Challenging SNP impact on caspofungin resistance by full-length *FKS1* allele replacement in *Candida lusitaniae*

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Objectives: A strain of the opportunistic pathogenic yeast *Candida lusitaniae* was genetically engineered for full-length replacement of the *FKS1* gene encoding the target of echinocandin antifungals in order to assess the impact of *FKS* mutations on echinocandin resistance and reduced echinocandin susceptibility (RES).

Methods: *FKS1* allelic exchange was achieved by transforming *C. lusitaniae* with two DNA fragments covering the entire *FKS1* ORF. Both fragments overlap a 40 bp region where SNPs or small indels of interest were inserted. To target integration at the *FKS1* locus, each DNA fragment was fused with split auxotrophic markers of which complementary truncated parts were previously inserted into the chromosomal regions flanking *FKS1*, allowing selection on minimal medium.

Results: Three SNPs described in the *FKS1* hotspot (HS) regions HS1 or HS2 of clinical isolates of *Candida albicans* were expressed at an equivalent position in *C. lusitaniae* and were confirmed to confer either reduced susceptibility (F641V) or full resistance (S645P and R1361G) to caspofungin. The F659 deletion reported in an *FKS2* allele of *Candida glabrata* and the naturally occurring P660A substitution in *FKS1* of *Candida parapsilosis* were shown to confer a 256-fold and 6-fold increase in caspofungin MIC, respectively, when introduced into an *FKS1* allele of *C. lusitaniae*.

Conclusions: We have successfully developed a *C. lusitaniae* strain for the expression of full-length *FKS1* alleles harbouring known mutations contributing to reduced susceptibility or resistance to caspofungin, thus opening the way for the screening of other *FKS1*/*FKS2* mutations potentially involved in RES.

Introduction

The echinocandins caspofungin, micafungin and anidulafungin are structurally related acylated cyclic hexapeptides having concentration-dependent antifungal activity against *Candida* spp. and *Aspergillus* spp.[1] They are now the first-line treatment for invasive candidiasis in most patients. They act as non-competitive inhibitors of the fungal β(1,3)-glucan synthase, thus disturbing and inhibiting fungal cell wall synthesis.[1] β(1,3)-Glucan synthase consists of a cytoplasmic regulatory protein and membrane-bound Fksp catalytic subunits, which may be encoded according to the fungal species by several *FKS* paralogue genes. In yeast species belonging to the CTG clade of *Candida*, e.g. *Candida albicans*, the *FKS1* gene that encodes the catalytic subunit targeted by echinocandins is essential,[2] whereas in *Candida glabrata*[3] and *Saccharomyces cerevisiae*,[4] the catalytic subunits are encoded by the *FKS1* and *FKS2* genes, which are at least partially functionally redundant. Besides a tolerance phenomenon mediated by calcineurin, high-osmolarity glycerol (HOG) and protein kinase C (PKC) cellular stress response pathways leading to increased chitin synthesis[5] and cell wall remodelling,[6] the main mechanism of echinocandin resistance involves non-synonymous mutations of the *FKS* genes, which decrease the binding affinity of the target protein to the echinocandin inhibitor.[7] The two so-called hotspot (HS) regions, HS1 and HS2, were identified as the regions of the *FKS* genes containing all resistance mutations.[8] However, in clinical strains resistant to echinocandins, the HS1 and HS2 regions are the only regions that have been screened for genetic variability because of the large size of the *FKS* genes (~6 kb). Accordingly, very little information is known about the genetic variability of *FKS* regions outside the HSs and about their possible contribution to echinocandin resistance. The use of next-generation sequencing for clinical molecular diagnosis in the near future is expected to provide extensive information about the overall genetic variability of *FKS* genes. This will raise the question of the contribution of new genetic polymorphisms to the resistance or reduced susceptibility to echinocandins (RES), as reported for a third HS region identified

