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ORIGINAL ARTICLE

High diversity of lysis-resistant cells upon the application of targeted physical and chemical lysis to environmental samples originating from three different water bodies

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Abstract

In most habitats, fluctuating environmental conditions create periods of compromised survival for metabolically active organisms. In response, various survival strategies have evolved, including the formation of resilient resting cells. We assessed the biodiversity of the lysis-resistant bacteria in three different environments by applying a harsh physicochemical treatment to the samples. The bacterial diversity of the lysis-resistant fraction was compared with the bacterial diversity from the same environmental samples without the application of the enrichment method. As expected, in the lysis-resistant fraction, a significantly higher relative abundance of endospore-forming Firmicutes (for instance, *Bacillus*, *Clostridium* and *Paenisporosarcina*) was observed in comparison with the untreated samples. However, genera from which the existence of a resistant cell form is not yet reported were also highly enriched in comparison with the untreated samples. Our results suggest a more diversified repertoire of bacterial resistant cellular structures than previously thought.

KEYWORDS
Bacillus, bacteria, biodiversity, DNA, ecosystem, environment

1 | INTRODUCTION

Natural environments can be unstable, and hence, organisms must have strategies to survive under environmental conditions that are not optimal for their growth and reproduction (Gray et al., 2019; Yang et al., 2016). One common strategy is dormancy (Logan et al., 2000; Wood et al., 2013). Dormancy consists of the decrease in the metabolic activity of the organism; it can be achieved through

different mechanisms (Cáceres & Tessier, 2003; Hice et al., 2018). In bacteria, one mechanism is the formation of resistant modified cells (Peberdy, 1980; Roszak & Colwell, 1987). The most resistant modified cells known to date are endospores, which are formed by members of the Firmicutes phylum (Higgins & Dworkin, 2012; Koch, 1876; Nicholson et al., 2000). Only four other bacterial phyla have been studied for the formation of similar resistant cell forms: Actinobacteria, which form exospores (Chater et al., 2010); Cyanobacteria, which form akinetes (Kaplan-Levy, Hadas, Summers, Rücker, & Sukenik, 2010a), members of the order Myxococcales (in the δ-Proteobacteria), which form myxospores (Julien et al., 2000; Kottel et al., 1975), and Azotobacteraceae, which form cysts (Socolofsky & Wyss, 1961). The extent to which model organisms from these five clades provide an adequate background to infer the properties of dormant cells, and the taxonomic prevalence of dormancy in the environment is far from clear. For instance, type strains might not be ideal representations of their environmental counterparts (Fux et al., 2005; Galperin et al., 2012). Also, in comparison with environmental strains, there is evidence that laboratory strains present lower resistance, reduced virulence (Fine et al., 1999), or even a decreased capability to produce spores (Maughan et al., 2007; Norris et al., 2020).

Reports of resistant cell forms in taxa other than these five thoroughly studied ones have in many cases been received with skepticism. This is due to the fact that microscopic evidence attesting to the existence of a modified cell may be misleading, as it is either insufficient in itself (Ajithkumar et al., 2003), raises questions about contamination (Ghosh et al., 2009), or might result from the mistaken interpretation of cellular inclusions as cysts or spores (Beskrovnaya et al., 2020; Girija et al., 2010). It is possible to hypothesize that most sporulating taxa are likely to produce resistant forms only in natural environments, since replicating the complex environmental conditions triggering dormancy or other physiological responses in the laboratory can be difficult. For instance, specific sporulation media have been proposed to induce sporulation in *Bacillus* (Hageman et al., 1984), *Streptomyces* (Ochi, 1986), or *Myxococcus* (Bragg et al., 2012), but they cannot be applied universally to induce dormancy in other species. This is well illustrated by the formation of akinetes in cyanobacteria, which are dormant, non-motile cells with thick walls (Adams & Duggan, 1999; Kaplan-Levy, Hadas, Summers, & Sukenik, 2010b; Kumar et al., 2010). As for many other species, nutrient depletion triggers the formation of akinetes, but triggers are species-specific (Perez et al., 2016). Furthermore, even if a "universal sporulation media" could be developed, low culturability of environmental bacterial communities limits the diversity of bacteria that can be assessed with these media (Staley & Konopka, 1985; Steen et al., 2019). Therefore, dormancy and the identification of dormant cells must be studied directly in environmental samples, which requires specialized methods.

In this study, we assessed the biodiversity of lysis-resistant bacteria by applying a harsh physicochemical treatment originally developed to enrich endospores (Wunderlin et al., 2016). First, for the validation of this approach to detect diverse lysis-resistant cells and not only endospores, the diversity of the lysis-resistant fraction was compared with the total diversity from the same environmental samples in which DNA was extracted without the application of the separation method. Following this validation, the diversity of lysis-resistant bacteria was investigated in different environments.

2 | METHODS

2.1 | Site description and sampling

This study focused on lacustrine sediments that were included in multiple sampling campaigns to evaluate the complementarity of bacterial spores as a paleoecological marker of human impact. For those environments in which soils or rivers might provide a strong contribution to sediment communities, soil and river samples were also included (see below). The validation of the approach as a general method to study lysis-resistant cells was carried out with sediment samples from Lake Geneva. The sediment core was recovered during a previous study in the Rhone River delta, on the eastern side of the lake. A sediment core was retrieved in August 2011 and has been previously dated and described for paleoecology (core CAN01). The characterization included dating and chemical analysis, as well as (for a subset of samples) the quantification of total bacteria and endospore-forming bacteria by qPCR, and the specific analysis of Firmicutes communities by sequencing of the *spo0A* gene (Wunderlin et al., 2014).

For the analysis of the diversity of lysis-resistant bacterial communities, three sampling sites with a variable impact of human activities were considered. First, sediment samples retrieved in the Vidy Bay, located on the shoreline near the city of Lausanne, Switzerland (high human impact site). Samples originated from ten sediment cores retrieved between July 2011 and May 2012. In samples from these cores, the total bacterial abundance and the total bacterial communities were investigated previously. The latter showed that the high abundance of Firmicutes is correlated with metal contamination (Sauvain et al., 2014).

The Joeri Lakes, located in the Eastern Swiss Alps in the canton of Graubünden (Switzerland, 46°/46′N and 9°/58′E), were the second sampling site. These high alpine lakes were formed by glacier retreat and have a restricted human impact. Lake I is situated at 2489 m above sea level, has a surface area of 93,700 m^2 and a maximum depth of 10.4 m. Lake XIII is located at an altitude of 2639 m above sea level and has a surface area of 15,400 m^2 and a maximum depth of 10 m (Gabathuler, 1999). Samples were collected in August 2016 from sediment cores and surface sediments. In addition, given the potential effect of soils for the establishment of the sediment communities, soil samples surrounding the lakes were also collected. Samples were stored at 4°C and treated in the following 3–20 days (depending on the sample).

Lake Liambezi is located in Namibia at the eastern side of the Caprivi Strip in the complex drainage system of the Kwando and Zambezi rivers. Its southern shore forms the border with

Botswana. The Okavango Delta is in northern Botswana and represents the second-largest inland delta in the world, with an area of ~18,000 km^2. This third system has a unique hydrology and cumulatively represents an intermediate human impact site. Samples from Lake Liambezi, from its affluents/effluents, and from the Okavango Delta, were collected in August 2016 and March 2017, and included sediment cores, surface sediments, and river water. These samples were stored in subzero conditions and treated in the shortest possible period before retrieval (10–20 days depending on the sample), including pre-filtering and separation of core layers in the field.

2.2 | DNA extraction

All the sediment and soil samples were initially processed for the separation of the biomass from the sediment and soils particles. The separation of the biomass from the sediment and soil was performed by adding Na-Hexa-meta-phosphate to 3 g of wet sediment/soil and homogenizing using Ultra-Thurrax® Tube Drive control (IKA). The supernatant was then filtered onto 0.2 µm pore-size nitrocellulose filters (Merck Millipore) to collect the biomass on the filters. Water samples were directly filtered onto 0.2 µm sterilized filters for the collection of biomass.

Half of the filter was cut (~1.5 g of sediment/soil) to be used for the separation of the lysis-resistant cell fraction as previously described in Wunderlin et al. (2016). Vegetative cells were lysed by a combination of treatments including heat, enzymatic agents (lysozyme), and disrupting chemicals (Tris-EDTA, NaOH, and SDS). Following cell lysis, a DNAse treatment was applied to remove DNA released from the lysed cells. After removal of DNAse and washing, the lysis-resistant cells were collected by filtration onto 0.2 µm sterilized filters.

DNA extraction was performed using the FastDNA®SPIN kit for soil (MP Biomedicals). A modified protocol including successive bead-beating steps was applied in order to ensure the retrieval of DNA from the lysis-resistant bacterial cells, as shown previously in the case of spores from endospore-forming Firmicutes (Wunderlin et al., 2013). DNA extracts from the different bead-beating steps were pooled by ethanol precipitation and resuspended in PCR-grade water. The same DNA extraction procedure was applied in the biomass collected directly from the samples (herein referred as to total community) and in the biomass collected after lyses (herein referred as lysis-resistant community). DNA quantification was performed using Qubit® dsDNA HS Assay Kit (for the lysis-resistant community) or Qubit® dsDNA BR Assay Kit (for the total community) on a Qubit® 2.0 Fluorometer (Invitrogen).

