Molecular Resistance Mechanisms in Rare Yeast

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Background

The resistance genes involved in antifungal resistance in *Candida spp.* were initialy studied and characterized in the most common pathogenic species *Candida albicans* or *Candida glabrata*. Antifungal resistance in rare species is less understood. *Candida inconspicua*, *Candida rugosa,* and *Candida ciferrii* are an emerging challenge for modern medicine because infections caused by these pathogens led to high mortality rates, partially due to their high antifungal resistance levels. The underlying molecular mechanisms behind are not fully understood and were therefore the objective of this study. We aim to characterize their primary resistance genes (*ERG11* and *FKS1*) and find SNPs (single nucleotide polymorphisms) that led to amino acid changes that impact on antifungal resistances.

Method

Reference strains of *Candida inconspicua* (CBS180), *Candida rugosa* (CBS613), and *Candida ciferrii* (CBS4856) were send to collaborators in the CRG (Center of genomic regulation, Toni Gabaldón group) in Barcelona. Whole genome sequencing was performed and the genes involved in resistance (*ERG11* and *FKS1*) were characterized in respective reference strains.

	Whole Genome length	*ERG11* gene length	*FKS1* gene length	*ERG11* protein length	*FKS1* protein length
Candida inconpicua (CBS180)	11Mb	1455bp	5616bp	812 aminoacids	1871 aminoacids
Candida rugosa (CBS613)	13Mb	1377bp	5673bp	458 aminoacids	1890 aminoacids
Candida ciferrii (CBS4856)	20Mb	1617bp	5733bp	538 aminoacids	1910 aminoacids

Table 1. Characteristics of reference strains.

Bit-Score	E Value	Grade	Hit start	Hit end	Name	Description	Sequence Le...	Topology	Molecule Type	# Sequences	% Pairwise I...
287.73	4.30e-89	62.1%	246	500	XP_015469227	Lanosterol 14-alpha demethylase [Debaryomyces fabryi]	262	linear	AA	2	58.1%
279.256	1.23e-85	59.4%	246	414	EMG46317	Lanosterol 14-alpha demethylase [Candida maltosa Xu...	169	linear	AA	2	75.8%
276.559	1.39e-84	60.0%	246	414	ADI76636	lanosterol 14-alpha-demethylase [Candida albicans]	169	linear	AA	2	76.9%
276.559	1.35e-84	60.0%	246	414	ADI76627	lanosterol 14-alpha-demethylase [Candida albicans]	169	linear	AA	2	76.9%
276.559	1.31e-84	60.0%	246	414	ADI76624	lanosterol 14-alpha-demethylase [Candida albicans]	169	linear	AA	2	76.9%
276.559	1.38e-84	60.0%	246	414	ADI76584	lanosterol 14-alpha-demethylase [Candida albicans]	169	linear	AA	2	76.9%
276.559	1.44e-84	60.0%	246	414	ADI76577	lanosterol 14-alpha-demethylase [Candida albicans]	169	linear	AA	2	76.9%
278.1	2.43e-85	60.9%	246	414	PVH23354	lanosterol 14-alpha demethylase [[Candida] haemulonis]	169	linear	AA	2	78.7%
282.722	4.64e-87	61.2%	246	414	PSK75255	lanosterol 14-alpha demethylase [[Candida] auris]	169	linear	AA	2	79.3%
283.878	1.62e-87	61.5%	246	414	PIS56902	lanosterol 14-alpha demethylase [[Candida] auris]	169	linear	AA	2	79.9%
283.878	1.62e-87	61.5%	246	414	PIS55918	lanosterol 14-alpha demethylase [[Candida] auris]	169	linear	AA	2	79.9%
276.174	3.58e-86	59.7%	105	273	XP_007375289	hypothetical protein SPAPADRAFT_61116 [Spathaspor...	169	linear	AA	2	76.4%
279.256	9.16e-86	60.6%	246	414	PVH15685	hypothetical protein CXQ87_003531 [[Candida] duobu...	169	linear	AA	2	78.1%
283.108	3.15e-87	61.5%	246	414	XP_024711630	hypothetical protein C7M61_004938 [[Candida] pseud...	169	linear	AA	2	79.9%
276.559	1.41e-84	60.0%	246	414	AAW50593	Erg11p [Candida albicans]	169	linear	AA	2	76.9%
276.559	1.42e-84	60.0%	246	414	AAW50592	Erg11p [Candida albicans]	169	linear	AA	2	76.9%
283.878	1.59e-87	61.5%	246	414	XP_018166924	Erg11p [[Candida] auris]	169	linear	AA	2	79.9%
276.174	1.50e-84	60.0%	246	414	AIX03622	Erg11 [Candida albicans]	169	linear	AA	2	76.9%
276.174	1.50e-84	60.0%	246	414	AIX03621	Erg11 [Candida albicans]	169	linear	AA	2	76.9%

Figure 2 Sequences was blasted against the NCBI database for confirmation.

