining 53% of variation; Figure 3b).

Microarray data were grouped and further analysed in a two-way ANOVA examining the effect of 'Environment' (that is, Sand or Liquid) and 'Growth Phase' (lag phase of 1-h contact, 'exponential' or 'stationary phase', Table 1). A total of 1418 genes were identified, whose expression reacted statistically significantly different to the condition 'Environment' ($P<0.001$). Two thousand and thirty-six genes were identified, which showed statistically significant interaction between the two terms ('Environment' and 'Growth Phase', $P<0.001$), of which 728 were shared with condition 'Environment' alone. Gene functions differentially expressed to the condition 'Environment' are implicated, among others, in cellular homeostasis, response to stress, protein secretion, a variety of biosynthetic and metabolic processes, inorganic ion scavenging or glutamine synthesis (Supplementary Table S5).

Compared with 1h- contact in sand with DBF, RW1 cells growing exponentially in sand (early phase: 12–28 h, Supplementary Figure S1) preferentially expressed genes related to energy generation (GO terms: ATP synthesis and oxidative phosphorylation) and biosynthesis of phospholipids, cell wall, ribonucleoproteins and fatty acids (Supplementary Table S6). In contrast, a wide range of genes decreased their expression in sand during exponential phase vs 1-h contact, which may have been particularly important for the first adaptation step after inoculation. GO analysis suggests that these are primarily functions in nutrient scavenging, transport, cellular homeostasis and oxidative damage repair (for example, antioxidant and electron carrier activity; Supplementary Table S6).

In comparison to cells growing exponentially in liquid suspended culture (*WS-DBF*) and at a relatively conservative cutoff level of more than fourfold expression difference, 142 genes were higher and 90 were lower expressed in sand (Supplementary Table S7). Among those are numerous genes for putative TonB-dependent receptors, which are indicative for scavenging of substrates, minerals and recycling of nitrogen from organo-N compounds (Lim, 2010). Interestingly, some genes putatively associated with adhesion were much more expressed

among exponentially growing cells in soil. This includes the 85-fold more highly expressed gene Swit_0615 (annotated as Flp/Fap pilin component) and the 38-fold more highly expressed Swit_0163 (annotated as Type IV secretory pathway TrbD component-like protein). The expression of a range of other genes is diminished among cells growing in sand with DBF compared with liquid culture (Supplementary Tables S7 and S8). This affects notably genes involved in stress response, in flagellar biosynthesis and a range of (two-component) regulatory systems (Supplementary Table S8).

RW1 cells sampled in late phase in sand with DBF (*SAND-DBF-STAT*) diminished expression of energy generation processes, biosynthesis and cellular metabolism and cell wall production (Supplementary Tables S9 and S10) but increased expression of functions implicated in oxidative stress response, cellular homeostasis, nutrient scavenging, turnover of proteins, polysaccharide production and transport and production of osmoprotectants (glutamate biosynthetic process, Supplementary Table S9). Interestingly, also expression of genes for DNA-modifying enzymes increased (Supplementary Table S9).

In comparison to RW1 cells growing in liquid suspension with DBF crystals (*LIQ-DBF-STAT*), cells in sand with DBF in late phase have a quite drastically different gene expression pattern (167 genes with higher and 161 with lower expression than in liquid), which can be interpreted by a variety of enriched GO terms (Supplementary Tables S11 and S12). As an example, cells in sand with DBF in late phase (*SAND-DBF-STAT*) showed consistently higher expression of genes involved in cellular homeostasis, stress response, glutamate biosynthesis and 'compound' binding (for example, GTP binding, NADP binding), suggestive for specific protection needed in the sand and osmotic balancing (Supplementary Table S11). On the other hand, genes involved in aromatic compound metabolism and flagellar synthesis decreased expression in soil. Frequently, differentially expressed genes also clustered in a number of potential co-transcribed regions, suggesting concerted regulation to specific environmental signals (Supplementary Table S12).

Aromatic compound metabolism

In order to analyse more specifically expression changes of RW1 genes potentially implicated in DBF and aromatic compound metabolism among the different growth conditions, we constructed a network with metabolites as nodes and known or predicted RW1 gene functions as edges (Supplementary Figure S2), based on previous analyses (Coronado *et al.*, 2012). Compared with exponential growth in liquid medium with DBF (*WS-DBF*, Figure 4a), cells in early growth phase in sand with DBF (*SAND-DBF-EXPO*) clearly changed the expression of genes implicated in DBF and aromatic compound metabolism (Figure 4b). Interestingly, expression of many of the known genes for DBF metabolism diminished in this phase in sand, whereas that of other genes with predicted function in aromatic compound metabolism but no known specificity increased (Figure 4c, Supplementary Figure S3, for example, Rieske-type dioxygenases). Expression of the known DBF network genes partially returned in later phase in the sand (Supplementary Figure S4), but overall, the expression of genes potentially implicated in aromatic compound metabolism in RW1 was strikingly different between exponential growth in liquid culture with DBF (*WS-DBF*) vs the other conditions (Figure 4c). Furthermore, expression of genes for aromatic compound metabolism was markedly different between the transition (*DBF-DBF*) and early phase in sand (*SAND-DBF-EXPO*) and the other conditions (*CTRL-DBF*, *LIQ-DBF-STAT* and *SAND-DBF-STAT*, Figure 4c; Supplementary Figure S3).

Discussion

The work presented here shows a comprehensive analysis of the global reactions of bacteria inoculated and growing in (contaminated) sand, compared with suspended batch growth in regular liquid culture. The types of global transcriptome global transcriptome reactions uncovered here may be representative for those which such cells would undergo when being deployed for targeted bioaugmentation processes of contaminated sites. We chose here to work with *S. wittichii* RW1 as an example of a strain that can degrade a number of pertinent aromatic hydrocarbons (DBF and dioxin) and which as such has been proposed in the past as a realistic strain to be inoculated with the purpose of achieving increased DBF and dioxin biodegradation rates in the environment (Wittich *et al.*, 1992; Wilkes *et al.*, 1996; Megharaj *et al.*, 1997; Halden *et al.*, 1999; Miller *et al.*, 2010). Globally speaking, and despite being mostly descriptive, we believe our results help to explain a number of common trivial practical observations, from which various crucial measures may be suggested to avoid immediate failure of future strain inoculation efforts.

We are aware that some compromises had to be made in our experimental setup in order to allow proper analysis and comparison of RW1 genome-wide transcriptional responses. For example, we used regular sand which was contaminated with DBF, rather than a more complex soil with more clay minerals and organic matter. This was crucial in order to obtain sufficient high-quality RW1 RNA but certainly has given strain RW1 some competitive advantage given the lower background of native microbiome in this sand compared with soils with higher organic matter. Notwithstanding the use of sand, other groups have shown that inoculated

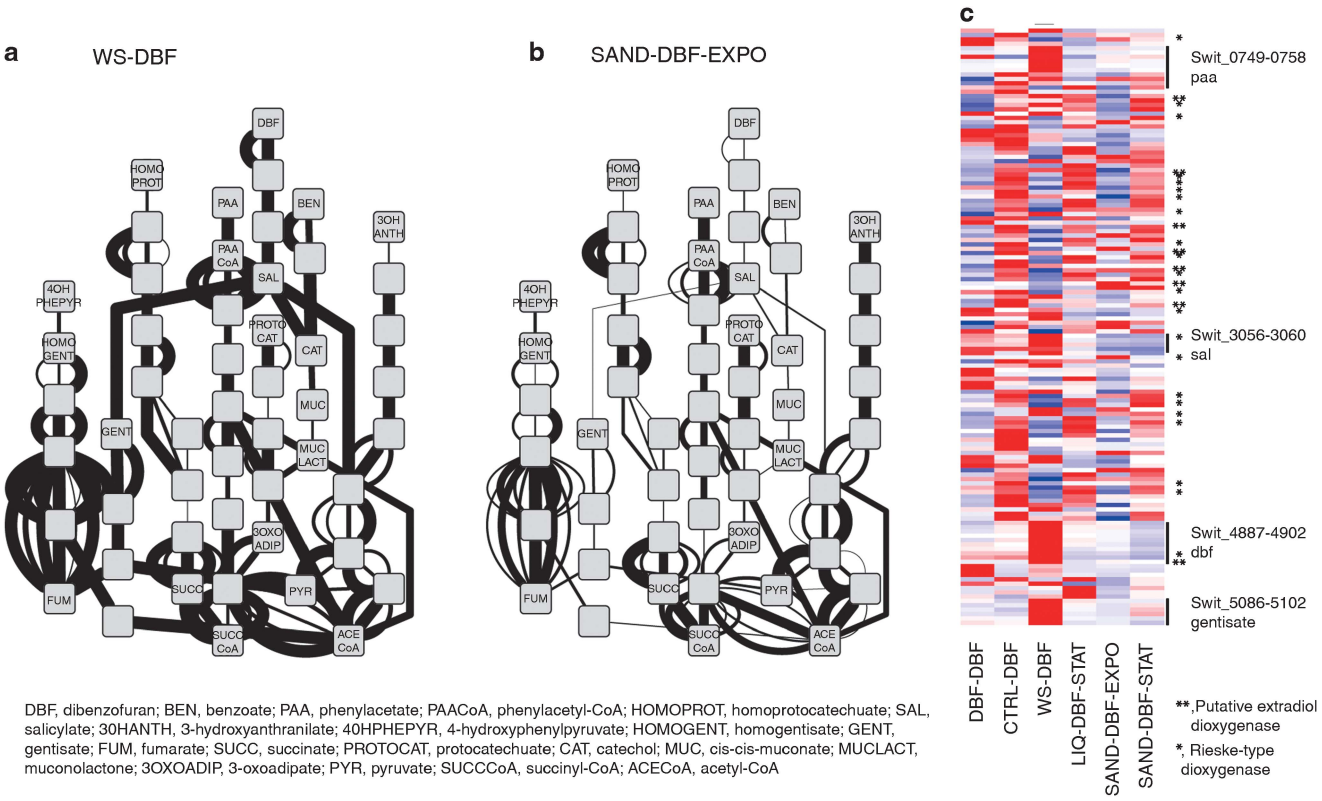

Figure 4 Network analysis of DBF and aromatic compound metabolism by *S. wittichii* RW1 under different growth conditions. (**a**, **b**) Inferred DBF and aromatic compound metabolic network in liquid cultures growing exponentially with DBF (*WS-DBF*) or in sand (*SAND-DBF-EXPO*), respectively. Nodes represent substrates and metabolites converted in RW1-predicted enzyme reactions (edges). Edge line width is a linear-scale representation of normalized expression of the gene coding for the particular enzyme. For a complete overview of all node names and edges, see Supplementary Figure S2, whereas Supplementary Figure S4 shows the expression networks under all six growth conditions. (**c**) Heat map of normalized expression of all RW1 genes predicted to be implicated in aromatic compound metabolism (including those of unknown specificity and not represented in the metabolic network) under the different growth conditions. Colours and heat map generation as in Figure 2b. For a full-scale version with all gene designations and predicted functions, see Supplementary Figure S3.

sphingomonads do grow in more complex soils and at the expense of polycyclic aromatic hydrocarbons (Megharaj *et al.*, 1997; Halden *et al.*, 1999; Fida *et al.*, 2013). In addition, in order to sample sufficient RNA for the immediate response of RW1 cells to a soil transition (the 1-h response), we had to inoculate 10^8 cells per g of material, which may have exacerbated the lack of nutrients and minerals on a per cell basis. Inoculation of high cell densities is not uncommon in bioaugmentation efforts, which, however, impedes such cells from actually growing and establishing themselves at the expense of their unique target substrate in the soil (here: DBF). At much lower starting cell concentrations (2×10^5 per g), we did observe specific growth in sand at the expense of DBF (Figures 1b and c) with similar growth rates as in liquid culture. Finally, by including a wide range of control conditions for each of the examined steps in the inoculation process (that is, lag transition, exponential growth and stationary phase), we are confident that our comparative analyses of the RW1 genome-wide responses correctly highlight the respective transcriptome changes.

Our observations during the first step of inoculation (1-h contact, lag phase) show that cells that have been precultured on the same carbon substrate as their target contaminant (here, as example, DBF) display the least amount of gene expression differences, compared with those that have been precultured on a different (aromatic) substrate (here: salicylate). This was somewhat surprising, given that salicylate is an intermediate of DBF metabolism (Wittich *et al.*, 1992). Cells prepared and inoculated under such circumstances do not have to readjust their primary metabolism, although we can still see evidence of increased scavenging reactions for nutrients. Interestingly, even inoculated cells precultured on DBF after 1 h in sand with DBF show a 'stationary phase' signature (Figure 3b), indicating they are going through a period of cell growth arrest after inoculation. Cells that had been pregrown on the DBF-related aromatic substrate salicylate showed major transitions of carbon metabolism and osmotic adaptations (Supplementary Table S2), which in a field situation might mean that their capacity for substrate competition is diminished compared with native bacteria. *In extremo*, when

cells are inoculated into sand which does not contain DBF, they display extreme carbon and nutrient shortage stress, even though they do grow to some extent (Figure 1b). This indicates that when the intended target substrate is not sufficiently bioavailable, the inoculation is likely to fail. We found some 40 genes commonly statistically significantly expressed during sand transitions compared with the controls, the majority of which also change expression during growth and stationary phase in sand with DBF (Table 2), which might therefore constitute essential elements governing this transition state.

One of the interesting basic questions our work may help to address is whether cells 'realise' that they are in a 'soil' rather than in liquid culture. Clearly, RW1 gene expression during the transition phase and during exponential growth in sand with DBF was very different from that in all liquid cultivations (Figures 2 and 4), whereas late growth phase in sand resembled more stationary phase and slow growth in liquid culture. This resemblance was also reflected in expression of genes potentially implicated in aromatic compound metabolism (Figure 4c). It has previously been suggested that behaviour in dry soil might be experimentally induced by lowering of the water potential in liquid culture through the addition of salt (solute potential (SP)) or of swelling agents, such as polyethylene glycol (matric potential (MP)) (Roberson and Firestone, 1992; Halverson and Firestone, 2000; Johnson *et al.*, 2011; van de Mortel and Halverson, 2004). When we compare, however, the RW1 differentially expressed genes during exponential growth in sand at 4.8% GWC with previous data on the differentially expressed genes in RW1 induced upon SP or MP stress (both not reducing the growth rate by >20%) (Johnson *et al.*, 2011), we find very little overall similarities (Figures 5a and b, Supplementary Table S13). For most of these genes, the response to MP and SP stress is mutually exclusive, but the expression of several genes for motility is consistently diminished and genes for polysaccharide biosynthesis are consistently more highly expressed under sand, MP and SP stress (Supplementary Table S13). Interesting is a gene for a putative glutathione-dependent formaldehyde-activating protein suspected in formaldehyde detoxification (Vorholt, 2002) (Swit_1412), which showed 56-fold higher expression in cells growing exponentially in sand with DBF compared with liquid, compared with fivefold under SP stress and twofold under MP stress (Supplementary Table S13). We conclude that, although MP and SP stress each produce a number of useful expression signatures related to that in sand, they are not quite representative for sand behaviour. The gene expression programme displayed in exponentially growing RW1 cells in sand at 4.8% GWC with DBF must, therefore, be a specific reaction to the sand physico-chemical environment.

As an extension to this question, it is interesting to determine whether there are specific gene functions that seem to be very important for the survival or growth of RW1 under sand conditions. As transcriptome data as recorded here remain essentially descriptive, we compared the genome-wide gene expression data with previous transposon scanning of RW1 for survival functions (Roggo *et al.* 2013). In Figure 5c, we plot hereto the ^{2}log difference of normalized expression of genes appearing in the ANOVA interaction terms against the ^{2}log

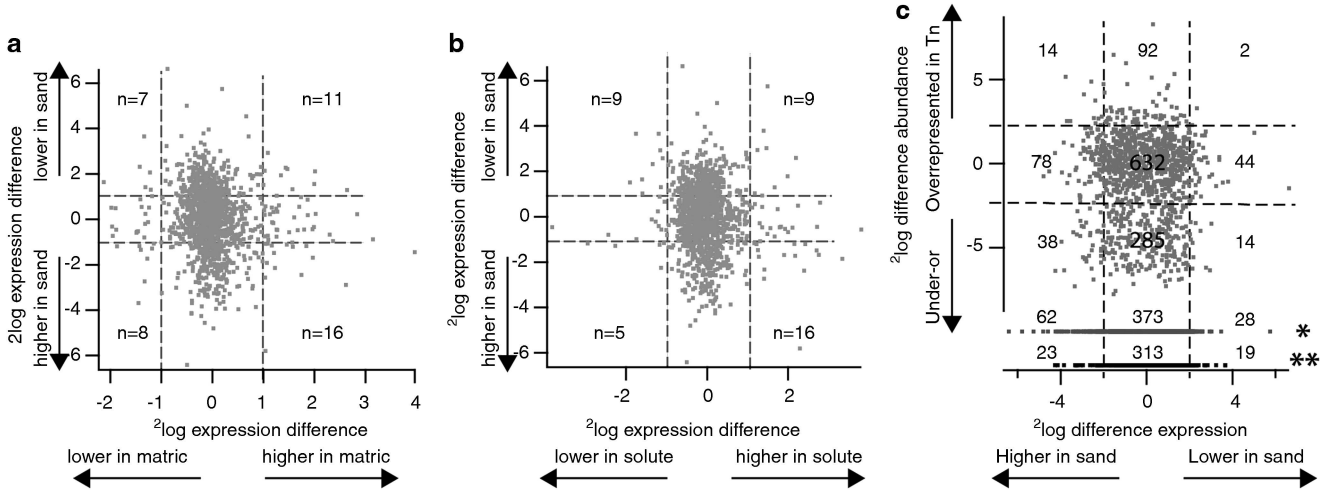

Figure 5 Correlation between genome-wide gene expression of *S. wittichii* RW1 growing exponentially in sand with DBF and (**a**) matric stress, (**b**) solute stress or (**c**) relative fitness cost of gene interruption on survival and growth in sand. Each dot is the average ^{2}log expression difference from triplicate treatments vs controls (for example, matric stress vs regular growth, expression in sand vs liquid culture). Numbers represent the number of genes in the respective quadrant. Dottes lines indicate twofold cutoff. Dots in panel (**c**) marked with a single (*) or a double asterisk (**) indicate genes judged to be essential in sand or under all growth conditions, respectively. Matric and solute stress transcriptome data from Johnson *et al.* (2011); fitness cost data taken from the transposon scanning approach in Roggo *et al.* (2013).

ratio of the abundance of transposon insertions in exponentially growing RW1 populations in sand (vs the control of the transposon abundance distribution in the starter transposon library), with gene lists appearing in Supplementary Tables S14 and S15. The transposon scanning approach is based on the premise that any transposon insertion in a gene affecting growth under a specific set of growth conditions will lead to a lower abundance (or even disappearance) of that transposon mutant population in the metapopulation of all mutants (Gawronski *et al.*, 2009; van Opijnen *et al.*, 2009; Roggo *et al.*, 2013). Under exclusion of essential genes and only focussing on those genes with higher expression in sand, one can conclude from GO classification that a wide range of metabolic processes is specific for growth in soil (Table 3), and a further range of gene functions is implicated in specific survival in soil upon cessation of growth (cellular homeostasis, nutrient scavenging, stress response, Table 3). The list of gene functions is too long to discuss in detail, but a few genes are worth highlighting given previous discussions (Johnson *et al.*, 2011; Roggo *et al.*, 2013). In contrast to previous conclusions on the role of a putative trehalose synthesis cluster (Swit_4523-4533), these genes are not particularly differentially expressed in sand; neither are genes for putative polysaccharide biosynthesis (Swit_3608-3613), which were both higher expressed during matric and solute stress (Johnson *et al.*, 2011) and disruption of which caused drastic fitness loss in sand (Roggo *et al.*, 2013). Rather, it seems that glutamate biosynthesis is used to compensate for osmolarity differences in sand (Swit_0656-0659; Supplementary Table S16). On the other hand, previously mentioned genes involved in fatty acid metabolism (Swit_3903-3908), for a 17-kDa 'surface antigen' (Swit_1507-1509), and for a cell wall hydrolase SleB (Swit_3463) are higher expressed in sand, as well as under solute or matric stress, and transposon insertion causes fitness loss (Supplementary Tables S14 and S15). Also, pertinently higher expressed in sand is a gene cluster for arsenic detoxification (Swit_2243-2244), interruption of which causes strong fitness loss (Supplementary Table S15). The cluster around Swit_0163 (type IV secretory pathway TrbD component-like protein) is also intriguing, because it is essential for growth in sand and is up to 38-fold higher expressed (Supplementary Table S16). A few further putative operons seem specific for sand growth, such as putative RND efflux systems (Swit_1152-1154; Swit_3230-3231), or alkyl hydroperoxidase activity (Swit_3585-3587) (Supplementary Table S16). Interestingly and similar as under solute or matric stress, expression of the flagellar cluster Swit_1260-1293 is decreased in soil, although insertions in this gene region mostly cause fitness loss (Supplementary Table S14). From recent work on *Pseudomonas putida*, it has been suggested

Table 3 Gene ontology interpretation of gene functions important for fitness in sand and higher expressed in sand than in liquid

Growth phase	*GOID*	*Ontology*	*Term*	*No. of probes*	*Total No. of probes*	*Log odds ratio*	P-*value*
Exponential	GO:0008152	BP	Metabolic process	111	2082	0.1	0.0678
	GO:0055114	BP	Oxidation–reduction process	38	573	0.4	0.0219
	GO:0006573	BP	Valine metabolic process	2	7	2.5	0.0424
	GO:0009276	CC	Gram-negative-bacterium-type cell wall	2	10	2.0	0.0403
	GO:0003995	MF	Acyl-CoA dehydrogenase activity	5	47	1.1	0.0819
	GO:0004491	MF	Methylmalonate-semialdehyde dehydrogenase (acylating) activity	2	5	3.0	0.0223
	GO:0016811	MF	Hydrolase activity, acting on carbon–nitrogen (but not peptide) bonds, in linear amides	5	35	1.5	0.0278
Stationary	GO:0019725	BP	Cellular homeostasis	8	43	2.7	0.0002
	GO:0006950	BP	Response to stress	7	79	1.7	0.0014
	GO:0030163	BP	Protein catabolic process	2	5	3.8	0.0081
	GO:0006508	BP	Proteolysis	9	96	1.7	0.0017
	GO:0006096	BP	Glycolysis	2	9	2.9	0.0270
	GO:0009306	BP	Protein secretion	4	32	2.1	0.0135
	GO:0006879	BP	Cellular iron ion homeostasis	2	10	2.8	0.0332
	GO:0043231	CC	Intracellular membrane-bounded organelle	2	12	2.5	0.0372
	GO:0008270	MF	Zinc ion binding	6	84	1.3	0.0336
	GO:0008233	MF	Peptidase activity	11	112	1.8	0.0429
	GO:0016624	MF	Oxidoreductase activity, acting on the aldehyde or oxo group of donors, disulphide as acceptor	2	14	2.3	0.0604
	GO:0008289	MF	Lipid binding	3	24	2.1	0.0386
	GO:0008236	MF	Serine-type peptidase activity	5	28	2.6	0.0031
	GO:0016668	MF	Oxidoreductase activity, acting on a sulphur group of donors, NAD(P) as acceptor	3	7	3.9	0.0008
	GO:0008565	MF	Protein transporter activity	4	38	1.9	0.0231
	GO:0004175	MF	Endopeptidase activity	4	42	1.7	0.0321
	GO:0016209	MF	Antioxidant activity	4	27	2.4	0.0070
	GO:0008199	MF	Ferric iron binding	2	11	2.7	0.0386

(Martinez-Garcia *et al.*, 2014) that flagella allow bacteria to explore the environment searching for nutrients and help escaping from predators or adverse conditions. But mutants without flagellar machinery are actually more resistant to oxidative stress and ultraviolet exposure (Martinez-Garcia *et al.*, 2014). In addition, cells without flagella have more metabolic energy in the form of ATP and reducing power in the form of NADPH, which could potentially be used to better cope with environmental stresses. Behaviour in soil may thus require low or temporarily restricted expression of flagellar systems, in order to optimize metabolic energy for survival and yet allow migration, when necessary. It is further interesting to note that a number of plasmid functions are differentially expressed in liquid or soil and have important fitness effects. For example, important for sand fitness and higher expressed in liquid in stationary phase are Swit_5005-5010 (a putative type IV secretion system encoded on pSWIT02) and Swit_5364-5467 (putative type IV functions encoded on pSWIT01; Supplementary Table S16). On the other hand, a clear polycistronic unit on pSWIT01 is higher expressed in exponentially growing cells in sand (Swit_5192-5196, mostly hypothetical function). Even though the molecular mechanisms of these effects are not immediately trivial, this underscores that natural plasmids can have important roles in general survival or growth in the environment.

Not only did introduced RW1 cells survive in sand but they also actively degraded DBF in the early and late phases (Figure 1c). Expression analysis of RW1 genes predicted to be implicated in aromatic compound metabolism showed that introduced cells adjust their metabolic network immediately after transition into sand with DBF, during the early (exponential) growth phase in sand and in later growth phases (Figure 4; Supplementary Figures S3 and S4). Interestingly, expression of the predicted aromatic compound network is quite dissimilar to that obtained during exponential growth on DBF in liquid culture (Figure 4). Although it cannot be excluded that RW1 in the early growth phase in sand profits from other available organic compounds, it is also possible that the strain deploys other unknown metabolic branches for DBF degradation, dependent on the growth environment (Supplementary Figure S3).

In conclusion, our results demonstrate for the first time the specific cellular reactions of a typical bacterial strain intended for environmental inoculation (*S. wittichii* RW1) to a contaminated environment (sand). We further conclude that such cellular reactions are mostly different from typical water stress achieved with SP or MP change in liquid cultures and, therefore, that sand or soil inoculations themselves should be used to understand cellular reactions to these environmental changes. Such experiments are necessary not only for the practise of bioaugmentation but also will help to more generally understand how single strains inoculated into complex microbiomes (for example, plant leaf surfaces, intestinal tract) are behaving. In the long term, this may help to better design and predict the success of inoculation efforts.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements

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Supplementary information to:

Genome-Wide Transcriptional Changes in the Dibenzofuran-Degrading *Sphingomonas wittichii* RW1 During Inoculation and Growth in Contaminated Sand

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Supplementary methods

Supplementary Figures S1-S4

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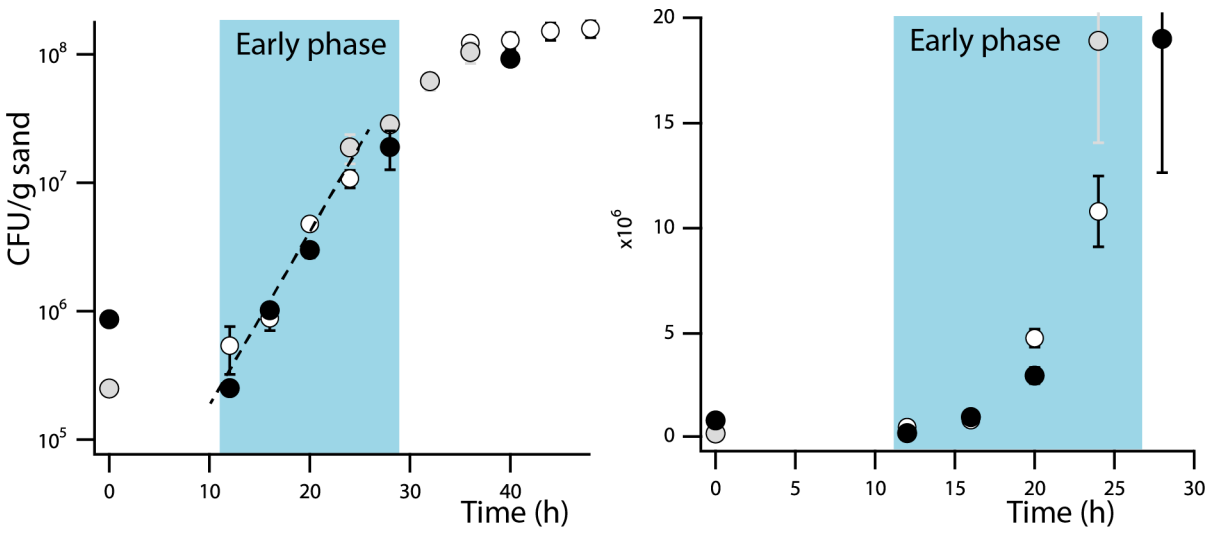
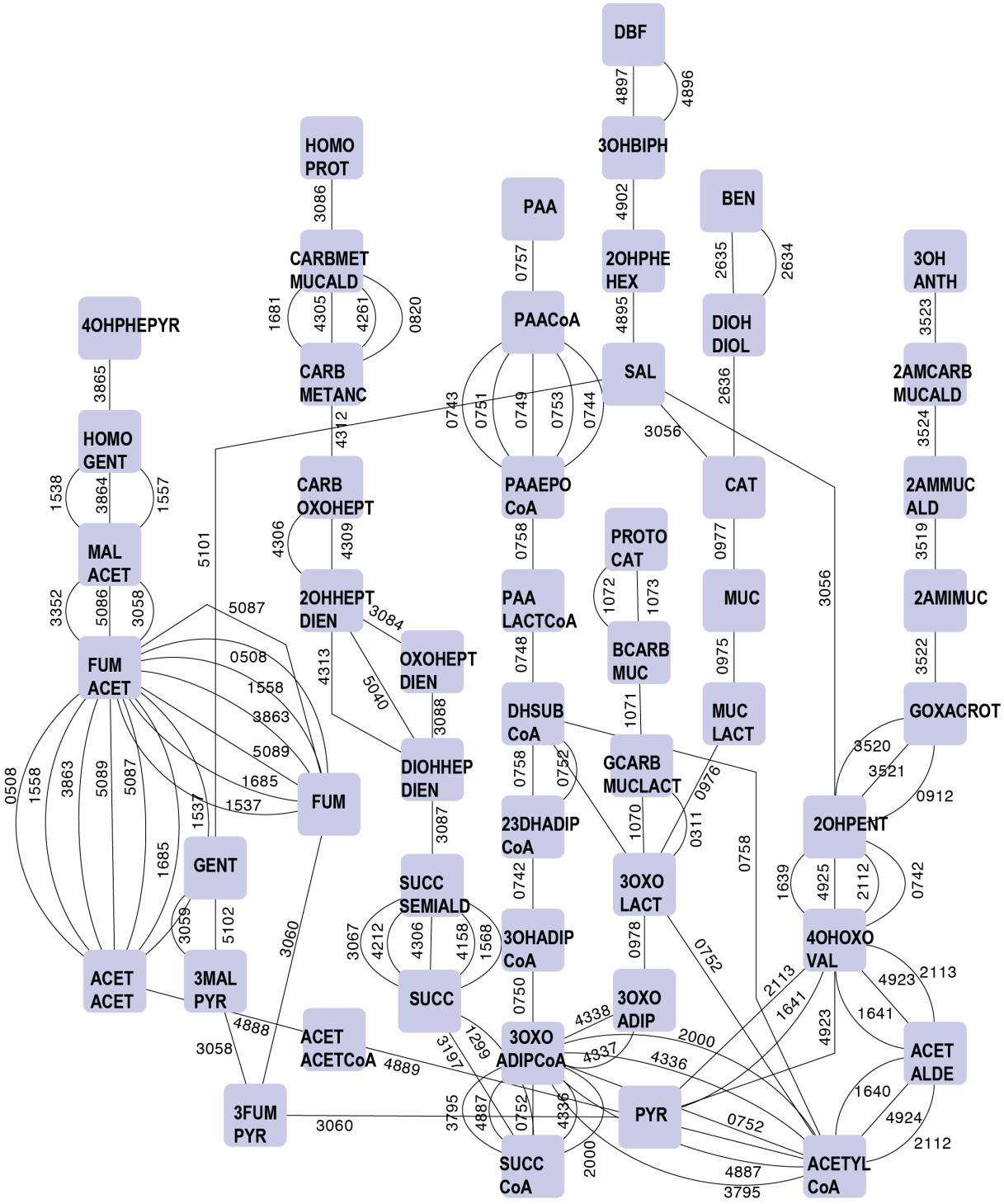
Supplementary methods

RNA isolation from cells incubated for 1 h in soil

We used a modified acid-phenol method (Johnson *et al.*, 2008). Briefly, each 2 ml tube that contained a frozen filter with RW1 cells (from – 80°C) was filled with 250 µl lysis buffer (containing 50 mM sodium acetate, 10 mM EDTA [pH 5.1]), 100 µl 10% sodium dodecyl sulfate and 1.0 ml buffer-equilibrated phenol (pH 4.3) (Aqua-Roti-Phenol, Roth Sochiel, FR). Cells were lysed by heating in a 70°C water bath for 2 min and then subjected to a bead-beating procedure with a FastPrep FP120 (Bio101, Thermo Fisher Scientific, Waltham, MA, USA). This consisted of bead-beating twice for 45 sec at maximum speed (6.0), followed by an incubation in a 70°C water bath for 10 min, and bead-beating twice again. Cellular debris was collected by centrifugation for 3 min at 15,000 × *g* and 4°C, after which the aqueous phase was transferred to a new nuclease-free microcentrifuge tube. Then, the aqueous phase was extracted twice with 1 volume of phenol-chloroform-isoamyl alcohol (pH 4.3) (25:24:1, vol/vol) and once with 1 volume of chloroform-isoamyl alcohol (24:1, vol/vol) (Sigma-Aldrich, CH). RNA was precipitated by addition of 0.1 volume of 3 M sodium acetate (pH 5.2) and 2 volumes of 100% ethanol, and incubated overnight at –20°C. The precipitate was collected by centrifugation for 30 min at maximum speed and 4°C, washed twice with 80% ethanol, and resuspended in nuclease-free water. Remaining DNA was removed by DNase I treatment using the DNA-free kit (Life technologies, Carlsbad, CA, USA) according to the manufacturer's instructions. An additional round of precipitation with sodium acetate (0.1 vol, 3 M, pH 5.2) and ethanol (2 vols, 100%) was performed and the RNA was finally purified using an RNeasy MinElute cleanup kit (Qiagen, Germany) in a final volume of 20 µl.



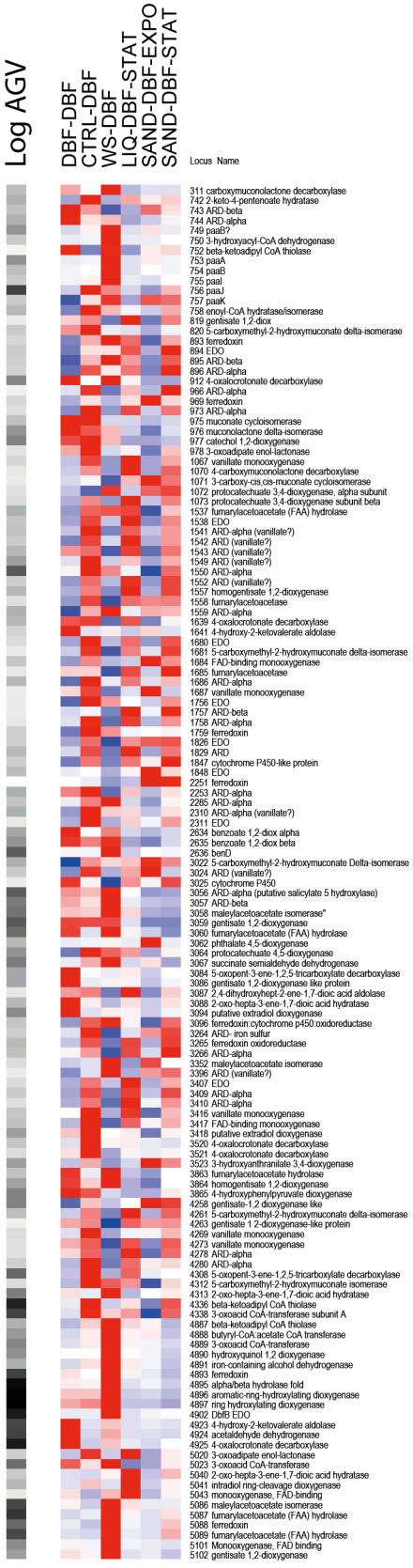
Supplementary Figure S1 *S. wittichii* RW1 population growth in sand with dibenzofuran (as colony forming units per g sand). Left panel, logarithmic representation. Right panel, linear representation. Note how the population development during the "early phase" in sand with dibenzofuran (12-28 h) is more accurately represented by exponential than by linear growth. Data from three independent inoculation experiments (different colored symbols), each carried out in quadruplates.



Supplementary Figure S2 Metabolic network for DBF and aromatic compound metabolism by *S. wittichii* RW1, displayed using Cytoskape 2.8.3. Nodes represent substrates and metabolic intermediates. Edges represent enzyme reactions converting the linked compounds. Numbers at the edges correspond to Swit_genes predicted to catalyze the indicated reaction. Network inferred from analysis as in Coronado et al., (Coronado *et al.*, 2012). Phenylacetate metabolism inferred by similarity to Teufel et al. (Teufel *et al.*, 2010). Node names, in alphabetical order:

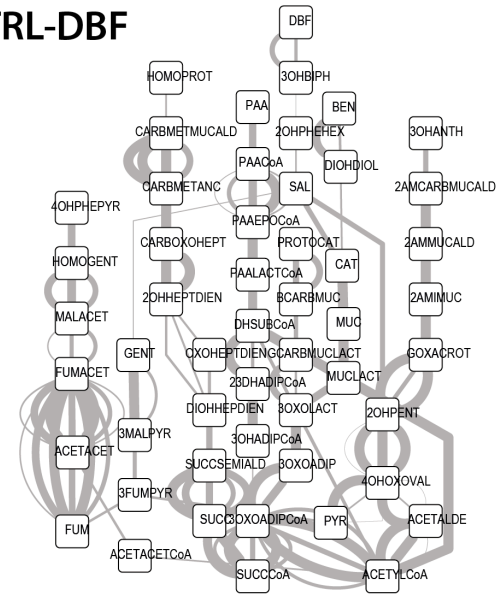
Abbreviation	Full name
23DHADIPCoA	2,3-dehydroadipyl-CoA
2AMCARBMUCALD	2-amino-3-carboxy-muconate-semialdehyde
2AMIMUC	2-aminomuconate
2AMMUCALD	2-amino-muconate-semialdehyde
2OHHEPTDIEN	2-Hydroxyhepta-2,4-dienedioate
2OHPENT	2-Hydroxy-2,4-pentadienoate
2OHPHEHEX	2-Hydroxy-6-oxo-6-(2-hydroxy-phenyl)-hexa-2,4-dienoate
3FUMPYR	3-fumarylpyruvate
3MALPYR	3-maleylpyruvate
3OHADIPCoA	3-hydroxyadipyl-CoA
3OHANTH	3-hydroxyanthranilate
3OHBIPH	2,2',3-Trihydroxybiphenyl
3OXOADIP	3-Oxoadipate
3OXOADIPCoA	3-oxo-adipyl-CoA
3OXOLACT	3-oxoadipate-enol-lactone
4OHOXOVAL	4-Hydroxy-2-oxo-valerate
4OHPHEPYR	4-Hydroxy-phenylpyruvate
ACETACET	Acetoacetate
ACETACETCoA	Acetoacetyl-CoA
ACETALDE	Acetaldehyde
ACETCoA	Acetyl-CoA
BCARBMUC	beta-carboxymuconate
BEN	Benzoate
CARBMETANC	1-Carboxy-methyl-2-hydroxy-anconate
CARBMETMUCALD	2-Hydroxy-5-carboxymethylmuconate semialdehyde
CARBOXOHEPT	5-Carboxy-2-oxo-hept-3-enedioate
CAT	Catechol
DBF	Dibenzofuran
DHSUBCoA	3-oxo-5,6-dehydrosuberyl-CoA
DIOHDIOL	cis-1,2-dihydroxycyclohexa-3,5-diene-1-carboxylate
DIOHHEPDIEN	2,4-Dihydroxy-hept-2-enedioate
FUMACET	4-Fumarylacetoacetate
GCARBMUCLACT	Gamma-carboxymuconolactone
GENT	Gentisate
GOXACROT	Gamma-oxalocrotonate
HOMOGENT	Homogentisate
HOMOPROT	Homoprotocatechuate
MALACET	4-Maleylacetoacetate
MUC	cis,cis-muconate
MUCLACT	(+)-muconolactone
OXOHEPTDIEN	2-Oxo-hept-3-dienedioate

PAA	Phenylacetate
PAACoA	Phenylacetyl-CoA
PAAEPOCoA	1,2-Epoxy-phenylacetyl-CoA
PAALACTCoA	2-oxepin-2(3H)-ylideneacetyl-CoA
PROTOCAT	Protocatechuate
PYR	Pyruvate
SAL	Salicylate
SUCC	Succinate
SUCCCoa	Succinyl-CoA
SUCCSEMIALD	Succinate semialdehyde

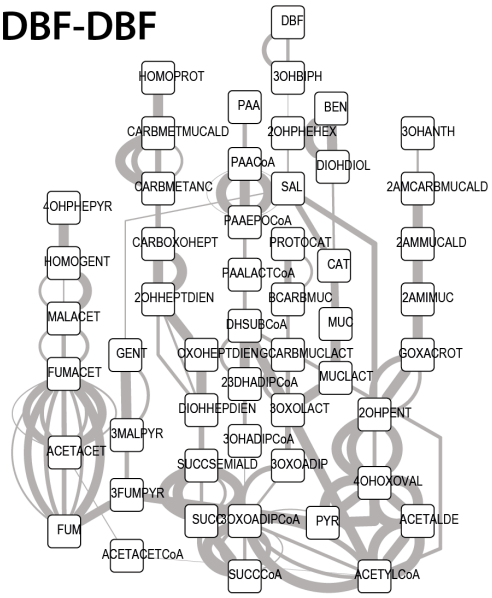


Supplementary Figure S3 Normalized gene expression of RW1 genes predicted to be implicated in aromatic compound metabolism, under different growth conditions. Colours indicate high (red), median (white) and low relative (blue) signal intensities. Log AVG, ^{10}log of the average absolute hybridisation intensity per indicated gene, on a scale from low (white) to high (black). Numbers and names on the right correspond to the Swit_gene notation and the predicted gene function. *DBF-DBF*, cells from DBF liquid cultures inoculated in sand contaminated with DBF; *CTRL-DBF*, cells from DBF liquid cultures not inoculated; *WS-DBF,* exponential growth in liquid with saturated amounts of DBF. *LIQ-DBF-STAT*, liquid cultures with crystals of dibenzofuran in late growth phase; *SAND-DBF-EXPO*, cells growing in sand in early (exponential) phase; *SAND-DBF-STAT*, cells from late growth phase in sand with DBF. Heatmap generated by Matrix2png (Pavlidis & Noble, 2003).

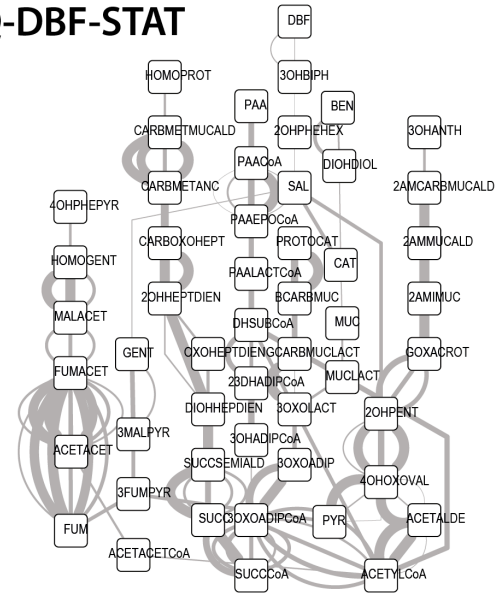
a) CTRL-DBF



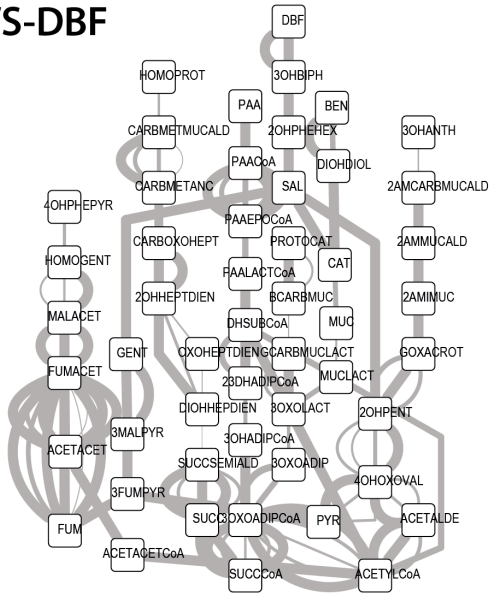
b) DBF-DBF



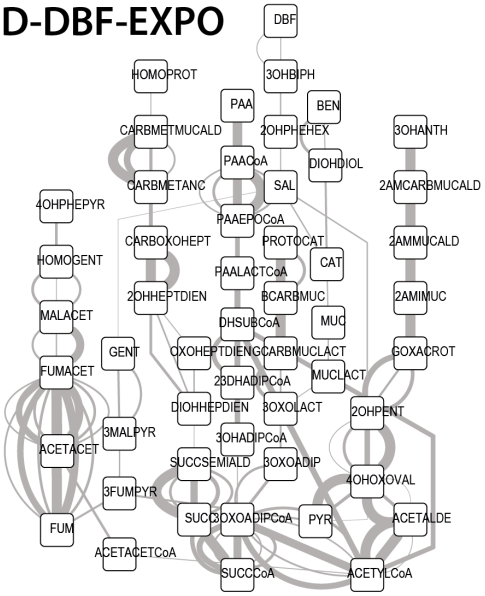
c) LIQ-DBF-STAT



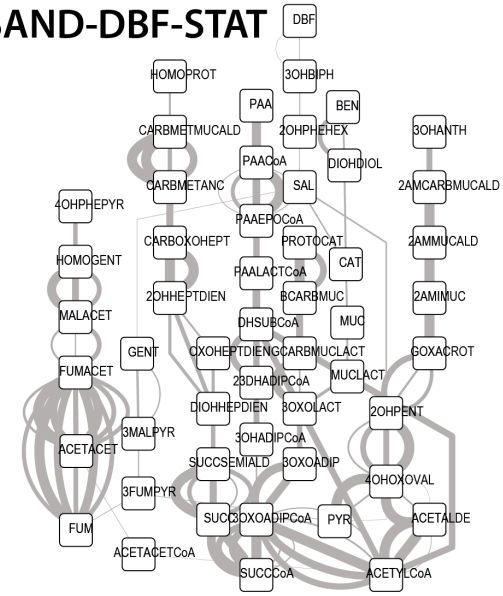
d) WS-DBF



e) SAND-DBF-EXPO



f) SAND-DBF-STAT



Supplementary Figure S4 Comparison of RW1 DBF and aromatic compound network expression under different growth conditions. For basic network description, see Supplementary Figure S2. Edge line thickness is a representation of normalized expression of the gene coding for the particular enzyme that carries out the reaction between two nodes. Normalization was carried out per gene among six conditions, taking the highest expression value as 100% (= line width 15). Line widths are a linear scale representation of normalized gene expression. notation and the predicted gene function.

DBF-DBF, cells from DBF liquid cultures inoculated in sand contaminated with DBF; *CTRL-DBF*, cells from DBF liquid cultures not inoculated; *WS-DBF,* exponential growth in liquid with saturated amounts of DBF. *LIQ-DBF-STAT*, liquid cultures with crystals of dibenzofuran in late growth phase; *SAND-DBF-EXPO*, cells growing in sand in early (exponential) phase; *SAND-DBF-STAT*, cells from late growth phase in sand with DBF.

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Table S1. **Enriched GO terms among the significantly differentially expressed genes in the comparison between in sand without DBF (SAL-NOTH) versus liquid culture grown with salicylate (control SAL).**

Genes higher expressed in cells of RW1 in soil without DBF after 1 h

GOID	Biological Process	log_odds_ratio	p-value	Genes
GO:0030416	methylamine metabolic process	2.78	2.40E-02	Swit_4363, Swit_4364
GO:0006573	valine metabolic process	2.29	4.80E-02	Swit_0647, Swit_0652
GO:0006265	DNA topological change	2.29	4.80E-02	Swit_4578, Swit_5285
GO:0006352	transcription initiation, DNA-dependent	1.36	8.30E-02	Swit_1109, Swit_3591, Swit_3924
GO:0019725	cellular homeostasis	1.26	2.00E-02	Swit_1124, Swit_2670, Swit_2779, Swit_3162, Swit_3587, Swit_4364
GO:0006281	DNA repair	1.26	6.40E-02	Swit_3206, Swit_3911, Swit_3979, Swit_3981, Swit_3982, Swit_5282
GO:0005976	polysaccharide metabolic process	1.21	4.30E-02	Swit_3438, Swit_3609, Swit_4514, Swit_4527, Swit_4528
GO:0007165	signal transduction	0.75	5.30E-02	Swit_0513, Swit_1090, Swit_1155, Swit_1160, Swit_1226, Swit_1954, Swit_1955, Swit_2540, Swit_3903, Swit_3925, Swit_3926, Swit_4432, Swit_5012, Swit_5270, Swit_5396
GO:0051171	regulation of nitrogen compound metabolic process	0.25	6.00E-02	Swit_0097, Swit_0278, Swit_0513, Swit_0654, Swit_0797, Swit_0953, Swit_1109, Swit_1160, Swit_1226, Swit_1476, Swit_1954, Swit_2385, Swit_2490, Swit_2540, Swit_2810, Swit_2921, Swit_3591, Swit_3604, Swit_3640, Swit_3791, Swit_3924, Swit_3925, Swit_4362, Swit_4432, Swit_4844, Swit_5012, Swit_5270, Swit_5337, Swit_5396
GO:0008152	metabolic process	-0.13	6.70E-02	Swit_0097, Swit_0142, Swit_0278, Swit_0513, Swit_0564, Swit_0617, Swit_0647, Swit_0649, Swit_0650, Swit_0652, Swit_0740, Swit_0762, Swit_0763, Swit_0780, Swit_0798, Swit_0944, Swit_0953, Swit_0956, Swit_0995, Swit_1051, Swit_1052, Swit_1109, Swit_1160, Swit_1179, Swit_1238, Swit_1412, Swit_1472, Swit_1473, Swit_1474, Swit_1476, Swit_1755, Swit_1842, Swit_1954, Swit_1955, Swit_2076, Swit_2119, Swit_2161, Swit_2261, Swit_2269, Swit_2270, Swit_2271, Swit_2277, Swit_2455, Swit_2559, Swit_2576, Swit_2577, Swit_2890, Swit_2891, Swit_2921, Swit_2926, Swit_2933, Swit_3015, Swit_3154, Swit_3162, Swit_3206, Swit_3279, Swit_3438, Swit_3475, Swit_3544, Swit_3586, Swit_3591, Swit_3593, Swit_3599, Swit_3600, Swit_3601, Swit_3602, Swit_3603, Swit_3604, Swit_3608, Swit_3609, Swit_3610, Swit_3640, Swit_3796, Swit_3803, Swit_3804, Swit_3835, Swit_3893, Swit_3907, Swit_3911, Swit_3924, Swit_3979, Swit_3981, Swit_3982, Swit_3983, Swit_4209, Swit_4227, Swit_4362, Swit_4363, Swit_4364, Swit_4365, Swit_4432, Swit_4514, Swit_4527, Swit_4528, Swit_4533, Swit_4578, Swit_4648, Swit_4764, Swit_4785, Swit_4790, Swit_4791, Swit_4896, Swit_4902, Swit_4903, Swit_5012, Swit_5248, Swit_5282, Swit_5285, Swit_5291, Swit_5315, Swit_5345

GOID	Cellular Component	log_odds_ratio	p-value	Genes
GO:0005694	chromosome	1.78	9.00E-02	Swit_4578, Swit_5285
GO:0016021	integral to membrane	0.3	9.40E-02	Swit_0786, Swit_1155, Swit_1172, Swit_1952, Swit_1955, Swit_2278, Swit_2322, Swit_2324, Swit_2334, Swit_2422, Swit_3455, Swit_3475, Swit_3926, Swit_4648, Swit_4749

GOID	Molecular Function	log_odds_ratio	p-value	Genes
GO:0003746	translation elongation factor activity	2.88	5.00E-03	Swit_2490, Swit_4844, Swit_5337
GO:0016209	antioxidant activity	2.34	6.90E-05	Swit_0038, Swit_2341, Swit_2933, Swit_3162, Swit_3586, Swit_3587, Swit_4101, Swit_5248
GO:0003916	DNA topoisomerase activity	2.29	5.40E-02	Swit_4578, Swit_5285
GO:0016987	sigma factor activity	1.36	9.60E-02	Swit_1109, Swit_3591, Swit_3924
GO:0004175	endopeptidase activity	1.03	8.10E-02	Swit_0617, Swit_0798, Swit_2119, Swit_3804, Swit_3835
GO:0000156	two-component response regulator activity	0.79	4.60E-02	Swit_0513, Swit_1160, Swit_1226, Swit_1954, Swit_2540, Swit_3925, Swit_4432, Swit_5012, Swit_5270, Swit_5396

Genes lower expressed in cells of RW1 in soil without DBF after 1 h

GOID	Biological Process	log_odds_ratio	p-value	Genes
GO:0006119	oxidative phosphorylation	3.46	3.20E-10	Swit_0620, Swit_0621, Swit_0622, Swit_0623, Swit_2991, Swit_2995, Swit_2996, Swit_2997, Swit_4483, Swit_4484, Swit_4485
GO:0045333	cellular respiration	3.06	5.90E-11	Swit_1297, Swit_1300, Swit_1311, Swit_1312, Swit_1395, Swit_1801, Swit_2732, Swit_2991, Swit_2995, Swit_2996, Swit_2997, Swit_3212, Swit_3875, Swit_3876, Swit_5200
GO:0022900	electron transport chain	2.99	8.70E-07	Swit_1395, Swit_1396, Swit_1801, Swit_2991, Swit_2995, Swit_2996, Swit_2997, Swit_3875, Swit_3876
GO:0046700	heterocycle catabolic process	2.91	6.90E-03	Swit_4630, Swit_4632, Swit_4633
GO:0009086	methionine biosynthetic process	2.74	2.80E-03	Swit_2399, Swit_2401, Swit_2664, Swit_4786
GO:0009296	flagellum assembly	2.59	4.80E-02	Swit_0212, Swit_0213
GO:0016226	iron-sulfur cluster assembly	2.59	4.80E-02	Swit_2380, Swit_2913
GO:0006096	glycolysis	2.33	2.50E-02	Swit_0446, Swit_1300, Swit_5154
GO:0015671	oxygen transport	2.33	6.80E-02	Swit_1200, Swit_5203
GO:0006412	translation	2.23	6.50E-05	Swit_1327, Swit_1343, Swit_1344, Swit_1345, Swit_1355, Swit_1356, Swit_1357, Swit_1377, Swit_3809, Swit_4045
GO:0006886	intracellular protein transport	2.1	9.10E-02	Swit_1454, Swit_2561
GO:0009082	branched chain family amino acid biosynthetic process	1.69	8.10E-02	Swit_0561, Swit_0609, Swit_4656
GO:0034404	nucleobase-containing small molecule biosynthetic process	1.59	5.00E-03	Swit_0620, Swit_0621, Swit_0622, Swit_0623, Swit_3786, Swit_4483, Swit_4484, Swit_4485, Swit_4682
GO:0046395	carboxylic acid catabolic process	1.42	2.00E-02	Swit_0975, Swit_3864, Swit_4630, Swit_4632, Swit_4633
GO:0019725	cellular homeostasis	1.29	7.60E-02	Swit_1296, Swit_1365, Swit_3139, Swit_3742, Swit_3991, Swit_4025, Swit_5151
GO:0046483	heterocycle metabolic process	0.92	3.80E-02	Swit_0620, Swit_0621, Swit_0622, Swit_0623, Swit_1398, Swit_2399, Swit_2880, Swit_3527, Swit_3877, Swit_4483, Swit_4484, Swit_4485, Swit_4630, Swit_4632, Swit_4633, Swit_4682, Swit_4754
GO:0006725	cellular aromatic compound metabolic process	0.9	9.50E-02	Swit_0743, Swit_0744, Swit_0976, Swit_0977, Swit_0978, Swit_1041, Swit_2399, Swit_2634, Swit_2635, Swit_2867, Swit_2880, Swit_3223, Swit_3818, Swit_3863, Swit_3864, Swit_3865, Swit_5035
GO:0046394	carboxylic acid biosynthetic process	0.78	5.10E-02	Swit_0561, Swit_0609, Swit_1413, Swit_2399, Swit_2401, Swit_2664, Swit_2970, Swit_3786, Swit_4656, Swit_4685, Swit_4786, Swit_4831
GO:0043436	oxoacid metabolic process	0.74	1.30E-02	Swit_0457, Swit_0561, Swit_0609, Swit_0807, Swit_0975, Swit_1300, Swit_1367, Swit_1413, Swit_2399, Swit_2401, Swit_2664, Swit_2970, Swit_3786, Swit_3863, Swit_3864, Swit_3865, Swit_4630, Swit_4632, Swit_4633, Swit_4656, Swit_4685, Swit_4786, Swit_4831, Swit_5055, Swit_5152

GOID	Cellular Component	log_odds_ratio	p-value	Genes
GO:0045259	proton-transporting ATP synthase complex	3.72	5.30E-07	Swit_0620, Swit_0621, Swit_0622, Swit_0623, Swit_4483, Swit_4484, Swit_4485
GO:0005840	ribosome	3.45	8.50E-07	Swit_1327, Swit_1343, Swit_1344, Swit_1345, Swit_1357, Swit_1377, Swit_2658, Swit_3809
GO:0009425	bacterial-type flagellum basal body	2.69	2.40E-02	Swit_1286, Swit_1287, Swit_1293
GO:0044424	intracellular part	1.5	6.50E-02	Swit_0212, Swit_0213, Swit_0446, Swit_0620, Swit_0621, Swit_0622, Swit_0623, Swit_1286, Swit_1287, Swit_1293, Swit_1296, Swit_1297, Swit_1327, Swit_1343, Swit_1344, Swit_1345, Swit_1355, Swit_1356, Swit_1357, Swit_1365, Swit_1367, Swit_1377, Swit_1801, Swit_2658, Swit_2664, Swit_2970, Swit_3128, Swit_3212, Swit_3373, Swit_3375, Swit_3809, Swit_3810, Swit_4376, Swit_4483, Swit_4484, Swit_4485, Swit_4630, Swit_4632, Swit_4633, Swit_4682, Swit_4831, Swit_5151, Swit_5152, Swit_5154, Swit_5339

Genes lower expressed in cells of RW1 in soil without DBF after 1 h cont…

GOID	Molecular Function	log_odds_ratio	p-value	Genes
GO:0019843	rRNA binding	3.91	8.80E-08	Swit_1327, Swit_1343, Swit_1344, Swit_1357, Swit_1377, Swit_3809
GO:0003735	structural constituent of ribosome	3.72	4.40E-08	Swit_1327, Swit_1343, Swit_1344, Swit_1345, Swit_1357, Swit_1377, Swit_3809
GO:0008172	S-methyltransferase activity	3.1	6.00E-04	Swit_1248, Swit_2399, Swit_2400, Swit_4786
GO:0015078	hydrogen ion transmembrane transporter activity	2.98	7.20E-12	Swit_0620, Swit_0621, Swit_0622, Swit_0623, Swit_1396, Swit_1801, Swit_3875, Swit_3876, Swit_4483, Swit_4484, Swit_4485
GO:0003954	NADH dehydrogenase activity	2.91	3.00E-06	Swit_2985, Swit_2988, Swit_2991, Swit_2992, Swit_2993, Swit_2994, Swit_2996, Swit_2997
GO:0050661	NADP binding	2.74	1.90E-03	Swit_1181, Swit_1707, Swit_2664, Swit_3786
GO:0003899	DNA-directed RNA polymerase activity	2.74	1.90E-03	Swit_1281, Swit_1326, Swit_3467, Swit_3468
GO:0046912	transferase activity, transferring acyl groups, acyl groups converted into alkyl on transfer	2.59	4.00E-02	Swit_0561, Swit_3212
GO:0015450	P-P-bond-hydrolysis-driven protein transmembrane transporter activity	2.59	4.00E-02	Swit_1454, Swit_2561
GO:0019825	oxygen binding	2.33	5.70E-02	Swit_1200, Swit_5203
GO:0048038	quinone binding	2.33	1.90E-02	Swit_2985, Swit_2992, Swit_2995
GO:0003746	translation elongation factor activity	2.1	7.60E-02	Swit_1355, Swit_1356
GO:0051539	4 iron, 4 sulfur cluster binding	1.96	7.70E-04	Swit_1707, Swit_2731, Swit_2732, Swit_2988, Swit_2991, Swit_2993, Swit_4058, Swit_4656
GO:0030976	thiamine pyrophosphate binding	1.91	9.70E-02	Swit_0609, Swit_1880
GO:0008239	dipeptidyl-peptidase activity	1.91	9.70E-02	Swit_0917, Swit_5053
GO:0003924	GTPase activity	1.91	9.70E-02	Swit_1355, Swit_1356
GO:0051287	NAD binding	1.79	6.50E-03	Swit_0457, Swit_2602, Swit_2664, Swit_2985, Swit_2988, Swit_4685
GO:0031405	lipoic acid binding	1.79	5.20E-02	Swit_1297, Swit_1367, Swit_5152
GO:0046906	tetrapyrrole binding	1.33	4.00E-03	Swit_1200, Swit_1394, Swit_1707, Swit_1800, Swit_1801, Swit_2399, Swit_3875, Swit_3876, Swit_4058, Swit_5200, Swit_5203
GO:0005515	protein binding	1.17	5.20E-02	Swit_1297, Swit_1326, Swit_1367, Swit_1454, Swit_2561, Swit_2664, Swit_2732, Swit_3128, Swit_5133, Swit_5152
GO:0051213	dioxygenase activity	1.1	6.20E-03	Swit_0743, Swit_0744, Swit_0977, Swit_1662, Swit_1680, Swit_2634, Swit_2635, Swit_2867, Swit_3086, Swit_3094, Swit_3223, Swit_3864, Swit_3865, Swit_5102, Swit_5203
GO:0046872	metal ion binding	0.75	4.40E-04	Swit_0312, Swit_0446, Swit_0457, Swit_0609, Swit_0744, Swit_0975, Swit_0977, Swit_1041, Swit_1200, Swit_1394, Swit_1396, Swit_1644, Swit_1648, Swit_1657, Swit_1707, Swit_1800, Swit_1801, Swit_1880, Swit_1939, Swit_2380, Swit_2399, Swit_2634, Swit_2702, Swit_2867, Swit_2988, Swit_2991, Swit_2993, Swit_3139, Swit_3223, Swit_3373, Swit_3729, Swit_3864, Swit_3875, Swit_3876, Swit_4025, Swit_4045, Swit_4058, Swit_4633, Swit_4656, Swit_4682, Swit_4786, Swit_5035, Swit_5200, Swit_5203
GO:0043169	cation binding	0.75	4.60E-04	Swit_0312, Swit_0446, Swit_0457, Swit_0609, Swit_0744, Swit_0975, Swit_0977, Swit_1041, Swit_1200, Swit_1394, Swit_1396, Swit_1644, Swit_1648, Swit_1657, Swit_1707, Swit_1800, Swit_1801, Swit_1880, Swit_1939, Swit_2380, Swit_2399, Swit_2634, Swit_2702, Swit_2867, Swit_2988, Swit_2991, Swit_2993, Swit_3139, Swit_3223, Swit_3373, Swit_3729, Swit_3864, Swit_3875, Swit_3876, Swit_4025, Swit_4045, Swit_4058, Swit_4633, Swit_4656, Swit_4682, Swit_4786, Swit_5035, Swit_5200, Swit_5203
GO:0000166	nucleotide binding	0.3	6.50E-02	Swit_0238, Swit_0267, Swit_0457, Swit_0609, Swit_0621, Swit_0622, Swit_0623, Swit_1181, Swit_1280, Swit_1296, Swit_1355, Swit_1356, Swit_1365, Swit_1536, Swit_1707, Swit_2602, Swit_2664, Swit_2970, Swit_2985, Swit_2988, Swit_3090, Swit_3128, Swit_3341, Swit_3373, Swit_3375, Swit_3786, Swit_3814, Swit_4682, Swit_4685, Swit_4754, Swit_4859, Swit_5133, Swit_5151

