Characterization of *Mucor circinelloides* sterol-14-α-demethylases expressed in *Saccharomyces cerevisiae*

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Aim

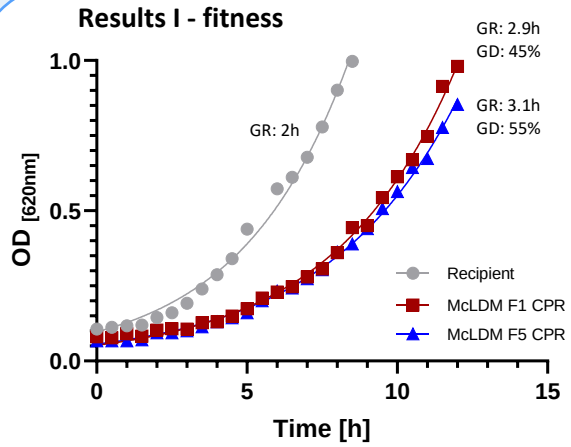
To understand the intrinsic resistance of mucormycetes to the short-tailed azoles, a property which limits treatment options.

Hypothesis: Amino acid substitutions **Y129F** and **V293A** in the ligand-binding pocket (LBP) of the azole target sterol-14α-demethylase (SDM) F5 isoform confer intrinsic resistance to short-tailed azole drugs such as voriconazole (VRC) but susceptibility to Posaconazole (PSC).

Table 1: Fold MIC change versus host strain for amphotericin B (AmB), short-(VRC) mid-(isavucinazole IVU) and long-tailed (PSC) azoles. = : < 5x; ↑: 10-20x; ↑↑↑: >100x

	AmB	VRC	IVU	PSC
McLDM F1 CPR	=	↑	↑	=
McLDM F5 CPR	=	↑↑↑	↑↑↑	=

Results I - fitness



Expression of the recombinant Mc*SDM* isoform and/or *CPR* genes appears to have a fitness cost

Fig. 1: Growth kinetics of recombinant strains using glucose as carbon source i.e. expression of the native *ScERG11* is blocked. GR: Growth rate (h), GD: growth rate reduction compared to the host strain (%).

Results III – sterol pathway inhibition

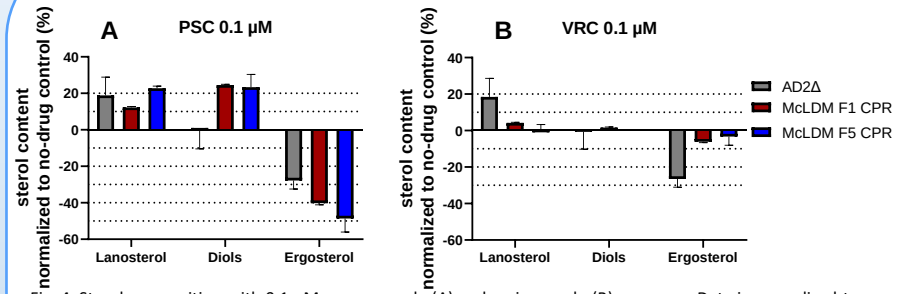


Fig. 4. Sterol composition with 0.1 µM posaconazole (A) and voriconazole (B) exposure. Data is normalized to the sterol composition of control samples without azole treatment.

Lanosterol ↑
Toxic Diols: ↑
Ergosterol: ↓
Growth inhibition: ✓

Lanosterol ↑
Toxic Diols: →
Ergosterol: →
Growth inhibition: X

Methods

Mucor circinelloides SDM homologs and their cognate NADPH-cytochrome-P450-reductase (CPR) were **expressed in an azole hypersensitive *Saccharomyces cerevisiae* strain.** The **hexhistidine tagged** gene of interest is overexpressed due to a pdr1-3 gain-of-function mutation causing constitutive expression from the the *PDR5* promotor [(1)]. The native ERG11 gene was also modified using the *Gal* promoter it galactose inducible and glucose suppressed.

Strain characterization:

I. Fitness (growth kinetics)
II. Protein expression (SDS-PAGE & Western blots)
III. Sterol pathway inhibition (sterol patterns, GCMS)

Results II – protein expression

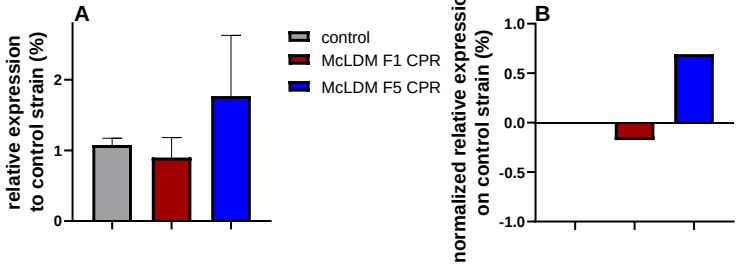
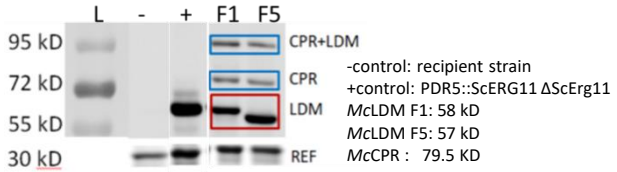


Fig. 2: Expression of +control, LDM F1/ LDM F5 CPR normalized to loading controls and to the +control.



-control: recipient strain
+control: PDR5::ScERG11 ΔScErg11
*Mc*LDM F1: 58 kD
*Mc*LDM F5: 57 kD
*Mc*CPR : 79.5 KD

Expression level in crude membranes: F5 > +control > F1

Fig. 3: Western blot using anti-6xHis HRP-antibody decoration and ECL of crude membrane fractions.

Interpretation and Conclusion

Wild type *M. circinelloides* SDM isoforms have been functionally expressed in *S. cerevisiae*. The strain expressing the **F5 isoform has a major fitness advantage during exposure to short- and medium- but not long- tailed azoles**. In the absence of azole drugs the overexpression of recombinant SDMs and their cognate reductase has a detectable fitness cost, causing a modest growth rate reduction in both recombinant strains. While 0.1 µM PCZ blocked ergosterol biosynthesis at both *Mc*SDM isoforms, **0.1 µM of the short-tailed azole drug VCZ has a modest impact on growth and ergosterol biosynthesis**. These effects are more pronounced for the F5 isoform. They is likely to be due to **lower affinity binding of the short-tailed azole drugs to the LDP of the SDM F5 isoform rather than differential overexpression.** This hypothesis remains to be clarified by using higher sub-MIC concentrations of VCZ and analysing the phenotypes of *Mc*SDM F5 F129Y and A293 revertant strains.

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REF.: 1: Lamping *et al.*, EUKARYOTIC CELL, 2007, 10.1128/EC.00091-07

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