Title: Site-Directed Mutagenesis of the 1,3-β-Glucan Synthase Catalytic Subunit of lt;igt;Pneumocystis jiroveciilt;/igt; and Susceptibility Assays Suggest Its Sensitivity to Caspofungin.

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Site-directed mutagenesis of the 1,3-β glucan synthase catalytic subunit of *Pneumocystis jirovecii* and susceptibility assays suggest its sensitivity to caspofungin

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Running title: Sensitivity of the *P. jirovecii* Gsc1 to caspofungin

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Abstract (~~248~~ 252 words)

The echinocandin caspofungin inhibits the catalytic subunit Gsc1 of the enzymatic complex synthetizing 1,3-β glucan, an essential compound of the fungal wall. Studies in rodents showed that caspofungin is effective against *Pneumocystis* asci. However, its efficacy against asci of *Pneumocystis jirovecii,* the species infecting exclusively humans, remains controversial. The aim of this study was to assess the sensitivity to caspofungin of the *P. jirovecii* Gsc1 subunit*,* as well as of those of *Pneumocystis carinii* and *Pneumocystis murina* infecting respectively rats and mice. In absence of an established *in vitro* culture method for *Pneumocystis* species*,* we used functional complementation of the *Saccharomyces cerevisiae gsc1* deletant. In the fungal pathogen *Candida albicans,* mutations leading to amino acid substitutions in Gsc1 confer resistance to caspofungin. We introduced the corresponding mutations into the *Pneumocystis gsc1* genes using site-directed mutagenesis. In spot dilution tests, the sensitivity to caspofungin of the complemented strains decreased with the number of mutations introduced, suggesting that the wild-type enzymes are sensitive. The minimum inhibitory concentrations of caspofungin determined by E-test® and Yeastone® for strains complemented with *Pneumocystis* enzymes (respectively 0.125 and 0.12 microg/ml) were identical to those upon complementation with the enzyme of *C. albicans* for which caspofungin presents low MICs. However, they were lower than the MICs upon complementation with the enzyme of the resistant species *Candida parapsilosis* (0.19 and 0.25). Sensitivity levels of Gsc1 enzymes of the three *Pneumocystis* species were similar. Our results suggest that *P. jirovecii* is sensitive to caspofungin during infections, as *P. carinii* and *P. murina*.

Keywords: echinocandins, drug sensitivity, drug resistance, heterologous functional complementation.

Main text: 3554 words

INTRODUCTION

The *Pneumocystis* genus comprises fungal species that colonize the lungs of mammals (1-4). Each of them displays strict host specificity for a single mammalian species. The species infecting humans is *Pneumocystis jirovecii,* an opportunistic pathogen that can cause fatal pneumonia [*Pneumocystis* pneumonia (PCP)] if not treated. The most effective drug against *P. jirovecii* is currently cotrimoxazole, a combination of sulfamethoxazole and trimethoprim, two inhibitors of enzymes that are involved in the folic acid biosynthesis pathway. However, potential resistance is emerging due to the selection of strains carrying specific mutations in the active site of the targets of both molecules (5-9). Moreover, cotrimoxazole can cause important side effects in some patients, such as intolerance and toxicity. For these reasons, it is crucial to find new drugs to treat PCP.

Echinocandins constitute an alternative class of antifungal drugs to consider for the treatment of PCP. This class includes caspofungin (CAS), anidulafungin, and micafungin. They are cyclic hexapeptides with fatty acyl side chains and act as non-competitive inhibitors of the catalytic subunit Gsc1 of the 1,3-β glucan synthase enzymatic complex (10). The decrease of the β-glucan synthesis results in the loss of cell integrity and rigidity that can lead to cell lysis. β-glucan molecules are components of the cell wall that are homopolymers of β-1,3 linked D- glucose with β -1,6 linked D-glucose side chains present in minority. The Gsc1 protein of *Pneumocystis carinii*, the species infecting rats, was first reported to be inhibited by the compound L-733,560, a molecule structurally close to echinocandins (11), but never used clinically. More recently, Cushion *et al*. (12) reported the efficacy of the echinocandins

(caspofungin, micafungin, and anidulafungin) in reducing cysts number within the lungs of the host. Recently, we identified and functionally ascertained the function of the Gsc1 subunit of *P. jirovecii* using complementation of the orthologous gene of *Saccharomyces cerevisiae* (13). The presence of a unique *gsc1* gene in the genome of *P. jirovecii,* as in that of *P. carinii*, further suggests that the Gsc1 subunit is a potential interesting drug target to fight PCP.

In *S. cerevisiae*, the 1,3-β glucan synthase catalytic subunit is encoded by two different genes, *GSC1* and *GSC2*. A third paralog, *GSC3*, is also present but it is involved only during sporulation. The two subunits *GSC1* and *GSC2* are functionally redundant, but their expression is differentially regulated. The expression of *GSC1* is constitutive and responsible for the cell wall synthesis during the vegetative growth, while that of *GSC2* is induced by glucose deprivation or pheromones and is also involved in cell wall synthesis during sporulation. *GSC1* and *GSC2* genes have an essential overlapping function, *i.e.*, only disruption of both genes is lethal. Importantly, the *GSC2* gene can replace the function of the *GSC1* gene during vegetative growth in the case of loss by mutation or deletion (14). The *S. cerevisiae* strain with a deletion of the *GSC1* gene shows a reduced and impaired growth in presence of CAS (15) or anidulafungin (16), but not of micafungin (16). On the other hand, the *S. cerevisiae* wild type shows a normal growth in presence of low doses of CAS and anidulafungin, but its growth is severely impaired in presence of micafungin. These observations showed that the *S. cerevisiae* Gsc1 and Gsc2 subunits have different sensitivities to each echinocandin despite that their identity at the amino acids sequence level is as high as 87% over the whole protein, with 81 and 94% identity at the level of the 1,3-β glucan synthase domains 1 and 2, respectively (Table 1). To our knowledge, the polymorphisms responsible for these different sensitivity have not been determined so far.

Spontaneous mutants resistant to echinocandins were initially isolated in *S. cerevisiae* and *Candida albicans* (17-19). Rare clinical isolates of *C. albicans* were also found to be resistant (20, 21). A specific substitution of a serine in position 645 to a proline (S645P) was identified in all spontaneous and most clinical resistant *C. albicans* isolates (21). It is localized within a highly conserved region of the Gsc1 protein in which other mutations conferring resistance to CAS were also identified in *C. albicans* (21). This "Hot Spot no. 1" of mutations starts at residue 641 and ends at residue 649 of *C. albicans* Gsc1. A second but less relevant hotspot of mutations conferring resistance has been identified in another region of the enzyme, from residue 1357 to residue 1364. The S645P substitution has been most frequently observed, a phenylalanine to serine substitution in position 641 (F641S) being the second most frequent substitution (22).The mutation corresponding to the *C. albicans* S645P substitution introduced by site-directed mutagenesis was found to confer reduced susceptibility to CAS *in vitro* to the mould *Aspergillus fumigatus* (23, 24).

Although demonstrated to reduce efficiently the asci during *P. carinii* and *P. murina* infections (10, 12, 25-28), the efficacy of CAS against *P. jirovecii* remains controversial. Indeed, clinical reports documented the clearance of PCP treated with CAS alone (29-31), or used in combination with cotrimoxazole (32-36) or clindamycin (37). However, failures of CAS treatment were also described (38, 39). Despite the generally high conservation of active sites among orthologous enzymes, one cannot exclude that the sensitivity to CAS may vary among *P. jirovecii* and the two *Pneumocystis* species infecting rodents because these species are relatively distant from each other (20% of mean divergence at nucleotide level in genomic coding sequences, 40). The *P. jirovecii* Gsc1 subunit bears 90 and 91% identity with those of *P. carinii* and *P. murina*,

105 respectively (Table 1). At the level of the 1,3-β glucan synthase domains 1 and 2, *i.e*., the active
106 sites, the identities are from 94 to 97%. These values are comparable to those between the Gsc1
107 and Gsc2 subunits of *S. cerevisiae* (see above), which present drastically different sensitivities to
108 the different echinocandins.

109 The aim of the present study was to determine if the Gsc1 subunit of *P. jirovecii* is sensitive
110 to the echinocandin CAS, as those of *P. carinii* and *P. murina*. To investigate the issue, we analysed
111 the level of sensitivity of *S. cerevisiae* strains functionally complemented by the expression of the
112 wild-type or mutated enzymes of the three *Pneumocystis* species.

MATERIALS AND METHODS

Strains and growth conditions. Y05251 is a *S. cerevisiae* haploid strain in which the 1,3-β glucan synthase catalytic subunit gene *GSC1* (also called *FKS1*) was deleted (*MAT***a** *his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 YLR342w::kanMX4*). It was obtained from Euroscarf (European *S. cerevisiae* Archive for Functional Analysis [http://www.euroscarf.de]). The strain, named the *gsc1* deletant hereafter, exhibits an impaired growth in the presence of low doses of CAS (14). The parental strain of the *gsc1* deletant is BY4741 (*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 uraΔ0*), and was also obtained from Euroscarf (hereafter named wild-type, WT). The latter was used as a control in the sensitivity tests and in MIC assays. Strains were grown on complete yeast extract-peptone-dextrose (YEPD) medium (1% wt/vol Difco yeast extract, 2% DIfco peptone, 2% glucose).

Single colonies of *Candida albicans* (ATCC 10231) and *Candida parapsilosis* (*sensu stricto*, *i.e.,* group I of the *C. parapsilosis* complex; ATCC 22019) were streaked on Sabouraud medium (0.5% wt/vol casein peptone, 0.5% meat extract peptone, 2% glucose), and then grown on minimal solid yeast nitrogen base (YNB) medium (0.67% wt/vol yeast nitrogen base, 2% glucose, 2% Gibco agar) supplemented with a complete supplement mixture (CSM, MP Biomedicals). *C. albicans* and *C. parapsilosis* were chosen because CAS presents respectively low and high MICs for them.

Cloning of the fungal *gsc1* genes. To identify the *P. murina gsc1* gene, the *P. carinii* Gsc1 protein (ID Q9HEZ4) was used as query sequence in BLASTp search against *P. murina* proteome at http://blast.ncbi.nlm.nih.gov/Blast.cgi. A single putative ortholog was detected (locus tag PNEG_03180). The *P. murina* gene sequence encoding the Gsc1 protein was then retrieved from

the European Nucleotide Archive (http://www.ebi.ac.uk/ena). The cloning of the *P. jirovecii* and *S. cerevisiae gsc1* genes was previously described (13). Since the *P. carinii* and *P. murina gsc1* genes include each three introns, their cDNAs were synthesized and cloned into the p416GPD vector (41) by GeneCust Europe (Ellange Luxemburg). Their size without introns is respectively 5835 bps and 5847 bps.

To perform a control of sensitivity of our heterologous expression model, the *GSC1* genes of *C. albicans* (GenBank D88815) and *C. parapsilosis* (EU221325) were amplified by PCR from yeast genomic DNA extracted as described previously (42). The detailed procedures of PCR amplification using the proofreading high-fidelity Expand polymerase (Roche Diagnostics) and cloning were described previously (43). Their sizes are respectively 5694 and 5730 pbs. PCR primers and conditions are listed in Tables S1 and S2. Because these primers were intended for oriented cloning, they were designed to create unique restriction sites at ends of the PCR products. After the PCR reactions, the products were extracted using the QIAquick gel extraction kit (Qiagen, Basel, Switzerland). For cloning each *Candida GSC1* gene into the p416GPD expression vector, the double restriction described in Table S1 were used.