Table S2. **Enriched GO terms among the significantly differentially expressed genes in the comparison between RW1 cells after 1 h in sand plus DBF (SAL-DBF) versus cells grown liquid cultures with salicylate (control SAL).**

Genes higher expressed in cells of RW1 in soil with DBF after 1 h

GOID	Biological Process	log_odds_ratio	p-value	Genes
GO:0006537	glutamate biosynthetic process	2.62	3.60E-02	Swit_0659, Swit_0657
GO:0009103	lipopolysaccharide biosynthetic process	2.62	9.50E-03	Swit_4542, Swit_0470, Swit_4528
GO:0009070	serine family amino acid biosynthetic process	2.39	4.90E-02	Swit_4685, Swit_4648
GO:0006261	DNA-dependent DNA replication	2.39	9.40E-02	Swit_5285, Swit_3982
GO:0006265	DNA topological change	2.39	4.90E-02	Swit_5285, Swit_4578
GO:0009712	catechol-containing compound metabolic process	1.98	7.70E-02	Swit_4890, Swit_5041, Swit_4887
GO:0034311	diol metabolic process	1.98	3.40E-02	Swit_4890, Swit_5041, Swit_4887
GO:0000271	polysaccharide biosynthetic process	1.83	9.10E-02	Swit_4527, Swit_2652, Swit_4542, Swit_0470, Swit_4528, Swit_4514
GO:0005976	polysaccharide metabolic process	1.8	4.20E-02	Swit_3609, Swit_4527, Swit_2652, Swit_4542, Swit_0470, Swit_4528, Swit_4514
GO:0015031	protein transport	1.67	1.30E-03	Swit_0118, Swit_3829, Swit_0120, Swit_4511, Swit_2135, Swit_0119, Swit_0117, Swit_2136, Swit_0452
GO:0045454	cell redox homeostasis	1.57	2.10E-02	Swit_3743, Swit_1124, Swit_3162, Swit_3587, Swit_0016
GO:0019439	aromatic compound catabolic process	1.29	7.00E-02	Swit_4923, Swit_4902, Swit_4924, Swit_1641
GOID	**Cellular Component**	**log_odds_ratio**	**p-value**	**Genes**
GO:0016021	integral to membrane	0.96	6.70E-03	Swit_2973, Swit_2322, Swit_3842, Swit_0118, Swit_3455, Swit_1952, Swit_0120, Swit_3926, Swit_2422, Swit_4648, Swit_1153, Swit_2135, Swit_3745, Swit_0119, Swit_3443, Swit_3475, Swit_2136, Swit_2334, Swit_0786, Swit_4749, Swit_2324, Swit_2278
GOID	**Molecular Function**	**log_odds_ratio**	**p-value**	**Genes**
GO:0018576	catechol 1,2-dioxygenase activity	2.88	2.40E-02	Swit_4890, Swit_5041
GO:0016209	antioxidant activity	2.62	4.40E-06	Swit_3586, Swit_5248, Swit_3743, Swit_3164, Swit_4101, Swit_3162, Swit_3587, Swit_2341, Swit_2933
GO:0003746	translation elongation factor activity	2.39	4.70E-02	Swit_0395, Swit_4844
GO:0003916	DNA topoisomerase activity	2.39	4.70E-02	Swit_5285, Swit_4578
GO:0003985	acetyl-CoA C-acetyltransferase activity	2.39	4.70E-02	Swit_3602, Swit_4887
GO:0003735	structural constituent of ribosome	2.2	6.00E-02	Swit_3869, Swit_1327
GO:0005507	copper ion binding	1.88	9.10E-02	Swit_2616, Swit_2221
GO:0051287	NAD binding	1.5	4.20E-02	Swit_4685, Swit_4534, Swit_1474, Swit_4924
GO:0008565	protein transporter activity	1.28	4.30E-02	Swit_0118, Swit_4511, Swit_0117, Swit_2136, Swit_0452
GO:0004175	endopeptidase activity	1.13	6.30E-02	Swit_0798, Swit_3835, Swit_3849, Swit_2546, Swit_2119

Genes lower expressed in cells of RW1 in soil with DBF after 1 h

GOID	Biological Process	log_odds_ratio	p-value	Genes
GO:0030416	methylamine metabolic process	3.27	2.40E-03	Swit_4363, Swit_3254, Swit_4364
GO:0009296	flagellum assembly	3.27	2.40E-03	Swit_1262, Swit_0212, Swit_0213
GO:0016226	iron-sulfur cluster assembly	2.69	3.60E-02	Swit_2913, Swit_2380
GO:0009060	aerobic respiration	2.52	1.30E-03	Swit_4614, Swit_1312, Swit_1801, Swit_5200, Swit_1311
GO:0046700	heterocycle catabolic process	2.42	5.20E-02	Swit_4633, Swit_4632
GO:0015671	oxygen transport	2.42	5.20E-02	Swit_1200, Swit_5203
GO:0001539	ciliary or flagellar motility	2.24	3.50E-03	Swit_1268, Swit_1262, Swit_1293, Swit_1287, Swit_1286
GO:0019614	catechol-containing compound catabolic process	2.01	9.00E-02	Swit_0978, Swit_0977
GO:0034313	diol catabolic process	2.01	9.00E-02	Swit_0978, Swit_0977
GO:0046395	carboxylic acid catabolic process	1.2	4.90E-02	Swit_3864, Swit_4633, Swit_4632, Swit_0975
GO:0006725	cellular aromatic compound metabolic process	1.08	1.10E-02	Swit_3056, Swit_3057, Swit_3046, Swit_3864, Swit_2867, Swit_3865, Swit_2635, Swit_0978, Swit_3863, Swit_0976, Swit_2880, Swit_0977, Swit_2634, Swit_3058, Swit_5035, Swit_3818, Swit_3223, Swit_1041
GO:0022607	cellular component assembly	0.8	3.80E-02	Swit_1262, Swit_0212, Swit_1281, Swit_0213
GO:0006950	response to stress	0.7	7.80E-02	Swit_1368, Swit_0619, Swit_0266, Swit_0203, Swit_3232, Swit_3730, Swit_3128, Swit_1248
GO:0051171	regulation of nitrogen compound metabolic process	0.43	5.60E-02	Swit_4239, Swit_1533, Swit_0733, Swit_1142, Swit_0153, Swit_3187, Swit_1918, Swit_0268, Swit_4296, Swit_2523, Swit_0806, Swit_3569, Swit_4931, Swit_4054, Swit_1275, Swit_4557, Swit_3257, Swit_0267, Swit_2402, Swit_0747, Swit_3233, Swit_0986, Swit_0816, Swit_2305, Swit_1945, Swit_1845, Swit_4866, Swit_3866, Swit_5296, Swit_1281, Swit_2042, Swit_2047, Swit_1882, Swit_5339, Swit_4721
GO:0055114	oxidation-reduction process	0.4	7.70E-02	Swit_1709, Swit_5055, Swit_4614, Swit_1027, Swit_3059, Swit_3056, Swit_3057, Swit_3046, Swit_1330, Swit_3786, Swit_3288, Swit_1166, Swit_1879, Swit_5033, Swit_3341, Swit_4155, Swit_1707, Swit_3068, Swit_3797, Swit_1312, Swit_3864, Swit_3254, Swit_1880, Swit_2602, Swit_5056, Swit_5102, Swit_2867, Swit_3865, Swit_3069, Swit_1801, Swit_2635, Swit_5200, Swit_2401, Swit_3722, Swit_1398, Swit_3730, Swit_0977, Swit_2634, Swit_3796, Swit_2816, Swit_1311, Swit_3236, Swit_1657, Swit_3067, Swit_5203, Swit_3223, Swit_1041

GOID	Cellular Component	log_odds_ratio	p-value	Genes
GO:0009425	bacterial-type flagellum basal body	3.2	6.60E-04	Swit_1268, Swit_1293, Swit_1287, Swit_1286
GO:0042995	cell projection	2.48	3.10E-02	Swit_1268, Swit_1262, Swit_1275, Swit_0212, Swit_1293, Swit_1287, Swit_1286, Swit_0213
GO:0043232	intracellular non-membrane-bounded organelle	1.86	1.10E-03	Swit_1268, Swit_1262, Swit_0212, Swit_1345, Swit_2827, Swit_1293, Swit_1287, Swit_1286, Swit_0213

Genes lower expressed in cells of RW1 in soil with DBF after 1 h cont…

GOID	Molecular Function	log_odds_ratio	p-value	Genes
GO:0019825	oxygen binding	2.42	4.70E-02	Swit_1200, Swit_5203
GO:0003857	3-hydroxyacyl-CoA dehydrogenase activity	2.2	6.40E-02	Swit_5055, Swit_3796
GO:0003774	motor activity	2.2	8.30E-03	Swit_1268, Swit_1262, Swit_1287, Swit_1286
GO:0005198	structural molecule activity	2.08	4.60E-03	Swit_1268, Swit_1262, Swit_2913, Swit_1345, Swit_1286
GO:0030976	thiamine pyrophosphate binding	2.01	8.10E-02	Swit_3237, Swit_1880
GO:0008198	ferrous iron binding	2.01	3.30E-02	Swit_3046, Swit_2867, Swit_1041
GO:0008239	dipeptidyl-peptidase activity	2.01	8.10E-02	Swit_5053, Swit_0917
GO:0020037	heme binding	1.59	3.80E-04	Swit_1798, Swit_4614, Swit_1027, Swit_1707, Swit_3250, Swit_3069, Swit_1801, Swit_1200, Swit_5200, Swit_3730, Swit_1800, Swit_5203
GO:0051213	dioxygenase activity	1.54	2.20E-05	Swit_3086, Swit_3094, Swit_3059, Swit_3056, Swit_3057, Swit_3046, Swit_1662, Swit_3864, Swit_5102, Swit_2867, Swit_3865, Swit_2635, Swit_3047, Swit_1680, Swit_0977, Swit_2634, Swit_4094, Swit_5203, Swit_3223

Table S3. **Enriched GO terms among the significantly differentially expressed genes in the comparison between RW1 cells after 1 h in sand plus DBF (DBF-DBF) versus cells grown liquid cultures with DBF (control DBF).**

Genes higher expressed in cells of RW1 in soil with DBF after 1 h

GOID	Biological Process	log_odds_ratio	p-value	Genes
GO:0006119	oxidative phosphorylation	4.16	1.50E-06	Swit_4483, Swit_0621, Swit_2997, Swit_2996, Swit_3880, Swit_2991, Swit_4485
GO:0006412	translation	3.43	2.20E-07	Swit_1357, Swit_1355, Swit_1343, Swit_1377, Swit_3845, Swit_1344, Swit_1345, Swit_1356, Swit_1327
GO:0045333	cellular respiration	3.09	2.30E-04	Swit_2997, Swit_1395, Swit_2996, Swit_3880, Swit_1299, Swit_2991
GO:0009086	methionine biosynthetic process	3.09	2.80E-02	Swit_2401, Swit_2664
GO:0019637	organophosphate metabolic process	3.09	2.80E-02	Swit_0736, Swit_0470
GO:0006457	protein folding	2.76	1.30E-02	Swit_3829, Swit_3531, Swit_1253
GO:0006396	RNA processing	2.56	7.00E-03	Swit_3615, Swit_3810, Swit_3848, Swit_2962
GO:0006006	glucose metabolic process	2.33	3.10E-02	Swit_2602, Swit_2606, Swit_2888
GO:0045454	cell redox homeostasis	2.31	1.30E-02	Swit_1124, Swit_1365, Swit_5151, Swit_3587
GO:0034404	nucleobase-containing small molecule biosynthetic process	2.09	1.10E-02	Swit_4483, Swit_0621, Swit_0030, Swit_0102, Swit_4485
GO:0006633	fatty acid biosynthetic process	2.09	8.20E-02	Swit_3556, Swit_0470
GO:0015031	protein transport	1.89	6.40E-02	Swit_3829, Swit_0120, Swit_4006, Swit_4007, Swit_1152

GOID	Cellular Component	log_odds_ratio	p-value	Genes
GO:0005840	ribosome	4.39	4.20E-06	Swit_1357, Swit_1343, Swit_1377, Swit_1344, Swit_1345, Swit_1327
GO:0044424	intracellular part	2.16	4.10E-05	Swit_4483, Swit_1357, Swit_3556, Swit_0621, Swit_3829, Swit_5152, Swit_1355, Swit_0030, Swit_1365, Swit_5151, Swit_2532, Swit_1343, Swit_1377, Swit_3490, Swit_3810, Swit_3845, Swit_0736, Swit_2664, Swit_1344, Swit_1345, Swit_1356, Swit_3848, Swit_2962, Swit_0470, Swit_1327, Swit_1253, Swit_3373, Swit_4485

GOID	Molecular Function	log_odds_ratio	p-value	Genes
GO:0003735	structural constituent of ribosome	4.85	8.50E-09	Swit_1357, Swit_1343, Swit_1377, Swit_1344, Swit_1345, Swit_1327
GO:0003954	NADH dehydrogenase activity	4.07	7.50E-08	Swit_2997, Swit_2986, Swit_2996, Swit_2992, Swit_2993, Swit_2991, Swit_2984
GOID	**Molecular Function**	**log_odds_ratio**	**p-value**	**Genes**
GO:0030234	enzyme regulator activity	3.68	1.00E-02	Swit_4832, Swit_1253
GO:0003723	RNA binding	3.46	6.00E-08	Swit_1357, Swit_0061, Swit_1355, Swit_3615, Swit_1343, Swit_1377, Swit_3810, Swit_3845, Swit_1344, Swit_1356, Swit_3848, Swit_1327
GO:0008408	3'-5' exonuclease activity	3.46	1.40E-02	Swit_0450, Swit_3810
GO:0008135	translation factor activity, nucleic acid binding	3.39	1.30E-02	Swit_1355, Swit_3845, Swit_1356
GO:0003924	GTPase activity	3.26	1.80E-02	Swit_1355, Swit_1356
GO:0003899	DNA-directed RNA polymerase activity	3.09	2.30E-02	Swit_3467, Swit_3468
GO:0050661	NADP binding	3.09	2.30E-02	Swit_2664, Swit_1181
GO:0005507	copper ion binding	2.94	2.80E-02	Swit_2616, Swit_3879
GO:0051287	NAD binding	2.89	5.30E-04	Swit_2986, Swit_2602, Swit_0457, Swit_0736, Swit_2664
GO:0015078	hydrogen ion transmembrane transporter activity	2.87	2.90E-04	Swit_4483, Swit_0621, Swit_3880, Swit_4485
GO:0008026	ATP-dependent helicase activity	2.46	5.30E-02	Swit_0140, Swit_3798
GO:0000287	magnesium ion binding	2.46	6.10E-03	Swit_0030, Swit_0457, Swit_2532, Swit_3373
GO:0051539	4 iron, 4 sulfur cluster binding	2.31	8.80E-03	Swit_2993, Swit_4707, Swit_2991, Swit_2962
GO:0008565	protein transporter activity	1.6	8.10E-02	Swit_4006, Swit_4007, Swit_1152
GO:0005515	protein binding	1.52	9.70E-02	Swit_3829, Swit_5152, Swit_0247, Swit_2664, Swit_1253
GO:0000166	nucleotide binding	1.14	2.70E-02	Swit_2973, Swit_0140, Swit_0621, Swit_4859, Swit_1355, Swit_0030, Swit_2986, Swit_1365, Swit_2602, Swit_0457, Swit_5151, Swit_0247, Swit_1299, Swit_0736, Swit_2664, Swit_1356, Swit_1181, Swit_4007, Swit_2917, Swit_1253, Swit_0102, Swit_3798, Swit_3373
GO:0005524	ATP binding	1.02	1.70E-02	Swit_2973, Swit_0140, Swit_0621, Swit_4859, Swit_0030, Swit_0247, Swit_1299, Swit_4007, Swit_2917, Swit_0102, Swit_3798, Swit_3373
GO:0001882	nucleoside binding	0.9	1.30E-02	Swit_2973, Swit_0140, Swit_0621, Swit_4859, Swit_0030, Swit_1365, Swit_5151, Swit_0247, Swit_1299, Swit_4007, Swit_2917, Swit_1253, Swit_0102, Swit_3468, Swit_3798, Swit_3373

Genes lower expressed in cells of RW1 in soil with DBF after 1 h

GOID	Biological Process	log_odds_ratio	p-value	Genes
GO:0030416	methylamine metabolic process	4.71	2.70E-03	Swit_4363, Swit_4364
GO:0019614	catechol-containing compound catabolic process	4.03	7.30E-03	Swit_0978, Swit_0977
GO:0034313	diol catabolic process	4.03	7.30E-03	Swit_0978, Swit_0977
GO:0010035	response to inorganic substance	3.71	8.10E-02	Swit_3730
GO:0015671	oxygen transport	3.44	9.70E-02	Swit_1200
GO:0009060	aerobic respiration	3.22	2.20E-02	Swit_4614, Swit_4790
GO:0019439	aromatic compound catabolic process	2.12	8.90E-02	Swit_3056, Swit_3046
GO:0007165	signal transduction	1.36	1.40E-02	Swit_4239, Swit_0268, Swit_0694, Swit_3504, Swit_0267, Swit_4721
GO:0006810	transport	0.94	9.80E-02	Swit_3256, Swit_3793, Swit_3144, Swit_1200, Swit_0964, Swit_3048, Swit_0692, Swit_3044, Swit_0687, Swit_0689, Swit_2826, Swit_2885
GOID	**Cellular Component**	**log_odds_ratio**	**p-value**	**Genes**
GO:0042597	periplasmic space	3.33	2.00E-03	Swit_4363, Swit_0693, Swit_5136, Swit_0692
GO:0031975	envelope	1.89	3.10E-03	Swit_3256, Swit_3144, Swit_0964, Swit_0693, Swit_3048, Swit_5136, Swit_0692, Swit_3044, Swit_0687, Swit_5295, Swit_2885
GO:0044462	external encapsulating structure part	1.86	4.50E-03	Swit_3256, Swit_3144, Swit_0964, Swit_0693, Swit_3048, Swit_5136, Swit_0692, Swit_3044, Swit_0687, Swit_2885
GOID	**Molecular Function**	**log_odds_ratio**	**p-value**	**Genes**
GO:0018576	catechol 1,2-dioxygenase activity	3.71	7.40E-02	Swit_0977
GO:0019825	oxygen binding	3.44	8.80E-02	Swit_1200
GO:0005509	calcium ion binding	3.03	2.40E-02	Swit_0693, Swit_5136
GO:0020037	heme binding	2.84	4.00E-05	Swit_4614, Swit_1200, Swit_5136, Swit_3730, Swit_4366, Swit_1800, Swit_0691
GO:0004871	signal transducer activity	1.48	4.40E-04	Swit_3256, Swit_4239, Swit_0268, Swit_0694, Swit_3504, Swit_0267, Swit_3144, Swit_0964, Swit_3048, Swit_3044, Swit_0687, Swit_2885, Swit_4721
GO:0005215	transporter activity	0.97	6.60E-03	Swit_3256, Swit_3793, Swit_3144, Swit_0964, Swit_3048, Swit_0692, Swit_3044, Swit_0687, Swit_0689, Swit_2826, Swit_2885
GO:0009055	electron carrier activity	0.87	6.40E-02	Swit_4614, Swit_3056, Swit_3797, Swit_0693, Swit_5136, Swit_4366, Swit_1800, Swit_0691

Table S4 **Enriched GO terms associated with the response to stress among significantly differentially expressed genes in pair-wise comparisons of RW1 cells after 1 h in soil compared to liquid controls**

Comparison	Expression pattern	Parent	GOID	Term	Genes
DBF-DBF versus control *DBF*	Lower expression in cells in sand with DBF after 1 h contact	Molecular Function	GO:0000156	two-component response regulator activity	Swit_0694, Swit_4239, Swit_0268, Swit_3504, Swit_4721
		Biological Process	GO:0006950	response to stress	Swit_3981, Swit_5282, Swit_3206, Swit_2948, Swit_3911, Swit_5248, Swit_2779, Swit_3979, Swit_3982, Swit_1147
			GO:0033554	cellular response to stress	Swit_3981, Swit_5282, Swit_3206, Swit_3911, Swit_5248, Swit_3979, Swit_3982
			GO:0051716	cellular response to stimulus	Swit_3981, Swit_5282, Swit_3206, Swit_3911, Swit_5248, Swit_3979, Swit_3982
SAL-NOTH versus control *SAL*	Higher expression in cells in sand without DBF after 1 h contact	Molecular Function	GO:0000156	two-component response regulator activity	Swit_2540, Swit_3925, Swit_5012, Swit_0513, Swit_1226, Swit_4432, Swit_1954, Swit_1160, Swit_5270, Swit_5396
		Biological Process	GO:0050896	response to stimulus	Swit_3927, Swit_3981, Swit_5282, Swit_3206, Swit_2948, Swit_3911, Swit_5248, Swit_2779, Swit_3979, Swit_3982, Swit_1147
			GO:0006974	response to DNA damage stimulus	Swit_3981, Swit_5282, Swit_3206, Swit_3911, Swit_3979, Swit_3982

Table S5. Genes commonly differentially expressed in all comparisons to cells in sand compared to liquid

GOID	Ontology	Term	No probes in class	Total No probes in class	Total No probes on array	No probes in comparison	log_odds_ratio	p-value	Genes
GO:0019725	biological process	cellular homeostasis	17	43	3458	901	0.60	8.96E-02	Swit_0016, Swit_0558, Swit_1124, Swit_1821, Swit_2617, Swit_2670, Swit_2722, Swit_2779, Swit_2865, Swit_3162, Swit_3225, Swit_3252, Swit_3587, Swit_3743, Swit_3974, Swit_4488, Swit_5247
GO:0006950	biological process	response to stress	28	79	3458	901	0.44	8.21E-02	Swit_0060, Swit_0266, Swit_0269, Swit_0601, Swit_0619, Swit_0626, Swit_1127, Swit_1212, Swit_1368, Swit_1465, Swit_2462, Swit_2536, Swit_2681, Swit_2779, Swit_2957, Swit_3128, Swit_3232, Swit_3597, Swit_3979, Swit_3982, Swit_4027, Swit_4493, Swit_4690, Swit_4727, Swit_4885, Swit_4948, Swit_5247, Swit_5351
GO:0009306	biological process	protein secretion	16	32	3458	901	0.94	3.52E-03	Swit_0216, Swit_0452, Swit_1144, Swit_1152, Swit_1953, Swit_2151, Swit_2349, Swit_2584, Swit_2608, Swit_3230, Swit_3724, Swit_3843, Swit_4053, Swit_4511, Swit_4651, Swit_4868
GO:0006541	biological process	glutamine metabolic process	6	9	3458	901	1.36	1.32E-02	Swit_0425, Swit_0435, Swit_1238, Swit_2796, Swit_3102, Swit_3227
GO:0009231	biological process	riboflavin biosynthetic process	5	9	3458	901	1.09	6.07E-02	Swit_1004, Swit_1194, Swit_2571, Swit_3785, Swit_3789
GO:0006099	biological process	tricarboxylic acid cycle	5	10	3458	901	0.94	9.56E-02	Swit_1299, Swit_2732, Swit_3212, Swit_3838, Swit_4790
GO:0034311	biological process	diol metabolic process	6	14	3458	901	0.72	6.08E-02	Swit_1782, Swit_4339, Swit_4887, Swit_4890, Swit_5041, Swit_5063
GO:0006313	biological process	transposition, DNA-mediated	12	16	3458	901	1.53	6.59E-05	Swit_4909, Swit_4911, Swit_4930, Swit_5067, Swit_5075, Swit_5080, Swit_5092, Swit_5109, Swit_5112, Swit_5125, Swit_5197, Swit_5398
GO:0003676	molecular function	nucleic acid binding	178	555	3458	901	0.30	7.26E-04	Swit_0045, Swit_0060, Swit_0154, Swit_0172, Swit_0188, Swit_0278, Swit_0314, Swit_0315, Swit_0374, Swit_0402, Swit_0431, Swit_0601, Swit_0625, Swit_0641, Swit_0663, Swit_0694, Swit_0741, Swit_0766, Swit_0774, Swit_0783, Swit_0797, Swit_0810, Swit_0833, Swit_0955, Swit_0974, Swit_0985, Swit_1017, Swit_1069, Swit_1079, Swit_1115, Swit_1121, Swit_1127, Swit_1165, Swit_1167, Swit_1173, Swit_1212, Swit_1326, Swit_1327, Swit_1355, Swit_1356, Swit_1357, Swit_1386, Swit_1464, Swit_1513, Swit_1533, Swit_1540, Swit_1565, Swit_1574, Swit_1578, Swit_1585, Swit_1678, Swit_1725, Swit_1777, Swit_1825, Swit_1880, Swit_1911, Swit_1923, Swit_1954, Swit_1962, Swit_2042, Swit_2047, Swit_2048, Swit_2110, Swit_2184, Swit_2191, Swit_2235, Swit_2244, Swit_2409, Swit_2413, Swit_2425, Swit_2460, Swit_2461, Swit_2462, Swit_2466, Swit_2534, Swit_2536, Swit_2633, Swit_2656, Swit_2681, Swit_2710, Swit_2775, Swit_2814, Swit_2828, Swit_2849, Swit_2901, Swit_2919, Swit_2980, Swit_3029, Swit_3054, Swit_3081, Swit_3085, Swit_3099, Swit_3315, Swit_3332, Swit_3353, Swit_3386, Swit_3411, Swit_3449, Swit_3467, Swit_3468, Swit_3528, Swit_3530, Swit_3537, Swit_3569, Swit_3591, Swit_3597, Swit_3615, Swit_3621, Swit_3622, Swit_3660, Swit_3704, Swit_3752, Swit_3791, Swit_3809, Swit_3810, Swit_3836, Swit_3848, Swit_3896, Swit_3972, Swit_4048, Swit_4054, Swit_4102, Swit_4170, Swit_4177, Swit_4191, Swit_4203, Swit_4205, Swit_4276, Swit_4316, Swit_4339, Swit_4510, Swit_4567, Swit_4626, Swit_4690, Swit_4717, Swit_4718, Swit_4720, Swit_4724, Swit_4727, Swit_4775, Swit_4798, Swit_4803, Swit_4844, Swit_4885, Swit_4908, Swit_4909, Swit_4911, Swit_4930, Swit_4931, Swit_4945, Swit_4948, Swit_4954, Swit_5060, Swit_5067, Swit_5075, Swit_5080, Swit_5092, Swit_5097, Swit_5106, Swit_5109, Swit_5112, Swit_5122, Swit_5125, Swit_5133, Swit_5155, Swit_5169, Swit_5190, Swit_5197, Swit_5204, Swit_5207, Swit_5211, Swit_5215, Swit_5233, Swit_5319, Swit_5325, Swit_5334, Swit_5398, Swit_5399

GO:0008270	molecular function	zinc ion binding	28	84	3458	901	0.36	8.51E-02	Swit_0060, Swit_0188, Swit_0240, Swit_0286, Swit_0627, Swit_1004, Swit_1127, Swit_1156, Swit_1179, Swit_1407, Swit_1779, Swit_1880, Swit_1933, Swit_2004, Swit_2119, Swit_2323, Swit_2841, Swit_3018, Swit_3300, Swit_3363, Swit_3849, Swit_4509, Swit_4633, Swit_4727, Swit_4786, Swit_4885, Swit_5190, Swit_5207
GO:0016639	molecular function	oxidoreductase activity, acting on the CH-NH2 group of donors, NAD or NADP as acceptor	5	10	3458	901	0.94	9.23E-02	Swit_0264, Swit_0657, Swit_0659, Swit_1330, Swit_3986
GO:0008565	molecular function	protein transporter activity	16	38	3458	901	0.69	2.31E-02	Swit_0452, Swit_1144, Swit_1152, Swit_1953, Swit_2151, Swit_2349, Swit_2584, Swit_2586, Swit_2608, Swit_3230, Swit_3724, Swit_3843, Swit_4047, Swit_4053, Swit_4511, Swit_4651
GO:0016616	molecular function	oxidoreductase activity, acting on the CH-OH group of donors, NAD or NADP as acceptor	18	43	3458	901	0.68	1.76E-02	Swit_0611, Swit_0647, Swit_0735, Swit_0736, Swit_1179, Swit_1194, Swit_2395, Swit_2571, Swit_2699, Swit_2830, Swit_2898, Swit_3270, Swit_3432, Swit_4534, Swit_4685, Swit_4731, Swit_4753, Swit_4927
GO:0008473	molecular function	ornithine cyclodeaminase activity	5	7	3458	901	1.45	1.57E-02	Swit_1052, Swit_1566, Swit_1715, Swit_3083, Swit_4262
GO:0005198	molecular function	structural molecule activity	9	19	3458	901	0.86	3.77E-02	Swit_1262, Swit_1270, Swit_1284, Swit_1286, Swit_1327, Swit_1357, Swit_3809, Swit_3869, Swit_3912
GO:0046912	molecular function	transferase activity, transferring acyl groups, acyl groups converted into alkyl on transfer	4	5	3458	901	1.62	1.85E-02	Swit_1299, Swit_3212, Swit_3838, Swit_4790
GO:0003899	molecular function	DNA-directed RNA polymerase activity	5	9	3458	901	1.09	5.84E-02	Swit_1326, Swit_3467, Swit_3468, Swit_3528, Swit_3982
GO:0003746	molecular function	translation elongation factor activity	4	7	3458	901	1.13	8.17E-02	Swit_1355, Swit_1356, Swit_2980, Swit_4844
GO:0016833	molecular function	oxo-acid-lyase activity	6	11	3458	901	1.07	4.22E-02	Swit_1641, Swit_2352, Swit_3227, Swit_3570, Swit_4791, Swit_4923
GO:0050661	molecular function	NADP binding	6	9	3458	901	1.36	1.26E-02	Swit_1707, Swit_2664, Swit_2699, Swit_2830, Swit_4731, Swit_4927
GO:0008199	molecular function	ferric iron binding	6	11	3458	901	1.07	4.22E-02	Swit_1782, Swit_2779, Swit_4890, Swit_5041, Swit_5063, Swit_5247
GO:0018576	molecular function	catechol 1,2-dioxygenase activity	4	5	3458	901	1.62	1.85E-02	Swit_1782, Swit_4890, Swit_5041, Swit_5063
GO:0016597	molecular function	amino acid binding	6	11	3458	901	1.07	9.20E-02	Swit_2394, Swit_3527, Swit_4685, Swit_4688, Swit_4731, Swit_5387
GO:0004359	molecular function	glutaminase activity	4	7	3458	901	1.13	8.17E-02	Swit_2796, Swit_4937, Swit_4955, Swit_5120
GO:0004803	molecular function	transposase activity	12	16	3458	901	1.53	5.98E-05	Swit_4909, Swit_4911, Swit_4930, Swit_5067, Swit_5075, Swit_5080, Swit_5092, Swit_5109, Swit_5112, Swit_5125, Swit_5197, Swit_5398

Table S6 **Enriched GO terms among the significantly differentially expressed genes in the comparison between RW1 cells growing exponentially in soil (*SAND-DBF-EXPO)* versus 1 h contact in soil with Dibenzofuran (*DBF-DBF*).**

Genes lower expressed in 1 h versus exponentially growing cells of RW1 in soil with DBF (Cutoff FC<-2)

GOID	Biological Process	No probes in class	Total No probes in class	Total No probes on array	No probes in comparison	log_odds_ratio	p-value	Genes
GO:0006573	valine metabolic process	4	7	3458	378	2.39	3.85E-03	Swit_5025, Swit_0652, Swit_0647, Swit_0611
GO:0006633	fatty acid biosynthetic process	6	18	3458	378	1.61	9.99E-03	Swit_4769, Swit_3555, Swit_0086, Swit_3556, Swit_4699, Swit_0470
GO:0006778	porphyrin-containing compound metabolic process	5	17	3458	378	1.43	6.65E-02	Swit_1710, Swit_0569, Swit_3877, Swit_4060, Swit_4955
GO:0006534	cysteine metabolic process	2	5	3458	378	1.87	9.66E-02	Swit_4648, Swit_2915
GO:0046677	response to antibiotic	5	8	3458	378	2.52	6.64E-04	Swit_2533, Swit_3846, Swit_4835, Swit_0660, Swit_3389
GO:0006412	translation	18	32	3458	378	2.36	4.95E-08	Swit_1356, Swit_1355, Swit_5127, Swit_3869, Swit_3809, Swit_1377, Swit_4508, Swit_1327, Swit_1092, Swit_1343, Swit_1345, Swit_3845, Swit_1344, Swit_3213, Swit_0434, Swit_1378, Swit_1357, Swit_2347
GO:0009252	peptidoglycan biosynthetic process	3	9	3458	378	1.61	6.72E-02	Swit_3846, Swit_0057, Swit_0660
GO:0006457	protein folding	5	17	3458	378	1.43	3.18E-02	Swit_1716, Swit_4040, Swit_3829, Swit_5351, Swit_3375
GO:0009084	glutamine family amino acid biosynthetic process	4	16	3458	378	1.19	9.04E-02	Swit_0659, Swit_4317, Swit_3896, Swit_2970
GO:0008033	tRNA processing	4	10	3458	378	1.87	1.77E-02	Swit_3615, Swit_0877, Swit_4508, Swit_2387
GO:0006541	glutamine metabolic process	3	9	3458	378	1.61	6.72E-02	Swit_3227, Swit_0435, Swit_2796
GO:0034404	nucleobase-containing small molecule biosynthetic process	13	45	3458	378	1.40	7.51E-04	Swit_4715, Swit_4483, Swit_0623, Swit_0231, Swit_4474, Swit_0030, Swit_4485, Swit_4484, Swit_3131, Swit_0621, Swit_0620, Swit_3970, Swit_0622
GO:0006119	oxidative phosphorylation	11	15	3458	378	2.75	2.22E-08	Swit_4483, Swit_0623, Swit_2996, Swit_4485, Swit_4484, Swit_2991, Swit_0621, Swit_0620, Swit_2995, Swit_0622, Swit_2997
GO:0015986	ATP synthesis coupled proton transport	7	9	3458	378	2.83	5.45E-06	Swit_4483, Swit_0623, Swit_4485, Swit_4484, Swit_0621, Swit_0620, Swit_0622
GO:0006096	glycolysis	3	9	3458	378	1.61	6.72E-02	Swit_0446, Swit_1300, Swit_2606
GOID	**Cellular Component**					**log_odds_ratio**	**p-value**	**Genes**
GO:0044424	intracellular part	58	240	3458	378	1.14	8.07E-03	Swit_2267, Swit_2656, Swit_0569, Swit_1356, Swit_2901, Swit_2664, Swit_1355, Swit_4317, Swit_5127, Swit_3528, Swit_3869, Swit_3809, Swit_4715, Swit_0877, Swit_4483, Swit_0623, Swit_0446, Swit_1271, Swit_3896, Swit_1377, Swit_2831, Swit_2532, Swit_1315, Swit_4508, Swit_1327, Swit_1092, Swit_2387, Swit_0030, Swit_3556, Swit_4485, Swit_4484, Swit_3848, Swit_1343, Swit_2658, Swit_1345, Swit_3845, Swit_1344, Swit_0621, Swit_3352, Swit_2970, Swit_0213, Swit_3213, Swit_0107, Swit_1378, Swit_1357, Swit_3810, Swit_2347, Swit_0620, Swit_3829, Swit_3970, Swit_3212, Swit_4563, Swit_0622, Swit_4699, Swit_0470, Swit_2795, Swit_3373, Swit_3375
GO:0016021	integral to membrane	36	208	3458	378	0.66	9.21E-02	Swit_3590, Swit_3617, Swit_2586, Swit_2146, Swit_0078, Swit_4648, Swit_2533, Swit_1912, Swit_0455, Swit_0423, Swit_2931, Swit_0057, Swit_4483, Swit_3532, Swit_2561, Swit_0440, Swit_0124, Swit_3877, Swit_2875, Swit_4835, Swit_2996, Swit_3374, Swit_4485, Swit_1395, Swit_4484, Swit_4006, Swit_0660, Swit_3389, Swit_2992, Swit_3905, Swit_0119, Swit_1181, Swit_1231, Swit_0120, Swit_2997, Swit_0118
GO:0005618	cell wall	6	15	3458	378	1.87	1.22E-02	Swit_2586, Swit_4648, Swit_3846, Swit_3463, Swit_0057, Swit_4006
GO:0030529	ribonucleoprotein complex	11	12	3458	378	3.07	3.51E-09	Swit_2656, Swit_3869, Swit_3809, Swit_1377, Swit_1315, Swit_1327, Swit_1343, Swit_2658, Swit_1345, Swit_1344, Swit_1357
GO:0045259	proton-transporting ATP synthase complex	7	8	3458	378	3.00	7.15E-06	Swit_4483, Swit_0623, Swit_4485, Swit_4484, Swit_0621, Swit_0620, Swit_0622

Genes lower expressed in 1 h versus exponentially growing cells of RW1 in soil with DBF (Cutoff FC<-2) cont…

GOID	Molecular Function					log_odds_ratio	p-value	Genes
GO:0016620	oxidoreductase activity, acting on the aldehyde or oxo group of donors	5	20	3458	378	1.19	5.74E-02	Swit_5025, Swit_0652, Swit_4306, Swit_2664, Swit_2602
GO:0016836	hydro-lyase activity	9	47	3458	378	0.81	6.17E-02	Swit_1011, Swit_3404, Swit_1968, Swit_0446, Swit_2726, Swit_4700, Swit_4265, Swit_4019, Swit_0470
GO:0004312	fatty acid synthase activity	3	6	3458	378	2.19	1.97E-02	Swit_4769, Swit_0086, Swit_3556
GO:0003697	single-stranded DNA binding	2	5	3458	378	1.87	9.40E-02	Swit_5233, Swit_2452
GO:0003723	RNA binding	17	42	3458	378	1.89	4.75E-03	Swit_2656, Swit_1356, Swit_4048, Swit_1355, Swit_3615, Swit_5127, Swit_3809, Swit_3896, Swit_1377, Swit_5337, Swit_1327, Swit_3848, Swit_1343, Swit_3845, Swit_1344, Swit_1357, Swit_3810
GO:0005525	GTP binding	9	21	3458	378	1.97	1.68E-04	Swit_2656, Swit_1356, Swit_5171, Swit_1355, Swit_3789, Swit_0399, Swit_5127, Swit_1389, Swit_2930
GO:0015078	hydrogen ion transmembrane transporter activity	12	21	3458	378	2.39	3.52E-05	Swit_2533, Swit_0455, Swit_4483, Swit_0623, Swit_4835, Swit_4485, Swit_4484, Swit_0621, Swit_0620, Swit_3389, Swit_1396, Swit_0622
GO:0003924	GTPase activity	3	8	3458	378	1.78	4.68E-02	Swit_1356, Swit_1355, Swit_5127
GO:0051287	NAD binding	7	26	3458	378	1.30	1.76E-02	Swit_0647, Swit_2664, Swit_2985, Swit_1201, Swit_2602, Swit_2988, Swit_0457
GO:0046983	protein dimerization activity	4	13	3458	378	1.49	4.40E-02	Swit_2664, Swit_4317, Swit_1049, Swit_1326
GO:0048038	quinone binding	3	9	3458	378	1.61	6.46E-02	Swit_2985, Swit_2995, Swit_2992
GO:0050136	NADH dehydrogenase (quinone) activity	6	14	3458	378	1.97	2.19E-03	Swit_2985, Swit_2996, Swit_2991, Swit_2988, Swit_2992, Swit_2997
GO:0003899	DNA-directed RNA polymerase activity	3	9	3458	378	1.61	6.46E-02	Swit_3528, Swit_3467, Swit_1326
GO:0003735	structural constituent of ribosome	8	8	3458	378	3.19	1.78E-08	Swit_3869, Swit_3809, Swit_1377, Swit_1327, Swit_1343, Swit_1345, Swit_1344, Swit_1357
GO:0019843	rRNA binding	6	6	3458	378	3.19	1.56E-06	Swit_3809, Swit_1377, Swit_1327, Swit_1343, Swit_1344, Swit_1357
GO:0008408	3'-5' exonuclease activity	3	7	3458	378	1.97	3.17E-02	Swit_0450, Swit_3810, Swit_2795
GO:0004812	aminoacyl-tRNA ligase activity	4	12	3458	378	1.61	3.32E-02	Swit_1092, Swit_3961, Swit_3213, Swit_2347

Genes higher expressed in 1 h versus exponentially growing cells of RW1 in soil with DBF (Cutoff FC >2)

GOID	Biological Process	No probes in class	Total No probes in class	Total No probes on array	No probes in comparison	log_odds_ratio	p-value	Genes
GO:0055114	oxidation-reduction process	76	573	3458	336	0.45	9.58E-03	Swit_2900, Swit_3743, Swit_4365, Swit_2253, Swit_1880, Swit_5055, Swit_5203, Swit_3865, Swit_3797, Swit_4335, Swit_1543, Swit_3324, Swit_5154, Swit_4273, Swit_1657, Swit_2722, Swit_3254, Swit_2270, Swit_1658, Swit_2271, Swit_5200, Swit_5033, Swit_4604, Swit_0744, Swit_3838, Swit_0996, Swit_5315, Swit_3162, Swit_3236, Swit_1474, Swit_0991, Swit_4345, Swit_3730, Swit_3716, Swit_1879, Swit_1550, Swit_3796, Swit_0672, Swit_5136, Swit_3736, Swit_1988, Swit_2635, Swit_1130, Swit_3307, Swit_3974, Swit_3735, Swit_0417, Swit_1045, Swit_1479, Swit_1174, Swit_0269, Swit_4370, Swit_3059, Swit_4924, Swit_3601, Swit_0977, Swit_2108, Swit_4155, Swit_3864, Swit_5135, Swit_3722, Swit_3291, Swit_2867, Swit_3741, Swit_2634, Swit_1472, Swit_3056, Swit_4790, Swit_4731, Swit_3093, Swit_1473, Swit_4897, Swit_3057, Swit_0693, Swit_1330, Swit_0703
GO:0051179	localization	57	426	3458	336	0.46	9.28E-02	Swit_1153, Swit_3230, Swit_3103, Swit_3724, Swit_3229, Swit_3241, Swit_1952, Swit_5203, Swit_3231, Swit_1154, Swit_4348, Swit_3738, Swit_4650, Swit_4350, Swit_4047, Swit_3475, Swit_4512, Swit_3725, Swit_1143, Swit_1450, Swit_5173, Swit_3720, Swit_1152, Swit_3857, Swit_0536, Swit_3737, Swit_3780, Swit_4053, Swit_0540, Swit_2407, Swit_3256, Swit_5297, Swit_2408, Swit_0215, Swit_1953, Swit_3833, Swit_3793, Swit_1631, Swit_1144, Swit_2441, Swit_1284, Swit_2826, Swit_4025, Swit_5129, Swit_1287, Swit_1283, Swit_1722, Swit_0028, Swit_3512, Swit_3044, Swit_0535, Swit_4894, Swit_3048, Swit_0687, Swit_0692, Swit_0689, Swit_3189
GO:0015031	protein transport	10	52	3458	336	0.98	5.38E-02	Swit_3230, Swit_3724, Swit_4047, Swit_3720, Swit_1152, Swit_4053, Swit_0215, Swit_1953, Swit_1144, Swit_2441
GO:0019725	cellular homeostasis	14	43	3458	336	1.74	1.21E-04	Swit_3743, Swit_0016, Swit_3742, Swit_2722, Swit_4364, Swit_3587, Swit_3162, Swit_3732, Swit_3974, Swit_3733, Swit_4025, Swit_3741, Swit_2779, Swit_2670
GO:0019439	aromatic compound catabolic process	11	46	3458	336	1.30	5.09E-03	Swit_1641, Swit_3087, Swit_0744, Swit_4633, Swit_4924, Swit_4923, Swit_0977, Swit_3864, Swit_2634, Swit_3056, Swit_4897
GO:0030416	methylamine metabolic process	3	5	3458	336	2.63	8.87E-03	Swit_3254, Swit_4364, Swit_4363
GO:0006879	cellular iron ion homeostasis	3	10	3458	336	1.63	5.36E-02	Swit_3732, Swit_4025, Swit_2779

Genes higher expressed in 1 h versus exponentially growing cells of RW1 in soil with DBF (Cutoff FC >2) cont…

GOID	Cellular Component					log_odds_ratio	p-value	Genes
GO:0016020	membrane	74	592	3458	336	0.36	1.54E-02	Swit_3698, Swit_1153, Swit_3230, Swit_3103, Swit_3724, Swit_3229, Swit_3241, Swit_1952, Swit_5365, Swit_3231, Swit_1154, Swit_4348, Swit_3738, Swit_4650, Swit_4350, Swit_4047, Swit_2778, Swit_2119, Swit_3475, Swit_4512, Swit_3745, Swit_3725, Swit_1143, Swit_1450, Swit_1955, Swit_5173, Swit_3720, Swit_1152, Swit_4740, Swit_4749, Swit_5200, Swit_3857, Swit_0536, Swit_3737, Swit_3780, Swit_4053, Swit_0540, Swit_2407, Swit_4345, Swit_3256, Swit_3716, Swit_2689, Swit_3190, Swit_5136, Swit_3736, Swit_2408, Swit_0215, Swit_4087, Swit_3516, Swit_3735, Swit_0417, Swit_5385, Swit_1953, Swit_0267, Swit_3833, Swit_3793, Swit_1631, Swit_1144, Swit_2441, Swit_4025, Swit_5129, Swit_2422, Swit_2132, Swit_1722, Swit_0028, Swit_3044, Swit_0535, Swit_4894, Swit_3048, Swit_0687, Swit_0693, Swit_0689, Swit_3973, Swit_3189
GO:0042597	periplasmic space	8	26	3458	336	1.66	3.96E-03	Swit_4365, Swit_4047, Swit_3254, Swit_4363, Swit_5136, Swit_2441, Swit_0693, Swit_0692
GOID	**Molecular Function**					**log_odds_ratio**	**p-value**	**Genes**
GO:0005215	transporter activity	49	366	3458	336	0.46	3.62E-02	Swit_1153, Swit_3230, Swit_3103, Swit_3724, Swit_3241, Swit_1952, Swit_5203, Swit_3231, Swit_1154, Swit_4348, Swit_3738, Swit_4650, Swit_4350, Swit_4047, Swit_3475, Swit_3725, Swit_1143, Swit_1450, Swit_5173, Swit_3720, Swit_1152, Swit_3857, Swit_0536, Swit_3737, Swit_3780, Swit_4053, Swit_0540, Swit_2407, Swit_3256, Swit_2408, Swit_1953, Swit_3833, Swit_3793, Swit_1631, Swit_1144, Swit_2441, Swit_2826, Swit_4025, Swit_5129, Swit_1722, Swit_0028, Swit_3044, Swit_0535, Swit_4894, Swit_3048, Swit_0687, Swit_0692, Swit_0689, Swit_3189
GO:0008484	sulfuric ester hydrolase activity	2	5	3458	336	2.04	7.50E-02	Swit_2518, Swit_3043
GO:0008565	protein transporter activity	8	38	3458	336	1.12	2.43E-02	Swit_3230, Swit_3724, Swit_4047, Swit_1152, Swit_4053, Swit_1953, Swit_1144, Swit_2441
GO:0016209	antioxidant activity	9	27	3458	336	1.78	5.88E-04	Swit_3743, Swit_2722, Swit_3587, Swit_3162, Swit_3730, Swit_3974, Swit_2341, Swit_0269, Swit_3741
GO:0016705	oxidoreductase activity, acting on paired donors, with incorporation o	14	57	3458	336	1.34	4.71E-04	Swit_3743, Swit_2253, Swit_5203, Swit_1543, Swit_4273, Swit_2271, Swit_0744, Swit_3162, Swit_2635, Swit_3974, Swit_3741, Swit_2634, Swit_3056, Swit_4897
GO:0005506	iron ion binding	38	224	3458	336	0.80	2.23E-04	Swit_2253, Swit_5203, Swit_4366, Swit_1543, Swit_3250, Swit_4273, Swit_2450, Swit_2271, Swit_5200, Swit_3255, Swit_4367, Swit_4656, Swit_1798, Swit_0744, Swit_1474, Swit_3730, Swit_4633, Swit_1550, Swit_5297, Swit_0672, Swit_5136, Swit_1988, Swit_3307, Swit_3732, Swit_3974, Swit_2341, Swit_0269, Swit_1800, Swit_0977, Swit_3864, Swit_4025, Swit_2867, Swit_2634, Swit_3056, Swit_1473, Swit_4897, Swit_0691, Swit_2779
GO:0009055	electron carrier activity	41	285	3458	336	0.57	3.87E-03	Swit_2253, Swit_3632, Swit_3742, Swit_3797, Swit_4366, Swit_1543, Swit_3250, Swit_4273, Swit_2722, Swit_0370, Swit_2271, Swit_5200, Swit_3255, Swit_4367, Swit_1798, Swit_0744, Swit_5315, Swit_3236, Swit_3716, Swit_1879, Swit_1550, Swit_0738, Swit_0672, Swit_5136, Swit_4295, Swit_3736, Swit_1988, Swit_3307, Swit_2341, Swit_1479, Swit_1174, Swit_1800, Swit_4155, Swit_4842, Swit_0995, Swit_2634, Swit_3056, Swit_1473, Swit_4897, Swit_0691, Swit_0693
GO:0051537	2 iron, 2 sulfur cluster binding	13	86	3458	336	0.64	6.24E-02	Swit_2253, Swit_1543, Swit_4273, Swit_2271, Swit_0744, Swit_1550, Swit_0672, Swit_1988, Swit_3307, Swit_4778, Swit_2634, Swit_3056, Swit_4897
GO:0016702	oxidoreductase activity, acting on single donors with incorporation of	15	97	3458	336	0.67	5.53E-02	Swit_5203, Swit_3865, Swit_0370, Swit_3418, Swit_2635, Swit_3086, Swit_3059, Swit_0977, Swit_3864, Swit_2867, Swit_2634, Swit_3047, Swit_4897, Swit_3094, Swit_3057
GO:0008289	lipid binding	6	24	3458	336	1.36	8.01E-02	Swit_3231, Swit_1154, Swit_1143, Swit_1038, Swit_2268, Swit_1055
GO:0005381	iron ion transmembrane transporter activity	3	10	3458	336	1.63	6.25E-02	Swit_4047, Swit_2441, Swit_4025
GO:0047437	4-oxalocrotonate decarboxylase activity	5	11	3458	336	2.23	2.20E-03	Swit_1639, Swit_3088, Swit_2111, Swit_0912, Swit_0911
GO:0046912	transferase activity, transferring acyl groups, acyl groups converted int	2	5	3458	336	2.04	7.50E-02	Swit_3838, Swit_4790

Table S7 RW1 genes differentially expressed between exponentially growing cells in liquid versus soil with DBF

yellow/green: possibly same operon

UP in sand DBF at > 4-fold

Systematic Name	FC ([Exp-Liq] vs [Exp Sand])	Annotation
Swit_0036	-4.8386354	ATPase involved in DNA replication initiation
Swit_0038	-11.689444	phosphoesterase, PA-phosphatase related
Swit_0066	-4.667946	CheW protein
Swit_0163	-38.303673	Type IV secretory pathway TrbD component-like protein
Swit_0166	-7.6873193	hypothetical protein Swit_0166
Swit_0181	-4.732045	lytic transglycosylase, catalytic
Swit_0271	-4.93061	ABC transporter related
Swit_0295	-7.768005	short-chain dehydrogenase/reductase SDR
Swit_0307	-5.444603	hypothetical protein Swit_0307
Swit_0347	-6.127131	transketolase, central region
Swit_0351	-19.187805	AMP-dependent synthetase and ligase
Swit_0368	-5.5210967	enoyl-CoA hydratase/isomerase
Swit_0478	-4.541134	TonB-dependent receptor
Swit_0495	-4.8783803	peptidoglycan glycosyltransferase
Swit_0606	-17.541544	hypothetical protein Swit_0606
Swit_0615	-85.320114	Flp/Fap pilin component
Swit_0641	-4.254797	XRE family transcriptional regulator
Swit_0652	-6.170913	methylmalonate-semialdehyde dehydrogenase
Swit_0669	-4.756028	AMP-dependent synthetase and ligase
Swit_0681	-6.2372236	amidohydrolase
Swit_0700	-4.94094	hypothetical protein Swit_0700
Swit_0724	-6.097148	methylenetetrahydromethanopterin reductase
Swit_0735	-4.796942	3-hydroxybutyryl-CoA dehydrogenase
Swit_0769	-4.3480334	short chain dehydrogenase
Swit_0787	-5.508536	ABC transporter related
Swit_0810	-6.101851	AraC family transcriptional regulator
Swit_0868	-6.90476	hypothetical protein Swit_0868
Swit_0927	-8.770806	amidase
Swit_0958	-4.761217	butyryl-CoA:acetate CoA transferase
Swit_0961	-5.3268967	hypothetical protein Swit_0961
Swit_1011	-7.6777744	enoyl-CoA hydratase
Swit_1022	-9.745878	TonB-dependent receptor
Swit_1042	-4.796334	luciferase family protein
Swit_1062	-15.364342	TPR repeat-containing protein
Swit_1071	-4.840094	fumarate lyase
Swit_1127	-4.149456	DNA-O6-methylguanine--protein-cysteine S-methyltransferase / transcriptional regulator Ada
Swit_1179	-5.4898243	S-(hydroxymethyl)glutathione dehydrogenase
Swit_1239	-4.8531384	hypothetical protein Swit_1239
Swit_1273	-8.302759	hypothetical protein Swit_1273
Swit_1364	-6.832562	hypothetical protein Swit_1364
Swit_1412	-55.747093	glutathione-dependent formaldehyde-activating, GFA
Swit_1433	-10.698508	gamma-glutamyltransferase
Swit_1540	-7.6076713	AraC family transcriptional regulator
Swit_1577	-5.2298284	hypothetical protein Swit_1577
Swit_1584	-4.023033	hypothetical protein Swit_1584
Swit_1633	-6.014156	hypothetical protein Swit_1633
Swit_1760	-4.047834	L-carnitine dehydratase/bile acid-inducible protein F
Swit_1777	-4.0201454	LysR family transcriptional regulator
Swit_1782	-8.271563	intradiol ring-cleavage dioxygenase
Swit_1814	-6.5392385	glycosyl transferase, group 1
Swit_1825	-8.125111	TetR family transcriptional regulator
Swit_1832	-14.215885	dehydratase
Swit_1848	-12.442711	glyoxalase/bleomycin resistance protein/dioxygenase
Swit_1895	-7.234636	hypothetical protein Swit_1895

UP in sand DBF at > 4-fold

Systematic Name	FC ([Exp-Liq] vs [Exp Sand])	Annotation
Swit_1915	-19.172344	hypothetical protein Swit_1915
Swit_1936	-5.5593686	porphobilinogen deaminase
Swit_1949	-4.8594704	spermidine synthase-like protein
Swit_1951	-4.2286534	RND efflux system outer membrane lipoprotein
Swit_1981	-8.507374	short-chain dehydrogenase/reductase SDR
Swit_2012	-5.9435263	FAD dependent oxidoreductase
Swit_2021	-5.125875	enoyl-CoA hydratase
Swit_2057	-7.6359954	hypothetical protein Swit_2057
Swit_2063	-5.0650506	alanine dehydrogenase
Swit_2072	-5.796795	coenzyme A transferase
Swit_2081	-5.3934374	hypothetical protein Swit_2081
Swit_2082	-4.3055224	short-chain dehydrogenase/reductase SDR
Swit_2110	-7.772354	transcriptional regulator IclR-like protein
Swit_2146	-8.451497	integral membrane sensor signal transduction histidine kinase
Swit_2241	-6.2726	arsenate resistance ArsH
Swit_2243	-4.694659	arsenate reductase
Swit_2244	-5.463514	ArsR family transcriptional regulator
Swit_2261	-11.9554615	Rieske (2Fe-2S) domain-containing protein
Swit_2267	-28.002712	antibiotic biosynthesis monooxygenase
Swit_2299	-7.2921224	beta-lactamase domain-containing protein
Swit_2357	-10.069049	ATPase involved in chromosome partitioning-like protein
Swit_2421	-5.3399076	peptidase M61 domain-containing protein
Swit_2437	-4.3346467	NAD-dependent epimerase/dehydratase
Swit_2478	-9.521657	hypothetical protein Swit_2478
Swit_2499	-8.438313	endoribonuclease L-PSP
Swit_2574	-6.587383	hypothetical protein Swit_2574
Swit_2586	-9.214667	general secretion pathway L
Swit_2656	-4.115893	signal recognition particle subunit FFH/SRP54 (srp54)
Swit_2783	-12.901178	hypothetical protein Swit_2783
Swit_2877	-5.822791	methyltransferase type 12
Swit_2900	-5.9301543	methionine-R-sulfoxide reductase
Swit_2916	-6.182212	SufBD protein
Swit_2950	-4.3347387	TonB-dependent receptor, plug
Swit_2965	-4.2591553	hypothetical protein Swit_2965
Swit_3017	-23.59648	aldehyde dehydrogenase
Swit_3029	-5.8602915	CRP/FNR family transcriptional regulator
Swit_3062	-5.918544	phthalate 4,5-dioxygenase
Swit_3089	-12.919083	Pseudo
Swit_3130	-4.4779286	Pseudo
Swit_3172	-4.0113726	hypothetical protein Swit_3172
Swit_3183	-4.6345816	oxidoreductase domain-containing protein
Swit_3285	-10.988785	hypothetical protein Swit_3285
Swit_3288	-8.598541	short-chain dehydrogenase/reductase SDR
Swit_3302	-5.9381294	hypothetical protein Swit_3302
Swit_3400	-9.917874	L-carnitine dehydratase/bile acid-inducible protein F
Swit_3425	-4.9236736	outer membrane-like protein
Swit_3445	-4.4065304	hypothetical protein Swit_3445
Swit_3496	-6.676804	hypothetical protein Swit_3496
Swit_3523	-9.575452	3-hydroxyanthranilate 3,4-dioxygenase
Swit_3590	-10.277944	hypothetical protein Swit_3590
Swit_3617	-9.273397	putative inner membrane protein translocase component YidC
Swit_3622	-4.445875	LuxR family transcriptional regulator
Swit_3648	-8.480915	5-oxoprolinase (ATP-hydrolyzing)
Swit_3659	-4.291931	rhodanese domain-containing protein
Swit_3684	-10.761387	hypothetical protein Swit_3684
Swit_3764	-8.893735	hypothetical protein Swit_3764
Swit_3814	-4.4848156	thymidine kinase
Swit_3836	-5.0506153	ECF subfamily RNA polymerase sigma-24 factor

UP in sand DBF at > 4-fold

Systematic Name	FC ([Exp-Liq] vs [Exp Sand])	Annotation
Swit_3849	-4.4233694	peptidase M48, Ste24p
Swit_3858	-13.159579	alpha/beta hydrolase fold
Swit_3899	-4.914494	hypothetical protein Swit_3899
Swit_3938	-10.620576	hypothetical protein Swit_3938
Swit_4024	-5.187571	Sel1 domain-containing protein
Swit_4026	-7.7904744	Pseudo
Swit_4072	-4.4222193	hypothetical protein Swit_4072
Swit_4121	-6.63462	AMP-dependent synthetase and ligase
Swit_4153	-6.9443045	hypothetical protein Swit_4153
Swit_4262	-5.9999676	ornithine cyclodeaminase
Swit_4339	-3.9844935	beta-ketoadipate pathway transcription regulator
Swit_4351	-17.915419	short-chain dehydrogenase/reductase SDR
Swit_4431	-9.795642	NUDIX hydrolase
Swit_4455	-4.3131523	hypothetical protein Swit_4455
Swit_4459	-3.9546757	hypothetical protein Swit_4459
Swit_4519	-9.5228195	methyltransferase-like protein
Swit_4539	-14.540476	NAD-dependent epimerase/dehydratase
Swit_4551	-15.315985	hypothetical protein Swit_4551
Swit_4646	-7.407447	hypothetical protein Swit_4646
Swit_4764	-5.1995463	2-octaprenylphenol hydroxylase
Swit_4769	-4.488214	enoyl-(acyl carrier protein) reductase
Swit_4821	-5.08162	glycosyl transferase, group 1
Swit_4844	-5.2477384	GreA/GreB family elongation factor
Swit_4874	-4.1949735	hypothetical protein Swit_4874
Swit_5084	-8.712486	hypothetical protein Swit_5084
Swit_5121	-4.1395664	hypothetical protein Swit_5121
Swit_5145	-4.17934	hypothetical protein Swit_5145
Swit_5195	-15.674349	hypothetical protein Swit_5195
Swit_5233	-4.1427736	single-strand binding protein/primosomal replication protein n
Swit_5241	-6.394356	hypothetical protein Swit_5241