Table 1. Origin and genotype of *C. lusitaniae* strains

Strain	Origin	Genotype[a]	Reference(s)
CBS 6936	citrus peel	WT	17,30
6936 *trp1*D, *ura3*D	CBS 6936	*trp1*D*[798]*, *ura3*D*[360]*	18
F1 *TRP1*, *ura3*D	6936 *trp1*D, *ura3*D	*IG5*^{0}*FKS1::TRP1*, *ura3*D	this study
F1 *TRP1*, *URA3*	F1 *TRP1*, *ura3*D	*IG5*^{0}*FKS1::TRP1*, *IG3*^{0}*FKS1::URA3*	this study
F1 *trp1*D*3*^{0}, *URA3*	F1 *TRP1*, *URA3*	*IG5*^{0}*FKS1::trp1*D*3*^{0}, *IG3*^{0}*FKS1::URA3*	this study
F1 *trp1*D*3*^{0}, *ura3*D*5*^{0}	F1 *trp1*D*3*^{0}, *URA3*	*IG5*^{0}*FKS1::trp1*D*3*^{0}, *IG3*^{0}*FKS1::ura3*D*5*^{0}	this study

[a]*IG5*^{0}*FKS1* and *IG3*^{0}*FKS1* correspond to the intergenic (IG) region located upstream (5^0) and downstream (3^0) of *FKS1*, respectively. D*3*^{0} and D*5*^{0} mean that the relevant gene was deleted in its 3^0 or in its 5^0 part, respectively.

in the *FKS1* gene of *S. cerevisiae* and *C. glabrata*.[9] Generally, the impact of a missense mutation is demonstrated at the molecular level by expressing an allele containing the suspected mutation in a biological model, such as *S. cerevisiae*. However, because of the large size of the *FKS* genes, the genetic models developed so far have been restricted to the extrachromosomal expression of chimeric *S. cerevisiae FKS* genes recombined with the HS1 region that is suspected of being involved in resistance.[10–13]

In this work, we report the development of a new expression system based on the replacement of the full-length *FKS1* allele in *Candida* (teleomorph *Clavispora*) *lusitaniae*, an opportunistic pathogenic yeast belonging to the CTG clade. Its haploid genome can be genetically engineered easily.[14,15] This recently allowed us to develop an expression system to screen the impact of mutations in the essential gene *ERG11* on azole antifungal susceptibility.[16] Since deletion of *FKS1* in *C. lusitaniae* is not viable, the replacement of *FKS1* was based on the recombination of two linear DNA fragments encompassing the entire ORF and overlapping a small 40 bp region where SNPs or small indels of interest were inserted. Correct integration at *FKS1* was selected by the functional reconstitution of auxotrophic markers implanted in the upstream and downstream regions of *FKS1*. As a proof of concept, we successfully expressed *FKS* mutations in *C. lusitaniae* that have already been described in clinical isolates of *C. albicans*, *C. glabrata* and *Candida parapsilosis*.

Materials and methods

Yeast strains and culture conditions

The different strains used and constructed in this study are listed in Table 1. The *C. lusitaniae* WT strain CBS 6936[17] was used for gene cloning and as a susceptible reference strain for antifungal susceptibility testing. The double auxotrophic mutant 6936 *trp1*D, *ura3*D,[14,18] bearing a 798 bp deletion in the phosphoribosyl-anthranilate isomerase gene and a 360 bp deletion in the orotidine 5′-phosphate decarboxylase gene, respectively, was used for transformation experiments.

Yeasts were cultivated in liquid YPD medium (1% yeast extract/2% peptone/2% dextrose) at 35°C under agitation (250 rpm). In transformation experiments, selection was achieved on synthetic YNBS medium [0.67% yeast nitrogen base without amino acids (Difco Laboratories)/2% glucose/1 M sorbitol]. When needed, uracil (50 mg/L), tryptophan (50 mg/L), 5-fluoroorotic acid (0.8 g/L) and/or 5-fluoroanthranilate (0.3 g/L) were added at the indicated concentration. RPMI 1640 (Sigma), supplemented with 2% glucose and buffered to pH 7.0 with 0.165 M MOPS, was used for antifungal susceptibility testing. Solid media were obtained with 2% agar (Sigma).

Antifungal susceptibility testing

In vitro caspofungin susceptibility was determined using Etest (bioMe´rieux, France) according to the manufacturer's instructions. For each *FKS* mutation expressed in *C. lusitaniae*, caspofungin MIC was determined for eight prototroph clones selected after transformation and confirmed for having acquired the mutation of interest by nucleotide sequencing. For the WT strain and clones transformed with an *FKS1* allele conferring RES (F634V and P642A), MIC was confirmed by the CLSI broth microdilution method[19] using RPMI supplemented with different concentrations of caspofungin (Merck & Co., Kenilworth, NJ, USA), from 0.03 to 16 mg/L.