Site-directed mutagenesis. The Gsc1 protein sequences of *C. albicans* (UniProt ID O13428), *S. cerevisiae* (P38631), *P. jirovecii* (L0PD34, locus tag PNEJI1_001061), *P. carinii* (Q9HEZ4), *P. murina* (M7P3D9, locus tag PNEG_03180), and *C. parapsilosis* (A9YLC3) were aligned using T-Coffee (44). This alignment allowed determining the positions within the *Pneumocystis* genes corresponding to the mutations F641S and S645P conferring resistance to CAS in *C. albicans* (Fig. 2; alignment of the complete proteins is shown in Fig. S1). To perform site-directed mutagenesis, two different

kits were used. The QuikChange II XL Site-Directed Mutagenesis Kit (Agilent Technologies) was used to create the mutation in the *P. jirovecii gsc1* gene leading to the substitution of the serine at position 718 of the Gsc1 protein into a proline (S718P). The Q5 Site-Directed Mutagenesis Kit (BioLabs) was used to introduce the F714S/S718P double substitution in *P. jirovecii*, the S715P substitution in *P. carinii*, and the S719P substitution in *P. murina*. Mutagenesis were performed according to manufacturers' instructions. Minipreparations of plasmid DNA were subsequently carried out (45). In order to verify the presence of the desired mutations, an internal segment of the *gsc1* genes was amplified and subsequently sequenced. Primers for mutagenic reactions and PCR amplifications are listed in Table S1. Mutagenesis amplification reactions and PCR conditions are described in Table S2. Sequencing of both strands was performed using to the two primers used for amplification, as well as the BigDye Terminator DNA sequencing kit and ABI PRISM 3100 automated sequencer (both from PerkinElmer Biosystems).

Transformation of the *S. cerevisiae gsc1* deletant. Transformation with plasmids containing the *P. jirovecii gsc1* or the *S. cerevisiae GSC1* gene were previously described (13). The *S. cerevisiae GSC1* gene could not be cloned in the p416GPD plasmid because of restriction sites issues, but into p415GPD (leu marker instead of ura). The recombinant p416GPD plasmids containing the *Pneumocystis* mutated *gsc1* alleles, as well as the *C. albicans* or *C. parapsilosis GSC1* gene were introduced into the *gsc1* deletant by transformation for uracil prototrophy using the one-step method (46). Transformants were selected on solid YNB medium supplemented with CSM (MP Biomedicals) lacking uracil. In order to be used as controls in the sensitivity tests and in the MIC assays, the *gsc1* deletant and the WT were transformed with the empty p416GPD plasmid. Three

179 transformants clones of each constructed strain were randomly chosen and purified by growth
180 on the same selective medium.
181
182 **Test of complementation and susceptibility to caspofungin.** Before studying the sensitivity to
183 echinocandins, we had to assess the function of the *P. carinii* and the *P. murina gsc1* genes, as
184 we previously carried out for the *P. jirovecii gsc1* gene (13). Functional complementation of the
185 *gsc1* deletant was proven by the spot dilution test on YNB selective medium lacking uracil and
186 supplemented with or without 150 ng/ml CAS (Fluka, Chemie AG). CAS appeared sensitive to
187 temperature variation in our hands. Consequently, the medium was cooled down to 54°C before
188 adding CAS. In addition, Petri dishes containing CAS were stored at room temperature, but not
189 at 4°C. The concentration of CAS used in our experiments was selected after several trials. To that
190 aim, transformant isolates carrying the *P. carinii gsc1* or *P. murina gsc1* gene were grown
191 overnight in selective medium YNB supplemented with CSM lacking uracil to avoid the loss of the
192 plasmid. Cells were then diluted at an optical density (OD) at 540 nm of 0.1 in NaCl 0.9% wt/vol
193 (ca. 7.5 x 10^5 cells/ml). Four serial 10-fold dilutions in NaCl 0.9% were prepared, and 3 µl of each
194 dilution were spotted on the medium. Spots were observed after 3 to 4 days of incubation at
195 30°C. The same procedure assessed the functionality and sensitivity to CAS of the strains
196 complemented with the mutated *gsc1* alleles. The sensitivity to micafungin could not be studied
197 because the *gsc1* deletant had no phenotype to complement on this drug, *i.e.*, the Gsc2 subunit
198 is resistant. Anidulafungin could also not be studied because, for underdetermined reasons, the
199 results were not reproducible in our hands.
200

MIC assessment using E-test®. Assays were performed according to manufacturer's instructions. Each strain was grown overnight in selective medium YNB + CSM lacking uracil, or leucine for *S. cerevisiae GSC1* gene, and then adjusted in NaCl 0.9 % to an OD540=0.2 (~1.5 x 10^6 cells/ml). One hundred microliters of this dilution was spread on fresh YNB solid medium + CSM lacking uracil or leucine. A single strip of E-test caspofungin (Biomérieux) was then applied on each petri dish. MICs were read after 2 days of incubation at 30°C, or 35°C for the *Candida* species. The MIC was defined as the concentration at which no growth was observed on both sides of the E-test strip.

MIC assessment using Sensititre YeastOne®. Assays were performed according to manufacturer's instructions, except that the incubation time was increased from 24 to 48 h for all *S. cerevisiae* strains because of their low growth rate. Each strain was grown overnight in selective medium YNB, then adjusted in NaCl 0.9 % to an OD540=0.2 (ca. 1.5 x 10^6 cells/ml). Twenty microliters of this dilution were then diluted into 11 ml of YeastOne® inoculum broth in order to obtain ca. 3 x 10^3 cells/ml. One hundred microliters was then transferred into each well of a YeastOne® plate (Thermofisher Scientific). Plates were observed and MICs determined after 24 or 48 h of incubation at 30°C, or 35°C for the *Candida* species. MIC was defined as the first well in which no pellet of cells was observable.

RESULTS

Functional ascertainment of the *P. carinii* and *P. murina gsc1* genes by complementation of the *S. cerevisiae gsc1* deletant. We identified a single Gsc1 protein within the *P. murina* proteome by a homology search using the Gsc1 protein of *P. carinii* as the query sequence. To ascertain the function of the *P. carinii* and *P. murina gsc1* genes, recombinant plasmids expressing them were introduced into the *S. cerevisiae gsc1* deletant. The identities of the Gsc1 proteins studied relatively to that of *S. cerevisiae* are given in Table S3. Serial dilution of the transformed strains were spotted on medium containing or not CAS (spot dilution test, Fig. 1). The deletion of the *GSC1* gene in *S. cerevisiae* causes a paradoxical effect, i.e., an increased susceptibility to CAS, though the target of CAS is absent (15). This is due to the replacement of Gsc1 by Gsc2, an enzyme that is more sensitive to CAS (14). On the other hand, this replacement allows the growth of the deletant strain on medium without CAS. On medium supplemented with CAS, a complete restoration of the wild-type growth was observed in presence of the control *S. cerevisiae GSC1* gene, but not in the presence of the empty vector (*S. cerevisiae gsc1* deletant + empty plasmid, Fig. 1). A partial restoration was observed in the presence of the *P. carinii* or *P. murina* gene, as we previously reported for *P. jirovecii* and reproduced here (Fig. 1). These observations demonstrated that the expression of *P. carinii* and *P. murina gsc1* genes rescued the function of the deleted *S. cerevisiae GSC1* gene, ascertaining their function. In order to investigate the sensitivity to CAS of the three *Pneumocystis* enzymes, we used site-directed mutagenesis to introduce mutations that correspond to those conferring resistance in other fungi.

Sensitivity to CAS of the *S. cerevisiae* strains complemented with the *Pneumocystis* Gsc1 mutated proteins. Mutants resistant to echinocandins carrying mutations F641S and S645P within the hotspot no. 1 of Gsc1 have been described in the pathogenic fungus *C. albicans* (17-21). The sequences of this hotspot of mutations of the *P. jirovecii*, *P. carinii*, and *P. murina* Gsc1 protein were aligned with those of *C. albicans*, *S. cerevisiae*, and *C. parapsilosis* (Fig. 2; alignment of the complete proteins is shown in Fig. S1). This alignment identified the positions in the three *Pneumocystis gsc1* genes corresponding to the *C. albicans* F641S and S645P substitutions. Site-directed mutagenesis was used to introduce one or two mutations encoding the corresponding substitutions within the *gsc1* gene of *P. jirovecii*, *P. carinii*, or *P. murina* (the polymorphisms introduced at the nucleotide sequence level are described in Table S1).

The partial restoration of the wild-type growth on CAS observed with the *P. jirovecii* Gsc1 enzyme increased in presence of one mutation (S718P compared to wild-type, Fig. 1), and increased more upon introduction of the two mutations simultaneously (S718P + F714S compared to S718P and wild-type). Similarly, the partial restoration with the *P. carinii* or *P. murina* enzyme increased in presence of a single mutation (S715P and S719P compared to their respective wild-type). This increase of complementation efficiency corresponds to a decrease of sensitivity to CAS. This demonstrated that the three wild-type *Pneumocystis* enzymes present a certain level of sensitivity to CAS.

Minimum inhibitory concentration (MIC) assessment using E-test® and Sensititre YeastOne®. We determined the MICs of CAS for the *S. cerevisiae* WT and complemented *gsc1* deletant

strains. To assess the sensitivity of the two methods, we also analysed *C. albicans* and *C. parapsilosis,* as well as the *S. cerevisiae gsc1* deletant complemented with the *GSC1* gene of these two *Candida* species. CAS presents low MICs for the former *Candida* species, whereas it presents high MICs for the latter (47, 48). The natural high MICs for *C. parapsilosis* are due to a polymorphism at the end of the hotspot no. 1 that has not been observed in *C. albicans* so far (49; proline to serine in position 660, Fig. 2). According to Espinel-Ingroff et al (47) and Canton et al (48), the MICs of CAS for *C. parapsilosis* that we obtained were below the epidemiological cutoff values for both E-test and YeastOne methods (0.5 versus 4 and 0.5 versus 2 μg/ml, respectively). The MICs for *C. albicans* we obtained were also below the epidemiological cutoff values of wild-type isolates (0.38 versus 0.5 and 0.12 versus 0.25 μg/ml, respectively). In agreement with the spot dilution tests described here above, we observed using both E-test® and YeastOne® a decreased MIC for the *S. cerevisiae gsc1* deletant compared to the WT (respectively 0.125 and 0.12 versus 0.250 and 0.25 μg/ml; Table 2; the E-test results are shown in Fig. S3). All *S. cerevisiae* strains complemented with the *Pneumocystis* wild-type or mutated genes had MICs identical to those of the *gsc1* deletant (0.125 for E-test and 0.12 for Yeastone). The increase of MIC of CAS conferred by the mutations introduced was not detected using E-test or YeastOne. Thus, these methods are less sensitive than the spot dilution test used above since the latter always allowed detection of this decrease in several experiments. The MIC values for *C. albicans* whole cells using E-test® and YeastOne® were similar to those for the *S. cerevisiae* WT strain (0.380 and 0.12 versus 0.250 and 0.25), whereas, consistently with its reported high MICs, *C. parapsilosis* had higher MICs also in our hands using both methods (0.500 and 0.50). The increased MICs for *C. parapsilosis* was also detected using both methods upon heterologous

expression of its Gsc1 subunit in *S. cerevisiae* (respectively 0.190 and 0.25 versus 0.125 and 0.12 for *C. albicans* Gsc1), despite that MICs were systematically lower using heterologous expression than whole cells. Using the heterologous expression system, the wild-type *Pneumocystis* Gsc1 subunits had identical MICs than the *C. albicans* Gsc1 (0.125 and 0.12), whereas *C. parapsilosis* Gsc1 presented higher MICs (0.190 and 0.25). These observations suggested that the sensitivity level to CAS of the three *Pneumocystis* enzymes is similar to that of *C. albicans*, which presents low MICs of CAS.

DISCUSSION

Because of the absence of an *in vitro* culture method, sensitivity to CAS cannot be performed directly on whole *Pneumocystis* cells. A study reported the effects of echinocandins against *P. murina* and *P. carinii* using suspension and biofilm culture methods (50). Unfortunately, these methods are not established for *P. jirovecii.* Consequently, we studied the Gsc1 enzymes of three *Pneumocystis* species in the heterologous system of expression of *S. cerevisiae*. We used site-directed mutagenesis to introduce into the *Pneumocystis* enzymes the substitutions corresponding to those conferring resistance to CAS in *C. albicans*. This revealed that, despite the divergence among their active sites, the three *Pneumocystis* Gsc1 enzymes present low MICs of CAS, and this to a similar level. Because CAS has been demonstrated to be effective in reducing *P. carinii* and *P. murina* asci during infections (12, 27), this observation suggested that CAS could also be effective against *P. jirovecii*. Moreover, MICs determination showed that the level of sensitivity of *Pneumocystis* Gsc1 was similar to that of the *C. albicans* enzyme, suggesting that

303 the sensitivity of the *Pneumocystis* enzymes is at a level that is usable clinically. It is of course
304 difficult to translate our results obtained at the enzyme level to the whole cell level. Nevertheless,
305 Gsc1 is a cell surface enzyme that is easily reachable by drugs, and thus more likely to behave
306 similarly among the three *Pneumocystis* species. A structural difference of the cell wall could
307 induce varying sensitivity to CAS of the Gsc1 subunit among the three *Pneumocystis* species.
308 However, there is presently no obvious reasons to think that the wall of *P. jirovecii* is different
309 from those of *P. carinii* and *P. murina*. The efficacy of echinocandins, and specifically of CAS, to
310 treat *P. jirovecii* infections remain controversial, and accordingly the American drug and
311 European medical agencies do not advise their use for that purpose. Our results bring new
312 arguments in favor of the use of this class of antifungals for the treatment of PCP, suggesting the
313 need to implement clinical trials in humans. Finally, our results support the high relevance of the
314 animal models as tools to understand the effect of CAS on the human pathogen *P. jirovecii*.