FC, fold-change
yellow/green: possibly same operon

yellow/green: possibly same operon

DOWN in sand DBF at >4-fold

Systematic Name	FC ([Exp-Liq] vs [Exp-Sand])	Annotation
Swit_0045	10.403069	histone family protein DNA-binding protein
Swit_0064	4.1585107	hypothetical protein Swit_0064
Swit_0127	4.9677434	hypothetical protein Swit_0127
Swit_0131	5.5963516	hypothetical protein Swit_0131
Swit_0143	4.70186	polysaccharide biosynthesis protein
Swit_0228	5.98996	acetolactate synthase
Swit_0521	4.836504	transglutaminase domain-containing protein
Swit_0601	5.239132	Holliday junction resolvase YqgF
Swit_0604	4.5178595	sporulation domain-containing protein
Swit_0610	5.6434255	acetolactate synthase 3 regulatory subunit
Swit_0687	5.9779234	TonB-dependent receptor
Swit_0689	13.849567	hypothetical protein Swit_0689
Swit_0690	7.0995464	YVTN beta-propeller repeat-containing protein
Swit_0691	5.8036237	hypothetical protein Swit_0691
Swit_0692	8.121806	extracellular solute-binding protein
Swit_0693	7.689245	Pyrrolo-quinoline quinone
Swit_0694	6.762993	two component LuxR family transcriptional regulator
Swit_0703	53.616516	aldehyde dehydrogenase
Swit_0739	4.8510723	hypothetical protein Swit_0739
Swit_0763	9.80466	aromatic amino acid aminotransferase
Swit_0822	4.924671	acyl-CoA dehydrogenase domain-containing protein
Swit_0823	4.0758023	endoribonuclease L-PSP
Swit_0893	4.7163286	Rieske (2Fe-2S) domain-containing protein
Swit_1014	4.0935755	enoyl-CoA hydratase/isomerase
Swit_1167	4.852177	AraC family transcriptional regulator
Swit_1236	5.4008656	beta-lactamase superfamily hydrolase
Swit_1270	4.9699655	flagellar basal-body rod protein FlgC
Swit_1286	12.573785	flagellar hook-basal body complex subunit FliE
Swit_1330	9.42759	glutamate dehydrogenase
Swit_1386	4.2630053	two component transcriptional regulator
Swit_1424	4.0694075	hypothetical protein Swit_1424
Swit_1787	4.3909373	endoribonuclease L-PSP
Swit_1893	4.410123	xylose isomerase domain-containing protein
Swit_2117	4.594805	hypothetical protein Swit_2117
Swit_2224	4.1861377	amidohydrolase
Swit_2526	4.612262	signal-transduction protein
Swit_2652	4.4769683	polysaccharide biosynthesis protein
Swit_2670	6.574093	redoxin domain-containing protein
Swit_2679	8.080796	hypothetical protein Swit_2679
Swit_2767/3(	8.827517	#N/A
Swit_2779	6.0052757	Ferritin, Dps family protein
Swit_2866	7.621762	glyoxalase/bleomycin resistance protein/dioxygenase
Swit_3043	4.700832	sulfatase
Swit_3047	4.0390897	phytanoyl-CoA dioxygenase
Swit_3048	5.8888106	TonB-dependent receptor
Swit_3056	4.1062746	Rieske (2Fe-2S) domain-containing protein
Swit_3057	6.6626315	aromatic-ring-hydroxylating dioxygenase, beta subunit
Swit_3058	4.157337	maleylacetoacetate isomerase
Swit_3081	4.1953964	GntR family transcriptional regulator
Swit_3085	4.843411	LysR family transcriptional regulator
Swit_3189	98.945946	TonB-dependent receptor, plug
Swit_3217	4.5863795	molybdopterin molybdochelatase
Swit_3433	5.0580893	hypothetical protein Swit_3433
Swit_3434	6.7508035	amidohydrolase

DOWN in sand DBF at >4-fold

Systematic Name	FC ([Exp-Liq] vs [Exp-Sand])	Annotation
Swit_3457	4.322173	glutathione S-transferase domain-containing protein
Swit_3478	4.3085275	cytochrome B561
Swit_3553	4.0705137	hypothetical protein Swit_3553
Swit_3799	5.6950955	hypothetical protein Swit_3799
Swit_3898	5.350966	hypothetical protein Swit_3898
Swit_3972	12.686013	ECF subfamily RNA polymerase sigma-24 factor
Swit_3973	32.171535	putative transmembrane anti-sigma factor
Swit_4385	3.9751613	hypothetical protein Swit_4385
Swit_4409	4.417549	peptidase M24
Swit_4427	4.6691957	hypothetical protein Swit_4427
Swit_4442	3.9813128	hypothetical protein Swit_4442
Swit_4567	5.3739815	AsnC family transcriptional regulator
Swit_4571	4.0563116	acetyltransferase-like protein
Swit_4731	4.570815	homoserine dehydrogenase
Swit_4786	12.61382	5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase
Swit_4788	7.5884223	methylitaconate delta2-delta3-isomerase
Swit_4789	23.156599	hypothetical protein Swit_4789
Swit_4790	4.4239907	methylcitrate synthase
Swit_4791	4.839826	2,3-dimethylmalate lyase
Swit_4889	6.8112283	3-oxoacid CoA-transferase, A subunit
Swit_4890	4.500789	hydroxyquinol 1,2-dioxygenase
Swit_4894	4.2440476	TonB-dependent receptor
Swit_4896	4.1108036	aromatic-ring-hydroxylating dioxygenase, beta subunit
Swit_4897	5.1642995	ring hydroxylating dioxygenase, alpha subunit
Swit_4937	4.6614227	cobyrinic acid a,c-diamide synthase
Swit_4961	7.186831	hypothetical protein Swit_4961
Swit_4963	4.417996	hypothetical protein Swit_4963
Swit_4989	5.3063545	hypothetical protein Swit_4989
Swit_5045	4.4676003	TonB-dependent receptor
Swit_5053	6.3709903	X-Pro dipeptidyl-peptidase domain-containing protein
Swit_5090	7.1183624	Pseudo
Swit_5100	15.539823	Pseudo
Swit_5103	4.710963	Pseudo
Swit_5207	6.081135	MucR family transcriptional regulator
Swit_5247	4.9390807	Ferritin, Dps family protein
Swit_5289	5.367324	hypothetical protein Swit_5289

FC, fold-change
yellow/green: possibly same operon

Table S8 **Enriched GO terms among the significantly differentially expressed genes in the comparison between RW1 cells growing exponentially in soil versus cells growing exponentially in batch suspension DBF.**

Genes lower expressed in exponentially growing cells of RW1 in liquid than in soil with DBF (Cutoff FC<-2)

GOID	Biological Process	No probes in class	Total No probes in class	Total No probes on array	No probes in comparison	log_odds_ratio	p-values	Genes
GO:0006098	pentose-phosphate shunt	2	8	3458	229	1.92	9.07E-02	Swit_0647, Swit_3270
GO:0006573	valine metabolic process	2	7	3458	229	2.11	7.10E-02	Swit_0647, Swit_0652
GO:0016226	iron-sulfur cluster assembly	2	5	3458	229	2.59	3.68E-02	Swit_2916, Swit_3912
GOID	**Cellular component**							
GO:0005618	cell wall	4	15	3458	229	2.01	7.76E-03	Swit_0057, Swit_2586, Swit_3463, Swit_4648
GOID	**Molecular function**							
GO:0003746	translation elongation factor activity	3	7	3458	229	2.69	8.67E-03	Swit_1355, Swit_1356, Swit_4844
GO:0003924	GTPase activity	2	8	3458	229	1.92	9.70E-02	Swit_1355, Swit_1356
GO:0005525	GTP binding	4	21	3458	229	1.52	4.87E-02	Swit_1355, Swit_1356, Swit_2656, Swit_3789
GO:0016841	ammonia-lyase activity	3	13	3458	229	1.80	5.24E-02	Swit_2394, Swit_4262, Swit_4632
GO:0003684	damaged DNA binding	2	6	3458	229	2.33	5.68E-02	Swit_3597, Swit_4885

Genes higher expressed in exponentially growing cells of RW1 in liquid than in soil with DBF (Cutoff FC<-2)

GOID	Biological Process	No probes in class	Total No probes in class	Total No probes on array	No probes in comparison	log_odds_ratio	p-values	Genes
GO:0006950	response to stress	9	79	3458	202	0.96	1.09E-02	Swit_2957, Swit_2779, Swit_0269, Swit_4027, Swit_0619, Swit_0266, Swit_5247, Swit_5351, Swit_0601
GO:0031326	regulation of cellular biosynthetic process	30	416	3458	202	0.30	5.59E-02	Swit_2947, Swit_4803, Swit_1678, Swit_1386, Swit_0135, Swit_0402, Swit_3085, Swit_4720, Swit_1167, Swit_1017, Swit_0188, Swit_2413, Swit_3081, Swit_3504, Swit_4567, Swit_5122, Swit_1585, Swit_0315, Swit_1477, Swit_1165, Swit_5207, Swit_4276, Swit_4177, Swit_0694, Swit_0797, Swit_0067, Swit_5319, Swit_3411, Swit_1533, Swit_3972
GO:0006779	porphyrin-containing compound biosynthetic process	3	17	3458	202	1.60	7.23E-02	Swit_4937, Swit_1398, Swit_4955
GO:0001539	ciliary or flagellar motility	3	17	3458	202	1.60	7.23E-02	Swit_1270, Swit_1286, Swit_1284
GO:0019439	aromatic compound catabolic process	6	46	3458	202	1.16	2.74E-02	Swit_4924, Swit_1538, Swit_4923, Swit_4887, Swit_3056, Swit_4897
GO:0009086	methionine biosynthetic process	2	9	3458	202	1.93	9.27E-02	Swit_4786, Swit_4731
GO:0006313	transposition, DNA-mediated	5	16	3458	202	2.42	1.63E-03	Swit_5197, Swit_4909, Swit_5075, Swit_4911, Swit_5125
GOID	**Cellular component**							
GO:0009288	bacterial-type flagellum	3	19	3458	202	1.43	5.16E-02	Swit_1270, Swit_1286, Swit_1284

Genes higher expressed in exponentially growing cells of RW1 in liquid than in soil with DBF (Cutoff FC<-2)

GOID	Molecular function	No probes in class	Total No probes in class	Total No probes on array	No probes in comparison	log_odds_ratio	p-values	Genes
GO:0008199	ferric iron binding	3	11	3458	202	2.22	2.37E-02	Swit_2779, Swit_5247, Swit_4890
GO:0000156	phosphorelay response regulator activity	10	99	3458	202	0.79	6.59E-02	Swit_2947, Swit_1386, Swit_0135, Swit_0402, Swit_4720, Swit_3504, Swit_0694, Swit_0067, Swit_5319, Swit_3411
GO:0003677	DNA binding	38	489	3458	202	0.41	4.17E-02	Swit_4803, Swit_1678, Swit_1386, Swit_0402, Swit_3085, Swit_4720, Swit_1167, Swit_1017, Swit_0188, Swit_2413, Swit_3081, Swit_4567, Swit_5325, Swit_5122, Swit_1585, Swit_0601, Swit_0315, Swit_0172, Swit_5197, Swit_1165, Swit_5207, Swit_4909, Swit_5075, Swit_5215, Swit_4276, Swit_4177, Swit_0694, Swit_0797, Swit_4954, Swit_5334, Swit_5319, Swit_3411, Swit_4911, Swit_5125, Swit_1533, Swit_4908, Swit_3972, Swit_0045
GO:0004359	glutaminase activity	3	7	3458	202	2.88	6.00E-03	Swit_4937, Swit_2796, Swit_4955
GO:0042242	cobyrinic acid a,c-diamide synthase activity	2	6	3458	202	2.51	4.47E-02	Swit_4937, Swit_4955
GO:0005198	structural molecule activity	3	19	3458	202	1.43	9.86E-02	Swit_1270, Swit_1286, Swit_1284
GO:0050661	NADP binding	2	9	3458	202	1.93	9.55E-02	Swit_2830, Swit_4731
GO:0004803	transposase activity	5	16	3458	202	2.42	1.77E-03	Swit_5197, Swit_4909, Swit_5075, Swit_4911, Swit_5125
GO:0008410	CoA-transferase activity	3	14	3458	202	1.88	4.59E-02	Swit_5021, Swit_4888, Swit_4889

Table S9 **Enriched GO terms among the significantly differentially expressed genes in the comparison between RW1 cells growing exponentially in soil (*SAND-EXPO-DBF)* versus stationary phase in soil with dibenzofuran (*SAND-STAT-DBF*).**

Genes lower expressed in exponentially growing cells of RW1 in soil with DBF

Cutoff <-2

GOID	Biological Process	No probes in class	Total No probes in class	Total No probes on array	No probes in comparison	log_odds_ratio	p-value	Genes
GO:0006537	glutamate biosynthetic process	2	6	3458	263	2.13	7.38E-02	Swit_0659, Swit_0657
GO:0009306	protein secretion	5	32	3458	263	1.04	9.89E-02	Swit_3230, Swit_3724, Swit_1152, Swit_4053, Swit_1144
GO:0007165	signal transduction	18	153	3458	263	0.63	4.80E-02	Swit_5027, Swit_3822, Swit_2974, Swit_3181, Swit_0400, Swit_3444, Swit_4568, Swit_5012, Swit_4721, Swit_3516, Swit_2781, Swit_0135, Swit_0394, Swit_5296, Swit_0073, Swit_0175, Swit_3504, Swit_0694
GO:0019725	cellular homeostasis	10	43	3458	263	1.61	7.87E-03	Swit_3743, Swit_1124, Swit_0016, Swit_3742, Swit_2722, Swit_3139, Swit_4488, Swit_3741, Swit_5247, Swit_2779
GO:0010468	regulation of gene expression	45	423	3458	263	0.48	1.38E-02	Swit_5337, Swit_1957, Swit_2701, Swit_0151, Swit_4344, Swit_3537, Swit_5018, Swit_1553, Swit_1962, Swit_2613, Swit_1891, Swit_0374, Swit_5163, Swit_2974, Swit_3181, Swit_4203, Swit_4191, Swit_1123, Swit_3444, Swit_3569, Swit_2042, Swit_5204, Swit_5066, Swit_5012, Swit_3740, Swit_4721, Swit_4078, Swit_2781, Swit_0135, Swit_0891, Swit_5122, Swit_0394, Swit_0830, Swit_1678, Swit_5296, Swit_3258, Swit_4803, Swit_0073, Swit_0654, Swit_0175, Swit_4177, Swit_3504, Swit_3085, Swit_1167, Swit_0694
GO:0080090	regulation of primary metabolic process	46	420	3458	263	0.53	6.40E-02	Swit_5337, Swit_1957, Swit_2701, Swit_0151, Swit_4344, Swit_3537, Swit_5018, Swit_1553, Swit_1962, Swit_2613, Swit_1891, Swit_0374, Swit_5163, Swit_2974, Swit_3181, Swit_4203, Swit_0400, Swit_4191, Swit_1123, Swit_3444, Swit_3569, Swit_2042, Swit_5204, Swit_5066, Swit_5012, Swit_3740, Swit_4721, Swit_4078, Swit_2781, Swit_0135, Swit_0891, Swit_5122, Swit_0394, Swit_0830, Swit_1678, Swit_5296, Swit_3258, Swit_4803, Swit_0073, Swit_0654, Swit_0175, Swit_4177, Swit_3504, Swit_3085, Swit_1167, Swit_0694
GO:0006310	DNA recombination	7	43	3458	263	1.10	4.60E-02	Swit_5092, Swit_3752, Swit_5398, Swit_2460, Swit_5066, Swit_5122, Swit_4911
GO:0015074	DNA integration	4	19	3458	263	1.47	5.52E-02	Swit_3752, Swit_5097, Swit_5071, Swit_2460
GO:0006879	cellular iron ion homeostasis	3	10	3458	263	1.98	3.73E-02	Swit_3139, Swit_5247, Swit_2779
GO:0006979	response to oxidative stress	2	7	3458	263	1.91	9.81E-02	Swit_4077, Swit_0269
GOID	**Molecular function**							
GO:0009055	electron carrier activity	28	285	3458	263	0.37	6.76E-02	Swit_0659, Swit_1124, Swit_5313, Swit_3265, Swit_1830, Swit_3409, Swit_0359, Swit_3632, Swit_3742, Swit_1543, Swit_1193, Swit_2722, Swit_4233, Swit_0890, Swit_5200, Swit_1801, Swit_1067, Swit_5315, Swit_1552, Swit_1527, Swit_3716, Swit_1550, Swit_0672, Swit_3736, Swit_1988, Swit_2341, Swit_1800, Swit_0995
GO:0008565	protein transporter activity	6	38	3458	263	1.05	5.74E-02	Swit_3230, Swit_3724, Swit_3748, Swit_1152, Swit_4053, Swit_1144
GO:0004601	peroxidase activity	6	15	3458	263	2.39	4.35E-04	Swit_3743, Swit_1193, Swit_2341, Swit_4077, Swit_0269, Swit_3741
GO:0015035	protein disulfide oxidoreductase activity	2	7	3458	263	1.91	8.92E-02	Swit_1124, Swit_3742
GO:0005506	iron ion binding	29	224	3458	263	0.77	1.65E-03	Swit_5313, Swit_3907, Swit_3265, Swit_3409, Swit_0359, Swit_1557, Swit_4198, Swit_1543, Swit_1193, Swit_3139, Swit_0890, Swit_5200, Swit_1801, Swit_4598, Swit_4656, Swit_1067, Swit_1552, Swit_1527, Swit_1550, Swit_5297, Swit_0672, Swit_3407, Swit_1988, Swit_2341, Swit_0269, Swit_1800, Swit_1538, Swit_5247, Swit_2779
GO:0051537	2 iron, 2 sulfur cluster binding	10	86	3458	263	0.61	9.95E-02	Swit_5313, Swit_3265, Swit_3409, Swit_1543, Swit_0890, Swit_1067, Swit_1552, Swit_1550, Swit_0672, Swit_1988

Genes lower expressed in exponentially growing cells of RW1 in soil with DBF cont…

GOID	Molecular function							
GO:0016769	transferase activity, transferring nitrogenous group	7	33	3458	263	1.48	8.94E-03	Swit_4079, Swit_4172, Swit_4192, Swit_4344, Swit_0864, Swit_5326, Swit_3900
GO:0003677	DNA binding	56	489	3458	263	0.59	4.11E-04	Swit_2828, Swit_5337, Swit_0192, Swit_1957, Swit_0156, Swit_3981, Swit_2425, Swit_5092, Swit_2701, Swit_3752, Swit_0151, Swit_4344, Swit_3537, Swit_5018, Swit_1553, Swit_5398, Swit_1962, Swit_5097, Swit_2613, Swit_1891, Swit_0374, Swit_5163, Swit_3181, Swit_4203, Swit_5071, Swit_3472, Swit_4191, Swit_1123, Swit_3444, Swit_3569, Swit_2409, Swit_2184, Swit_2042, Swit_2460, Swit_5204, Swit_5066, Swit_5012, Swit_3740, Swit_2198, Swit_4078, Swit_0891, Swit_5122, Swit_0394, Swit_0830, Swit_1678, Swit_3258, Swit_4803, Swit_5215, Swit_0654, Swit_0175, Swit_0172, Swit_4177, Swit_4911, Swit_3085, Swit_1167, Swit_0694
GO:0003700	sequence-specific DNA binding transcription factor	30	291	3458	263	0.44	3.45E-02	Swit_1957, Swit_2701, Swit_0151, Swit_4344, Swit_3537, Swit_5018, Swit_1553, Swit_1962, Swit_2613, Swit_1891, Swit_0374, Swit_5163, Swit_3181, Swit_4203, Swit_1123, Swit_3569, Swit_2042, Swit_5204, Swit_5012, Swit_3740, Swit_4078, Swit_0891, Swit_0394, Swit_0830, Swit_1678, Swit_3258, Swit_0175, Swit_4177, Swit_3085, Swit_0694
GO:0000156	phosphorelay response regulator activity	13	99	3458	263	0.79	2.86E-02	Swit_2974, Swit_3181, Swit_3444, Swit_5012, Swit_4721, Swit_2781, Swit_0135, Swit_0394, Swit_5296, Swit_0073, Swit_0175, Swit_3504, Swit_0694

Genes higher expressed in exponentially growing cells of RW1 in soil with DBF

Cutoff >2

GOID	Biological Process	No probes in class	Total No probes in class	Total No probes on array	No probes in comparison	log_odds_ratio	p-value	Genes
GO:0006573	valine metabolic process	3	7	3458	369	2.01	3.19E-02	Swit_5025, Swit_0652, Swit_0647
GO:0046677	response to antibiotic	3	8	3458	369	1.81	4.70E-02	Swit_2533, Swit_3846, Swit_0660
GO:0006412	translation	16	32	3458	369	2.23	3.04E-08	Swit_1356, Swit_1355, Swit_3809, Swit_1377, Swit_4508, Swit_1327, Swit_1092, Swit_3801, Swit_1343, Swit_1345, Swit_3845, Swit_1344, Swit_3213, Swit_1378, Swit_1357, Swit_2347
GO:0019439	aromatic compound catabolic process	10	46	3458	369	1.03	3.76E-02	Swit_4632, Swit_3046, Swit_4270, Swit_3298, Swit_1641, Swit_3810, Swit_4887, Swit_4902, Swit_4923, Swit_4897
GO:0009252	peptidoglycan biosynthetic process	3	9	3458	369	1.64	6.49E-02	Swit_3846, Swit_0057, Swit_0660
GO:0034404	nucleobase-containing small molecule biosynthetic	10	45	3458	369	1.06	1.97E-02	Swit_4715, Swit_4483, Swit_0623, Swit_0231, Swit_4474, Swit_4485, Swit_4484, Swit_0621, Swit_2485, Swit_0622
GO:0006119	oxidative phosphorylation	10	15	3458	369	2.64	3.63E-07	Swit_4483, Swit_0623, Swit_2996, Swit_4485, Swit_4484, Swit_2991, Swit_0621, Swit_2995, Swit_0622, Swit_2997
GO:0015986	ATP synthesis coupled proton transport	6	9	3458	369	2.64	9.91E-05	Swit_4483, Swit_0623, Swit_4485, Swit_4484, Swit_0621, Swit_0622
GO:0006096	glycolysis	4	9	3458	369	2.06	1.10E-02	Swit_0446, Swit_1300, Swit_3121, Swit_2606
GO:0001539	ciliary or flagellar motility	5	17	3458	369	1.46	3.02E-02	Swit_1271, Swit_1261, Swit_1268, Swit_1293, Swit_1283
GO:0009060	aerobic respiration	4	14	3458	369	1.42	5.67E-02	Swit_1299, Swit_3212, Swit_1300, Swit_3876
GOID	**Cellular Component**							
GO:0044424	intracellular part	52	240	3458	369	1.02	1.75E-02	Swit_2267, Swit_2656, Swit_0569, Swit_1356, Swit_4632, Swit_2901, Swit_1355, Swit_4317, Swit_3809, Swit_4715, Swit_0877, Swit_4483, Swit_0623, Swit_0446, Swit_1271, Swit_1377, Swit_2831, Swit_2532, Swit_1407, Swit_1315, Swit_4508, Swit_1327, Swit_1092, Swit_4505, Swit_4485, Swit_3801, Swit_4484, Swit_1343, Swit_2658, Swit_1345, Swit_3845, Swit_1344, Swit_0621, Swit_1261, Swit_3352, Swit_2970, Swit_0213, Swit_3213, Swit_1296, Swit_1378, Swit_1357, Swit_3810, Swit_2347, Swit_3212, Swit_0622, Swit_1268, Swit_0470, Swit_1293, Swit_3373, Swit_0403, Swit_3375, Swit_1283
GO:0030529	ribonucleoprotein complex	10	12	3458	369	2.97	3.66E-08	Swit_2656, Swit_3809, Swit_1377, Swit_1315, Swit_1327, Swit_1343, Swit_2658, Swit_1345, Swit_1344, Swit_1357
GO:0045259	proton-transporting ATP synthase complex	6	8	3458	369	2.81	7.84E-05	Swit_4483, Swit_0623, Swit_4485, Swit_4484, Swit_0621, Swit_0622

Genes higher expressed in exponentially growing cells of RW1 in soil with DBF cont…

GOID	Molecular function	No probes in class	Total No probes in class	Total No probes on array	No probes in comparison	log_odds_ratio	p-value	Genes
GO:0016620	oxidoreductase activity, acting on the aldehyde or c	5	20	3458	369	1.23	5.57E-02	Swit_5025, Swit_0652, Swit_4306, Swit_2602, Swit_3067
GO:0018576	catechol 1,2-dioxygenase activity	2	5	3458	369	1.91	9.26E-02	Swit_1782, Swit_4890
GO:0016671	oxidoreductase activity, acting on a sulfur group of	2	5	3458	369	1.91	9.26E-02	Swit_2900, Swit_4056
GO:0008135	translation factor activity, nucleic acid binding	5	11	3458	369	2.09	3.06E-02	Swit_4844, Swit_1356, Swit_1355, Swit_3801, Swit_3845
GO:0005525	GTP binding	9	21	3458	369	2.01	1.57E-04	Swit_2656, Swit_1356, Swit_1355, Swit_3789, Swit_0399, Swit_3801, Swit_1389, Swit_2930, Swit_3342
GO:0015078	hydrogen ion transmembrane transporter activity	9	21	3458	369	2.01	2.70E-04	Swit_2533, Swit_0455, Swit_4483, Swit_0623, Swit_4485, Swit_4484, Swit_0621, Swit_0622, Swit_3876
GO:0003924	GTPase activity	4	8	3458	369	2.23	6.47E-03	Swit_1356, Swit_1355, Swit_3801, Swit_3342
GO:0051287	NAD binding	7	26	3458	369	1.34	1.69E-02	Swit_0647, Swit_2985, Swit_1201, Swit_2602, Swit_2988, Swit_2986, Swit_0087
GO:0003954	NADH dehydrogenase activity	11	16	3458	369	2.69	5.09E-08	Swit_2985, Swit_2996, Swit_2991, Swit_2984, Swit_2988, Swit_2986, Swit_2992, Swit_2983, Swit_2994, Swit_2993, Swit_2997
GO:0048038	quinone binding	4	9	3458	369	2.06	1.07E-02	Swit_2985, Swit_2995, Swit_2992, Swit_2983
GO:0005198	structural molecule activity	11	19	3458	369	2.44	6.52E-07	Swit_3809, Swit_1271, Swit_1377, Swit_1327, Swit_1343, Swit_1345, Swit_1344, Swit_1261, Swit_1357, Swit_1268, Swit_1283
GO:0019843	rRNA binding	6	6	3458	369	3.23	1.49E-06	Swit_3809, Swit_1377, Swit_1327, Swit_1343, Swit_1344, Swit_1357
GO:0051539	4 iron, 4 sulfur cluster binding	7	31	3458	369	1.08	4.22E-02	Swit_4715, Swit_2991, Swit_2988, Swit_4707, Swit_2983, Swit_4058, Swit_2993
GO:0000287	magnesium ion binding	6	28	3458	369	1.01	7.25E-02	Swit_0446, Swit_2532, Swit_4505, Swit_3121, Swit_3373, Swit_5035
GO:0004812	aminoacyl-tRNA ligase activity	4	12	3458	369	1.64	3.23E-02	Swit_1092, Swit_3961, Swit_3213, Swit_2347
GO:0046912	transferase activity, transferring acyl groups, acyl gr	2	5	3458	369	1.91	9.26E-02	Swit_1299, Swit_3212

Table S10 **RW1 genes differentially expressed between exponentially growing and stationary phase cells in soil with DBF**

yellow/green: possibly same operon

Cutoff <-4 **Higher in stationary phase**

Systematic Name	FC ([Exp-Sand] vs [Stat-Sand])	Log FC ([Exp-Sand] vs [Stat-Sand])	Annotation
Swit_0016	-6.7872143	-2.7628195	redoxin domain-containing protein
Swit_0147	-5.9794755	-2.580019	hypothetical protein Swit_0147
Swit_0156	-4.7309237	-2.242122	XRE family transcriptional regulator
Swit_0387	-5.5566516	-2.4742157	hypothetical protein Swit_0387
Swit_0393	-4.5005956	-2.170116	hypothetical protein Swit_0393
Swit_0394	-4.3575497	-2.123517	regulatory protein, LuxR
Swit_0545	-7.8014164	-2.963736	hypothetical protein Swit_0545
Swit_0654	-5.727758	-2.5179706	CRP/FNR family transcriptional regulator
Swit_0658	-6.5298314	-2.7070458	hypothetical protein Swit_0658
Swit_0862	-10.745027	-3.4255972	phasin family protein
Swit_0900	-6.522447	-2.7054133	hypothetical protein Swit_0900
Swit_1055	-5.6205206	-2.4907038	biotin/lipoyl attachment domain-containing protein
Swit_1056	-7.1907573	-2.8461437	transketolase domain-containing protein
Swit_1106	-8.239007	-3.0424705	hypothetical protein Swit_1106
Swit_1107	-7.003087	-2.807991	hypothetical protein Swit_1107
Swit_1123	-4.8650303	-2.2824488	MerR family transcriptional regulator
Swit_1124	-12.162883	-3.6044133	glutaredoxin
Swit_1144	-4.309206	-2.107422	secretion protein HlyD family protein
Swit_1152	-26.37638	-4.7211747	RND family efflux transporter MFP subunit
Swit_1153	-6.6112523	-2.7249236	hydrophobe/amphiphile efflux-1 (HAE1) family protein
Swit_1167	-4.429437	-2.1471233	AraC family transcriptional regulator
Swit_1406	-4.580464	-2.1954937	hypothetical protein Swit_1406
Swit_1451	-5.9967756	-2.584187	hypothetical protein Swit_1451
Swit_1538	-6.9907503	-2.8054473	glyoxalase/bleomycin resistance protein/dioxygenase
Swit_1644	-4.35266	-2.1218975	methionine aminopeptidase, type I
Swit_1654	-7.507402	-2.9083138	NAD-dependent aldehyde dehydrogenase-like protein
Swit_1658	-4.1989408	-2.0700254	short-chain dehydrogenase/reductase SDR
Swit_1664	-4.1664166	-2.0588071	glutathione S-transferase domain-containing protein
Swit_2042	-4.193267	-2.0680747	AraC family transcriptional regulator
Swit_2183	-6.393087	-2.6765127	plasmid maintenance system killer
Swit_2184	-7.1040626	-2.8286443	XRE family plasmid maintenance system antidote protein
Swit_2198	-4.116188	-2.0413089	single-strand binding protein
Swit_2353	-4.030326	-2.0108964	hypothetical protein Swit_2353
Swit_2422	-5.057081	-2.338305	transglycosylase-associated protein
Swit_2631	-4.369768	-2.1275568	PilT domain-containing protein
Swit_2779	-7.062576	-2.8201945	Ferritin, Dps family protein
Swit_2934	-4.073956	-2.0264304	membrane-like protein
Swit_3087	-7.8278365	-2.9686136	2,4-dihydroxyhept-2-ene-1,7-dioic acid aldolase
Swit_3093	-6.27742	-2.6501718	hypothetical protein Swit_3093
Swit_3095	-4.453643	-2.154986	hypothetical protein Swit_3095
Swit_3139	-7.9549127	-2.991846	bacterioferritin
Swit_3140	-4.4003444	-2.1376164	hypothetical protein Swit_3140
Swit_3193	-4.7688584	-2.253644	hypothetical protein Swit_3193
Swit_3232	-43.3802	-5.438965	OsmC family protein
Swit_3451	-7.057581	-2.8191738	NUDIX hydrolase
Swit_3471	-5.9452963	-2.5717487	hypothetical protein Swit_3471
Swit_3537	-4.1460342	-2.051732	CarD family transcriptional regulator
Swit_3569	-4.0735707	-2.026294	TetR family transcriptional regulator
Swit_3716	-4.435799	-2.149194	cytochrome B561
Swit_3721	-4.8129487	-2.266921	hypothetical protein Swit_3721
Swit_3722	-13.77086	-3.7835467	FAD-dependent pyridine nucleotide-disulphide oxidoreductase
Swit_3723	-18.33254	-4.196335	rhodanese domain-containing protein
Swit_3729	-9.819743	-3.2956853	hypothetical protein Swit_3729
Swit_3748	-5.1535106	-2.3655555	TonB family protein
Swit_3752	-7.270856	-2.8621252	phage integrase family protein

Cutoff <-4	**Higher in stationary phase**		
Systematic Name	**FC ([Exp-Sand] vs [Stat-Sand])**	**Log FC ([Exp-Sand] vs [Stat-Sand])**	**Annotation**
Swit_3778	-6.5564923	-2.7129242	hypothetical protein Swit_3778
Swit_3904	-23.996286	-4.584739	hypothetical protein Swit_3904
Swit_3907	-6.41995	-2.682562	fatty acid hydroxylase
Swit_4193	-4.928275	-2.3010828	hypothetical protein Swit_4193
Swit_4198	-4.2777596	-2.0968554	formate dehydrogenase
Swit_4345	-5.424257	-2.4394255	thiosulfate reductase cytochrome B subunit (membrane anchoring protein)-like protein
Swit_4374	-5.3948483	-2.4315825	hypothetical protein Swit_4374
Swit_4409	-5.22581	-2.3856547	peptidase M24
Swit_4413	-5.4151464	-2.4370003	type IV pilus assembly PilZ
Swit_4490	-4.4571176	-2.156111	hypothetical protein Swit_4490
Swit_4532	-11.457161	-3.5181777	sugar transferase
Swit_4591	-7.5901856	-2.9241352	hypothetical protein Swit_4591
Swit_4749	-6.7512155	-2.7551472	transglycosylase-associated protein
Swit_4803	-4.270658	-2.0944583	helix-turn-helix domain-containing protein
Swit_4811	-25.199505	-4.6553235	hypothetical protein Swit_4811
Swit_4814	-4.526603	-2.178429	hypothetical protein Swit_4814
Swit_4872	-11.35862	-3.5057156	hypothetical protein Swit_4872
Swit_4876	-9.663258	-3.2725096	hypothetical protein Swit_4876
Swit_4933	-5.9526944	-2.5735428	Pseudo
Swit_4939	-4.3916636	-2.1347675	hypothetical protein Swit_4939
Swit_4940	-7.0357823	-2.8147109	hypothetical protein Swit_4940
Swit_4942	-8.110729	-3.0198317	hypothetical protein Swit_4942
Swit_4958/4983/5139	-4.269898	-2.0942016	transposase
Swit_4987	-4.3891525	-2.1339424	Pseudo
Swit_5003	-4.104256	-2.0371208	P-type conjugative transfer protein VirB9
Swit_5065	-5.9679866	-2.5772443	coenzyme A transferase
Swit_5082	-5.5900016	-2.4828486	Pseudo
Swit_5083	-5.5762887	-2.4793053	hypothetical protein Swit_5083
Swit_5100	-7.8242865	-2.9679592	Pseudo
Swit_5114	-4.1659274	-2.0586376	CopG/Arc/MetJ family transcriptional regulator
Swit_5119	-4.272878	-2.0952082	hypothetical protein Swit_5119
Swit_5146	-4.2391677	-2.083781	hypothetical protein Swit_5146
Swit_5147	-4.6281776	-2.2104442	hypothetical protein Swit_5147
Swit_5158	-4.4590683	-2.1567423	hypothetical protein Swit_5158
Swit_5163	-4.260209	-2.0909243	hypothetical protein Swit_5163
Swit_5165	-5.3578606	-2.421657	hypothetical protein Swit_5165
Swit_5178	-6.681952	-2.7402697	hypothetical protein Swit_5178
Swit_5180	-6.5092626	-2.7024941	hypothetical protein Swit_5180
Swit_5181	-4.0744934	-2.0266206	PilT domain-containing protein
Swit_5182	-4.590638	-2.1986947	prevent-host-death family protein
Swit_5221	-5.549043	-2.472239	hypothetical protein Swit_5221
Swit_5230	-4.184179	-2.0649445	hypothetical protein Swit_5230
Swit_5258	-4.0869994	-2.031042	nuclease
Swit_5259	-8.432809	-3.0760133	hypothetical protein Swit_5259
Swit_5260	-8.40942	-3.0720062	hypothetical protein Swit_5260
Swit_5267	-4.9784455	-2.3156953	hypothetical protein Swit_5267
Swit_5275	-5.058586	-2.3387341	hypothetical protein Swit_5275
Swit_5295	-6.464352	-2.6925058	alternative oxidase
Swit_5296	-7.133331	-2.834576	response regulator receiver protein
Swit_5297	-7.9433627	-2.98975	sec-independent protein translocase protein TatC
Swit_5298	-4.695533	-2.231289	hypothetical protein Swit_5298
Swit_5318	-16.429695	-4.0382338	hypothetical protein Swit_5318
Swit_5322	-4.173932	-2.061407	hypothetical protein Swit_5322
Swit_5340	-6.525011	-2.7059803	hypothetical protein Swit_5340
Swit_5358	-9.215555	-3.204071	hypothetical protein Swit_5358
Swit_5365	-4.417413	-2.1432018	Type IV conjugative transfer system protein TraL
Swit_5380	-4.151328	-2.053573	hypothetical protein Swit_5380
Swit_5395	-4.482681	-2.1643617	hypothetical protein Swit_5395

FC, fold-change
yellow/green: possibly same operon

Cutoff >4 UP	Higher in exponential phase		
Systematic Name	FC ([Exp-Sand] vs [Stat-Sand])	Log FC ([Exp-Sand] vs [Stat-Sand])	Annotation
Swit_0036	6.7719073	2.7595623	ATPase involved in DNA replication initiation
Swit_0038	7.924328	2.9862885	phosphoesterase, PA-phosphatase related
Swit_0052	4.824411	2.2703528	aminotransferase, class V
Swit_0057	5.8481016	2.5479684	monofunctional biosynthetic peptidoglycan transglycosylase
Swit_0083	4.0649605	2.0232413	50S ribosomal protein L9
Swit_0085	8.361208	3.0637114	Pseudo
Swit_0119	4.9395194	2.3043706	biopolymer transport-like protein
Swit_0163	4.3452163	2.119428	Type IV secretory pathway TrbD component-like protein
Swit_0231	4.036732	2.013188	putative adenylate/guanylate cyclase
Swit_0399	4.011537	2.0041552	hypothetical protein Swit_0399
Swit_0438	5.737318	2.5203764	carbamoyl phosphate synthase large subunit
Swit_0446	4.7175775	2.2380462	enolase
Swit_0460	4.0135665	2.0048847	30S ribosomal protein S2
Swit_0461	4.623019	2.2088354	elongation factor Ts
Swit_0469	4.465296	2.1587558	outer membrane chaperone Skp (OmpH)
Swit_0470	7.2017198	2.8483415	3-hydroxyacyl-[acyl-carrier-protein] dehydratase
Swit_0478	4.389057	2.1339111	TonB-dependent receptor
Swit_0606	31.140755	4.960732	hypothetical protein Swit_0606
Swit_0616	10.8919935	3.4451962	Flp/Fap pilin component
Swit_0621	4.7790413	2.2567213	F0F1 ATP synthase subunit alpha
Swit_0622	4.0987153	2.0351717	F0F1 ATP synthase subunit gamma
Swit_0623	4.980598	2.316319	F0F1 ATP synthase subunit beta
Swit_0700	5.3715076	2.425327	hypothetical protein Swit_0700
Swit_0771	6.195942	2.6313236	molecular chaperone-like protein
Swit_0810	5.899607	2.5606189	AraC family transcriptional regulator
Swit_0951	5.5191445	2.4644446	LysR family transcriptional regulator
Swit_0958	4.5929246	2.199413	butyryl-CoA:acetate CoA transferase
Swit_0961	7.3226166	2.8723593	hypothetical protein Swit_0961
Swit_0967	4.0149884	2.005396	hypothetical protein Swit_0967
Swit_0969	4.457652	2.156284	Rieske (2Fe-2S) domain-containing protein
Swit_0981	4.519821	2.1762657	TonB-dependent receptor
Swit_1011	5.6530976	2.4990416	enoyl-CoA hydratase
Swit_1022	6.6774044	2.7392874	TonB-dependent receptor
Swit_1029	22.530104	4.493782	hypothetical protein Swit_1029
Swit_1042	4.6249757	2.2094457	luciferase family protein
Swit_1062	17.706741	4.146227	TPR repeat-containing protein
Swit_1179	7.8755636	2.9773831	S-(hydroxymethyl)glutathione dehydrogenase
Swit_1271	4.0960245	2.0342243	flagellar basal-body rod protein FlgB
Swit_1273	4.844291	2.2762856	hypothetical protein Swit_1273
Swit_1333	4.0331182	2.0118957	preprotein translocase subunit SecY
Swit_1335	10.136058	3.3414247	50S ribosomal protein L30
Swit_1336	5.5102377	2.4621146	30S ribosomal protein S5
Swit_1337	6.984809	2.8042207	50S ribosomal protein L18
Swit_1338	4.5379186	2.1820307	50S ribosomal protein L6
Swit_1339	4.5376363	2.181941	30S ribosomal protein S8
Swit_1340	5.495285	2.4581943	30S ribosomal protein S14
Swit_1341	7.159755	2.8399103	50S ribosomal protein L5
Swit_1342	6.747905	2.7544396	50S ribosomal protein L24
Swit_1343	8.451873	3.079271	50S ribosomal protein MRPL14P
Swit_1344	6.337196	2.6638446	SSU ribosomal protein S17P
Swit_1345	4.960181	2.3103929	50S ribosomal protein L29
Swit_1346	6.438099	2.6866348	50S ribosomal protein L16
Swit_1347	4.465127	2.1587012	30S ribosomal protein S3
Swit_1349	15.777176	3.979767	SSU ribosomal protein S19P
Swit_1350	4.707545	2.2349749	50S ribosomal protein L2
Swit_1351	4.09537	2.0339937	50S ribosomal protein L23
Swit_1352	5.214205	2.3824472	50S ribosomal protein L4
Swit_1354	5.999171	2.584763	30S ribosomal protein S10
Swit_1355	7.4673095	2.9005885	elongation factor Tu
Swit_1356	4.8992286	2.2925546	elongation factor G
Swit_1364	8.32243	3.0570047	hypothetical protein Swit_1364
Swit_1377	4.331482	2.1148608	50S ribosomal protein L25/general stress protein Ctc

Cutoff >4 UP	Higher in exponential phase		
Systematic Name	FC ([Exp-Sand] vs [Stat-Sand])	Log FC ([Exp-Sand] vs [Stat-Sand])	Annotation
Swit_1412	62.203354	5.9589205	glutathione-dependent formaldehyde-activating, GFA
Swit_1572	4.001479	2.0005333	alpha/beta hydrolase fold
Swit_1576	6.622859	2.7274542	hypothetical protein Swit_1576
Swit_1599	5.5506415	2.4726546	AMP-dependent synthetase and ligase
Swit_1725	4.8252378	2.2706	LysR family transcriptional regulator
Swit_1805	8.669051	3.115874	hypothetical protein Swit_1805
Swit_1825	17.0632	4.0928164	TetR family transcriptional regulator
Swit_1895	7.5918	2.924442	hypothetical protein Swit_1895
Swit_1915	12.732026	3.6703901	hypothetical protein Swit_1915
Swit_1936	5.095637	2.3492625	porphobilinogen deaminase
Swit_1981	7.770958	2.9580925	short-chain dehydrogenase/reductase SDR
Swit_2035	7.560161	2.918417	5-oxoprolinase (ATP-hydrolyzing)
Swit_2063	4.9001856	2.2928364	alanine dehydrogenase
Swit_2110	4.2675085	2.093394	transcriptional regulator IclR-like protein
Swit_2125	4.33511	2.1160686	hypothetical protein Swit_2125
Swit_2146	11.957952	3.5798984	integral membrane sensor signal transduction histidine kinase
Swit_2188	4.312468	2.1085138	hypothetical protein Swit_2188
Swit_2267	54.98104	5.7808623	antibiotic biosynthesis monooxygenase
Swit_2299	10.155673	3.344214	beta-lactamase domain-containing protein
Swit_2357	11.767903	3.5567853	ATPase involved in chromosome partitioning-like protein
Swit_2437	5.1649704	2.36876	NAD-dependent epimerase/dehydratase
Swit_2499	16.807219	4.071009	endoribonuclease L-PSP
Swit_2563	5.9616137	2.575703	50S ribosomal protein L11
Swit_2564	5.13176	2.3594537	50S ribosomal protein L1
Swit_2593	4.2892365	2.100721	hypothetical protein Swit_2593
Swit_2656	4.0467234	2.0167542	signal recognition particle subunit FFH/SRP54 (srp54)
Swit_2658	7.5229635	2.9113011	16S rRNA processing protein RimM
Swit_2783	18.566711	4.2146463	hypothetical protein Swit_2783
Swit_2916	6.9924364	2.8057952	SufBD protein
Swit_2985	7.196692	2.847334	NADH dehydrogenase subunit D
Swit_2991	6.0963116	2.6079366	NADH dehydrogenase subunit G
Swit_2992	4.297712	2.1035688	NADH dehydrogenase (quinone)
Swit_2996	7.036409	2.8148394	NADH dehydrogenase subunit L
Swit_2997	4.388895	2.1338577	proton-translocating NADH-quinone oxidoreductase, chain M
Swit_3006	4.2619534	2.0915148	histidinol-phosphate aminotransferase
Swit_3008	26.206738	4.711866	hypothetical protein Swit_3008
Swit_3016	13.861044	3.792964	hypothetical protein Swit_3016
Swit_3043	5.7158713	2.5149734	sulfatase
Swit_3044	8.781525	3.1344714	TonB-dependent receptor
Swit_3046	39.142025	5.2906466	glyoxalase/bleomycin resistance protein/dioxygenase
Swit_3047	4.6056266	2.2033975	phytanoyl-CoA dioxygenase
Swit_3048	6.3627715	2.6696553	TonB-dependent receptor
Swit_3060	4.8562355	2.2798383	fumarylacetoacetate (FAA) hydrolase
Swit_3062	9.314376	3.219459	phthalate 4,5-dioxygenase
Swit_3068	5.0892563	2.3474548	short-chain dehydrogenase/reductase SDR
Swit_3089	17.783846	4.1524954	Pseudo
Swit_3183	5.4018445	2.4334521	oxidoreductase domain-containing protein
Swit_3191	4.6504536	2.2173715	filamentous haemagglutinin outer membrane protein
Swit_3215	8.056135	3.010088	GCN5-related N-acetyltransferase
Swit_3249	4.860659	2.281152	hypothetical protein Swit_3249
Swit_3288	5.6300254	2.4931414	short-chain dehydrogenase/reductase SDR
Swit_3315	4.4040036	2.1388156	AraC family transcriptional regulator
Swit_3317	10.599509	3.4059255	acyl-CoA dehydrogenase domain-containing protein
Swit_3341	7.4245887	2.892311	sulfate adenylyltransferase subunit 2
Swit_3373	5.9719696	2.5782068	S-adenosylmethionine synthetase
Swit_3375	10.963038	3.4545758	chaperonin Cpn10
Swit_3376	9.905525	3.3082335	chaperonin GroEL
Swit_3404	4.065094	2.0232887	3-alpha,7-alpha,12-alpha-trihydroxy-5-beta-cholest-24-enoyl-CoAhydratase
Swit_3476	10.976459	3.4563408	50S ribosomal protein L7/L12
Swit_3477	10.648162	3.4125326	50S ribosomal protein L10
Swit_3555	5.0369263	2.3325436	putative glycerol-3-phosphate acyltransferase PlsX
Swit_3590	16.564734	4.050043	hypothetical protein Swit_3590
Swit_3617	6.557142	2.713067	putative inner membrane protein translocase component YidC

Cutoff >4 UP	Higher in exponential phase		
Systematic Name	FC ([Exp-Sand] vs [Stat-Sand])	Log FC ([Exp-Sand] vs [Stat-Sand])	Annotation
Swit_3622	5.021236	2.3280425	LuxR family transcriptional regulator
Swit_3630	5.0802774	2.3449073	acetyl-CoA acetyltransferase
Swit_3764	5.1776085	2.3722858	hypothetical protein Swit_3764
Swit_3810	4.689833	2.2295365	polynucleotide phosphorylase/polyadenylase
Swit_3814	6.0333776	2.5929658	thymidine kinase
Swit_3821	4.2904353	2.101124	hypothetical protein Swit_3821
Swit_3846	5.57855	2.47989	1A family penicillin-binding protein
Swit_3849	4.44059	2.1507514	peptidase M48, Ste24p
Swit_3862	4.9290395	2.3013065	radical SAM domain-containing protein
Swit_3938	5.49132	2.457153	hypothetical protein Swit_3938
Swit_4026	13.821833	3.788877	Pseudo
Swit_4034	6.3002095	2.6553998	hypothetical protein Swit_4034
Swit_4121	5.0503325	2.3363783	AMP-dependent synthetase and ligase
Swit_4153	5.358246	2.4217608	hypothetical protein Swit_4153
Swit_4306	4.7916074	2.2605097	succinate semialdehyde dehydrogenase
Swit_4319	5.284563	2.4017842	peptidase S10, serine carboxypeptidase
Swit_4351	13.378327	3.7418258	short-chain dehydrogenase/reductase SDR
Swit_4431	4.2159944	2.075873	NUDIX hydrolase
Swit_4483	5.077667	2.3441658	F0F1 ATP synthase subunit A
Swit_4484	5.3021593	2.40658	H+-transporting two-sector ATPase, C subunit
Swit_4485	7.9505086	2.9910471	H+-transporting two-sector ATPase, B/B' subunit
Swit_4519	6.9943223	2.8061843	methyltransferase-like protein
Swit_4539	6.472176	2.6942508	NAD-dependent epimerase/dehydratase
Swit_4551	12.1316	3.600698	hypothetical protein Swit_4551
Swit_4594	6.841445	2.774301	30S ribosomal protein S9
Swit_4595	8.574289	3.100017	50S ribosomal protein L13
Swit_4605	4.013074	2.0047078	hypothetical protein Swit_4605
Swit_4709	5.922111	2.5661116	3-isopropylmalate dehydratase, small subunit
Swit_4750	4.938686	2.3041272	hypothetical protein Swit_4750
Swit_4821	6.0381503	2.5941067	glycosyl transferase, group 1
Swit_4825	5.0748014	2.3433514	hypothetical protein Swit_4825
Swit_4888	8.97126	3.1653106	butyryl-CoA:acetate CoA transferase
Swit_4889	5.9749527	2.5789273	3-oxoacid CoA-transferase, A subunit
Swit_4890	6.271271	2.648758	hydroxyquinol 1,2-dioxygenase
Swit_4891	4.868268	2.2834086	iron-containing alcohol dehydrogenase
Swit_4892	6.950316	2.7970786	hypothetical protein Swit_4892
Swit_4893	13.268302	3.7299118	ferredoxin
Swit_4894	15.147681	3.921025	TonB-dependent receptor
Swit_4895	33.970055	5.0861917	alpha/beta hydrolase fold
Swit_4896	18.478382	4.2077665	aromatic-ring-hydroxylating dioxygenase, beta subunit
Swit_4897	6.4719653	2.6942039	ring hydroxylating dioxygenase, alpha subunit
Swit_4921	7.8670497	2.9758227	3-keto-5-aminohexanoate cleavage enzyme
Swit_4922	4.765633	2.252668	pyruvate, phosphate dikinase
Swit_5033	4.8041453	2.2642798	short-chain dehydrogenase/reductase SDR
Swit_5084	5.618176	2.4901018	hypothetical protein Swit_5084
Swit_5129	6.548825	2.711236	TonB-dependent receptor
Swit_5134	4.8101225	2.2660737	AMP-binding domain protein
Swit_5135	4.674995	2.2249649	aldehyde dehydrogenase
Swit_5168	7.552459	2.9169464	hypothetical protein Swit_5168
Swit_5193	6.9691606	2.8009849	hypothetical protein Swit_5193

FC, fold-change

yellow/green: possibly same operon

Table S11 **Enriched GO terms among the significantly differentially expressed genes in the comparison between stationary phase RW1 cells in soil (*SAND-STAT-DBF*) versus cells growing in batch suspension with crystals of Dibenzofuran.**

Genes higher expressed in stationary phase cells of RW1 in soil with DBF (Cutoff FC >2)

GOID	Biological Process	No probes in class	Total No probes in class	Total No probes on array	No probes in comparison	log_odds_ratio	p-value	Genes
GO:0015031	protein transport	14	52	3458	167	2.48	5.09E-07	Swit_1152, Swit_3724, Swit_2586, Swit_4511, Swit_1096, Swit_4053, Swit_3230, Swit_2656, Swit_3720, Swit_1953, Swit_4651, Swit_2151, Swit_2541, Swit_3843
GO:0019725	cellular homeostasis	6	43	3458	167	1.53	1.92E-02	Swit_1124, Swit_0016, Swit_3587, Swit_2722, Swit_2779, Swit_3743
GO:0006537	glutamate biosynthetic process	2	6	3458	167	2.79	3.45E-02	Swit_0659, Swit_0657
GO:0006950	response to stress	8	79	3458	167	1.07	2.11E-02	Swit_2957, Swit_0060, Swit_3232, Swit_2779, Swit_0269, Swit_3597, Swit_1127, Swit_3128
GO:0030163	protein catabolic process	2	5	3458	167	3.05	9.98E-02	Swit_4509, Swit_4376
GO:0006099	tricarboxylic acid cycle	2	10	3458	167	2.05	9.04E-02	Swit_3212, Swit_2732
GO:0009231	riboflavin biosynthetic process	2	9	3458	167	2.20	7.48E-02	Swit_1004, Swit_3785
GOID	**Cellular Component**							
GO:0030529	ribonucleoprotein complex	3	12	3458	167	2.37	3.64E-02	Swit_2656, Swit_3809, Swit_1327
GOID	**Molecular Function**							
GO:0008565	protein transporter activity	10	38	3458	167	2.45	8.01E-06	Swit_1152, Swit_3724, Swit_2586, Swit_4511, Swit_4053, Swit_3230, Swit_1953, Swit_4651, Swit_2151, Swit_3843
GO:0016209	antioxidant activity	6	27	3458	167	2.20	1.51E-03	Swit_3586, Swit_3587, Swit_2722, Swit_0269, Swit_3743, Swit_3164
GO:0016668	oxidoreductase activity, acting on a sulfur group of donors, NAD	2	7	3458	167	2.56	4.19E-02	Swit_3586, Swit_2722
GO:0032549	ribonucleoside binding	19	250	3458	167	0.65	4.86E-02	Swit_4724, Swit_4509, Swit_0249, Swit_0252, Swit_4845, Swit_1121, Swit_2656, Swit_2906, Swit_1355, Swit_4859, Swit_4416, Swit_1004, Swit_2917, Swit_0262, Swit_3468, Swit_0102, Swit_0425, Swit_3128, Swit_1513
GO:0008270	zinc ion binding	9	84	3458	167	1.15	1.93E-02	Swit_0060, Swit_4509, Swit_2004, Swit_1156, Swit_0286, Swit_1004, Swit_2119, Swit_1179, Swit_1127
GO:0016597	amino acid binding	2	11	3458	167	1.91	9.67E-02	Swit_4685, Swit_3527
GO:0051287	NAD binding	4	26	3458	167	1.67	3.47E-02	Swit_4685, Swit_2664, Swit_4534, Swit_0095
GO:0008237	metallopeptidase activity	5	43	3458	167	1.27	5.51E-02	Swit_4509, Swit_0514, Swit_1156, Swit_2119, Swit_0917
GO:0008289	lipid binding	4	24	3458	167	1.79	1.35E-03	Swit_3231, Swit_1951, Swit_1143, Swit_1154
GO:0005515	protein binding	7	67	3458	167	1.11	4.19E-02	Swit_2664, Swit_2656, Swit_2421, Swit_2918, Swit_2732, Swit_1326, Swit_3128
GO:0050661	NADP binding	2	9	3458	167	2.20	6.74E-02	Swit_2664, Swit_1707
GO:0005525	GTP binding	5	21	3458	167	2.30	2.74E-03	Swit_0252, Swit_2656, Swit_1355, Swit_1004, Swit_0262
GO:0030234	enzyme regulator activity	2	6	3458	167	2.79	3.09E-02	Swit_4832, Swit_4528
GO:0003723	RNA binding	7	42	3458	167	1.79	7.75E-03	Swit_2656, Swit_3809, Swit_1355, Swit_1327, Swit_4717, Swit_3810, Swit_1513
GO:0003735	structural constituent of ribosome	2	8	3458	167	2.37	5.41E-02	Swit_3809, Swit_1327
GO:0003924	GTPase activity	2	8	3458	167	2.37	5.41E-02	Swit_1355, Swit_0262
GO:0003899	DNA-directed RNA polymerase activity	3	9	3458	167	2.79	7.60E-03	Swit_1326, Swit_3468, Swit_3528

Genes lower expressed in stationary phase cells of RW1 in soil with DBF (cutoff FC <-2)

GOID	Biological Process	No probes in class	Total No probes in class	Total No probes on array	No probes in comparison	log_odds_ratio	p-value	Genes
GO:0006725	cellular aromatic compound metabolic process	51	684	3458	161	0.68	3.55E-02	Swit_4909, Swit_5075, Swit_2536, Swit_0082, Swit_4276, Swit_4931, Swit_4177, Swit_4955, Swit_0694, Swit_2460, Swit_3407, Swit_4948, Swit_5106, Swit_4753, Swit_5276, Swit_5155, Swit_4923, Swit_5211, Swit_5092, Swit_2466, Swit_5063, Swit_3054, Swit_2047, Swit_5319, Swit_3411, Swit_4930, Swit_5204, Swit_4911, Swit_1212, Swit_4175, Swit_0833, Swit_5112, Swit_3217, Swit_5097, Swit_5041, Swit_5125, Swit_5398, Swit_1533, Swit_4887, Swit_3056, Swit_5080, Swit_5067, Swit_3057, Swit_0115, Swit_4908, Swit_3058, Swit_5133, Swit_4890, Swit_3972, Swit_4897, Swit_4896
GO:0006313	transposition, DNA-mediated	10	16	3458	161	3.75	2.36E-10	Swit_4909, Swit_5075, Swit_5092, Swit_4930, Swit_4911, Swit_5112, Swit_5125, Swit_5398, Swit_5080, Swit_5067
GO:0001539	ciliary or flagellar motility	3	17	3458	161	1.92	4.27E-02	Swit_1286, Swit_1284, Swit_1293
GO:0015074	DNA integration	6	19	3458	161	2.76	1.58E-04	Swit_2460, Swit_5106, Swit_5211, Swit_4930, Swit_5097, Swit_4908
GO:0034311	diol metabolic process	4	14	3458	161	2.62	3.05E-03	Swit_5063, Swit_5041, Swit_4887, Swit_4890
GOID	**Cellular Component**							
GO:0044461	bacterial-type flagellum part	3	13	3458	161	2.31	1.10E-02	Swit_1286, Swit_1284, Swit_1293
GO:0030288	outer membrane-bounded periplasmic space	3	19	3458	161	1.76	3.20E-02	Swit_0692, Swit_0693, Swit_5136
GOID	**Molecular Function**							
GO:0003677	DNA binding	40	489	3458	161	0.81	2.08E-04	Swit_5207, Swit_4909, Swit_5075, Swit_5215, Swit_2536, Swit_4276, Swit_4931, Swit_4177, Swit_1574, Swit_0694, Swit_2460, Swit_4948, Swit_5106, Swit_5155, Swit_0797, Swit_4954, Swit_5211, Swit_5092, Swit_2466, Swit_3054, Swit_5334, Swit_2047, Swit_5319, Swit_3411, Swit_4930, Swit_5204, Swit_4911, Swit_1212, Swit_0833, Swit_5112, Swit_5097, Swit_5125, Swit_5398, Swit_1533, Swit_5080, Swit_5067, Swit_4908, Swit_5133, Swit_3972, Swit_0045
GO:0004803	transposase activity	10	16	3458	161	3.75	2.67E-10	Swit_4909, Swit_5075, Swit_5092, Swit_4930, Swit_4911, Swit_5112, Swit_5125, Swit_5398, Swit_5080, Swit_5067
GO:0051213	dioxygenase activity	12	105	3458	161	1.30	4.09E-02	Swit_3407, Swit_5063, Swit_2771, Swit_4175, Swit_3059, Swit_5041, Swit_3056, Swit_3057, Swit_4890, Swit_4897, Swit_4896, Swit_3047
GO:0003887	DNA-directed DNA polymerase activity	2	11	3458	161	1.97	9.25E-02	Swit_4948, Swit_5276
GO:0008199	ferric iron binding	3	11	3458	161	2.55	1.30E-02	Swit_5063, Swit_5041, Swit_4890
GO:0018576	catechol 1,2-dioxygenase activity	3	5	3458	161	3.69	9.70E-04	Swit_5063, Swit_5041, Swit_4890
GO:0050661	NADP binding	2	9	3458	161	2.25	6.44E-02	Swit_2699, Swit_4927
GO:0008410	CoA-transferase activity	3	14	3458	161	2.20	2.58E-02	Swit_5021, Swit_4888, Swit_4889

Table S12 RW1 genes differentially expressed between stationary phase cells in liquid versus soil with DBF

yellow/green: possibly same operon

Cutoff <-4 lower in liquid

Systematic Name	FC ([Stat-Liq] vs [Stat-Sand])	Log FC ([Stat-Liq] vs [Stat-Sand])	Annotation
Swit_0016	-8.003047	-3.0005493	redoxin domain-containing protein
Swit_0060	-9.304142	-3.217873	RNA polymerase factor sigma-32
Swit_0084	-6.3145795	-2.6586866	30S ribosomal protein S18
Swit_0251	-6.0025325	-2.5855713	RNA-binding protein Hfq
Swit_0278	-4.2218103	-2.0778618	GntR family transcriptional regulator
Swit_0347	-7.0953565	-2.8268752	transketolase, central region
Swit_0630	-6.0518994	-2.597388	polyhydroxyalkonate synthesis repressor, PhaR
Swit_0657	-6.05948	-2.599194	glutamate synthase (NADPH) large subunit
Swit_0658	-9.036772	-3.1758075	hypothetical protein Swit_0658
Swit_0659	-12.331679	-3.6242974	putative oxidoreductase
Swit_0773	-4.2095113	-2.0736527	hypothetical protein Swit_0773
Swit_0862	-14.859949	-3.8933573	phasin family protein
Swit_1107	-7.059017	-2.8194673	hypothetical protein Swit_1107
Swit_1124	-33.923077	-5.084195	glutaredoxin
Swit_1152	-55.87332	-5.8040876	RND family efflux transporter MFP subunit
Swit_1153	-26.56731	-4.7315803	hydrophobe/amphiphile efflux-1 (HAE1) family protein
Swit_1388	-6.347179	-2.6661155	hypothetical protein Swit_1388
Swit_1406	-4.290255	-2.1010635	hypothetical protein Swit_1406
Swit_2183	-4.3925977	-2.1350744	plasmid maintenance system killer
Swit_2243	-17.691708	-4.1450014	arsenate reductase
Swit_2244	-19.085625	-4.2544146	ArsR family transcriptional regulator
Swit_2339	-6.0593853	-2.5991714	hypothetical protein Swit_2339
Swit_2411	-10.396318	-3.3780007	cytochrome c, class I
Swit_2456	-4.100661	-2.0358565	30S ribosomal protein S1
Swit_2501	-5.3693686	-2.4247525	hypothetical protein Swit_2501
Swit_2551	-5.2374845	-2.388874	hypothetical protein Swit_2551
Swit_2559	-16.453306	-4.0403056	acyl-CoA synthetase
Swit_2586	-8.477817	-3.0836928	general secretion pathway L
Swit_2616	-5.523031	-2.4654603	copper resistance protein CopC
Swit_2664	-4.1282763	-2.0455396	aspartate-semialdehyde dehydrogenase
Swit_2722	-5.3112097	-2.4090405	NADPH-glutathione reductase
Swit_2779	-4.478123	-2.1628942	Ferritin, Dps family protein
Swit_2794	-9.963336	-3.316629	opacity protein and related surface antigens-like protein
Swit_2821	-4.455735	-2.1556635	ankyrin
Swit_2877	-4.326262	-2.113121	methyltransferase type 12
Swit_2900	-4.8138943	-2.2672045	methionine-R-sulfoxide reductase
Swit_2940	-4.0266094	-2.0095656	hypothetical protein Swit_2940
Swit_2941	-4.440438	-2.150702	hypothetical protein Swit_2941
Swit_2955	-8.847023	-3.1451921	hypothetical protein Swit_2955
Swit_2957	-10.744423	-3.4255161	OsmC family protein
Swit_3212	-4.776555	-2.2559705	citrate synthase
Swit_3231	-4.7693796	-2.2538016	RND efflux system outer membrane lipoprotein
Swit_3232	-4.5423484	-2.1834383	OsmC family protein