2.3 | Sequencing and data analysis

Amplicon sequencing of the 16S rRNA gene was performed by Fasteris, using Illumina MiSeq platform (Illumina). Universal primers Bakt_341F (5′-CCTACGGGNGGCWGCAG-3′) and Bakt_805R (5′-GACTACHVGGGTATCTAATCC-3′) were used for the selection of the hypervariable V3–V4 region (Herlemann et al., 2011). Sequencing data were analyzed using Mothur (Schloss et al., 2009), following the standard procedure of MiSeq SOP (Kozich et al., 2013), with an additional step of singleton removal prior to the clustering in OTUs. The alignment of amplicons and the taxonomic assignment of representative OTUs was performed using the SILVA NR v123 reference database (Quast et al., 2013). A total of 11,387,677 amplicons (2,585,518 unique sequences) were retained after quality filtering and removal of chimeras. At this point, singletons were removed (2,332,236 sequences), as well as unclassifiable sequences or sequences belonging to undesirable lineages (chloroplast, mitochondria, archaea, and eukaryote; 99,150 sequences; 2778 unique sequences). The remaining 8,956,291 reads (250,504 unique sequences) were clustered into OTUs, using the OptiClust method (Westcott & Schloss, 2017) with an identity threshold of 97%, leading to the identification of 43,151 OTUs.

The same data treatment for the comparison of lysis-resistant and total community in Lake Geneva gave the following results: 5,026,205 reads after quality filtering and chimera removal (1,016,871 unique sequences), 900,038 singletons, 22,622 (1110 unique sequences) unclassified sequences or from undesirable lineages, and 4,103,545 retained sequences (115,723 unique sequences) clustered in 17,129 OTUs.

The sequences were deposited in GenBank under BioProject accession numbers PRJNA 396429, 396276, and 396277.

2.4 | Statistical and multivariate analysis

Community and multivariate analyses were performed using R version 3.4.0 (Team, 2014) and the phyloseq and vegan packages (McMurdie & Holmes, 2013; Oksanen et al., 2020). Principal coordinates analysis (PCoA) was calculated based on Bray–Curtis dissimilarity and Hellinger transformation of the OTU table. Rare OTUs (less than 10 reads) were removed prior to the analysis of the lysis-resistant communities, as a conservative measure to reduce the background of OTUs representing contamination by abundant members of the non-resistant total community. For the validation experiment (Lake Geneva samples), the threshold was reduced to four reads, as in the case of the validation, evaluating the extent of this potential contamination was important. Difference between groups based on community composition was tested using permutational multivariate analysis of variance (PERMANOVA) using the Adonis function from the vegan package, based on the same Hellinger transformed matrix as described above, with 999 permutations. The distribution pattern of bacterial phyla was analyzed using contour plot of 2D Kernel density estimates, with the MASS package (Venables & Ripley, 2013). The limma package was used for the display of Venn diagrams (Ritchie et al., 2015).

Additional analyses were performed to evaluate the contribution of incomplete lysis (i.e., contamination by members of the total community not producing a lysis-resistant cell) to the

composition of the lysis-resistant community. For this, an "enrichment index" was calculated for each individual OTU by comparing the relative contribution of the abundance in the lysis-resistant fraction (based on absolute total sequence counts) as compared to the total abundance of the OTU (lysis/lysis + total fraction). This index ranges between 0 (OTUs only detected in the total community fraction) and 1 (OTUs only detected in the lysis-resistant fraction). The results were represented as cumulative graphs, in which the proportion of OTUs displaying the same distribution were grouped together. This was done for all the OTUs in a given sample, as well as separating the OTUs by phyla. In addition, the same index was calculated for the most abundant OTUs and the results represented as a heatmap. Except for the heatmap, which was produced in R, all the analyses, were produced with the Julia language (Bezanson et al., 2017) using the Gadfly graphics package (Jones et al., 2021).

3 | RESULTS

3.1 | Validation of the analysis comparing total and lysis-resistant bacterial communities

To first validate that the harsh physicochemical treatment is indeed selecting for lysis-resistant cells and that the community investigated does not simply represent a subsampling of abundant vegetative cells that are inefficiently lysed, a subset of the samples was used to compare the total versus the lysis-resistant community obtained after the lysis treatment from the same sample. In the case of subsampling, lysis-resistant and total communities originating from the same sample can be expected to be more similar to each other compared with other communities corresponding to the lysis-resistant or total communities originating from different samples. Also, the most dominant species would be expected to be the same between the two extraction methods.

The validation was conducted in sediment samples originated from a sediment core used for a palaeoecological study in Lake Geneva and covering the period between 1921 and 2011. In this sediment core, dating and chemical analysis, quantification of total bacteria and endospore-forming bacteria by qPCR, and the specific analysis of Firmicutes communities by sequencing of the *spo0A* gene, were previously performed in a subset of samples (Wunderlin et al., 2014). However, the total or lysis-resistant communities were never investigated. The comparison of the community composition showed a clear separation between the lysis-resistant and total bacterial communities (PCoA Axis 1, explaining 33.2% of the variance; Figure 1a). Moreover, PERMANOVA test shows that the total and the lysis-resistant communities were significantly different (p-value < 0.001).

To better visualize the groups explaining the separation of the total and lysis-resistant communities, the distribution and density of OTUs separated by phyla were plotted on the PCoA (Figure 1b). This analysis allowed identifying clades for which the density of OTUs is higher in the total community, higher in the lysis-resistant community, or equally distributed. Acidobacteria is an example of a group containing mainly OTUs associated with the total community fraction. OTUs belonging to Proteobacteria were distributed in both communities, although represented by individual sets of OTUs for each type of DNA extraction method (total versus lysis-resistant). In contrast, OTUs belonging to phyla Actinobacteria, Firmicutes, and Chlamydiae were found in the lysis-resistant fraction. This was expected in the first two groups, as survival in the form of highly specialized lysis-resistant spores is well-documented in the literature for species within Actinobacteria and Firmicutes. A similar type of dormant cell called elementary bodies is also known for Chlamydiae (Hoare et al., 2008; Korhonen et al., 2019), but it was never shown to provide resistance to lysis.

The composition of the communities at the genus level was analyzed as well. The shift in dominance and community structure of Firmicutes in the lysis-resistant community was clearly observed (Figure S1). The relative abundance of the 37 most abundant OTUs in the dataset was compared for the total and lysis-resistant community fractions (Table S1). These 37 OTUs corresponded to 17 OTUs assigned to genera not known for the formation of spores (non-spore forming), six corresponding to previously known spore-formers, and 14 OTUs in which spore formation could not be unambiguously assessed. The six known spore-forming bacteria were between five and twenty-five times more abundant in the lysis-resistant community (Table S1). The same is the case for three out of seventeen of the genera that unambiguously correspond to organisms so far not known for the production of spores or other lysis-resistant cellular structures (i.e., *Mesorhizobium*, *Gaiella*, and *Rhizobium*). Analogously, OTUs from seven clades that cannot be precisely classified into a specific genus were between two and twenty-five times more abundant in the lysis-resistant community. Those OTUs were affiliated Peptostreptococcaceae (Firmicutes), Gaiellales (Actinobacteria), Ruminococcaceae (Firmicutes), Pir4 (Planctomycetales), D8A-2 (Peptococcaceae), MB-A2-108 (Actinobacteria), and PeM15 (Actinobacteria). Furthermore, the communities of the two most abundant phyla, Proteobacteria and Firmicutes, were compared (Figure S1). In the case of Proteobacteria, OTUs classified as *Anaeromyxobacter* and *Mesorhizobium* are clearly dominant among lysis-resistant structures. In contrast, OTUs classified in these two genera were only rarely found in the total bacterial community. This was particularly remarkable for the most abundant OTU, which was affiliated to *Mesorhizobium* and was more than a thousand times more abundant in the lysis-resistant community, as compared to the total community (Table S1). In the case of Firmicutes, the vast majority of the OTUs was detected in the lysis-resistant community. The only exception was an OTU affiliated to the genus *Bacillus*, which was more prevalent in the total community. Overall, the analysis strongly suggests that a different fraction of the community is analyzed by applying the enrichment method.