315 Studies in animal models showed that echinocandins provoke the disappearance of *P.*
316 *carinii* and *P. murina* asci but not of the trophic forms, probably because the latter cells have no
317 or little cell wall made of 1,3-β glucan (12). Thus, the treatment did not eradicate the infection,
318 and its cessation resulted in the repopulation in asci from the remaining trophic cells.
319 Consequently, it is likely that CAS is useful only if used in combination with another therapy
320 targeting trophic forms, or both cellular forms such as cotrimoxazole. CAS inhibited efficiently
321 the dissemination of the pathogen in animal models (12), which is consistent with the fact that
322 asci are believed to be the transmission particles (12, 50).

323 In conclusion, our results demonstrate that the human pathogen *P. jirovecii* Gsc1 enzyme
324 is sensitive to caspofungin, similarly to the enzymes of the animal pathogens *P. carinii* and *P.*

325 *murina.* This suggests that echinocandins might be a good alternative to treat PCP in humans
326 when used in combination with an established treatment. The use of echinocandins to fight
327 *Pneumocystis* infections deserves further investigation.

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LEGENDS

FIG 1. Sensitivity to caspofungin (CAS) of *S. cerevisiae* WT and functionally complemented *gsc1* deletant strains using the spot dilution test. The complementing genes expressed on plasmid, wild-type or encoding the indicated amino acid substitution, are described on the left. The growth of the *S. cerevisiae* deletant strain on medium without CAS is due to the activity of the orthologous gene *GSC2*, that replaces the function of the deleted *GSC1* gene. Log dilution of a suspension of cells at ca. 7.5 x10^5/ml were spotted on minimal selective medium without (left) or with (right) 150 ng/ml of CAS, and incubated for 3 days at 30°C. The most concentrated suspension is on the left. The complementing gene was expressed on plasmid p416GPD except that of *S. cerevisiae* on p415GPD because of restriction sites issues. Minimal selective medium YNB supplemented with CSM without uracil was used to select for p416GPD, while YNB supplemented with CSM without leucin was used to select p415GPD. Three independent isolates of each strain were analyzed, one representative isolate is shown here.

FIG 2. Multiple-sequence alignment of the region encompassing the hot spot no. 1 of mutations of Gsc1 proteins. T-Coffee was used (44). The identical, strongly, and weakly conserved residues are indicated by asterisks, double points, and single points, respectively. Dashes indicate gaps. The hot spot no. 1 of mutations (21) is shown above the alignment by the dashed line. Residues F641 and S645 conferring CAS resistance in *C. albicans* and the corresponding residues in the other proteins are in bold. The natural resistance of *C. parapsilosis* is due to the polymorphism

499 P660A at the end of the same hotspot of mutations. The alignment of the complete proteins is

500 show in Figure S1.

501 ## SUPPLEMENTAL MATERIAL

502 **Table S1.** Oligonucleotide primers used for mutagenesis reactions and PCR amplifications
503
504 **Table S2.** Conditions of mutagenesis and PCR reactions
505
506 **Table S3.** Sequence identity (%) of the *S. cerevisiae* Gsc1 protein to the ortholog proteins studied
507 in the complementation assays and MIC determinations
508

509 **Fig. S1.** Multiple sequence alignment of Gsc1 proteins of relevant fungi. T-Coffee was used (44).
510 The identical, strongly, and weakly conserved residues are indicated by asterisks, double points,
511 and single points, respectively. Dashes indicate gaps. The hot spots no. 1 (21) and 2 (49) of
512 mutations, as well as the 1,3-β glucan synthase domains 1 and 2, are indicated above the
513 alignment. Residues F641 and S645 conferring CAS resistance in *C. albicans* and the
514 corresponding residues in the other proteins are shown in bold. The natural resistance of *C.*
515 *parapsilosis* is due to a polymorphism at the end of the hotspot no. 1 (P660A).
516

517 **Fig. S2.** Multiple sequence alignment of *S. cerevisiae* Gsc proteins. The Uniprot accession
518 numbers of Gsc1, Gsc2, and Gsc3 are respectively P38631, P40989, and Q04952. T-Coffee was
519 used (44). The identical, strongly, and weakly conserved residues are indicated by asterisks,
520 double points, and single points, respectively. Dashes indicate gaps. The 1,3-β glucan synthase
521 domains 1 and 2 are indicated above the alignment.

Fig S3. E-test determination of the minimum inhibitory concentration (MIC) of caspofungin (CAS) for the *S. cerevisiae* WT and functionally complemented *gsc1* deletant strains, as well as for *Candida* species. One hundred microliters of a suspension of cells at ca. 1.5 x 10^6 cells/ml were spread on minimal selective medium lacking uracil, or lacking leucin for the *S. cerevisiae GSC1* gene. The CAS E-test strip was deposited, and the plate was incubated at 30°C, or 35°C for the *Candida* species. The concentration at which no growth was observed on both sides of the E-test strip was defined as the MIC.

TABLE 1. Sequence identity (%) of Gsc proteins to their orthologs and paralogs

		Whole protein	1,3-β glucan synthase domain 1	1,3-β glucan synthase domain 2
P. jirovecii Gsc1[a] to	*P. carinii* Gsc1[a]	90	94	97
	P. murina Gsc1[a]	91	95	96
	S. cerevisiae Gsc1[a]	59	70	73
S. cerevisiae Gsc1[b] to	*S. cerevisiae* Gsc2[b]	87	81	94
	S. cerevisiae Gsc3[b]	51	57	60

[a] Alignment of these proteins is shown in Figure S1.

[b] Alignment of these proteins is shown in Figure S2.

TABLE 2. Minimum inhibitory concentrations (MIC) of caspofungin (CAS) for the *S. cerevisiae* WT and functionally complemented *gsc1* deletant strains, as well as for *Candida* species [a]

Strain		MIC (μg/ml) E-test	MIC (μg/ml) YeastOne
S. cerevisiae WT + empty plasmid		0.250	0.25
S. cerevisiae gsc1 deletant + empty plasmid		0.125	0.12
S. cerevisiae gsc1 deletant + plasmid +	*S. cerevisiae GSC1*	0.250	0.25
	P. jirovecii gsc1 wild-type	0.125	0.12
	S718P	0.125	0.12
	S718P + F714S	0.125	0.12
	P. carinii gsc1 wild-type	0.125	0.12
	S715P	0.125	0.12
	P. murina gsc1 wild-type	0.125	0.12
	S719P	0.125	0.12
	C. albicans GSC1	0.125	0.12
	C. parapsilosis GSC1	0.190	0.25
C. albicans		0.380	0.12
C. parapsilosis		0.500	0.50

[a] One isolate among three of each complemented strain was chosen randomly for analysis. One out of two experiments that gave similar results is reported here. Although obtained using various methods that have various sensitivities, the MIC values previously published correspond roughly to ours for *S. cerevisiae* WT (0.25 versus 0.03-0.4; 15, 16, 21) and *gsc1* deletant (0.12-0.125 versus 0.0015-0.1; 15, 16, 21), as well as for *C. albicans* (0.12-0.380 versus 0.12-0.25; 22, 47) and *C. parapsilosis* (0.50 versus 0.25-8; 47-49).

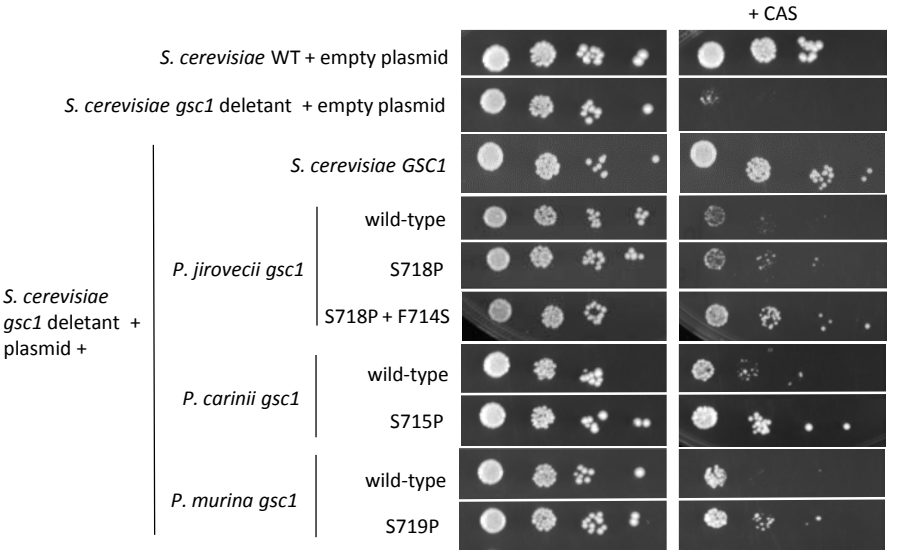

+ CAS
S. cerevisiae WT + empty plasmid
S. cerevisiae gsc1 deletant + empty plasmid
S. cerevisiae GSC1
S. cerevisiae gsc1 deletant + plasmid +
P. jirovecii gsc1
wild-type
S718P
S718P + F714S
P. carinii gsc1
wild-type
S715P
P. murina gsc1
wild-type
S719P

FIG 1.

```
                                                    ---------
C.albicans          611 NYIKLKGLDMWMSYLLWFLVFLAKLVESYFFLTLSLRDPIRNLSTMTMR-CVGEVWYKDIVCRNQAKIVLGL  681
S.cerevisiae        609 AFAPLHGLDRWMSYLVWVTVFAAKYSESYYFLVLSLRDPIRILSTTAMR-CTGEYWWGAVLCKVQPKIVLGL  679
A.fumigatus         644 SFPRLHGNDMWMSYGLWVCVFGAKLAESYFFLTLSFKDPIRILSPMQIHQCAGVKYIGNVLCHKQPQILLGL  715
P.jirovecii         684 NFAKLKGNDLWLSYGLWIAVFACKFAESYFFLSLSLRDPIRYLNTMTIG-HCGIRYLGSILCPYQAKITLGI  754
P.carinii           681 NFAKLKGNDLWLSYGLWIAVFACKFAESYFFLSLSLRDPIRYLNTMTIG-HCGIRYLGSSLCPYQAKITLGI  751
P.murina            685 NFAKLKGNDLWLSYGLWIAVFACKFAESYFFLSLSLRDPIRYLNTMTIG-HCGIRYLGSALCPYQAKITLGI  755
C.parapsilosis      622 NFVKLRGLDMWMSYLLWVLVFLAKLVESYFFLTLSLRDAIRNLSKTTMR-CTGEVWYGDIVCRQQAKIVLGL  692
                         :  *:* * *:** :*. ** .*  ***:** **::*.** *.   :    *  :    :*  *.:* **:
```

FIG 2.