DNA extraction, PCR amplifications and nucleotide sequencing

Genomic DNA was extracted by a glass bead method for yeast cell disruption, as previously described.[14] The high-fidelity DNA polymerase Pfu (Promega) was used to amplify DNA fragments by PCR for cloning steps and for overlapping PCR. Routine PCRs were performed with GoTaq DNA polymerase or GoTaq Long for PCR targets up to 5 kb (Promega), as recommended by the supplier. All primers used in this study (Table S1, available as Supplementary data at *JAC* Online) were synthesized by Eurofins MWG Operon (Europe). When required, PCR products were sequenced by Eurofins MWG Operon using the BigDye Terminator v3.1 kit (Applied Biosystems).

Molecular constructions and yeast transformations

To construct the DNA cassettes, DNA fragments were generated by PCR using primers having a 15 bp overlap homology at their ends and were assembled and cloned using the In-Fusion™ HD Cloning Kit (Clontech), as previously described.[16] Yeast strains were transformed by electroporation,[15] slightly modified by adding 1 M sorbitol to the lithium acetate buffer, using 1 to 2 μg of transforming DNA per experiment. Prototrophic transformants were selected on YNBS selective medium after 3 days of incubation at 35°C.

Southern blotting

Approximately 10 μg of *C. lusitaniae* DNA was digested with the appropriate restriction enzyme, separated by electrophoresis in a 1% agarose gel and transferred onto nylon membranes (Hybond N+; Roche Molecular Biochemicals). Hybridization was carried out with digoxigenin (DIG)-labelled probes synthesized with a PCR DIG probe synthesis kit (Roche Molecular Biochemicals), as specified by the supplier.

Results

Accurate annotation of the FKS1 gene of C. lusitaniae

Comparison of BLAST sequences using the Fks1 proteins of *C. albicans* and *C. glabrata* allowed identification of the *FKS1* gene

