Characterization and functional analysis of sterol 14 α -demethylase genes (*cyp51*) in *Mucor circinelloides*

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Introduction

Several members of Mucoromycotina are considered as opportunistic human pathogens, which can cause frequently fatal systemic infections in immunocompromised patients. In the recent years, the number of patients with mucormycosis has significantly increased worldwide. In clinics, triazoles are frequently used for treatment of invasive fungal infection. These compounds inhibit the activity of the enzyme, sterol 14 α -demethylase (Cyp51), which participated in the ergosterol biosynthesis. However, Mucoral fungi are generally resistant to the majority of the presently available azoles. *M. circinelloides* genome encodes 2 *cyp51* genes (*cyp51a* and *cyp51b*) and the aim of the present study has been the functional characterization of this genes.

Methodes

1. Relative transcript levels of *cyp51* genes were measured under different growth conditions (i.e. different cultivation temperatures and azole treatments) using the qRT-PCR method.
2. A CRISPR-Cas9 system was used to disrupt the two *cyp51* genes of *M. circinelloides*. The isolated mutants were characterized and their ergosterol content and sensitivity to various azoles were measured.

Results II.

Disruption of *cyp51* genes using the CRISPR/Cas9 system

Genome editing strategy designed to disrupt the *cyp51* genes of *M. circinelloides* by the CRISPR/Cas9 method and positions of the primers used in the study. TGG indicates the PAM sequence; arrows show the orientations of the primers.

PCR analysis of the transformants using the McP450/1_7 and McP450/1_8 ($\Delta cyp51a$) and McP450/2_7 and McP450/2_8 ($\Delta cyp51b$) primers.
M - 1kb marker (Thermo Scientific)
1 - MS12 *cyp51a*
2 - MS12 *cyp51b*
3 - $\Delta cyp51a$ *cyp51a*
4 - $\Delta cyp51b$ *cyp51b*

Minimal inhibitory concentration (MIC90) of the different strains against azoles

Strains/ Azoles	MIC90				
	ketocon- azol	itracon- azol	flucona- zol	posacon- azol	ravucon- azol
MS12	>64	>64	>64	2	16
$\Delta cyp51a$	64	>64	>64	2	16
$\Delta cyp51b$	>64	1	>64	0,5	8

Ergosterol content of the different strains

ergosterol content mg/g (dry weight)	
MS12	68
$\Delta cyp51a$	19
$\Delta cyp51b$	13

Results I.

Relative transcript levels of the *cyp51* genes during the cultivation period

Relative transcript levels of the *cyp51* genes at different temperature

Relative transcript levels of the *cyp51* genes after azole-treatment

Conclusion

The genes *cyp51a* and *cyp51b* showed a constitutively high and a moderate transcription level, respectively, under the tested conditions. Fluconazole had no effect on the transcription level of *cyp51* genes while the other azole increased the relative transcript level of both genes. Ergosterol content of the disrupted mutant significantly decreased compared to the wild type. Interestingly, disruption of *cyp51b* resulted increased sensitivity against to itra-, posa-, and ravuconazole, while disruption of *cyp51a* had no effect.

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