TABLE S1. Oligonucleotide primers used for mutagenesis reactions and PCR amplifications [a]

Experiment	Target [b]	Primer	5'-3' nucleotide sequence	Fragment amplified size (bp)	Description
S718P single substitution introduction	*Pj gsc1*	Pjgsc1t2152cStart Pjgsc1t2152cEnd	TAGGATCTCGCAGAG**G**AAGAGATAGAAAAAAGTATGATTCTGCA [c, d] TGCAGAATCATACTTTTTTCTATCTCTT**C**CTCTGCGAGATCCTA [b, c]	11564	*Pj gsc1* position 2124 to 2167 containing the T2152C substitution (S718P in the protein)
S718P + F714S substitutions introduction	*Pj gsc1*	Pjgsc1t2141c/t2152cStart Pjgsc1t2141c/t2152cEnd	CTCTT**C**CTCTGCGAGATCCTATTAG [e] ATAGA**G**AAAAGTATGATTCTGCAAAC [c, e]	11564	*Pj gsc1* position 2147 to 2171 containing the T2152C substitution (S718P in the protein) *Pj gsc1* position 2121 to 2146 containing the T2141C substitution (F714S in the protein)
Control mutagenesis	*Pj gsc1* internal fragment	Pjgsc1control mutation A Pjgsc1control mutation B	CTCCATGGAAACCGAACCTC TATGTTTCTCCAAGGCGTCC	511	*Pj gsc1* position 1880 to 1894 *Pj gsc1* position 2372 to 2391
S715P single substitution introduction	*Pc gsc1*	Pcgsc1t2143cStart Pcgsc1t2143cEnd	CTTGTCTCTT**C**CTTTGAGAGATC [c, e] AAGAAATAGGATTCTGCAAATTTG [e]	11413	*Pc gsc1* position 2133 to 2155 containing the T2143C substitution (S715P in the protein) *Pc gsc1* position 2109 to 2132
Control mutagenesis	*Pc gsc1* internal fragment	Pcgsc1 control mutation A Pcgsc1 control mutation B	TGCTCCATGGAAACCGAATGTTAG TGACCTAGCGACTGAACAAATAG	471	*Pc gsc1* position 1869 to 1892 *Pc gsc1* position 2318 to 2340
S719P single substitution introduction	*Pm gsc1*	Pmgsc1t2155cStart Pmgsc1t2155cEnd	CTTGTCTCTT**C**CTTTGAGAGATC [c, e] AAGAAATAGGATTCAGCAAAC [e]	11625	*Pm gsc1* position 2145 to 2167 containing the T2155C substitution (S719P in the protein) *Pm gsc1* position 2124 to 2144
Control mutagenesis	*Pm gsc1* internal fragment	Pmgsc1 control mutation A Pmgsc1 control mutation B	GGAAACCGAATGTCAGCGGTC GATCTGGCGACAGAACAAATAG	462	*Pm gsc1* position 1889 to 1905 *Pm gsc1* position 2330 to 2351
Gene oriented cloning	*Ca GSC1*	CaGSC1 SmaI start CaGC1 ClaI end	CGCCCCGGGATGTCGTATAACGATAATAATAATC [f] CCCCCCATCGATTTAAACTCTGAATGGATTTGTAG [f]	5715	*Ca GSC1* first 22 nt, SmaI restriction site is underlined *Ca GSC1* last 23 nt, ClaI restriction site is underlined

Gene oriented cloning	*Cp GSC1*	CpGSC1 SpeI start CpGSC1 SalI end	GCGACTAGTATGTCGTATAACGATAACAAC [f] CCCCCCGTCGACTTATCTGAACGCATTCGTAG [f]	5751	*Cp GSC1* first 21 nt, SpeI restriction site is underlined *Cp GSC1* last 20 nt, SalI restriction site is underlined

[a] Primers were synthesized by Microsynth (Balgach, Switzerland).

[b] *Pj, P. jirovecii ; Pc, P. carinii, Pm, P. murina; Ca, C. albicans; Cp, C. parapsilosis.*

[c] Bold residues represent the nucleotide to be substituted.

[d] Designed with the QuikChange Primer Design Program (http://www.agilent.com/genomics/qcpd).

[e] Designed with the NEBaseChanger program (http://nebasechanger.neb.com/).

[f] These primers include the underlined restriction sites for oriented cloning and three or six upstream bases allowing restriction.

TABLE S2. Conditions of mutagenesis and PCR reactions

Experiment	Target[a]	Initial denaturation		Denaturation		Annealing		Elongation		Final extension	
		sec	°C	sec	°C	sec	°C	sec	°C	sec	°C
S718P single substitution introduction[b]	*Pj gsc1*	60	95	50	95	50	60	700	68	420	68
S718P + F714S substitutions introduction[c]	*Pj gsc1*	30	98	10	98	30	56	360	72	120	72
Control mutagenesis[d]	*Pj gsc1* internal fragment	180	94	30	94	30	57	40	72	600	72
S715P single substitution introduction[c]	*Pc gsc1*	30	98	10	98	30	57	360	72	120	72
Control mutagenesis[d]	*Pc gsc1* internal fragment	180	94	30	94	30	63	40	72	600	72
S719P single substitution introduction[c]	*Pm gsc1*	30	98	10	98	30	57	360	72	120	72
Control mutagenesis[d]	*Pm gsc1* internal fragment	180	94	30	94	30	60	60	72	600	72
Gene oriented cloning[e]	*Ca GSC1*	180	94	25	94	30	57	360	68	360	72
Gene oriented cloning[e]	*Cp GSC1*	180	94	25	94	30	52	360	68	360	72

[a] *Pj, P. jirovecii ; Pc, P. carinii; Pm, P. murina, Ca, C. albicans; Cp, C. parapsilosis.*

[b] Using the Quickchange II XL Site-Directed Mutagenesis Kit (Agilent Technologies).

[c] Using the Q5 Site-Directed Mutagenesis Kit (BioLabs).

[d] Amplification using the proofreading high-fidelity Expand polymerase (Roche Diagnostics), 35 cycles.

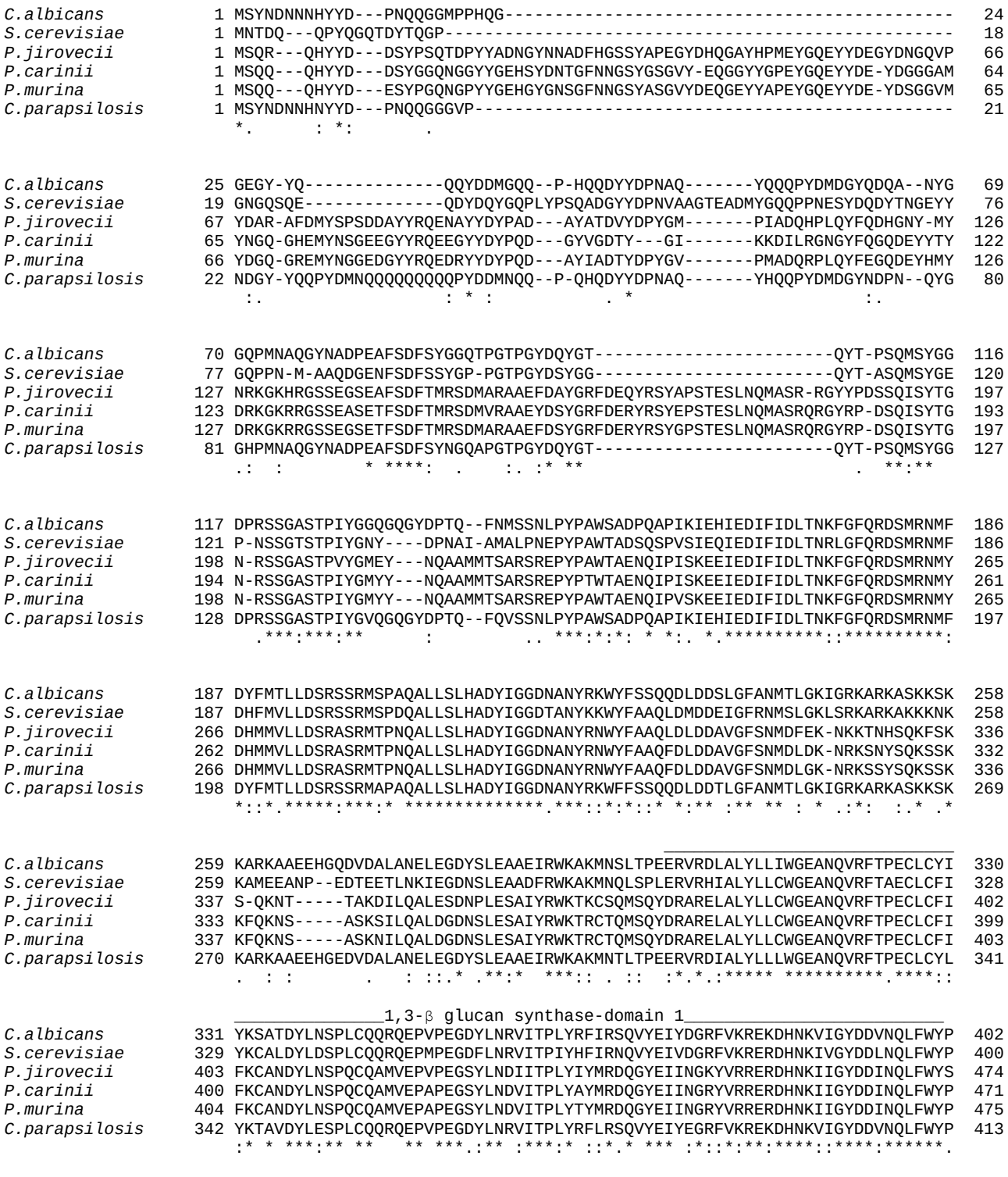
[e] Amplification using the Kapa Long Range HotStart polymerase (kappa Biosystem), 35 cycles.

TABLE S3. Sequence identity (%) of the *S. cerevisiae* Gsc1 protein to the ortholog proteins studied in the complementation assays and minimum inhibitory concentration (MIC) determinations [a]

		Whole protein	1,3-β glucan synthase domain 1	1,3-β glucan synthase domain 2
S. cerevisiae to	*P. jirovecii*	59	69	70
	P. carinii	59	71	70
	P. murina	59	71	70
	C. albicans	72	80	80
	C. parapsilosis	71	76	81

[a] Alignment of Gsc1 proteins is shown in Figure S1.

FIG S1.