Cutoff <-4 **lower in liquid**

Systematic Name	FC ([Stat-Liq] vs [Stat-Sand])	Log FC ([Stat-Liq] vs [Stat-Sand])	Annotation
Swit_3346	-6.085828	-2.6054535	hypothetical protein Swit_3346
Swit_3432	-4.488849	-2.1663456	3-hydroxybutyrate dehydrogenase
Swit_3456	-4.4389706	-2.1502252	hypothetical protein Swit_3456
Swit_3457	-10.484248	-3.3901515	glutathione S-transferase domain-containing protein
Swit_3463	-9.561334	-3.257212	cell wall hydrolase, SleB
Swit_3471	-8.134087	-3.0239804	hypothetical protein Swit_3471
Swit_3537	-5.548477	-2.472092	CarD family transcriptional regulator
Swit_3586	-11.95283	-3.5792804	alkylhydroperoxidase
Swit_3587	-7.355136	-2.878752	alkyl hydroperoxide reductase/ Thiol specific antioxidant/ Mal allergen
Swit_3723	-24.936882	-4.640209	rhodanese domain-containing protein
Swit_3724	-9.140846	-3.1923277	RND family efflux transporter MFP subunit
Swit_3752	-5.0274715	-2.329833	phage integrase family protein
Swit_3778	-24.675348	-4.6249986	hypothetical protein Swit_3778
Swit_3813	-5.6059422	-2.4869568	hypothetical protein Swit_3813
Swit_3858	-4.4156523	-2.1426265	alpha/beta hydrolase fold
Swit_3892	-4.2339277	-2.0819967	50S ribosomal protein L27
Swit_3903	-38.542934	-5.2683945	diacylglycerol kinase, catalytic region
Swit_3904	-31.275879	-4.9669785	hypothetical protein Swit_3904
Swit_3907	-18.230745	-4.1883016	fatty acid hydroxylase
Swit_3960	-5.0264306	-2.3295343	hypothetical protein Swit_3960
Swit_4072	-4.0500116	-2.017926	hypothetical protein Swit_4072
Swit_4509	-7.262746	-2.860515	membrane protease FtsH catalytic subunit
Swit_4510	-5.708911	-2.5132155	MarR family transcriptional regulator
Swit_4511	-4.080698	-2.028816	secretion protein HlyD family protein
Swit_4533	-4.023753	-2.0085418	glycoside hydrolase family protein
Swit_4592	-5.7496767	-2.523481	hypothetical protein Swit_4592
Swit_4656	-4.398166	-2.136902	dihydroxy-acid dehydratase
Swit_4685	-7.5820518	-2.9225883	D-3-phosphoglycerate dehydrogenase
Swit_4686	-15.979713	-3.9981697	phosphoserine aminotransferase
Swit_4696	-9.26833	-3.2123094	TonB-dependent receptor
Swit_4724	-11.304775	-3.4988604	DEAD/DEAH box helicase domain-containing protein
Swit_4744	-4.21119	-2.074228	acriflavin resistance protein
Swit_4763	-4.6917586	-2.2301288	hypothetical protein Swit_4763
Swit_4764	-5.235468	-2.3883185	2-octaprenylphenol hydroxylase
Swit_4811	-4.3445735	-2.1192145	hypothetical protein Swit_4811
Swit_4822	-4.2319417	-2.0813198	hypothetical protein Swit_4822

Cutoff >4 **higher in liquid**

Systematic Name	FC ([Stat-Liq] vs [Stat-Sand])	Log FC ([Stat-Liq] vs [Stat-Sand])	Annotation
Swit_0045	11.991867	3.5839844	histone family protein DNA-binding protein
Swit_0115	4.6578383	2.2196605	dihydroneopterin aldolase
Swit_1293	4.5305886	2.1796985	flagellar basal body-associated protein FliL
Swit_1515	4.523225	2.1773517	hypothetical protein Swit_1515
Swit_2168/2	9.29314	3.2161663	IS4 family transposase
Swit_2652	4.55098	2.1861773	polysaccharide biosynthesis protein
Swit_3043	17.330902	4.115275	sulfatase
Swit_3044	18.71713	4.2262874	TonB-dependent receptor
Swit_3045	7.173713	2.84272	monooxygenase, FAD-binding
Swit_3047	20.031973	4.3242326	phytanoyl-CoA dioxygenase
Swit_3048	22.937475	4.5196347	TonB-dependent receptor
Swit_3056	4.071869	2.025691	Rieske (2Fe-2S) domain-containing protein
Swit_3057	4.566606	2.1911223	aromatic-ring-hydroxylating dioxygenase, beta subunit
Swit_3058	5.538316	2.4694474	maleylacetoacetate isomerase
Swit_3060	9.843534	3.2991765	fumarylacetoacetate (FAA) hydrolase
Swit_3068	5.0107455	2.3250253	short-chain dehydrogenase/reductase SDR
Swit_3506	4.5063696	2.1719656	Flp/Fap pilin component
Swit_3972	8.8162	3.140157	ECF subfamily RNA polymerase sigma-24 factor
Swit_3973	16.773235	4.068089	putative transmembrane anti-sigma factor
Swit_4389	17.873774	4.1597724	hypothetical protein Swit_4389
Swit_4788	4.422886	2.144988	methylitaconate delta2-delta3-isomerase
Swit_4789	5.439244	2.443406	hypothetical protein Swit_4789
Swit_4790	4.359639	2.1242087	methylcitrate synthase
Swit_4791	8.593313	3.1032145	2,3-dimethylmalate lyase
Swit_4888	6.8383694	2.7736523	butyryl-CoA:acetate CoA transferase
Swit_4889	9.065831	3.1804392	3-oxoacid CoA-transferase, A subunit
Swit_4890	6.9934926	2.806013	hydroxyquinol 1,2-dioxygenase
Swit_4891	6.0058646	2.586372	iron-containing alcohol dehydrogenase
Swit_4892	4.820333	2.2691329	hypothetical protein Swit_4892
Swit_4893	23.7665	4.5708575	ferredoxin
Swit_4894	17.222692	4.106239	TonB-dependent receptor
Swit_4895	13.01523	3.702129	alpha/beta hydrolase fold
Swit_4896	11.5056305	3.5242682	aromatic-ring-hydroxylating dioxygenase, beta subunit
Swit_4897	10.63846	3.4112175	ring hydroxylating dioxygenase, alpha subunit
Swit_4908	5.1949873	2.3771203	integrase catalytic subunit
Swit_4919	4.1389494	2.0492647	hypothetical protein Swit_4919
Swit_4921	7.0630207	2.8202853	3-keto-5-aminohexanoate cleavage enzyme
Swit_4927	5.0920615	2.34825	2-dehydropantoate 2-reductase
Swit_4934	4.0754867	2.0269723	relaxase/mobilization nuclease family protein
Swit_4957/4	4.946167	2.306311	IstB ATP binding domain-containing protein
Swit_4958/4	5.3409166	2.4170873	transposase
Swit_4959	4.327639	2.1135802	hypothetical protein Swit_4959
Swit_4985	4.897501	2.2920458	hypothetical protein Swit_4985

Cutoff >4 **higher in liquid**

Systematic Name	FC ([Stat-Liq] vs [Stat-Sand])	Log FC ([Stat-Liq] vs [Stat-Sand])	Annotation
Swit_4987	5.6509023	2.4984813	Pseudo
Swit_4988	6.093185	2.6071966	glycoside hydrolase family protein
Swit_4989	5.689802	2.5083785	hypothetical protein Swit_4989
Swit_4991	5.334179	2.4152663	carbamoyltransferase
Swit_4994	4.1983566	2.0698247	Abi family protein
Swit_4996	4.146083	2.051749	hypothetical protein Swit_4996
Swit_5010	4.2945223	2.1024976	lytic transglycosylase, catalytic
Swit_5026	5.744852	2.5222697	TonB-dependent receptor
Swit_5053	6.8229146	2.7703881	X-Pro dipeptidyl-peptidase domain-containing protein
Swit_5056	4.204939	2.072085	short-chain dehydrogenase/reductase SDR
Swit_5057	4.123634	2.0439162	cupin 2 domain-containing protein
Swit_5067	4.394549	2.135715	transposase Tn3 family protein
Swit_5078	4.86631	2.2828283	transposase IS66
Swit_5079	12.027494	3.5882642	IS66 Orf2 family protein
Swit_5080	4.2963357	2.1031067	transposase IS3/IS911 family protein
Swit_5083	4.3339634	2.115687	hypothetical protein Swit_5083
Swit_5100	8.33809	3.059717	Pseudo
Swit_5108	4.1708994	2.0603585	hypothetical protein Swit_5108
Swit_5129	13.806104	3.7872343	TonB-dependent receptor
Swit_5133	6.6744275	2.7386441	Fis family GAF modulated sigma54 specific transcriptional regulator
Swit_5134	8.643752	3.1116576	AMP-binding domain protein
Swit_5135	9.01574	3.172446	aldehyde dehydrogenase
Swit_5136	10.898441	3.44605	Pyrrolo-quinoline quinone
Swit_5201	4.0666556	2.0238428	hypothetical protein Swit_5201
Swit_5362	5.552125	2.47304	hypothetical protein Swit_5362
Swit_5364	93.8107	6.5516806	hypothetical protein Swit_5364

FC, fold-change
yellow/green: possibly same operon

Table S13 RW1 genes commonly differentially expressed in sand and under matric (MP) or solute (SP) stress

Log FC 2log fold-change

Systematic Name	Log FC ([Exp-Liq] vs [Exp-Sand])	log FC matric vs ctrl	log FC solute vs ctrl	Annotation	common to SP<-1	common to SP>1
MP<-1, higher in sand						
Swit_0993	-1.0641931	-1.1484665	0.27427006	hypothetical protein Swit_0993		
Swit_2221	-1.346249	-1.3974959	-0.9471436	CopA family copper resistance protein		
Swit_2559	-1.1789513	-1.4283413	-2.9505682	acyl-CoA synthetase	Swit_2559	
Swit_2765	-1.1891165	-1.86938	0.15969849	hypothetical protein Swit_2765		
Swit_3089	-3.6914318	-1.4492712	0.18535995	Pseudo		
Swit_3607	-1.730648	-1.2732693	0.2898032	hypothetical protein Swit_3607		
Swit_3904	-1.289468	-1.1796455	-2.2715492	hypothetical protein Swit_3904	Swit_3904	
Swit_4121	-2.7300138	-1.3255043	1.4125366	AMP-dependent synthetase and ligase		Swit_4121
MP<-1, lower in sand						
Swit_0067	1.6389477	-2.1282878	-1.6079805	response regulator receiver protein	Swit_0067	
Swit_1141	1.0235746	-1.181398	-1.5989063	acyltransferase 3	Swit_1141	
Swit_1264	1.3033333	-1.2081774	-1.1408257	flagellar basal body P-ring protein	Swit_1264	
Swit_1270	2.3132358	-1.424581	-1.3436236	flagellar basal-body rod protein FlgC	Swit_1270	
Swit_1286	3.652347	-1.3350072	-1.1942	flagellar hook-basal body complex subunit FliE	Swit_1286	
Swit_3081	2.0688071	-1.2189627	-0.1215499	GntR family transcriptional regulator		
Swit_4412	1.4186821	-1.3129455	-0.8605351	type IV pilus assembly PilZ		
MP>1, lower in sand						
Swit_0619	1.296579	2.6354098	1.6739931	heat shock protein Hsp20		Swit_0619
Swit_1472	1.9356753	1.3426143	0.22178397	formate dehydrogenase delta subunit		
Swit_3457	2.1117568	2.0106878	0.14327653	glutathione S-transferase domain-containing protein		
Swit_4785	1.510539	1.0024226	-1.9045944	dihydroneopterin aldolase	Swit_4785	
Swit_4788	2.9238	1.08962	-1.1537533	methylitaconate delta2-delta3-isomerase	Swit_4788	
Swit_4790	2.1453483	1.0267143	-0.8382232	methylcitrate synthase		
Swit_4791	2.2749553	1.4350176	-0.9604915	2,3-dimethylmalate lyase		
Swit_4937	2.2207704	1.470136	0.8917214	cobyrinic acid a,c-diamide synthase		
Swit_5006	1.0423673	1.2598798	0.5645499	type IV secretion system family protein		
Swit_5307	1.3263168	1.570239	0.542521	hypothetical protein Swit_5307		
Swit_5351	1.5506263	1.9962654	0.36499247	heat shock protein 90		
MP>1, higher in sand						
Swit_0103	-1.0710404	1.0838509	0.7629487	hypothetical protein Swit_0103		
Swit_1124	-1.1116177	1.4581789	0.18329921	glutaredoxin		
Swit_1153	-1.4533236	3.9647849	0.11325185	hydrophobe/amphiphile efflux-1 (HAE1) family protein		
Swit_1179	-2.45676	1.7432117	-0.6034057	S-(hydroxymethyl)glutathione dehydrogenase		
Swit_1412	-5.8008246	1.0387304	2.2917979	glutathione-dependent formaldehyde-activating, GFA		Swit_1412
Swit_2577	-1.4855897	1.2067108	2.7061682	glycosyl transferase, group 1		Swit_2577
Swit_2900	-2.5680697	1.0333722	0.93518335	methionine-R-sulfoxide reductase		
Swit_3463	-1.1765318	1.8399417	0.95428663	cell wall hydrolase, SleB		
Swit_3568	-1.2120476	1.5738084	2.4260013	hypothetical protein Swit_3568		Swit_3568
Swit_3813	-1.0833435	1.0391781	1.3135626	hypothetical protein Swit_3813		Swit_3813
Swit_3986	-1.1550602	1.1663582	-1.0652927	Glu/Leu/Phe/Val dehydrogenase, dimerisation region	Swit_3986	
Swit_4072	-2.1447706	1.2260072	0.4454557	hypothetical protein Swit_4072		
Swit_4076	-1.025672	1.565676	-0.2362239	hypothetical protein Swit_4076		
Swit_4527	-1.6904943	1.9661431	1.9044608	polysaccharide biosynthesis protein		Swit_4527
Swit_4646	-2.8889763	2.6192355	-0.2509572	hypothetical protein Swit_4646		
Swit_4724	-1.9210398	1.0671196	-0.6900927	DEAD/DEAH box helicase domain-containing protein		
SP<-1, higher in sand						
Swit_0958	-2.2513304	0.2766126	-1.134206	butyryl-CoA:acetate CoA transferase		
Swit_1309	-1.2359688	-0.81217545	-1.1699104	nucleotidyl transferase		
Swit_2559	-1.1789513	-1.4283413	-2.9505682	acyl-CoA synthetase		
Swit_3904	-1.289468	-1.1796455	-2.2715492	hypothetical protein Swit_3904		
Swit_3986	-1.1550602	1.1663582	-1.0652927	Glu/Leu/Phe/Val dehydrogenase, dimerisation region		
SP<-1, lower in sand						
Swit_0067	1.6389477	-2.1282878	-1.6079805	response regulator receiver protein		
Swit_0228	2.5825465	-0.92148906	-1.101311	acetolactate synthase		
Swit_1141	1.0235746	-1.181398	-1.5989063	acyltransferase 3		
Swit_1264	1.3033333	-1.2081774	-1.1408257	flagellar basal body P-ring protein		
Swit_1270	2.3132358	-1.424581	-1.3436236	flagellar basal-body rod protein FlgC		
Swit_1286	3.652347	-1.3350072	-1.1942	flagellar hook-basal body complex subunit FliE		
Swit_4785	1.510539	1.0024226	-1.9045944	dihydroneopterin aldolase		
Swit_4786	3.6569333	0.712471	-1.7560667	5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase		
Swit_4788	2.9238	1.08962	-1.1537533	methylitaconate delta2-delta3-isomerase		

SP>1, lower in sand

Systematic Name	Log FC ([Exp-Liq] vs [Exp-Sand])	log FC matric vs ctrl	log FC solute vs ctrl	Annotation
Swit_0619	1.296579	2.6354098	1.6739931	heat shock protein Hsp20
Swit_0689	3.791769	-0.17088461	1.395556	hypothetical protein Swit_0689
Swit_0692	3.0218005	-0.13040575	1.4650612	extracellular solute-binding protein
Swit_0693	2.942842	-0.076296486	1.2435277	Pyrrolo-quinoline quinone
Swit_0703	5.7446055	0.18930116	1.4931444	aldehyde dehydrogenase
Swit_2779	2.5862305	0.30576578	2.193915	Ferritin, Dps family protein
Swit_3529	1.0317284	0.5128418	1.175732	methyltransferase type 12
Swit_3794	1.7807587	0.39410627	1.9869463	hypothetical protein Swit_3794
Swit_5045	2.1595001	0.46136475	1.0428365	TonB-dependent receptor

SP>1, higher in sand

Swit_0862	-1.2098986	0.7104355	1.2222347	phasin family protein
Swit_1412	-5.8008246	1.0387304	2.2917979	glutathione-dependent formaldehyde-activating, GFA
Swit_2577	-1.4855897	1.2067108	2.7061682	glycosyl transferase, group 1
Swit_3568	-1.2120476	1.5738084	2.4260013	hypothetical protein Swit_3568
Swit_3743	-1.164305	0.18384139	1.1668255	1-Cys peroxiredoxin
Swit_3813	-1.0833435	1.0391781	1.3135626	hypothetical protein Swit_3813
Swit_3836	-2.3364592	0.33106646	1.417153	ECF subfamily RNA polymerase sigma-24 factor
Swit_3839	-1.4714622	0.8340451	1.2577919	hypothetical protein Swit_3839
Swit_3979	-1.7686875	0.23283608	1.2829963	ATP-dependent DNA ligase
Swit_3982	-1.2034788	0.70398265	2.157645	DNA ligase D
Swit_4121	-2.7300138	-1.3255043	1.4125366	AMP-dependent synthetase and ligase
Swit_4527	-1.6904943	1.9661431	1.9044608	polysaccharide biosynthesis protein
Swit_4575	-1.7209163	0.74580574	2.1754785	hypothetical protein Swit_4575
Swit_4648	-1.8531834	0.9822388	3.339261	hypothetical protein Swit_4648
Swit_4764	-2.3783858	0.6706613	2.398944	2-octaprenylphenol hydroxylase
Swit_5249	-1.6751572	0.9031625	2.7591934	ankyrin

Table S14 **RW1 genes differentially expressed in sand and liquid exponentially growing culture, and judged essential or causing fitness loss upon interruption by transposon marker insertion**

Data from Roggo et al., 2013

>Fourfold	Lower in sand			No reads		2log ratio
Location	SystematicName	FC ([Exp-Liq] vs [Exp-Sand])	Annotation	SAND4	TN01	Prop SAND4/TN01
Xsome	Swit_0064	4.16	hypothetical protein Swit_0064	0	66	Ess sand
Xsome	Swit_0127	4.97	hypothetical protein Swit_0127	0	0	Essential
Xsome	Swit_0143	4.70	polysaccharide biosynthesis protein	19	491	-2.85
Xsome	Swit_0228	5.99	acetolactate synthase	0	113	Ess sand
Xsome	Swit_0601	5.24	Holliday junction resolvase YqgF	0	89	Ess sand
Xsome	Swit_0604	4.52	sporulation domain-containing protein	2	340	-5.57
Xsome	Swit_0610	5.64	acetolactate synthase 3 regulatory subunit	0	0	Essential
Xsome	Swit_0690	7.10	YVTN beta-propeller repeat-containing protein	1	0	Essential
Xsome	Swit_0692	8.12	extracellular solute-binding protein	0	204	Ess sand
Xsome	Swit_0693	7.69	Pyrrolo-quinoline quinone	1	292	-6.35
Xsome	Swit_0694	6.76	two component LuxR family transcriptional regulator	1	261	-6.19
Xsome	Swit_0703	53.62	aldehyde dehydrogenase	0	264	Ess sand
Xsome	Swit_0739	4.85	hypothetical protein Swit_0739	0	1	Ess sand
Xsome	Swit_0823	4.08	endoribonuclease L-PSP	0	0	Essential
Xsome	Swit_0893	4.72	Rieske (2Fe-2S) domain-containing protein	0	0	Essential
Xsome	Swit_1270	4.97	flagellar basal-body rod protein FlgC	0	379	Ess sand
Xsome	Swit_1286	12.57	flagellar hook-basal body complex subunit FliE	1	145	-5.34
Xsome	Swit_1386	4.26	two component transcriptional regulator	0	49	Ess sand
Xsome	Swit_1424	4.07	hypothetical protein Swit_1424	0	60	Ess sand
Xsome	Swit_1787	4.39	endoribonuclease L-PSP	0	1	Ess sand
Xsome	Swit_1893	4.41	xylose isomerase domain-containing protein	0	79	Ess sand
Xsome	Swit_2117	4.59	hypothetical protein Swit_2117	0	0	Essential
Xsome	Swit_2224	4.19	amidohydrolase	1	82	-4.52
Xsome	Swit_2679	8.08	hypothetical protein Swit_2679	2	85	-3.57
Xsome	Swit_2779	6.01	Ferritin, Dps family protein	0	65	Ess sand
Xsome	Swit_2866	7.62	glyoxalase/bleomycin resistance protein/dioxygenase	0	14	Ess sand
Xsome	Swit_3047	4.04	phytanoyl-CoA dioxygenase	1	66	-4.21
Xsome	Swit_3057	6.66	aromatic-ring-hydroxylating dioxygenase, beta subunit	0	0	Essential
Xsome	Swit_3058	4.16	maleylacetoacetate isomerase	1	40	-3.48
Xsome	Swit_3081	4.20	GntR family transcriptional regulator	0	88	Ess sand
Xsome	Swit_3085	4.84	LysR family transcriptional regulator	8	335	-3.55
Xsome	Swit_3217	4.59	molybdopterin molybdochelatase	1	117	-5.03
Xsome	Swit_3478	4.31	cytochrome B561	0	266	Ess sand
Xsome	Swit_3553	4.07	hypothetical protein Swit_3553	0	104	Ess sand
Xsome	Swit_3799	5.70	hypothetical protein Swit_3799	5	287	-4.00
Xsome	Swit_3898	5.35	hypothetical protein Swit_3898	0	0	Essential
Xsome	Swit_3972	12.69	ECF subfamily RNA polymerase sigma-24 factor	0	0	Essential
Xsome	Swit_4385	3.98	hypothetical protein Swit_4385	0	1	Ess sand
Xsome	Swit_4409	4.42	peptidase M24	28	438	-2.13
Xsome	Swit_4427	4.67	hypothetical protein Swit_4427	0	47	Ess sand
Xsome	Swit_4442	3.98	hypothetical protein Swit_4442	8	139	-2.28
Xsome	Swit_4731	4.57	homoserine dehydrogenase	0	0	Essential
Xsome	Swit_4786	12.61	5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase	0	0	Essential
Xsome	Swit_4789	23.16	hypothetical protein Swit_4789	0	0	Essential
Xsome	Swit_4791	4.84	2,3-dimethylmalate lyase	0	4	Ess sand
pSWIT02	Swit_4896	4.11	aromatic-ring-hydroxylating dioxygenase, beta subunit	0	2	Ess sand
pSWIT02	Swit_4897	5.16	ring hydroxylating dioxygenase, alpha subunit	0	0	Essential
pSWIT02	Swit_4989	5.31	hypothetical protein Swit_4989	1	94	-4.87
pSWIT01	Swit_5207	6.08	MucR family transcriptional regulator	0	116	Ess sand
pSWIT01	Swit_5247	4.94	Ferritin, Dps family protein	0	244	Ess sand
pSWIT01	Swit_5289	5.37	hypothetical protein Swit_5289	0	2	Ess sand

>Fourfold higher in sand				No Reads		2log ratio
Location	Systematic Name	FC ([Exp-Liq] vs [Exp-Sand])	Annotation	SAND4	TN01	Prop SAND4/TN01
Xsome	Swit_0036	-4.84	ATPase involved in DNA replication initiation	0	0	Essential
Xsome	Swit_0066	-4.67	CheW protein	0	0	Essential
Xsome	Swit_0163	-38.30	Type IV secretory pathway TrbD component-like protein	0	63	Ess sand
Xsome	Swit_0166	-7.69	hypothetical protein Swit_0166	0	39	Ess sand
Xsome	Swit_0271	-4.93	ABC transporter related	52	769	-2.05
Xsome	Swit_0295	-7.77	short-chain dehydrogenase/reductase SDR	0	0	Essential
Xsome	Swit_0307	-5.44	hypothetical protein Swit_0307	0	239	Ess sand
Xsome	Swit_0347	-6.13	transketolase, central region	2	125	-4.13
Xsome	Swit_0368	-5.52	enoyl-CoA hydratase/isomerase	1	426	-6.90
Xsome	Swit_0606	-17.54	hypothetical protein Swit_0606	0	0	Essential
Xsome	Swit_0615	-85.32	Flp/Fap pilin component	0	1	Ess sand
Xsome	Swit_0641	-4.25	XRE family transcriptional regulator	0	0	Essential
Xsome	Swit_0652	-6.17	methylmalonate-semialdehyde dehydrogenase	1	69	-4.27
Xsome	Swit_0669	-4.76	AMP-dependent synthetase and ligase	0	447	Ess sand
Xsome	Swit_0681	-6.24	amidohydrolase	7	160	-2.68
Xsome	Swit_0724	-6.10	methylenetetrahydromethanopterin reductase	0	194	Ess sand
Xsome	Swit_0735	-4.80	3-hydroxybutyryl-CoA dehydrogenase	0	78	Ess sand
Xsome	Swit_0769	-4.35	short chain dehydrogenase	1	160	-5.48
Xsome	Swit_0810	-6.10	AraC family transcriptional regulator	0	130	Ess sand
Xsome	Swit_0868	-6.90	hypothetical protein Swit_0868	0	113	Ess sand
Xsome	Swit_0927	-8.77	amidase	0	380	Ess sand
Xsome	Swit_0958	-4.76	butyryl-CoA:acetate CoA transferase	3	350	-5.03
Xsome	Swit_1022	-9.75	TonB-dependent receptor	0	310	Ess sand
Xsome	Swit_1071	-4.84	fumarate lyase	0	126	Ess sand
Xsome	Swit_1179	-5.49	S-(hydroxymethyl)glutathione dehydrogenase	3	54	-2.33
Xsome	Swit_1239	-4.85	hypothetical protein Swit_1239	0	64	Ess sand
Xsome	Swit_1273	-8.30	hypothetical protein Swit_1273	0	0	Essential
Xsome	Swit_1412	-55.75	glutathione-dependent formaldehyde-activating, GFA	0	18	Ess sand
Xsome	Swit_1433	-11.69	gamma-glutamyltransferase	0	184	Ess sand
Xsome	Swit_1584	-4.02	hypothetical protein Swit_1584	0	312	Ess sand
Xsome	Swit_1633	-6.01	hypothetical protein Swit_1633	0	32	Ess sand
Xsome	Swit_1760	-4.05	L-carnitine dehydratase/bile acid-inducible protein F	0	24	Ess sand
Xsome	Swit_1777	-4.02	LysR family transcriptional regulator	2	221	-4.95
Xsome	Swit_1782	-8.27	intradiol ring-cleavage dioxygenase	0	133	Ess sand
Xsome	Swit_1825	-8.13	TetR family transcriptional regulator	4	189	-3.72
Xsome	Swit_1832	-14.22	dehydratase	1	292	-6.35
Xsome	Swit_1848	-13.23	glyoxalase/bleomycin resistance protein/dioxygenase	0	264	Ess sand
Xsome	Swit_1915	-19.17	hypothetical protein Swit_1915	0	0	Essential
Xsome	Swit_1936	-5.56	porphobilinogen deaminase	0	0	Essential
Xsome	Swit_1981	-8.51	short-chain dehydrogenase/reductase SDR	18	317	-2.30
Xsome	Swit_2021	-5.13	enoyl-CoA hydratase	0	73	Ess sand
Xsome	Swit_2057	-7.64	hypothetical protein Swit_2057	0	273	Ess sand
Xsome	Swit_2063	-5.07	alanine dehydrogenase	0	109	Ess sand
Xsome	Swit_2072	-5.80	coenzyme A transferase	0	324	Ess sand
Xsome	Swit_2110	-7.77	transcriptional regulator IclR-like protein	1	171	-5.58
Xsome	Swit_2241	-6.27	arsenate resistance ArsH	0	203	Ess sand
Xsome	Swit_2267	-28.00	putative monooxygenase	0	1	Ess sand
Xsome	Swit_2357	-11.10	ATPase involved in chromosome partitioning-like protein	0	0	Essential
Xsome	Swit_2421	-5.34	peptidase M61 domain-containing protein	0	218	Ess sand
Xsome	Swit_2437	-4.33	NAD-dependent epimerase/dehydratase	1	210	-5.88
Xsome	Swit_2478	-9.52	hypothetical protein Swit_2478	1	67	-4.23
Xsome	Swit_2586	-9.21	general secretion pathway L	0	74	Ess sand
Xsome	Swit_2656	-4.12	signal recognition particle subunit FFH/SRP54 (srp54)	0	0	Essential
Xsome	Swit_2900	-5.93	methionine-R-sulfoxide reductase	2	87	-3.60
Xsome	Swit_2916	-6.18	SufBD protein	0	0	Essential
Xsome	Swit_2965	-4.26	hypothetical protein Swit_2965	0	229	Ess sand
Xsome	Swit_3029	-5.86	CRP/FNR family transcriptional regulator	5	640	-5.16
Xsome	Swit_3062	-5.92	phthalate 4,5-dioxygenase	1	273	-6.25
Xsome	Swit_3183	-4.63	oxidoreductase domain-containing protein	0	1	Ess sand
Xsome	Swit_3288	-8.60	short-chain dehydrogenase/reductase SDR	27	403	-2.06
Xsome	Swit_3425	-4.92	outer membrane-like protein	4	237	-4.05
Xsome	Swit_3590	-11.28	hypothetical protein Swit_3590	0	94	Ess sand
Xsome	Swit_3617	-9.27	putative inner membrane protein translocase component YidC	2	51	-2.83
Xsome	Swit_3648	-8.48	5-oxoprolinase (ATP-hydrolyzing)	2	142	-4.31
Xsome	Swit_3684	-11.76	hypothetical protein Swit_3684	0	63	Ess sand
Xsome	Swit_3814	-4.48	thymidine kinase	0	98	Ess sand
Xsome	Swit_3849	-4.42	peptidase M48, Ste24p	0	0	Essential
Xsome	Swit_3858	-13.16	alpha/beta hydrolase fold	0	144	Ess sand
Xsome	Swit_3899	-4.91	hypothetical protein Swit_3899	0	0	Essential
Xsome	Swit_3938	-11.62	hypothetical protein Swit_3938	1	61	-4.09
Xsome	Swit_4072	-4.42	hypothetical protein Swit_4072	0	0	Essential
Xsome	Swit_4153	-6.94	hypothetical protein Swit_4153	0	246	Ess sand

>Fourfold higher in sand				No Reads		2log ratio
Location	Systematic Name	FC ([Exp-Liq] vs [Exp-Sand])	Annotation	SAND4	TN01	Prop SAND4/TN01
Xsome	Swit_4339	-3.98	beta-ketoadipate pathway transcription regulator	6	276	-3.68
Xsome	Swit_4351	-17.92	short-chain dehydrogenase/reductase SDR	0	21	Ess sand
Xsome	Swit_4431	-9.80	NUDIX hydrolase	0	0	Essential
Xsome	Swit_4455	-4.31	hypothetical protein Swit_4455	0	0	Essential
Xsome	Swit_4551	-15.32	hypothetical protein Swit_4551	0	299	Ess sand
Xsome	Swit_4646	-7.41	hypothetical protein Swit_4646	0	1	Ess sand
Xsome	Swit_4769	-4.49	enoyl-(acyl carrier protein) reductase	0	0	Essential
Xsome	Swit_4844	-5.25	GreA/GreB family elongation factor	0	52	Ess sand
Xsome	Swit_4874	-4.19	hypothetical protein Swit_4874	0	4	Ess sand
pSWIT01	Swit_5195	-15.67	hypothetical protein Swit_5195	0	158	Ess sand
pSWIT01	Swit_5233	-4.14	single-strand binding protein/primosomal replication protein n	0	17	Ess sand
pSWIT01	Swit_5241	-6.39	hypothetical protein Swit_5241	2	278	-5.49

Table S15 **RW1 genes differentially expressed in sand and liquid stationary phase culture, and judged essential or causing fitness loss upon interruption by transposon marker insertion**

Data from Roggo et al., 2013

>Fourfold lower expressed in sand **No reads**

Location	Systematic Name	Log FC ([Stat-Liq] vs [Stat-Sand])	Annotation	SAND10	TN01	2 log ratio prop SAND10/ TN01
Xsome	Swit_0616	2.87	Flp/Fap pilin component	0	12	Ess sand
Xsome	Swit_2527	3.56	hypothetical protein Swit_2527	0	1	Ess sand
Xsome	Swit_3043	4.12	sulfatase	2	917	-6.35
Xsome	Swit_3047	4.32	phytanoyl-CoA dioxygenase	1	66	-3.55
Xsome	Swit_3056	2.03	Rieske (2Fe-2S) domain-containing protein	1	175	-4.96
Xsome	Swit_3057	2.19	aromatic-ring-hydroxylating dioxygenase, b	0	0	Essential
Xsome	Swit_3058	2.47	maleylacetoacetate isomerase	1	40	-2.83
Xsome	Swit_3060	3.30	fumarylacetoacetate (FAA) hydrolase	0	66	Ess sand
Xsome	Swit_3972	3.14	ECF subfamily RNA polymerase sigma-24 fa	0	0	Essential
Xsome	Swit_3973	4.07	putative transmembrane anti-sigma factor	0	154	Ess sand
Xsome	Swit_4308	2.42	5-oxopent-3-ene-1,2,5-tricarboxylate decar	0	0	Essential
Xsome	Swit_4389	4.16	hypothetical protein Swit_4389	0	51	Ess sand
Xsome	Swit_4788	2.14	methylitaconate delta2-delta3-isomerase	0	80	Ess sand
Xsome	Swit_4791	3.10	2,3-dimethylmalate lyase	0	4	Ess sand
pSWIT02	Swit_4895	3.70	alpha/beta hydrolase fold	0	0	Essential
pSWIT02	Swit_4896	3.52	aromatic-ring-hydroxylating dioxygenase, b	0	2	Ess sand
pSWIT02	Swit_4921	2.82	3-keto-5-aminohexanoate cleavage enzyme	8	545	-3.60
pSWIT02	Swit_5010	2.10	lytic transglycosylase, catalytic	2	411	-5.20
pSWIT02	Swit_5036	3.06	cupin 2 domain-containing protein	3	772	-5.52
pSWIT02	Swit_5053	2.77	X-Pro dipeptidyl-peptidase domain-containi	84	3189	-2.76
pSWIT02	Swit_5056	2.07	short-chain dehydrogenase/reductase SDR	11	1347	-4.45
pSWIT02	Swit_5057	2.04	cupin 2 domain-containing protein	1	65	-3.54
pSWIT02	Swit_5108	2.06	hypothetical protein Swit_5108	0	347	Ess sand
pSWIT01	Swit_5365	4.31	Type IV conjugative transfer system protein	12	791	-3.79

>Fourfold higher expressed in sand

Location	Systematic Name	Log FC ([Stat-Liq] vs [Stat-Sand])	Annotation	SAND10	TN01	2 log ratio prop SAND10/TN01
Xsome	Swit_0016	-3.00	redoxin domain-containing protein	0	245	Ess sand
Xsome	Swit_0060	-3.22	RNA polymerase factor sigma-32	0	3	Ess sand
Xsome	Swit_0251	-2.59	RNA-binding protein Hfq	0	0	Essential
Xsome	Swit_0630	-2.60	polyhydroxyalkonate synthesis repressor, P	1	0	Essential
Xsome	Swit_0656	-2.50	hypothetical protein Swit_0656	0	37	Ess sand
Xsome	Swit_0657	-2.60	glutamate synthase (NADPH) large subunit	0	72	Ess sand
Xsome	Swit_0658	-3.18	hypothetical protein Swit_0658	0	35	Ess sand
Xsome	Swit_0659	-3.62	putative oxidoreductase	0	0	Essential
Xsome	Swit_1107	-2.82	hypothetical protein Swit_1107	0	4	Ess sand
Xsome	Swit_1124	-5.08	glutaredoxin	1	363	-6.01
Xsome	Swit_1153	-4.73	hydrophobe/amphiphile efflux-1 (HAE1) far	6	739	-4.45
Xsome	Swit_1367	-2.31	pyruvate dehydrogenase complex dihydroli	0	0	Essential
Xsome	Swit_1388	-2.67	hypothetical protein Swit_1388	2	245	-4.45
Xsome	Swit_1509	-2.02	17 kDa surface antigen	0	35	Ess sand
Xsome	Swit_2243	-4.15	arsenate reductase	0	32	Ess sand
Xsome	Swit_2244	-4.25	ArsR family transcriptional regulator	1	240	-5.42
Xsome	Swit_2339	-2.60	hypothetical protein Swit_2339	6	142	-2.07
Xsome	Swit_2411	-3.38	cytochrome c, class I	0	42	Ess sand
Xsome	Swit_2501	-2.42	hypothetical protein Swit_2501	0	294	Ess sand
Xsome	Swit_2559	-4.04	acyl-CoA synthetase	0	0	Essential
Xsome	Swit_2722	-2.41	NADPH-glutathione reductase	0	101	Ess sand

>Fourfold higher expressed in sand

Location	Systematic Name	Log FC ([Stat-Liq] vs [Stat-Sand])	Annotation	SAND10	TN01	2 log ratio prop SAND10/TN01
Xsome	Swit_2779	-2.16	Ferritin, Dps family protein	1	65	-3.53
Xsome	Swit_2794	-3.32	opacity protein and related surface antigen:	0	17	Ess sand
Xsome	Swit_2877	-2.11	methyltransferase type 12	0	308	Ess sand
Xsome	Swit_2941	-2.15	hypothetical protein Swit_2941	1	130	-4.53
Xsome	Swit_2956	-2.39	MarR family transcriptional regulator	0	0	Essential
Xsome	Swit_2957	-3.43	OsmC family protein	1	101	-4.17
Xsome	Swit_3201	-2.15	thiamine biosynthesis protein ThiC	0	0	Essential
Xsome	Swit_3212	-2.26	citrate synthase	0	0	Essential
Xsome	Swit_3216	-2.53	LexA repressor	0	76	Ess sand
Xsome	Swit_3231	-2.25	RND efflux system outer membrane lipopro	0	31	Ess sand
Xsome	Swit_3232	-2.18	OsmC family protein	0	82	Ess sand
Xsome	Swit_3346	-2.61	hypothetical protein Swit_3346	0	0	Essential
Xsome	Swit_3463	-3.26	cell wall hydrolase, SleB	0	56	Ess sand
Xsome	Swit_3471	-3.02	hypothetical protein Swit_3471	1	136	-4.60
Xsome	Swit_3537	-2.47	CarD family transcriptional regulator	0	0	Essential
Xsome	Swit_3586	-3.58	alkylhydroperoxidase	0	118	Ess sand
Xsome	Swit_3587	-2.88	alkyl hydroperoxide reductase/ Thiol specifi	1	279	-5.63
Xsome	Swit_3723	-4.64	rhodanese domain-containing protein	0	0	Essential
Xsome	Swit_3778	-4.62	hypothetical protein Swit_3778	1	66	-3.55
Xsome	Swit_3858	-2.14	alpha/beta hydrolase fold	1	144	-4.68
Xsome	Swit_3903	-5.27	diacylglycerol kinase, catalytic region	0	0	Essential
Xsome	Swit_3904	-4.97	hypothetical protein Swit_3904	0	0	Essential
Xsome	Swit_3907	-4.19	fatty acid hydroxylase	0	0	Essential
Xsome	Swit_3960	-2.33	hypothetical protein Swit_3960	0	1	Ess sand
Xsome	Swit_3977	-2.54	2Fe-2S iron-sulfur cluster binding domain-c	0	0	Essential
Xsome	Swit_4271	-3.37	2-oxo-hepta-3-ene-1,7-dioic acid hydratase	0	36	Ess sand
Xsome	Swit_4509	-2.86	membrane protease FtsH catalytic subunit	1	102	-4.18
Xsome	Swit_4511	-2.03	secretion protein HlyD family protein	6	492	-3.87
Xsome	Swit_4532	-2.75	sugar transferase	0	153	Ess sand
Xsome	Swit_4656	-2.14	dihydroxy-acid dehydratase	0	0	Essential
Xsome	Swit_4685	-2.92	D-3-phosphoglycerate dehydrogenase	0	0	Essential
Xsome	Swit_4686	-4.00	phosphoserine aminotransferase	0	0	Essential
Xsome	Swit_4764	-2.39	2-octaprenylphenol hydroxylase	0	55	Ess sand
Xsome	Swit_4811	-2.12	hypothetical protein Swit_4811	0	16	Ess sand
Xsome	Swit_4822	-2.08	hypothetical protein Swit_4822	0	98	Ess sand
Xsome	Swit_4872	-2.80	hypothetical protein Swit_4872	0	74	Ess sand
pSWIT01	Swit_5318	-2.96	hypothetical protein Swit_5318	0	167	Ess sand

Table S16 RW1 operons coherently differentially expressed in sand and liquid culture, and judged essential or causing fitness loss upon interruption by transposon marker insertion

Growth phase	Systematic Name	Coding region	Log FC ([Exp-Liq] vs [Exp-Sand])	No Reads SAND4	No Reads TN01	2log ratio	Annotation
Expo phase	Swit_0117	112986..113633	1.28	0	0	essential	TonB family protein
	Swit_0118	113719..114444	1.63	1	412	-6.85	MotA/TolQ/ExbB proton channel
	Swit_0163	155454..155720	-5.26	0	63	essential sand	Type IV secretory pathway TrbD component-like protein
	Swit_0164	155727..158195	-1.42	26	891	-3.26	AAA ATPase
	Swit_0165	158192..158926	-1.57	0	151	essential sand	P-type conjugative transfer protein TrbJ
	Swit_0166	158919..159284	-2.94	0	39	essential sand	hypothetical protein Swit_0166
	Swit_0282	298877..300355	-1.21	61	440	-1.01	AMP-dependent synthetase and ligase
	Swit_0283	300348..301424	-1.65	0	80	essential sand	L-carnitine dehydratase/bile acid-inducible protein F
	Swit_0284	301409..302227	-1.52	0	10	essential sand	xylose isomerase domain-containing protein
	Swit_0285	complement(302373.	-1.06	0	0	essential	ferredoxin
	Swit_0286	302847..303860	-1.46	0	250	essential sand	alcohol dehydrogenase
	Swit_0287	303857..305008	-1.42	2	193	-4.75	hypothetical protein Swit_0287
	Swit_0288	305011..305514	-1.71	0	1	essential sand	hypothetical protein Swit_0288
	Swit_0692	757860..758717	3.02	0	204	essential sand	extracellular solute-binding protein
	Swit_0693	758813..760552	2.94	1	292	-6.35	Pyrrolo-quinoline quinone
	Swit_0694	760566..761324	2.76	1	261	-6.19	two component LuxR family transcriptional regulator
	Swit_1682	1873779..1874339	-1.32	4	312	-4.45	hypothetical protein Swit_1682
	Swit_1683	1874336..1875454	#N/A	#N/A	#N/A	#N/A	amidohydrolase 2
	Swit_1684	1875460..1876581	-1.64	0	172	essential sand	monooxygenase, FAD-binding
	Swit_2013	2253443..2255143	-2.76	0	234	essential sand	dihydroorotase
	Swit_2014	2255145..2256524	#N/A	#N/A	#N/A	#N/A	major facilitator transporter
	Swit_2015	2256533..2256943	#N/A	#N/A	#N/A	#N/A	hypothetical protein Swit_2015
	Swit_2016	2256940..2258106	-1.54	1	787	-7.78	lipid-transfer protein
	Swit_2380	2646488..2647066	1.29	0	0	essential	scaffold protein Nfu/NifU-like protein
	Swit_2381	2647105..2647710	1.92	0	120	essential sand	hypothetical protein Swit_2381
	Swit_2382	2647717..2648331	1.89	0	0	essential	peptidase M22, glycoprotease
	Swit_2383	2648328..2648789	1.21	0	172	essential sand	ribosomal-protein-alanine acetyltransferase
	Swit_2634	2917282..2918634	1.76	6	657	-4.94	benzoate 1,2-dioxygenase, alpha subunit
	Swit_2635	2918631..2919146	1.01	0	36	essential sand	2-chlorobenzoate 1,2-dioxygenase
	Swit_2636	2919160..2919942	1.41	1	47	-3.72	1,6-dihydroxycyclohexa-2,4-diene-1-carboxylate dehydrogenase
	Swit_3056	3354158..3355420	2.04	67	175	0.45	Rieske (2Fe-2S) domain-containing protein
	Swit_3057	3355417..3355893	2.74	0	0	essential	aromatic-ring-hydroxylating dioxygenase, beta subunit
	Swit_3058	3355913..3356533	2.06	1	40	-3.48	maleylacetoacetate isomerase
	Swit_3059	3356580..3357629	1.21	0	216	essential sand	gentisate 1,2-dioxygenase
	Swit_3060	3357651..3358334	1.39	2	66	-3.21	fumarylacetoacetate (FAA) hydrolase
	Swit_3504	complement(3861261	1.69	1	50	-3.81	response regulator receiver protein
	Swit_3505	complement(386238(	0.57	1	77	-4.43	TadE family protein
	Swit_3506	complement(386281(	1.53	0	21	essential sand	Flp/Fap pilin component
	Swit_3507	complement(3863087	1.01	7	320	-3.68	membrane-like protein
	Swit_3508	complement(386480(	2.34	0	0	essential	hypothetical protein Swit_3508
	Swit_3509	complement(3865041	1.80	24	107	-0.32	tetratricopeptide TPR_4
	Swit_3510	complement(3865733	1.79	0	44	essential sand	type II secretion system protein
	Swit_3511	complement(386662(	0.89	1	1	1.84	type II secretion system protein
	Swit_3512	complement(3867528	1.88	1	106	-4.89	type II secretion system protein E
	Swit_3513	complement(3868874	0.92	1	208	-5.86	response regulator receiver protein
	Swit_3514	complement(3870037	-0.07	0	163	essential sand	type II and III secretion system protein
	Swit_3743	4106588..4107232	-1.16	0	61	essential sand	1-Cys peroxiredoxin
	Swit_3744	4107313..4108233	-1.91	0	33	essential sand	beta-lactamase domain-containing protein
	Swit_4189	4610184..4610666	-1.04	0	106	essential sand	aromatic-ring-hydroxylating dioxygenase, beta subunit
	Swit_4190	4610663..4611421	-1.58	0	5	essential sand	short-chain dehydrogenase/reductase SDR
	Swit_4210	4638404..4639288	-1.06	0	14	essential sand	LysR family transcriptional regulator
	Swit_4211	4639396..4640673	-1.22	0	121	essential sand	4-aminobutyrate aminotransferase
	Swit_4249	4682481..4682774	-2.13	0	219	essential sand	antibiotic biosynthesis monooxygenase
	Swit_4250	4682786..4683394	-1.38	7	201	-3.01	glutathione S-transferase domain-containing protein
	Swit_5192	complement(81316..8	-2.40	0	110	essential sand	hypothetical protein Swit_5192
	Swit_5193	complement(82068..8	-3.26	0	147	essential sand	hypothetical protein Swit_5193
	Swit_5194	complement(82900..8	#N/A	#N/A	#N/A	#N/A	hypothetical protein Swit_5194
	Swit_5195	complement(84322..8	-3.97	0	158	essential sand	hypothetical protein Swit_5195
	Swit_5196	complement(84783..8	-4.80	203	1056	-0.75	ParB family protein

Growth phase	Systematic Name	Coding region	Log FC ([Exp-Liq] vs [Exp-Sand])	SAND4	TN01	2log ratio	Annotation
Stationary	Swit_0656	complement(710482.	-2.50	0	37	essential sand	hypothetical protein Swit_0656
	Swit_0657	complement(710992.	-2.60	0	72	essential sand	glutamate synthase (NADPH) large subunit
	Swit_0658	complement(715531.	-3.18	0	35	essential sand	hypothetical protein Swit_0658
	Swit_0659	complement(716073.	-3.62	0	0	essential	putative oxidoreductase
	Swit_0691	757366..757863	1.58	0	346	essential sand	hypothetical protein Swit_0691
	Swit_0692	757860..758717	1.23	0	204	essential sand	extracellular solute-binding protein
	Swit_1152	1298624..1299904	-5.80	81	946	-1.06	RND family efflux transporter MFP subunit
	Swit_1153	1300000..1303188	-4.73	6	739	-4.45	hydrophobe/amphiphile efflux-1 (HAE1) family protein
	Swit_1154	1303200..1304621	-1.18	0	158	essential sand	RND efflux system outer membrane lipoprotein
	Swit_1155	1304708..1306525	#N/A	#N/A	#N/A	#N/A	diguanylate cyclase
	Swit_1156	1306601..1309228	-1.48	2	267	-4.57	peptidase M1, membrane alanine aminopeptidase-like protein
	Swit_1394	complement(1537701	-1.33	0	8	essential sand	cytochrome c1
	Swit_1395	complement(153856(	#N/A	#N/A	#N/A	#N/A	cytochrome b/b6 domain-containing protein
	Swit_1396	complement(1539837	-1.37	0	10	essential sand	ubiquinol-cytochrome c reductase, iron-sulfur subunit
	Swit_3056	3354158..3355420	2.03	1	175	-4.96	Rieske (2Fe-2S) domain-containing protein
	Swit_3057	3355417..3355893	2.19	0	0	essential	aromatic-ring-hydroxylating dioxygenase, beta subunit
	Swit_3058	3355913..3356533	2.47	1	40	-2.83	maleylacetoacetate isomerase
	Swit_3059	3356580..3357629	1.63	13	216	-1.56	gentisate 1,2-dioxygenase
	Swit_3060	3357651..3358334	3.30	0	66	essential sand	fumarylacetoacetate (FAA) hydrolase
	Swit_3230	complement(3551168	-1.73	0	113	essential sand	secretion protein HlyD family protein
	Swit_3231	complement(3552359	-2.25	0	31	essential sand	RND efflux system outer membrane lipoprotein
	Swit_3585	complement(3948148	-1.95	0	0	essential	Serine O-acetyltransferase
	Swit_3586	complement(3948998	-3.58	0	118	essential sand	alkylhydroperoxidase
	Swit_3587	complement(3949662	-2.88	1	279	-5.63	alkyl hydroperoxide reductase/ Thiol specific antioxidant/ Mal allergen
	Swit_3972	4360680..4361207	3.14	0	0	essential	ECF subfamily RNA polymerase sigma-24 factor
	Swit_3973	4361197..4361952	4.07	0	154	essential sand	putative transmembrane anti-sigma factor
	Swit_3976	complement(4363731	-1.67	3	82	-2.28	aldehyde oxidase and xanthine dehydrogenase, molybdopterin binding
	Swit_3977	complement(4365908	-2.54	0	0	essential	2Fe-2S iron-sulfur cluster binding domain-containing protein
	Swit_4691	5159424..5160017	-1.44	0	191	essential sand	hypothetical protein Swit_4691
	Swit_4692	5160014..5160739	#N/A	#N/A	#N/A	#N/A	protein-disulfide isomerase-like protein
	Swit_4693	5160843..5161586	-1.61	1	292	-5.70	protein-disulfide isomerase-like protein
	Swit_5005	complement(122433.	1.10	17	489	-2.36	TrbL/VirB6 plasmid conjugal transfer protein
	Swit_5006	complement(123607.	#N/A	#N/A	#N/A	#N/A	type IV secretion system family protein
	Swit_5007	complement(124328.	1.56	136	1847	-1.28	type IV secretion/conjugal transfer ATPase
	Swit_5008	complement(126738.	1.91	1	261	-5.54	type IV secretory pathway, VirB3 family protein
	Swit_5009	complement(127084.	#N/A	#N/A	#N/A	#N/A	VIRB2 type IV secretion fmaily protein
	Swit_5010	complement(127422.	2.10	2	411	-5.20	lytic transglycosylase, catalytic
	Swit_5056	complement(171717.	2.07	11	1347	-4.45	short-chain dehydrogenase/reductase SDR
	Swit_5057	complement(172514.	2.04	1	65	-3.54	cupin 2 domain-containing protein
	Swit_5058	complement(172949.	1.90	24	1407	-3.39	cyclase family protein
	Swit_5364	272080..272508	6.55	3	49	-1.78	hypothetical protein Swit_5364
	Swit_5365	272550..272855	4.31	12	791	-3.79	Type IV conjugative transfer system protein TraL
	Swit_5366	272857..273423	1.89	3	359	-4.65	TraE family protein
	Swit_5367	273420..274223	1.37	6	587	-4.36	hypothetical protein Swit_5367

CHAPTER IV

Behaviour of *Arthrobacter chlorophenolicus* A6 in liquid cultures and sand inoculations.

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CHAPTER IV. Behaviour of *Arthrobacter chlorophenolicus* A6 in liquid cultures and sand inoculations.

Summary

In order to study survival and activity of *Arthrobacter chlorophenolicus* inoculated into dry sand, experiments were conducted in which sand was contaminated or not with 4-chlorophenol (4CP) in concentrations slightly lower than the maximum tolerable concentration (0.1 mmol per g sand). When inoculated at low cell densities of 2.5x 10^5 CFU per g growth of strain A6 was observed, but as well as in sand without or with 4CP. Interestingly, growth rates in sand on 4CP were higher than in liquid culture, suggesting that A6 can use other carbon compounds from the sand for its growth. Comparison of transcriptomes in exponentially and stationary phase cells in liquid culture helped to identify a series of specific genes and pathways. Further transcriptomes recorded of cells introduced from liquid into sand identified factors important for the immediate reaction of the cells to 4CP. Unfortunately, transcriptomes from cells growing on sand on 4CP were not significantly different from those of non-inoculated sand, and hybridisations displayed very weak signals, suggesting RNA was lost. Further studies are necessary to compare the growth behavior of *A. chlorophenolicus* in sand to that of previous work with *Sphingomonas wittichi*.

Introduction

The genus *Arthrobacter* is commonly found in soil in a broad range of environments. *Arthrobacter* spp are known to resist particularly hard conditions like changes in temperature, dryness, or exposure to toxic contaminants (Mongodin et al 2006). *A. chlorophenolicus* A6 is a soil microorganism, which is able to use a wide number of carbon sources and resist extreme environments (Unell et al 2007, Westerberg et al 2000). The main interest of the strain in biodegradation lies in the efficient mineralization of high concentrations of chloro- and nitrophenols (Elvang et al 2001, Westerberg et al 2000), para-nitrophenol (Sahoo et al 2011) and bromophenols (Sahoo et al 2014).

Chlorophenols, nitrophenols and bromophenols are toxic substances produced either during treatment of wastewaters with chlorine, during bleaching of wood pulps for the manufacture of paper, or in industries implicated in the production of insecticides, explosives, dyes, drugs and treatment of leather (Agency for Toxic Substances and Disease Registry (ATSDR) http://www.atsdr.cdc.gov/). All these compounds are highly recalcitrant and can accumulate in the environment, leading to risks for human, animal and plant health.

Strain A6 was isolated from soil enrichments with increasing concentrations of 4-chlorophenol (4CP) (Westerberg et al 2000). Since then several studies have focused on behaviour of strain A6 in soil environments. Changes in native soil communities after contamination with 4CP and/or inoculation of A6 were followed by Jernberg and Jansson (2002), showing rapid fluctuations in the communities in response to the disturbances applied, probably in order to favour well-adapted populations. The effects of soil temperature on the performance of A6 was studied by Backman et al (2004) and Backman and Jansson (2004). While the first found that

A6 survives better when inoculated at 5°C, the second demonstrates that *Arthrobacter* can degrade 4CP equally efficiently at 28°C or 5°C after 17 days. Unell and collaborators (2008) have shown the capability of A6 in degrading high concentrations of nitrophenols, chlorophenols and phenols in sandy loam soil slurries and with the help of a mutant strain, they suggest a different catabolic pathway for the degradation of phenol. Other studies have analysed the genes implicated in the pathways of degradation of 4CP, which differed from the degradation pathway of this compound by aerobic microorganisms (Nordin et al 2005), and the adaptation of A6 cell membrane fatty acids to different concentrations of phenolic compounds and extreme temperatures by the alteration of their anteiso/iso ratios to counterbalance the fluidity increase caused by the organic solvents or temperature (Unell et al 2007). Finally, the A6 proteome was characterized in the wild type strain and a mutant impaired in growth on 4CP and 4-nitrophenol (4NP) under different chemical exposures and temperatures, this study confirmed the 4CP degradation pathway but not the phenol one (Unell et al 2009).

Since the genome sequence is available from the DOE Joint Genome Institute we created custom-made microarrays to study the transcriptome of A6. Previously, we studied genome-wide transcriptomic changes of strain A6 in conditions of water stress (Chapter II). We also studied transcriptome changes of A6 in the phyllosphere under different moisture conditions (Chapter V) and compared this with experiments of A6 growing on agar surfaces (Scheublin et al 2014). Here the goal was to characterize the A6 genome-wide response in cells growing or not in sand contaminated with or without 4CP, and compare this response to the transcriptional behaviour in liquid cultures growing on 4CP. This approach was previously successfully applied to *Sphingomonas wittichii* RW1 (Moreno-Forero and van der

Meer 2015). The final goal was to eventually compare responses of strain A6 and RW1 in similar sandy soil conditions in presence or absence of contamination to detect differences or commonalities in their response to inoculation.

Materials and Methods

Survival in sand

The strain *A.chlorophenolicus* A6 was initially isolated from soil and found to resist high (up to 2.7 mM) concentrations of 4 chlorophenol (4CP) in slurries of soil (Westerberg et al 2000). Here we used non-sterilized sand collected on a beach of lake Leman (46.5079741 N, 6.545103 E) in the spring of 2013, with a gravimetric water content (GWC) of 4.8%, similar to that used for RW1 growth (50 µl liquid/ g sand, Moreno-Forero and van der Meer 2015). The survival of A6 was measured in bare sand and sand contaminated with decreasing amounts of 4CP (1, 0.5, 0.1, or 0.01 mmols per g sand). The strain was inoculated at a low cell density (2.5 x 10^5 cells in GM (minimal media in $g \cdot l^{-1}$: K_2HPO_4, 2.10; KH_2PO_4, 0.40; NH_4NO_3, 0.50; $MgSO_4 \cdot 7H_2O$, 0.20; $CaCl_2 \cdot 2H_2O$, 0.023; and $FeCl_3 \cdot 6H_2O$, 0.002 (Alexander and Lustigman 1966) without carbon source per g sand) in microcosms consisting of a 15 ml plastic Greiner tube filled with 1 g sand. After 16 h incubation at room temperature (24-26°C) the cells were recovered from the sand by adding 1.5 ml of saline to the microcosm, vortexing during 30 sec, after which tenfold serial dilutions were prepared that were plated on GM agar plates containing 1 mM of 4CP. As plates frequently showed two different colony morphotypes, we performed colony PCR for 10 colonies of each morphotype with primers specific to genes responsible of degradation of 4CP and unique for A6 to differentiate the colonies and identify "true" A6 colonies (Table 1). Once the morphotypes were identified, all counts were restricted to them.

Table 1 Primer specifications

Primer name	Target gene	Sequence	Annealing Temp
140501- pl-1fw	Achl_4564	ctg agc tgt act acg agc cg	59.5
140502-pl-1rev	Achl_4565	Ctg tac ggg cta tgt cgg tc	59.5

Colony forming units (CFU) of verified A6 colonies were plotted as a function of time to establish a "growth curve" of A6 in sand with or without 4CP contamination. In separate microcosms we inoculated 2.5 x 10^5 cells per g of sand, and every 3 h three microcosms were sacrificed to extract the cells as described before to follow the growth of *Arthrobacter* in sand during 60 h. Growth curves of both “treatments” were then "overlaid". Growth of A6 was also followed in liquid cultures, containing GM medium and 1 mM 4CP at 28°C and 180 rpm.

Transcriptional responses of A6 growing in sand and liquid cultures

Once we established the exponential and the stationary phase of growth of A6 either in sand or liquid, we repeated the experiments to extract RNA from samples in the two phases of growth and both environments. Purified RNAs were then used to measure the genome-wide gene expression of *A. chlorophenolicus* under the different conditions. This was further complemented with an analysis of the "immediate" reaction of A6 cells transferred from liquid culture to sand (see below).

For the liquid cultures 80 ml of GM with 4CP (1 mM) were inoculated in quadruplicates at an initial OD of 0.02. 18 hours (exponential phase, OD≈0.15, "LIQ-4CP-EXPO") and 48 h later (stationary, OD≈0.3, "LIQ-4CP-STAT"), 8 ml of stop solution was added (containing 5% buffer-equilibrated phenol, pH 7.4 in ethanol, (Gulez et al 2014, Rhodius and Wade 2009)). The liquid and stop solution were vigorously mixed, after which batches of 20 ml liquid solution were decanted and

filtered over a 0.2 µm membrane filter by vacuum suction. The filter was immediately frozen in liquid nitrogen and put in a bead tube of the Power-soil RNA kit of Mobio maintained in a dry-ice bath with ethanol until all four filters of the same replicate were in the tube. The tubes were then transferred to the freezer at -80°C until RNA extraction.

For sand, we inoculated 160 tubes with 10 g of sand each, with 2.5 x 10^5 cells of A6 per g sand. For each phase of growth (exponential and stationary) 4 replicates of 20 tubes each (6 hours after inoculation for exponential, "SOE", and 12 hours for stationary, "SOS") were taken for extraction of RNA from cells. 15 ml of saline solution (0.9% NaCl) and 1.5 ml of stop solution was added to each tube, vortexed for 30", after which the suspension was filtered over a cell strainer of 40 µm pore size to remove sand grains. The filtrate obtained was then filtered and frozen as described previously for liquid samples.