Additional analyses were performed to validate the unique composition of the lysis-resistant community. For this, an enrichment

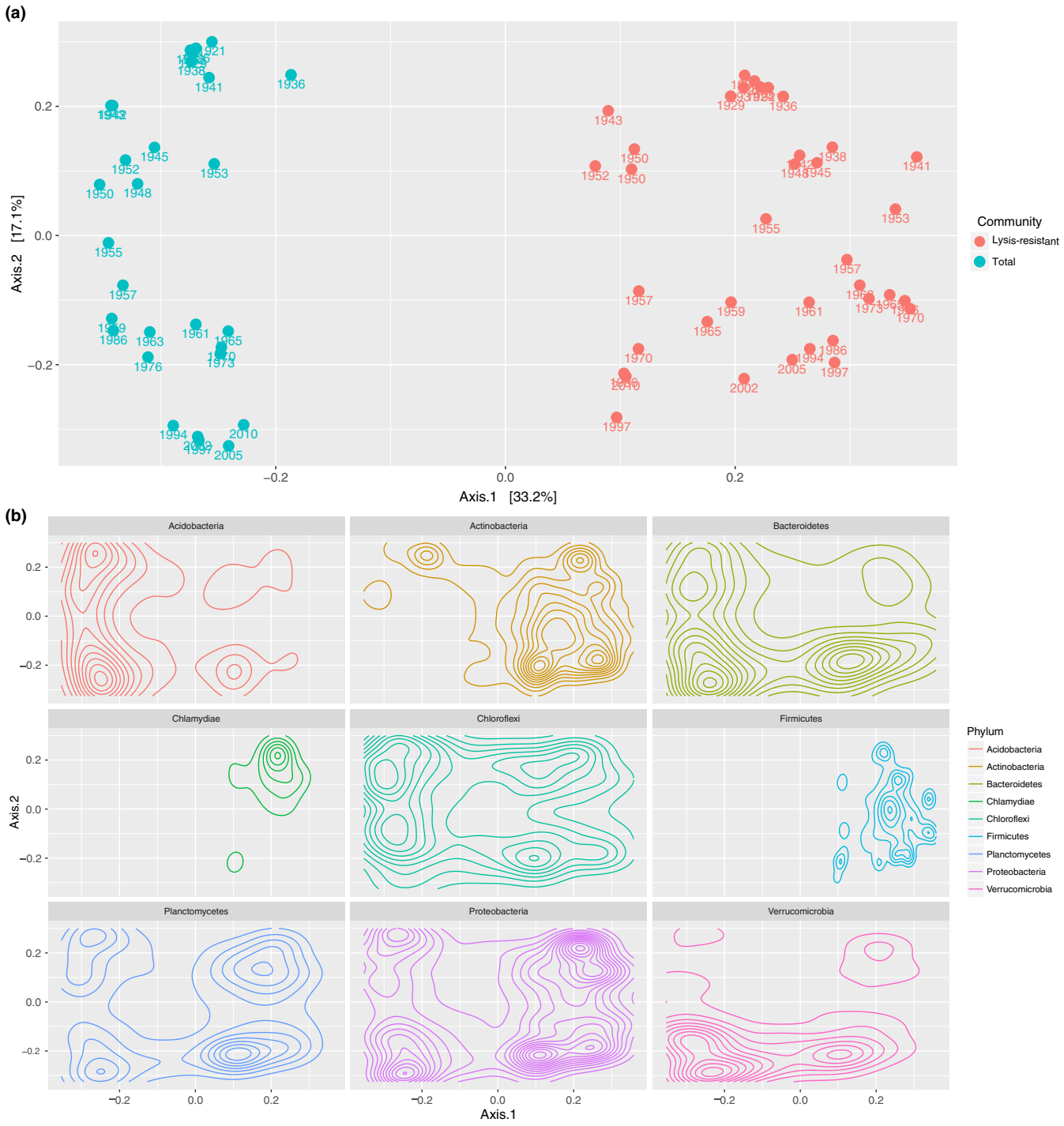

FIGURE 1 Comparison of the lysis-resistant and total bacterial communities in a sediment core obtained from Lake Geneva, Switzerland. (a) Principal coordinate analysis (PCoA) of the communities showing the separation of the lysis-resistant from the total bacterial community. (b) Density distribution of OTUs from the 9 most abundant phyla in the dataset projected on the PCoA presented in a (OTUs with minimum 4 reads and phyla >1% mean relative abundance in the entire dataset)

index in the lysis-resistant fraction was calculated for each individual OTU detected in a given sample (Table S2). The hypothesis tested with this analysis was that in the absence of cross-contamination of the lysis-resistant fraction, a bimodal distribution of the OTUs was to be expected with OTUs enriched either in the lysis-resistant or total fraction. A different distribution (i.e., large fraction of the community presenting and intermediate enrichment index) would suggest contamination. Although it can be considered that contamination is more likely to occur for OTUs with high relative abundance in the total fraction, in the first part of the analysis, we did not filter the data by relative abundance, but rather considered all OTUs with sequence counts in either group. The results indicate a bimodal enrichment distribution for the OTUs in all the samples (Figure 2a; Figure S3). Moreover, a breakdown by phyla confirmed that specific

OTUs constitute the vast majority of lysis-resistant community in each one of the samples (Figure 2b; Dataset S1). This unique composition can also be observed for the majority of those OTUs that represented the most prevalent members of the community (OTUs present in all the samples; Figure 2c). A taxonomic breakdown by phylum once more indicates that Firmicutes are particularly enriched in the lysis-resistant fraction among these highly prevalent OTUs (Figure S4).

3.2 | Diversity of the environmental bacterial community forming lysis-resistant cells

To investigate the diversity of environmental bacteria forming lysis-resistant cells, the spore enrichment method described above was applied to a variety of mostly sediment samples from aquatic systems in which different levels of human impact could be expected. In addition, river and soil samples in the vicinity of water bodies were also collected for those systems with low and intermediate human impact, as in those case, the soil and river bacterial communities were considered as important contributors of the lysis-resistant fraction in sediments. A total of 8,956,291 16S rRNA gene sequences were obtained. The sequences were grouped (clustering at 97% identity) into more than 90,000 operational taxonomic units (OTUs). A ranking of the OTUs by sequence counts (Table S3) shows that a large fraction (94%) of the dataset corresponds to OTUs represented by less than 100 reads.

Although a variety of bacterial phyla were represented in the lysis-resistant communities from multiple environments, representatives of Actinobacteria, Firmicutes, and Proteobacteria were notably enriched, representing on average 86% of the relative abundance of the bacterial community (Figure 3a; Table S4). At first sight, this broadly corresponds to phyla in which spore-producing representatives are known. Indeed, OTUs affiliated to known spore-forming genera were detected and included *Bacillus*, *Clostridium*, *Paenibacillus*, *Lysinibacillus* (Firmicutes), and *Anaeromyxobacter* (Proteobacteria [Sanford et al., 2002]), confirming the performance of the enrichment approach to select spore-forming bacteria. However, in many samples, Proteobacteria were largely as abundant (or more so) than Firmicutes; an unexpected finding since only a limited number of Proteobacterial genera are known to produce spores or similar durable cellular structures (Barton, 2005). Closer inspection of the Proteobacteria genera detected provides additional insight into the diversity of groups that resist the cell lysis treatment. Notably, among the twenty-five most abundant OTUs, several genera were not previously known to form resistant structures. These included the Proteobacteria *Mesorhizobium*, *Burkholderia*, *Thiobacillus*, and *Pseudorhodoferax* (W.-M. Chen et al., 2013); and the Actinobacteria *Arthrobacter* (Ding et al., 2009; Gobbetti & Rizzello, 2014) and *Cryobacterium* (Bajerski et al., 2011). These groups represented 42% of the total sequence counts within the twenty-five most abundant OTUs (Figure 3b). The enrichment of putatively asporogenic (non-spore former) or poorly spore-forming genera even extended to Firmicutes, in which OTUs affiliated to the genera *Turicibacter* (Auchtung et al., 2016) and *Intestinibacter* (Gerritsen et al., 2014) were detected in these environmental samples.

3.3 | Environment-specific distribution of the bacterial community forming lysis-resistant cells

The sampling sites corresponded to three types of environments, which were selected based on different levels of human impact, from very high (low-altitude temperate lake in Switzerland), to intermediate (a tropical river system in Namibia/Botswana), and low (high-altitude temperate lake in Switzerland) human impacts. These three sites were clearly discernible in a principal coordinate analysis (PCoA) based on the structure of the lysis-resistant community (Figure 4a). This geographical/environmental signature was still visible for different types of samples (sediment, soil, and water) belonging to the same environment (soil and river samples were grouped closer to sediment samples from their respective sampling site). The distribution pattern of OTUs affiliated to different bacterial phyla was also analyzed (Figure 4b). Density plots of OTUs belonging to the most abundant phyla (>0.1% mean relative abundance in the entire dataset) were calculated and displayed in the axis selected in the PCoA of the lysis-resistant communities. Although some phyla (for instance, Actinobacteria, Cyanobacteria, Planctomycetales, Proteobacteria, and Saccharibacteria) appear at high density in all environments, the density plots suggest a site-specific distribution pattern of OTUs. In addition, some groups appear to be diagnostic of the environment. For example, the distribution of OTUs affiliated to Chlamydiae, was denser in samples originating from the low-altitude temperate Lake Geneva. The density of OTUs belonging to Spirochaeta also suggested a site-specific distribution pattern in the case of samples from the tropical river system in Namibia/Botswana.