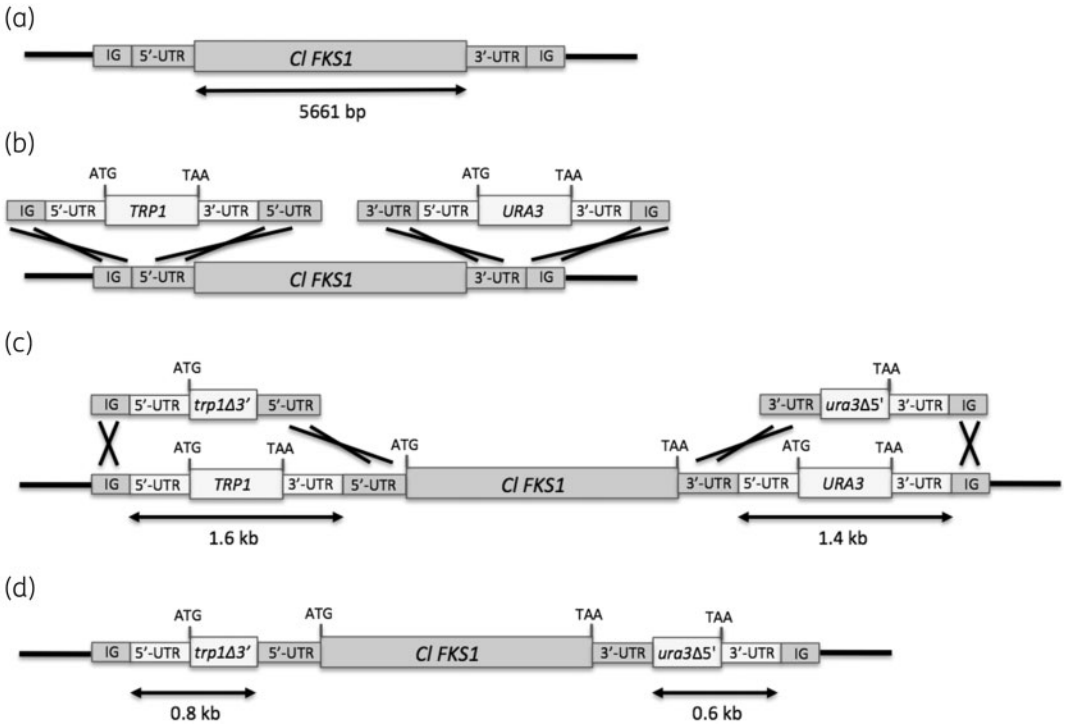

Figure 1. Schematic representation of the molecular modifications performed at the *FKS1* locus of *C. lusitaniae* to obtain the strain F1 *trp1*D3^0, *ura3*D5^0. (a) *FKS1* locus of the strain 6936 *trp1*D, *ura3*D. (b) Successive integration of the *TRP1* and *URA3* genes in the upstream and downstream regions flanking *FKS1*, respectively. (c) Deletion of the 3^0 coding region and the 3^0-UTR of the *TRP1* gene, and of the 5^0-UTR and the 5^0 coding region of the *URA3* gene. (d) Resulting genetic map of the *FKS1* locus of strain F1 *trp1*D3^0, *ura3*D5^0. Genes are not represented at the same scale.

of *C. lusitaniae* (GenBank accession number OVF07437) in the genome of the CBS 6936 strain (GenBank accession number LYUB02000000[17]) on scaffold 13 (locus tag A9F13_13g01430). The intronless ORF of 5661 nt encodes a predicted protein of 1886 amino acids showing 82.9% and 71.8% identity with the orthologous protein of *C. albicans* (strain SC5314, GenBank accession number XP_721429) and of *C. glabrata* (strain CBS 138, GenBank accession number XP_446406), respectively. The genomic map surrounding the *FKS1* gene of strain CBS 6936 was comparable to that of the *C. lusitaniae* strain ATCC 42720 (GenBank accession number NW_003101577, scaffold 2, *FKS1* encompasses CLUG_01697 to CLUG_01702 due to sequence errors). Synteny with *C. albicans* SC5314 was conserved. A gene encoding the putative eukaryotic translation initiation factor 2A is located 2221 bp upstream of the *C. lusitaniae FKS1* gene and a gene encoding a putative a-1,6-mannosyltransferase is located 382 bp downstream.

Design of a C. lusitaniae strain for the homologous expression of full-length mutated FKS1 alleles

An initial difficulty for replacing the *FKS1* allele in *C. lusitaniae* is that the gene is essential, as in *C. albicans*.[2] Attempts to obtain a deletion mutant in *C. lusitaniae* have failed until now, in spite of generally having a good rate of homologous recombination during transformation experiments, from 25% to 100% according to the locus targeted.[14,15] The haploidy of *C. lusitaniae* represents an additional obstacle for working with essential genes.

A second difficulty is the large size of *FKS1*, which requires the generation and manipulation of large DNA fragments *in vitro*; in the present case, 5661 bp for the ORF, plus ~0.4 kb for each 5^0-UTR and 3^0-UTR (where UTR stands for untranslated transcribed region), and 1.5 kb for the functional genetic marker allowing the selection of the yeasts having achieved *FKS1* allele replacement. Using the 'split-marker' strategy developed in *S. cerevisiae*,[20] we conducted a preliminary experiment in *C. lusitaniae* where we successfully repaired a large 990 bp deletion in the *URA3* gene of a mutant strain after transformation with two independent linear DNA fragments. Each fragment carried half of the *URA3* coding region, and had extremities allowing the fragments to first recombine with each other before integrating the locus by recombination with the chromosomal regions flanking the deletion (data not shown). We therefore decided to replace the *FKS1* allele with two recombinant DNA fragments, targeting their integration at the *FKS1* locus by the concomitant reconstitution of two auxotrophic markers in the upstream and downstream intergenic (IG) regions of *FKS1* (Figure 1).

Engineering the C. lusitaniae strain for FKS1 allele replacement

All genetic modifications were performed in the strain 6936 *trp1*D, *ura3*D. First, the *C. lusitaniae TRP1* gene was integrated into the *FKS1* upstream IG region of strain 6936 *trp1*D, *ura3*D (Figure 1a), using a DNA cassette made of the *TRP1* gene with its own 5^0 and 3^0 regulatory regions flanked on both sides by DNA fragments (~400 bp) homologous to the *FKS1* upstream IG region. One transformant, named F1 *TRP1*, *ura3*D, was selected. Second, according to the same principle, the *URA3* gene was introduced into the *FKS1*