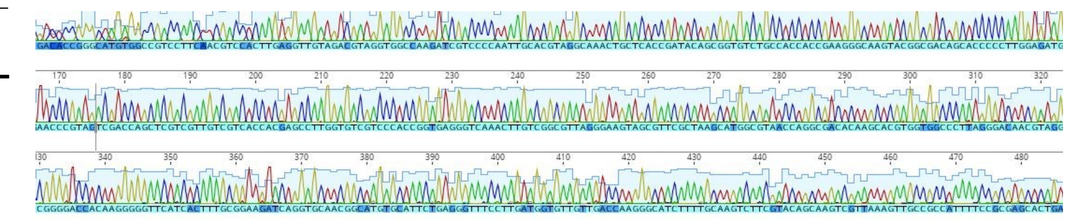

Figure 1. SANGER sequencing was performed for *ERG11* and *FKS1* of our clinical isolate using primers designed on the basis of our reference strains.

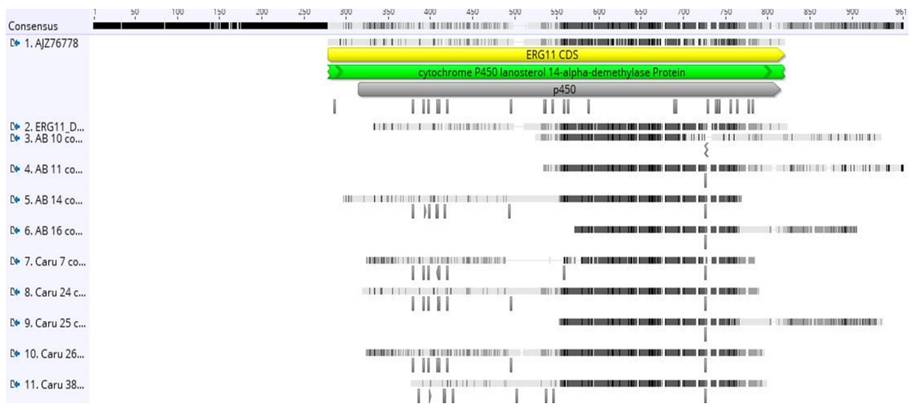

Figure 3. Alignment with reference for confirmation

Results

Figure 4. ERG11 alignment of *Candida rugosa* isolates sequences for determination of SNPs.

Figure 5. *FKS1* alignment of *Candida inconspicua* isolates sequences for determination of SNPs.

Species (*ERG11*)	N	1 Hotspot	2 Hotspot	3 Hotspot
C. albicans (AJZ76778)	1	QAFYKFD	TYMEDHG	LSVDYGFGGIIV
C. rugosa (consensus)	24	-AFYKFD	TYMEDHG	LG-EYGFGGIIV
C. ciferrii (consensus)	8	GAFYKFE	KYLKDNG	MSSEYGFGGIII

Table 2. ERG11 mutation hotspots, in red SNPs.

Species (*FKS1*)	N	1 Hotspot	2 Hotspot
C. albicans (ADB43260)	1	FLTLPLRDP	DWIRRYTL
C. rugosa (consensus)	24	FLTLSLRDP	DWVRRYTL
C. ciferrii (consensus)	8	FLTLSIRDP	GWLQRCIL
C. inconspicua (consensus)	160	FLILSIRDP	DWIRRYTL

Table 3. FKS1 mutation hotspots, in red SNPs.

Conclusion

Erg11 and *FKS1* are the genes that code for the target of the most commonly used antifungal families azoles and echinocandins. Azoles binds to lanosterol 14-alpha-demethylase an important enzyme in the ergosterol pathway wich is a key molecule in the cell menbrane. On the other hand, *FKS1* codes for β-1,3-glucan synthase wich produces the main component β-D-glucan of the fungal cell wall. The main resistance mechanism in *Candida albicans* are mutation in these genes and drug transporters. Mutations in these genes, particularly hotspot (hs) regions of these genes lead to a lower antifungal susceptibility. Other mechanishm are described in azole resistance such as up regulation of the *ERG11* gene and up regulation of membran transporters also known as *efflux pumps*. *ERG11* mutation at position D153E (*Erg11* HS1) were perviously associated with low azole susceptibility (*Marichal et al. 1999*). The mutation H283N has not been described yet, but other aminoacid substitution (AA) in the same position (e.g., H283D, H283R– *ERG11* HS2) were previously associated with fluconazole resistance (*Goldman et al. 2004*, *Chau et al. 2004*). In addition some of mutations found in *Erg11* HS3 are located at the same position as other already describes AA, but only D446N was associated with fluconazole resistance (*Morio et al. 2009)*. Mutations at position P649 (HS1) in the *FKS1* gene were previously linked with a moderate increase in echinocandin resistance. Most described mutations are located in *FKS1 HS1,* but mutations in FKS1 HS2 occuring at positions W1358 and R1361 were linked with a variable decrease of echinocandin susceptibility (*Lackner et al. 2014)*. Nevertheless, the mutations occuring in FKS1 HS2 identified in this study have not yet been linked to echinocandin resitance The high intraspecific conservation of these AA show their key role for protein function. As these organisms are resistance certain drug classes the selection pressure on these genes is limited. The interspecific variation of these protein could might be linked with intrinsic resistance that is a species specific feature. The role of these intrinsic resistances in adaptation to certain enviromental niches is described in literature (*Bretagne et al. 2017*). The SNPs found could explain the interspecific variation and the difference in susceptibility to azoles and echinocandins although the impact of these SNPs remains to be evaluated in a protein model and mutant strains.