4 | DISCUSSION

The molecular assessment on the diversity of lysis-resistant bacterial communities using environmental DNA extracted from diverse environmental samples shows that the community forming structures that withstand lysis includes many genera from which the existence of a resistant cell form was either entirely unknown or not known to provide persistence in the environment. The validation experiment based on the comparison of the total and lysis-resistant communities indicated that contamination of the lysis-resistant community with abundant members of lysis-susceptible total bacterial community is an unlikely explanation for the diversity of previously unknown lysis-resistant species observed in these environmental samples. This was even the case for highly abundant members of the total bacterial community for which contamination is more likely to occur.

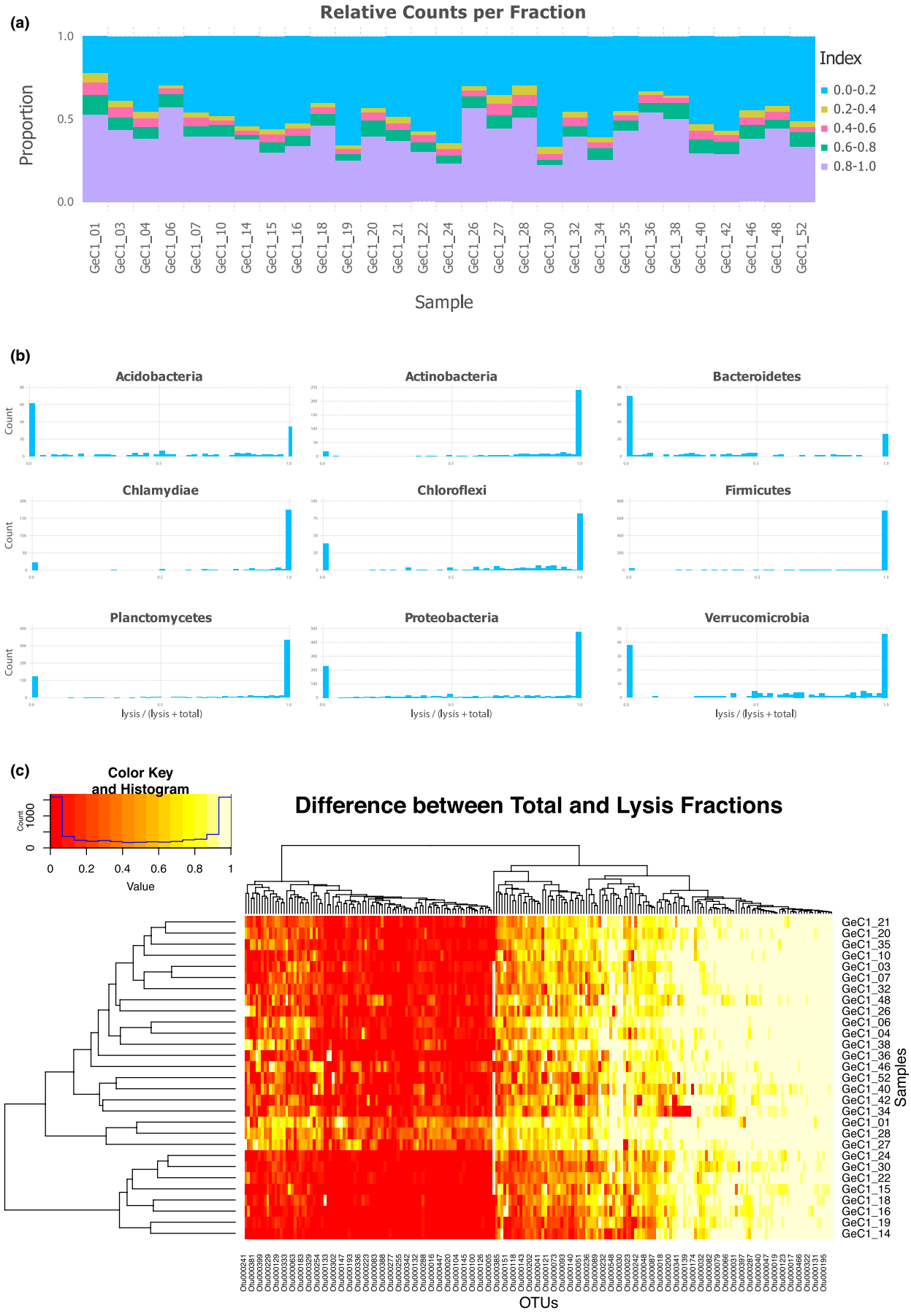

FIGURE 2 Evaluation of the composition of the lysis-resistant community calculated by a lysis-enrichment index. The enrichment index was calculated by comparing the abundance (sequence counts) in the lysis-resistant fraction over the total abundance (sequence counts in lysis+ total fraction). In the distribution, OTUs enriched in the lysis-resistant fraction had an index value of 1, while those enriched in the total community fraction had an index value of 0. (a) Bar plots representing the relative fraction of the OTUs enriched in the lysis-resistant (enrichment index 0.8–1.0), the total community (enrichment index 0.0–0.2), or those shared between the two fractions. The distribution for all individual samples can be found in Figure S3. (b) Example of the distribution of the enrichment index for OTUs separated by phyla in the most superficial sample (GeC1_01). The graphs for all the samples can be found in Dataset S1. (c) Heatmap representing the enrichment index calculated for the 216 OTUs for which the enrichment index can be calculated in all individual samples (most prevalent OTUs)

Our results suggest the presence of a large diversity of lysis-resistant, non-canonical sporulating bacterial genera in various environments. One of these genera is *Arthrobacter*. Even though the genus *Arthrobacter* is recognized as asporogenic, its resistance to desiccation and long periods of starvation is widely accepted (Boylen, 1973; Chen & Alexander, 1973; Ensign, 1970; Mongodin et al., 2006). Moreover, highly resistant *Arthrobacter* spp. have been isolated from extreme environments such as the deep ocean sediment surface (Chen et al., 2005), arctic ice (Demkina, Soina, El'-Registan, & Zvyagintsev, 2000b; Dsouza et al., 2015), and chemical-contaminated sites (Wang et al., 2013). In addition to these highly resistant isolates of *Arthrobacter* from natural environments, several studies have shown that the different morphological cellular stages produced by representatives of this genus do not have the same resistance to various stressors. One example of this is the salt-induced myceloids of *Arthrobacter globiformis* that show a higher resistance to UV radiation and heat shock than its bacilli-shaped cells (exponential-phase) or its cocci-shaped cells (stationary-phase; Malwane & Deutch, 1999). There is also evidence of dormant cells of *A. globiformis* showing long-term viability and a differential cyst-like ultrastructure when grown in a unbalanced medium (Demkina, Soina, & El'-Registan, 2000a; Duxbury et al., 1977). These examples show the diversity of strategies favoring resistance of the genus *Arthrobacter*. Moreover, the relative abundance of this genus in the lysis-resistant fraction suggests the presence of *Arthrobacter* species with a potentially higher resistance to lysis in natural environments than the laboratory type strains.

Significantly, Chlamydiae were among the groups not known for the existence of a lysis-resistant cell providing persistence in the environment, which were diagnostic of samples in a temperate lake (Lake Geneva). This lake is one of the most human-impacted environments in Western Europe and lysis-resistant Chlamydiae appear to be diagnostic of this anthropogenic impact. Chlamydiae are strictly obligate intracellular pathogens that require an eukaryotic host to complete their developmental cycle (Abdelrahman & Belland, 2005). As part of their life cycle, Chlamydiae form dormant structures called elementary bodies, sometimes referred as "spore-like" cells. These elementary bodies are non-replicative infectious cells forms, that are released from ruptured infected cells and can be transmitted from one individual to another (Elwell et al., 2016; Grieshaber et al., 2018). However, their persistence in the environment as elementary bodies has, to the best of our knowledge, not yet been studied. Furthermore, recent studies have shown the widespread distribution of environmental Chlamydiae (Collingro et al., 2020; Taylor-Brown et al., 2015), which have been isolated from within protist and various animal hosts, suggesting that persistence in the environment is ecologically relevant for this group.

Another group for which environmentally persistent lysis-resistant cells was not known was Spirochaeta, reported as common in natural aquatic environments (Harwood & Canale-Parola, 1984). Spirochaeta also presented a site-specific distribution pattern. In this case, the highest density of OTUs from this group appears to be a signature of sediment samples and rivers from Namibia/Botswana. Literature on Spirochaeta also suggests the existence of ovoid bodies or spore-like spherical bodies, which are resistant and vary in their ability to produce (Thomson & Thomson, 1914) or not (Noguchi, 1912) new cells. In both cases, Chlamydiae and Spirochaeta, the results obtained here indicate that elementary and ovoid bodies might provide a lysis-resistant cellular adaptation to persist in the environment, but in the case of Spirochaeta, this should be further validated by establishing its relative abundance in the total community fraction, which was not done here.