3 Kg of sand was contaminated with 0.1 mmol 4CP per g and distributed in tubes with 10 g of sand each. These were inoculated with the same starting amount of A6 cells as for the non-contaminated sand control above. The samples were recovered 10, 24 and 55 hours after inoculation to have exponential (10 h, SAND-4CP-EXPO), stationary (24 h, SAND-4CP-STAT) and late stationary (55 h, SAND-4CP-LATE) phase, respectively. Four replicates were taken for each time point, and each replicate consisted of 25 tubes. The procedure of extraction was the same as described previously for non-contaminated sand.

Two negative controls were carried out, each one consisting of 20 tubes of 10 g of 4CP contaminated sand ("NEG-4CP") or bare sand ("NEG-Sand"). The same volume of GM media but without A6 cells was added, after which tubes were left for 24 h. RNA was extracted from the tubes with the same procedure applied above.

To measure the immediate response of A6 after inoculation into sand, 600 ml of exponential phase cells were centrifuged three times for 5 min each, to finally collect 1 ml of cells. These were inoculated into sandy microcosms of 2 g of sand. A set of four replicates (two tubes each per replicate) with sand contaminated with 4CP was inoculated with 80 µl of pellet cells precultured in GM and 4CP as carbon source ("SAND-1H-4CP"). Another set of four replicates ("SAND-1H") was inoculated with cells coming from precultures in GM with yeast extract (0.1%). One hour after inoculation the cells were recovered with 5 ml of saline, vortexing and filtering as described above, after which RNA was extracted.

RNA extraction, labelling and microarray hybridization

The procedure for RNA extraction with the Power-Soil total RNA isolation kit (Mobio laboratories, Carlsbad, CA, USA) was described previously (Moreno-Forero and van der Meer 2015). We used a Power clean Pro RNA clean up kit (Mobio laboratories, Carlsbad, CA, USA) for an extra purification step with the samples in sand due to the more important amount of sand necessary to extract enough RNA for the microarray. The procedure for labelling and microarray hybridization and scanning was performed as described by Moreno-Forero and van der Meer (2015).

Data analysis

For each pairwise comparison we analysed genome-wide expression separately in order to find the genes statistically different between the groups using a t-test with unequal variance (Welch's t-test) to calculate p-values. p-values were then corrected into false discovery rates (FDRs) using the Benjamini and Hochberg procedure for multiple hypotheses testing. Genes statistically significantly different expressed were

considered when the FDR was less than 0.05 and the fold-difference between normalized data was higher than 2.

A principal component analysis was performed with all the samples to verify the reproducibility of replicates and distribution of conditions.

The list of genes differentially expressed was then analysed by Gene Ontology (GO) terminology (GOConsortium et al 2000). The GOEAST tool (Zheng and Wang 2008) under Alexa's algorithm (Alexa et al 2006) was used to further compare the statistical relevance of the identified groups of differentially expressed genes. Unfortunately, several isolations failed to hybridize and resulted in very poor quality signals. These could not be properly compared by statistical analyses.

Results and discussion

Behaviour of A. chlorophenolicus in dry-sand conditions

Figure 1 shows the number of A6 cells recovered 16 h after inoculation of 2.5 x 10^5 cells per g of sand. A6 grows well in non-contaminated soil and at a concentration of 0.01 mmol 4CP per g of sand. In that case, we measured at least an increase of two logs of the number of inoculated cells.

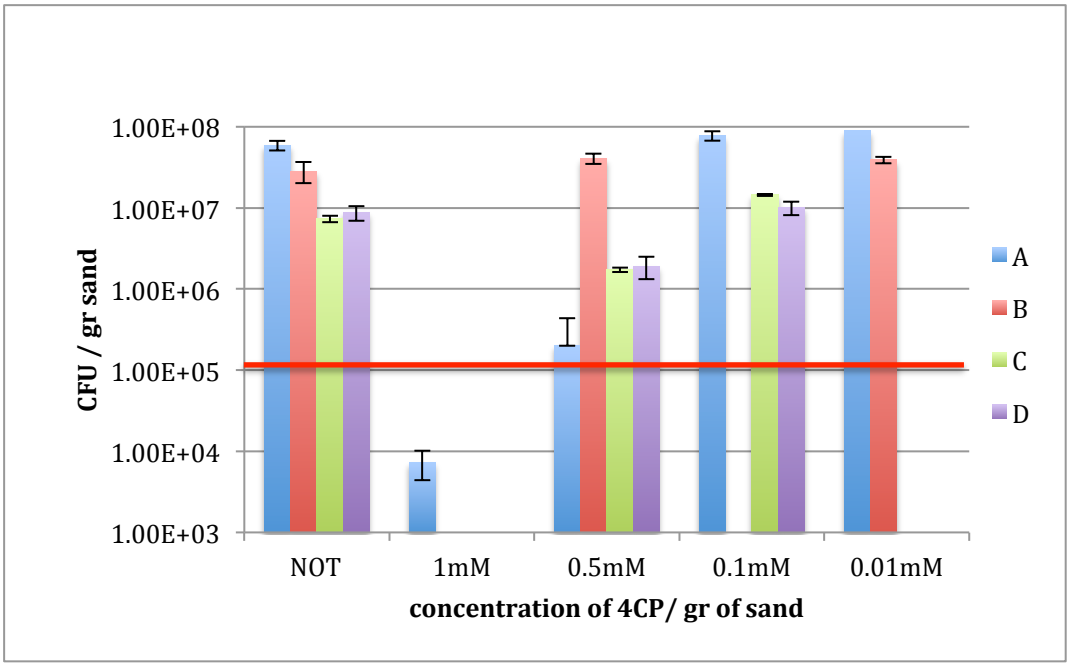

Figure 1: Growth of *A. chlorophenolicus* in clean sand (NOT) or in sand dosed with different amounts of 4CP (mM, mmol dosed per g sand). Initial inoculum size was 2.5 $\cdot 10^5$ cells (CFU) per g of sand (red line). CFU were counted in sand extracts after 16 h of growth. Colonies were verified for being *A. chlorophenolicus* by specific PCR. A,B.C,D refer to independently carried out experiments.

In contrast, at 1 mM *A. chlorophenolicus* seems to not have survived from the inoculation. Only in a single experiment we detected 10^3 cells per g of sand. A dosage of 1 mmol 4CP per g sand corresponds to 128.6 µg per g of sand. Strain A6 has been reported to degrade until 175 µg per g of soil (Elvang et al 2001). But considering the low amount of liquid added to the sand in our case, the 4CP concentration at a dosage of 1 mmol per g sand can represent as high as 2.6 mg per ml. In the Elvang paper the amount of liquid in the soil is not exactly specified, apart from having 20% of moisture. In liquid cultures the maximum 4CP concentration tolerated by *A. chlorophenolicus* is 350 µg per ml, which could explain why A6 did not survive in sand with 1 mM dosage of 4CP, but can grow at dosages of 10 times lower. In the following, we chose a dosage of 0.1 mM 4CP per g sand to follow the growth of A6 and study transcriptome changes.

Growth in sand

The A6 population in uncontaminated sand developed until a population size of at least two logs more than the initial inoculum (up to 2.7 x 10^7 cells per g, Figure 2, white squares).

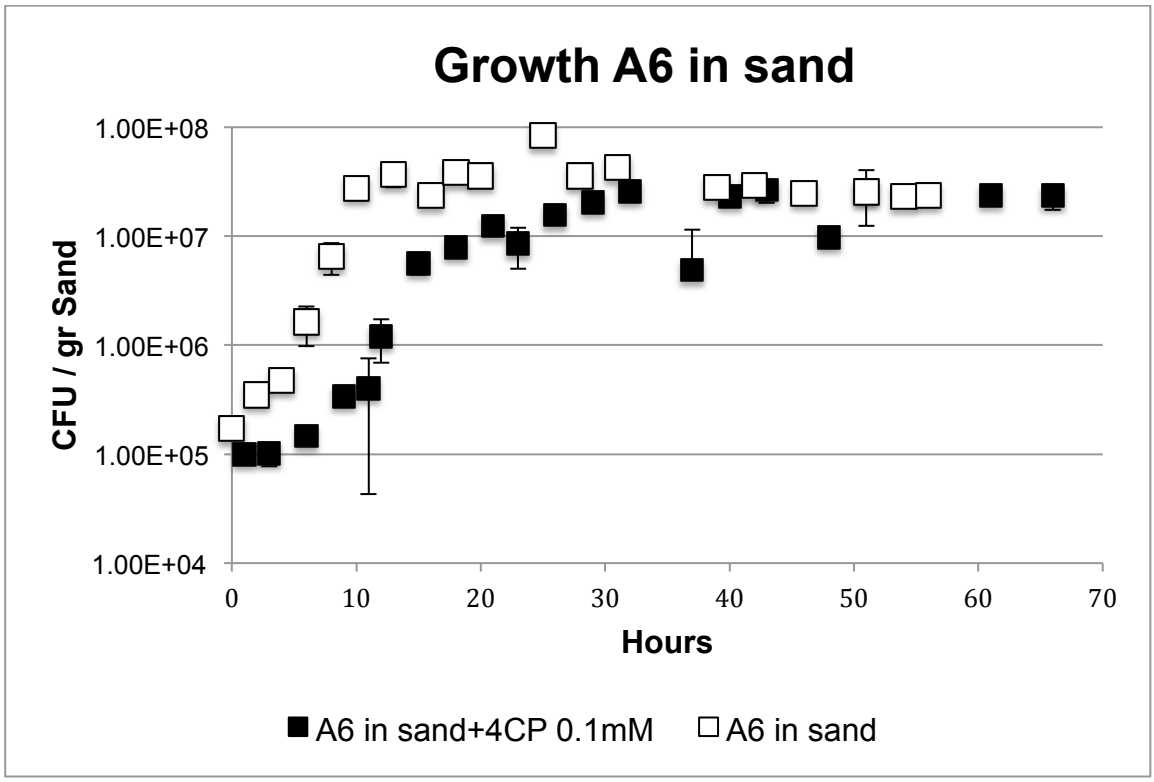

Figure 2. Growth curve of *A. chlorophenolicus* A6 in uncontaminated sand (white squares) or contaminated sand (black squares) with 0.1 mmol 4CP per g of sand.

Cells reached stationary phase after 10 h and remained without further growth afterwards. In 4CP-contaminated sand (0.1 mmol per g dosage) the population of *A. chlorophenolicus* increased more slowly than in non-contaminated sand (e.g., an estimated μ=0.679 in uncontaminated and μ=0.3153 in 4-CP contaminated sand). The final population size was similar (i.e., 2.6 x 10^7 cells per g) after 30 h of growth (Figure 2, black squares). Interestingly, growth in sand was faster than in liquid cultures with 4CP as sole carbon and energy source (Figure 3). A6 is known to have a great versatility to uses different carbon sources. Perhaps the soil environment offers a greater variety of carbon sources, even though the percentage of organic

matter is only 0.028 ± 0.005, allowing faster growth than in liquid (Westerberg et al 2000). This could explain the increase of growth rate in 4CP-contaminated sand compared with 4CP in liquid (μ=0.0944 in liquid, μ= 0.3153 in 4CP-contaminated sand). In order to address the question whether *A. chlorophenolicus* really degraded 4CP in soil, it would thus be necessary to quantify the amount of 4CP over time. It has been shown that A6 can degrade 4CP in soil microcosms with 25% of moisture from 180 μg per g of soil to less than 10 μg per g after 8 days with a starting inoculum of 2.8 x 10^8 cells per g (Jernberg and Jansson 2002). Degradation in soil slurries consisting of 30 g soil plus 300 ml of buffer, attained from a concentration of 5 mM to 0 in 14 h. However in this case, the exact starting number of cells was not described (Unell et al 2008).

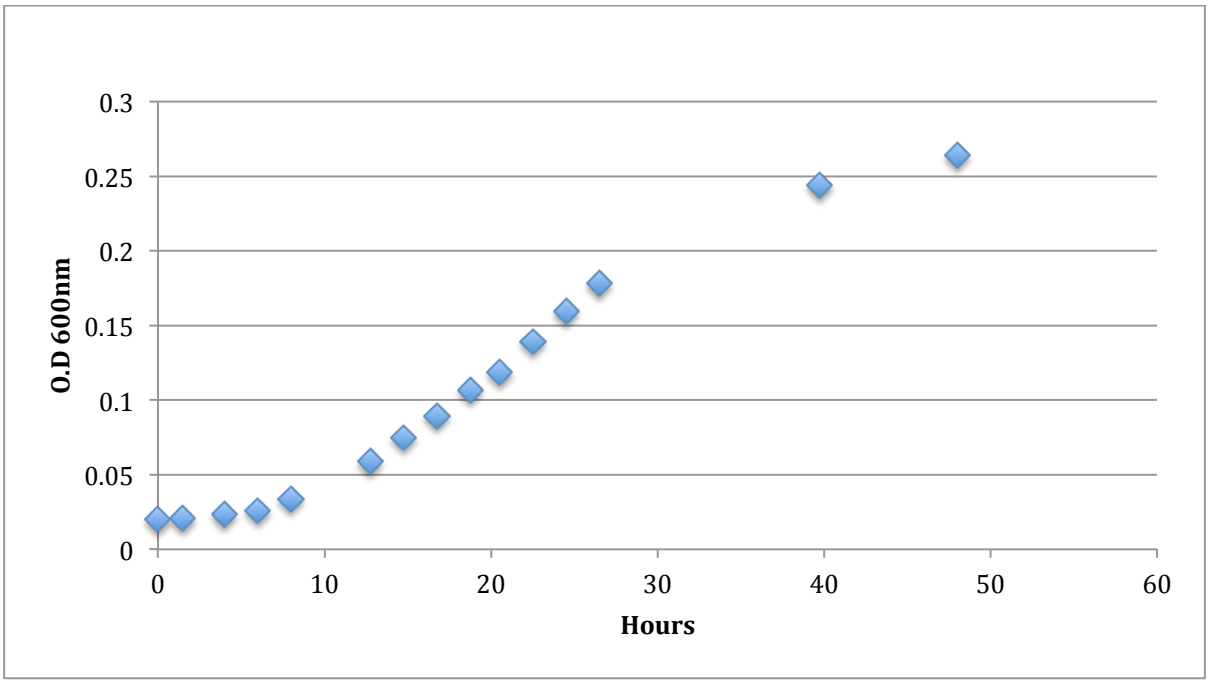

Figure 3. Growth curve of *A. chlorophenolicus* in liquid GM medium with 1 mM 4CP.

Transcriptome analysis

In order to compare the transcriptional responses of A6 both in sand and liquid growth, we performed an extraction of cells in exponential and stationary phases in each environment. In addition, we included one more sample in late stationary phase in sand to measure possible changes in transcriptional state after the presumed degradation of 4CP.

Unfortunately, we experienced severe problems to collect A6 cells in cultures with 4CP. Although in other experiments cells could be rapidly recovered from suspension by filtration, for unknown reasons *A. chlorophenolicus* cultures growing in minimal media with 4CP filtered extremely slowly (e.g., more than 10 min for a 10 ml culture in contrast to 1-2 min for cultures without 4CP). The reasons for this have not become clear. Cells looked similar in phase-contrast microscopy in 4CP as in in GM plus yeast extract cultures. Somehow it seemed that cultures became extremely hydrophobic after growth on 4CP, causing repulsion to the filter. We tried to fix the cells during the filtration process to avoid RNA degradation and changes in transcriptome due to the time of filtration, by adding a stop solution of 5% of phenol in ethanol (Gulez et al 2014, Rhodius and Wade 2009). The same procedure was then applied for sand samples after extraction with saline.

The other surprise was that the hybridisation signals on micro-arrays with RNA isolated from sand-growth experiments (either contaminated with 4CP or not) were extremely low and hardly distinguishable from non-inoculated negative controls. Table 2 shows raw representative hybridisation values for a set of genes in A6 responsible for the degradation of 4CP for the different RNA samples. Clearly, the values obtained in RNA isolated from sand in exponential and stationary phases are

extremely low compared with liquid cultures and sometimes even lower than the negative controls. These results could therefore not be interpreted further.

Figure 5 shows a two dimensional PCA of micro-array hybridisations with RNA from liquid and contaminated sand. We did observe a net separation between the samples from contaminated sand in exponential phase (SAND-4CP-EXPO) and stationary (SAND-4CP-STAT), from the negative controls (NEG). However, this seems mainly due to highly expressed genes like ribosomal RNAs, which were still detectable on the micro-arrays despite the overall low signals. The NEG control hybridisations also showed signals from ribosomal RNAs even though no A6 was inoculated. Those signals may therefore come from other *Arthrobacter* in the soil. A clear separation was detected between samples taken from exponential growth in liquid (LIQ-4CP-EXPO) versus stationary phase (LIQ-4CP-STAT). Also samples isolated from 1 h contact of cells in sand (SAND-1H and SAND-1H-4CP) grouped together and were very close to samples in liquid exponential phase.

Table 2. Raw signal values of some genes of *Arthrobacter chlorophenolicus* implicated in degradation of 4CP in the different arrays performed in sand and liquid

	Negative controls		Sand + 4CP- Exponential				Sand + 4CP- Stationary				Liquide + 4CP exponential			Liquide + 4CP-stationary			Sand 1 hour			Sand + 4CP 1 hour		
Locus Tag	Neg-4CP	Neg-Sand	10h-1	10h-2	10h-3	10h-4	24h-1	24h-2	24h-3	24h-4	LE1	LE3	LE4	LS1	LS3	LS4	S-1h-1	S-1h-3	S-1h-4	1h4CP-1	1h4CP-2	1h4CP-3
Achl_4564	83.2	58.7	10.0	7.2	3.8	13.4	38.9	107.7	101.1	100.3	4953.0	6425.5	3610.8	86.4	42.6	75.3	34.3	62.3	61.4	2976.9	2199.6	1377.2
Achl_4565	290.1	175.3	7.4	10.2	6.5	6.0	39.8	93.3	107.3	150.0	3506.6	4946.4	2888.9	103.7	52.8	59.6	42.6	78.2	79.4	1503.9	1203.2	723.0
Achl_4566	59.0	34.8	7.8	8.4	8.2	9.4	56.9	112.4	134.8	188.5	4638.5	5844.6	3973.4	60.2	32.1	26.4	42.3	91.0	61.4	1910.9	1414.7	1156.2
Achl_4567	175.8	187.1	3.2	3.7	3.8	4.8	6.8	6.7	5.6	8.8	489.0	620.1	442.2	218.9	91.3	135.1	16.2	44.7	58.1	124.4	122.3	84.2
Achl_4568	143.3	33.3	5.3	6.9	7.0	6.6	38.5	70.0	75.2	118.0	2179.8	3320.4	2196.9	34.0	28.7	24.7	17.3	44.6	31.1	2706.9	2788.6	1665.0
Achl_4569	132.7	31.1	3.3	9.3	9.1	3.6	94.9	137.2	142.0	366.1	4810.3	6785.0	5048.0	106.8	59.3	59.3	39.0	87.4	62.3	9287.6	7648.0	5171.9
Achl_4570	46.5	26.7	4.3	6.2	4.6	5.7	38.1	190.9	198.2	269.6	5340.1	11668.2	6672.8	89.4	43.3	59.1	32.1	66.9	48.6	11807.2	8580.0	6161.7
Achl_4571	596.0	96.8	3.5	5.1	3.9	6.9	4.8	14.3	10.8	10.9	136.8	203.2	195.5	122.6	68.0	88.4	192.2	373.1	328.5	339.3	303.3	178.1
Achl_4572	88.4	21.3	8.0	8.9	4.7	7.8	275.2	869.7	1097.8	976.5	63155.8	75304.4	47251.5	198.4	127.2	175.1	56.8	109.3	91.4	22681.7	22453.3	14691.8
Achl_4573	89.6	25.1	20.5	36.0	10.0	16.5	704.1	1321.8	1539.1	3004.4	73087.1	147718.3	92250.0	1999.1	1095.5	1962.6	124.9	239.2	172.8	36329.8	29376.8	22648.2
Achl_4574	291.9	195.4	4.9	6.0	5.8	4.3	27.1	87.6	78.3	151.5	7027.9	10276.7	6742.4	513.3	211.4	243.1	160.4	280.4	255.8	3542.2	3533.2	2144.1

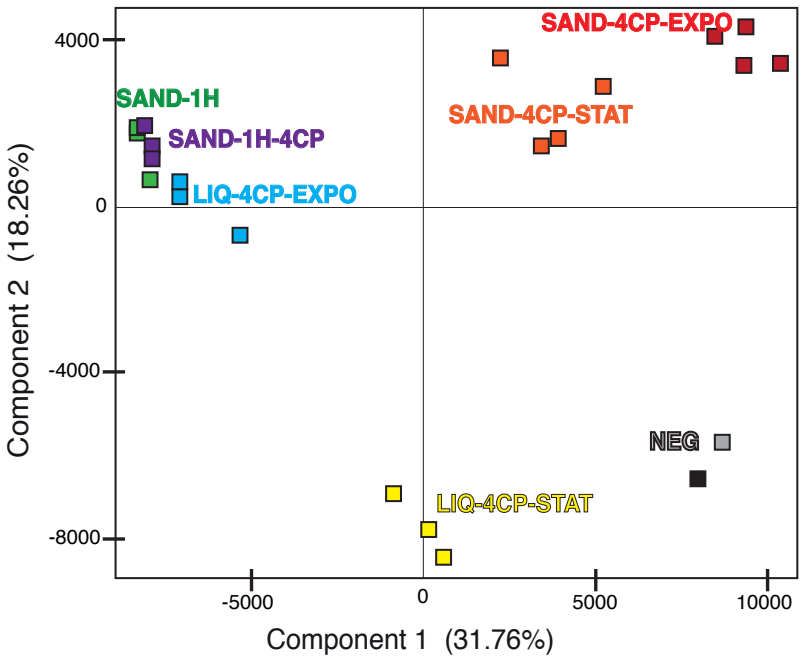

Figure 5. Two dimensional PCA of the microarray samples of *A. chlorophenolicus*. LIQ-4CP-EXPO, liquid cultures on GM with 4CP in exponential phase. LIQ-4CP-STAT, idem stationary phase. SAND-4CP-EXPO, Sand contaminated with 4CP in exponential phase; SAND-4CP-STAT, early stationary phase, SAND-1H-4CP, cells from sand with 4CP after 1 h of incubation. SAND-1H, cells from clean sand after 1 h incubation. NEG, negative controls of uninoculated sand with (black symbol) or without 4CP (grey symbol).

Due to the poor quality of the data sets for growing A6 cells in contaminated and uncontaminated sand, we did not use those data for further analysis. Samples from liquid media in exponential versus stationary phase, and the differences for the one-hour inoculation between contaminated and uncontaminated sand are described in the following in phenomenological terms.

Without surprise the comparison between the two phases of growth in liquid cultures showed that cells are highly active during exponential phase (649 genes were found higher expressed than in stationary phase). A large part of those are genes related to

energy generation (Table 3). GO analysis of the genes higher expressed under exponential phase showed preponderance of terms related with metabolic process (GO:0009225, GO:0006541, GO:0032787, GO:0006568, GO:0042180, GO:0009308, GO:0019318), translation (GO:0006412), gluconeogenesis (GO:0006094), cellular respiration (GO:0045333), and oxidation-reduction processes (GO:0055114, Table 4). Other terms showed activation of cellular membranes (GO:0016998, GO:0008610), and motility (GO:0001539, GO:0030031).

575 genes were found lower expressed in exponential than in stationary phase (Table 3 and 4). Most of those are related to transport (GO:0006810, GO:0008643) or response to stress (GO:0006950), whereas others are related with amino acid metabolism (GO:0006547, GO:0006544) and breakdown of organic compounds (GO:0009310). This indicates that *A. chlorophenolicus* does not need to make great efforts to obtain enough energy during the exponential phase of growth in liquid cultures.

Similar as for *S. wittichii* RW1 and sand with dibenzofuran (Moreno-Forero and van der Meer 2015), strain A6 is very active after 1 h of incubation in sand with 4CP compared with to uncontaminated sand. 167 genes were higher expressed in contaminated sand (Table 5), among which a number of dehydogenases, dioxygenases and monooxygenases. In addition, almost all of the genes within the cluster implicated in degradation of 4CP are highly expressed in cells inoculated in sand with 4CP compared to uncontaminated sand. This behaviour is confirmed by GO analysis (Table 6), where the terms related to energy generation are highly enriched in 4CP compared to uncontaminated sand (e.g., glucogenesis GO:0006094, tricarboxilic acid cycle GO:0006099, oxidation reduction process GO:0055114, monocarboxylic acid metabolic process GO:0032787). In absence of 4CP 162 genes

were higher expressed (Table 5). These are related with transport or flagella, but we also find quite a few of genes for hypothetical proteins.

Unfortunately the work remains a little incomplete, because of the difficulties to obtain a reliable print of the behaviour of *Arthrobacter* during long-term growth in contaminated sand. Therefore, we could not analyze A6 genes specifically induced or repressed during growth in sand compared to liquid culture. It might be necessary to repeat these experiments while trying to improve the filtration speed of cultures grown on 4CP. Perhaps the poor hybridization signals obtained from soil cultures were due to important losses of A6 cells during extraction and filtration, which might be related to the capability of the strain to rapidly change cell shape, like observed in presence of matric stress (Chapter II). Changes in cell shape have been reported for A6 when entering stationary phase (van Elsas et al 2007). It will be further necessary to study the importance of water content in soil for the biodegradation of high amounts of 4CP by *Arthrobacter*, because it seems an important factor to consider more carefully if the strain is intended for 4CP biodegradation in soils.

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Table 3. Liste de genes differentially expressed for *A. chlorophenolicus* in the comparison liquid cultures with 4CP in exponetial vs stationary phase

Gene ID	Fold change	Regulation LE vs LS	annotation	Gene ID	Fold change	Regulation LE vs LS	annotation
Achl_0001	2.58	up	chromosomal replication initiator protein DnaA	Achl_0005	2.20	down	protein of unknown function DUF721
Achl_0002	4.47	up	DNA polymerase III, beta subunit	Achl_0030	3.01	down	binding-protein-dependent transport systems inner membrane component
Achl_0006	4.66	up	DNA gyrase, B subunit	Achl_0045	4.89	down	hypothetical protein Achl_0045
Achl_0009	5.78	up	hypothetical protein Achl_0009	Achl_0049	2.59	down	hypothetical protein Achl_0049
Achl_0012	2.16	up	hypothetical protein Achl_0012	Achl_0050	4.26	down	hypothetical protein Achl_0050
Achl_0013	3.02	up	Peptidylprolyl isomerase	Achl_0051	6.93	down	protein of unknown function DUF1469
Achl_0014	2.35	up	Rhomboid family protein	Achl_0052	3.34	down	hypothetical protein Achl_0052
Achl_0017	5.27	up	sortase family protein	Achl_0093	2.49	down	protein of unknown function DUF112 transmembrane
Achl_0018	3.10	up	hypothetical protein Achl_0018	Achl_0100	2.82	down	protein of unknown function DUF6 transmembrane
Achl_0019	4.45	up	glutamine amidotransferase of anthranilate synth	Achl_0104	3.44	down	major facilitator superfamily MFS_1
Achl_0024	4.58	up	protein serine/threonine phosphatase	Achl_0111	2.44	down	hypothetical protein Achl_0111
Achl_0046	3.45	up	Glyoxalase/bleomycin resistance protein/dioxyger	Achl_0125	2.77	down	transcriptional regulator, AraC family
Achl_0076	4.04	up	putative integral membrane protein	Achl_0127	3.25	down	hypothetical protein Achl_0127
Achl_0082	2.33	up	protein of unknown function DUF1185	Achl_0129	2.30	down	transcriptional regulator, TetR family
Achl_0083	2.35	up	protein of unknown function DUF1185	Achl_0137	2.99	down	oxidoreductase domain protein
Achl_0086	5.34	up	4-oxalocrotonate tautomerase	Achl_0156	2.61	down	hypothetical protein Achl_0156
Achl_0087	5.02	up	Luciferase-like monooxygenase	Achl_0166	5.50	down	Resolvase domain protein
Achl_0105	3.64	up	hypothetical protein Achl_0105	Achl_0182	2.65	down	Peptidase M1 membrane alanine aminopeptidase
Achl_0121	20.66	up	phosphoenolpyruvate synthase	Achl_0198	2.41	down	PfkB domain protein
Achl_0122	5.70	up	protein of unknown function DUF299	Achl_0220	4.61	down	DNA-directed DNA polymerase
Achl_0176	2.91	up	major facilitator superfamily MFS_1	Achl_0231	5.33	down	hypothetical protein Achl_0231
Achl_0211	2.18	up	protein of unknown function DUF125 transmembr	Achl_0237	15.94	down	hypothetical protein Achl_0237
Achl_0235	3.89	up	hypothetical protein Achl_0235	Achl_0238	13.97	down	hypothetical protein Achl_0238
Achl_0266	12.38	up	nitroreductase	Achl_0252	2.24	down	Xylose isomerase domain protein TIM barrel
Achl_0267	2.66	up	phosphoglucomutase, alpha-D-glucose phosphate	Achl_0255	2.01	down	extracellular solute-binding protein family 1
Achl_0281	3.22	up	Rhodanese domain protein	Achl_0261	3.40	down	Pseudogene
Achl_0285	2.64	up	seryl-tRNA synthetase	Achl_0268	3.33	down	NERD domain protein
Achl_0299	2.80	up	Cof-like hydrolase	Achl_0276	5.42	down	uncharacterized integral membrane protein-like protein
Achl_0306	3.42	up	Inorganic diphosphatase	Achl_0277	4.80	down	hypothetical protein Achl_0277
Achl_0312	2.74	up	Glyoxalase/bleomycin resistance protein/dioxyger	Achl_0278	15.92	down	hypothetical protein Achl_0278
Achl_0315	3.80	up	GTP cyclohydrolase I	Achl_0286	4.48	down	hypothetical protein Achl_0286
Achl_0316	3.70	up	dihydropteroate synthase	Achl_0290	3.84	down	major facilitator superfamily MFS_1
Achl_0327	2.51	up	alpha/beta hydrolase fold protein	Achl_0297	2.77	down	binding-protein-dependent transport systems inner membrane component
Achl_0329	5.31	up	lysyl-tRNA synthetase	Achl_0302	2.16	down	hypothetical protein Achl_0302
Achl_0330	7.13	up	hypothetical protein Achl_0330	Achl_0338	5.50	down	hypothetical protein Achl_0338
Achl_0346	3.43	up	peptide chain release factor 3	Achl_0353	2.00	down	hypothetical protein Achl_0353
Achl_0361	2.55	up	hypothetical protein Achl_0361	Achl_0373	3.58	down	peptidase C60 sortase A and B
Achl_0456	2.20	up	Xylose isomerase domain protein TIM barrel	Achl_0391	2.22	down	urease, beta subunit
Achl_0518	2.96	up	General substrate transporter	Achl_0394	2.21	down	Urease accessory protein UreF
Achl_0591	2.93	up	hypothetical protein Achl_0591	Achl_0416	3.52	down	ANTAR domain protein with unknown sensor
Achl_0593	5.69	up	UspA domain protein	Achl_0420	3.47	down	twin-arginine translocation pathway signal
Achl_0594	6.19	up	protein of unknown function DUF112 transmembr	Achl_0428	2.03	down	hypothetical protein Achl_0428
Achl_0595	8.01	up	putative integral membrane protein	Achl_0449	3.32	down	Alpha/beta hydrolase fold-3 domain protein
Achl_0596	13.89	up	hypothetical protein Achl_0596	Achl_0458	2.55	down	hypothetical protein Achl_0458
Achl_0601	2.54	up	Xanthine/uracil/vitamin C permease	Achl_0467	2.02	down	Allophanate hydrolase subunit 2
Achl_0628	2.01	up	hypothetical protein Achl_0628	Achl_0480	2.51	down	alpha amylase catalytic region
Achl_0629	4.35	up	exodeoxyribonuclease III Xth	Achl_0487	3.66	down	aldo/keto reductase
Achl_0651	3.95	up	NAD+ synthetase	Achl_0509	6.03	down	hypothetical protein Achl_0509

Gene ID	Fold change	Regulation LE vs LS	annotation	Gene ID	Fold change	Regulation LE vs LS	annotation
Achl_0652	3.07	up	orotate phosphoribosyltransferase	Achl_0514	3.02	down	aceytltranferase
Achl_0656	2.51	up	HAD-superfamily hydrolase, subfamily IIA	Achl_0519	2.96	down	hypothetical protein Achl_0519
Achl_0658	2.91	up	fructose-bisphosphate aldolase, class II	Achl_0522	3.53	down	Methyltransferase type 11
Achl_0659	4.33	up	hypothetical protein Achl_0659	Achl_0523	3.50	down	Activator of Hsp90 ATPase 1 family protein
Achl_0660	2.35	up	hypothetical protein Achl_0660	Achl_0525	3.76	down	PTS system, fructose subfamily, IIC subunit
Achl_0666	4.54	up	peptidase M14 carboxypeptidase A	Achl_0526	2.40	down	1-phosphofructokinase
Achl_0667	2.32	up	Adenylosuccinate synthase	Achl_0527	3.75	down	transcriptional regulator, DeoR family
Achl_0675	3.15	up	Antibiotic biosynthesis monooxygenase	Achl_0528	3.13	down	hypothetical protein Achl_0528
Achl_0678	5.56	up	phosphoribosylformylglycinamidine synthase II	Achl_0553	2.30	down	O-methyltransferase family 3
Achl_0679	3.43	up	phosphoribosylformylglycinamidine synthase I	Achl_0554	2.52	down	Ferritin Dps family protein
Achl_0689	6.03	up	aspartate kinase	Achl_0559	5.61	down	ribonuclease BN
Achl_0704	8.87	up	DSBA oxidoreductase	Achl_0562	7.29	down	hypothetical protein Achl_0562
Achl_0710	12.48	up	malate synthase A	Achl_0563	3.60	down	hypothetical protein Achl_0563
Achl_0711	21.47	up	isocitrate lyase	Achl_0565	3.10	down	RNA polymerase, sigma 28 subunit, FliA/WhiG subfamily
Achl_0717	3.64	up	hypothetical protein Achl_0717	Achl_0577	3.05	down	General substrate transporter
Achl_0785	2.03	up	oxidoreductase domain protein	Achl_0579	2.21	down	hypothetical protein Achl_0579
Achl_0796	3.94	up	hypothetical protein Achl_0796	Achl_0582	3.42	down	Lysine exporter protein (LYSE/YGGA)
Achl_0802	4.81	up	hypothetical protein Achl_0802	Achl_0588	2.45	down	urocanate hydratase
Achl_0804	2.32	up	two component transcriptional regulator, winged	Achl_0604	2.45	down	UspA domain protein
Achl_0805	2.35	up	putative two component transcriptional regulator	Achl_0606	5.28	down	Pseudogene
Achl_0806	2.57	up	putative phosphohistidine phosphatase, SixA	Achl_0607	2.44	down	ABC transporter related
Achl_0811	2.18	up	YhgE/Pip C-terminal domain protein	Achl_0608	2.08	down	putative ABC transporter integral membrane protein
Achl_0814	3.04	up	Phosphoenolpyruvate carboxykinase (GTP)	Achl_0610	2.23	down	cation diffusion facilitator family transporter
Achl_0818	3.40	up	Phosphomannomutase	Achl_0623	5.09	down	extracellular solute-binding protein family 1
Achl_0825	3.56	up	5'-Nucleotidase domain protein	Achl_0624	5.48	down	binding-protein-dependent transport systems inner membrane component
Achl_0826	2.33	up	Glyoxalase/bleomycin resistance protein/dioxyger	Achl_0626	5.51	down	hypothetical protein Achl_0626
Achl_0832	3.06	up	hypothetical protein Achl_0832	Achl_0672	4.50	down	2OG-Fe(II) oxygenase
Achl_0835	6.34	up	aldo/keto reductase	Achl_0673	2.43	down	protein of unknown function DUF419
Achl_0841	3.29	up	TAP domain protein	Achl_0685	2.55	down	hypothetical protein Achl_0685
Achl_0847	5.22	up	phosphoglycerate mutase 1 family	Achl_0686	4.84	down	transcriptional regulator, MarR family
Achl_0848	2.09	up	phosphate uptake regulator, PhoU	Achl_0687	6.83	down	hypothetical protein Achl_0687
Achl_0851	3.68	up	hypothetical protein Achl_0851	Achl_0688	23.59	down	hypothetical protein Achl_0688
Achl_0861	6.07	up	alpha amylase catalytic region	Achl_0692	3.56	down	putative integral membrane protein
Achl_0862	7.55	up	trehalose synthase	Achl_0718	3.66	down	transcriptional regulator, TetR family
Achl_0869	2.70	up	Alpha,alpha-trehalose-phosphate synthase (UDP-f	Achl_0731	2.69	down	hypothetical protein Achl_0731
Achl_0870	9.53	up	DSBA oxidoreductase	Achl_0732	2.85	down	hypothetical protein Achl_0732
Achl_0890	4.68	up	hypothetical protein Achl_0890	Achl_0738	5.04	down	short-chain dehydrogenase/reductase SDR
Achl_0891	3.17	up	hypothetical protein Achl_0891	Achl_0739	3.61	down	acyl-CoA dehydrogenase domain protein
Achl_0894	5.89	up	chaperonin GroEL	Achl_0740	2.09	down	protein of unknown function DUF1684
Achl_0910	7.71	up	phosphoserine aminotransferase	Achl_0752	6.74	down	hypothetical protein Achl_0752
Achl_0911	5.35	up	iron (metal) dependent repressor, DtxR family	Achl_0763	2.28	down	aminoglycoside phosphotransferase
Achl_0912	2.71	up	Peptidase M23	Achl_0766	3.82	down	hypothetical protein Achl_0766
Achl_0913	34.33	up	NLP/P60 protein	Achl_0779	2.41	down	oxidoreductase domain protein
Achl_0914	23.65	up	NLP/P60 protein	Achl_0808	2.86	down	protein of unknown function DUF6 transmembrane
Achl_0932	14.02	up	succinyl-CoA synthetase, beta subunit	Achl_0829	2.60	down	ABC transporter related
Achl_0933	10.06	up	succinyl-CoA synthetase, alpha subunit	Achl_0839	2.35	down	FAD-dependent pyridine nucleotide-disulphide oxidoreductase
Achl_0973	2.83	up	hypothetical protein Achl_0973	Achl_0846	3.05	down	hypothetical protein Achl_0846
Achl_0982	2.73	up	hypothetical protein Achl_0982	Achl_0864	2.29	down	hypothetical protein Achl_0864

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Achl_0983	3.35	up	hypothetical protein Achl_0983	Achl_0865	2.17	down	D-lactate dehydrogenase (cytochrome)
Achl_0984	3.63	up	biotin synthase	Achl_0871	2.82	down	ChaB family protein
Achl_0988	3.50	up	NADH:flavin oxidoreductase/NADH oxidase	Achl_0872	3.01	down	Elongation factor G
Achl_0996	2.01	up	Transglycosylase domain protein	Achl_0873	3.63	down	major facilitator superfamily MFS_1
Achl_0998	4.02	up	hypothetical protein Achl_0998	Achl_0876	13.78	down	hypothetical protein Achl_0876
Achl_1045	4.20	up	major facilitator superfamily MFS_1	Achl_0877	2.73	down	amino acid permease-associated region
Achl_1094	3.84	up	protein of unknown function DUF1016	Achl_0881	2.11	down	glycine dehydrogenase
Achl_1135	4.93	up	carbohydrate kinase, thermoresistant glucokinase	Achl_0882	2.09	down	glycine cleavage system T protein
Achl_1164	4.57	up	phosphoribosylglycinamide formyltransferase	Achl_0892	2.93	down	cold-shock DNA-binding domain protein
Achl_1167	3.50	up	phosphoribosylaminoimidazolecarboxamide form	Achl_0897	2.23	down	protein of unknown function DUF909
Achl_1189	3.68	up	Glycine hydroxymethyltransferase	Achl_0900	2.12	down	polysaccharide deacetylase
Achl_1190	5.34	up	Methenyltetrahydrofolate cyclohydrolase	Achl_0901	3.51	down	hypothetical protein Achl_0901
Achl_1195	2.69	up	exodeoxyribonuclease III Xth	Achl_0902	3.69	down	protein of unknown function DUF427
Achl_1196	2.76	up	tryptophanyl-tRNA synthetase	Achl_0937	3.27	down	PfkB domain protein
Achl_1197	2.21	up	Phosphoesterase HXTX	Achl_0938	2.56	down	hypothetical protein Achl_0938
Achl_1201	17.57	up	succinate dehydrogenase and fumarate reductase	Achl_0941	2.90	down	Xylose isomerase domain protein TIM barrel
Achl_1202	13.38	up	succinate dehydrogenase, flavoprotein subunit	Achl_0942	2.46	down	Inositol 2-dehydrogenase
Achl_1203	4.00	up	putative succinate dehydrogenase, membrane sul	Achl_0944	2.21	down	sugar transporter
Achl_1206	2.67	up	amidohydrolase	Achl_0947	4.44	down	hypothetical protein Achl_0947
Achl_1213	2.12	up	inner-membrane translocator	Achl_0954	2.63	down	hypothetical protein Achl_0954
Achl_1218	2.22	up	adenosine deaminase	Achl_0955	2.08	down	Resolvase domain protein
Achl_1220	6.13	up	Phosphopyruvate hydratase	Achl_0958	2.27	down	transcriptional regulator, GntR family
Achl_1221	3.68	up	Septum formation initiator	Achl_0966	2.92	down	hypothetical protein Achl_0966
Achl_1225	3.19	up	FAD-dependent pyridine nucleotide-disulphide ox	Achl_0968	2.24	down	thiamine pyrophosphate protein central region
Achl_1235	2.46	up	Aldose 1-epimerase	Achl_0971	2.40	down	ABC transporter related
Achl_1236	2.72	up	protein of unknown function UPF0118	Achl_0985	3.34	down	permease for cytosine/purines uracil thiamine allantoin
Achl_1252	7.32	up	fumarate lyase	Achl_0989	2.40	down	hypothetical protein Achl_0989
Achl_1253	3.38	up	carbonic anhydrase	Achl_1001	3.70	down	transcriptional regulator, GntR family
Achl_1255	3.52	up	fructose-1,6-bisphosphatase, class II	Achl_1007	2.90	down	2-dehydro-3-deoxyphosphogluconate aldolase/4-hydroxy-2-oxoglutarate al
Achl_1257	3.99	up	mannose-6-phosphate isomerase, class I	Achl_1016	3.41	down	Carboxymethylenebutenolidase
Achl_1259	4.03	up	phosphoribosylaminoimidazole carboxylase, catal	Achl_1022	2.36	down	Cupin 2 conserved barrel domain protein
Achl_1260	4.14	up	phosphoribosylaminoimidazole carboxylase, ATPa	Achl_1027	2.09	down	hypothetical protein Achl_1027
Achl_1263	2.13	up	transcription factor WhiB	Achl_1028	4.15	down	Glyoxalase/bleomycin resistance protein/dioxygenase
Achl_1264	2.54	up	glycosyl transferase, family 2	Achl_1033	2.78	down	SOUL heme-binding protein
Achl_1265	2.22	up	hypothetical protein Achl_1265	Achl_1038	2.74	down	hypothetical protein Achl_1038
Achl_1269	5.32	up	adenosylhomocysteinase	Achl_1039	6.78	down	protein of unknown function DUF1486
Achl_1273	2.03	up	integral membrane protein TIGR01906	Achl_1040	2.41	down	hypothetical protein Achl_1040
Achl_1282	5.13	up	Transglycosylase domain protein	Achl_1053	3.51	down	protein of unknown function DUF1275
Achl_1288	3.26	up	ribose-phosphate pyrophosphokinase	Achl_1056	3.69	down	Integral membrane protein TerC
Achl_1289	7.76	up	ribosomal 5S rRNA E-loop binding protein Ctc/L25	Achl_1060	2.96	down	hypothetical protein Achl_1060
Achl_1295	5.17	up	integral membrane protein	Achl_1063	2.49	down	putative integral membrane protein
Achl_1296	4.97	up	proteinase inhibitor I25 cystatin	Achl_1064	2.37	down	beta-lactamase
Achl_1308	2.08	up	purine nucleotide phosphorylase	Achl_1065	4.04	down	major facilitator superfamily MFS_1
Achl_1309	3.22	up	FAD-dependent pyridine nucleotide-disulphide ox	Achl_1068	7.42	down	hydrophobic
Achl_1314	3.59	up	General substrate transporter	Achl_1069	2.22	down	PHP domain protein
Achl_1317	4.00	up	Pseudogene	Achl_1071	2.82	down	alpha/beta hydrolase fold protein
Achl_1325	3.61	up	carboxyl transferase	Achl_1077	3.23	down	histidinol-phosphate aminotransferase
Achl_1331	2.33	up	Fibronectin type III domain protein	Achl_1085	2.64	down	hypothetical protein Achl_1085
Achl_1332	3.40	up	ATPase associated with various cellular activities A	Achl_1086	4.07	down	Pseudogene

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AchI_1333	2.80	up	protein of unknown function DUF58	AchI_1090	3.24	down	hypothetical protein AchI_1090
AchI_1335	2.67	up	RDD domain containing protein	AchI_1100	2.56	down	YCII-related
AchI_1337	2.60	up	FHA domain containing protein	AchI_1102	2.74	down	4-hydroxythreonine-4-phosphate dehydrogenase
AchI_1349	2.39	up	glutamyl-tRNA(Gln) amidotransferase, C subunit	AchI_1105	2.12	down	binding-protein-dependent transport systems inner membrane component
AchI_1350	7.22	up	glutamyl-tRNA(Gln) amidotransferase, A subunit	AchI_1106	2.72	down	ABC transporter related
AchI_1359	6.54	up	hypothetical protein AchI_1359	AchI_1107	2.26	down	ABC transporter related
AchI_1360	12.50	up	O-acetylhomoserine/O-acetylserine sulfhydrylase	AchI_1113	5.44	down	hypothetical protein AchI_1113
AchI_1367	2.68	up	glycyl-tRNA synthetase	AchI_1115	3.25	down	hemerythrin HHE cation binding domain protein
AchI_1371	2.72	up	YibE/F family protein	AchI_1116	2.50	down	hypothetical protein AchI_1116
AchI_1378	3.88	up	protein of unknown function DUF1469	AchI_1120	4.29	down	ABC transporter related
AchI_1384	4.59	up	ribosomal protein S2	AchI_1132	4.63	down	transcriptional regulator, SARP family
AchI_1385	7.95	up	translation elongation factor Ts	AchI_1149	2.21	down	diguanylate cyclase/phosphodiesterase with PAS/PAC sensor(s)
AchI_1387	2.74	up	ribosome recycling factor	AchI_1150	4.39	down	Transglycosylase-associated protein
AchI_1389	2.13	up	hypothetical protein AchI_1389	AchI_1156	2.41	down	hypothetical protein AchI_1156
AchI_1396	4.32	up	SSS sodium solute transporter superfamily	AchI_1157	2.37	down	hypothetical protein AchI_1157
AchI_1415	3.13	up	YCII-related	AchI_1158	2.40	down	transcriptional regulator, SARP family
AchI_1416	3.28	up	1-hydroxy-2-methyl-2-(E)-butenyl 4-diphosphate s	AchI_1161	3.93	down	hypothetical protein AchI_1161
AchI_1419	2.48	up	prolyl-tRNA synthetase	AchI_1162	3.12	down	hypothetical protein AchI_1162
AchI_1427	2.50	up	ribosome-binding factor A	AchI_1173	4.39	down	hypothetical protein AchI_1173
AchI_1432	2.88	up	tRNA pseudouridine synthase B	AchI_1174	5.45	down	hypothetical protein AchI_1174
AchI_1439	5.11	up	guanosine pentaphosphate synthetase I/polyribor	AchI_1179	9.98	down	multiple sugar-binding periplasmic receptor
AchI_1447	2.06	up	molybdenum cofactor biosynthesis protein C	AchI_1180	8.14	down	Monosaccharide-transporting ATPase
AchI_1450	3.94	up	Dihydrodipicolinate reductase	AchI_1181	12.25	down	ABC transporter related
AchI_1454	3.18	up	dihydrodipicolinate synthase	AchI_1182	2.39	down	ROK family protein
AchI_1455	3.54	up	beta-lactamase domain protein	AchI_1207	3.88	down	transcriptional regulator, MarR family
AchI_1466	2.55	up	Diaminopimelate epimerase	AchI_1227	3.39	down	Extracellular ligand-binding receptor
AchI_1467	2.74	up	methyltransferase small	AchI_1228	2.93	down	ABC transporter related
AchI_1472	3.43	up	histidinol-phosphate aminotransferase	AchI_1229	2.40	down	ABC transporter related
AchI_1473	2.03	up	Imidazoleglycerol-phosphate dehydratase	AchI_1240	2.14	down	hypothetical protein AchI_1240
AchI_1476	6.15	up	bifunctional HisA/TrpF protein	AchI_1245	2.93	down	PhoH family protein
AchI_1480	2.94	up	translation initiation factor IF-3	AchI_1247	3.04	down	hypothetical protein AchI_1247
AchI_1481	2.91	up	ribosomal protein L35	AchI_1278	4.06	down	transglutaminase domain protein
AchI_1482	3.42	up	ribosomal protein L20	AchI_1310	2.85	down	phage shock protein C, PspC
AchI_1491	4.57	up	phenylalanyl-tRNA synthetase, alpha subunit	AchI_1324	3.04	down	hypothetical protein AchI_1324
AchI_1492	2.79	up	phenylalanyl-tRNA synthetase, beta subunit	AchI_1344	2.24	down	GCN5-related N-acetyltransferase
AchI_1498	3.00	up	arginine biosynthesis bifunctional protein ArgJ	AchI_1347	2.39	down	signal transduction histidine kinase regulating citrate/malate metabolism
AchI_1500	2.06	up	acetylornithine and succinylornithine aminotransf	AchI_1353	2.58	down	Alcohol dehydrogenase GroES domain protein
AchI_1501	2.09	up	ornithine carbamoyltransferase	AchI_1355	2.29	down	FAD dependent oxidoreductase
AchI_1502	2.58	up	arginine repressor, ArgR	AchI_1356	3.28	down	imidazolonepropionase
AchI_1503	4.64	up	Argininosuccinate synthase	AchI_1377	2.64	down	drug resistance transporter, Bcr/CflA subfamily
AchI_1504	2.01	up	argininosuccinate lyase	AchI_1390	3.68	down	hypothetical protein AchI_1390
AchI_1517	2.77	up	DNA repair protein RecN	AchI_1399	9.59	down	pyruvate dehydrogenase (acetyl-transferring) E1 component, alpha subunit
AchI_1534	2.18	up	pseudouridine synthase	AchI_1400	9.16	down	Transketolase central region
AchI_1538	3.52	up	small GTP-binding protein	AchI_1401	7.57	down	catalytic domain of components of various dehydrogenase complexes
AchI_1541	4.07	up	glycine cleavage system H protein	AchI_1403	4.85	down	Propionyl-CoA carboxylase
AchI_1544	2.68	up	protein of unknown function DUF151	AchI_1404	2.66	down	Carbamoyl-phosphate synthase L chain ATP-binding
AchI_1547	13.42	up	pyruvate carboxylase	AchI_1405	3.75	down	acyl-CoA dehydrogenase domain protein
AchI_1548	2.18	up	AMP-dependent synthetase and ligase	AchI_1410	3.02	down	protein of unknown function DUF1222
AchI_1556	2.93	up	Lytic transglycosylase catalytic	AchI_1418	2.65	down	hypothetical protein AchI_1418

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Achl_1559	2.23	up	hypothetical protein Achl_1559	Achl_1441	2.65	down	hypothetical protein Achl_1441
Achl_1568	5.01	up	phospho-N-acetylmuramoyl-pentapeptide-transfe	Achl_1460	3.40	down	transcriptional regulator, MarR family
Achl_1570	2.10	up	cell division protein FtsW	Achl_1484	6.44	down	hypothetical protein Achl_1484
Achl_1574	5.66	up	cell division protein FtsZ	Achl_1493	2.12	down	4'-phosphopantetheinyl transferase
Achl_1575	2.64	up	protein of unknown function DUF152	Achl_1509	2.05	down	DNA-3-methyladenine glycosylase
Achl_1577	2.99	up	protein of unknown function DUF552	Achl_1552	2.76	down	putative esterase/lipase
Achl_1578	3.01	up	protein of unknown function YGGT	Achl_1561	3.28	down	membrane protein
Achl_1579	3.72	up	DivIVA family protein	Achl_1633	2.22	down	TrkA-N domain protein
Achl_1586	2.50	up	ATP-cone domain protein	Achl_1646	2.71	down	ABC transporter related
Achl_1591	3.24	up	glutamine synthetase, type I	Achl_1653	3.22	down	hypothetical protein Achl_1653
Achl_1594	5.88	up	glutamine synthetase, type I	Achl_1662	8.31	down	hypothetical protein Achl_1662
Achl_1599	3.05	up	lipoate-protein ligase B	Achl_1691	2.24	down	diguanylate phosphodiesterase
Achl_1603	2.25	up	OsmC family protein	Achl_1693	2.25	down	RNA polymerase, sigma-24 subunit, ECF subfamily
Achl_1604	3.93	up	hypothetical protein Achl_1604	Achl_1694	3.47	down	hypothetical protein Achl_1694
Achl_1605	9.61	up	2-oxoglutarate dehydrogenase, E2 component, dil	Achl_1698	5.61	down	heat shock protein Hsp20
Achl_1606	6.80	up	dihydrolipoamide dehydrogenase	Achl_1702	2.46	down	hypothetical protein Achl_1702
Achl_1607	2.67	up	Leucyl aminopeptidase	Achl_1704	2.99	down	potassium-transporting ATPase, A subunit
Achl_1615	2.35	up	transcriptional repressor, CopY family	Achl_1711	2.08	down	extracellular solute-binding protein family 1
Achl_1627	6.79	up	hypothetical protein Achl_1627	Achl_1722	2.95	down	NAD(P) transhydrogenase, alpha subunit
Achl_1630	5.01	up	hypothetical protein Achl_1630	Achl_1738	2.59	down	type III effector Hrp-dependent outers
Achl_1631	3.83	up	hypothetical protein Achl_1631	Achl_1745	2.04	down	MbtH domain protein
Achl_1637	8.37	up	Pseudogene	Achl_1748	2.12	down	Pseudogene
Achl_1641	3.22	up	aldo/keto reductase	Achl_1749	2.34	down	glycoside hydrolase clan GH-D
Achl_1650	2.90	up	hypothetical protein Achl_1650	Achl_1761	4.53	down	band 7 protein
Achl_1659	3.30	up	Rhodanese domain protein	Achl_1762	5.00	down	FAD linked oxidase domain protein
Achl_1667	3.17	up	Ribulose-phosphate 3-epimerase	Achl_1763	2.51	down	sodium/hydrogen exchanger
Achl_1674	14.52	up	phosphoribosyl-ATP diphosphatase	Achl_1765	2.90	down	protein of unknown function DUF1206
Achl_1675	9.66	up	ATP phosphoribosyltransferase	Achl_1771	5.47	down	Amylo-alpha-16-glucosidase
Achl_1676	3.24	up	imidazoleglycerol phosphate synthase, cyclase sut	Achl_1772	3.37	down	bifunctional deaminase-reductase domain protein
Achl_1680	3.37	up	hypothetical protein Achl_1680	Achl_1779	2.50	down	extracellular solute-binding protein family 5
Achl_1682	3.84	up	Indole-3-glycerol-phosphate synthase	Achl_1782	2.39	down	hypothetical protein Achl_1782
Achl_1683	2.33	up	tryptophan synthase, beta subunit	Achl_1783	2.65	down	hypothetical protein Achl_1783
Achl_1684	3.19	up	tryptophan synthase, alpha subunit	Achl_1786	2.10	down	dienelactone hydrolase
Achl_1689	2.80	up	response regulator receiver and ANTAR domain pr	Achl_1790	4.95	down	Pseudogene
Achl_1726	9.55	up	2-hydroxypropyl-CoM lyase	Achl_1794	3.15	down	transcriptional regulator, LuxR family
Achl_1727	9.69	up	Domain of unknown function DUF1852	Achl_1800	2.10	down	bifunctional deaminase-reductase domain protein
Achl_1755	2.07	up	aldo/keto reductase	Achl_1812	2.56	down	major facilitator superfamily MFS_1
Achl_1792	2.66	up	Glyoxalase/bleomycin resistance protein/dioxyger	Achl_1867	2.97	down	Helix-turn-helix type 11 domain protein
Achl_1802	3.00	up	DNA polymerase I	Achl_1900	2.41	down	glycosyl transferase family 9
Achl_1813	2.10	up	alpha/beta hydrolase fold protein	Achl_1901	6.02	down	hypothetical protein Achl_1901
Achl_1820	2.95	up	excinuclease ABC, A subunit	Achl_1902	2.49	down	protein of unknown function DUF805
Achl_1824	4.28	up	hypothetical protein Achl_1824	Achl_1932	2.90	down	band 7 protein
Achl_1827	5.22	up	Superoxide dismutase	Achl_2005	3.64	down	putative integral membrane protein
Achl_1830	2.39	up	Triose-phosphate isomerase	Achl_2021	4.33	down	GCN5-related N-acetyltransferase
Achl_1834	3.18	up	oxppcycle protein	Achl_2038	2.39	down	hypothetical protein Achl_2038
Achl_1835	2.57	up	glucose-6-phosphate 1-dehydrogenase	Achl_2041	2.59	down	CDP-alcohol phosphatidyltransferase
Achl_1836	4.25	up	phosphoglucose isomerase (PGI)	Achl_2046	4.77	down	hypothetical protein Achl_2046
Achl_1837	5.03	up	transaldolase	Achl_2049	2.70	down	protein of unknown function DUF159
Achl_1838	3.90	up	transketolase	Achl_2051	2.12	down	hypothetical protein Achl_2051

Gene ID	Fold change	Regulation LE vs LS	annotation	Gene ID	Fold change	Regulation LE vs LS	annotation
AchI_1840	3.37	up	sulphate transporter	AchI_2052	2.53	down	ABC-1 domain protein
AchI_1841	2.14	up	cytochrome oxidase assembly	AchI_2053	2.48	down	transcriptional regulator, PadR-like family
AchI_1843	4.39	up	ABC transporter related	AchI_2056	2.16	down	NUDIX hydrolase
AchI_1853	2.43	up	cobalt transport protein	AchI_2057	7.26	down	Domain of unknown function DUF1918
AchI_1854	3.74	up	ABC transporter related	AchI_2068	3.07	down	hypothetical protein AchI_2068
AchI_1855	4.70	up	BioY protein	AchI_2069	5.66	down	UspA domain protein
AchI_1856	2.53	up	hypothetical protein AchI_1856	AchI_2070	3.20	down	hypothetical protein AchI_2070
AchI_1872	6.38	up	3-oxoacyl-(acyl-carrier-protein) reductase	AchI_2072	3.05	down	UspA domain protein
AchI_1873	4.30	up	short-chain dehydrogenase/reductase SDR	AchI_2076	3.29	down	UspA domain protein
AchI_1875	6.30	up	ABC transporter related	AchI_2077	2.18	down	ATPase, P-type (transporting), HAD superfamily, subfamily IC
AchI_1878	3.44	up	ribosomal protein L31	AchI_2079	2.58	down	Pseudogene
AchI_1882	3.33	up	protein of unknown function DUF404	AchI_2093	3.56	down	NAD-dependent epimerase/dehydratase
AchI_1883	3.38	up	protein of unknown function DUF403	AchI_2098	2.36	down	conserved hypothetical alanine-rich protein
AchI_1910	5.83	up	undecaprenol kinase	AchI_2103	2.33	down	hypothetical protein AchI_2103
AchI_1911	2.81	up	cysteine/1-D-myo-inosityl 2-amino-2-deoxy-alpha-	AchI_2108	2.75	down	Pseudogene
AchI_1912	2.22	up	protein of unknown function DUF75	AchI_2119	2.67	down	glutaredoxin-like protein NrdH
AchI_1913	2.09	up	HAD-superfamily hydrolase, subfamily IA, variant	AchI_2132	10.74	down	hypothetical protein AchI_2132
AchI_1922	3.33	up	peptidylprolyl isomerase FKBP-type	AchI_2136	2.35	down	Cyclopropane-fatty-acyl-phospholipid synthase
AchI_1925	5.09	up	hypothetical protein AchI_1925	AchI_2153	2.66	down	Endonuclease/exonuclease/phosphatase
AchI_1926	2.13	up	sec-independent translocation protein mttA/Hcf1	AchI_2169	2.42	down	hypothetical protein AchI_2169
AchI_1944	3.26	up	transcriptional regulator, AsnC family	AchI_2183	3.37	down	DNA alkylation repair enzyme
AchI_1946	2.34	up	cytochrome c oxidase subunit III	AchI_2188	2.03	down	hypothetical protein AchI_2188
AchI_1948	2.27	up	Rieske (2Fe-2S) domain protein	AchI_2199	4.57	down	hypothetical protein AchI_2199
AchI_1953	3.92	up	cytochrome c oxidase, subunit I	AchI_2302	8.54	down	General substrate transporter
AchI_1954	4.88	up	cytochrome c oxidase, subunit II	AchI_2304	2.19	down	major facilitator superfamily MFS_1
AchI_1957	2.54	up	hypothetical protein AchI_1957	AchI_2306	2.26	down	nuclease SbcCD, D subunit
AchI_1958	3.95	up	Dihydroorotate oxidase	AchI_2309	3.79	down	hypothetical protein AchI_2309
AchI_1964	3.77	up	Endothelin-converting enzyme 1	AchI_2323	2.34	down	hypothetical protein AchI_2323
AchI_1965	2.57	up	cell envelope-related transcriptional attenuator	AchI_2347	2.52	down	glycosyl transferase family 4
AchI_1972	2.21	up	chaperone protein DnaJ	AchI_2365	3.10	down	hypothetical protein AchI_2365
AchI_1977	3.82	up	GTP-binding protein LepA	AchI_2402	2.35	down	hypothetical protein AchI_2402
AchI_1979	6.53	up	ribosomal protein S20	AchI_2406	2.48	down	type II secretion system protein
AchI_1990	2.00	up	alpha/beta hydrolase fold protein	AchI_2408	2.27	down	type II secretion system protein E
AchI_1993	3.68	up	Methionine adenosyltransferase	AchI_2425	2.56	down	putative acetyltransferase
AchI_1994	2.63	up	phosphopantothenoylcysteine decarboxylase/pho	AchI_2432	2.10	down	hypothetical protein AchI_2432
AchI_1999	3.50	up	carbamoyl-phosphate synthase, large subunit	AchI_2440	2.90	down	phosphoribosyltransferase
AchI_2000	7.86	up	carbamoyl-phosphate synthase, small subunit	AchI_2448	2.19	down	protein of unknown function DUF58
AchI_2001	2.27	up	hypothetical protein AchI_2001	AchI_2455	3.03	down	extracellular solute-binding protein family 5
AchI_2002	2.62	up	dihydroorotase, multifunctional complex type	AchI_2457	2.37	down	aminotransferase class V
AchI_2003	2.19	up	aspartate carbamoyltransferase	AchI_2545	4.12	down	hypothetical protein AchI_2545
AchI_2006	5.09	up	NusB antitermination factor	AchI_2560	2.53	down	transcriptional regulator, LysR family
AchI_2007	4.91	up	translation elongation factor P	AchI_2570	2.45	down	GCN5-related N-acetyltransferase
AchI_2008	4.39	up	hypothetical protein AchI_2008	AchI_2590	2.21	down	FAD-dependent pyridine nucleotide-disulphide oxidoreductase
AchI_2010	4.90	up	Shikimate kinase	AchI_2591	4.94	down	transcriptional regulator, LuxR family
AchI_2011	3.86	up	Chorismate synthase	AchI_2592	3.32	down	DNA ligase I, ATP-dependent Dnl1
AchI_2013	8.91	up	aminodeoxychorismate lyase	AchI_2593	3.29	down	bifunctional deaminase-reductase domain protein
AchI_2016	4.06	up	putative secreted protein	AchI_2607	3.41	down	PfkB domain protein
AchI_2017	2.70	up	secreted protein	AchI_2627	3.71	down	glycosyl transferase family 2