C.albicans
S.cerevisiae
P.jirovecii
P.carinii
P.murina
C.parapsilosis
1,3-β glucan synthase-domain 1

```
                 _________
C.albicans       403 EGISRIIFEDGTRLVDIPQEERFLKLGEVEWKNVFFKTYKEIRTWLHFVTNFNRIWIIHGTIYWMYTAYNSP  474
S.cerevisiae     401 EGIAKIVLEDGTKLIELPLEERYLRLGDVVWDDVFFKTYKETRTWLHLVTNFNRIWVMHISIFWMYFAYNSP  472
P.jirovecii      475 EGIERIVLSDKTRIIDLPPEQRYLRLKDVVWKKVFFKTYRETRSWFHLFTNFNRIWIIHITVYWFYTAANSP  546
P.carinii        472 EGIQRIVLSDKTRMVDLPLDQRYPRFKDVVWKKAFFKTYRETRSWFHLFTNFNRIWIIHITVYWFYTAANSP  543
P.murina         476 EGIERIVLSDKTRMVDLPLDQRYPRFKDVVWKKAFFKTYRETRSWFHLFTNFNRIWIIHITVYWFYTAANSP  547
C.parapsilosis   414 EGVSRIIFTDGTRLIDIPKEERYLRLGEVEWSNVFFKTYKEIRTWLHFVTNFNRIWIIHGSIYWMYTAYNSP  485
                     **: :*:: * *:::::* ::*: :: :* *...*****:* *:*:*:.*******:* :::*:* * ***

C.albicans       475 TLYTKHYVQTINQQPLASSRWAACAIGGVLASFIQILATLFEWIFVPREWAGAQHLSRRMLFLVLIFLLNLV  546
S.cerevisiae     473 TFYTHNYQQLVDNQPLAAYKWASCALGGTVASLIQIVATLCEWSFVPRKWAGAQHLSRRFWFLCIIFGINLG  544
P.jirovecii      547 TVYTHNYQQSLDNQPPFAYRMSAVSFGGGVASLLMIIATLAEWAYVPRKWAGAQHLTRRLLFLILFLIINVA  618
P.carinii        544 TVYTHNYQQSLDNQPPFAYRMSAVGFGGGVASLLMIVATLAEWAYVPRKWPGAQHLTRRLLFLILFFIINVA  615
P.murina         548 TVYTHNYQQSLDNQPPFAYRMSAVGFGGGVASLLMIIATLAEWAYVPRRWPGAQHLTRRLLFLILFLIINVA  619
C.parapsilosis   486 TLYTKNYVQTINQQPLASSRWAACAIGGIIAAFLQILATIFEWMFVPREWAGAQHLTRRLMFLILIFLVNLA  557
                     *.**::* * :::**  : : :: .:** :*::: *:**: ** :***.*.*****:**: ** ::: :*:

C.albicans       547 PPVYTFQITKLV-IYSKSAYAVSIVGFFIAVATLVFFAVMPLGGLFTSYMNKRSRRYIASQTFTANYIKLKG  617
S.cerevisiae     545 PIIFVFAYDKDT-VYSTAAHVVAAVMFFVAVATIIFFSIMPLGGLFTSYMKKSTRRYVASQTFTAAFAPLHG  615
P.jirovecii      619 PGVYVIKFAPWKPKVSVVTTLISIIHFLIAMFTFLFFAIMPLGGLFGNYLYKKTRRYVASQTFTANFAKLKG  690
P.carinii        616 PGVYVIKFAPWKPNVSIVTTLISIMHFLIAIFTFLFFAIMPLGGLFGNYLYKKTRRYVASQTFTANFAKLKG  687
P.murina         620 PGVYVIKFAQWKPNVSVVTTLISIMHFLIAIFTFLFFAIMPLGGLFGNYLYKKTRRYVASQTFTANFAKLKG  691
C.parapsilosis   558 PVVYTFKVAGLT-LYSKSSYALSVVGFFIAVATLVFFAVMPLGGLFTSYMNKRSRRYISSHTFTANFVKLRG  628
                     * ::.:         *  :  :: : *::*: *::**::******* .*: * :***::*:**** :  *:*

                                          Hot spot 1
                                          ---------
C.albicans       618 LDMWMSYLLWFLVFLAKLVESYFFLTLSLRDPIRNLSTMTMRCVGEVWYKDIVCRNQAKIVLGLMYLVDLLL  689
S.cerevisiae     616 LDRWMSYLVWVTVFAAKYSESYYFLVLSLRDPIRILSTTAMRCTGEYWWGAVLCKVQPKIVLGLVIATDFIL  687
P.jirovecii      691 NDLWLSYGLWIAVFACKFAESYFFLSLSLRDPIRYLNTMTIGHCGIRYLGSILCPYQAKITLGIMYITDLVL  762
P.carinii        688 NDLWLSYGLWIAVFACKFAESYFFLSLSLRDPIRYLNTMTIGHCGIRYLGSSLCPYQAKITLGIMYITDLVL  759
P.murina         692 NDLWLSYGLWIAVFACKFAESYFFLSLSLRDPIRYLNTMTIGHCGIRYLGSALCPYQAKITLGIMYITDLVL  763
C.parapsilosis   629 LDMWMSYLLWVLVFLAKLVESYFFLTLSLRDAIRNLSKTTMRCTGEVWYGDIVCRQQAKIVLGLMYAVDLLL  700
                      * *:** :*. ** .*  ***:** *****.** *.. ::   *  :    :*  *.**.**::  .*::*

C.albicans       690 FFLDTYMWYIICNCIFSIGRSFYLGISILTPWRNIFTRLPKRIYSKILATTEMEIKYKPKVLISQIWNAIVI  761
S.cerevisiae     688 FFLDTYLWYIIVNTIFSVGKSFYLGISILTPWRNIFTRLPKRIYSKILATTDMEIKYKPKVLISQVWNAIII  759
P.jirovecii      763 FFLDTYLWYIIWNTICSVARSFYLGVSIWTPWRNIFSRMPKRIYSKILATNDMEIKYKPKVLISQ-------  827
P.carinii        760 FFLDTYLWYIIWNTICSVARSFYLGVSIWTPWRNIFSRMPKRIYSKILATNDMEIKYKPKVLISQVWNAVVI  831
P.murina         764 FFLDTYLWYIIWNTICSVARSFYLGVSIWTPWRNIFSRMPKRIYSKILATNDMEIKYKPKVLISQVWNAVVI  835
C.parapsilosis   701 FFLDTYLWYIICNCIFSIGRSFYLGISILTPWRNIFTRLPKRIYSKILATTEMEIKYKPKVLISQIWNAIVI  772
                     ******:**** * * *:.:*****:** *******:*:***********.:**********

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C.albicans       762 SMYREHLLAIDHVQKLLYHQVPSEIEGKRTLRAPTFFVSQDDNNFETEFFPRNSEAERRISFFAQSLATPMP  833
S.cerevisiae     760 SMYREHLLAIDHVQKLLYHQVPSEIEGKRTLRAPTFFVSQDDNNFETEFFPRDSEAERRISFFAQSLSTPIP  831
P.jirovecii      828 -------------------VPSEQEGKRTLRAPTFFISQEDHSFKTEFFPSHSEAERRISFFAQSLSTPIP  879
P.carinii        832 SMYREHLLAIDHVQKLLYHQVPSEQEGKRTLRAPTFFISQEDHSFKTEFFPSHSEAERRISFFAQSLSTPIP  903
P.murina         836 SMYREHLLAIDHVQKLLYHQVPSEQEGKRTLRAPTFFISQEDHSFKTEFFPSHSEAERRISFFAQSLSTPIP  907
C.parapsilosis   773 SMYREHLLAIDHVQKLLYHQVPSEIEGKRTLRAPTFFASQDDNNFETEFFPRNSEAERRISFFAQSLATPMP  844
                                      **** ************ **:*:.*:***** .**************:**:*

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C.albicans       834 EPLPVDNMPTFTVFTPHYSEKILLSLREIIREDDQFSRVTLLEYLKQLHPVEWDCFVKDTKILAEETAAYEN  905
S.cerevisiae     832 EPLPVDNMPTFTVLTPHYAERILLSLREIIREDDQFSRVTLLEYLKQLHPVEWECFVKDTKILAEETAAYEG  903
P.jirovecii      880 EPLPVDNMPTFTVLVPHYGEKILYSLREIIREDDQLSRVTLLEYLKQLHPVEWDCFVKDTKILAEETSLYNG  951
P.carinii        904 EPLPVDNMPTFTVLVPHYGEKILYSLREIIREDDQLSRVTLLEYLKQLHPVEWDCFVKDTKILAEETSLYNG  975
P.murina         908 EPLPVDNMPTFTVLVPHYGEKILYSLREIIREDDQLSRVTLLEYLKQLHPVEWDCFVKDTKILAEETSLYNG  979
C.parapsilosis   845 EPVPVDNMPTFTVFTPHYSEKILLSLREIIREDDQFSRVTLLEYLKQLHPVEWECFVKDTKILAEETAAYEN  916
                     **:**********:.***.*:** **********:*****************:************: *:.

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C.albicans       906 GDDSEKLSEDGLKSKIDDLPFYCIGFKSAAPEYTLRTRIWASLRSQTLYRTVSGFMNYARAIKLLYRVENPE  977
S.cerevisiae     904 NE-NEAEKEDALKSQIDDLPFYCIGFKSAAPEYTLRTRIWASLRSQTLYRTISGFMNYSRAIKLLYRVENPE  974
P.jirovecii      952 GSSFDKDEKDTVKSKIDDLPFYCVGFKSAAPEYTLRTRIWASLRSQTLYRTVSGFMNYSRAIKLLYRVENPD 1023
P.carinii        976 GVPFDKDEKDTVKSKIDDLPFYCVGFKSSAPEYTLRTRIWASLRSQTLYRTVSGFMNYSRAIKLLYRVENPD 1047
P.murina         980 GVPFDKDEKDTVKSKIDDLPFYCVGFKSSAPEYTLRTRIWASLRSQTLYRTVSGFMNYSRAIKLLYRVENPD 1051
C.parapsilosis   917 GEDAEKASEDGLKSKIDDLPFYCIGFKSAAPEYTLRTRIWASLRSQTLYRTVSGFMNYARAIKLLYRVENPE  988
                     .   :  .:* :**:********:****:**********************:******:***********:

                     ____________________________________________________________________________
C.albicans       978 LVQYFGGDPEGLELALERMARRKFRFLVSMQRLSKFKDDEMENAEFLLRAYPDLQIAYLDEEPALNEDEEPR 1049
S.cerevisiae     975 IVQMFGGNAEGLERELEKMARRKFKFLVSMQRLAKFKPHELENAEFLLRAYPDLQIAYLDEEPPLTEGEEPR 1046
P.jirovecii     1024 VVQMFGGNTDKLEHELERMARRKFKFVISMQRFFKFNKEEQENTEFLLRAYPDLQIAYLDEEPPSHEGDEPK 1095
P.carinii       1048 VVQMFGGNTDKLEHELERMARRKFKFDISMQRFFKFSKEELENTEFLLRAYPDLQIAYLDEEPPMNEGDEPK 1119
P.murina        1052 VVQMFGGNTDKLEHELERMARRKFKFVISMQRFFKFSKEELENTEFLLRAYPDLQIAYLDEEPPMNEGDEPK 1123
C.parapsilosis   989 LVQYFGGDPEGLELALEKMARRKFRFLVSMQRLSKFKDDEMENAEFLLRAYPDLQIAFLDEEPALNEDEEPR 1060
                     :** ***:.: **  **:******:* :****: **. .* **:*************:*****.  *.:**:
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C.albicans        1050 VYSALIDGHCEMLENGRRRPKFRVQLSGNPILGDGKSDNQNHAVIFHRGEYIQLIDANQDNYLEECLKIRSV 1121
S.cerevisiae      1047 IYSALIDGHCEILDNGRRRPKFRVQLSGNPILGDGKSDNQNHALIFYRGEYIQLIDANQDNYLEECLKIRSV 1118
P.jirovecii       1096 IYSSLIDGYSEIMEDGRRRPKFRIQLSGNPILGDGKSDNQNHAIIFYRGEYIQLIDANQDNYLEECLKIRSV 1167
P.carinii         1120 IYSSLIDGYSEIMENGKRRPKFRIQLSGNPILGDGKSDNQNHAIIFYRGEYIQLIDANQDNYLEECLKIRSV 1191
P.murina          1124 IYSSLIDGYSEIMENGKRRPKFRIQLSGNPILGDGKSDNQNHAIIFYRGEYIQLIDANQDNYLEECLKIRSV 1195
C.parapsilosis    1061 VYSSLIDGHCEMLENGRRRPKFRVQLSGNPILGDGKSDNQNHAIIFHRGEYIQLIDANQDNYLEECLKIRSV 1132
                       :**:****:.*::::*:******:******************:**:*************************

C.albicans        1122 LAEFEEMNVEHVNPYAPNLKSED-NNTKKDPVAFLGAREYIFSENSGVLGDVAAGKEQTFGTLFARTLAQIG 1192
S.cerevisiae      1119 LAEFEELNVEQVNPYAPGLRYEE--QTTNHPVAIVGAREYIFSENSGVLGDVAAGKEQTFGTLFARTLSQIG 1188
P.jirovecii       1168 LAEFEEMSPLEEFPYNPNENS-----KVNNPVAILGAREYIFSENIGVLGDVAAGKEQTFGTLFARTLAQIG 1234
P.carinii         1192 LAEFEEMTPTEESPYNPNEIS-----SATNPVAILGAREYIFSENIGVLGDVAAGKEQTFGTLFARTLAQIG 1258
P.murina          1196 LAEFEEMTPIEESPYNPNEVS-----SAANPVAILGAREYIFSENIGVLGDVAAGKEQTFGTLFARTLAQIG 1262
C.parapsilosis    1133 LAEFEELNVEHVNPYSPDLKSENPLHEKKAPVAILGAREYIFSENSGVLGDVAAGKEQTFGTLFARTLAQIG 1204
                       ******:.  .  ** *.            ***::********** **********************:***

C.albicans        1193 GKLHYGHPDFLNATFMLTRGGVSKAQKGLHLNEDIYAGMNAMMRGGKIKHCEYYQCGKGRDLGFGSILNFTT 1264
S.cerevisiae      1189 GKLHYGHPDFINATFMTTRGGVSKAQKGLHLNEDIYAGMNAMLRGGRIKHCEYYQCGKGRDLGFGTILNFTT 1260