Persistence in the environment is not the only ecological advantage expected to be provided by a lysis-resistant cell. Dissemination is also another property expected to be enhanced in a lysis-resistant population (Lennon & Jones, 2011). Therefore, the number of OTUs shared among the three environments studied here was established (putative cosmopolitan species; Figure S5a). Population density is expected to play an important role in the biogeographic distribution pattern of a species, with highly abundant species being more prone to having a cosmopolitan distribution (Lennon & Jones, 2011). When both rare and abundant OTUs (18,515 OTUs) are considered, only 9% display a cosmopolitan distribution. In contrast, when the 1000 most abundant OTUs were analyzed, the percentage of cosmopolitan OTUs increases to 42% (Table S5; Figure S5b). At first glance, this supports the hypothesis of a relationship between species abundance and dispersal potential proposed in the literature for dormant cells (Lennon & Jones, 2011). However, a caveat to this result is that we did not establish the absolute abundance of the different OTUs in the samples, but considered instead sequence counts as a proxy to abundance, which is known to be biased (Kumar et al., 2018). Hence, these results should be validated by quantifying the absolute abundance of expected cosmopolitan and rare species by an independent method such as quantitative PCR.

Our results point to a high diversity of lysis-resistant bacteria only through their DNA. However, a non-destructive method

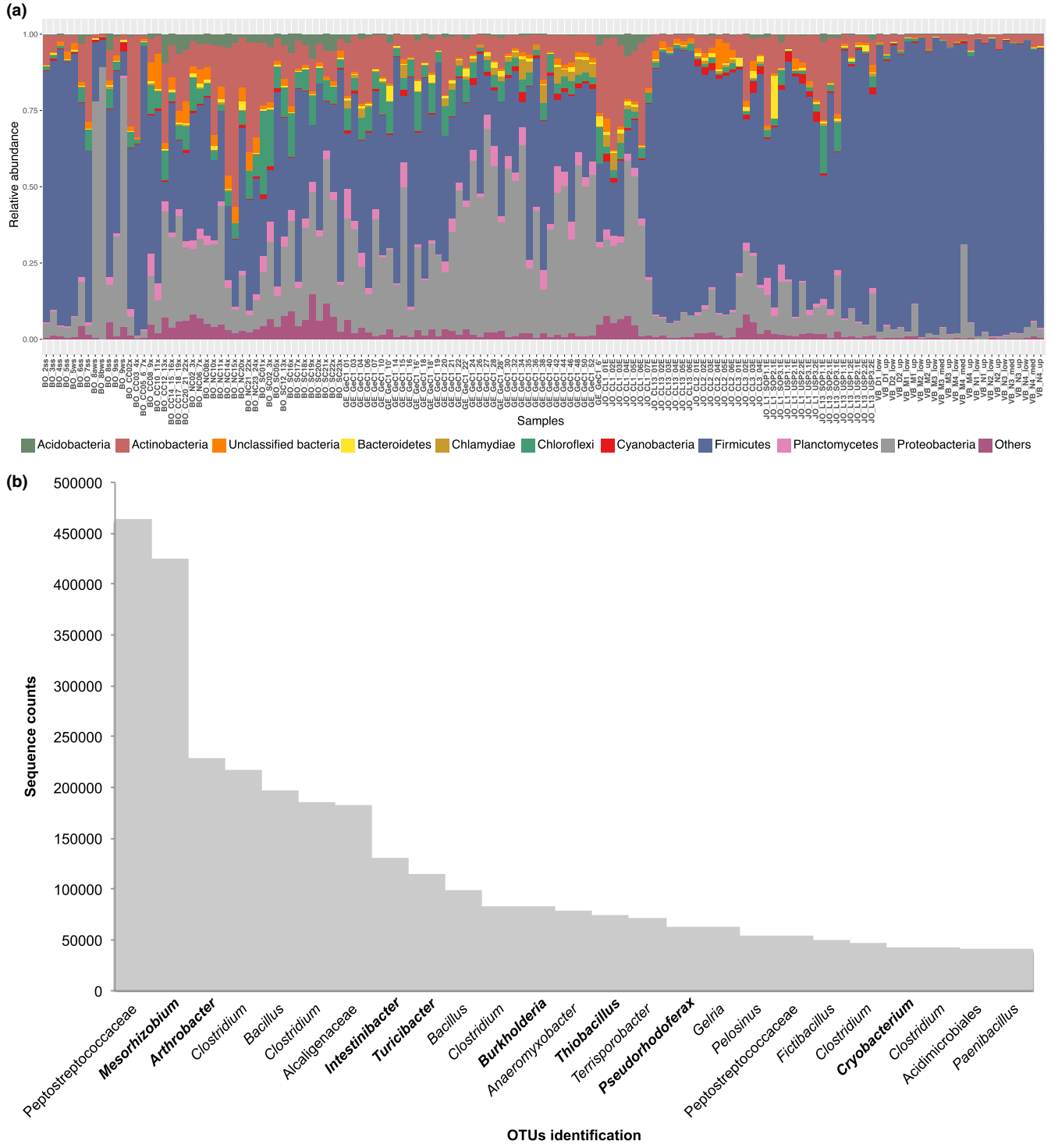

FIGURE 3 Composition of the lysis-resistant bacterial community in different environments. (a) Relative abundance of the most abundant OTUs (over 1% relative abundance) grouped to a phylum level showing the dominance of Firmicutes, Proteobacteria, and Actinobacteria. The relative abundance of these three groups varied significantly between different environmental samples. (b) Absolute frequency (in sequence counts) of the 25 most abundant lysis-resistant OTUs. OTU identification is given in the highest taxonomic range (up to genus level) in which the OTUs could be classified. Genera in which spore formation has not been reported are highlighted in bold

FIGURE 4 Biogeographical distribution patterns of the lysis-resistant communities. (a) Principal coordinate analysis (PCoA) showing the similarities of the lysis-resistant communities between different sampling sites. The type of sample (sediment, soil, or water sample) is also indicated. (b) Gradients indicating the density distribution of OTUs for the most abundant phyla (OTUs with minimum 10 reads and phyla >0.5% mean relative abundance in the entire dataset) projected on the PCoA presented in a

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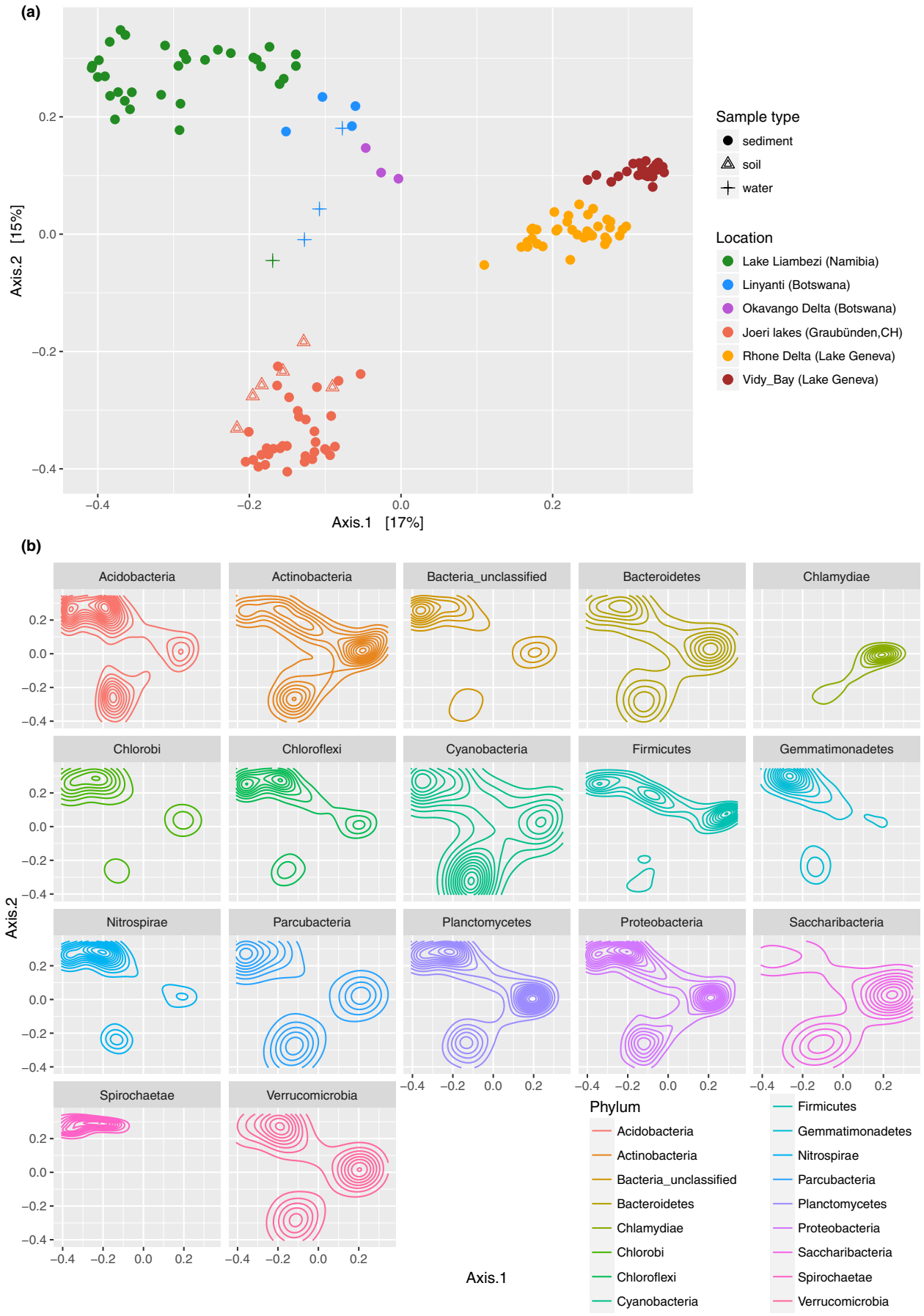

