RNA INTERFERENCE OF CA²⁺-ATPASE GENE IN ASPERGILLUS FUMIGATUS

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The saprophytic species Aspergillus fumigatus is a deuteromycete found worldwide, having essential role in recycling carbon and nitrogen. However, in the past 20 years it has gone from being a saprophytic fungus of minor interest to becoming one important fungal pathogen. The knowledge about the regulation of calcium and manganese levels in Aspergillus fumigatus is very limited; the homeostasis of these ions could be directly controlled by the function of specific ATPases like the PMR1 calcium ATPase. A fragment of the Afpmr1 gene, showing low identity with others calcium ATPase genes, was cloned in an A. fumigatus expression vector (pALB1) for RNAi. After induction of gene expression, a double strand RNA construct for RNAi has properly silenced either the alb1 gene alone or the double silencing with the gene of interest Afpmr1, leading to constructions white colored colonies. After confirmation of the gene silencing by quantitative RT-PCR techniques, the selected clones were used in macrophages killing and phagocytosis assays. The Afpmr1 silenced clone showed a decrease in the phagocytosis and killing percentage compared with control groups, as well as in the mean number of internalized conidia. These results indicate that the Afpmr1 gene alters cellular processes that can be related with maintenance of the cell wall structure and composition, as well as promotes alterations in the macrophages phagocytosis and killing. Support by FAPESP and CNPg