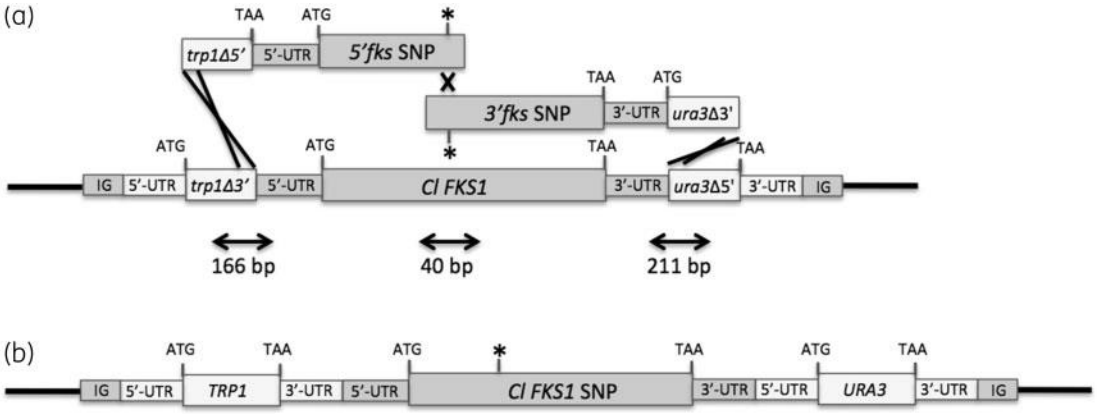

Figure 2. Strategy used to replace the resident *FKS1* allele of the strain F1 *trp1*D3^0, *ura3*D5^0 by an *FKS1* allele bearing a mutation. (a) Replacement was performed by transforming strain F1 *trp1*D3^0, *ura3*D5^0 simultaneously with two linear DNA fragments covering the entire *FKS1* ORF with its regulatory regions, each fragment being fused at one extremity to the complementary part of the genetic marker implanted in the IG region of *FKS1*. (b) Resulting genetic map of the *FKS1* locus after *FKS1* allelic exchange. The size of the overlapping regions that were used to allow crossover to take place is indicated. An SNP is represented by an asterisk.

downstream IG region of the strain F1 *TRP1*, *ura3*D (Figure 1b), leading to the selection of a prototrophic transformant F1 *TRP1*, *URA3*. Third, the 3^0 half part of the *TRP1* gene was removed from the strain F1 *TRP1*, *URA3* by replacing *TRP1* with a DNA cassette made of the 5^0 half part of *TRP1* flanked by DNA fragments homologous to the *FKS1* upstream IG region (Figure 1c). Tryptophan auxotrophic clones were selected by 5-fluoroanthranilic acid resistance and one of them, named F1 *trp1*D3^0, *URA3*, was retained for further manipulation. Fourth, using the same strategy, the 5^0 half part of the *URA3* gene was removed from the strain F1 *trp1*D3^0, *URA3*, to yield the strain F1 *trp1*D3^0, *ura3*D5^0, which was selected among the clones resistant to 5-fluoroorotic acid (Figure 1d).

At each step of the molecular modifications, correct targeted integration of the different DNA cassettes was verified by PCR and nucleotide sequencing. The genotype of the selected transformants (Table 1) was also verified by Southern blotting analysis of EcoRV-digested genomic DNA using the 3^0*TRP1*, 5^0*URA3*, 3^0*URA3* and *FKS1* probes (Figure S1) to exclude clones exhibiting any additional or incorrect integration events. Finally, we verified that the molecular modifications achieved in the strain F1 *trp1*D3^0, *ura3*D5^0 did not alter its susceptibility to echinocandin drugs; both CLSI and Etest methods gave a caspofungin MIC of 0.125 mg/L, which was identical to that of the WT strain CBS 6936. Accordingly, strain F1 *trp1*D3^0, *ura3*D5^0 was used in the rest of the study for all *FKS1* allele replacement experiments.