is still required for a better characterization of the morphological diversity of these resistant cells. The incontrovertible evidence that non-canonical sporulating bacteria are able to produce specialized lysis-resistant cells would be the isolation of such cells. Furthermore, enrichment and culturing of these organisms would allow to assess the physiology and function of lysis-resistant cells. Progress in this area is slow and suffers from setbacks such as misleading microscopic evidence, risk of contamination and difficulty to recreate environmental conditions that induce the formation of lysis-resistant cells in the laboratory. Even though all DNA extraction methods are intrinsically biased, enriching resistant structures and then characterizing their diversity should provide a more comprehensive insight into community members that are able to generate lysis-resistant cells in the environment. This is the first step toward cataloguing the diversity of structures and mechanisms allowing bacterial survival under adverse conditions, with the future goal of understanding the environmental cues involved in both dormancy and revival.

We inhabit a planet that is expected to undergo increasing episodes of punctuated environmental stress. Under these conditions, the production of resistant cells might be an adaptive trait that will be under strong positive selection with unknown consequences in the dynamics of microbial populations in the environment (Shoemaker & Lennon, 2018). Moreover, dormant populations constitute a seed bank from which new communities emerge after perturbation. Microorganisms are at the base of the functioning of the biosphere; therefore, the unique community of bacteria forming lysis-resistant cells cannot be ignored if we want to improve our ability to predict the microbial response to environmental change.

AUTHOR CONTRIBUTIONS

TJ and CP contributed equally to this work. PJ, TV, PHV, AL, and CP made major contributions to the conception or design of the study. TJ, CP, MF, AL, PSC, DA, TV, PHV, and PJ made major contributions to the acquisition, analysis, or interpretation of the data. TJ, CP, AC, and PJ made major contributions to the writing of the manuscript. All the authors proofread and approved of the manuscript.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The sequences were deposited in GenBank under BioProject accession numbers PRJNA 396429, 396276, and 396277.

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REFERENCES

Abdelrahman, Y. M., & Belland, R. J. (2005). The chlamydial developmental cycle. *FEMS Microbiology Reviews*, *29*, 949–959. https://doi.org/10.1016/j.femsre.2005.03.002

Adams, D. G., & Duggan, P. S. (1999). Heterocyst and akinete differentiation in cyanobacteria. *New Phytologist*, *144*(1), 3–33. https://doi.org/10.1046/j.1469-8137.1999.00505.x

Ajithkumar, B., Ajithkumar, V. P., Iriye, R., Doi, Y., & Sakai, T. (2003). Spore-forming *Serratia marcescens* subsp. *sakuensis* subsp. nov., isolated from a domestic wastewater treatment tank. *International Journal of Systematic and Evolutionary Microbiology*, *53*(1), 253–258. https://doi.org/10.1099/ijs.0.02158-0

Auchtung, T. A., Holder, M. E., Gesell, J. R., Ajami, N. J., Duarte, R. T. D., Itoh, K., Caspi, R. R., Petrosino, J. F., Horai, R., & Zárate-Bladés, C. R. (2016). Complete genome sequence of *Turicibacter* sp. strain H121, isolated from the feces of a contaminated germ-free mouse. *Genome Announcements*, *4*(2), 2015–2016. https://doi.org/10.1128/genomeA.00114-16

Bajerski, F., Ganzert, L., Mangelsdorf, K., Lipski, A., & Wagner, D. (2011). *Cryobacterium arcticum* sp. nov., a psychrotolerant bacterium from an Arctic soil. *International Journal of Systematic and Evolutionary Microbiology*, *61*(8), 1849–1853. https://doi.org/10.1099/ijs.0.027128-0

Barton, L. (2005). *Structural and functional relationship in prokaryotes*. Springer.

Beskrovnaya, P., Fakih, D., Morneau, I., Hashimi, A., Guadarrama Bello, D., Xing, S., Nanci, A., Huan, T., & Tocheva, E. I. (2020). No endospore formation confirmed in members of the phylum proteobacteria. *Applied and Environmental Microbiology*, *87*(5), 1–11. https://doi.org/10.1128/aem.02312-20

Bezanson, J., Edelman, A., Karpinski, S., & Shah, V. B. (2017). Julia: A fresh approach to numerical computing. *SIAM Review*, *59*(1), 65–98.

Boylen, C. W. (1973). Survival of *Arthrobacter crystallopoietes* during prolonged periods of extreme desiccation. *Journal of Bacteriology*, *113*(1), 33–37.

Bragg, J., Rajkovic, A., Anderson, C., Curtis, R., Van Houten, J., Begres, B., Naples, C., Snider, M., Fraga, D., & Singer, M. (2012). Identification and characterization of a putative arginine kinase homolog from *Myxococcus xanthus* required for fruiting body formation and cell differentiation. *Journal of Bacteriology*, *194*(10), 2668–2676. https://doi.org/10.1128/JB.06435-11

Cáceres, C. E., & Tessier, A. J. (2003). How long to rest: The ecology of optimal dormancy and environmental constraint. *Ecology*, *84*(5), 1189–1198. https://doi.org/10.1890/0012-9658(2003)084[1189:HLTRTE]2.0.CO;2

Chater, K. F., Biró, S., Lee, K. J., Palmer, T., & Schrempf, H. (2010). The complex extracellular biology of *Streptomyces*: Review article. *FEMS Microbiology Reviews*, *34*(2), 171–198. https://doi.org/10.1111/j.1574-6976.2009.00206.x

Chen, M., & Alexander, M. (1973). Survival of soil bacteria during prolonged desiccation. *Soil Biology and Biochemistry*, *5*(2), 213–221. https://doi.org/10.1016/0038-0717(73)90004-7

Chen, M., Xiao, X., Wang, P., Zeng, X., & Wang, F. (2005). *Arthrobacter ardleyensis* sp. nov., isolated from Antarctic lake sediment and deep-sea sediment. *Archives of Microbiology*, *183*(4), 301–305. https://doi.org/10.1007/s00203-005-0772-y

Chen, W.-M., Lin, Y.-S., Young, C.-C., & Sheu, S.-Y. (2013). *Pseudorhodoferax aquiterrae* sp. nov., isolated from groundwater. *International Journal of Systematic and Evolutionary Microbiology*, *63*(Pt_1), 169–174. https://doi.org/10.1099/ijs.0.039842-0

Collingro, A., Köstlbacher, S., & Horn, M. (2020). Chlamydiae in the environment. *Trends in Microbiology*, *28*(11), 877–888. https://doi.org/10.1016/j.tim.2020.05.020

Demkina, E. B., Soina, V. S., & El'-Registan, G. L. (2000a). Formation of resting forms of *Arthrobacter globiformis* in autolyzing cell suspensions. *Mikrobiologiya*, *69*(3), 383–388.

Demkina, E. V., Soina, V. S., El'-Registan, G. I., & Zvyagintsev, D. G. (2000b). Reproductive resting forms of *Arthrobacter globiformis*. *Microbiology*, *69*(3), 309–313. https://doi.org/10.1007/BF02756739

Ding, L., Hirose, T., & Yokota, A. (2009). Four novel Arthrobacter species isolated from filtration substrate. *International Journal of Systematic and Evolutionary Microbiology*, *59*(4), 856–862. https://doi.org/10.1099/ijs.0.65301-0

Dsouza, M., Taylor, M. W., Turner, S. J., & Aislabie, J. (2015). Genomic and phenotypic insights into the ecology of *Arthrobacter* from Antarctic soils. *BMC Genomics*, *16*(1), 1–18. https://doi.org/10.1186/s12864-015-1220-2

Duxbury, T., Gray, T. R. G., & Sharples, G. P. (1977). Structure and chemistry of walls of rods, cocci and cystites of *Arthrobacter globiformis*. *Journal of General Microbiology*, *103*(1), 91–99. https://doi.org/10.1099/00221287-103-1-91

Elwell, C., Mirrashidi, K., & Engel, J. (2016). Chlamydia cell biology and pathogenesis. *Nature Reviews Microbiology*, *14*(6), 385–400. https://doi.org/10.1038/nrmicro.2016.30

Ensign, J. C. (1970). Long-term starvation survival of rod and spherical cells of *Arthrobacter crystallopoietes*. *Journal of Bacteriology*, *103*(3), 569–577.