Gene ID	Fold change	Regulation LE vs LS	annotation	Gene ID	Fold change	Regulation LE vs LS	annotation
Achl_2018	8.05	up	RNA-binding S4 domain protein	Achl_2660	3.24	down	peptidase S58 DmpA
Achl_2022	4.30	up	aspartyl-tRNA synthetase	Achl_2662	2.56	down	binding-protein-dependent transport systems inner membrane component
Achl_2025	5.10	up	protein of unknown function DUF349	Achl_2663	2.34	down	extracellular solute-binding protein family 1
Achl_2028	3.32	up	protein-export membrane protein SecF	Achl_2699	2.25	down	acetyl-CoA acetyltransferase
Achl_2029	3.12	up	protein-export membrane protein SecD	Achl_2711	4.95	down	putative signal transduction histidine kinase
Achl_2033	2.22	up	crossover junction endodeoxyribonuclease RuvC	Achl_2712	4.33	down	phage shock protein C, PspC
Achl_2040	3.43	up	pyridoxine biosynthesis protein	Achl_2713	3.19	down	hypothetical protein Achl_2713
Achl_2043	3.51	up	threonyl-tRNA synthetase	Achl_2723	2.63	down	hypothetical protein Achl_2723
Achl_2055	3.21	up	Endonuclease/exonuclease/phosphatase	Achl_2747	2.74	down	Na+/H+ antiporter
Achl_2065	4.99	up	Peptidoglycan-binding LysM	Achl_2749	4.38	down	bifunctional deaminase-reductase domain protein
Achl_2116	2.78	up	Ribonucleoside-diphosphate reductase	Achl_2750	4.92	down	hypothetical protein Achl_2750
Achl_2137	11.40	up	iojap-like protein	Achl_2763	2.29	down	Arsenical pump membrane protein
Achl_2138	2.41	up	hypothetical protein Achl_2138	Achl_2764	7.49	down	hypothetical protein Achl_2764
Achl_2139	4.49	up	nicotinate (nicotinamide) nucleotide adenylyltrans	Achl_2765	6.51	down	Glyoxalase/bleomycin resistance protein/dioxygenase
Achl_2145	4.40	up	ribosomal protein L21	Achl_2766	2.81	down	hypothetical protein Achl_2766
Achl_2147	2.34	up	ribonuclease, Rne/Rng family	Achl_2771	2.11	down	ABC transporter related
Achl_2158	2.84	up	Endopeptidase Clp	Achl_2776	23.58	down	putative signal transduction protein with CBS domains
Achl_2159	3.05	up	Endopeptidase Clp	Achl_2799	2.30	down	Aldehyde Dehydrogenase
Achl_2160	2.92	up	trigger factor	Achl_2801	4.30	down	helix-turn-helix domain protein
Achl_2162	2.12	up	ribose 5-phosphate isomerase	Achl_2805	2.59	down	GntR domain protein
Achl_2189	20.03	up	glyceraldehyde-3-phosphate dehydrogenase, type	Achl_2809	2.06	down	protein of unknown function DUF1206
Achl_2195	6.28	up	cysteine synthase A	Achl_2813	3.35	down	hypothetical protein Achl_2813
Achl_2196	6.08	up	serine O-acetyltransferase	Achl_2832	3.41	down	aminotransferase class I and II
Achl_2203	3.61	up	2-oxo-acid dehydrogenase E1 subunit, homodime	Achl_2833	2.28	down	Glyoxalase/bleomycin resistance protein/dioxygenase
Achl_2205	4.21	up	putative (acyl-carrier-protein) S-malonyltransferas	Achl_2837	4.96	down	Mg2 transporter protein CorA family protein
Achl_2206	3.11	up	3-oxoacyl-(acyl-carrier-protein) synthase III	Achl_2838	4.51	down	transcriptional regulator, ArsR family
Achl_2207	6.65	up	phosphopantetheine-binding	Achl_2850	3.05	down	hypothetical protein Achl_2850
Achl_2208	5.73	up	3-oxoacyl-(acyl-carrier-protein) synthase 2	Achl_2851	10.21	down	hypothetical protein Achl_2851
Achl_2219	3.28	up	ribosomal protein L19	Achl_2867	3.15	down	response regulator receiver protein
Achl_2223	2.58	up	ribosomal protein S16	Achl_2871	2.56	down	Animal heme peroxidase
Achl_2239	3.91	up	Ribonuclease III	Achl_2872	3.48	down	Pseudogene
Achl_2240	2.88	up	ribosomal protein L32	Achl_2876	2.87	down	hypothetical protein Achl_2876
Achl_2251	4.71	up	D-alanine/D-alanine ligase	Achl_2883	6.44	down	hypothetical protein Achl_2883
Achl_2252	3.41	up	Glycerol-3-phosphate dehydrogenase (NAD(P)(+))	Achl_2884	3.69	down	hypothetical protein Achl_2884
Achl_2253	3.79	up	phospholipid/glycerol acyltransferase	Achl_2886	2.71	down	lipolytic protein G-D-S-L family
Achl_2254	4.58	up	UDP-N-acetylglucosamine 1-carboxyvinyltransfera	Achl_2887	2.03	down	putative integral membrane protein
Achl_2255	3.88	up	3-isopropylmalate dehydratase, small subunit	Achl_2905	2.89	down	Pyruvate dehydrogenase (acetyl-transferring)
Achl_2268	2.88	up	3-isopropylmalate dehydrogenase	Achl_2919	2.74	down	hypothetical protein Achl_2919
Achl_2273	2.78	up	methionyl-tRNA synthetase	Achl_2929	4.27	down	Pseudogene
Achl_2274	2.62	up	ABC transporter related	Achl_2939	3.54	down	Pseudogene
Achl_2276	7.97	up	D-3-phosphoglycerate dehydrogenase	Achl_2956	3.71	down	beta-mannanase-like protein
Achl_2277	7.92	up	ketol-acid reductoisomerase	Achl_2958	2.89	down	glycosyl transferase family 2
Achl_2278	18.96	up	acetolactate synthase, small subunit	Achl_2960	2.41	down	phenylacetate-CoA oxygenase/reductase, PaaK subunit
Achl_2279	6.37	up	acetolactate synthase, large subunit, biosynthetic	Achl_2961	4.64	down	phenylacetate-CoA oxygenase, PaaJ subunit
Achl_2286	3.48	up	malate/quinone oxidoreductase	Achl_2962	2.74	down	phenylacetate-CoA oxygenase, PaaI subunit
Achl_2288	5.08	up	Luciferase-like monooxygenase	Achl_2963	4.63	down	phenylacetate-CoA oxygenase, PaaH subunit
Achl_2289	4.29	up	transcriptional regulator, MarR family	Achl_2964	7.70	down	phenylacetate-CoA oxygenase, PaaG subunit
Achl_2313	2.71	up	ribonuclease PH	Achl_2967	3.79	down	protein of unknown function DUF661
Achl_2318	2.77	up	nicotinate phosphoribosyltransferase	Achl_2970	4.33	down	carbon storage regulator, CsrA
Achl_2328	3.23	up	hypothetical protein Achl_2328	Achl_3011	3.80	down	hypothetical protein Achl_3011

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Achl_2336	15.82	up	hypothetical protein Achl_2336	Achl_3012	11.31	down	hypothetical protein Achl_3012
Achl_2337	13.15	up	H+transporting two-sector ATPase delta/epsilon s	Achl_3013	3.19	down	transcriptional regulator, PadR-like family
Achl_2338	7.55	up	ATP synthase F1, beta subunit	Achl_3014	2.70	down	hypothetical protein Achl_3014
Achl_2339	19.04	up	ATP synthase F1, gamma subunit	Achl_3015	3.19	down	ABC transporter related
Achl_2340	16.84	up	ATP synthase F1, alpha subunit	Achl_3018	2.01	down	alpha/beta hydrolase fold protein
Achl_2341	8.52	up	ATP synthase F1, delta subunit	Achl_3020	2.94	down	glutamate--cysteine ligase GCS2
Achl_2342	8.25	up	ATP synthase F0, B subunit	Achl_3023	2.23	down	chromate transporter, chromate ion transporter (CHR) family
Achl_2343	12.75	up	H+transporting two-sector ATPase C subunit	Achl_3026	2.43	down	hypothetical protein Achl_3026
Achl_2344	18.84	up	ATP synthase F0, A subunit	Achl_3028	2.54	down	alpha/beta hydrolase fold protein
Achl_2345	3.73	up	hypothetical protein Achl_2345	Achl_3029	3.38	down	putative PAS/PAC sensor protein
Achl_2346	10.07	up	hypothetical protein Achl_2346	Achl_3034	5.91	down	cell envelope-related transcriptional attenuator
Achl_2352	2.35	up	peptide chain release factor 1	Achl_3037	2.50	down	carbohydrate kinase, YjeF related protein
Achl_2353	4.04	up	transcription termination factor Rho	Achl_3038	3.52	down	heat shock protein Hsp20
Achl_2354	6.17	up	homoserine kinase	Achl_3039	3.11	down	hypothetical protein Achl_3039
Achl_2355	3.94	up	threonine synthase	Achl_3041	4.29	down	hypothetical protein Achl_3041
Achl_2356	4.91	up	Homoserine dehydrogenase	Achl_3042	4.11	down	FAD-dependent pyridine nucleotide-disulphide oxidoreductase
Achl_2367	2.19	up	3-oxoacyl-(acyl-carrier-protein) synthase III	Achl_3047	11.95	down	phenylacetic acid degradation protein PaaD
Achl_2374	4.23	up	ABC-3 protein	Achl_3048	10.34	down	phenylacetate-CoA ligase
Achl_2376	3.12	up	periplasmic solute binding protein	Achl_3050	3.95	down	hypothetical protein Achl_3050
Achl_2379	7.51	up	2-oxoglutarate dehydrogenase, E1 subunit	Achl_3052	12.31	down	Glyoxalase/bleomycin resistance protein/dioxygenase
Achl_2380	2.27	up	hypothetical protein Achl_2380	Achl_3053	3.73	down	phenylacetic acid degradation protein paaN
Achl_2387	3.25	up	GTPase EngC	Achl_3054	3.07	down	thioesterase superfamily protein
Achl_2389	4.21	up	Cysteine desulfurase	Achl_3058	2.29	down	hypothetical protein Achl_3058
Achl_2398	3.04	up	Peptidase M23	Achl_3063	3.74	down	acetyl-CoA acetyltransferase
Achl_2399	3.22	up	protein of unknown function DUF214	Achl_3066	3.02	down	CsbD family protein
Achl_2411	3.38	up	dTDP-glucose 4,6-dehydratase	Achl_3067	3.85	down	DNA-directed DNA polymerase
Achl_2412	4.08	up	dTDP-4-dehydrorhamnose reductase	Achl_3068	2.55	down	dienelactone hydrolase
Achl_2413	3.02	up	glycosyl transferase family 2	Achl_3070	2.82	down	Mn2+/Fe2+ transporter, NRAMP family
Achl_2415	3.51	up	glycosyl transferase family 2	Achl_3075	2.51	down	extracellular solute-binding protein family 1
Achl_2418	2.77	up	glycosyl transferase family 2	Achl_3082	2.30	down	hypothetical protein Achl_3082
Achl_2423	5.31	up	glycosyl transferase, family 2	Achl_3084	3.08	down	hypothetical protein Achl_3084
Achl_2427	2.19	up	hypothetical protein Achl_2427	Achl_3094	4.34	down	hypothetical protein Achl_3094
Achl_2428	5.18	up	transcription factor WhiB	Achl_3099	2.65	down	excision promoter, Xis
Achl_2445	2.08	up	integral membrane protein	Achl_3118	2.03	down	alpha-L-rhamnosidase
Achl_2464	2.61	up	Electron transfer flavoprotein alpha subunit	Achl_3123	2.97	down	binding-protein-dependent transport systems inner membrane component
Achl_2465	4.48	up	Electron transfer flavoprotein alpha/beta-subunit	Achl_3134	2.29	down	hypothetical protein Achl_3134
Achl_2470	3.01	up	hypothetical protein Achl_2470	Achl_3136	2.79	down	Mandelate racemase/muconate lactonizing protein
Achl_2471	2.70	up	protein of unknown function UPF0182	Achl_3137	3.05	down	dihydrodipicolinate synthetase
Achl_2475	2.18	up	UvrD/REP helicase	Achl_3152	3.03	down	hypothetical protein Achl_3152
Achl_2477	3.60	up	aminoglycoside phosphotransferase	Achl_3156	2.95	down	hypothetical protein Achl_3156
Achl_2482	7.45	up	extracellular solute-binding protein family 3	Achl_3157	3.86	down	TadE family protein
Achl_2483	7.30	up	polar amino acid ABC transporter, inner membran	Achl_3169	2.93	down	extracellular solute-binding protein family 1
Achl_2484	2.88	up	ABC transporter related	Achl_3171	2.52	down	binding-protein-dependent transport systems inner membrane component
Achl_2488	3.91	up	Uroporphyrinogen III synthase HEM4	Achl_3184	2.72	down	hypothetical protein Achl_3184
Achl_2489	3.87	up	porphobilinogen deaminase	Achl_3185	6.34	down	transcriptional regulator, XRE family
Achl_2490	3.55	up	Ferrochelatase	Achl_3205	2.15	down	amidohydrolase
Achl_2491	6.03	up	Chlorite dismutase	Achl_3208	3.78	down	urate oxidase
Achl_2505	2.92	up	DEAD/DEAH box helicase domain protein	Achl_3217	2.28	down	hypothetical protein Achl_3217
Achl_2506	2.65	up	DNA methylase N-4/N-6 domain protein	Achl_3219	3.28	down	hypothetical protein Achl_3219

Gene ID	Fold change	Regulation LE vs LS	annotation	Gene ID	Fold change	Regulation LE vs LS	annotation
Achl_2511	2.09	up	protein of unknown function DUF1003	Achl_3229	3.43	down	hypothetical protein Achl_3229
Achl_2520	4.10	up	hypothetical protein Achl_2520	Achl_3238	2.34	down	hypothetical protein Achl_3238
Achl_2526	2.79	up	putative transferase	Achl_3246	3.47	down	glycosyl transferase family 51
Achl_2530	2.22	up	UDP-glucose 4-epimerase	Achl_3249	3.14	down	hypothetical protein Achl_3249
Achl_2531	7.75	up	citrate synthase I	Achl_3258	3.43	down	protein of unknown function DUF322
Achl_2533	3.20	up	4Fe-4S ferredoxin iron-sulfur binding domain prot	Achl_3259	6.15	down	hypothetical protein Achl_3259
Achl_2535	2.39	up	GTP-binding protein TypA	Achl_3263	2.54	down	CsbD family protein
Achl_2546	3.96	up	GTP-binding protein YchF	Achl_3266	2.34	down	hypothetical protein Achl_3266
Achl_2548	2.44	up	hydroxymethylbutenyl pyrophosphate reductase	Achl_3274	4.03	down	hypothetical protein Achl_3274
Achl_2553	5.01	up	aminotransferase class I and II	Achl_3294	4.45	down	hypothetical protein Achl_3294
Achl_2569	6.36	up	hypothetical protein Achl_2569	Achl_3297	2.24	down	permease for cytosine/purines uracil thiamine allantoin
Achl_2571	3.03	up	UTP-glucose-1-phosphate uridylyltransferase	Achl_3298	2.78	down	transcriptional regulator, CdaR
Achl_2573	2.31	up	regulatory protein, FmdB family	Achl_3314	4.44	down	hypothetical protein Achl_3314
Achl_2575	4.68	up	hypothetical protein Achl_2575	Achl_3315	2.05	down	hypothetical protein Achl_3315
Achl_2576	5.45	up	GMP synthase, large subunit	Achl_3320	2.90	down	hypothetical protein Achl_3320
Achl_2577	4.47	up	hypothetical protein Achl_2577	Achl_3324	2.96	down	hypothetical protein Achl_3324
Achl_2583	2.06	up	hypothetical protein Achl_2583	Achl_3327	2.05	down	Pseudogene
Achl_2594	7.07	up	chaperonin GroEL	Achl_3333	2.60	down	Aldehyde Dehydrogenase
Achl_2595	4.15	up	chaperonin Cpn10	Achl_3336	3.27	down	protoporphyrinogen oxidase
Achl_2614	5.35	up	inositol 1-phosphate synthase	Achl_3337	2.54	down	putative integral membrane protein
Achl_2615	2.96	up	aldo/keto reductase	Achl_3343	3.27	down	ABC transporter related
Achl_2618	3.92	up	glycogen debranching enzyme GlgX	Achl_3347	4.46	down	Pseudogene
Achl_2621	3.40	up	glucosamine/fructose-6-phosphate aminotransfer	Achl_3355	2.85	down	ABC transporter transmembrane region
Achl_2624	6.31	up	large conductance mechanosensitive channel prot	Achl_3356	2.51	down	ABC transporter related
Achl_2625	2.33	up	phosphoglucosamine mutase	Achl_3359	2.10	down	amidohydrolase 2
Achl_2630	6.69	up	ribosomal protein S9	Achl_3374	2.87	down	transcriptional regulator, XRE family
Achl_2631	8.59	up	ribosomal protein L13	Achl_3378	2.60	down	natural resistance-associated macrophage protein
Achl_2654	9.17	up	ribosomal protein L17	Achl_3379	2.73	down	hypothetical protein Achl_3379
Achl_2655	6.05	up	DNA-directed RNA polymerase, alpha subunit	Achl_3380	2.59	down	two component transcriptional regulator, LuxR family
Achl_2656	4.87	up	ribosomal protein S11	Achl_3381	2.76	down	histidine kinase
Achl_2657	4.31	up	ribosomal protein S13	Achl_3382	2.98	down	protein of unknown function DUF214
Achl_2658	4.35	up	ribosomal protein L36	Achl_3383	4.67	down	ABC transporter related
Achl_2659	2.51	up	translation initiation factor IF-1	Achl_3394	2.62	down	N-acetylglucosamine-6-phosphate deacetylase
Achl_2667	24.86	up	adenylate kinase	Achl_3401	2.36	down	hypothetical protein Achl_3401
Achl_2668	12.82	up	preprotein translocase, SecY subunit	Achl_3402	3.64	down	hypothetical protein Achl_3402
Achl_2669	7.92	up	ribosomal protein L15	Achl_3408	4.25	down	histidine kinase
Achl_2670	9.99	up	ribosomal protein L30	Achl_3411	4.23	down	polysaccharide deacetylase
Achl_2671	4.66	up	ribosomal protein S5	Achl_3415	2.46	down	peptidase M20
Achl_2672	6.45	up	ribosomal protein L18	Achl_3418	8.69	down	hypothetical protein Achl_3418
Achl_2673	4.06	up	ribosomal protein L6 signature 1	Achl_3424	4.28	down	transcriptional regulator, IclR family
Achl_2674	6.92	up	ribosomal protein S8	Achl_3430	9.48	down	hypothetical protein Achl_3430
Achl_2675	5.80	up	ribosomal protein L5	Achl_3431	19.59	down	hypothetical protein Achl_3431
Achl_2676	6.37	up	ribosomal protein L24	Achl_3443	3.71	down	Spore coat protein CotH
Achl_2677	4.38	up	ribosomal protein L14	Achl_3445	2.59	down	GCN5-related N-acetyltransferase
Achl_2678	2.86	up	ribosomal protein S17	Achl_3459	2.99	down	Pseudogene
Achl_2679	3.86	up	ribosomal protein L29	Achl_3460	3.56	down	hypothetical protein Achl_3460
Achl_2680	4.79	up	ribosomal protein L16	Achl_3469	2.09	down	FAD dependent oxidoreductase
Achl_2681	3.16	up	ribosomal protein S3	Achl_3482	2.40	down	NifC-like ABC-type porter
Achl_2682	4.81	up	ribosomal protein L22	Achl_3483	2.88	down	molybdenum ABC transporter, periplasmic molybdate-binding protein
Achl_2683	4.74	up	ribosomal protein S19	Achl_3484	8.04	down	DNA binding domain protein, excisionase family

Gene ID	Fold change	Regulation LE vs LS	annotation	Gene ID	Fold change	Regulation LE vs LS	annotation
Achl_2684	3.39	up	ribosomal protein L2	Achl_3487	2.09	down	protein of unknown function DUF307
Achl_2685	4.86	up	Ribosomal protein L25/L23	Achl_3489	4.36	down	protein of unknown function DUF886
Achl_2686	4.48	up	ribosomal protein L4/L1e	Achl_3503	2.81	down	hypothetical protein Achl_3503
Achl_2687	3.75	up	ribosomal protein L3	Achl_3505	2.57	down	hypothetical protein Achl_3505
Achl_2688	3.53	up	ribosomal protein S10	Achl_3509	3.05	down	putative transmembrane anti-sigma factor
Achl_2690	2.05	up	hypothetical protein Achl_2690	Achl_3511	2.03	down	Secreted repeat of unknown function
Achl_2692	4.28	up	translation elongation factor Tu	Achl_3527	3.27	down	hypothetical protein Achl_3527
Achl_2693	4.09	up	translation elongation factor G	Achl_3531	2.33	down	glycoside hydrolase family 43
Achl_2694	3.57	up	ribosomal protein S7	Achl_3537	3.03	down	flavin reductase domain protein FMN-binding
Achl_2701	8.18	up	ribosomal protein L7/L12	Achl_3539	2.44	down	Luciferase-like monooxygenase
Achl_2702	7.94	up	ribosomal protein L10	Achl_3542	2.06	down	hypothetical protein Achl_3542
Achl_2705	9.13	up	ribosomal protein L1	Achl_3545	4.02	down	fumarylacetoacetate (FAA) hydrolase
Achl_2706	9.01	up	ribosomal protein L11	Achl_3574	6.21	down	CsbD family protein
Achl_2707	3.43	up	NusG antitermination factor	Achl_3596	2.39	down	Glycine hydroxymethyltransferase
Achl_2708	3.43	up	preprotein translocase, SecE subunit	Achl_3600	2.40	down	extracellular solute-binding protein family 1
Achl_2709	4.32	up	aminotransferase class I and II	Achl_3608	2.36	down	5-oxopent-3-ene-1,2,5-tricarboxylate decarboxylase
Achl_2722	4.27	up	hypothetical protein Achl_2722	Achl_3615	4.31	down	pyruvate phosphate dikinase PEP/pyruvate-binding
Achl_2731	3.03	up	Dihydrofolate reductase	Achl_3625	3.05	down	hypothetical protein Achl_3625
Achl_2732	3.29	up	hypothetical protein Achl_2732	Achl_3634	3.20	down	protein tyrosine phosphatase
Achl_2733	4.46	up	aspartate-semialdehyde dehydrogenase	Achl_3648	2.67	down	hypothetical protein Achl_3648
Achl_2739	3.84	up	hypothetical protein Achl_2739	Achl_3651	2.51	down	ABC transporter, permease protein
Achl_2742	2.11	up	HpcH/HpaI aldolase	Achl_3672	2.17	down	hypothetical protein Achl_3672
Achl_2752	3.59	up	DEAD/DEAH box helicase domain protein	Achl_3673	4.08	down	Aldehyde Dehydrogenase
Achl_2761	2.70	up	Cystathionine gamma-synthase	Achl_3695	3.90	down	hypothetical protein Achl_3695
Achl_2781	6.24	up	ferric uptake regulator, Fur family	Achl_3710	2.39	down	polar amino acid ABC transporter, inner membrane subunit
Achl_2782	15.15	up	Catalase	Achl_3715	3.34	down	hypothetical protein Achl_3715
Achl_2811	6.63	up	Ferredoxin--NADP(+) reductase	Achl_3730	3.05	down	lipolytic protein G-D-S-L family
Achl_2812	2.42	up	uroporphyrin-III C-methyltransferase	Achl_3741	15.66	down	hypothetical protein Achl_3741
Achl_2814	2.10	up	binding-protein-dependent transport systems inn	Achl_3743	2.88	down	hypothetical protein Achl_3743
Achl_2816	2.58	up	aliphatic sulfonates family ABC transporter, peripl	Achl_3748	4.28	down	oxidoreductase molybdopterin binding
Achl_2817	5.79	up	sulfate adenylyltransferase, large subunit	Achl_3751	3.15	down	hypothetical protein Achl_3751
Achl_2818	4.85	up	sulfate adenylyltransferase, small subunit	Achl_3754	3.60	down	HipA N-terminal domain protein
Achl_2820	14.77	up	Sulfite reductase (ferredoxin)	Achl_3760	6.56	down	gamma-glutamyltransferase
Achl_2823	4.65	up	hypothetical protein Achl_2823	Achl_3771	2.37	down	hypothetical protein Achl_3771
Achl_2825	2.27	up	Polyprenyl synthetase	Achl_3778	2.20	down	sugar transporter
Achl_2826	3.17	up	geranylgeranyl reductase	Achl_3788	4.03	down	Mannitol dehydrogenase domain protein
Achl_2829	2.49	up	extracellular solute-binding protein family 3	Achl_3789	7.57	down	PTS system, mannitol-specific IIC subunit
Achl_2830	5.09	up	polar amino acid ABC transporter, inner membran	Achl_3790	8.39	down	transcriptional regulator, TetR family
Achl_2831	2.73	up	ABC transporter related	Achl_3791	3.00	down	phosphoenolpyruvate-protein phosphotransferase
Achl_2834	2.30	up	2-oxoglutarate decarboxylase	Achl_3799	2.28	down	transcriptional regulator, LacI family
Achl_2854	12.69	up	alpha/beta hydrolase fold protein	Achl_3805	2.02	down	extracellular solute-binding protein family 5
Achl_2855	8.74	up	hypothetical protein Achl_2855	Achl_3806	2.94	down	oligopeptide/dipeptide ABC transporter, ATPase subunit
Achl_2856	7.45	up	periplasmic solute binding protein	Achl_3807	2.98	down	binding-protein-dependent transport systems inner membrane component
Achl_2857	4.60	up	lipoprotein	Achl_3808	2.63	down	binding-protein-dependent transport systems inner membrane component
Achl_2858	4.83	up	ABC-3 protein	Achl_3811	2.25	down	putative lipoprotein
Achl_2907	3.22	up	UTP-glucose-1-phosphate uridylyltransferase	Achl_3819	2.03	down	aminotransferase class I and II
Achl_2941	2.52	up	glycosyl transferase group 1	Achl_3824	3.67	down	hypothetical protein Achl_3824
Achl_2942	3.08	up	glycosyltransferase-like protein	Achl_3836	5.81	down	monooxygenase FAD-binding
Achl_2943	3.71	up	polysaccharide pyruvyl transferase	Achl_3843	3.22	down	NAD(P)(+) transhydrogenase (AB-specific)

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Achl_2945	7.18	up	Acetyltransferase (isoleucine patch superfamily)-li	Achl_3866	2.56	down	hypothetical protein Achl_3866
Achl_2946	5.35	up	hypothetical protein Achl_2946	Achl_3870	4.52	down	UspA domain protein
Achl_2947	9.42	up	glycosyl transferase group 1	Achl_3876	5.08	down	protein of unknown function DUF6 transmembrane
Achl_2948	2.31	up	capsular exopolysaccharide family	Achl_3877	2.65	down	protein of unknown function DUF1470
Achl_2950	6.38	up	hypothetical protein Achl_2950	Achl_3890	2.10	down	single-strand binding protein
Achl_2979	5.92	up	surface presentation of antigens (SPOA) protein	Achl_3895	2.41	down	exopolysaccharide biosynthesis polyprenyl glycosylphosphotransferase
Achl_2982	2.59	up	flagellar FlbD family protein	Achl_3898	2.43	down	DegT/DnrJ/EryC1/StrS aminotransferase
Achl_2983	4.81	up	protein of unknown function DUF1078 domain pro	Achl_3899	2.11	down	putative acetyltransferase protein
Achl_2984	10.97	up	flagellar hook capping protein	Achl_3900	3.55	down	glycosyl transferase group 1
Achl_2993	3.86	up	flagellar basal-body rod protein FlgC	Achl_3902	3.48	down	DegT/DnrJ/EryC1/StrS aminotransferase
Achl_2994	7.32	up	flagellar basal-body rod protein FlgB	Achl_3907	2.23	down	hypothetical protein Achl_3907
Achl_2996	3.14	up	flagellar protein FliS	Achl_3913	2.90	down	histidine kinase
Achl_2997	7.12	up	flagellar hook-associated 2 domain protein	Achl_3944	2.34	down	hypothetical protein Achl_3944
Achl_2998	7.13	up	flagellin domain protein	Achl_3945	2.19	down	Dihydrofolate reductase
Achl_3000	2.99	up	flagellar hook-associated protein FlgK	Achl_3960	2.34	down	hypothetical protein Achl_3960
Achl_3001	2.45	up	flagellar hook-associated protein 3	Achl_3963	2.16	down	hypothetical protein Achl_3963
Achl_3003	2.10	up	hypothetical protein Achl_3003	Achl_3967	4.74	down	hypothetical protein Achl_3967
Achl_3033	2.63	up	protein tyrosine phosphatase	Achl_3971	2.53	down	NUDIX hydrolase
Achl_3069	2.57	up	amino acid permease-associated region	Achl_3978	2.42	down	filamentation induced by cAMP protein Fic
Achl_3078	2.32	up	AMP-dependent synthetase and ligase	Achl_3980	2.39	down	hypothetical protein Achl_3980
Achl_3088	3.19	up	cytochrome c-type biogenesis protein CcsB	Achl_3981	5.06	down	hypothetical protein Achl_3981
Achl_3093	29.81	up	YceI family protein	Achl_3983	2.48	down	hypothetical protein Achl_3983
Achl_3129	2.50	up	binding-protein-dependent transport systems inn	Achl_3997	3.93	down	hypothetical protein Achl_3997
Achl_3138	2.10	up	NAD-dependent epimerase/dehydratase	Achl_4002	5.09	down	hypothetical protein Achl_4002
Achl_3148	3.77	up	hypothetical protein Achl_3148	Achl_4008	2.73	down	protein of unknown function DUF1112
Achl_3168	10.04	up	acetate/CoA ligase	Achl_4028	2.61	down	hypothetical protein Achl_4028
Achl_3176	2.72	up	transcriptional regulator, Crp/Fnr family	Achl_4038	2.66	down	LGFP repeat protein
Achl_3178	2.93	up	Endoribonuclease L-PSP	Achl_4039	2.03	down	hypothetical protein Achl_4039
Achl_3179	2.12	up	hypothetical protein Achl_3179	Achl_4051	2.28	down	hypothetical protein Achl_4051
Achl_3180	3.01	up	glycosyl transferase family 51	Achl_4058	2.28	down	hypothetical protein Achl_4058
Achl_3181	3.30	up	metallophosphoesterase	Achl_4065	2.61	down	hypothetical protein Achl_4065
Achl_3187	3.18	up	phosphoribosylamine/glycine ligase	Achl_4076	3.10	down	hypothetical protein Achl_4076
Achl_3191	3.20	up	hypothetical protein Achl_3191	Achl_4086	3.80	down	hypothetical protein Achl_4086
Achl_3193	2.08	up	Asp/Glu/hydantoin racemase	Achl_4096	2.78	down	hypothetical protein Achl_4096
Achl_3194	3.87	up	permease for cytosine/purines uracil thiamine alla	Achl_4101	3.57	down	hypothetical protein Achl_4101
Achl_3198	3.64	up	aldo/keto reductase	Achl_4103	4.68	down	hypothetical protein Achl_4103
Achl_3210	4.48	up	transcriptional regulator, IclR family	Achl_4128	2.22	down	Resolvase domain protein
Achl_3211	5.54	up	Malate dehydrogenase (oxaloacetate-decarboxyla	Achl_4131	2.56	down	hypothetical protein Achl_4131
Achl_3212	5.22	up	malate synthase A	Achl_4139	2.41	down	hypothetical protein Achl_4139
Achl_3213	4.10	up	hypothetical protein Achl_3213	Achl_4146	2.17	down	hypothetical protein Achl_4146
Achl_3214	6.25	up	allantoin catabolism protein	Achl_4158	2.32	down	peptidase A24A prepilin type IV
Achl_3228	2.30	up	alpha/beta hydrolase fold protein	Achl_4165	2.51	down	hypothetical protein Achl_4165
Achl_3230	4.23	up	fatty acid desaturase	Achl_4168	5.41	down	hypothetical protein Achl_4168
Achl_3256	56.76	up	hypothetical protein Achl_3256	Achl_4170	2.75	down	hypothetical protein Achl_4170
Achl_3275	2.18	up	Alcohol dehydrogenase GroES domain protein	Achl_4195	3.04	down	metallophosphoesterase
Achl_3285	3.35	up	UspA domain protein	Achl_4198	2.23	down	hypothetical protein Achl_4198
Achl_3342	2.38	up	hypothetical protein Achl_3342	Achl_4201	2.10	down	hypothetical protein Achl_4201

Gene ID	Fold change	Regulation LE vs LS	annotation
Achl_3344	2.49	up	peptidase S1 and S6 chymotrypsin/Hap
Achl_3350	3.75	up	protein of unknown function DUF450
Achl_3351	3.15	up	hypothetical protein Achl_3351
Achl_3365	2.13	up	catalase/peroxidase HPI
Achl_3372	2.14	up	lipolytic protein G-D-S-L family
Achl_3419	4.34	up	hypothetical protein Achl_3419
Achl_3423	6.43	up	sodium:dicarboxylate symporter
Achl_3425	2.62	up	major facilitator superfamily MFS_1
Achl_3436	5.82	up	Xylose isomerase domain protein TIM barrel
Achl_3437	3.60	up	2-hydroxy-3-oxopropionate reductase
Achl_3438	6.06	up	glyoxylate carboligase
Achl_3440	2.63	up	allantoinase
Achl_3441	4.07	up	putative two component transcriptional regulator
Achl_3442	5.20	up	alkyl hydroperoxide reductase/ Thiol specific antic
Achl_3449	2.47	up	response regulator receiver protein
Achl_3450	3.42	up	Cellulose synthase (UDP-forming)
Achl_3452	2.82	up	putative anti-sigma regulatory factor, serine/threc
Achl_3454	4.99	up	OHCU decarboxylase
Achl_3455	6.23	up	hydroxyisourate hydrolase
Achl_3456	2.44	up	transcriptional regulator, IclR family
Achl_3470	3.20	up	oxidoreductase alpha (molybdopterin) subunit
Achl_3471	6.29	up	beta-lactamase domain protein
Achl_3472	5.77	up	mycothiol-dependent formaldehyde dehydrogena
Achl_3474	2.26	up	amidophosphoribosyltransferase
Achl_3572	2.77	up	Amine oxidase (copper-containing)
Achl_3594	2.10	up	sarcosine oxidase, delta subunit family
Achl_3643	3.50	up	domain of unknown function DUF1737
Achl_3644	5.62	up	Rhodanese domain protein
Achl_3645	3.17	up	O-succinylhomoserine sulfhydrylase
Achl_3654	3.21	up	hypothetical protein Achl_3654
Achl_3676	2.18	up	aspartate 1-decarboxylase
Achl_3718	10.77	up	histone family protein DNA-binding protein
Achl_3721	3.28	up	ribosomal protein S14
Achl_3722	3.47	up	ribosomal protein L33
Achl_3723	3.16	up	ribosomal protein L28
Achl_3728	3.57	up	transcriptional regulator, MarR family
Achl_3747	2.40	up	NmrA family protein
Achl_3758	6.45	up	fumarate lyase
Achl_3759	2.93	up	amino acid permease-associated region
Achl_3773	80.45	up	Malate dehydrogenase (oxaloacetate-decarboxylating) (NADP(+))
Achl_3782	4.13	up	transcriptional regulator, MarR family
Achl_3783	2.32	up	GCN5-related N-acetyltransferase
Achl_3817	5.48	up	Transketolase central region
Achl_3818	3.56	up	pyruvate dehydrogenase (acetyl-transferring) E1 component, alpha subunit
Achl_3822	2.44	up	membrane protein of unknown function
Achl_3826	3.52	up	adenylosuccinate lyase
Achl_3832	5.69	up	thioesterase superfamily protein
Achl_3846	2.91	up	transcriptional regulator, IclR family
Achl_3848	3.40	up	3-oxoacid CoA-transferase, A subunit
Achl_4217	2.90	down	hypothetical protein Achl_4217
Achl_4223	2.59	down	hypothetical protein Achl_4223
Achl_4230	2.58	down	hypothetical protein Achl_4230
Achl_4237	2.21	down	hypothetical protein Achl_4237
Achl_4254	2.93	down	CMP/dCMP deaminase zinc-binding
Achl_4258	2.98	down	hypothetical protein Achl_4258
Achl_4268	3.20	down	hypothetical protein Achl_4268
Achl_4271	2.20	down	hypothetical protein Achl_4271
Achl_4274	3.26	down	hypothetical protein Achl_4274
Achl_4279	2.18	down	hypothetical protein Achl_4279
Achl_4290	2.74	down	hypothetical protein Achl_4290
Achl_4317	4.23	down	hypothetical protein Achl_4317
Achl_4333	3.12	down	hypothetical protein Achl_4333
Achl_4342	2.52	down	hypothetical protein Achl_4342
Achl_4361	4.61	down	hypothetical protein Achl_4361
Achl_4364	2.05	down	hypothetical protein Achl_4364
Achl_4366	2.31	down	hypothetical protein Achl_4366
Achl_4397	2.49	down	hypothetical protein Achl_4397
Achl_4411	2.09	down	hypothetical protein Achl_4411
Achl_4414	3.66	down	hypothetical protein Achl_4414
Achl_4416	3.17	down	hypothetical protein Achl_4416
Achl_4417	2.14	down	putative Trp operon repressor
Achl_4438	2.73	down	hypothetical protein Achl_4438
Achl_4440	2.96	down	TadE family protein
Achl_4443	2.17	down	Cobyrinic acid ac-diamide synthase
Achl_4452	2.33	down	peptidoglycan-binding LysM
Achl_4455	3.21	down	hypothetical protein Achl_4455
Achl_4476	3.63	down	heat shock protein DnaJ domain protein
Achl_4484	4.45	down	hypothetical protein Achl_4484
Achl_4487	2.61	down	lipoprotein
Achl_4498	2.64	down	DNA-directed DNA polymerase
Achl_4508	2.44	down	hypothetical protein Achl_4508
Achl_4551	2.14	down	CHAP domain containing protein
Achl_4553	2.50	down	hypothetical protein Achl_4553
Achl_4556	2.83	down	hypothetical protein Achl_4556
Achl_4585	4.71	down	hypothetical protein Achl_4585
Achl_4620	2.12	down	transcriptional regulator, LacI family
Achl_4634	3.27	down	hypothetical protein Achl_4634
Achl_4638	3.22	down	single-strand binding protein

Gene ID	Fold change	Regulation LE vs LS	annotation
AchI_3852	2.12	up	fumarate lyase
AchI_3854	3.15	up	protocatechuate 3,4-dioxygenase, beta subunit
AchI_3872	2.38	up	Heavy metal transport/detoxification protein
AchI_3875	2.79	up	hypothetical protein AchI_3875
AchI_3878	3.67	up	hypothetical protein AchI_3878
AchI_3881	5.19	up	DoxX family protein
AchI_3883	2.70	up	replicative DNA helicase
AchI_3888	2.30	up	ribosomal protein L9
AchI_3891	3.83	up	ribosomal protein S6
AchI_3909	2.07	up	hypothetical protein AchI_3909
AchI_3910	2.05	up	Aspartyl aminopeptidase
AchI_3921	2.38	up	hypothetical protein AchI_3921
AchI_3929	2.10	up	thioredoxin
AchI_3930	4.26	up	parB-like partition protein
AchI_3931	3.75	up	Cobyrinic acid ac-diamide synthase
AchI_3932	3.48	up	methyltransferase GidB
AchI_3933	7.09	up	single-stranded nucleic acid binding R3H domain p
AchI_3934	5.62	up	60 kDa inner membrane insertion protein
AchI_3936	3.06	up	ribonuclease P protein component
AchI_3977	2.96	up	hypothetical protein AchI_3977
AchI_3984	5.86	up	hypothetical protein AchI_3984
AchI_3996	9.94	up	putative lysyl tRNA synthetase-like protein
AchI_4057	2.45	up	hypothetical protein AchI_4057
AchI_4126	2.90	up	hypothetical protein AchI_4126
AchI_4155	3.43	up	hypothetical protein AchI_4155
AchI_4179	3.50	up	hypothetical protein AchI_4179
AchI_4190	3.28	up	Cobyrinic acid ac-diamide synthase
AchI_4209	5.77	up	hypothetical protein AchI_4209
AchI_4210	2.93	up	hypothetical protein AchI_4210
AchI_4234	2.93	up	hypothetical protein AchI_4234
AchI_4282	2.88	up	hypothetical protein AchI_4282
AchI_4383	5.84	up	hypothetical protein AchI_4383
AchI_4424	8.25	up	hypothetical protein AchI_4424
AchI_4450	2.86	up	hypothetical protein AchI_4450
AchI_4472	7.01	up	histone family protein DNA-binding protein
AchI_4481	2.30	up	hypothetical protein AchI_4481
AchI_4501	2.82	up	helicase domain protein
AchI_4502	2.51	up	hypothetical protein AchI_4502
AchI_4546	2.18	up	hypothetical protein AchI_4546
AchI_4547	6.39	up	enoyl-(acyl carrier protein) reductase
AchI_4548	13.08	up	hypothetical protein AchI_4548
AchI_4549	5.10	up	transcriptional regulator, ArsR family
AchI_4564	14.09	up	monooxygenase FAD-binding
AchI_4565	9.90	up	iron-containing alcohol dehydrogenase
AchI_4566	24.99	up	intradiol ring-cleavage dioxygenase
AchI_4568	20.30	up	hypothetical protein AchI_4568
AchI_4569	14.18	up	intradiol ring-cleavage dioxygenase
AchI_4570	25.26	up	flavin reductase domain protein FMN-binding
AchI_4572	71.27	up	protein of unknown function DUF1486
AchI_4573	21.26	up	4-hydroxyphenylacetate 3-hydroxylase
AchI_4574	4.91	up	iron-containing alcohol dehydrogenase
AchI_4578	3.23	up	Succinate dehydrogenase
AchI_4581	17.62	up	hypothetical protein AchI_4581
AchI_4586	6.58	up	FMN adenylyltransferase
AchI_4590	2.37	up	phospholipase/Carboxylesterase
AchI_4594	45.29	up	3-oxoacid CoA-transferase, A subunit
AchI_4595	30.60	up	3-oxoacid CoA-transferase, B subunit
AchI_4612	2.38	up	extracellular solute-binding protein family 1
AchI_4613	2.39	up	binding-protein-dependent transport systems inner membrane component
AchI_4614	2.55	up	binding-protein-dependent transport systems inner membrane component
AchI_4615	3.44	up	hypothetical protein AchI_4615
AchI_4616	7.18	up	PfkB domain protein
AchI_4619	5.15	up	hypothetical protein AchI_4619
AchI_4626	2.09	up	protein of unknown function DUF1016

Table 4. **Enriched GO terms among the differentially expressed genes in the comparison of liquid cultures with 4CP in exponential versus stationary phases**

GO terms with genes Up regulated in Liquid exponential-A6

GOID	Biological Process	No probes in class	Total No probes in class	Total No probes on array	No probes in comparison	log_odds_ratio	p-value	Genes
GO:0009225	nucleotide-sugar metabolic process	4	5	2535	364	2.48	2.97E-03	Achl_2254 , Achl_2411 , Achl_2907 , Achl_2571
GO:0006412	translation	15	22	2535	364	2.25	1.18E-08	Achl_2701 , Achl_2669 , Achl_1289 , Achl_2672 , Achl_1491 , Achl_2686 , Achl_2679 , Achl_0346 , Achl_1480 , Achl_2678 , Achl_1911 , Achl_2273 , Achl_1196 , Achl_1367 , Achl_1419
GO:0006094	gluconeogenesis	5	8	2535	364	2.12	2.35E-03	Achl_0121 , Achl_1547 , Achl_1836 , Achl_0814 , Achl_1830
GO:0009109	coenzyme catabolic process	6	10	2535	364	2.06	1.09E-03	Achl_0710 , Achl_0933 , Achl_2531 , Achl_3758 , Achl_3212 , Achl_1994
GO:0007155	cell adhesion	3	5	2535	364	2.06	2.39E-02	Achl_2856 , Achl_2997 , Achl_2376
GO:0009089	lysine biosynthetic process via diaminopimelate	3	5	2535	364	2.06	2.39E-02	Achl_2733 , Achl_1450 , Achl_1454
GO:0006188	IMP biosynthetic process	5	9	2535	364	1.95	4.66E-03	Achl_0678 , Achl_1260 , Achl_1259 , Achl_3826 , Achl_0679
GO:0045333	cellular respiration	9	17	2535	364	1.88	7.59E-04	Achl_0710 , Achl_0933 , Achl_2531 , Achl_3758 , Achl_3212 , Achl_1954 , Achl_1953 , Achl_2470 , Achl_1946
GO:0006541	glutamine metabolic process	5	10	2535	364	1.80	8.24E-03	Achl_2000 , Achl_1594 , Achl_0019 , Achl_0679 , Achl_1591
GO:0001539	ciliary or flagellar motility	5	10	2535	364	1.80	8.24E-03	Achl_2994 , Achl_2998 , Achl_2979 , Achl_2983 , Achl_3001
GO:0030031	cell projection assembly	3	6	2535	364	1.80	4.27E-02	Achl_2997 , Achl_2996 , Achl_3000
GO:0006091	generation of precursor metabolites and energy	17	36	2535	364	1.72	3.75E-03	Achl_2337 , Achl_0710 , Achl_0933 , Achl_2342 , Achl_2531 , Achl_3758 , Achl_3212 , Achl_1954 , Achl_1836 , Achl_2353 , Achl_1953 , Achl_2618 , Achl_3818 , Achl_2470 , Achl_0658 , Achl_1830 , Achl_1946
GO:0032787	monocarboxylic acid metabolic process	22	48	2535	364	1.67	1.43E-05	Achl_4573 , Achl_0121 , Achl_1547 , Achl_0710 , Achl_4547 , Achl_1872 , Achl_3438 , Achl_2208 , Achl_1190 , Achl_3212 , Achl_1836 , Achl_0315 , Achl_0316 , Achl_1325 , Achl_3437 , Achl_3854 , Achl_2206 , Achl_0814 , Achl_3846 , Achl_1830 , Achl_2367 , Achl_3594
GO:0034637	cellular carbohydrate biosynthetic process	11	24	2535	364	1.67	9.14E-04	Achl_0121 , Achl_1547 , Achl_2614 , Achl_2254 , Achl_1836 , Achl_2412 , Achl_3450 , Achl_0814 , Achl_0869 , Achl_1830 , Achl_2948
GO:0016998	cell wall macromolecule catabolic process	3	7	2535	364	1.58	6.68E-02	Achl_2065 , Achl_1556 , Achl_0996

GO:0008610	lipid biosynthetic process	11	27	2535	364	1.50	7.15E-04	Achl_4547 , Achl_1872 , Achl_2208 , Achl_2614 , Achl_2412 , Achl_1325 , Achl_2252 , Achl_2206 , Achl_2948 , Achl_2825 , Achl_2367
GO:1901607	alpha-amino acid biosynthetic process	19	49	2535	364	1.43	6.82E-02	Achl_1726 , Achl_2276 , Achl_2195 , Achl_1476 , Achl_2196 , Achl_1594 , Achl_1190 , Achl_2356 , Achl_2733 , Achl_2355 , Achl_1450 , Achl_0315 , Achl_0316 , Achl_1472 , Achl_1591 , Achl_1454 , Achl_2731 , Achl_1683 , Achl_1504
GO:0006568	tryptophan metabolic process	3	8	2535	364	1.38	9.58E-02	Achl_1476 , Achl_2040 , Achl_1683
GO:0042180	cellular ketone metabolic process	58	158	2535	364	1.35	2.31E-13	Achl_0711 , Achl_0121 , Achl_1547 , Achl_1360 , Achl_0710 , Achl_1726 , Achl_2276 , Achl_2000 , Achl_4547 , Achl_1872 , Achl_2279 , Achl_2195 , Achl_2354 , Achl_1476 , Achl_2196 , Achl_3438 , Achl_0689 , Achl_1594 , Achl_2208 , Achl_3211 , Achl_1190 , Achl_3212 , Achl_2356 , Achl_1491 , Achl_2733 , Achl_0019 , Achl_1836 , Achl_1541 , Achl_2355 , Achl_1450 , Achl_0315 , Achl_0316 , Achl_1325 , Achl_3437 , Achl_1472 , Achl_0679 , Achl_2040 , Achl_1591 , Achl_1454 , Achl_3645 , Achl_3854 , Achl_2206 , Achl_0814 , Achl_2731 , Achl_3846 , Achl_1911 , Achl_2273 , Achl_1196 , Achl_2761 , Achl_1367 , Achl_1419 , Achl_1830 , Achl_1683 , Achl_2834 , Achl_2367 , Achl_3594 , Achl_1501 , Achl_1504
GO:0044283	small molecule biosynthetic process	53	163	2535	364	1.18	2.84E-04	Achl_0121 , Achl_1547 , Achl_2337 , Achl_1726 , Achl_2342 , Achl_2276 , Achl_4586 , Achl_4547 , Achl_1872 , Achl_2279 , Achl_2195 , Achl_1476 , Achl_2196 , Achl_0689 , Achl_1594 , Achl_2208 , Achl_0678 , Achl_2614 , Achl_1190 , Achl_2356 , Achl_2254 , Achl_2733 , Achl_1836 , Achl_1260 , Achl_2353 , Achl_1259 , Achl_2355 , Achl_1450 , Achl_0315 , Achl_3931 , Achl_0316 , Achl_1325 , Achl_3826 , Achl_1472 , Achl_0679 , Achl_4190 , Achl_1288 , Achl_1591 , Achl_3187 , Achl_1454 , Achl_2206 , Achl_0814 , Achl_2731 , Achl_2318 , Achl_1853 , Achl_1830 , Achl_3872 , Achl_1683 , Achl_2834 , Achl_3474 , Achl_1218 , Achl_2367 , Achl_1504
GO:0009308	amine metabolic process	39	125	2535	364	1.12	1.65E-06	Achl_1360 , Achl_1726 , Achl_2276 , Achl_2000 , Achl_2279 , Achl_2195 , Achl_2354 , Achl_1476 , Achl_2196 , Achl_0689 , Achl_1594 , Achl_2356 , Achl_2254 , Achl_1491 , Achl_2733 , Achl_0019 , Achl_1541 , Achl_2355 , Achl_1450 , Achl_3437 , Achl_1472 , Achl_0679 , Achl_2040 , Achl_1591 , Achl_1454 , Achl_3645 , Achl_2731 , Achl_3180 , Achl_1911 , Achl_2273 , Achl_1196 , Achl_2761 , Achl_1367 , Achl_3440 , Achl_1419 , Achl_1683 , Achl_1570 , Achl_1501 , Achl_1504

GO:0019318	hexose metabolic process	14	45	2535	364	1.12	6.59E-02	Achl_0121 , Achl_2189 , Achl_1547 , Achl_1836 , Achl_2618 , Achl_3437 , Achl_3818 , Achl_2907 , Achl_0814 , Achl_2571 , Achl_0658 , Achl_1835 , Achl_1830 , Achl_2530
GO:0055114	oxidation-reduction process	51	257	2535	364	0.47	7.12E-02	Achl_3773 , Achl_4566 , Achl_2189 , Achl_2782 , Achl_2820 , Achl_4569 , Achl_0710 , Achl_0933 , Achl_4565 , Achl_2276 , Achl_2531 , Achl_1606 , Achl_2811 , Achl_3758 , Achl_4547 , Achl_1872 , Achl_0835 , Achl_3472 , Achl_3211 , Achl_1827 , Achl_3212 , Achl_2288 , Achl_0087 , Achl_4574 , Achl_2356 , Achl_1954 , Achl_2818 , Achl_2733 , Achl_1450 , Achl_1953 , Achl_2618 , Achl_3198 , Achl_3437 , Achl_3818 , Achl_2252 , Achl_4578 , Achl_1641 , Achl_3470 , Achl_3854 , Achl_2731 , Achl_2470 , Achl_2615 , Achl_2268 , Achl_1835 , Achl_2812 , Achl_1830 , Achl_1946 , Achl_1948 , Achl_3275 , Achl_3365 , Achl_1755

GOID	Cellular Components	No probes in class	Total No probes in class	Total No probes on array	No probes in comparison	log_odds_ratio	p-value	Genes
GO:0005840	ribosome	7	8	2535	364	2.61	8.43E-05	Achl_2701 , Achl_2669 , Achl_1289 , Achl_2672 , Achl_2686 , Achl_2679 , Achl_2678
GO:0009288	bacterial-type flagellum	8	15	2535	364	1.89	3.68E-02	Achl_2994 , Achl_2998 , Achl_2997 , Achl_2979 , Achl_2983 , Achl_2996 , Achl_3000 , Achl_3001
GO:0042995	cell projection	11	24	2535	364	1.67	6.21E-03	Achl_2984 , Achl_2994 , Achl_2998 , Achl_2997 , Achl_2979 , Achl_2983 , Achl_2993 , Achl_2996 , Achl_3000 , Achl_2982 , Achl_3001
GO:0030288	outer membrane-bounded periplasmic space	5	13	2535	364	1.42	4.11E-02	Achl_0596 , Achl_0870 , Achl_0704 , Achl_2482 , Achl_2829
GO:0044424	intracellular part	66	187	2535	364	1.30	3.15E-10	Achl_1547 , Achl_2337 , Achl_2984 , Achl_2342 , Achl_2701 , Achl_2669 , Achl_1289 , Achl_2531 , Achl_2994 , Achl_2998 , Achl_2997 , Achl_1606 , Achl_2672 , Achl_1476 , Achl_2196 , Achl_2979 , Achl_1594 , Achl_1574 , Achl_0678 , Achl_2983 , Achl_2254 , Achl_1491 , Achl_2686 , Achl_0002 , Achl_2733 , Achl_1836 , Achl_1541 , Achl_1450 , Achl_2239 , Achl_2679 , Achl_2993 , Achl_0315 , Achl_1579 , Achl_1993 , Achl_1325 , Achl_0346 , Achl_0679 , Achl_0306 , Achl_2252 , Achl_2621 , Achl_1591 , Achl_1309 , Achl_1454 , Achl_0675 , Achl_2996 , Achl_2206 , Achl_3000 , Achl_2678 , Achl_1911 , Achl_2273 , Achl_1517 , Achl_1196 , Achl_3176 , Achl_1367 , Achl_1607 , Achl_2982 , Achl_0001 , Achl_1502 , Achl_1419 , Achl_3001 , Achl_2147 , Achl_1972 , Achl_2367 , Achl_2742 , Achl_1501 , Achl_3910

GOID	Molecular Function	No probes in class	Total No probes in class	Total No probes on array	No probes in comparison	log_odds_ratio	p-value	Genes
GO:0005198	structural molecule activity	12	15	2535	364	2.48	1.81E-08	Achl_2701 , Achl_2669 , Achl_1289 , Achl_2994 , Achl_2998 , Achl_2672 , Achl_2983 , Achl_2686 , Achl_2679 , Achl_3000 , Achl_2678 , Achl_3001
GO:0046912	transferase activity, transferring acyl groups, acyl groups converted into alkyl on transfer	4	6	2535	364	2.22	1.02E-02	Achl_0710 , Achl_0933 , Achl_2531 , Achl_3212
GO:0004812	aminoacyl-tRNA ligase activity	7	11	2535	364	2.15	2.23E-04	Achl_3996 , Achl_1491 , Achl_1911 , Achl_2273 , Achl_1196 , Achl_1367 , Achl_1419
GO:0003924	GTPase activity	5	8	2535	364	2.12	2.22E-03	Achl_2817 , Achl_1574 , Achl_0346 , Achl_2387 , Achl_2535
GO:0004312	fatty acid synthase activity	4	7	2535	364	1.99	9.97E-03	Achl_4547 , Achl_1872 , Achl_2206 , Achl_2367
GO:0016857	racemase and epimerase activity, acting on carbohydrates and derivatives	3	6	2535	364	1.80	4.12E-02	Achl_2412 , Achl_1667 , Achl_2530
GO:0016861	intramolecular oxidoreductase activity, interconverting aldoses and ketoses	5	11	2535	364	1.66	1.27E-02	Achl_1476 , Achl_1836 , Achl_1257 , Achl_1830 , Achl_2162
GO:0005525	GTP binding	7	16	2535	364	1.61	4.05E-03	Achl_2817 , Achl_1574 , Achl_2546 , Achl_0346 , Achl_2387 , Achl_0814 , Achl_2535
GO:0016833	oxo-acid-lyase activity	3	7	2535	364	1.58	6.47E-02	Achl_0711 , Achl_0019 , Achl_2742
GO:0020037	heme binding	5	12	2535	364	1.54	1.92E-02	Achl_2782 , Achl_2820 , Achl_1954 , Achl_1953 , Achl_3365
GO:0016765	transferase activity, transferring alkyl or aryl (other than methyl) groups	6	15	2535	364	1.48	1.29E-02	Achl_1360 , Achl_2254 , Achl_0316 , Achl_1993 , Achl_3645 , Achl_2761
GO:0016782	transferase activity, transferring sulfur-containing groups	5	13	2535	364	1.42	2.76E-02	Achl_4594 , Achl_4595 , Achl_2389 , Achl_3848 , Achl_1659
GO:0016763	transferase activity, transferring pentosyl groups	3	8	2535	364	1.38	9.28E-02	Achl_2318 , Achl_3474 , Achl_1308
GO:0016597	amino acid binding	5	14	2535	364	1.31	3.81E-02	Achl_2276 , Achl_0689 , Achl_3211 , Achl_2356 , Achl_1501
GO:0043176	amine binding	5	14	2535	364	1.31	3.81E-02	Achl_2276 , Achl_0689 , Achl_3211 , Achl_2356 , Achl_1501
GO:0051287	NAD binding	8	23	2535	364	1.28	1.09E-02	Achl_3773 , Achl_2189 , Achl_2276 , Achl_1872 , Achl_3211 , Achl_2733 , Achl_2252 , Achl_2268
GO:0005506	iron ion binding	11	44	2535	364	0.80	3.99E-02	Achl_4566 , Achl_2782 , Achl_2820 , Achl_4569 , Achl_0911 , Achl_1954 , Achl_1953 , Achl_2533 , Achl_3854 , Achl_1948 , Achl_3365
GO:0019842	vitamin binding	16	64	2535	364	0.80	1.47E-02	Achl_1547 , Achl_1360 , Achl_0910 , Achl_2279 , Achl_2195 , Achl_3438 , Achl_2553 , Achl_2709 , Achl_2389 , Achl_2355 , Achl_3818 , Achl_1472 , Achl_3645 , Achl_2761 , Achl_1683 , Achl_2834
GO:0030170	pyridoxal phosphate binding	11	49	2535	364	0.64	7.91E-02	Achl_1360 , Achl_0910 , Achl_2195 , Achl_2553 , Achl_2709 , Achl_2389 , Achl_2355 , Achl_1472 , Achl_3645 , Achl_2761 , Achl_1683

GO:0046872	metal ion binding	43	192	2535	364	0.64	5.52E-03	Achl_3773 , Achl_4566 , Achl_2782 , Achl_2820 , Achl_4569 , Achl_4565 , Achl_2856 , Achl_2279 , Achl_3438 , Achl_3472 , Achl_3211 , Achl_0911 , Achl_1827 , Achl_4574 , Achl_1954 , Achl_1491 , Achl_0666 , Achl_1257 , Achl_0796 , Achl_1953 , Achl_1993 , Achl_0306 , Achl_1253 , Achl_1840 , Achl_1288 , Achl_2387 , Achl_3470 , Achl_2533 , Achl_3854 , Achl_2376 , Achl_0658 , Achl_2268 , Achl_1607 , Achl_0267 , Achl_3440 , Achl_3872 , Achl_1948 , Achl_1972 , Achl_3275 , Achl_3365 , Achl_2742 , Achl_3909 , Achl_3910

GO terms with genes DOWN regulated in Liquid exponential-A6

GOID	Biological Process	No probes in class	Total No probes in class	Total No probes on array	No probes in comparison	log_odds_ratio	p-value	Genes
GO:0006547	histidine metabolic process	3	9	2535	274	1.62	5.08E-02	Achl_1356 , Achl_1077 , Achl_0588
GO:0006544	glycine metabolic process	4	12	2535	274	1.62	2.41E-02	Achl_3596 , Achl_3945 , Achl_0881 , Achl_3469
GO:0009231	riboflavin biosynthetic process	4	13	2535	274	1.51	3.22E-02	Achl_2749 , Achl_1772 , Achl_2593 , Achl_1800
GO:0009310	amine catabolic process	5	17	2535	274	1.44	2.05E-02	Achl_3673 , Achl_1356 , Achl_3136 , Achl_0588 , Achl_3469
GO:0008643	carbohydrate transport	5	19	2535	274	1.28	3.28E-02	Achl_3789 , Achl_0525 , Achl_3791 , Achl_0944 , Achl_3778
GO:0006950	response to stress	16	92	2535	274	0.69	5.77E-03	Achl_2069 , Achl_1698 , Achl_0220 , Achl_3870 , Achl_3067 , Achl_4476 , Achl_3038 , Achl_0523 , Achl_2076 , Achl_2072 , Achl_2766 , Achl_4498 , Achl_2871 , Achl_0554 , Achl_0604 , Achl_1509
GO:0006810	transport	55	379	2535	274	0.43	1.45E-02	Achl_2302 , Achl_1180 , Achl_3484 , Achl_3789 , Achl_0624 , Achl_0623 , Achl_2837 , Achl_1065 , Achl_0525 , Achl_2627 , Achl_0873 , Achl_0582 , Achl_0985 , Achl_0577 , Achl_2455 , Achl_0030 , Achl_3791 , Achl_1704 , Achl_3807 , Achl_3123 , Achl_3806 , Achl_3169 , Achl_3483 , Achl_3355 , Achl_3070 , Achl_0297 , Achl_2747 , Achl_0877 , Achl_1106 , Achl_1377 , Achl_3808 , Achl_3378 , Achl_2662 , Achl_3171 , Achl_3356 , Achl_1763 , Achl_3075 , Achl_1779 , Achl_2960 , Achl_3600 , Achl_3482 , Achl_0971 , Achl_3710 , Achl_2663 , Achl_2408 , Achl_3297 , Achl_0610 , Achl_3023 , Achl_1633 , Achl_0944 , Achl_3778 , Achl_1105 , Achl_1711 , Achl_3805 , Achl_0255
GOID	**Cellular Components**							

