

Molecular Resistance Mechanisms in Rare Yeast

Antonio Pérez-Hansen¹, Cornelia Lass-Flörl¹ and Michaela Lackner¹ #Rare yeast study group ¹Division of Hygiene and Medical Microbiology, Medical University of Innsbruck, Austria.

Background

The resistance genes involved in antifungal resistance in *Candida spp.* were initially 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

Results

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 ERG11 gene FKS1 gene ERG11 protein FKS1 protein

A A A A A A A A A A A A A A A A A A A		length	length	length	length	length	
	Candida inconpicua	11Mb	1455bn	5616bp	812 aminoacide	1871 aminoacide	
A. A. S.	(CBS180)	TTIMD	110000	001055		10/1 ammoacius	
Lilli Jan	Candida rugosa	13Mb	1377bp	5673bp	458 aminoacids	1890 aminoacide	
in a	(CBS613)	TOIND		F	-Jo annio acius	1000 ammoacius	
	Candida ciferrii	20Mb	1617bp	5733bp	538 aminoacids	1910 aminoacids	
Lilling	(CBS4856)		•		550 annio dellas	1510 animodelu5	

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

Table 1. Characteristics of reference strains.

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Bit-Score	E Value	Grade	Hit start	Hit end		Name	Description w	Sequence Le	Topology	Molecule Type	# Sequences	% Pairwise I	%:	D
287.73	4.30e-89	62.1%	246	500	3	XP_015469227	Lanosterol 14-alpha demethylase [Debaryomyces fabryi]	262	linear	AA	2	58.1%	57.E	
279.256	1.23e-85	59.4%	246	414	3	EMG46317	Lanosterol 14-alpha demethylase [Candida maltosa Xu	169	linear	AA	2	75.8%	75.7	
276.559	1.39e-84	60.0%	246	414	3	AD176636	lanosterol 14-alpha-demethylase [Candida albicans]	169	linear	AA	2	76.9%	76.5	
276.559	1.35e-84	60.0%	246	414	3	ADI76627	lanosterol 14-alpha-demethylase [Candida albicans]	169	linear	AA	2	76.9%	76.5	
276.559	1.31e-84	60.0%	246	414	50	AD176624	lanosterol 14-alpha-demethylase [Candida albicans]	169	linear	AA	2	76.9%	76.5	
276.559	1.38e-84	60.0%	246	414	39	ADI76584	lanosterol 14-alpha-demethylase [Candida albicans]	169	linear	AA	2	76.9%	76.5	87
276.559	1.44e-84	60.0%	246	414	32	AD176577	lanosterol 14-alpha-demethylase [Candida albicans]	169	linear	AA	2	76.9%	76.5	C
278.1	2.43e-85	60.9%	246	414	3	PVH23354	lanosterol 14-alpha demethylase [[Candida] haemulonis]	169	linear	AA	2	78.7%	78.7	
282.722	4.64e-87	61.2%	246	414	50	PSK75255	lanosterol 14-alpha demethylase [[Candida] auris]	169	linear	AA	2	79.3%	79.3	C
83.878	1.62e-87	61.5%	246	414	3	PIS56902	lanosterol 14-alpha demethylase [[Candida] auris]	169	linear	AA	2	79.9%	79.5	
283.878	1.62e-87	61.5%	246	414	50	PIS55918	lanosterol 14-alpha demethylase [[Candida] auris]	169	linear	AA	2	79.9%	79.5	C
276.174	3.58e-86	59.7%	105	273	3	XP_007375289	hypothetical protein SPAPADRAFT_61116 [Spathaspor	. 169	linear	AA	2	76.4%	76.3	
279.256	9.16e-86	60.6%	246	414	3	PVH15685	hypothetical protein CXQ87_003531 [[Candida] duobu	. 169	linear	AA	2	78.1%	78.1	C
283.108	3.15e-87	61.5%	246	414	3	XP_024711630	hypothetical protein C7M61_004938 [[Candida] pseud	169	linear	AA	2	79.9%	79.5	
276.559	1.41e-84	60.0%	246	414	39	AAW50593	Erg11p [Candida albicans]	169	linear	AA	2	76.9%	76.5	C
276.559	1.42e-84	60.0%	246	414	3	AAW50592	Erg11p [Candida albicans]	169	linear	AA	2	76.9%	76.5	
283.878	1.59e-87	61.5%	246	414	3	XP_018166924	Erg11p [[Candida] auris]	169	linear	AA	2	79.9%	79.5	C
276.174	1.50e-84	60.0%	246	414	39	AIX03622	Erg11 [Candida albicans]	169	linear	AA	2	76.9%	76.5	
276.174	1.50e-84	60.0%	246	414	3	AIX03621	Erg11 [Candida albicans]	169	linear	AA	2	76.9%	76.5	1