Fine, D. H., Furgang, D., Schreiner, H. C., Goncharoff, P., Charlesworth, J., Ghazwan, G., Fitzgerald-Bocarsly, P., & Figurski, D. H. (1999). Phenotypic variation in *Actinobacillus actinomycetemcomitans* during laboratory growth: Implications for virulence. *Microbiology*, *145*(6), 1335–1347. https://doi.org/10.1099/13500872-145-6-1335

Fux, C. A., Shirtliff, M., Stoodley, P., & Costerton, J. W. (2005). Can laboratory reference strains mirror "real-world" pathogenesis? *Trends in Microbiology*, *13*(2), 58–63. https://doi.org/10.1016/j.tim.2004.11.001

Gabathuler, M. (1999). *Physical Ecosystem Determinants in High Mountain Lakes (The Jori Lakes, Switzerland). 13449.*

Galperin, M. Y., Mekhedov, S. L., Puigbo, P., Smirnov, S., Wolf, Y. I., & Rigden, D. J. (2012). Genomic determinants of sporulation in bacilli and clostridia: Towards the minimal set of sporulation-specific genes. *Environmental Microbiology*, *14*(11), 2870–2890. https://doi.org/10.1111/j.1462-2920.2012.02841.x

Gerritsen, J., Fuentes, S., Grievink, W., van Niftrik, L., Tindall, B. J., Timmerman, H. M., Rijkers, G. T., & Smidt, H. (2014). Characterization of *Romboutsia ilealis* gen. nov., sp. nov., isolated from the gastrointestinal tract of a rat, and proposal for the reclassification of five closely related members of the genus *Clostridium* into the genera *Romboutsia* gen. nov. intestinib. *International Journal of Systematic and Evolutionary Microbiology*, *64*(Part 5), 1600–1616. https://doi.org/10.1099/ijs.0.059543-0

Ghosh, J., Larsson, P., Singh, B., Pettersson, B. M. F., Islam, N. M., Sarkar, S. N., Dasgupta, S., & Kirsebom, L. A. (2009). Sporulation in mycobacteria. *Proceedings of the National Academy of Sciences*, *106*(26), 10781–10786. https://doi.org/10.1073/pnas.0904104106

Girija, K. R., Sasikala, C., Ramana, C. V., Spröer, C., Takaichi, S., Thiel, V., & Imhoff, J. F. (2010). *Rhodobacter johrii* sp. nov., an endospore-producing cryptic species isolated from semi-arid tropical soils. *International Journal of Systematic and Evolutionary Microbiology*, *60*(9), 2099–2107. https://doi.org/10.1099/ijs.0.011718-0

Gobbetti, M., & Rizzello, C. G. (2014). *Arthrobacter*. In *Encyclopedia of food microbiology* (Vol. *1*, 2nd ed., pp. 69–76). Elsevier. https://doi.org/10.1016/B978-0-12-384730-0.00009-4

Gray, D. A., Dugar, G., Gamba, P., Strahl, H., Jonker, M. J., & Hamoen, L. W. (2019). Extreme slow growth as alternative strategy to survive deep starvation in bacteria. *Nature Communications*, *10*(1), 1–12. https://doi.org/10.1038/s41467-019-08719-8

Grieshaber, S., Grieshaber, N., Yang, H., Baxter, B., Hackstadt, T., & Omsland, A. (2018). Impact of active metabolism on *Chlamydia trachomatis* elementary body transcript profile and infectivity. *Journal of Bacteriology*, *200*(14), e00065-18. https://doi.org/10.1128/JB.00065-18

Hageman, J. H., Shankweiler, G. W., Wall, P. R., Franich, K., McCowan, G. W., Cauble, S. M., Grajeda, J., & Quinones, C. (1984). Single, chemically defined sporulation medium for *Bacillus subtilis*: Growth, sporulation, and extracellular protease production. *Journal of Bacteriology*, *160*(1), 438–441. https://doi.org/10.1128/jb.160.1.438-441.1984

Harwood, C. S., & Canale-Parola, E. (1984). Ecology of spirochetes. *Annual Review of Microbiology*, *38*, 161–192. https://doi.org/10.1146/annurev.mi.38.100184.001113

Herlemann, D. P. R., Labrenz, M., Jürgens, K., Bertilsson, S., Waniek, J. J., & Andersson, A. F. (2011). Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea. *ISME Journal*, *5*(10), 1571–1579. https://doi.org/10.1038/ismej.2011.41

Hice, S. A., Santoscoy, M. C., Soupir, M. L., & Cademartiri, R. (2018). Distinguishing between metabolically active and dormant bacteria on paper. *Applied Microbiology and Biotechnology*, *102*(1), 367–375. https://doi.org/10.1007/s00253-017-8604-y

Higgins, D., & Dworkin, J. (2012). Recent progress in *Bacillus subtilis* sporulation. *FEMS Microbiology Reviews*, *36*(1), 131–148. https://doi.org/10.1111/j.1574-6976.2011.00310.x

Hoare, A., Timms, P., Bavoil, P. M., & Wilson, D. P. (2008). Spatial constraints within the chlamydial host cell inclusion predict interrupted development and persistence. *BMC Microbiology*, *8*, 1–9. https://doi.org/10.1186/1471-2180-8-5

Jones, D. C., Arthur, B., Nagy, T., Mattriks, Gowda, S., Godisemo, Holy, T., Noack, A., Sengupta, A., Darakananda, D., Adam, B., Dunning, I., Leblanc, S., Huijzer, R., Fischer, K., Chudzicki, D., Piibeleht, M., Mellnik, A., Kleinschmidt, D., … Saba, E. (2021). GiovineItalia/Gadfly.jl: v1.3.4 (v1.3.4). Zenodo. https://doi.org/10.5281/zenodo.5559613

Julien, B., Kaiser, A. D., & Garza, A. (2000). Spatial control of cell differentiation in *Myxococcus xanthus*. *Proceedings of the National Academy of Sciences of the United States of America*, *97*(16), 9098–9103. https://doi.org/10.1073/pnas.97.16.9098

Kaplan-Levy, R. N., Hadas, O., Summers, M. L., Rücker, J., & Sukenik, A. (2010a). Akinetes: Dormant cells of cyanobacteria. In *Dormancy and resistance in harsh environments* (Vol. *21*, pp. 5–27). Springer. https://doi.org/10.1007/978-3-642-12422-8_2

Kaplan-Levy, R. N., Hadas, O., Summers, M. L., & Sukenik, A. (2010b). Dormancy and resistance in harsh. *Environments*, *21*(November), 189–202. https://doi.org/10.1007/978-3-642-12422-8

Koch, R. (1876). *Die Ätiologie der Milzbrand-Krankheit, begründet auf die Entwicklungsgeschichte des Bacillus Anthracis (1876)* (pp. 19–43). https://doi.org/10.1007/978-3-662-56454-7_2

Korhonen, S., Hokynar, K., Mannonen, L., Paavonen, J., Hiltunen-Back, E., & Puolakkainen, M. (2019). Transcriptional expression of the *ompA*, *cpaf*, *tarp*, and *tox* genes of *Chlamydia trachomatis* clinical isolates at different stages of the developmental cycle. *Microorganisms*, *7*(6), 1–11. https://doi.org/10.3390/microorganisms7060153

Kottel, R. H., Bacon, K., Clutter, D., & White, D. (1975). Coats from *Myxococcus xanthus*: Characterization and synthesis during myxospore differentiation. *Journal of Bacteriology*, *124*(1), 550–557. https://doi.org/10.1128/jb.124.1.550-557.1975

Kozich, J. J., Westcott, S. L., Baxter, N. T., Highlander, S. K., & Schloss, P. D. (2013). Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the miseq illumina sequencing platform. *Applied and Environmental*

Microbiology, *79*(17), 5112–5120. https://doi.org/10.1128/AEM.01043-13

Kumar, K., Mella-Herrera, R. A., & Golden, J. W. (2010). Cyanobacterial heterocysts. *Cold Spring Harbor Perspectives in Biology*, *2*(4), 1–20. https://doi.org/10.1101/cshperspect.a000315

Kumar, M. S., Slud, E. V., Okrah, K., Hicks, S. C., Hannenhalli, S., & Corrada Bravo, H. (2018). Analysis and correction of compositional bias in sparse sequencing count data. *BMC Genomics*, *19*(1), 799. https://doi.org/10.1186/s12864-018-5160-5

Lennon, J. T., & Jones, S. E. (2011). Microbial seed banks: The ecological and evolutionary implications of dormancy. *Nature Reviews Microbiology*, *9*(2), 119–130. https://doi.org/10.1038/nrmicro2504