Expression of full-length mutated FKS1 alleles in C. lusitaniae

Replacement of the resident WT *FKS1* allele of the strain F1 *trp1*D3^0, *ura3*D5^0 by an *FKS1* allele containing any SNP of interest was achieved by co-transformation with two recombinable DNA fragments overlapping the *FKS1* gene and its regulatory sequences. To ensure their correct targeted integration at the *FKS1* locus, each fragment was fused to the complementary part of one of the auxotrophic markers inserted in the upstream and downstream regions flanking *FKS1*. To allow crossover to take place and to reconstitute functional *TRP1*, *FKS1* and *URA3* genes, the transforming DNA fragments contained an overlapping region of 166 bp in the *TRP1* region or 211 bp in the *URA3* region, and 40 bp in the *FKS1* region where the SNP was introduced (Figure 2). When the two DNA fragments were co-transformed into the F1 *trp1*D3^0, *ura3*D5^0 strain, they recombined *in cellulo* to integrate the *FKS1* locus, thereby allowing selection on YNBS medium of prototroph transformant yeast cells that had integrated the new *FKS1* allele containing the SNP of interest. Selected clones were then screened for the presence of the point mutation by DNA sequencing and their caspofungin susceptibility pattern was determined. According to the position of the SNP of interest, 40% to 75% of the transformants tested carried the desired point mutation. The other clones were prototrophic, but the molecular events leading to integration of the DNA fragments took place outside the overlapping region where the mutation was introduced.

Proof of concept: impact of FKS mutations known to be associated with in vitro caspofungin resistance or RES

Our genetic model was first challenged with three mutations previously reported in the *FKS1* alleles of clinical isolates of *C. albicans*, resulting in amino acid changes phenylalanine to valine at position 641 (F641V) in HS1, serine to proline at position 645 (S645P) in HS1 and arginine to glycine at position 1361 (R1361G) in HS2 (Figure 3).

Expression of the mutated *FKS1* allele bearing the F634V mutation (equivalent to F641V) in the *C. lusitaniae* strain F1 *trp1*D3^0, *ura3*D5^0 conferred an intermediate level of resistance to caspofungin (MIC" 1.5 mg/L), while introduction of S638P (equivalent to S645P) or R1352G (equivalent to R1361G) mutations conferred full resistance (MICs ≥32 mg/L; Figure 4).

Our model was then challenged with a mutation reported in *C. glabrata*. Unlike other *Candida* species, mutations conferring resistance to caspofungin also affect *FKS2*, with a 2-fold greater frequency than that of *FKS1* mutations. Accordingly, we introduced a mutation specific to the *FKS2* gene of *C. glabrata* into the *FKS1* gene of *C. lusitaniae* at the equivalent position. The mutation consisted of the deletion of a phenylalanine at position 659 (Figure 3). Expression of the *FKS1* F634del allele in *C. lusitaniae* resulted in an increase in caspofungin MIC from 0.125 to 32 mg/L (Figure 4).

