

Amplification and molecular cloning of the ERG11 (lanosterol-14 α -demethylase) and ERG24 (c-14-reductase) genes from *Moniliophthora perniciosa*

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The causal agent of *Theobroma cacao* witches' broom disease, *Moniliophthora perniciosa*, has caused countless damages to cacao production in South Bahia. Host colonization by *M. perniciosa* leads to tissue degeneration and pod fruits. Many efforts have been carried out to reduce the damage caused by this disease, such as chemical control with systemic azole fungicides, which inhibit ergosterol biosynthesis in fungi. Molecular studies have been used to identify genes that play crucial roles in fungal survival and virulence. Following this trend, this study aimed at the amplification and molecular cloning of *M. perniciosa* lanosterol-14 α -demethylase (ERG11) and c-14-reductase (ERG24) genes. The enzymes coded by these genes are involved in the ergosterol biosynthesis pathway, which is a known biological target of azol-derivative fungicides. Thus, sequences deposited in database of Genome Sequencing Project of *C. perniciosa* (previous denomination) as well as bioinformatic tools were employed to locate consensus genomic sequences that were used to draw specific ERG11 and ERG24 primers. Polymerase Chain Reaction, from genomic DNA, afforded PCR fragments with 2.000bp (ERG11) and 1.600pb (ERG24), approximately, as expected. PCR products were then cloned in Invitrogen TOPO vector and sequenced for identity confirmation. The results reported herein are the first step to heterologous expression and purification studies that shall be useful for the design of novel inhibitors of *M. perniciosa* ergosterol biosynthesis pathway.

Keywords: Ergosterol biosynthesis; *Moniliophthora perniciosa*; witches' broom disease.

Supported by: FAPESB and FINEP.