P.jirovecii       1235 GKLHYGHPDFLNGPFMTTRGGVSKAQKGLHLNEDIYAGMTALLRGGRIKHCEYYQCGKGRDLGFGSILNFTT 1306
P.carinii         1259 GKLHYGHPDFLNGPFMTTRGGVSKAQKGLHLNEDIYAGMTALLRGGRIKHCEYYQCGKGRDLGFGSILNFTT 1330
P.murina          1263 GKLHYGHPDFLNGPFMTTRGGVSKAQKGLHLNEDIYAGMTALLRGGRIKHCEYYQCGKGRDLGFGSILNFTT 1334
C.parapsilosis    1205 GKLHYGHPDFLNATFMLTRGGVSKAQKGLHLNEDIYAGMNAMMRGGKIKHCEYYQCGKGRDMGFGSILNFTT 1276
                       **********:*..** *********************.*::***:**************:***:******

                       _______________1,3-β glucan synthase domain 2___________________________
C.albicans        1265 KIGAGMGEQMLSREYFYLGTQLPLDRFLSFYYGHPGFHINNLFIQLSLQVFILVLANLNSLAHEAIMCSYNK 1336
S.cerevisiae      1261 KIGAGMGEQMLSREYYYLGTQLPVDRFLTFYYAHPGFHLNNLFIQLSLQMFMLTLVNLSSLAHESIMCIYDR 1332
P.jirovecii       1307 KVGTGMGEQMLSREYYYLGTQLPLDRFLSFYYAHPGFHINNLFIILSVQLLMIVMINLGSMYNILLICKPRR 1378
P.carinii         1331 KVGTGMGEQMLSREYYYLGTQLPLDRFLSFYYAHPGFHINNLFIILSVQLLMIVMINLGSMYNILLICRPRR 1402
P.murina          1335 KVGTGMGEQMLSREYYYLGTQLPLDRFLSFYYAHPGFHINNLFIILSVQLLMIVMINLGSMYNILLICRPRR 1406
C.parapsilosis    1277 KIGAGMGEQMLSREYYYLSTQLPLDRFLSFYYGHPGFHINNLFIQLSLQVFMLVLANLNSLAHESIICSYDR 1348
                       *:*:***********:**.****:****:***.*****:***** **:*:::::.: **.*: :  ::*   :

                                   Hot spot 2
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C.albicans        1337 DVPVTDVLYPFGCYNIAPAVDWIRRYTLSIFIVFFISFIPLVVQELIERGVWKAFQRFVRHFISMSPFFEVF 1408
S.cerevisiae      1333 NKPKTDVLVPIGCYNFQPAVDWVRRYTLSIFIVFWIAFVPIVVQELIERGLWKATQRFFCHLLSLSPMFEVF 1404
P.jirovecii       1379 GQPITDPFLPVGCYSLAPVLDWIKRSIISIFIVFFIAFIPLVVQELTERGVWRASTRLAKHFGSLSPLFEVF 1450
P.carinii         1403 GQPITDPYLPVGCYSIAPVLDWIKRSIISIFIVFFIAFIPLVVQELTERGVWRASTRLAKHFGSLSPLFEVF 1474
P.murina          1407 GQPITDPYLPVGCYSLAPVLDWIKRSIISIFIVFFIAFIPLVVQELTERGVWRASTRLAKHFGSLSPLFEVF 1478
C.parapsilosis    1349 DVPVTDVLYPFGCYNIAPAVDWIRRYTLSIFIVFFISFIPLVVQELIERGVWKACQRFVRHFISLSPMFEVF 1420
                       . * **   *.***.: *.:**::*  :******:*:*:*:***** ***:*:*  *:  *: *:**:****

C.albicans        1409 VAQIYSSSVFTDLTVGGARYISTGRGFATSRIPFSILYSRFADSSIYMGARLMLILLFGTVSHWQAPLLWFW 1480
S.cerevisiae      1405 AGQIYSSALLSDLAIGGARYISTGRGFATSRIPFSILYSRFAGSAIYMGARSMLMLLFGTVAHWQAPLLWFW 1476
P.jirovecii       1451 VSQIYANSLLQNLAFGGARYIGTGRGFATTRIPFSILFSRFAGASIYLGSRTLIMLLFATVTMWIPHLVYFW 1522
P.carinii         1475 VSQIYANSLLQNLAFGGARYIGTGRGFATTRIPFSILFSRFAGASIYLGSRTLIMLLFATVTMWIPHLVYFW 1546
P.murina          1479 VSQIYANSLLQNLAFGGARYIGTGRGFATTRIPFSILFSRFAGASIYLGSRTLIMLLFATVTMWIPHLVYFW 1550
C.parapsilosis    1421 VAQIYSSSVFTDLTVGGARYISTGRGFATSRIPFSILYSRFADSSIYMGARLMLILLFGTVAHWQAPLLWFW 1492
                       ..***:.::: :*:.******.*******:*******:****.::**:*:* :::***.**: * . *::**

C.albicans        1481 ASLSALMFSPFIFNPHQFAWEDFFLDYRDFIRWLSRGNTKWHRNSWIGYVRLSRSRITGFKRKLTGDVSEKA 1552
S.cerevisiae      1477 ASLSSLIFAPFVFNPHQFAWEDFFLDYRDYIRWLSRGNNQYHRNSWIGYVRMSRARITGFKRKLVGDESEKA 1548
P.jirovecii       1523 VSVLALCICPFIFNPHQFSWTDFFVDYREFIRWLSRGNSRSHANSWIGYCRLSRTRITGFKRKALGQPSEKL 1594
P.carinii         1547 VSVLALCISPFIFNPHQFSWTDFFVDYREFIRWLSRGNSRSHANSWIGYCRLSRTRITGFKRKALGQPSEKL 1618
P.murina          1551 VSVLALCISPFIFNPHQFSWTDFFVDYREFIRWLSRGNSRSHANSWIGYCRLSRTRITGFKRKALGQPSEKL 1622
C.parapsilosis    1493 ASLSSLMFSPFIFNPHQFAWEDFFIDYRDFIRWLSRGNTKWHRNSWIGYVRLSRSRITGFKRKLTGDISEKA 1564
                       .*: :* :.**:******:* ***:***::********.: * ****** *:**:********  *: ***

C.albicans        1553 AGDASRAHRSNVLFADFLPTLIYTAGLYVAYTFINAQTGVTSYPYEINGSTDPQPVNSTLRLIICALAPVVI 1624
S.cerevisiae      1549 AGDASRAHRTNLIMAEIIPCAIYAAGCFIAFTFINAQTGVKT--------TDDDRVNSVLRIIICTLAPIAV 1612
P.jirovecii       1595 SGDIPRAGFNNVFFSEVIGPLILVILSLVPFCFMNSRPGFEPFGKSN---PARNGSNPLIRIAIVSFAPICV 1663
P.carinii         1619 SGDIPRAGFSNVFFSEVIGPMILVLLSLVPYCFINSRPGFEPFGKSN---PAKNGSNPLIRIAIVSFAPICV 1687
P.murina          1623 SGDIPRAGFSNVFFSEVIGPMILVLLSLVPYCFINSRPGFEPFGKSN---PAKNGSNPLIRIAIVSFAPICV 1691
C.parapsilosis    1565 AGDASRAHRSNILFADFLPTLIYTAGLYVAFTFINAQTGVTQYPYEIDGSTDVQEVNSVLRLIVCSLAPVVI 1636
                       :** .**  .*:::::.:   * .    :.: *:*::.*.          .  :  *. :*: : ::**: :
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                          ____________________
C.albicans          1625 DMGCLGVCLAMACCAGPMLGLCCKKTGAVIAGVAHGVAVIVHIIFFIVMWVTEGFNFARLMLGIATMIYVQR 1696
S.cerevisiae        1613 NLGVLFFCMGMSCCSGPLFGMCCKKTGSVMAGIAHGVAVIVHIAFFIVMWVLESFNFVRMLIGVVTCIQCQR 1684
P.jirovecii         1664 NALVAFVFFGMACCMGPILTICCKKFGAVLATISHAIAVIVLVAFFEVLWFLEGWSFSKTILGLVTMISLQR 1735
P.carinii           1688 NAMVAFVFFGMACCMGPILTICCKKFAAVLATISHAIAVIILVTFFEVLWFLEGWSFSKTILGLVTMISLQR 1759
P.murina            1692 NAMVAFVFFGMACCMGPILTICCKKFGAVLATISHAIAVIILVTFFEVLWFLEGWSFSKTILGLVTMISLQR 1763
C.parapsilosis      1637 DCGVLFGCVGMACCAGPMMGLCCKKTGAVIAGIAHGIAVIVHIVFFIVMWVMEGFNFARMLLGFATMIYVQR 1708
                           :       ..*:** **:: :**** .:*:* ::*.:***: : ** *:*. *.::* : ::*..* *  **


C.albicans          1697 LLFKFLTLCFLTREFKNDKANTAFWTGKWYNTGMGWMAFTQPSREFVAKIIEMSEFAGDFVLAHIILFCQLP 1768
S.cerevisiae        1685 LIFHCMTALMLTREFKNDHANTAFWTGKWYGKGMGYMAWTQPSRELTAKVIELSEFAADFVLGHVILICQLP 1756
P.jirovecii         1736 AFLKMLTIMILTREFKHDGSNLAWWTGRWYSNNLGVHAMSQPAREFVCKVIELSLFAADFCLGHLLLFILTP 1807
P.carinii           1760 AFLKILTIMILTREFKHDGSNLAWWTGRWYSNNLGVYAMSQPAREFVCKVIELSLFAADFCLGHLLLFILTP 1831
P.murina            1764 AFLKMLTIMILTREFKHDGSNLAWWTGRWYSNNLGVYAMSQPAREFVCKVIELSLFAADFCLGHLLLFILTP 1835
C.parapsilosis      1709 LLFKFLTLAFLTREFKNDKANTAFWTGKWYNTGMGWMAFTQPSREFVAKIVEMSEFAGDFMLAHIILFCQLP 1780
                           ::: :*  :******:* :* *:***:**...:*  * :**:**:..*::*:* **.** *.*::*:   *


C.albicans          1769 FLFIPLVDRWHSMMLFWLKPSRLIRPPIYSLKQARLRKRMVRKYCVLYFAVLILFIVIIVAPAVASGQIPVD 1840
S.cerevisiae        1757 LIIIPKIDKFHSIMLFWLKPSRQIRPPIYSLKQTRLRKRMVKKYCSLYFLVLAIFAGCIIGPAVASAKIHK- 1827
P.jirovecii         1808 ILAIPYIDRWHSMLLFWLRPSRQIRPPIFSLKQNKLRKRIVRRYATLFFGLFLLFLMIILVPAVGHSKFPK- 1878
P.carinii           1832 ILAIPYIDRWHSMLLFWLRPSRQIRPPIFSLKQNKLRKRIVRRYATLFFGLFLLFLMIILVPALGHSKFPK- 1902
P.murina            1836 ILAIPYIDRWHSMLLFWLRPSRQIRPPIFSLKQNKLRKRIVRRYATLFFGLFLLFLMIILVPALGHSKFPK- 1906
C.parapsilosis      1781 ILAVPLIDRWHSMMLFWLKPSRLIRPPIYSLKQARLRKRMVRKYVTLYFAVLLLFVVIIAAPAAASGSIKVD 1852
                           :: :* :*::**::****:*** *****:**** :****:*::*  *:* :: :*   *  ** . ..:

C.albicans          1841 QFANIGGSGSIADGLFQPRNVSNNDTGN-HRPKTYTWSYLSTRFTGTTTPYSTNPFRV 1897
S.cerevisiae        1828 HIGDS--LDGVVHNLFQPINTTNNDTGS-Q-----MSTYQSHYYTHTPSLKTWSTI-K 1876
P.jirovecii         1879 SLNNIPAL--KNLGLIQPSNDPRGATGRTTRPANSNGTYKMFT--------------- 1919
P.carinii           1903 SLNNIAFL--KNLGLIQPSNDPRGATGRTTRPGNSNGTYKLFIY------------- 1944
P.murina            1907 SLNNIAFL--KNLGLIQPSNDPRGATGRTTRPGNSNGTYKLFIY------------- 1948
C.parapsilosis      1853 QFADIGSKGSIAYGLFQPRNVSNNDTGPTNRPKSYTWSYLSEKYSGHTTAYSTNAF-R 1909
                           : :         .*:** * ... **          :*
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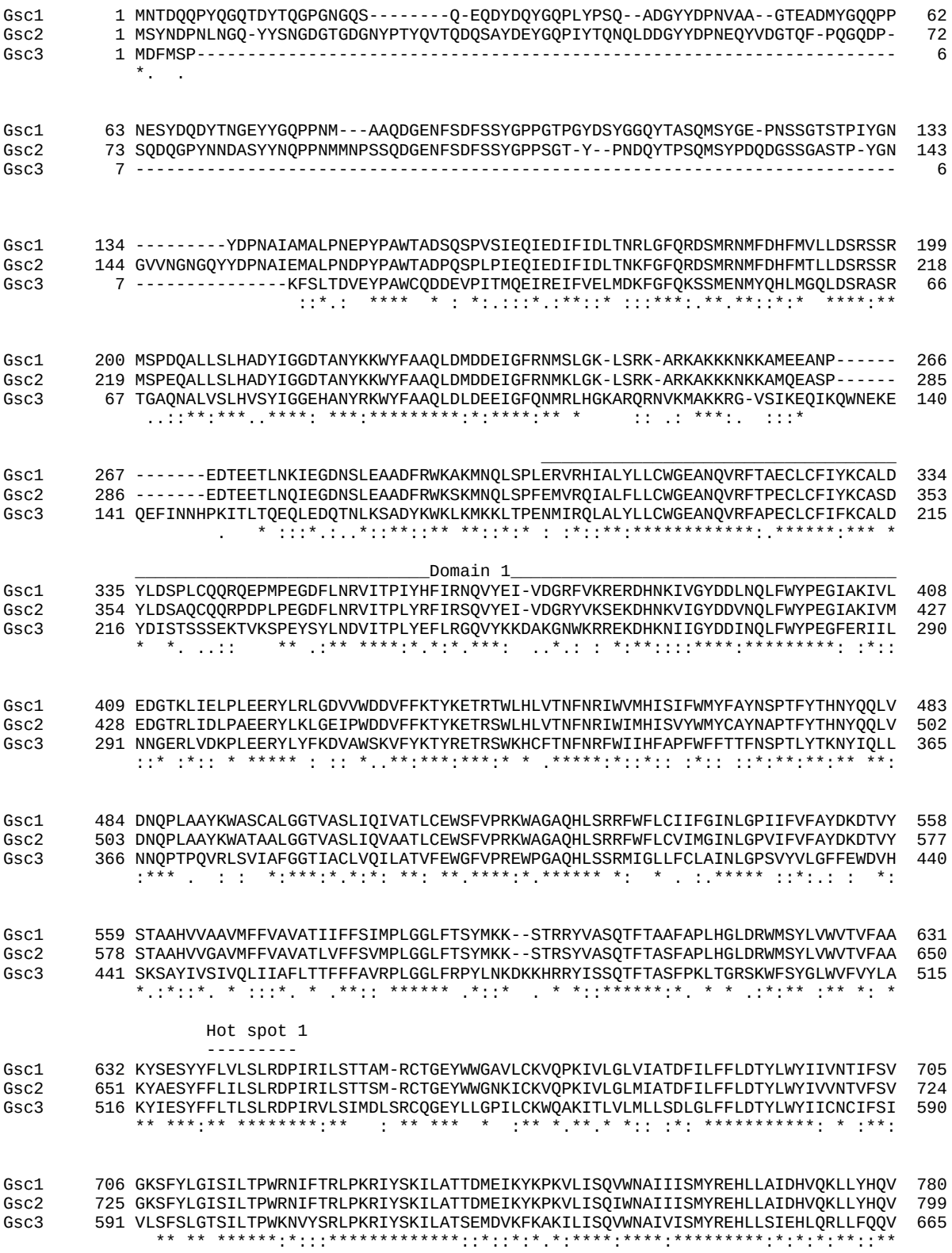
FIG S2.


Gsc1 1 MNTDQQPYQGQTDYTQGPGNGQS--------Q-EQDYDQYGQPLYPSQ--ADGYYDPNVAA--GTEADMYGQQPP 62
Gsc2 1 MSYNDPNLNGQ-YYSNGDGTGDGNYPTYQVTQDQSAYDEYGQPIYTQNQLDDGYYDPNEQYVDGTQF-PQGQDP- 72
Gsc3 1 MDFMSP---------- 6
Gsc1 63 NESYDQDYTNGEYYGQPPNM---AAQDGENFSDFSSYGPPGTPGYDSYGGQYTASQMSYGE-PNSSGTSTPIYGN 133
Gsc2 73 SQDQGPYNNDASYYNQPPNMMNPSSQDGENFSDFSSYGPPSGT-Y--PNDQYTPSQMSYPDQDGSSGASTP-YGN 143
Gsc3 7 ---------- 6
Gsc1 134 ---------YDPNAIAMALPNEPYPAWTADSQSPVSIEQIEDIFIDLTNRLGFQRDSMRNMFDHFMVLLDSRSSR 199
Gsc2 144 GVVNGNGQYYDPNAIEMALPNDPYPAWTADPQSPLPIEQIEDIFIDLTNKFGFQRDSMRNMFDHFMTLLDSRSSR 218
Gsc3 7 ---------------KFSLTDVEYPAWCQDDEVPITMQEIREIFVELMDKFGFQKSSMENMYQHLMGQLDSRASR 66
Gsc1 200 MSPDQALLSLHADYIGGDTANYKKWYFAAQLDMDDEIGFRNMSLGK-LSRK-ARKAKKKNKKAMEEANP------ 266
Gsc2 219 MSPEQALLSLHADYIGGDTANYKKWYFAAQLDMDDEIGFRNMKLGK-LSRK-ARKAKKKNKKAMQEASP------ 285
Gsc3 67 TGAQNALVSLHVSYIGGEHANYRKWYFAAQLDLDEEIGFQNMRLHGKARQRNVKMAKKRG-VSIKEQIKQWNEKE 140
Gsc1 267 -------EDTEETLNKIEGDNSLEAADFRWKAKMNQLSPLERVRHIALYLLCWGEANQVRFTAECLCFIYKCALD 334
Gsc2 286 -------EDTEETLNQIEGDNSLEAADFRWKSKMNQLSPFEMVRQIALFLLCWGEANQVRFTPECLCFIYKCASD 353
Gsc3 141 QEFINNHPKITLTQEQLEDQTNLKSADYKWKLKMKKLTPENMIRQLALYLLCWGEANQVRFAPECLCFIFKCALD 215