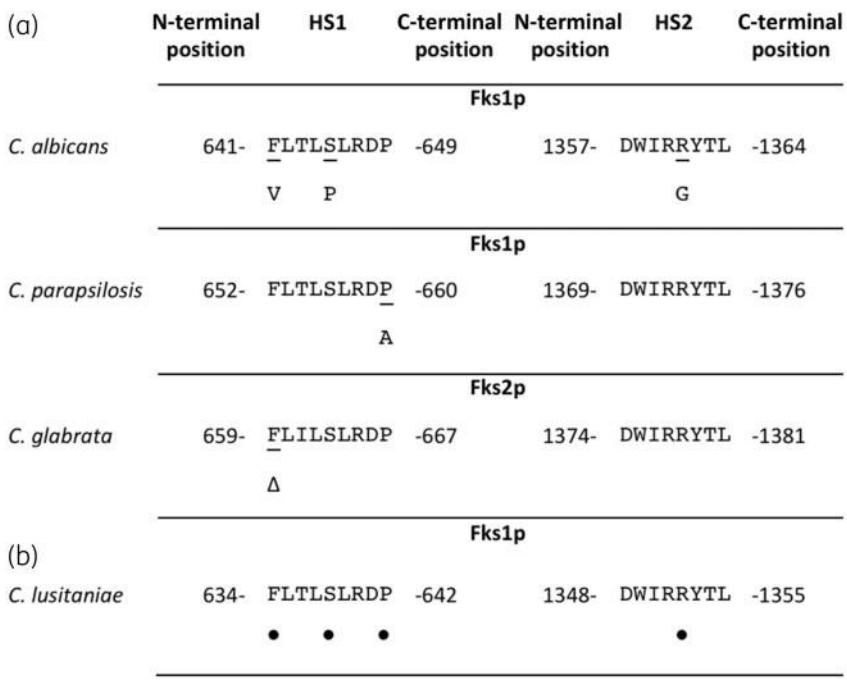

Figure 3. HS1 and HS2 amino acid sequences of Fks proteins. (a) Fks1p from *C. albicans* and *C. parapsilosis* and Fks2p from *C. glabrata*. The amino acid substitutions expressed in Fks1p of *C. lusitaniae* are shown below the underlined WT residue. A deletion is represented by D. Numbers flanking the HS sequence correspond to the positions of the first and last amino acids of the HS relative to the whole Fksp sequence. (b) Corresponding positions of the amino acids that were substituted in the *C. lusitaniae* Fks1p in this study are indicated by filled circles.

Lastly, our system was tested with the naturally occurring P660A polymorphism reported in the *FKS1* alleles of all isolates of the *C. parapsilosis* species complex.[10] This mutation was shown to be responsible for higher MICs of all echinocandins for *C. parapsilosis*, compared with other *Candida* species (Figure 3).[10] Introduction of the equivalent mutation P642A into the *C. lusitaniae FKS1* allele gave a 6-fold increase in caspofungin MIC (0.125 to 0.75 mg/L; Figure 4). This confirmed the role of the P660A substitution in RES and showed that this reduced susceptibility can be transferred to other species of the CTG clade.

Discussion

The main molecular mechanism leading to resistance to echinocandins or RES is mediated by non-synonymous mutations affecting the Fksp subunits of b(1,3)-glucan synthase.[2,8] The resistance mutations known so far are located within two HS regions.[7,8] However, echinocandins are non-competitive inhibitors and do not interact directly with the catalytic site. This suggests that mutations outside HS regions can contribute to resistance and RES, as already shown in *S. cerevisiae*.[9]

One way to demonstrate the involvement of a mutation in resistance or RES is to express the mutated allele and the WT allele from which it derives in cells having an isogenic background and to compare echinocandin MICs. The *S. cerevisiae* expression system was based on an *fks1* deletion mutant that was complemented by the expression of a WT or mutated *FKS1* allele cloned in a 2l ori episomal plasmid.[8] Nucleotide modifications were introduced into the *FKS* allele as point mutations, or as a chimeric construction involving a *S. cerevisiae FKS* allele recombined with the HS region of an *FKS* allele suspected of conferring resistance to a clinical isolate.[8,9,11–13] *FKS1* expression in *S. cerevisiae* was facilitated by the fact that an *fks1*D mutant is viable owing to the presence of the functionally redundant paralogue gene *GSC2* (alias *FKS2*).[21] Functional redundancy has also been reported in the related opportunistic pathogenic yeast species, *C. glabrata*,[3,22] whose prevalence of clinical isolates resistant to echinocandins has increased in recent years.[7,23] This made allelic replacement possible at the chromosomal level in *C. glabrata* by integrating one *FKS* copy fused to an auxotrophic marker to select the transformants.[22] However, interpreting the effect of an *FKS* mutation on RES in *C. glabrata* can be hampered by the presence of the WT *FKS* paralogue.[3]

Most of the invasive yeast infections are caused by *Candida* species belonging to the CTG clade, of which *C. albicans* is the most prevalent. This phylogenic group of yeasts is characterized by the fact that *FKS1* is an essential gene whose deletion is not compensated by *FKS2*.[24] Engineering *C. albicans* to express a mutated *FKS1* allele was achieved with an integrative plasmid carrying a copy of the mutated *FKS1* allele designed to recombine to the chromosomal *FKS1* locus.[8] However, the diploidy of *C. albicans* can constitute an issue for testing echinocandin resistance because of the difficulty of obtaining homozygous mutants.