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



Figure 3. Alignment with reference for confirmation

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nsus	JDI		VMNPEVVPGTTYVVPKGHHVEVSPGYAM	SERY FPNADK FDPHRWI Consensu	IS	SRRYLPQLTF	TSSFAPEKGIDRYESYLLWVAVFAA	K S S Y F F L I L S I R D P I R N L S
ndida albicans (AJZ76778)		ETYEDLOK LPSVNNTIKETERMHMPLHSIFRK ERG11 CDS cytochrome P450 lanosterol 14-alpha-demeth		SERVEDNPEDEDPTRWI	DB43260	SRRY IASQTF	TANYIKLKGLDMWMSYLLWFLWFLA	KLVESYFFETEPLRDPIRNES GSC1CDS B-glucan synthase catalytic subunit Protein
511_DIURU tein 9.25 CDRU tein 7974 tein AB 10 tein AB 11 tein AB 14 tein AB 14 tein Caru 7 otein Caru 24 otein Caru 25 otein Caru 25 otein Caru 38 otein Caru 38 otein CBS 613 otein CBS 613 otein LL1158 otein NRZ BK 495 otein NRZ BK 495 otein PEU 866 otein PEU 868 otein PEU 868		P450 SYEDLOKMPLYNNTIK ETERMHMPLHSIFRK LYEDLOKMPLYNNTIK ETERMHMPLHLIFRK LYEDLOKMPLYNNTIK ETERMHMPLHLIFRK	VMNPEVVPGTTYVVPKGHHVEVSPGYAMI VMNPEVVPGTTYVVPKGHHVEVSPGYAMI VMNPEVVPGTTYVVPKGHHVEVSPGYAMI VMNPEVVPGTTYVVPKGHHVEVSPGYAMI VMNPEVVPGTTYVVPKGHHVEVSPGYAMI VMNPEVVPGTTYVVPKGHHVEVSPGYAMI VMNPEVVPGTTYVVPKGHHVEVSPGYAMI VMNPEVVPGTTYVVPKGHHVEVSPGYAMI VMNPEVVPGTTYVVPKGHHVEVSPGYAMI VMNPEVVPGTTYVVPKGHHVEVSPGYAMI VMNPEVVPGTTYVVPKGHHVEVSPGYAMI VMNPEVVPGTTYVVPKGHHVEVSPGYAMI VMNPEVVPGTTYVVPKGHHVEVSPGYAMI VMNPEVVPGTTYVVPKGHHVEVSPGYAMI VMNPEVVPGTTYVVPKGHHVEVSPGYAMI VMNPEVVPGTTYVVPKGHHVEVSPGYAMI VMNPEVVPGTTYVVPKGHHVEVSPGYAMI VMNPEVVPGTTYVVPKGHHVEVSPGYAMI VMNPEVVPGTTYVVPKGHHVEVSPGYAMI	C* 2. Proj SERYFPNADKFDPHRWI C* 3. Proj SERYFPNADKFDPHRWI C* 5. Proj SERYFPNADKFDPHRWI C* 5. Proj SERYFPNADKFDPHRWI C* 6. Proj SERYFPNADKFDPHRWI C* 7. Proj SERYFPNADKFDPHRWI C* 9. Proj SERYFPNADKFDPHRWI C* 10. Pri SERYFPNADKFDPHRWI C* 10. Pri SERYFPNADKFDPHRWI C* 10. Pri SERYFPNADKFDPHRWI C* 11. Pri SERYFPNADKFDPHRWI C* 13. Pri SERYFPNADKFDPHRWI C* 13. Pri SERYFPNADKFDPHRWI C* 14. Pri SERYFPNADKFDPHRWI C* 15. Pri SERYFPNADKFDPHRWI C* 15. Pri SERYFPNADKFDPHRWI C* 15. Pri SERYFPNADKFDPHRWI C* 16. Pri SERYFPNADKFDPHRWI C* 17. Pri SERYFPNADKFDPHRWI C* 18. Pri SERYFPNADKFDPHRWI C* 19. Pri SERYFPNADKFDPHRWI <th>tein 9.16 CDIN FKS1 H1 tein 9 FKS1 H1 tein 12.73 CDIN