Domain 1
Gsc1 335 YLDSPLCQQRQEPMPEGDFLNRVITPIYHFIRNQVYEI-VDGRFVKRERDHNKIVGYDDLNQLFWYPEGIAKIVL 408
Gsc2 354 YLDSAQCQQRPDPLPEGDFLNRVITPLYRFIRSQVYEI-VDGRYVKSEKDHNKVIGYDDVNQLFWYPEGIAKIVM 427
Gsc3 216 YDISTSSSEKTVKSPEYSYLNDVITPLYEFLRGQVYKKDAKGNWKRREKDHKNIIGYDDINQLFWYPEGFERIIL 290
Gsc1 409 EDGTKLIELPLEERYLRLGDVVWDDVFFKTYKETRTWLHLVTNFNRIWVMHISIFWMYFAYNSPTFYTHNYQQLV 483
Gsc2 428 EDGTRLIDLPAEERYLKLGEIPWDDVFFKTYKETRSWLHLVTNFNRIWIMHISVYWMYCAYNAPTFYTHNYQQLV 502
Gsc3 291 NNGERLVDKPLEERYLYFKDVAWSKVFYKTYRETRSWKHCFTNFNRFWIIHFAPFWFFTTFNSPTLYTKNYIQLL 365
Gsc1 484 DNQPLAAYKWASCALGGTVASLIQIVATLCEWSFVPRKWAGAQHLSRRFWFLCIIFGINLGPIIFVFAYDKDTVY 558
Gsc2 503 DNQPLAAYKWATAALGGTVASLIQVAATLCEWSFVPRKWAGAQHLSRRFWFLCVIMGINLGPVIFVFAYDKDTVY 577
Gsc3 366 NNQPTPQVRLSVIAFGGTIACLVQILATVFEWGFVPREWPGAQHLSSRMIGLLFCLAINLGPSVYVLGFFEWDVH 440
Gsc1 559 STAAHVVAAVMFFVAVATIIFFSIMPLGGLFTSYMKK--STRRYVASQTFTAAFAPLHGLDRWMSYLVWVTVFAA 631
Gsc2 578 STAAHVVGAVMFFVAVATLVFFSVMPLGGLFTSYMKK--STRSYVASQTFTASFAPLHGLDRWMSYLVWVTVFAA 650
Gsc3 441 SKSAYIVSIVQLIIAFLTTFFFAVRPLGGLFRPYLNKDKKHRRYISSQTFTASFPKLTGRSKWFSYGLWVFVYLA 515
Hot spot 1
Gsc1 632 KYSESYYFLVLSLRDPIRILSTTAM-RCTGEYWWGAVLCKVQPKIVLGLVIATDFILFFLDTYLWYIIVNTIFSV 705
Gsc2 651 KYAESYFFLILSLRDPIRILSTTSM-RCTGEYWWGNKICKVQPKIVLGLMIATDFILFFLDTYLWYIVVNTVFSV 724
Gsc3 516 KYIESYFFLTLSLRDPIRVLSIMDLSRCQGEYLLGPILCKWQAKITLVLMLLSDLGLFFLDTYLWYIICNCIFSI 590
Gsc1 706 GKSFYLGISILTPWRNIFTRLPKRIYSKILATTDMEIKYKPKVLISQVWNAIIISMYREHLLAIDHVQKLLYHQV 780
Gsc2 725 GKSFYLGISILTPWRNIFTRLPKRIYSKILATTDMEIKYKPKVLISQIWNAIIISMYREHLLAIDHVQKLLYHQV 799
Gsc3 591 VLSFSLGTSILTPWKNVYSRLPKRIYSKILATSEMDVKFKAKILISQVWNAIVISMYREHLLSIEHLQRLLFQQV 665

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                                           __________________________________________________
Gsc1     781 PSEIEGKRTLRAPTFFVSQDDNNFET-EFFPRDSEAERRISFFAQSLSTPIPEPLPVDNMPTFTVLTPHYAERIL  854
Gsc2     800 PSEIEGKRTLRAPTFFVSQDDNNFET-EFFPRDSEAERRISFFAQSLSTPIPEPLPVDNMPTFTVLTPHYAERIL  873
Gsc3     666 DSLMGDTRTLKSPTFFVAQDDSTFKSMEFFPSNSEAKRRISFFAQSLATPISEPVPVDCMPTFTVLVPHYSEKIL  740
              * : ..***:.*****:***..*:: **** :***:*********:***.**:*** *******.***:*:**

             ___________________________________________________________________________
Gsc1     855 LSLREIIREDDQFSRVTLLEYLKQLHPVEWECFVKDTKILAEETAAYEGN-------------------------  904
Gsc2     874 LSLREIIREDDQFSRVTLLEYLKQLHPVEWDCFVKDTKILAEETAAYENN-------------------------  923
Gsc3     741 LGLKEIIREESPKSKITVLEYLKHLHPTEWECFVKDTKLLSMEKSFLKEAESSHDEDRLEIPDALYDPRSSPLSD  815
             *.*:*****:.  *::*:*****:***.**:*******:*: *.:  :

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Gsc1     905 ----ENEAEKEDALKSQIDDLPFYCIGFKSAAPEYTLRTRIWASLRSQTLYRTISGFMNYSRAIKLLYRVENPEI  975
Gsc2     924 ----EDEPEKEDALKSQIDDLPFYCIGFKSAAPEYTLRTRIWASLRSQTLYRTISGFMNYSRAIKLLYRVENPEI  994
Gsc3     816 HTESRKLPTEDDLIKEKINDLPFSYFGFNSSEPSYTLRTRIWASLRTQTLYRTLSGFMNYSKAIKLLYRIENPSL  890
                 .. . ::* :*.:*:****  :**:*: *.***********:******:*******:*******:***.:

             ___________________________________________________________________________
Gsc1     976 VQMFGGNAEGLERELEKMARRKFKFLVSMQRLAKFKPHELENAEFLLRAYPDLQIAYLDEEPPLTEGEEPRIYSA 1050
Gsc2     995 VQMFGGNADGLERELEKMARRKFKFLVSMQRLAKFKPHELENAEFLLRAYPDLQIAYLDEEPPLNEGEEPRIYSA 1069
Gsc3     891 VSLYRGNNEALENDLENMASRKFRMVVAMQRYAKFNKDEVEATELLLRAYPNMFISYLLEELEQNESEK-TYYSC  964
             *.:: ** :.**.:**:** ***:::*:*** ***: .*:* :*:******:: *:** **    .*.*:   **.

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Gsc1    1051 LIDGHCEIL-DNGRRRPKFRVQLSGNPILGDGKSDNQNHALIFYRGEYIQLIDANQDNYLEECLKIRSVLAEFEE 1124
Gsc2    1070 LIDGHCEIL-ENGRRRPKFRVQLSGNPILGDGKSDNQNHALIFYRGEYIQLIDANQDNYLEECLKIRSVLAEFEE 1143
Gsc3     965 LTNGYAEFDEESGLRKPIFKIRLSGNPILGDGKSDNQNHSIIFYRGEYIQVIDANQDNYLEECLKIRSVLSEFEE 1039
             * :*:.*:  :.* *:* *:::*****************::*********:*******************:****

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Gsc1    1125 LNVEQVNPYAPGLRYEEQTTNHPVAIVGAREYIFSENSGVLGDVAAGKEQTFGTLFARTLSQIGGKLHYGHPDFI 1199
Gsc2    1144 LGIEQIHPYTPGLKYEDQSTNHPVAIVGAREYIFSENSGVLGDVAAGKEQTFGTLFARTLAQIGGKLHYGHPDFI 1218
Gsc3    1040 LELNPTIPYIPGIEYEEEP--PPIAIVGSREYIFSENIGVLGDIAAGKEQTFGTLFARTLAEIGGKLHYGHPDFL 1112
             * ::   ** **:.**::.   *:****:******** *****:****************::************:

             ___________Domain 2________________________________________________________
Gsc1    1200 NATFMTTRGGVSKAQKGLHLNEDIYAGMNAMLRGGRIKHCEYYQCGKGRDLGFGTILNFTTKIGAGMGEQMLSRE 1274
Gsc2    1219 NATFMTTRGGVSKAQKGLHLNEDIYAGMNAVLRGGRIKHCEYYQCGKGRDLGFGTILNFTTKIGAGMGEQMLSRE 1293
Gsc3    1113 NGIFMTTRGGLSKAQRGLHLNEDIYAGMNAICRGGKIKHSDYYQCGKGRDLGFGSILNFTTKIGAGMGEQLLSRE 1187
             *. *******:****:**************: ***:***.:*************:***************:****

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Gsc1    1275 YYYLGTQLPVDRFLTFYYAHPGFHLNNLFIQLSLQMFMLTLVNLSSLAHESIMCIYDRNKPKTDVLVPIGCYNFQ 1349
Gsc2    1294 YYYLGTQLPIDRFLTFYYAHPGFHLNNLFIQLSLQMFMLTLVNLHALAHESILCVYDRDKPITDVLYPIGCYNFH 1368
Gsc3    1188 YYYLGTQLPMDRFLSFFYAHPGFHLNNLFISFSVQLFFVLLLNLGALNHEIIACFYDKDAPITNLETPVGCYNIQ 1262
             *********:****:*:*************.:*:*:*:: *:** :* ** * *.**:: * *::  *:****::

             Hot spot 2
             -----------________________________________________________________________
Gsc1    1350 PAVDWVRRYTLSIFIVFWIAFVPIVVQELIERGLWKATQRFFCHLLSLSPMFEVFAGQIYSSALLSDLAIGGARY 1424
Gsc2    1369 PAIDWVRRYTLSIFIVFWIAFVPIVVQELIERGLWKATQRFFRHILSLSPMFEVFAGQIYSSALLSDIAVGGARY 1443
Gsc3    1263 PALHWVSIFVLSIFIVFFIAFAPLLIQEVLEKGIWRAASRFLHHLLSMAPLFEVFVCQVYSNSLLMDLTFGGAKY 1337
             **:.**  :.*******:***.*:::**::*:*:*:*:.**: *:**::*:****. *:**.:** *::.***:*

             ___________________________________________________________________________
Gsc1    1425 ISTGRGFATSRIPFSILYSRFAGSAIYMGARSMLMLLFGTVAHWQAPLLWFWASLSSLIFAPFVFNPHQFAWEDF 1499
Gsc2    1444 ISTGRGFATSRIPFSILYSRFAGSAIYMGSRSMLMLLFGTVAHWQAPLLWFWASLSALIFAPFIFNPHQFAWEDF 1518
Gsc3    1338 ISTGRGFAITRLDFFTLYSRFVNISIYSGFQVFFMLLFAIISMWQPALLWFWITVISMCFAPFIFNPHQFAFMDF 1412
             ******** :*: *  *****.. :** * : ::****. :: **..***** :: :: ****:*******: **

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Gsc1    1500 FLDYRDYIRWLSRGNNQYHRNSWIGYVRMSRARITGFKRKLVGDESEKAAGDASRAHRTNLIMAEI-IPCAIYAA 1573
Gsc2    1519 FLDYRDYIRWLSRGNNKYHRNSWIGYVRMSRSRVTGFKRKLVGDESEKSAGDASRAHRTNLIMAEI-IPCAIYAA 1592
Gsc3    1413 FIDYKTFIHWLFSGNTKYQKESWANFVKSSRSRFTGYKSKTVDDISEDSGHDSKKARFWNVFFAELFLPFCVFL- 1486
             *:**: :*:**  **.:*:::** .:*: **:*.**:*:* * *.* **.:. *:.:*:  *:::**: :* .::

             ___________________________________________________________
Gsc1    1574 GCFIAFTFINAQTGVKTTDDDRVNSVLRIIICTLAPIAVNLGVLFFCMGMSCCSGPLFGMCCKKTGSVMAGIAHG 1648
Gsc2    1593 GCFIAFTFINAQTGVKTTDEDRVNSTLRIIICTLAPIVIDIGVLFFCMGLSCCSGPLLGMCCKKTGSVMAGIAHG 1667
Gsc3    1487 FNFTAFSFINAQTGVS--DSTPTSAVFRLLLVTFLPIFLNSIVLFLLFWVSLFVVPGLSYCCKDAGAVIAFIAHT 1559
               * **:********.  *.  ..:.:*::: *: ** ::  ***: : :*    * :. ***.:*:*:* ***
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Gsc1    1649 VAVIVHIAFFIVMWVLESFNFVRMLIGVVTCIQCQRLIFHCMTALMLTREFKNDHANTAFWTGKWYGKGMGYMAW 1723
Gsc2    1668 IAVVVHIVFFIVMWVLEGFSFVRMLIGVVTCIQCQRLIFHCMTVLLLTREFKNDHANTAFWTGKWYSTGLGYMAW 1742
Gsc3    1560 FSVLVYLLDFELMWFLQGWNFTRTLILLITCINMHLILFKVFTTIFLTREYKNNKAHLAWWNGKWYNTGMGWSII 1634
             .:*:*::  * :**.*:.:.*.* ** ::***: : ::*: :*.::****:**::*: *:*.****..*:*:


Gsc1    1724 TQPSRELTAKVIELSEFAADFVLGHVILICQLPLIIIPKIDKFHSIMLFWLKPSRQI-RPPIYSLKQTRLRKRMV 1797
Gsc2    1743 TQPTRELTAKVIELSEFAADFVLGHVILIFQLPVICIPKIDKFHSIMLFWLKPSRQI-RPPIYSLKQARLRKRMV 1816
Gsc3    1635 LQPIREYFVKIMESSYFAADFFLGHFLLFIQTPIILLPFIDYWHTMVLFWMNPRSIIAHKRILTRKQRALRSRIV 1709
              ** **  .*::* * *****.***.:*: * *:* :* ** :*:::***::*   * :  * : **  **.*:*


Gsc1    1798 KKYCSLYFLVLAIFAGCIIGPAVASAKIHKHIGDSLDGV-VHNLFQPINTTNNDTGSQMSTYQSHYYTHTPSLKT 1871
Gsc2    1817 RRYCSLYFLVLIIFAGCIVGPAVASAHVPKDLGSGLTGT-FHNLVQPRNVSNNDTGSQMSTYKSHYYTHTPSLKT 1890
Gsc3    1710 SKYFSLYFVMLGVLLFMLIAPFFAGDFVSS-PQELLEGTLFEGIFQPNNQNNNDTGPNA---PSTILTTTPTLPT 1780
              :* ****::* ::   ::.* .*.  : .   . * *. ...:.** * .*****.:     *   * **:* *

Gsc1    1872 WSTIK 1876
Gsc2    1891 WSTIK 1895
Gsc3    1781 FRTVA 1785
             : *:
```
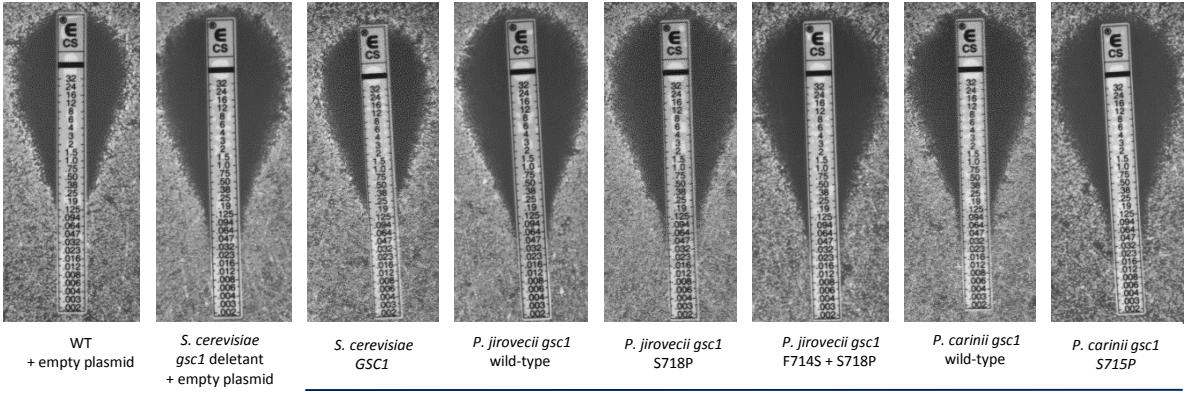