To overcome the problems resulting from *FKS* redundancy or diploidy, we developed in this work a strain of the CTG clade haploid yeast species *C. lusitaniae* as an alternative model for full-length *FKS1* replacement. Several studies previously showed that echinocandin MICs were from 4-fold to 8-fold higher for *C. lusitaniae* than for *C. albicans*.[25,26] However, most clinical isolates exhibit low echinocandin MICs with more than 90% of the isolates being

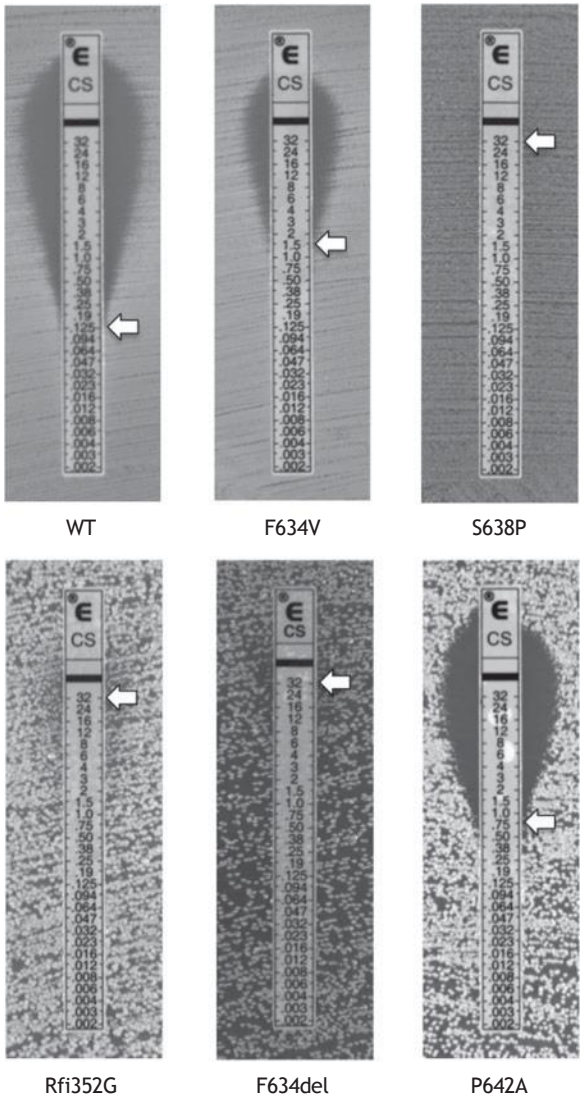

Figure 4. Etest determination of caspofungin susceptibility of the *C. lusitaniae* strain F1 *trp1*Δ*3*^{0} , *ura3*Δ*5*^{0} expressing a WT Fks1 protein and expressing Fks1 proteins containing different amino acid substitutions. For each *FKS1* allele expressed, caspofungin MIC was determined for eight independent clones after having controlled at the molecular level that they had integrated the SNP of interest. The MIC is indicated by a white arrow.

inhibited by concentrations lower or equal to 1 mg/L for anidulafungin and 0.5 mg/L for caspofungin and micafungin.[27] Here, we used a strain derived from the type strain CBS 6936 of *C. lusitaniae*,[17] which has a low caspofungin MIC of 0.125 mg/L and whose susceptibility remained unchanged after the genetic modifications performed in both IG regions flanking the *FKS1* gene. We used caspofungin because of its availability in powder form that allowed MIC determination to be performed both by the CLSI microdilution method and by Etest, notably in the case of RES.

Our expression system was challenged with known mutations reported and validated from different genes (*FKS1* or *FKS2*), different regions (HS1 or HS2) and different *Candida* species. When introduced at an equivalent position into the *FKS1* gene of *C. lusitaniae*, the *FKS1* S645P and R1361G of *C. albicans*,[8] the latter mutation being initially discovered in the *FKS1* R1361G of *Candida krusei*,[8] and the *FKS2* F659del of *C. glabrata*[28] conferred high caspofungin MICs, while the *FKS2* F641V of *C. glabrata*[29] and the *FKS1* P660A of *C. parapsilosis*[10] conferred RES. Most importantly, the caspofungin MICs conferred to *C. lusitaniae* were fully comparable to those for the clinical isolates from which they were originally reported. In conclusion, we have successfully developed a *C. lusitaniae* strain for the expression of a single chromosomal copy of an essential *FKS1* essential gene that allows any new genetic polymorphism involved in echinocandin resistance or RES in *Candida* species to be screened.

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Transparency declarations

None to declare.

Author contributions

I. A., C. C. and T. N. designed the experiments. C. C., V. F.-O. and N. B. performed the experiments. I. A., C. C., N. B. and T. N. analysed the results. I. A. and T. N. wrote the manuscript.

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