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THE *Aspergillus fumigatus* DNA MISMATCH REPAIR SYSTEM AND ITS RELATION WITH AZOLE RESISTANCE

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INTRODUCTION

The increasing detection of azole resistant *Aspergillus fumigatus* isolates is threatening the azole class effectiveness in the aspergillosis management (1). In a previous WGS analysis, a collection of azole susceptible and resistant *A. fumigatus* genomes from very diverse geographical origins were differentiated in four clusters (2). All genomes harboring the azole resistance mechanism TR34/L98H in *cyp51A* were included within the same cluster. The genetic closeness of the strains harboring TR insertions suggests additional genetic mechanisms operating in them that in turn, results in selection of genotypes that fit better to the environment. Some studies have suggested the genetic instability in *A. fumigatus* as a possible mechanism of evolving azole resistance. One of the systems in charge of recognition and repairing the mistakes during cell replication is the DNA mismatch repair (MMR) system (3, 4, 5).

OBJECTIVE

Investigate four *A. fumigatus* MMR genes and their relation with azole resistance:

msh6 ***msh2***
pms1 ***mlh1***

1 Whole genome sequencing (WGS) analysis

The genes *msh6*, *msh2*, *pms1* and *mlh1* were analyzed in a collection of 161 *A. fumigatus* strains that were whole genome sequenced.

Table 1. Mutations detected in the WGS analysis of the genes *msh6*, *msh2*, *pms1*, *mlh1* and the percentage of strains harboring them.

Gene (Gene code)	Mutations	% of strains
msh6 Afu4g08300	A55V	0,62
	V118A	0,62
	D121E	0,62
	G178A	1,86
	I183R	10,56
	G240A	**42,86**
	N289S	2,48
msh2 Afu3g09850	A45T	3,73
	P329T	3,73
	E467D	0,62
	E812G	1,24
	A889E	0,62
pms1 Afu2g13410	G286C	0,62
	P401A, V438A, K464R, Q611E, E687K, E760K	4,35
	E444G	2,48
	S758Y	1,24
	D1013Y	0,62
mlh1 Afu5g11700	K310R	4,35
	S368N	4,35
	I510T	1,86
	A641S	4,35

The mutation G240A in Msh6 was the most prevalent, only harbored by strains from Cluster II. All the strains with the TR34/L98H azole resistance mechanism had the G240A *msh6* mutation.

2 Generation of an *A. fumigatus* Δ*msh6* strain

In order to assess the role of the *msh6* G240A mutation in azole drug susceptibility, the corresponding *msh6* gene was deleted from an *akuB*^{KU80} strain and replaced by the resistance marker pyrithiamine (*ptrA*).

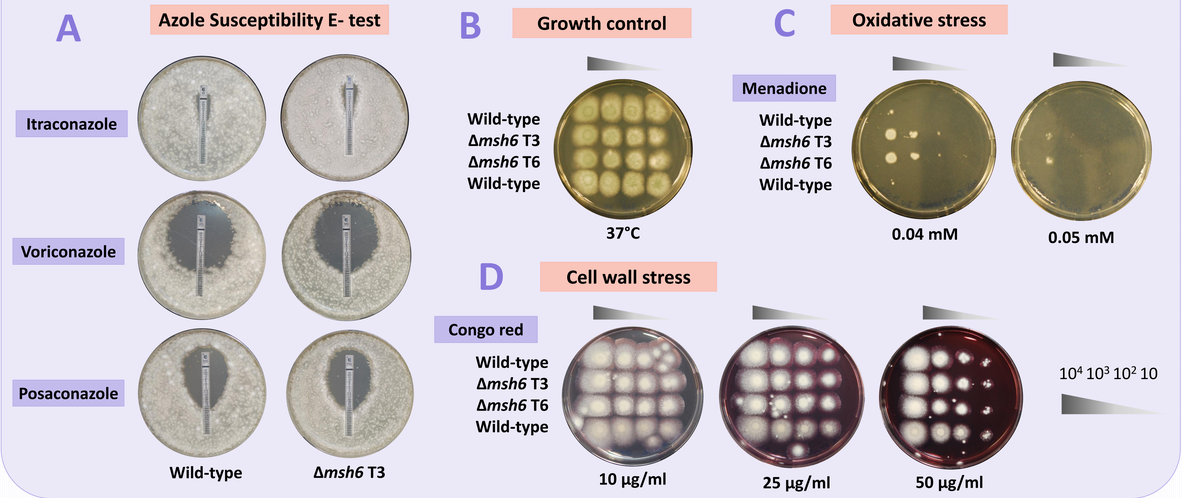
1 **Construction of the *A. fumigatus* Δ*msh6* fusion cassette by overlapping PCR**

2 **Generation of Δ*msh6 A. fumigatus* strains by transformation with the fusion cassette using protoplasts**

3 **Knock-out verification by PCR amplification**

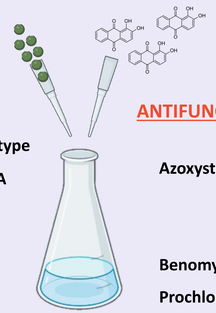
3 Phenotypic tests on the *A. fumigatus* Δ*msh6* strains

Two independent Δ*msh6* strains (T3 and T6) and its parental wild-type strain (*akuB*^{KU80}) were subjected to azole susceptibility E-tests (A) that confirmed that the azole susceptibility of the mutant strain did not change with respect to the parental strain. In addition, mutants were subjected to growth controls at a range of different temperatures (37°C-45°C-50°C-60°C), showing no differences; results are only shown for 37°C (B). Strains were also subjected to oxidative stress with menadione (C) and cell wall stress with Congo Red (D) and Calcofluor white (data not shown). Differences were only seen in the menadione tests, being the mutant strains (T3 and T6) more resistant (0.5 mM) than the wild-type.



4 Mutagenesis experiments

Mutagenesis experiments were performed using a strain harboring the mutation G240A in *msh6* and a wild-type strain. Both strains were growth in liquid MM media, with shaking and heat conditions, under stepwise concentrations of benomyl, prochloraz and azoxystrobin drugs alone and in combination.



STRAINS

***msh6*-Wild-type**

***msh6*-G240A**

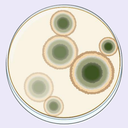
ANTIFUNGALS

Azoxystrobin

Benomyl

Prochloraz

Strains that grew under 32 mg/L of each antifungal were transferred to plates with 32 mg/L of the same antifungal



Strains grown in the plates were picked and isolated for further analysis of the target genes

RESULTS

In the background strains with the **G240A** mutation in Msh6, we recovered several **azoxystrobin** resistant isolates harboring the mutations **F129L** or **G143A** in the *cytB* gene.

We were unable to recover antifungal resistant mutants from the *msh6* wild-type strain.

We were unable to recover any mutant strain grown under the pressure of the drugs benomyl or prochloraz.

**Mutagenesis using other antifungal drugs including boscalid or imazalil and the Δmsh6 strain are currently in progress.*

CONCLUSIONS

1. **Modifications in genes involved in the MMR system could be related to a higher *A. fumigatus* mutation rate and contribute to resistance acquisition.**
2. **This study suggests a possible link between alterations in Msh6 and azole resistance in *A. fumigatus*.**

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