FKS1 H1 tein 14ANR23920 FKS1 H1 tein 14ANR23920 FKS1 H1 tein 110.10 FKS1 H1 tein 1175 FKS1 H1 tein 1282 FKS1 H1 tein 1394 FKS1 H1 otein 1408 FKS1 H1 otein 1618 FKS1 H1 otein 4001 FKS1 H1 otein 8760 FKS1 H1 otein 1222 FKS1 H1 otein 12573 FKS1 H1 otein 14098 FKS1 H1 otein 14126 FKS1 H1 otein 14233 FKS1 H1</th> <th>SRRYLPQLTF SRRYLPQLTF SRRYLPQLTF SRRYLPQLTF SRRYLPQLTF SRRYLPQLTF SRRYLPQLTF SRRYLPQLTF SRRYLPQLTF SRRYLPQLTF SRRYLPQLTF SRRYLPQLTF SRRYLPQLTF SRRYLPQLTF SRRYLPQLTF SRRYLPQLTF SRRYLPQLTF SRRYLPQLTF SRRYLPQLTF SRRYLPQLTF</th> <th>TSSFAPLKGIDRYLSYLLWVAVFAA TSSFAPLKGIDRYLSYLLWVAVFAA</th> <th>K S S Y F I S R P R I S K S S Y F I S R P R I S K S S Y F I S R P R I S K S S Y F I S R P R I S K S S Y F I S R P R I S K S S Y F I S I D P R I S K S S Y F I S I D P R I S K S S Y F I S I D P R I S K I S X D P R I S K I S</th>	tein 9.16 CDIN FKS1 H1 tein 9 FKS1 H1 tein 12.73 CDIN FKS1 H1 tein 14ANR23920 FKS1 H1 tein 14ANR23920 FKS1 H1 tein 110.10 FKS1 H1 tein 1175 FKS1 H1 tein 1282 FKS1 H1 tein 1394 FKS1 H1 otein 1408 FKS1 H1 otein 1618 FKS1 H1 otein 4001 FKS1 H1 otein 8760 FKS1 H1 otein 1222 FKS1 H1 otein 12573 FKS1 H1 otein 14098 FKS1 H1 otein 14126 FKS1 H1 otein 14233 FKS1 H1	SRRYLPQLTF SRRYLPQLTF SRRYLPQLTF SRRYLPQLTF SRRYLPQLTF SRRYLPQLTF SRRYLPQLTF SRRYLPQLTF SRRYLPQLTF SRRYLPQLTF SRRYLPQLTF SRRYLPQLTF SRRYLPQLTF SRRYLPQLTF SRRYLPQLTF SRRYLPQLTF SRRYLPQLTF SRRYLPQLTF SRRYLPQLTF SRRYLPQLTF	TSSFAPLKGIDRYLSYLLWVAVFAA TSSFAPLKGIDRYLSYLLWVAVFAA	K S S Y F I S R P R I S K S S Y F I S R P R I S K S S Y F I S R P R I S K S S Y F I S R P R I S K S S Y F I S R P R I S K S S Y F I S I D P R I S K S S Y F I S I D P R I S K S S Y F I S I D P R I S K I S X D P R I S K I S
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Table 2. ERG11 mutation hotspots, in red SNPs.

Table 3. FKS1 mutation hotspots, in red SNPs.

Conclusion

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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 membrane. 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 occuring 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 resistance The high intraspecific conservation 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 descri