Logan, N. A., Lebbe, L., Hoste, B., Goris, J., Forsyth, G., Heyndrickx, M., Murray, B. L., Syme, N., Wynn-Williams, D. D., & De Vos, P. (2000). Aerobic endospore-forming bacteria from geothermal environments in northern Victoria land, Antarctica, and Candlemas Island, South Sandwich archipelago, with the proposal of *Bacillus fumarioli* sp. nov. *International Journal of Systematic and Evolutionary Microbiology*, *50*(5), 1741–1753. https://doi.org/10.1099/00207713-50-5-1741

Malwane, S., & Deutch, C. E. (1999). Adaptive characteristics of salt-induced myceloids of *Arthrobacter globiformis*. *Antonie van Leeuwenhoek*, *International Journal of General and Molecular Microbiology*, *75*(4), 335–344. https://doi.org/10.1023/A:1002017105413

Maughan, H., Masel, J., Birky, C. W., & Nicholson, W. L. (2007). The roles of mutation accumulation and selection in loss of sporulation in experimental populations of *Bacillus subtilis*. *Genetics*, *177*(2), 937–948. https://doi.org/10.1534/genetics.107.075663

McMurdie, P. J., & Holmes, S. (2013). Phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One*, *8*(4), e61217. https://doi.org/10.1371/journal.pone.0061217

Mongodin, E. F., Shapir, N., Daugherty, S. C., DeBoy, R. T., Emerson, J. B., Shvartzbeyn, A., Radune, D., Vamathevan, J., Riggs, F., Grinberg, V., Khouri, H., Wackett, L. P., Nelson, K. E., & Sadowsky, M. J. (2006). Secrets of soil survival revealed by the genome sequence of *Arthrobacter aurescens* TC1. *PLoS Genetics*, *2*(12), 2094–2106. https://doi.org/10.1371/journal.pgen.0020214

Nicholson, W. L., Munakata, N., Horneck, G., Melosh, H. J., & Setlow, P. (2000). Resistance of bacillus endospores to extreme terrestrial and extraterrestrial environments. *Microbiology and Molecular Biology Reviews*, *64*(3), 548–572. https://doi.org/10.1128/mmbr.64.3.548-572.2000

Noguchi, H. (1912). Cultivation of *Spirochcta gallinarum*. *Journal of Experimental Medicine*, *16*, 620–629.

Norris, M. H., Zincke, D., Leiser, O. P., Kreuzer, H., Hadfied, T. L., & Blackburn, J. K. (2020). Laboratory strains of *Bacillus anthracis* lose their ability to rapidly grow and sporulate compared to wildlife outbreak strains. *PLoS One*, *15*(1), 1–11. https://doi.org/10.1371/journal.pone.0228270

Ochi, K. (1986). A decrease in GTP content is associated with aerial mycelium formation in *Streptomyces* MA406-A-1. *Journal of General Microbiology*, *132*(2), 299–305. https://doi.org/10.1099/00221287-132-2-299

Oksanen, A. J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., Mcglinn, D., Minchin, P. R., Hara, R. B. O., Simpson, G. L., Solymos, P., Stevens, M. H. H., & Szoecs, E. (2020). *Package 'vegan.'*

Peberdy, J. F. (1980). Spore formation in bacteria. In *Developmental microbiology* (pp. 88–114). Springer US. https://doi.org/10.1007/978-1-4613-3927-4_5

Perez, R., Forchhammer, K., Salerno, G., & Maldener, I. (2016). Clear differences in metabolic and morphological adaptations of akinetes of two Nostocales living in different habitats. *Microbiology*, *162*(2), 214–223. https://doi.org/10.1099/mic.0.000230

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., & Glöckner, F. O. (2013). The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Research*, *41*(D1), 590–596. https://doi.org/10.1093/nar/gks1219

Ritchie, M. E., Phipson, B., Wu, D., Hu, Y., Law, C. W., Shi, W., & Smyth, G. K. (2015). *limma* powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Research*, *43*(7), e47. https://doi.org/10.1093/nar/gkv007

Roszak, D. B., & Colwell, R. R. (1987). Survival strategies of bacteria in the natural environment. *Microbiological Reviews*, *51*(3), 365–379. https://pubmed.ncbi.nlm.nih.gov/3312987

Sanford, R. A., Cole, J. R., & Tiedje, J. M. (2002). Characterization and description of *Anaeromyxobacter dehalogenans* gen. nov., sp. nov., an aryl-halorespiring facultative anaerobic myxobacterium. *Applied and Environmental Microbiology*, *68*(2), 893–900. https://doi.org/10.1128/AEM.68.2.893-900.2002

Sauvain, L., Bueche, M., Junier, T., Masson, M., Wunderlin, T., Kohler-Milleret, R., Gascon Diez, E., Loizeau, J. L., Tercier-Waeber, M. L., & Junier, P. (2014). Bacterial communities in trace metal contaminated lake sediments are dominated by endospore-forming bacteria. *Aquatic Sciences*, *76*(S1), 33–46. https://doi.org/10.1007/s00027-013-0313-8

Schloss, P. D., Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B., Lesniewski, R. A., Oakley, B. B., Parks, D. H., Robinson, C. J., Sahl, J. W., Stres, B., Thallinger, G. G., Van Horn, D. J., & Weber, C. F. (2009). Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology*, *75*(23), 7537–7541. https://doi.org/10.1128/AEM.01541-09

Shoemaker, W. R., & Lennon, J. T. (2018). Evolution with a seed bank: The population genetic consequences of microbial dormancy. *Evolutionary Applications*, *11*(1), 60–75. https://doi.org/10.1111/eva.12557

Socolofsky, M. D., & Wyss, O. (1961). Cysts of *Azotobacter*. *Journal of Bacteriology*, *81*(6), 946–954. https://doi.org/10.1128/JB.81.6.946-954.1961

Staley, J. T., & Konopka, A. (1985). Measurement of in situ activities of nonphotosynthetic microorganisms in aquatic and terrestrial habitats. *Annual Review of Microbiology*, *39*(1), 321–346. https://doi.org/10.1146/annurev.mi.39.100185.001541

Steen, A. D., Crits-Christoph, A., Carini, P., DeAngelis, K. M., Fierer, N., Lloyd, K. G., & Cameron Thrash, J. (2019). High proportions of bacteria and archaea across most biomes remain uncultured. *The ISME Journal*, *13*(12), 3126–3130. https://doi.org/10.1038/s41396-019-0484-y

Taylor-Brown, A., Vaughan, L., Greub, G., Timms, P., & Polkinghorne, A. (2015). Twenty years of research into chlamydia-like organisms: A revolution in our understanding of the biology and pathogenicity of members of the phylum Chlamydiae. *Pathogens and Disease*, *73*(1), 1–15. https://doi.org/10.1093/femspd/ftu009

Team, R. C. (2014). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Thomson, J. G., & Thomson, D. T. (1914). Some researches on spirochetes occurring in the alimentary tract of man and some of the lower animals. *The Royal Society of Medicine*, *3*, 47–70.

Venables, W. N., & Ripley, B. D. (2013). *Modern applied statistics with S-PLUS*. Springer Science & Business Media.

Wang, Q., Xie, S., & Hu, R. (2013). Bioaugmentation with *Arthrobacter* sp. strain DAT1 for remediation of heavily atrazine-contaminated soil. *International Biodeterioration and Biodegradation*, *77*, 63–67. https://doi.org/10.1016/j.ibiod.2012.11.003

Westcott, S. L., & Schloss, P. D. (2017). OptiClust, an improved method for assigning amplicon-based sequence data to operational

taxonomic units. *MSphere*, *2*(2), 1–11. https://doi.org/10.1128/mspheredirect.00073-17

Wood, T. K., Knabel, S. J., & Kwan, B. W. (2013). Bacterial persister cell formation and dormancy. *Applied and Environmental Microbiology*, *79*(23), 7116–7121. https://doi.org/10.1128/AEM.02636-13

Wunderlin, T., Corella, J. P., Junier, T., Bueche, M., Loizeau, J. L., Girardclos, S., & Junier, P. (2014). Endospore-forming bacteria as new proxies to assess impact of eutrophication in Lake Geneva (Switzerland-France). *Aquatic Sciences*, *76*(S1), 103–116. https://doi.org/10.1007/s00027-013-0329-0

Wunderlin, T., Junier, T., Paul, C., Jeanneret, N., & Junier, P. (2016). Physical isolation of endospores from environmental samples by targeted lysis of vegetative cells. *Journal of Visualized Experiments*, *107*, 1–7. https://doi.org/10.3791/53411

Wunderlin, T., Junier, T., Roussel-Delif, L., Jeanneret, N., & Junier, P. (2013). Stage 0 sporulation gene a as a molecular marker to study diversity of endospore-forming firmicutes. *Environmental Microbiology Reports*, *5*(6), 911–924. https://doi.org/10.1111/1758-2229.12094

Yang, D. C., Blair, K. M., & Salama, N. R. (2016). Staying in shape: The impact of cell shape on bacterial survival in diverse environments. *Microbiology and Molecular Biology Reviews*, *80*(1), 187–203. https://doi.org/10.1128/mmbr.00031-15

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