GO:0016021	integral to membrane	39	297	2535	274	0.28	4.22E-02	Achl_2302 , Achl_1180 , Achl_3789 , Achl_0559 , Achl_0624 , Achl_1150 , Achl_0525 , Achl_2627 , Achl_1056 , Achl_0582 , Achl_3843 , Achl_0577 , Achl_3509 , Achl_0030 , Achl_1704 , Achl_3382 , Achl_3807 , Achl_3123 , Achl_3913 , Achl_3355 , Achl_0297 , Achl_3381 , Achl_2747 , Achl_0877 , Achl_1377 , Achl_3808 , Achl_2662 , Achl_3171 , Achl_2347 , Achl_3356 , Achl_1763 , Achl_1902 , Achl_3482 , Achl_0971 , Achl_3710 , Achl_2763 , Achl_0944 , Achl_3778 , Achl_1105

GOID	Molecular Function	No probes in class	Total No probes in class	Total No probes on array	No probes in comparison	log_odds_ratio	p-value	Genes
GO:0008703	5-amino-6-(5-phosphoribosylamino)uracil reductase activity	4	8	2535	274	2.21	6.27E-03	Achl_2749 , Achl_1772 , Achl_2593 , Achl_1800
GO:0016780	phosphotransferase activity, for other substituted phosphate groups	4	9	2535	274	2.04	1.04E-02	Achl_2041 , Achl_2347 , Achl_3895 , Achl_1493
GO:0000150	recombinase activity	3	7	2535	274	1.99	3.04E-02	Achl_0166 , Achl_4128 , Achl_0955
GO:0003985	acetyl-CoA C-acetyltransferase activity	2	5	2535	274	1.89	9.13E-02	Achl_3063 , Achl_2699
GO:0032440	2-alkenal reductase [NAD(P)] activity	3	8	2535	274	1.79	4.48E-02	Achl_0944 , Achl_3778 , Achl_0881
GO:0005351	sugar:hydrogen symporter activity	5	15	2535	274	1.62	1.63E-02	Achl_3789 , Achl_0525 , Achl_3791 , Achl_0944 , Achl_3778
GO:0008483	transaminase activity	7	26	2535	274	1.32	1.61E-02	Achl_3902 , Achl_2832 , Achl_1077 , Achl_3898 , Achl_2457 , Achl_0881 , Achl_3819
GO:0003887	DNA-directed DNA polymerase activity	4	17	2535	274	1.12	9.91E-02	Achl_0220 , Achl_3067 , Achl_4498 , Achl_1069
GO:0019842	vitamin binding	11	64	2535	274	0.67	7.25E-02	Achl_1399 , Achl_3902 , Achl_2832 , Achl_1077 , Achl_1404 , Achl_3898 , Achl_3596 , Achl_2457 , Achl_0968 , Achl_0881 , Achl_3819
GO:0005215	transporter activity	52	331	2535	274	0.54	2.84E-02	Achl_2302 , Achl_1180 , Achl_3484 , Achl_3789 , Achl_0624 , Achl_0623 , Achl_2837 , Achl_1065 , Achl_0525 , Achl_0873 , Achl_0985 , Achl_0577 , Achl_2455 , Achl_0030 , Achl_3791 , Achl_1704 , Achl_3807 , Achl_3123 , Achl_3806 , Achl_3169 , Achl_3483 , Achl_3355 , Achl_3070 , Achl_0297 , Achl_2747 , Achl_0877 , Achl_1377 , Achl_3808 , Achl_3378 , Achl_2662 , Achl_3171 , Achl_3356 , Achl_1763 , Achl_3075 , Achl_1779 , Achl_3600 , Achl_3482 , Achl_0971 , Achl_3710 , Achl_2663 , Achl_2763 , Achl_3297 , Achl_0610 , Achl_3023 , Achl_1633 , Achl_0944 , Achl_3778 , Achl_2077 , Achl_1105 , Achl_1711 , Achl_3805 , Achl_0255

Table 5. **Liste de genes differentially expressed for *A. chlorophenolicus* in the comparison of 1 hour after inoculation in sand contaminated with 4CP versus non contaminated**

Gene ID	Fold change	Regulation ([4cp] vs [non])	Annotation	Gene ID	Fold change	Regulation ([4cp] vs [non])	Annotation
Achl_0028	3.07	down	extracellular solute-binding protein family 5	Achl_0042	4.96	up	protein of unknown function DUF1348
Achl_0046	2.21	down	Glyoxalase/bleomycin resistance protein/dioxyg	Achl_0088	50.94	up	aminotransferase class I and II
Achl_0077	3.15	down	hypothetical protein Achl_0077	Achl_0089	7.61	up	Glyoxylate reductase
Achl_0109	2.01	down	Glyoxalase/bleomycin resistance protein/dioxyg	Achl_0091	6.03	up	transcriptional regulator, IclR family
Achl_0110	4.08	down	transcriptional regulator, AraC family	Achl_0094	3.23	up	hypothetical protein Achl_0094
Achl_0128	2.29	down	hypothetical protein Achl_0128	Achl_0121	7.44	up	phosphoenolpyruvate synthase
Achl_0137	2.10	down	oxidoreductase domain protein	Achl_0122	3.50	up	protein of unknown function DUF299
Achl_0166	4.50	down	Resolvase domain protein	Achl_0239	2.27	up	Glycerate kinase
Achl_0179	2.74	down	CMP/dCMP deaminase zinc-binding	Achl_0266	10.02	up	nitroreductase
Achl_0180	4.94	down	hypothetical protein Achl_0180	Achl_0274	2.60	up	L-carnitine dehydratase/bile acid-inducible protein F
Achl_0192	2.24	down	pyruvate carboxyltransferase	Achl_0299	3.35	up	Cof-like hydrolase
Achl_0245	2.83	down	protein of unknown function DUF336	Achl_0404	2.01	up	transferase hexapeptide repeat containing protein
Achl_0249	2.37	down	Xylose isomerase domain protein TIM barrel	Achl_0591	5.69	up	hypothetical protein Achl_0591
Achl_0254	2.26	down	binding-protein-dependent transport systems i	Achl_0662	3.82	up	hypothetical protein Achl_0662
Achl_0333	2.06	down	peptidase S9 prolyl oligopeptidase active site d	Achl_0663	2.56	up	peptidase S26B, signal peptidase
Achl_0350	2.32	down	Aldehyde Dehydrogenase	Achl_0686	8.79	up	transcriptional regulator, MarR family
Achl_0390	4.15	down	urease, gamma subunit	Achl_0710	137.84	up	malate synthase A
Achl_0391	3.19	down	urease, beta subunit	Achl_0711	171.62	up	isocitrate lyase
Achl_0392	4.01	down	urease, alpha subunit	Achl_0712	3.34	up	transcriptional regulator, XRE family
Achl_0393	3.66	down	UreE urease accessory domain protein	Achl_0717	9.12	up	hypothetical protein Achl_0717
Achl_0394	2.20	down	Urease accessory protein UreF	Achl_0749	2.17	up	protein of unknown function DUF161
Achl_0395	2.38	down	urease accessory protein UreG	Achl_0767	5.09	up	cytochrome P450
Achl_0396	3.41	down	Urease accessory protein UreD	Achl_0768	6.13	up	major facilitator superfamily MFS_1
Achl_0397	3.78	down	high-affinity nickel-transporter	Achl_0814	4.66	up	Phosphoenolpyruvate carboxykinase (GTP)
Achl_0398	2.54	down	secreted protein	Achl_0957	2.58	up	Myo-inositol catabolism IolB domain protein
Achl_0476	2.58	down	putative integral membrane protein	Achl_0961	2.31	up	peptidase M10A and M12B matrixin and adamalysin
Achl_0480	10.27	down	alpha amylase catalytic region	Achl_0969	3.44	up	transcriptional regulator, MarR family
Achl_0510	3.13	down	acetyltransferase-like protein	Achl_0982	8.67	up	hypothetical protein Achl_0982
Achl_0512	2.25	down	Pyridoxal-dependent decarboxylase	Achl_1081	2.84	up	hypothetical protein Achl_1081
Achl_0513	11.45	down	L-lysine 6-monooxygenase (NADPH)	Achl_1112	2.34	up	lipase class 2
Achl_0515	6.15	down	IucA/IucC family protein	Achl_1123	3.16	up	Amidase
Achl_0522	2.76	down	Methyltransferase type 11	Achl_1182	2.22	up	ROK family protein
Achl_0523	3.15	down	Activator of Hsp90 ATPase 1 family protein	Achl_1183	3.06	up	Nitrilase/cyanide hydratase and apolipoprotein N-acyltrans
Achl_0553	2.42	down	O-methyltransferase family 3	Achl_1243	2.19	up	Hly-III family protein
Achl_0596	2.71	down	hypothetical protein Achl_0596	Achl_1382	3.90	up	acyl-CoA dehydrogenase domain protein
Achl_0630	2.69	down	methylmalonate-semialdehyde dehydrogenase	Achl_1395	2.22	up	protein of unknown function DUF485
Achl_0631	3.21	down	Enoyl-CoA hydratase/isomerase	Achl_1396	2.86	up	SSS sodium solute transporter superfamily
Achl_0632	2.55	down	3-hydroxyisobutyrate dehydrogenase	Achl_1453	2.94	up	hypothetical protein Achl_1453

Gene ID	Fold change	Regulation ([4cp] vs [non])	Annotation	Gene ID	Fold change	Regulation ([4cp] vs [non])	Annotation
Achl_0643	2.10	down	histidine kinase	Achl_1471	5.88	up	Peptidoglycan-binding LysM
Achl_0647	2.21	down	3-oxoacid CoA-transferase, B subunit	Achl_1527	5.22	up	AMP-dependent synthetase and ligase
Achl_0677	2.02	down	hypothetical protein Achl_0677	Achl_1556	2.43	up	Lytic transglycosylase catalytic
Achl_0682	2.77	down	2-alkenal reductase	Achl_1599	2.02	up	lipoate-protein ligase B
Achl_0696	2.03	down	Peptidase M23	Achl_1604	2.86	up	hypothetical protein Achl_1604
Achl_0728	2.01	down	hypothetical protein Achl_0728	Achl_1615	4.03	up	transcriptional repressor, CopY family
Achl_0736	3.36	down	extracellular solute-binding protein family 1	Achl_1659	2.65	up	Rhodanese domain protein
Achl_0801	2.06	down	Glyoxalase/bleomycin resistance protein/dioxyg	Achl_1721	3.40	up	Putative gen
Achl_0825	3.95	down	5'-Nucleotidase domain protein	Achl_1723	3.79	up	NAD(P)(+) transhydrogenase (AB-specific)
Achl_0839	2.22	down	FAD-dependent pyridine nucleotide-disulphide	Achl_1728	4.21	up	transcriptional regulator, LysR family
Achl_0867	2.15	down	ABC transporter related	Achl_1747	2.90	up	hypothetical protein Achl_1747
Achl_0875	3.00	down	hypothetical protein Achl_0875	Achl_1767	7.09	up	short-chain dehydrogenase/reductase SDR
Achl_0903	5.25	down	hypothetical protein Achl_0903	Achl_1768	2.42	up	Alcohol dehydrogenase GroES domain protein
Achl_0904	4.96	down	hypothetical protein Achl_0904	Achl_1773	19.95	up	GTP cyclohydrolase II
Achl_0947	2.59	down	hypothetical protein Achl_0947	Achl_1889	2.00	up	acyl-CoA oxidase domain protein
Achl_0950	3.21	down	hypothetical protein Achl_0950	Achl_1890	2.62	up	transcriptional regulator, TetR family
Achl_0980	2.61	down	glycoside hydrolase family 26	Achl_1892	2.08	up	short-chain dehydrogenase/reductase SDR
Achl_1010	2.27	down	oxidoreductase alpha (molybdopterin) subunit	Achl_2189	5.82	up	glyceraldehyde-3-phosphate dehydrogenase, type I
Achl_1013	5.55	down	formaldehyde dehydrogenase, glutathione-inde	Achl_2241	3.96	up	hypothetical protein Achl_2241
Achl_1033	2.19	down	SOUL heme-binding protein	Achl_2266	2.24	up	hypothetical protein Achl_2266
Achl_1065	3.07	down	major facilitator superfamily MFS_1	Achl_2288	7.72	up	Luciferase-like monooxygenase
Achl_1113	4.25	down	hypothetical protein Achl_1113	Achl_2289	7.69	up	transcriptional regulator, MarR family
Achl_1116	4.21	down	hypothetical protein Achl_1116	Achl_2297	2.36	up	quinolinate synthetase complex, A subunit
Achl_1117	2.54	down	Putative gen	Achl_2298	2.69	up	NUDIX hydrolase
Achl_1158	3.78	down	transcriptional regulator, SARP family	Achl_2315	2.04	up	glutamate racemase
Achl_1168	2.38	down	hypothetical protein Achl_1168	Achl_2376	4.14	up	periplasmic solute binding protein
Achl_1169	3.02	down	hypothetical protein Achl_1169	Achl_2378	2.08	up	protein of unknown function DUF21
Achl_1178	8.05	down	Pectate lyase/Amb allergen	Achl_2543	2.12	up	Luciferase-like monooxygenase
Achl_1191	2.03	down	ABC transporter related	Achl_2553	2.46	up	aminotransferase class I and II
Achl_1313	11.74	down	major facilitator superfamily MFS_1	Achl_2566	2.76	up	NUDIX hydrolase
Achl_1315	2.24	down	glutamine amidotransferase	Achl_2567	5.53	up	cytidylyltransferase
Achl_1331	2.46	down	Fibronectin type III domain protein	Achl_2568	3.05	up	nicotinamide mononucleotide transporter PnuC
Achl_1399	5.25	down	pyruvate dehydrogenase (acetyl-transferring) E	Achl_2647	3.51	up	Flp pilus assembly protein ATPase CpaE-like protein
Achl_1401	3.32	down	catalytic domain of components of various deh	Achl_2786	217.63	up	protein of unknown function DUF779
Achl_1530	3.28	down	sodium:dicarboxylate symporter	Achl_2787	161.01	up	Alcohol dehydrogenase GroES domain protein
Achl_1702	2.28	down	hypothetical protein Achl_1702	Achl_2788	199.41	up	Aldehyde Dehydrogenase
Achl_1724	2.08	down	transcriptional coactivator/pterin dehydratase	Achl_2812	3.47	up	uroporphyrin-III C-methyltransferase
Achl_1760	3.66	down	hypothetical protein Achl_1760	Achl_2816	3.95	up	aliphatic sulfonates family ABC transporter, periplsmic ligar
Achl_1761	2.23	down	band 7 protein	Achl_2817	3.77	up	sulfate adenylyltransferase, large subunit

Gene ID	Fold change	Regulation ([4cp] vs [non])	Annotation
Achl_1763	2.38	down	sodium/hydrogen exchanger
Achl_1817	2.13	down	ABC transporter related
Achl_1859	2.42	down	hypothetical protein Achl_1859
Achl_1894	2.23	down	membrane protein-like protein
Achl_1898	3.31	down	hypothetical protein Achl_1898
Achl_2132	3.75	down	hypothetical protein Achl_2132
Achl_2232	10.48	down	ammonium transporter
Achl_2322	2.14	down	glucosamine-6-phosphate isomerase
Achl_2409	2.86	down	Lysozyme
Achl_2453	2.47	down	binding-protein-dependent transport systems i
Achl_2454	2.51	down	binding-protein-dependent transport systems i
Achl_2455	3.03	down	extracellular solute-binding protein family 5
Achl_2540	2.06	down	4-phytase
Achl_2636	2.16	down	hypothetical protein Achl_2636
Achl_2652	2.80	down	two component transcriptional regulator, winge
Achl_2743	2.01	down	Glyoxalase/bleomycin resistance protein/dioxyg
Achl_2749	3.21	down	bifunctional deaminase-reductase domain prot
Achl_2750	2.90	down	hypothetical protein Achl_2750
Achl_2764	2.63	down	hypothetical protein Achl_2764
Achl_2771	2.12	down	ABC transporter related
Achl_2833	2.17	down	Glyoxalase/bleomycin resistance protein/dioxyg
Achl_2852	4.07	down	hypothetical protein Achl_2852
Achl_2883	2.37	down	hypothetical protein Achl_2883
Achl_2884	2.12	down	hypothetical protein Achl_2884
Achl_2956	2.17	down	beta-mannanase-like protein
Achl_2984	2.13	down	flagellar hook capping protein
Achl_2993	2.38	down	flagellar basal-body rod protein FlgC
Achl_2994	3.30	down	flagellar basal-body rod protein FlgB
Achl_3005	3.53	down	ABC transporter related
Achl_3025	2.02	down	hypothetical protein Achl_3025
Achl_3039	2.42	down	hypothetical protein Achl_3039
Achl_3058	2.98	down	hypothetical protein Achl_3058
Achl_3119	2.92	down	extracellular solute-binding protein family 1
Achl_3126	3.16	down	hypothetical protein Achl_3126
Achl_3199	2.17	down	hypothetical protein Achl_3199
Achl_3221	2.27	down	protein of unknown function DUF72
Achl_3292	2.47	down	hypothetical protein Achl_3292
Achl_3297	2.26	down	permease for cytosine/purines uracil thiamine a
Achl_3305	3.14	down	hypothetical protein Achl_3305
Achl_3314	3.82	down	hypothetical protein Achl_3314
Achl_3323	2.13	down	Glyoxalase/bleomycin resistance protein/dioxyg
Achl_2818	3.76	up	sulfate adenylyltransferase, small subunit
Achl_2819	2.51	up	phosphoadenosine phosphosulfate reductase
Achl_2820	4.01	up	Sulfite reductase (ferredoxin)
Achl_2822	2.03	up	protein of unknown function UPF0126
Achl_2864	4.98	up	hypothetical protein Achl_2864
Achl_2933	2.17	up	hypothetical protein Achl_2933
Achl_2957	5.76	up	hypothetical protein Achl_2957
Achl_3074	2.22	up	binding-protein-dependent transport systems inner memb
Achl_3093	55.32	up	YceI family protein
Achl_3168	2.31	up	acetate/CoA ligase
Achl_3192	8.70	up	short-chain dehydrogenase/reductase SDR
Achl_3193	26.60	up	Asp/Glu/hydantoin racemase
Achl_3195	12.02	up	2-oxo-acid dehydrogenase E1 subunit, homodimeric type
Achl_3196	3.78	up	molybdenum cofactor cytidylyltransferase
Achl_3203	3.79	up	Xanthine/uracil/vitamin C permease
Achl_3204	2.62	up	CMP/dCMP deaminase zinc-binding
Achl_3207	5.05	up	hypothetical protein Achl_3207
Achl_3210	2.71	up	transcriptional regulator, IclR family
Achl_3211	52.73	up	Malate dehydrogenase (oxaloacetate-decarboxylating)
Achl_3212	18.62	up	malate synthase A
Achl_3213	16.99	up	hypothetical protein Achl_3213
Achl_3214	16.30	up	allantoin catabolism protein
Achl_3215	3.47	up	Serine-type D-Ala-D-Ala carboxypeptidase
Achl_3232	2.50	up	Luciferase-like monooxygenase
Achl_3245	2.48	up	transcriptional regulator, PadR-like family
Achl_3256	4.08	up	hypothetical protein Achl_3256
Achl_3417	4.08	up	NADP oxidoreductase coenzyme F420-dependent
Achl_3423	15.89	up	sodium:dicarboxylate symporter
Achl_3436	26.85	up	Xylose isomerase domain protein TIM barrel
Achl_3437	12.80	up	2-hydroxy-3-oxopropionate reductase
Achl_3438	36.79	up	glyoxylate carboligase
Achl_3439	38.75	up	Glycerate kinase
Achl_3440	11.32	up	allantoinase
Achl_3441	14.05	up	putative two component transcriptional regulator, winged
Achl_3443	3.93	up	Spore coat protein CotH
Achl_3460	4.40	up	hypothetical protein Achl_3460
Achl_3461	3.02	up	transcriptional regulator, GntR family
Achl_3462	47.36	up	formyltetrahydrofolate deformylase
Achl_3463	14.43	up	glycine cleavage T protein (aminomethyl transferase)
Achl_3464	5.90	up	Formiminotransferase-cyclodeaminase
Achl_3465	3.41	up	Methylenetetrahydrofolate dehydrogenase (NADP(+))

Gene ID	Fold change	Regulation ([4cp] vs [non])	Annotation	Gene ID	Fold change	Regulation ([4cp] vs [non])	Annotation
Achl_3329	2.84	down	hypothetical protein Achl_3329	Achl_3466	3.76	up	MoeA domain protein domain I and II
Achl_3365	2.09	down	catalase/peroxidase HPI	Achl_3469	2.28	up	FAD dependent oxidoreductase
Achl_3366	2.47	down	beta-lactamase domain protein	Achl_3481	3.36	up	ABC transporter related
Achl_3375	2.40	down	Glyoxalase/bleomycin resistance protein/dioxy	Achl_3483	2.09	up	molybdenum ABC transporter, periplasmic molybdate-binc
Achl_3380	2.35	down	two component transcriptional regulator, LuxR	Achl_3484	2.63	up	DNA binding domain protein, excisionase family
Achl_3381	2.50	down	histidine kinase	Achl_3525	2.63	up	glutamine synthetase, type III
Achl_3433	2.32	down	amidohydrolase	Achl_3572	4.81	up	Amine oxidase (copper-containing)
Achl_3445	2.55	down	GCN5-related N-acetyltransferase	Achl_3577	4.04	up	Glutamate dehydrogenase (NADP(+))
Achl_3480	8.26	down	peptidase S1 and S6 chymotrypsin/Hap	Achl_3589	11.92	up	hypothetical protein Achl_3589
Achl_3490	2.61	down	Ferritin Dps family protein	Achl_3590	11.28	up	formyltetrahydrofolate deformylase
Achl_3634	2.63	down	protein tyrosine phosphatase	Achl_3591	13.70	up	L-serine dehydratase 1
Achl_3638	2.15	down	pyridoxamine 5'-phosphate oxidase-related FM	Achl_3592	22.84	up	Sarcosine oxidase
Achl_3665	2.86	down	SCP-like extracellular protein	Achl_3593	26.41	up	sarcosine oxidase, alpha subunit family
Achl_3672	2.37	down	hypothetical protein Achl_3672	Achl_3594	28.28	up	sarcosine oxidase, delta subunit family
Achl_3729	2.08	down	Putative gen	Achl_3595	53.58	up	sarcosine oxidase, beta subunit family
Achl_3731	4.70	down	Siderophore-interacting protein	Achl_3596	21.52	up	Glycine hydroxymethyltransferase
Achl_3732	2.82	down	ABC transporter related	Achl_3597	9.33	up	transcriptional regulator, GntR family
Achl_3733	3.15	down	transport system permease protein	Achl_3619	2.82	up	chaperone DnaJ domain protein
Achl_3734	4.27	down	transport system permease protein	Achl_3676	2.37	up	aspartate 1-decarboxylase
Achl_3735	4.60	down	periplasmic binding protein	Achl_3690	2.15	up	Oxidoreductase FAD-binding domain protein
Achl_3760	4.89	down	gamma-glutamyltransferase	Achl_3691	2.06	up	formaldehyde dehydrogenase, glutathione-independent
Achl_3771	3.51	down	hypothetical protein Achl_3771	Achl_3728	2.94	up	transcriptional regulator, MarR family
Achl_3789	3.75	down	PTS system, mannitol-specific IIC subunit	Achl_3761	11.20	up	AAA ATPase central domain protein
Achl_3790	3.15	down	transcriptional regulator, TetR family	Achl_3773	5.47	up	Malate dehydrogenase (oxaloacetate-decarboxylating) (NA
Achl_3791	2.53	down	phosphoenolpyruvate-protein phosphotransfer	Achl_3810	6.92	up	NADPH-dependent FMN reductase
Achl_3806	2.82	down	oligopeptide/dipeptide ABC transporter, ATPas	Achl_3848	3.20	up	3-oxoacid CoA-transferase, A subunit
Achl_3807	2.63	down	binding-protein-dependent transport systems i	Achl_3881	20.89	up	DoxX family protein
Achl_3808	3.45	down	binding-protein-dependent transport systems i	Achl_3885	3.69	up	NADPH-dependent FMN reductase
Achl_3821	3.09	down	Pectate lyase/Amb allergen	Achl_4059	2.03	up	hypothetical protein Achl_4059
Achl_3866	2.08	down	hypothetical protein Achl_3866	Achl_4221	5.48	up	5 nucleotidase deoxy cytosolic type C
Achl_3875	2.21	down	hypothetical protein Achl_3875	Achl_4321	2.06	up	DNA-directed DNA polymerase

Gene ID	Fold change	Regulation ([4cp] vs [non])	Annotation
Achl_3914	2.75	down	ABC transporter, integral membrane subunit
Achl_3915	2.56	down	ABC transporter related
Achl_3960	3.18	down	hypothetical protein Achl_3960
Achl_3977	2.11	down	hypothetical protein Achl_3977
Achl_4135	2.25	down	hypothetical protein Achl_4135
Achl_4157	2.19	down	hypothetical protein Achl_4157
Achl_4225	5.72	down	hypothetical protein Achl_4225
Achl_4328	2.71	down	hypothetical protein Achl_4328
Achl_4513	2.55	down	hypothetical protein Achl_4513
Achl_4524	2.84	down	hypothetical protein Achl_4524
Achl_4598	2.99	down	Resolvase domain protein
Achl_4612	2.12	down	extracellular solute-binding protein family 1
Achl_4621	4.09	down	extracellular solute-binding protein family 1
Achl_4398	2.37	up	hypothetical protein Achl_4398
Achl_4528	2.95	up	transcriptional regulator, MerR family
Achl_4564	32.40	up	monooxygenase FAD-binding
Achl_4565	13.47	up	iron-containing alcohol dehydrogenase
Achl_4566	18.83	up	intradiol ring-cleavage dioxygenase
Achl_4568	65.80	up	hypothetical protein Achl_4568
Achl_4569	99.05	up	intradiol ring-cleavage dioxygenase
Achl_4570	148.00	up	flavin reductase domain protein FMN-binding
Achl_4572	202.00	up	protein of unknown function DUF1486
Achl_4573	125.06	up	4-hydroxyphenylacetate 3-hydroxylase
Achl_4574	10.33	up	iron-containing alcohol dehydrogenase
Achl_4578	4.13	up	Succinate dehydrogenase
Achl_4581	61.88	up	hypothetical protein Achl_4581
Achl_4586	7.75	up	FMN adenylyltransferase
Achl_4593	18.26	up	acetyl-CoA acetyltransferase
Achl_4594	27.81	up	3-oxoacid CoA-transferase, A subunit
Achl_4595	18.24	up	3-oxoacid CoA-transferase, B subunit
Achl_4599	3.26	up	hypothetical protein Achl_4599

Table 6. **Enriched GO terms among the differentially expressed genes in the comparison between A6 cells after 1h in sand contaminated with 4-Chlorophenol (4CP) versus cells in only sand**

Genes higher expressed in cells o f A6 in sand with 4 CP after 1 h

GOID	Biological Process	No probes in class	Total No probes in class	Total No probes on array	No probes in comparison	log_odds_ratio	p-value	Genes
GO:0006546	glycine catabolic process	3	6	2535	123	3.37	2.56E-03	Achl_3593 , Achl_3463 , Achl_3469
GO:0006094	gluconeogenesis	3	8	2535	123	2.95	6.62E-03	Achl_3591 , Achl_0121 , Achl_0814
GO:0016998	cell wall macromolecule catabolic process	2	7	2535	123	2.56	4.88E-02	Achl_1471 , Achl_1556
GO:0046165	alcohol biosynthetic process	3	11	2535	123	2.49	1.74E-02	Achl_3591 , Achl_0121 , Achl_0814
GO:0032787	monocarboxylic acid metabolic process	13	48	2535	123	2.48	2.00E-06	Achl_0710 , Achl_4573 , Achl_3595 , Achl_3438 , Achl_3594 , Achl_3593 , Achl_3212 , Achl_3591 , Achl_3437 , Achl_0121 , Achl_0814 , Achl_3465 , Achl_1889
GO:0006189	'de novo' IMP biosynthetic process	2	8	2535	123	2.37	6.29E-02	Achl_3462 , Achl_3590
GO:0006099	tricarboxylic acid cycle	2	9	2535	123	2.20	7.82E-02	Achl_0710 , Achl_3212
GO:0046356	acetyl-CoA catabolic process	2	9	2535	123	2.20	7.82E-02	Achl_0710 , Achl_3212
GO:0009310	amine catabolic process	3	17	2535	123	1.86	5.69E-02	Achl_3593 , Achl_3463 , Achl_3469
GO:0055114	oxidation-reduction process	34	257	2535	123	1.45	6.82E-07	Achl_2788 , Achl_2787 , Achl_0710 , Achl_4569 , Achl_3211 , Achl_3592 , Achl_4566 , Achl_3212 , Achl_4565 , Achl_3437 , Achl_3195 , Achl_4574 , Achl_3192 , Achl_2288 , Achl_0089 , Achl_1767 , Achl_2189 , Achl_3773 , Achl_0767 , Achl_4578 , Achl_3577 , Achl_2820 , Achl_1382 , Achl_1723 , Achl_2818 , Achl_2812 , Achl_3465 , Achl_2819 , Achl_3232 , Achl_1768 , Achl_3690 , Achl_2543 , Achl_3691 , Achl_1889
GO:0042180	cellular ketone metabolic process	19	158	2535	123	1.31	3.99E-04	Achl_0711 , Achl_0710 , Achl_3595 , Achl_3211 , Achl_3438 , Achl_3594 , Achl_3593 , Achl_3596 , Achl_3212 , Achl_3463 , Achl_3591 , Achl_3437 , Achl_0121 , Achl_0814 , Achl_3577 , Achl_3465 , Achl_3525 , Achl_3469 , Achl_1889
GOID	**Cellular Components**							
GO:0043190	ATP-binding cassette (ABC) transporter complex	2	5	2535	123	3.04	1.12E-02	Achl_3481 , Achl_3484
GO:0030288	outer membrane-bounded periplasmic space	2	13	2535	123	1.66	7.36E-02	Achl_0094 , Achl_3483

GOID	Molecular Function							
GO:0008115	sarcosine oxidase activity	4	6	2535	123	3.78	7.33E-05	Achl_3595 , Achl_3594 , Achl_3593 , Achl_3592
GO:0004372	glycine hydroxymethyltransferase activity	3	5	2535	123	3.63	1.04E-03	Achl_3462 , Achl_3596 , Achl_3590
GO:0008410	CoA-transferase activity	3	8	2535	123	2.95	5.21E-03	Achl_4594 , Achl_4595 , Achl_3848
GO:0003995	acyl-CoA dehydrogenase activity	3	10	2535	123	2.63	1.04E-02	Achl_4573 , Achl_1382 , Achl_1889
GO:0051539	4 iron, 4 sulfur cluster binding	3	14	2535	123	2.14	2.74E-02	Achl_3591 , Achl_2820 , Achl_2297
GO:0051287	NAD binding	4	23	2535	123	1.84	2.28E-02	Achl_3211 , Achl_0089 , Achl_2189 , Achl_3773
GO:0004497	monooxygenase activity	6	39	2535	123	1.66	1.00E-02	Achl_4573 , Achl_4564 , Achl_2288 , Achl_0767 , Achl_3232 , Achl_2543
GO:0005506	iron ion binding	6	44	2535	123	1.49	1.78E-02	Achl_4569 , Achl_4566 , Achl_0767 , Achl_2820 , Achl_2297 , Achl_3690
GO:0009055	electron carrier activity	8	94	2535	123	0.81	9.06E-02	Achl_4573 , Achl_3593 , Achl_0767 , Achl_4578 , Achl_2820 , Achl_1382 , Achl_3690 , Achl_1889

Genes lower expressed in cells o f A6 in sand with 4 CP after 1 h

GOID	Biological Process	No probes in class	Total No probes in class	Total No probes on array	No probes in comparison	log_odds_ratio	p-value	Genes
GO:0006573	valine metabolic process	2	6	2535	99	3.09	1.68E-02	Achl_0630 , Achl_0632
GO:0009401	phosphoenolpyruvate-dependent sugar phosphotransferase system	2	9	2535	99	2.51	3.76E-02	Achl_3789 , Achl_3791
GO:0006810	transport	27	379	2535	99	0.87	6.64E-04	Achl_2232 , Achl_3735 , Achl_3734 , Achl_4621 , Achl_0397 , Achl_3789 , Achl_3808 , Achl_0736 , Achl_1530 , Achl_3733 , Achl_0028 , Achl_1065 , Achl_2455 , Achl_3119 , Achl_3806 , Achl_3807 , Achl_3490 , Achl_3791 , Achl_2454 , Achl_2453 , Achl_1763 , Achl_0254 , Achl_3297 , Achl_0867 , Achl_1817 , Achl_4612 , Achl_2540

GOID	Cellular Components							
GO:0042995	cell projection	3	24	2535	99	1.68	6.25E-02	Achl_2994 , Achl_2993 , Achl_2984

GOID	Molecular Function							
GO:0000150	recombinase activity	2	7	2535	99	2.87	2.87E-02	Achl_0166 , Achl_4598
GO:0016701	oxidoreductase activity, acting on single donors with incorporation of molecular oxygen	8	32	2535	99	2.68	1.39E-04	Achl_0513 , Achl_3375 , Achl_0046 , Achl_2833 , Achl_3323 , Achl_0801 , Achl_0109 , Achl_2743
GO:0016903	oxidoreductase activity, acting on the aldehyde or oxo group of donors	3	14	2535	99	2.46	6.67E-02	Achl_1399 , Achl_0630 , Achl_1010
GO:0046983	protein dimerization activity	3	17	2535	99	2.18	2.74E-02	Achl_3381 , Achl_3433 , Achl_0643
GO:0008236	serine-type peptidase activity	3	20	2535	99	1.94	4.22E-02	Achl_3480 , Achl_0682 , Achl_0333
GO:0005215	transporter activity	27	331	2535	99	1.06	5.52E-04	Achl_2232 , Achl_0515 , Achl_3735 , Achl_3734 , Achl_4621 , Achl_3789 , Achl_3808 , Achl_0736 , Achl_1530 , Achl_3733 , Achl_0028 , Achl_1065 , Achl_2455 , Achl_3119 , Achl_3806 , Achl_3807 , Achl_3791 , Achl_2454 , Achl_2453 , Achl_1763 , Achl_0254 , Achl_3297 , Achl_0867 , Achl_1817 , Achl_4612 , Achl_2540 , Achl_1191

CHAPTER V

Transcriptional profiling of Gram-positive Arthrobacter in the phyllosphere: induction of pollutant degradation genes by natural plant phenolic compounds.

And

Annex comparison with water stress

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SK M-F contributed by microarray hybridisations and preliminary data analysis.

This chapter includes an annex with the comparison with the results of water stress.

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Transcriptional profiling of Gram-positive *Arthrobacter* in the phyllosphere: induction of pollutant degradation genes by natural plant phenolic compounds

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Summary

Arthrobacter chlorophenolicus **A6 is a Gram-positive, 4-chlorophenol-degrading soil bacterium that was recently shown to be an effective colonizer of plant leaf surfaces. The genetic basis for this phyllosphere competency is unknown. In this paper, we describe the genome-wide expression profile of *A. chlorophenolicus* on leaves of common bean (*Phaseolus vulgaris*) compared with growth on agar surfaces. In phyllosphere-grown cells, we found elevated expression of several genes known to contribute to epiphytic fitness, for example those involved in nutrient acquisition, attachment, stress response and horizontal gene transfer. A surprising result was the leaf-induced expression of a subset of the so-called *cph* genes for the degradation of 4-chlorophenol. This subset encodes the conversion of the phenolic compound hydroquinone to 3-oxoadipate, and was shown to be induced not only by 4-chlorophenol but also hydroquinone, its glycosylated derivative arbutin, and phenol. Small amounts of hydroquinone, but not arbutin or phenol, were detected in leaf surface washes of *P. vulgaris* by gas chromatography-mass spectrometry. Our findings illustrate the utility of genomics approaches for exploration and improved understanding of a microbial habitat. Also, they highlight the potential for phyllosphere-based priming of bacteria to stimulate pollutant degradation, which holds promise for the application of phylloremediation.**

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Introduction

Plant leaf surfaces (collectively referred to as the phyllosphere) provide a large and unique habitat for microbial life. Even though the phyllosphere can be a harsh and stressful environment with rapid changes in temperature, relative humidity and harmful ultraviolet radiation, it is typically colonized by large populations and diverse communities of bacteria, fungi and other microorganisms (Leveau, 2006; Meyer and Leveau, 2012; Vorholt, 2012; Rastogi *et al*., 2013). A relatively understudied aspect of phyllosphere microbiology is the ability of several phyllosphere bacteria to degrade aromatic pollutants, such as toluene, phenol and phenanthrene (De Kempeneer *et al*., 2004; Sandhu *et al*., 2007; 2009; Waight *et al*., 2007; Yutthammo *et al*., 2010), as well as various foliar pesticides (Ning *et al*., 2010; Zhou *et al*., 2011). Such bacteria have potential towards phylloremediation (Sandhu *et al*., 2007), i.e. the removal of foliage-associated organic pollutants by members of the phyllosphere community.

Representatives of the genus *Arthrobacter* (high GC Gram-positive, family Micrococcaceae, order Actinomycetales, class Actinobacteria, phylum Actinobacteria) are well known for their exceptional resistance to various stresses and their ability to degrade a wide variety of organic pollutants (Mongodin *et al*., 2006). *Arthrobacter* species are common members of phyllosphere communities (Rastogi *et al*., 2012), and they were recently shown to exhibit a high level of epiphytic fitness (Scheublin and Leveau, 2013). This combination of properties makes *Arthrobacter* a target genus for studies on phylloremediation.

Little is known about the genes underlying phyllosphere competency in *Arthrobacter*. From the few studies that are available for other bacterial genera (Marco *et al*., 2005; Gourion *et al*., 2006; Fink *et al*., 2012; Yu *et al*., 2013), it

has become clear that phyllosphere exposure affects the expression of genes involved in motility, chemotaxis, biofilm formation and attachment, as well as genes related to nutrient starvation, and osmotic, oxidative and desiccation stresses. For the plant pathogen *Pseudomonas syringae*, genes involved in virulence, such as toxin production genes also showed different transcript levels (Yu *et al*., 2013). The proteome of *Methylobacterium extorquens* featured several induced proteins during epiphytic growth, including enzymes involved in methanol utilization, stress proteins and regulatory proteins (Gourion *et al*., 2006). In a metaproteomic study of the total phyllosphere community, proteins related to carbohydrate transport, carbon and nitrogen metabolism, motility, and stress were among the most abundantly expressed (Delmotte *et al*., 2009).

In the study we describe here, we employed whole-genome transcriptome arrays of *Arthrobacter chlorophenolicus* A6 to gain a better understanding of its phyllosphere competency. Strain A6 is a 4-chlorophenol-degrading isolate from soil (Westerberg *et al*., 2000). Recently, strain A6 was demonstrated to be an excellent phyllosphere colonizer (Scheublin and Leveau, 2013). It has been studied extensively with regard to the genes that contribute to 4-chlorophenol degradation, and its complete genome sequence is available (Nordin *et al*., 2005; Unell *et al*., 2009). We designed transcriptome arrays to investigate which genes were induced in the phyllosphere of common bean (*Phaseolus vulgaris*) as compared with growth on agar surfaces. Since water availability is an important factor in phyllosphere survival and activity (Beattie, 2011), we included both high and low relative humidity treatments for the phyllosphere-grown cells. In addition, we compared the expression profiles of strain A6 on agar surfaces with or without 4-chlorophenol. These analyses revealed an unexpected connection between epiphytic growth and 4-chlorophenol exposure, which we followed up on in more detail by quantifying the expression of 4-chlorophenol degradative genes in response to plant phenolic compounds using reverse-transcriptase real-time PCR and identification of naturally occurring phenolic compounds on bean leaf surfaces by gas chromatography-mass spectrometry (GC-MS).

Results

Using custom-made microarrays, we determined and compared the transcriptional profiles of *A. chlorophenolicus* A6 cells that were recovered in quadruplicate from (i) bean leaf surfaces after incubation for 48 h at 97% relative humidity (PhylH, for phyllosphere high humidity), (ii) bean leaf surfaces after incubation for 48 h, of which the first 24 h were at 97% relative humidity and the second 24 h at 50% relative humidity (PhylL, for phyllosphere low humidity), (iii) the surface of a 1/10 strength tryptic soy agar plate supplemented with 1 mM 4-chlorophenol after incubation for 48 h at 97% relative humidity (A+CP, for agar plus 4-chlorophenol), and (iv) the surface of a 1/10 strength tryptic soy agar plate after incubation for 48 h at 97% relative humidity (A-CP, for agar without 4-chlorophenol).

Clustering of the transcriptome microarray data showed a clear separation between phyllosphere samples (PhylH and PhylL) on the one hand and agar samples (A+CP and A-CP) on the other (Fig. 1). Among the agar samples, A+CP replicates also clearly separated from A-CP replicates. Such a separation was less obvious for the PhylH and PhylL samples (Fig. 1). We observed a strongly positive correlation between the expression of individual genes at high and low relative humidity in the phyllosphere (Fig. 2A), which suggests a similar bacterial experience under these two conditions. In fact, for only three genes that were differentially expressed in the phyllosphere compared with growth on agar, the expression was significantly different ($P < 0.05$) between the PhylH and PhylL samples. The first, Achl_4566, is part of the so-called *cph* gene cluster for 4-chlorophenol degradation and will be discussed in more detail below. Like Achl_4566, Achl_0518 was expressed more highly under conditions of low humidity. It encodes a putative substrate transporter belonging to the major facilitator superfamily MFS_1, with high sequence similarity to proline/betaine transporters of other *Arthrobacter* species. The expression of the third gene, Achl_2563, was lower under conditions of low humidity compared with high humidity. Its predicted product is also annotated as an MFS_1 protein. With 41% sequence similarity to a valanimycin resistance gene of *Streptomyces viridifaciens* (accession number AAN10244), Achl_2563 might be involved in antibiotic efflux, but why its expression is suppressed at low relative humidity is not evident.

A weak but significant positive correlation was observed between the PhylH and A+CP treatment (Fig. 2B), and between the PhylL and A+CP treatment (not shown), suggesting that there were more genes that responded in the same way to these two conditions than there were genes that responded in opposite directions. Included in this list of genes are several that contribute to the degradation of 4-chlorophenol, as will be detailed below. A complete list of differential gene expression is given in Supporting Information Table S1. A number of specific differences (Table 1) will be highlighted further below, organized primarily by gene function. Unless otherwise noted, any reference to up- or down-regulation of genes is relative to gene expression on agar surfaces in the absence of 4-chlorophenol (i.e. A-CP).

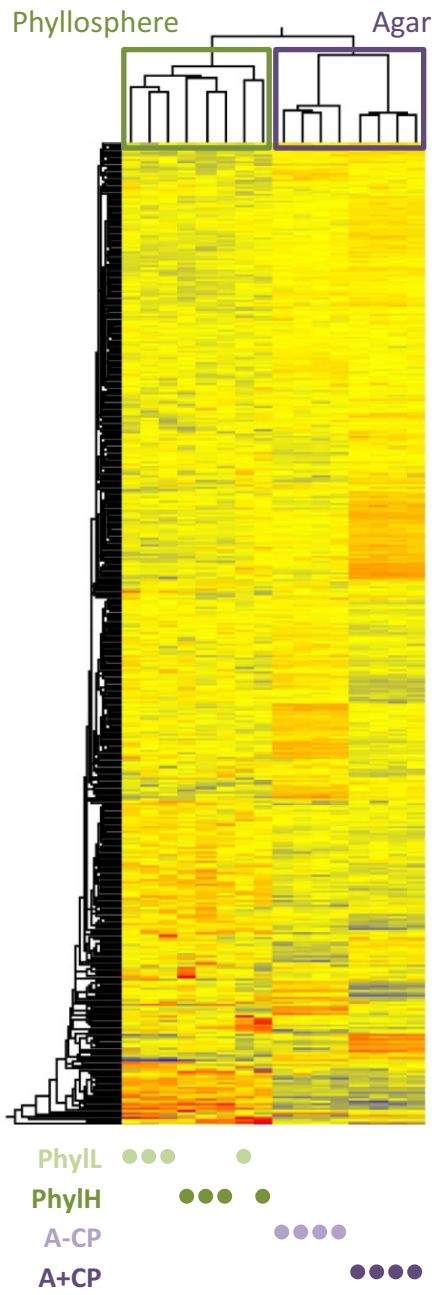

Fig. 1. Cluster diagram of *A. chlorophenolicus* A6 transcriptome array samples. Samples were organized by hierarchical clustering using Euclidian distances and the average linkage rule. PhylH, high-humidity phyllosphere treatment; PhylL, low-humidity phyllosphere treatment; A-CP, agar surface treatment; A+CP, agar plus 4-chlorophenol treatment.

Chlorophenol degradation genes

All but one of the 11 genes in the *cph* gene cluster (Achl_4564–4574) (Nordin *et al*., 2005) were induced in response to 4-chlorophenol (Fig. 3, black bars). The exception was Achl_4571 (*cphR*), which is annotated as a transcriptional activator protein. We found that three genes in the cluster, Achl_4564, Achl_4565 and Achl_4566, were also induced during growth on bean leaf surfaces (Fig. 2; Fig. 3, white and grey bars). These genes form a putative operon coding for the three-step conversion of hydroquinone to 3-oxoadipate as follows: Achl_4564 codes for CphC-II, converting hydroquinone to hydroxyquinol (Nordin *et al*., 2005); Achl_4566 codes for CphA-II, which is a predicted hydroxyquinol 1,2-dioxygenase; and the product of Achl_4565 is CphF-II, which is predicted to catalyse the conversion of maleylacetate to 3-oxoadipate. Not induced in the phyllosphere were Achl_4570 (*cphB*) and Achl_4573 (*cphC-I*), both of which are predicted to code for the production of hydroquinone from 4-chlorophenol, or Achl_4569 and Achl_4574, whose gene products are CphA-I and CphF-I, presumed paralogs of CphA-II and CphF-II respectively.

We confirmed by reverse transcription quantitative PCR (RT-qPCR) that Achl_4564 and Achl_4566, but not Achl_4569, were induced during epiphytic growth (Fig. 4A). In liquid culture, the expression of Achl_4569 was stimulated only by 4-chlorophenol (1 mM), while Achl_4564 and Achl_4566 were induced by 4-chlorophenol (1 mM), phenol (1 mM), hydroquinone (1 mM, 10 μM and 100 nM, but not 10 nM) and arbutin (1 mM and 10 μM, but not 100 nM). No induction was observed upon exposure to 1 mM concentrations of the following (plant) phenolic compounds: 4-hydroxybenzoic acid, protocatechuic acid, coumaric acid, caffeic acid, ferulic acid, quercetin, catechol or resorcinol (Fig. 4B). Arbutin is a glycosylated form of hydroquinone and has been identified in leaf extracts of several plant species (see *Discussion*). Using GC-MS, we were unable to detect arbutin in leaf washes from bean plants that were used in our experiments. However, we consistently found hydroquinone in these leaf washes in the amount of 1.5 ng per leaf averaged (Supporting Information Fig. S1). Other phenolic compounds that we identified in at least one of three replicate samples included caffeic acid, ferulic acid, 4-hydroxybenzoic acid, and protocatechuic acid; we did not detect 4-CP in the leaf washes.

Nutrient acquisition

Besides various phenolics, several other compounds were detected by GC-MS analysis of the bean leaf surface washes. These included compounds that one expects to find in the phyllosphere environment, such as intermediates of the citric acid cycle, tartrate, glycol, pentoses, hexoses, disaccharides, polar amino acids, long-chain alcohols and fatty acids (Supporting Information Table S2). Glucose, along with fructose and sucrose, is known to be the most abundant carbon source on bean leaf surfaces (Leveau and Lindow, 2001), but the

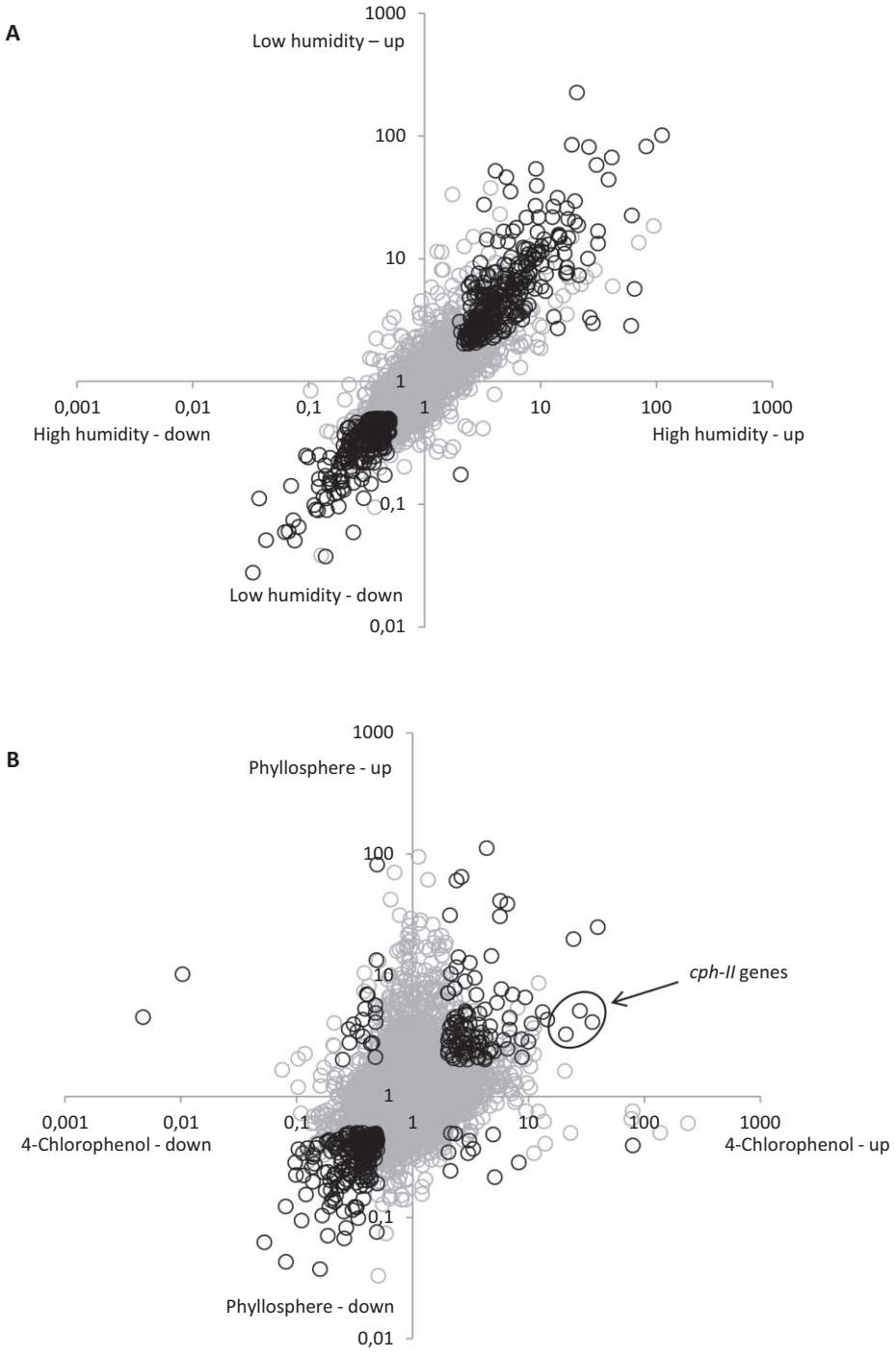

Fig. 2. Correlations between the fold change in gene expression under high- and low-humidity conditions in the phyllosphere (A), and between high humidity in the phyllosphere and 4-chlorophenol exposure (B). All changes in gene expression are relative to agar surface without chlorophenol. Black symbols represent genes that were significantly ($P < 0.05$) more than twofold differentially expressed in both treatments. The other genes are indicated in grey symbols. Indicated by a circle and arrow are the *cph-II* genes, i.e. *cphC-II*, *cphA-II* and *cphF-II*, which form a putative operon and have been implicated in 4-CP degradation, coding for the conversion of hydroquinone to oxoadipate.

Table 1. Differential expression of selected genes in *A. chlorophenolicus* A6.

Locus Tag[a]	PhylH[b]	PhylL[b]	A+4CP[b]	Predicted function
Achl_0049	**7.6**	**21.6**	1.6	Hypothetical protein
Achl_0050	**16.8**	**25.7**	–1.0	Hypothetical protein
Achl_0051	**26.2**	**81.1**	1.5	Protein of unknown function DUF1469
Achl_0052	**18.6**	**84.5**	1.9	Hypothetical protein
Achl_0159	**4.1**	**2.4**	**–2.1**	Beta-Ig-H3/fasciclin
Achl_0362	**111.8**	**101.3**	**4.4**	Phosphate ABC transporter, periplasmic phosphate-binding protein
Achl_0363	**21.1**	**18.7**	1.8	Phosphate ABC transporter, inner membrane subunit PstC
Achl_0364	**14.6**	**14.9**	1.8	Phosphate ABC transporter, inner membrane subunit PstA
Achl_0365	2.4	2.9	**–2.5**	Phosphate ABC transporter, ATPase subunit(EC:3.6.3.27)
Achl_0518	1.4	**11.3**	–1.5	General substrate transporter
Achl_0710	**10.2**	**5.9**	–96	Malate synthase A (EC:2.3.3.9)
Achl_0711	**4.5**	**4.3**	–211	Isocitrate lyase (EC4.1.3.1)
Achl_0848	**7.4**	**8.8**	1.5	Phosphate uptake regulator, PhoU
Achl_1321	**81.6**	**81.9**	**–2.0**	Hypothetical protein
Achl_1726	3.6	**11.0**	1.6	2-hydroxypropyl-CoM lyase (EC:2.1.1.14)
Achl_1744	**6.2**	**6.7**	1.8	Daunorubicin resistance ABC transporter ATPase subunit
Achl_1844	**3.2**	**3.1**	**3.0**	Putative transcriptional regulator
Achl_1845	**2.6**	**2.2**	**2.3**	FeS assembly protein SufB
Achl_1846	**2.7**	2.2	**2.7**	FeS assembly protein SufD
Achl_1847	**2.1**	1.9	**3.6**	Rieske (2Fe-2S) domain protein
Achl_1848	1.8	1.6	**2.3**	FeS assembly ATPase SufC
Achl_1849	2.1	2.1	**2.5**	Protein of unknown function DUF59
Achl_2231	**22.3**	6.2	–1.1	Nitrogen regulatory protein P-II
Achl_2232	**70.3**	13.5	–1.4	Ammonium transporter
Achl_2284	**2.5**	**2.3**	–1.7	Beta-Ig-H3/fasciclin
Achl_2389	2.0	**7.3**	1.6	Cysteine desulfurase
Achl_2563	**–4.1**	**–17.0**	**2.1**	Major facilitator superfamily MFS_1
Achl_2643	**20.6**	**225.1**	1.6	Flp/Fap pilin component
Achl_2644	**9.1**	**53.9**	–1.6	TadE family protein
Achl_2645	**5.5**	**35.2**	–1.8	Hypothetical protein
Achl_2646	4.5	**22.9**	–1.3	SAF domain protein
Achl_2647	**2.7**	**5.7**	–1.1	Flp pilus assembly protein ATPase CpaE-like protein
Achl_2648	2.2	**12.6**	1.2	Type II secretion system protein E
Achl_2649	1.0	**3.5**	–1.6	Type II secretion system protein
Achl_2650	1.7	**33.3**	1.2	Type II secretion system protein
Achl_2712	**13.0**	**3.4**	–2.0	Phage shock protein C, PspC
Achl_2713	**6.8**	1.9	**–2.4**	Hypothetical protein
Achl_2714	**3.2**	1.6	–1.7	Phage shock protein C, PspC
Achl_2817	4.3	**8.0**	**6.7**	Sulfate adenylyltransferase, large subunit(EC:2.7.7.4)
Achl_2818	5.4	**9.5**	**8.0**	Sulfate adenylyltransferase, small subunit(EC:2.7.7.4)
Achl_2819	**3.3**	**5.1**	**2.8**	Phosphoadenosine phosphosulfate reductase (EC:1.8.4.8)
Achl_2820	8.6	**12.9**	**12.2**	Sulfite reductase (ferredoxin) (EC:1.7.7.1)
Achl_2971	1.8	1.7	**2.6**	Hypothetical protein
Achl_2972	1.1	–1.1	**7.2**	Flagellar biosynthesis protein FlhA
Achl_2973	–2.3	**–2.3**	–1.2	Type III secretion exporter
Achl_2974	1.8	1.8	**3.4**	Flagellar biosynthetic protein FliR
Achl_2975	1.1	–1.2	**4.4**	Flagellar biosynthetic protein FliQ
Achl_2976	1.5	–1.0	1.9	Flagellar biosynthetic protein FliP
Achl_2977	1.3	1.1	**2.8**	Flagellar biosynthesis protein FliO
Achl_2978	1.7	2.0	**2.8**	Flagellar motor switch protein FliN
Achl_2979	1.1	–1.1	**7.2**	Surface presentation of antigens (SPOA) protein
Achl_2980	2.4	1.9	**2.9**	OmpA/MotB domain protein
Achl_2981	1.4	–1.1	**4.5**	MotA/TolQ/ExbB proton channel
Achl_2982	1.4	1.3	**3.2**	Flagellar FlbD family protein
Achl_2983	1.2	1.2	**5.4**	Protein of unknown function DUF1078 domain protein
Achl_2984	–1.1	–1.2	**5.3**	Flagellar hook capping protein
Achl_2985	2.3	2.5	**2.2**	Hypothetical protein
Achl_2986	1.3	–1.1	**4.3**	NLP/P60 protein
Achl_2987	2.2	1.8	1.8	Flagellar export protein FliJ
Achl_2988	2.7	1.9	**6.8**	ATPase, FliI/YscN family(EC:3.6.3.14)

Table 1. *cont.*

Locus Tag[a]	PhylH[b]	PhylL[b]	A+4CP[b]	Predicted function gene product
Achl_2989	1.2	1.3	**3.2**	Hypothetical protein
Achl_2990	**2.2**	1.9	**2.7**	Flagellar motor switch protein FliG
Achl_2991	**4.4**	**4.0**	**6.9**	Flagellar M-ring protein FliF
Achl_2992	2.4	2.1	**2.3**	Flagellar hook-basal body complex subunit FliE
Achl_2993	1.8	1.3	**3.6**	Flagellar basal-body rod protein FlgC
Achl_2994	1.8	1.3	**5.3**	Flagellar basal-body rod protein FlgB
Achl_2995	1.4	–1.1	**4.6**	Hypothetical protein
Achl_2996	–1.0	–1.2	**3.8**	Flagellar protein FliS
Achl_2997	–1.6	–2.0	**3.6**	Flagellar hook-associated 2 domain protein
Achl_2998	–1.1	–1.6	**7.6**	Flagellin domain protein
Achl_2999	1.2	–1.1	**5.7**	FlgN family protein
Achl_3000	2.0	1.3	**6.3**	Flagellar hook-associated protein FlgK
Achl_3001	1.3	1.3	**5.6**	Flagellar hook-associated protein 3
Achl_3258	**28.2**	**3.0**	1.2	Protein of unknown function DUF322
Achl_3259	**10.0**	1.9	–1.6	Hypothetical protein
Achl_3260	**64.7**	**5.7**	**2.6**	Hypothetical protein
Achl_3261	**26.6**	**3.3**	1.1	Hypothetical protein
Achl_3262	**60.3**	**2.8**	**2.4**	Hypothetical protein
Achl_3263	**14.1**	**2.7**	–1.2	CsbD family protein
Achl_3264	**9.4**	1.9	–1.3	RNA polymerase, sigma-24 subunit, ECF subfamily
Achl_3265	**6.7**	1.3	–1.6	Hypothetical protein
Achl_3266	**4.4**	1.5	–1.4	Hypothetical protein
Achl_3518	**17.4**	7.0	1.7	Ammonium transporter
Achl_3525	**7.6**	5.4	1.5	Glutamine synthetase, type III(EC:6.3.1.2)
Achl_3724	**31.2**	**16.7**	–1.3	Ferric reductase domain protein transmembrane component domain protein
Achl_3725	**25.7**	**10.0**	–1.1	FMN-binding domain protein
Achl_3726	**17.0**	**7.7**	1.3	ApbE family lipoprotein
Achl_3731	**3.8**	**4.3**	**2.6**	Siderophore-interacting protein
Achl_3732	**3.1**	**3.0**	**2.4**	ABC transporter related(EC:3.6.3.34)
Achl_3733	**4.0**	**4.5**	**3.1**	Transport system permease protein
Achl_3734	**5.9**	**6.7**	**5.3**	Transport system permease protein
Achl_3735	**6.9**	**7.0**	**7.3**	Periplasmic binding protein
Achl_3864	**94.6**	18.4	1.1	Hypothetical protein
Achl_4158	**3.7**	2.8	–1.1	Peptidase A24A prepilin type IV(EC:2.1.1.-,EC:3.4.23.43)
Achl_4451	**–30.3**	**–36.1**	–2.0	Hypothetical protein
Achl_4564	**5.1**	**46.0**	**27.7**	Monooxygenase FAD-binding(EC:1.14.13.20)
Achl_4565	**3.3**	**27.5**	**21.0**	Iron-containing alcohol dehydrogenase (EC:1.3.1.32)
Achl_4566	**4.1**	**51.9**	**35.5**	Intradiol ring-cleavage dioxygenase (EC:1.13.11.1)
Achl_4567	1.3	**5.2**	**3.1**	Hypothetical protein
Achl_4568	–1.3	–1.3	**79.0**	Hypothetical protein
Achl_4569	–1.5	–2.0	**77.1**	Intradiol ring-cleavage dioxygenase (EC:1.13.11.1)
Achl_4570	**–2.5**	**–2.7**	**79.5**	Flavin reductase domain protein FMN-binding
Achl_4571	–1.9	–1.9	–1.8	Transcriptional activator domain protein
Achl_4572	–1.7	–1.1	**237.6**	Protein of unknown function DUF1486
Achl_4573	–2.0	–1.7	**137.0**	4-hydroxyphenylacetate 3-hydroxylase (EC:1.14.13.3)
Achl_4574	1.6	1.9	**20.6**	Iron-containing alcohol dehydrogenase
Achl_4629	**3.7**	**2.7**	**2.1**	Relaxase/mobilization nuclease family protein
Achl_4630	**2.1**	1.8	**3.7**	Mobilization protein

a. Only those genes referred to explicitly in the text are listed here. For a complete list, see Supporting Information Table S1.
b. The values shown are fold changes in the expression of *A. chlorophenolicus* A6 genes on leaves at high relative humidity (PhylH), on leaves at low relative humidity (PhylL) or on agar plates supplemented with 4-chlorophenol (A+4CP), compared with agar plates without 4-CP. Genes with a more than twofold change in gene expression and a corrected P value < 0.05 are indicated in bold.

microarray data did not support the notion that *A. chlorophenolicus* A6 utilizes these sugars during epiphytic growth. Perhaps the expression of genes for catabolism of glucose went undetected due to the fact that growth on leaf surfaces was compared with growth on tryptic soy agar, which features glucose as the main carbon source. However, the microarray data did show phyllosphere-induced expression of genes involved in the acquisition of other nutrients, specifically phosphate, nitrogen, iron and sulphur, as explained below.