FIG S3.



WT
+ empty plasmid

S. cerevisiae gsc1 deletant
+ empty plasmid

S. cerevisiae GSC1

P. jirovecii gsc1 wild-type

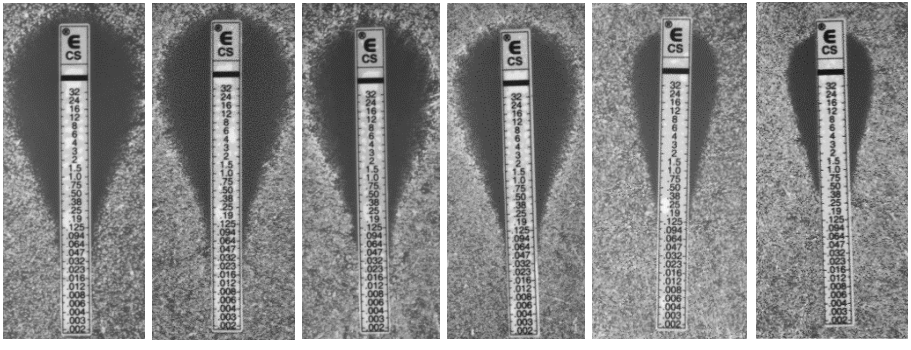
P. jirovecii gsc1 S718P

P. jirovecii gsc1 F714S + S718P

P. carinii gsc1 wild-type

P. carinii gsc1 S715P

S. cerevisiae gsc1 deletant + plasmid +



P. murina gsc1 wild-type

P. murina gsc1 S719P

C. albicans GSC1

C. parapsilosis GSC1

C. albicans

C. parapsilosis

S. cerevisiae gsc1 deletant + plasmid +