
EXTRA COPIES OF THE ASPERGILLUS FUMIGATUS SQUALENE EPOXIDASE GENE CONFER RESISTANCE TO TERBINAFINE: A GENETIC APPROACH TO STUDY GENE-DOSE DEPENDENT RESISTANCE TO ANTIFUNGALS IN ASPERGILLUS FUMIGATUS

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With the increasing use of antifungals such as amphotericin B, itraconazole, voriconazole, caspofungin, and terbinafine (TRB) in patients at high risk for invasive aspergillosis, resistance of *Aspergillus fumigatus* to these agents will ultimately emerge. Due to the lack of genetics in *A. fumigatus*, limited studies have addressed its mechanisms of resistance to antifungals. We transformed *A. fumigatus* protoplasts with a *pyrG*-based *A. fumigatus* genomic DNA library (constructed in the multi-copy nonintegrating vector pRG3-AMA1-NotI that also has the *pyr-4* gene for selection). We obtained one TRB-resistant *pyrG*⁺ transformant in minimal medium minus uracil plates that contained a fungicidal concentration (0.625 µg/ml) of TRB. To determine whether TRB resistance in that transformant was plasmid-dependent, we evicted the plasmid from it by subculturing it on a nutrient-rich medium. We found that loss of the plasmid resulted in loss of both uracil prototrophy and TRB resistance in that transformant. We further identified the gene conferring TRB resistance as the *A. fumigatus* squalene epoxidase gene, which encodes for the target enzyme of TRB. We then subcloned the complete gene into the vector pRG3-AMA1-NotI and transformed it into *A. fumigatus* protoplasts. Again, squalene epoxidase, in increased copies, resulted in TRB-specific resistance in *A. fumigatus*. This molecular approach has the potential to enhance our knowledge of the mechanisms of *A. fumigatus* resistance to modern antifungals.