Cluster Achl_0362–0365 codes for subunits of a phosphate ABC transporter system and was highly expressed in the phyllosphere. The fold-change in transcript level for Achl_0362, which encodes a periplasmic phosphate-binding protein, was highest of all differentially expressed phyllosphere genes (Supporting Information Table S1).

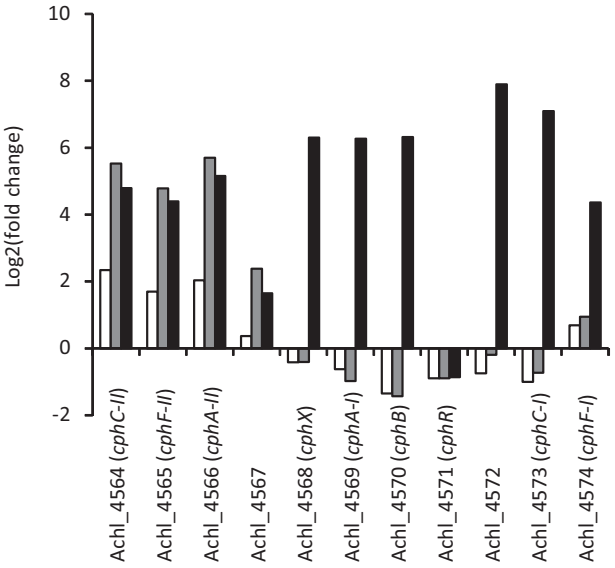

Fig. 3. Fold change in the expression of *A. chlorophenolicus* 4-chlorophenol degradation genes on the transcriptome arrays. White bars represent the comparison between the high-humidity phyllosphere and agar surface treatment, grey bars between the low-humidity phyllosphere and agar surface treatment, and black bars between the A+CP and A-CP agar surface treatment.

Achl_2231 and Achl_2232, which encode a nitrogen regulatory protein P-II and an ammonium uptake transporter, respectively, were also induced in the phyllosphere. Under nitrogen-limiting conditions, the P-II protein is involved in deadenylation of glutamine synthetase type I, which activates the enzyme. Glutamine synthetase type III, encoded by Achl_3525, also had an increased expression in the phyllosphere, as did the gene for a second ammonium transporter (Achl_3518), located several genes upstream. Ammonium transporters, the P-II protein and glutamine synthetases are all key enzymes in the acquisition of nitrogen at low ammonium concentrations (Javelle *et al*., 2004).

Cluster Achl_3731–3735 includes genes involved in iron uptake, and showed increased expression in the phyllosphere as well as in response to 4-chlorophenol. The cluster features a periplasmic binding protein, two transport system permease proteins and an ABC transporter, which are all part of an iron complex transport system. In addition, the cluster codes for a siderophore-interacting protein. In close proximity, three genes predicted to encode for a membrane-associated ferric iron reductase (Achl_3724–3726) also showed increased levels of expression in the phyllosphere.

Several sulphur assimilation genes were induced in the phyllosphere, as well as on agar with 4-chlorophenol. These genes included Achl_1844–1849 which code for components of the sulphur assimilation (SUF) system iron-sulphur (FeS) cluster. The SUF system operates under iron starvation and oxidative stress (Outten *et al*., 2004). Genes Achl_2817–2820 code for assimilatory sulphate reduction via 3′-phosphoadenylylsulfate (PAPS) and were expressed more highly in the phyllosphere, as were genes coding for incorporation of sulphide, the product of the PAPS assimilation pathway, i.e. 5-methyltetrahydropteroyltriglutamate/homocysteine S-methyltransferase (Achl_1726) and cysteine desulfurase (Achl_2389).

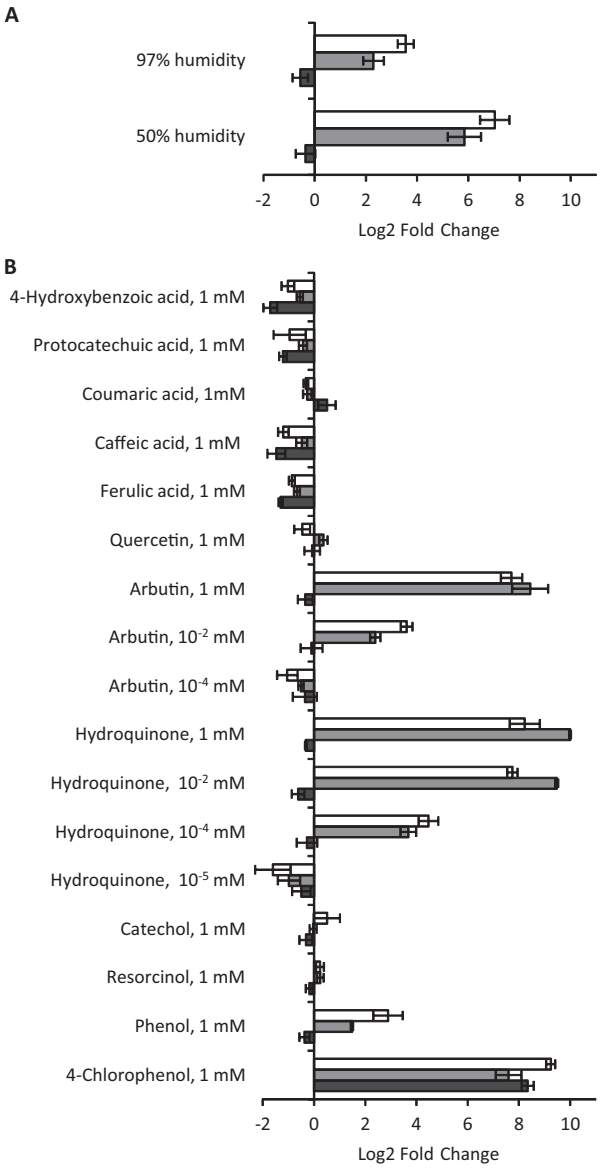

Fig. 4. Fold change in expression of *cph* genes in *A. chlorophenolicus* A6 as determined by RT-qPCR. Gene expression in the phyllosphere at high and low air humidity as compared with an agar surface (A), and gene expression in mineral medium in presence versus absence of several phenolic compounds (B). White bars, Achl_4564 (*cphC-II*); grey bars, Achl_4566 (*cphA-II*); black bars, Achl_4569 (*cphA-I*).

Attachment and motility

The Achl_2643–2650 gene cluster is predicted to be involved in surface attachment. The genes encode Flp pilus assembly proteins. Flp pili belong to a subfamily of the type IV pilin family, which mediates unspecific attachment to surfaces and the formation of microcolonies (Kachlany *et al*., 2001; Pelicic, 2008). Most genes of this cluster were highly expressed in the phyllosphere. Interestingly, this gene cluster is flanked upstream by another Flp pili cluster with a paralogous set of genes, but those genes were not induced in the phyllosphere. Achl_4158, coding for a prepilin peptidase, was expressed fourfold higher in the phyllosphere than on agar surfaces, while other pilin-associated genes were not differentially expressed. There is evidence that the presence of type IV pili increases the phyllosphere fitness of bacteria (Suoniemi *et al*., 1995; Roine *et al*., 1998).

Achl_0159 and Achl_2284 are two other genes with elevated expression in the phyllosphere and with involvement in attachment. Their predicted gene products contain a fasciclin-like (FAS1) domain, which is found in proteins from bacteria to mammals and considered an ancient cell adhesion domain (Ulstrup *et al*., 1995). This finding is in agreement with a metaproteomic study where bacterial proteins with a fasciclin domain were consistently recovered from the phyllosphere of soybean, clover and *Arabidopsis* (Delmotte *et al*., 2009).

The *A. chlorophenolicus* genome contains a cluster of 31 genes involved in flagellar synthesis (Achl_2971–3001). These genes were collectively induced in response to 4-chlorophenol, while only two of them were significantly higher expressed in the phyllosphere, namely Achl_2990 and Achl_2991, encoding a flagellar motor switch protein and a flagellar M-ring protein respectively.

Stress

Gene cluster Achl_3258–3266 was one of the most highly expressed in the phyllosphere. This cluster consists mainly of genes coding for hypothetical proteins, two of which (Achl_3258 and Achl_3265) contain an Asp23 domain, which is an alkaline shock protein family (Kuroda *et al*., 1995). The same cluster also codes for a CsbD family protein (Achl_3263) and a sigma-24 factor (Achl_3264). CsbD is a bacterial general stress response protein, but its role in stress response is unclear (Pragai and Harwood, 2002). The A6 genome contains three *csbD* homologs, all of which showed increased expression in the phyllosphere. The RNA polymerase sigma-24 subunit belongs to the extracytoplasmic function (ECF) subfamily of sigma factors. These sigma factors are involved in responses to extracytoplasmic stresses, such as oxidative stress and desiccation (Testerman *et al*., 2002; Cytryn *et al*., 2007). Nine such sigma-24 genes are present in the *A. chlorophenolicus* genome, and four of them were expressed significantly higher in the phyllosphere than on agar.

Another stress-related gene is *phoU* (Achl_0848), which showed elevated expression in the phyllosphere. PhoU acts as a global negative regulator that increases resistance against multiple antibiotics and stresses by a decrease in cellular metabolism (Li and Zhang, 2007). PhoU, as well as CsbD and Asp23, are expressed in a sigma B-dependent manner. The alternative sigma factor SigB is a master regulator in general stress response in *Bacillus subtilis* and related gram-positive bacteria (Hecker *et al*., 2007). However, the *A. chlorophenolicus* A6 genome does not appear to contain genes annotated as sigma B factors.

Also induced under stress is the phage shock protein C (Darwin, 2005). Cluster Achl_2712–2714 encodes two such phage shock C proteins and one hypothetical protein, all three of which were higher expressed on leaf surfaces than on agar surfaces. Like the ECF sigma factors, the phage–shock–protein system reacts to extracytoplasmic stress (Darwin, 2005).

The *A. chlorophenolicus* genome contains six drug resistance transporters of the EmrB/QacA subfamily. Four of these were significantly higher expressed in the phyllosphere (14-, 7- and two times 3-fold). In addition, Achl_1744, which is annotated to encode for a daunorubicin resistance ABC transporter, showed sixfold higher expression in the phyllosphere. These data suggest that *A. chlorophenolicus* A6 cells encountered adverse compounds in the phyllosphere that needed to be transported out of the cell.

Horizontal gene transfer

It has been shown previously for *Pseudomonas syringae* and for *Pseudomonas putida* that the phyllosphere stimulates horizontal gene transfer (Normander *et al*., 1998; Bjorklof *et al*., 2000). We observed phyllosphere-induced expression of genes encoding relaxase and mobilization proteins (Achl_4629 and Achl_4630). Both genes were also expressed to a higher level in the presence of 4-chlorophenol. They are located on plasmid pACHL02, which is the same plasmid that harbours the *cph* gene cluster for 4-chlorophenol degradation.

Glyoxylate bypass

The Achl_0710 and Achl_0711 genes coding for key enzymes (malate synthase and isocitrate lyase, respectively) in the glyoxylate bypass were induced in the

phyllosphere. Elevated levels of the same two enzymes were found during the growth of *A. chlorophenolicus* A6 on phenol (Unell *et al*., 2009), which the authors took as an indication of 'insufficient energy'. Activation of the glyoxylate bypass on plant leaf surfaces suggests that strain A6 is assimilating carbon from C2 compounds, possibly acetyl-CoA acquired through catabolism of plant-derived compounds.

Hypothetical proteins

The genes with the second and third most highly increased expression levels in the phyllosphere, Achl_3864 (95-fold) and Achl_1321 (82-fold), are annotated to encode for hypothetical proteins conserved in *Arthrobacter* species but with unknown function. They are not part of an operon with known genes. Achl_1321 has a transmembrane helix and a signal peptide cleavage site outside the cell, suggesting that this protein is secreted by the cell. Similarly, the gene with the highest degree of repression in the phyllosphere, Achl_4451, is a hypothetical protein. Investigation of the function of these three genes would be particularly interesting in the context of phyllosphere colonization.

Another gene cluster of interest is Achl_0049 to Achl_0052. These genes were induced in the phyllosphere compared with agar and expressed higher at high relative humidity. Again, these genes are all hypothetical proteins with unknown functions. Upstream of this cluster is a MarR regulatory gene, which was also higher expressed in the phyllosphere. MarR regulators can control a variety of functions, such as resistance to antibiotics, organic solvents and oxidative stress (Alekshun and Levy, 1999).

Discussion

Where previous studies of the phyllosphere transcriptome have focused on Gram-negative plant and human pathogens (Fink *et al*., 2012; Yu *et al*., 2013), we here present to the best of our knowledge the first transcriptional profile of a Gram-positive phyllosphere-competent strain. Our data set highlights many similarities and differences to the transcriptional profiles of leaf-associated bacteria published to date. We found an increased expression of genes related to nutrient acquisition, attachment, stress response and horizontal gene transfer in the phyllosphere, which is to a large extent in accordance with previous studies that investigated gene and protein expression in the phyllosphere (Marco *et al*., 2005; Delmotte *et al*., 2009; Fink *et al*., 2012; Yu *et al*., 2013). A surprising finding of the present study was the leaf-induced expression of part of the chlorophenol degradation pathway. This will be discussed in greater detail below. Another unique finding was the induction of Flp pili genes. Although attachment is considered to be an important factor in phyllosphere colonization, and a role of pili has been suggested (Leveau, 2006), this is the first time that Flp pili genes have been identified as phyllosphere-inducible. In contrast, leaf exposure of *P. syringae* increased the expression of a large number of genes involved in flagellar synthesis and chemotaxis (Yu *et al*., 2013). Therefore, attachment could be an important phyllosphere survival strategy for *Arthrobacter*, as opposed to motility for *P. syringae*. The expression profile of *A. chlorophenolicus* A6 in the phyllosphere was minimally affected by humidity levels. Moreover, we did not find evidence for (increased) production of osmoprotectants, such as trehalose in the phyllosphere, which is in contrast with the findings for *P. syringae* (Yu *et al*., 2013). However, one of the two significantly higher expressed genes under low versus high relative humidity is annotated to code for a transporter that may aid in osmoadaptation by allowing uptake of compatible solutes, including betaine and proline (Axtell and Beattie, 2002). Many epiphytes can produce these types of compounds, which would suggest that survival of A6 on leaf surfaces may be hardwired to depend in part on the presence and activity of other microbes on the leaf surfaces.

We observed substantial similarities between the transcriptional response of *A. chlorophenolicus* to the phyllosphere and upon exposure to 4-chlorophenol. Genes involved in phosphate and iron uptake, sulphur assimilation, plasmid mobilization genes and several 4-chlorophenol degradation genes were significantly higher expressed under both conditions. Out of 337 genes with a significantly altered expression under both conditions, 91% changed in the same direction, i.e. either up or down, compared with growth on agar in the absence of chlorophenol. Those similarities suggest a priming effect, where exposure to the phyllosphere could lead to a pre-adaptation of bacteria for growth on organic pollutants, such as 4-chlorophenol. Interestingly, a higher expression of genes involved in xenobiotic degradation in the phyllosphere compared with liquid growth medium was also found for the *P. syringae* transcriptome, although this species is not known as a pollutant degrader (Yu *et al*., 2013). In *A. chlorophenolicus* A6, stress-related genes that were induced in the phyllosphere were not responsive to the presence of 4-chlorophenol. This indicates that the stress response to 4-chlorophenol was different from phyllosphere-induced stresses. Under water stress induced by sodium chloride or polyethylene glycol, none of the genes for 4-chlorophenol degradation were induced (S.K.. Moreno and J.R. van der Meer, unpubl. data), suggesting that osmotic stress is not a trigger for their expression. A more likely explanation is that these genes react to

phenolic compounds that are naturally present in the phyllosphere.

Our data demonstrated the phyllosphere-induced expression of three genes within the *cph* gene cluster for 4-chlorophenol degradation. These three genes are thought to constitute a subcluster (cluster II) that evolved independently from the rest of the *cph* gene cluster and were combined by a more recent horizontal gene transfer event (Nordin *et al*., 2005). Based on our qPCR results (Fig. 4), we hypothesize that leaf surface-induced expression of the cluster II genes was triggered by the presence of hydroquinone or derivatives thereof on leaf surfaces. Indeed, we could detect hydroquinone in bean leaf washes, and our data suggest that hydroquinone is available to bacteria such as *A. chlorophenolicus* A6 on the leaf surface in concentrations sufficiently high to stimulate the expression of genes that code for the degradation of hydroquinone. Hydroquinone and its glycosylated form, arbutin, have been identified in the leaves of a broad range of plant species, such as pear, bearberry, *Polygonella myriophylla* and several species in the family Lamiaceae (Pedersen, 2000; Parejo *et al*., 2001; Jin and Sato, 2003; Weidenhamer and Romeo, 2004). In the resurrection fern, hydroquinone and arbutin are important for the plant to deal with desiccation stress (Suau *et al*., 1991). Hydroquinone has also been listed as a compound with antimicrobial (Jin and Sato, 2003) and surface-wetting (Wieckowska *et al*., 2007) properties.

Our findings are of potential interest for phyllosphere-based bioremediation studies. The ability of phyllosphere bacteria to degrade airborne aromatic pollutants, such as phenol, has been previously established (Sandhu *et al*., 2007; 2009). Although the exact relationship between genes involved in (or induced by) hydroquinone and phenol remains to be investigated, our observation that exposure to the phyllosphere induces many of the same degradation genes as exposure to phenol indicates that the phyllosphere might prime bacteria for pollutant degradation. Such priming, for example through a process of 'phyllo-augmentation', could potentially result in increased degradation of and/or a faster response to aromatic pollutants in bio-based environmental clean-up operations.

In summary, we demonstrated that the phyllosphere competency of *A. chlorophenolicus* A6 is linked to the expression of a number of specific gene functions that support epiphytic survival. Most unforeseen and exciting was the discovery that the best studied genes of this bacterium so far, namely the *cph* genes for the degradation of the pollutant 4-chlorophenol, were induced during leaf colonization, and that their elevated expression *in planta* concurs with our demonstration that hydroquinone, an inducer of these genes, is present in the leaf environment.

Experimental procedures

Experimental set-up and sample preparation for transcriptome microarrays

Arthrobacter chlorophenolicus strain A6 was grown at 28°C and 250 r.p.m. in lysogeny broth (LB). At mid-exponential phase, the bacterial culture was centrifuged for 10 min at 3838 *g*. The pellet was resuspended in sterile demineralized water to obtain a bacterial suspension with an optical density at 600 nm (OD_{600}) of 0.15, which corresponded to approximately 8×10^7 colony-forming units (CFU) per millilitre. The above-ground portion of 2-week old bean plants (*Phaseolus vulgaris*, green snap bean, variety Blue Lake Bush 274) with the first two leaves fully expanded were dipped into this bacterial suspension. Five plants were incubated at 97% air humidity for 2 days in a growth chamber; we refer to this as the high-humidity phyllosphere or PhylH treatment. Five other plants were incubated for 1 day at 97% air humidity, followed by a second day at 50% air humidity; we refer to this as the reduced- or low-humidity phyllosphere or PhylL treatment. In addition, 500 µl of bacterial suspension was spread on 1/10 strength Tryptone Soy Agar (TSA; Oxoid, Cambridge, UK) with 15 g agar per litre and supplemented with 1 mM 4-chorophenol (4-CP); we refer to this as the agar plus 4-CP (or A+CP) treatment. Another 500 µl was spread on 1/10 strength TSA without the 4-chlorophenol; we refer to this as the A-CP treatment. Both agar treatments were incubated at 97% humidity for 2 days. We chose agar surfaces as a control (rather than liquid cultures) so as to avoid picking up genes that are surface-induced but not phyllosphere-specific. All plants and plates were incubated in a growth chamber that was maintained a day–night cycle of 16 h and 8 h at 21°C and 16°C respectively. Bacterial population sizes on leaves were estimated at 0, 24 and 48 h post-inoculation to show that they increased during the first 24 h but stabilized during the second 24 h following inoculation (Supporting Information Fig. S2). Similarly, the bacteria on plate established a lawn during the first 24 h, without apparent growth after that and until time of harvest. For each treatment, we prepared four independent replicate experiments. For each replicated experiment, bacteria were recovered from leaves by sequentially putting five leaves from five different plants in 20 ml RNA protection solution [two parts RNAprotect bacteria reagent (Qiagen, Venlo, The Netherlands) and one part phosphate-buffered saline] with 5 s vortexing, 7 min sonication and 5 s vortexing for each leaf. The solution was centrifuged for 20 min at 3838 *g*, and bacterial pellets were frozen at −80°C until RNA extraction. Bacteria from agar plates were washed from the surface with 1 ml RNAprotect solution according to the manufacturer's instructions (Qiagen) and frozen at −80°C until RNA extraction.

Microarray design

YODA software (Nordberg, 2005) was used to design 50-mer probes that target genes from the chromosome and both plasmids of *A. chlorophenolicus* A6. The microarray design has been deposited in the NCBI Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo) under accession number GSE48198 (platform GPL17332). The majority of probes (99.5%) were designed with the following parameters: 1–3

non-overlapping probes per gene, a maximum of 70% identity to non-target sequences, a maximum of 15 consecutive matches to non-target sequences, a melting temperature range of 8°C and a GC content range of 12%. The remaining 0.5% of probes were designed with the following less stringent parameters: a maximum of 80% identity to non-target sequences, a melting temperature range of 15°C and a GC content range of 30%. In total, 13 589 probes were designed that target 99.8% of the predicted protein-coding A6 genes (4581 out of 4590). An additional seven positive control probes were included in the design. Probes were synthesized on microarrays by Agilent Technologies (Santa Clara, CA, USA) using the 8 × 15 000 format.

RNA extraction and microarray procedures

Bacterial pellets were thawed at room temperature and resuspended in 200 μl TE buffer (30 mM Tris-Cl, 1 mM EDTA, pH 8.0) containing 15 mg of lysozyme and 2 mg of proteinase K per millilitre. The suspension was incubated at room temperature for 30 min with regular vortexing. Then, 700 μl lysis solution of the Aurum Total RNA Mini Kit (Bio-Rad Laboratories, Veenendaal, The Netherlands) was added, and the total volume was transferred to a 2 ml screw cap tube containing 100 mg of 0.1:0.5 mm beads (1:1) (Merlin Bioproducts, Breda, The Netherlands). Tubes were shaken in a Mini-Beadbeater (Biospec Products, Breukelen, The Netherlands) twice for 1 min at 5000 r.p.m. with a 1 min interval on ice. Tubes were centrifuged for 10 s, and the solution without beads was transferred to a fresh tube. After the addition of 500 μl 70% isopropanol, samples were further treated according to the Aurum Total RNA Mini Kit protocol. Columns were eluted with 80 μl elution buffer. An additional 30 min DNase treatment was performed with Ambion TURBO DNA-*free* (Applied Biosystems, Nieuwerkerk a/d ijssel, The Netherlands). After the DNase treatment, the RNA was precipitated with 1/10 volume of 7.5 M ammonium acetate (Sigma-Aldrich, Zwijndrecht, The Netherlands), 1/50 volume glycogen (5 mg ml^{-1}) (Fermentas, St. Leon-Rot, Germany) and 2.5 volumes of ethanol. RNA pellets were washed with 80% ethanol and resuspended in 12 μl nuclease-free water. RNA quality was verified with Experion RNA StdSens (Bio-Rad Laboratories).

The procedures for cDNA synthesis and labelling, and for array hybridization, were based on a protocol described elsewhere, with slight modifications (Johnson *et al*., 2011). An amount of 2–5 μg of RNA was mixed with 1.25 μl random primers (500 μg ml^{-1}; Promega, Madison, WI, USA) in a total volume of 12 μl and incubated at 70°C for 10 min, followed by 4°C for 5 min. Each tube received 13 μl of mastermix containing 0.6 μl Cyanine 3-dCTP (1 mM; Perkin-Elmer, Waltham, MA, USA), 0.6 μl Superase-In (20 U μl^{-1}; Ambion), 1 μl Superscript II (200 U μl^{-1}; Life Technologies, Carlsbad, CA, USA), 5 μl 5× 1st strand buffer, 2.5 μl DTT (100 mM), 0.25 μl dATP-dGTP-dTTP mixture (10 mM each), 0.1 μl dCTP (5 mM) and 2.9 μl nuclease-free water. Labelled cDNA was produced by incubation at 42°C for 120 min, followed by 70°C for 10 min and 4°C for 5 min. RNA was hydrolysed under alkaline conditions as follows: After the addition of 2.5 μl 1 M NaOH, samples were heated at 65°C for 20 min, allowed to cool to room temperature for 10 min and neutralized by the addition of 2.5 μl 3 M Na-acetate (pH 5.2) and 2.5 μl 1 M HCl. The labelled cDNA was then purified using the MinElute PCR Purification Kit (Qiagen) according to the manufacturer's instructions. The product was eluted in 20 μl EB buffer, and quantity and incorporation efficiency were determined using the MICROARRAY function on a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Sixty nanogram of labelled cDNA with incorporation efficiencies between 2% and 3% was loaded onto each microarray, hybridized for 17 h at 65°C, and washed and scanned as described for labelled cRNA in the One-Color Microarray-Based Gene Expression Analysis Manual (Agilent Technologies). The fragmentation step (heating to 60°C for 30 min) was omitted. Hybridization signal intensities were extracted from scanned images using the Agilent Feature Extraction software package (version 10.7.1.1; Agilent Technologies).

To corroborate the array results, three replicate RNA samples each were obtained following the procedures as described above for the agar surface treatments, the high-humidity phyllosphere treatment and the low-humidity phyllosphere treatment. These RNA samples were subjected to RT-qPCR, as described below.

Microarray data analyses

The expression data were analysed using the Genespring GX software version 11 (Agilent Technologies) as described elsewhere (Johnson *et al*., 2011). In short, data were log2-transformed, normalized by quantile and scaled with the baseline to the median of all samples. All genes were filtered by expression level and were retained when the signal intensity was above the 20th percentile in at least one of the samples. Samples were clustered by hierarchical clustering using Euclidian distances and the average linkage rule; they were clustered on both entities and conditions. All treatments were compared pairwise with the agar surface control treatment using a Welch's *t* test with asymptotic *P* value computation and Benjamini–Hochberg false discovery rate for multiple testing correction. In addition, the high- and low-humidity phyllosphere treatments were compared with each other in a Welch's *t* test. Genes that were at least twofold differentially expressed between treatments with a corrected *P* value lower than 0.05 were considered statistically different. Gene annotations were retrieved from the IMG database (http://img.jgi.doe.gov/).

Array information is available in the NCBI Gene Expression Omnibus database under accession number GSE48198.

Gene expression in the presence of plant phenolic compounds

A. chlorophenolicus A6 cells were harvested from an LB overnight culture by centrifugation for 10 min at 3838 *g*. Cells were resuspended in Brunner mineral medium (MM; DSMZ medium no. 457, Braunschweig, Germany) with 5 mM fructose, adjusted to an OD_{600} of 0.15 and incubated at 28°C and 150 r.p.m. When the OD_{600} reached 0.35, 1 mM of phenolic compound (see below) was added, and the flasks were incubated for another 2 h at 28°C and 150 r.p.m. There were triplicates for each of the 18 treatments. We tested 12 phenolic compounds: phenol, 4-chlorophenol, 4-hydroxybenzoic

Table 2. Primer specifications.

Primer name	Target gene	Sequence	Annealing temperature
1611-f	Achl_1611	GTGGAAGTCATCAACAAG	54
1611-r	Achl_1611	GTCATGTCCAGTTCTAGTG	54
4564-f	Achl_4564	ATATCCCTCAGACGTACC	59
4564-r	Achl_4564	AGACATACTCAGTGGAGAAC	59
4566-f	Achl_4566	GCTTCTATGACGTCCAAT	59
4566-r	Achl_4566	AGTCCCCAGAAGGAGTAT	59
4569-f	Achl_4569	CCAGCACCTACACAACTTCG	59
4569-r	Achl_4569	AAGGCCTAGAACGTCAGAAAG	59

acid, protocatechuic acid, coumaric acid, caffeic acid, ferulic acid, quercetin, arbutin, hydroquinone, catechol and resorcinol. In the controls, no phenolic compound was added. Arbutin was also tested at concentrations of 10^{-2} and 10^{-4} mM, and hydroquinone at 10^{-2}, 10^{-4} and 10^{-5} mM. After 2 h, 3 ml culture was treated with RNAprotect (Qiagen) according to the manufacturer's instructions, and cells were frozen at –80°C until RNA extraction. In addition, the OD_{600} and the number of CFUs per millilitre (CFU ml^{-1}) of the cultures were determined. RNA was extracted according to the protocol described above and resuspended in 35 μl nuclease-free water.

RT-qPCR

Table 2 summarizes the qPCR primers that were designed for the *A. chlorophenolicus* A6 genes Achl_4564, Achl_4566, Achl_4569 and Achl_1611, using the primer3 software (http://primer3.sourceforge.net/) or SciTools of Integrated DNA Technologies (http://eu.idtdna.com/scitools/Applications/RealTimePCR/). Plasmid-encoded genes Achl_4564, Achl_4566 and Achl_4569 are part of the *A. chlorophenolicus* gene cluster for 4-chlorophenol degradation, while Achl_1611 serves as a reference gene; it is chromosomally located and encodes the RNA polymerase sigma factor RpoD. Appropriate annealing temperatures (Table 2) were optimized by testing a range of temperatures.

Two-step RT-qPCR was performed on RNA samples from phyllosphere bacteria (see *RNA extraction and microarray procedures*) and RNA samples from bacteria that were exposed to different phenolic compounds (see *Gene expression in the presence of plant phenolic compounds*). An amount of 420 ng RNA was converted to cDNA with random hexamer primers in a reaction volume of 20 μl (RevertAid First Strand cDNA Synthesis Kit, Fermentas). The cDNA product was diluted 50 times. qPCR mixtures contained 12.5 μl ABsolute™ QPCR SYBR® green mix (ABgene, Fisher Scientific, Landsmeer, The Netherlands), 10 μg BSA, 6.25 pmol of each primer and 5 μl of diluted cDNA template in a total volume of 25 μl. The qPCRs were performed on a Corbett Research Rotor-Gene 3000 thermal cycler (Westburg, Leusden, The Netherlands) with a regime of one step of 15 min at 95°C, and 40 cycles of 60 s at 95°C, 40 s at the respective annealing temperature (Table 2) and 60 s at 72°C. Gene expression was calculated relative to the Achl_1611 *rpoD* gene using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

Analysis of leaf surface washes by GC-MS

For each of the three independent samples, 32 primary leaves of 2-week-old *P. vulgaris* plants were sequentially dipped into 100% methanol for 30 s. The methanol extract was then divided in two parts, both parts received 50 ng of 3,5-dihydroxybenzoic acid as an internal standard and one part was spiked with 50 ng of hydroquinone. The methanol extract was filtered over Whatman filter paper, and the solvent evaporated under a flow of nitrogen. Dried samples were dissolved in 50 μl acetonitrile and subsequently derivatized with 100 μl BSTFA [N,O-bis(trimethylsilyl) trifluoroacetamide] and 10 μl TMCS (trimethylchlorosilane) overnight at room temperature. Samples were diluted with 500 μl acetonitrile and 1–10 μl were injected in an Agilent GC 7890 (Agilent) with BPX-5 column (30 m × 0.32 m × 0.25 μm; SGE, Darmstadt, Germany) in either split or split-less mode. The temperature programme from 50°C to 300°C was as follows: 50°C (5 min) to 100°C (30 min) at 30°C min^{-1}, to 175°C (5 min) at 10°C min^{-1}, to 250°C (5 min) at 10°C min^{-1}, and to 300°C (15 min) at 30°C min^{-1}. The injector was set at 280°C and the He flow was 1.7 ml min^{-1}. Mass spectra were recorded using an Agilent MS 5975C inert XL MSD and analysed with an MSD ChemStation G1701 EA.E.02.00.493. External standards were run for arbutin, caffeic acid, ferulic acid, 4-hydroxybenzoic acid, protocatechuic acid, hydroquinone, catechol, resorcinol, and 4-CP.

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Fig. S1. GC-MS analysis of *Phaseolus vulgaris* leaf surface wash extracts. A peak with the retention time (19.6 min) of silated hydroquinone is indicated in a chromatogram section by an arrow (A). The corresponding mass spectrum (B) matched that of silated hydroquinone from the NIST Mass Spectrometry Data Center (C) and of an authentic standard (not shown).

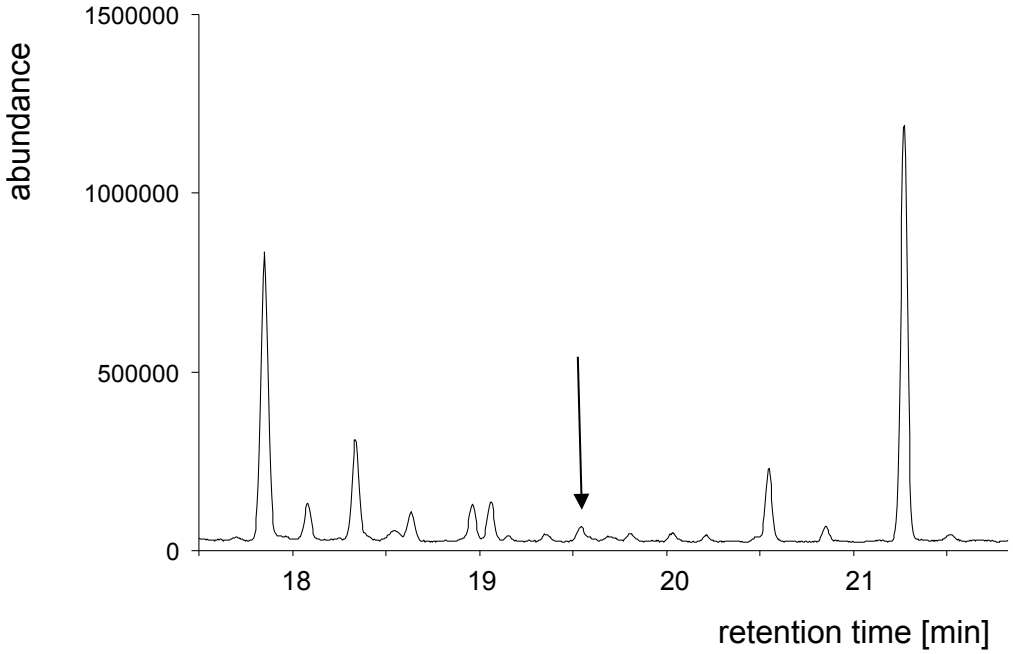
Fig. S2. Population sizes of *A. chlorophenolicus* A6 on bean leaves that were sampled for RNA extraction and subsequent microarray analysis. 'Wet' refers to the PhylH treatment, while 'dry' refers to the PhylL treatment.

Table S1. Fold change in gene expression and corrected *P* values of *A. chlorophenolicus* genes on transcriptome arrays. Genes with a more than twofold change in gene expression and a corrected *P* value lower than 0.05 are indicated in bold. Comparisons were made between (i) high-humidity phyllosphere and agar surface treatment, (ii) low-humidity phyllosphere and agar surface treatment, and (iii) chlorophenol and agar surface treatment. Genes with a significantly different expression between high and low phyllosphere humidity treatments are indicated with asterisks (** = $P < 0.05$, * = $P < 0.1$).

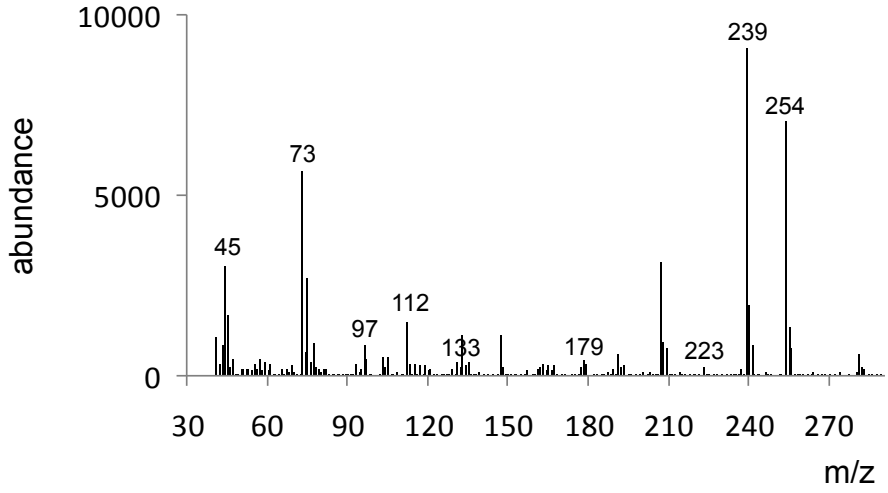
Table S2. Compounds identified in three independent methanol extracts from the surface of bean leaves.

Figure S1

A



B



C

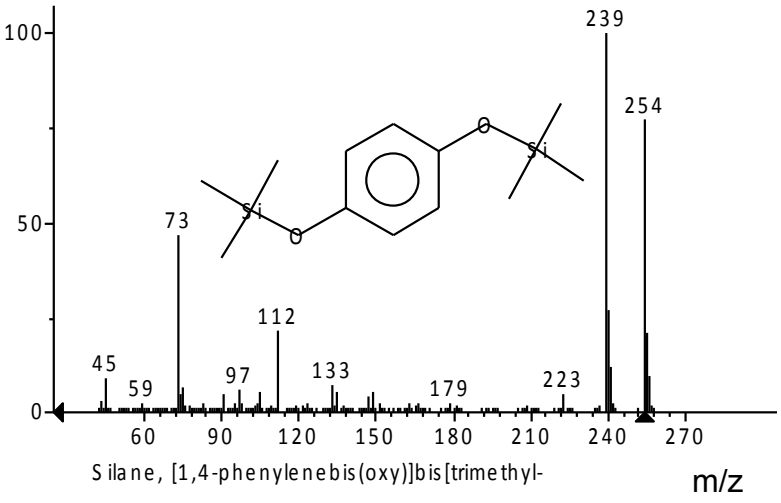
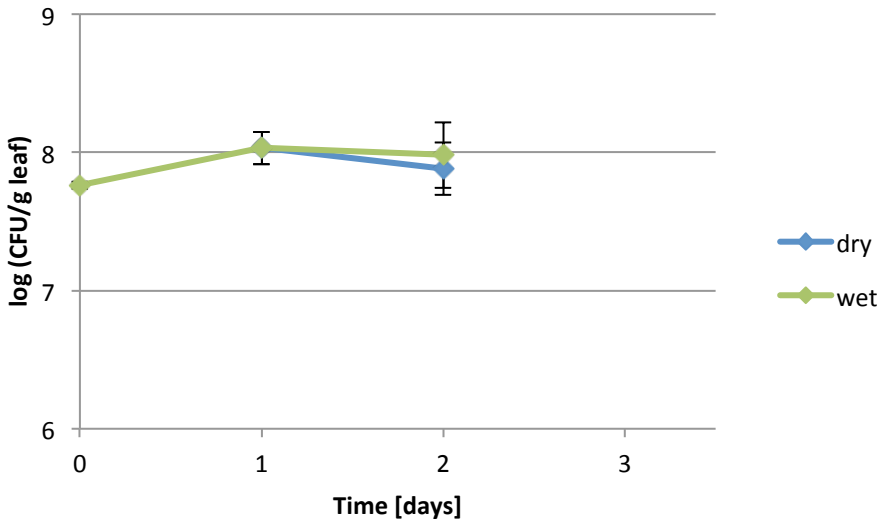


Figure S2



Annex: Comparison with water stress responses

As demonstrated by Scheublin and Leveau (2013) *A. chlorophenolicus* adapts very well to the leaf environment and increased its population size over the course of two days. Unexpectedly, no significant differences were detected in genome-wide gene expression between cells in the phyllosphere subjected to high (96%) or low (50%) humidity, because the variability between the sample replicates was higher than between sample conditions. In contrast, strong differences occurred between phyllosphere incubated cells and cells incubated in liquid culture under matric and solute stress (Chapter II).

Hierarchical clustering of phyllosphere, matric and solute stress transcriptomes shows mainly three defined groups of genes being differentially regulated. Group 1 (209 genes) and Group 3 (764 genes) contain genes that are mainly higher expressed in the phyllosphere compared to water stress conditions, whereas Group 2 (860 genes) comprises genes that are lower expressed in the phyllosphere (Figure 1).

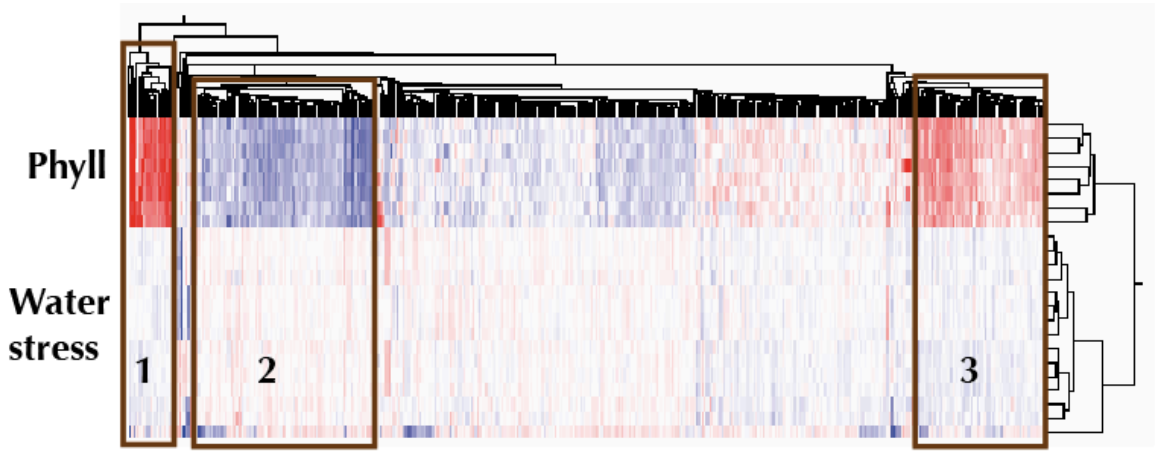


Figure 1 Hierarchical clustering of samples from phyllosphere experiments (Phyll) vs matric and solute stress experiments (Water stress). The numbers indicate groups of genes used to describe the differences between the two states. Red: increased expression. Blue: decreased expression.

GO-term analysis of enriched genes in Group 1 (Table 1) suggests an implication of genes at the membrane level (GO *cellular components*: membrane and integral to membrane), and transport. Other enriched terms are related to general metabolism (GO *biological process*: regulation of nitrogen utilization, glycine catabolic process, oxidation-reduction process). Genes enriched in Group 3 (Table 2) show, similarly, GO terms related to metabolism (e.g., valine metabolic process, penthose-phosphate shunt), signal transduction and transport. In contrast, genes in Group 2 (Table 3) display an enriched number of GO terms related to cellular division (e.g., cell wall formation, regulation of cell shape, cell cycle, and cell division) and metabolism (e.g., oxidative phosphorylation, oxoacid metabolic process, carboxylic acid biosynthetic process). These results suggest a general maintenance of functionality for cells in the phyllosphere, but without much active growth of the population.

Principal Component Analysis (Figure 2) groups all transcriptomes separately, suggesting that phyllosphere, agar surfaces with or without 4-chlorophenol, and water stress experiments (included matric and solute stress) provoke very different reactions from the cells.

Responses were averaged per treatment and subsequently again plotted across the four treatments by hierarchical clustering (Figure 3). The obtained clustering suggests that agar surface is perceived similarly by the cells as plant surface, although differing in important details. Addition of 4-CP as carbon source to agar-grown cells drastically changes the transcriptome, which suggests that cells on the plant leaf surface perceive no specific carbon substrate (as on agar surface without carbon). Important to note is that induced water stress by addition of salt or PEG is completely different than cells exposed to air on a surface.

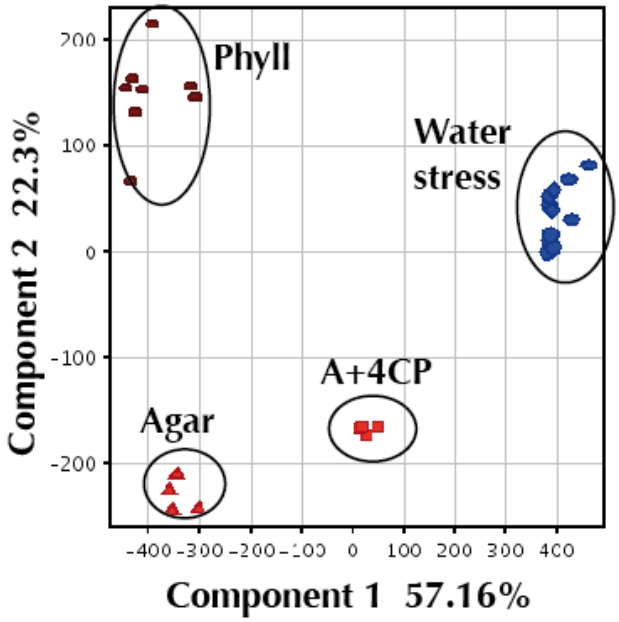


Figure 2 Two dimensional PCA of *A. chlorophenolicus* transcriptomes coming from cells in phyllosphere (Phyll), from cells on agar plates without or with 4-chlorophenol (A+4CP), and from cells under water stress.

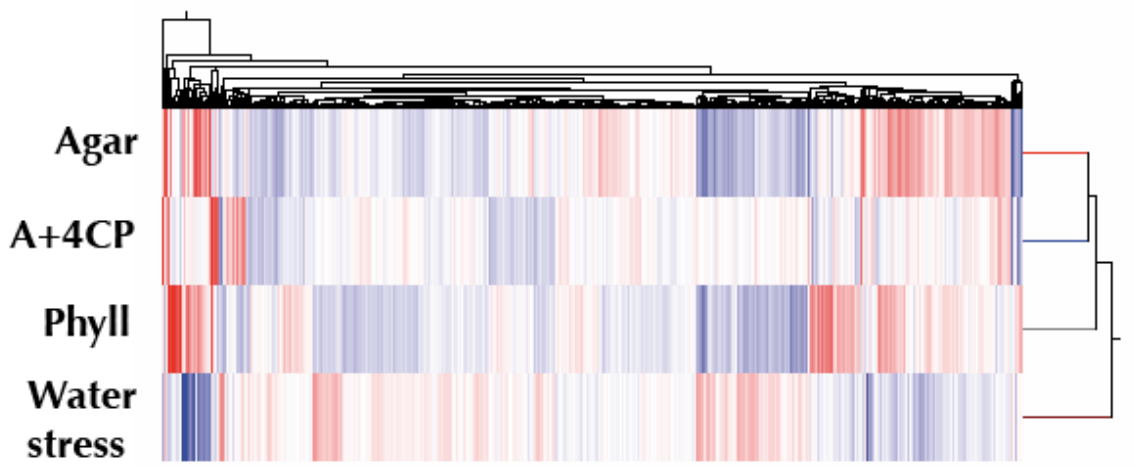


Figure 3 Hierarchical clustering of gene expression averaged across the groups formed in the PCA: cells on agar plates, or on agar plates with 4-chlorophenol (A+4CP), cells in the phyllosphere (Phyll) and during water stress. Each column shows the average of normalised signal per treatment group. Red colours indicate high and blue ones low signal levels.

Table 1. GO terms associated to group 1 differentially expressed genes of *A. chlorophenolicus* in Phyllosphere (increased) versus Water Stress (decreased, Figure 1) identified using GOEAST with Alexia's algorithm.

GOID	Biological Process	p-value
GO:0006817	phosphate ion transport	6.57E-05
GO:0006810	transport	9.23E-04
GO:0042558	pteridine-containing compound metabolic process	1.65E-02
GO:0006808	regulation of nitrogen utilization	2.02E-02
GO:0006352	transcription initiation, DNA-dependent	2.08E-02
GO:0055114	oxidation-reduction process	2.44E-02
GO:0006546	glycine catabolic process	2.94E-02
GO:0006461	protein complex assembly	6.53E-02
	Cellular Component	
GO:0016020	membrane	1.61E-04
GO:0016021	integral to membrane	6.44E-03
	Molecular Function	
GO:0008115	sarcosine oxidase activity	6.35E-05
GO:0005315	inorganic phosphate transmembrane transporter activity	6.35E-05
GO:0005215	transporter activity	1.33E-03
GO:0008410	CoA-transferase activity	4.71E-03
GO:0005506	iron ion binding	1.52E-02
GO:0016987	sigma factor activity	2.03E-02
GO:0050660	flavin adenine dinucleotide binding	4.79E-02
GO:0009055	electron carrier activity	6.97E-02
GO:0030976	thiamine pyrophosphate binding	7.65E-02
GO:0003995	acyl-CoA dehydrogenase activity	7.65E-02

Table 2. GO terms associated with group 3 differentially expressed genes of *A. chlorophenolicus* in Phyllosphere (increased) versus Water Stress (decreased, Figure 5) identified using GOEAST with Alexia's algorithm.

GOID	Biological Process	p-value
GO:0007165	signal transduction	3.56E-03
GO:0006810	transport	1.59E-02
GO:0006573	valine metabolic process	5.91E-02
GO:0006098	pentose-phosphate shunt	8.99E-02
	Cellular Component	
GO:0005886	plasma membrane	3.46E-03
GO:0016021	integral to membrane	5.14E-02
	Molecular Function	
GO:0008199	ferric iron binding	4.63E-03
GO:0000156	two-component response regulator activity	7.79E-03
GO:0016705	oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen	8.70E-03
GO:0005215	transporter activity	9.33E-03
GO:0016701	oxidoreductase activity, acting on single donors with incorporation of molecular oxygen	1.49E-02
GO:0003995	acyl-CoA dehydrogenase activity	1.56E-02
GO:0008703	5-amino-6-(5-phosphoribosylamino) uracil reductase activity	3.09E-02
GO:0050660	flavin adenine dinucleotide binding	4.44E-02
GO:0004616	phosphogluconate dehydrogenase (decarboxylating) activity	4.84E-02
GO:0046912	transferase activity, transferring acyl groups, acyl groups converted into alkyl on transfer	6.28E-02
GO:0009055	electron carrier activity	9.29E-02

Table 3. GO terms associated with group 2 differentially expressed genes of *A. chlorophenolicus* in Phyllosphere (decrased) versus Water Stress (increased, Figure 1) identified using GOEAST with Alexia's algorithm.

GOID	Biological Process	p-value
GO:0006412	translation	2.89E-09
GO:0043436	oxoacid metabolic process	2.33E-07
GO:0046394	carboxylic acid biosynthetic process	2.43E-06
GO:0006024	glycosaminoglycan biosynthetic process	2.16E-05
GO:0009252	peptidoglycan biosynthetic process	2.16E-05
GO:0044038	cell wall macromolecule biosynthetic process	2.16E-05
GO:0009168	purine ribonucleoside monophosphate biosynthetic process	3.13E-05
GO:0034641	cellular nitrogen compound metabolic process	3.41E-05
GO:0046483	heterocycle metabolic process	1.62E-04
GO:0010382	cellular cell wall macromolecule metabolic process	2.45E-04
GO:0034404	nucleobase-containing small molecule biosynthetic process	3.89E-04
GO:0000271	polysaccharide biosynthetic process	7.62E-04
GO:0007049	cell cycle	2.39E-03
GO:0051301	cell division	2.39E-03
GO:0008360	regulation of cell shape	4.23E-03
GO:0006119	oxidative phosphorylation	7.39E-03
GO:0005976	polysaccharide metabolic process	1.10E-02
GO:0006284	base-excision repair	3.74E-02
GO:0000105	histidine biosynthetic process	4.74E-02
GO:0006265	DNA topological change	4.74E-02
GO:0009394	2'-deoxyribonucleotide metabolic process	4.74E-02
GO:0009089	lysine biosynthetic process via diaminopimelate	4.74E-02
GO:0009064	glutamine family amino acid metabolic process	5.29E-02
GO:0009396	folic acid-containing compound biosynthetic process	5.46E-02
GO:0006508	proteolysis	5.78E-02
GO:0019637	organophosphate metabolic process	6.76E-02
GO:0008152	metabolic process	6.99E-02
GO:0008654	phospholipid biosynthetic process	8.19E-02
GO:0009225	nucleotide-sugar metabolic process	9.03E-02
GO:0033014	tetrapyrrole biosynthetic process	9.97E-02
GOID	**Cellular Component**	**p-value**
GO:0044424	intracellular part	4.21E-09
GO:0005840	ribosome	1.96E-04
GO:0005694	chromosome	6.66E-03
GOID	**Molecular Function**	**p-value**
GO:0003735	structural constituent of ribosome	7.06E-06
GO:0004812	aminoacyl-tRNA ligase activity	1.25E-04
GO:0016597	amino acid binding	1.35E-03
GO:0016881	acid-amino acid ligase activity	1.95E-03
GO:0005524	ATP binding	2.38E-03
GO:0004312	fatty acid synthase activity	3.19E-03

GO:0003723	RNA binding	3.35E-03
GO:0031406	carboxylic acid binding	3.82E-03
GO:0001883	purine nucleoside binding	9.19E-03
GO:0016835	carbon-oxygen lyase activity	1.38E-02
GO:0016747	transferase activity, transferring acyl groups other than amino-acyl groups	2.11E-02
GO:0000287	magnesium ion binding	2.33E-02
GO:0016866	intramolecular transferase activity	2.33E-02
GO:0016884	carbon-nitrogen ligase activity, with glutamine as amido-N-donor	2.51E-02
GO:0016763	transferase activity, transferring pentosyl groups	4.31E-02
GO:0003916	DNA topoisomerase activity	4.67E-02
GO:0004222	metalloendopeptidase activity	5.82E-02
GO:0003678	DNA helicase activity	6.65E-02
GO:0016857	racemase and epimerase activity, acting on carbohydrates and derivatives	8.08E-02
GO:0016628	oxidoreductase activity, acting on the CH-CH group of donors, NAD or NADP as acceptor	9.46E-02

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CHAPTER VI

General discussion

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The study of microorganisms under laboratory conditions has contributed to the discovery of important and relevant capabilities of bacteria that can be applied to the environment in order to clean up the toxic mess left by human activities.

However, the observations made under such artificial conditions fail to reflect the real reactions of bacteria in the environment. The purpose of my work was to obtain a better understanding of the behaviour of different bacteria under more real environments.

As starting point I studied the general responses of three different strains with recognized biodegradative capacities for toxic compounds under conditions considered as relevant for their survival when intended for use in bioremediation: mainly water stress (Chapter II). Using a metagenomic approach I studied the reactions of the strains to two main sources of water stress that is solute stress, by addition of salt to the growth media, and matric stress, by addition of PEG8000, both under sub-inhibitory growth conditions. The results indicated that the three strains had different sensitivities to the exposure to water stress. The strain that was most affected was *P. veronii* 1YdBTEX2 while *S. wittichii* RW1 showed the best tolerance. *A. chlorophenolicus* A6 presented only minor transcriptome changes when facing either solute or matric stress in comparison to "no" stress. Regarding the number of genes affected by the water stress I observed fewer changes in the expression of A6 genes compared with RW1 or 1YdBTEX2. This can indicate two separate strategies to cope with stress: on the one hand a modulation of few genes for a rapid adaptation when faced with stress, which would be involved with less requirements of energy (case of strain A6). On the other hand more flexibility to modulate gene expression

response but with energetic disadvantages (strains RW1 and 1YdBTEX2). Very few functions were shared by the three strains under water stress. These include decrease of flagellar motility and increase of synthesis of strain-specific compatible solutes (osmoprotectants) and catalases. A small set of well-conserved genes was found among the three strains, but their precise functions are still unclear; these were ABC transporters and aldehyde dehydrogenases.

When comparing these responses to a more real environment of dry sand (Chapter III) I found the adaptations observed to be fundamentally different as under liquid conditions with solute or matric stress (Moreno-Forero and van der Meer 2015). Some of the few common traits between water stress and soil inoculations were the transcriptome increase of genes for glutamate biosynthesis and the inactivation of genes related with motility. It has been suggested that the absence of flagella contributes to an increase in the energy available for the cell to cope with environmental stresses (Martinez-Garcia et al 2014).

In conclusion, although matric and solute stress provoke a number of useful expression signatures related to drought they are not really representative for sand environment at least regarding RW1 behaviour. The gene expression observed in exponentially growing RW1 cells in sand at 4.8% of gravimetric water content (GWC) with DBF must, therefore, be a specific reaction to the sand physico-chemical environment.

As I showed (Moreno-Forero and van der Meer 2015), *S. wittichii* can survive well inoculations in sand with low content water. Also, Coronado et al (2015) successfully obtained growth of RW1 in mixtures of sand with PAH-contaminated soil and dibenzofuran (DBF), even though with the maximal RW1 population size was smaller than upon inoculation in clean sand with DBF. In contrast, in absence of DBF the

growth of RW1 was very limited even with extra nutrients introduced from agricultural soil. I found that RW1 can adapt more quickly to sand with DBF if the cells have been precultured in DBF rather than on salicylate. Precultures grown on salicylate led to a more drastically changed transcriptome response than on DBF, presumably in order to adapt the cells to the new carbon source (Chapter III).

In a field situation the bioavailability of the toxic compound plays an important role. I demonstrated that RW1 in absence of DBF displays an extreme carbon and nutrient shortage stress when inoculated in high densities in bare sand. Interestingly, I found that the strain can even grow a little bit in bare sand when inoculated at low cell density. Chemical analysis suggested that cells may have been taking trace amounts of DBF in the sand. These results indicate the crucial importance of sufficient bioavailability of the target compound with strains and applications intended for bioremediation purpose, in order to avoid failures in the process.

The initial response of RW1 after inoculation of high amounts of cells (10^8 cells/gr of sand) causes growth arrest similar to a stationary phase of growth. When inoculation is made with lower densities (10^5 cells/gr) a clear increase of the population was detected when DBF is present in the soil (Coronado et al 2015, Moreno-Forero and van der Meer 2015). RW1 managed to degrade 20.4% of the DBF present in sand within 40 hours after low-density inoculations, then they enter in a stationary phase of growth, maybe as a result of depletion of other nutrients like nitrogen or phosphorous. It could be interesting to see if it is possible to relaunch the growth by addition of only basic nutrients to the soil.

Unfortunately, my attempts to measure the responses of *A. chlorophenolicus* A6 under the same dry-sand conditions were frustrated by the very low hybridization signals obtained with microarrays (Chapter IV). This was probably due to problems

experienced during filtration of cells. It may be possible that I lost an important amount of A6 cells during filtration, perhaps as a result of the extraordinary capability of strain A6 to change diminish cell shape, allowing them to escape through the filter.

A6 as a typical soil bacterium has been demonstrated to degrade efficiently high concentrations (175 µg/gr of soil) of 4-chlorophenols (4CP) in soil slurries (Elvang et al 2001). In my case and probably due to the low water content, A6 did "only" grow with 12.6 µg/gr of 4CP in semi dry-sand. These results pointed out the particular conditions that some strains need to efficiently degrade contaminants, and there are probably no key common factors in all strains intended for bioremediation which can be addressed to make bioaugmentation more successful.

A. chlorophenolicus is well known to resist harsh environments (Unell et al 2007, Westerberg et al 2000), and they showed very few transcriptomic changes under different humidity levels in the phyllosphere (Scheublin et al 2014) and when exposed to laboratory mimic drought stress (Chapter II). It was very curious to see such a dramatic change in cell shape under a minor change of matric stress but without almost any transcriptome changes. In presence of PEG8000 (as little as 0.25MPa decrease) A6 cells experienced a cell volume decrease of at least tenfold.

The general responses of A6 to the phyllosphere seem to be fundamentally different than the transcriptome signature observed under water stress (Chapter V), however compared with agar surface *Arthrobacter* activates many of the genes responsible of the degradation of 4CP (precultures in absence of 4CP). This seems to indicate that the presence of phenolic compounds in the leaves could potentially result in increased degradation properties or faster reaction to contaminants in a process of phylloremediation.

In conclusion, my work showed that direct environmental targets (soil, phyllosphere) should be used to interpret the specific cellular reactions to these environmental changes, helping to understand how single strains react to inoculations in more complex microbiomes and finally improve the practise of bioremediation.

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Curriculum vitae

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EDUCATION

1986-1990 University Jorge Tadeo Lozano, Santafé de Bogotá and Cartagena, Colombia
Marine Biologist
Best student of the graduating class, **Jorge Tadeo Lozano Prize**.
Title of the thesis: Caracterizacion estructural de la comunidad bentonica asociada a *Acropora palmate* (Lamarck, 1816) muerto, Isla Grande, Islas del Rosario, Caribe Colombiano.

2000-2001 University of Lausanne
Master in systematics and biodiversity management
Travail de diplôme : "Identification moléculaire des algues endosymbiotiques des grands foraminifères benthiques – étude d'une spécificité intra-individuelle", Professor Jan Pawlowski, director of the zoological station of the University of Geneva.

2009-2015 University of Lausanne
PhD in life sciences
Thesis: Environmental activity of bacteria degrading aromatic pollutants.
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1990-1993 Jorge Tadeo Lozano University, Cartagena, Colombia
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1992 Jorge Tadeo Lozano University, Santa Marta, Colombia
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1994-1996 Jorge Tadeo Lozano University, Santa Marta, Colombia
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Courses in Ichthyology and field research

1997 Instituto de Investigaciones Marinas y Costeras INVEMAR-COLCIENCIAS, Santa Marta, Colombia
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Taxonomy project « Organismos de la macroepifauna de la plataforma continental del Caribe Colombiano »

2001 University of Geneva, Switzerland
Station zoologique
Internship in the Molecular Systematics group

2002 Harvard Medical School, Massachusetts General Hospital, Boston, USA
Laboratory technician

2002-2003 Department of Biochemistry, University of Lausanne
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Research project: “Regulation of p21 (Cip1/WAF1) expression in keratinocyte differentiation”.

2005-2006 Centre Integratif de Genome CIG, University of Lausanne
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Research projects: Screen Identification of new ligands PPAR
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2008 Department of Fundamental Microbiology (DMF), University of Lausanne
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