28ª REGEM
Reunião de Genética de Microrganismos
09 a 11 de setembro de 2012 - Rafain Palace Hotel & Convention Center - Foz do Iguaçu - PR

Rational bioprospecting and identification of genes from sugarcane endophytic fungi *Epicoccum nigrum* and *Epicoccum* sp. involved in the biosynthesis of bioactive metabolites

Fávaro, LCL[1]; Melo, FL[2]; Araújo, FDS[3]; Marsaioli, AJ[3]; Silva, MA[4]; Rojas, RD[5]; Araújo, WL[5].

[1]Empresa Brasileira de Pesquisa Agropecuária, Embrapa Agroenergia, Brasília, DF
[2]Instituto de Biologia, Universidade de Brasília, UnB, Brasília, DF
[3]Instituto de Química, Universidade Estadual de Campinas, Unicamp, Campinas, SP
[4]Universidade de Mogi das Cruzes, UMC, Mogi das Cruzes, SP
[5]Instituto de Ciências Biomédicas, Universidade de São Paulo, USP, São Paulo, SP

leia.favaro@embrapa.br

Keywords: antimicrobial activity, AFLP markers, PKS genes, physiological variability, *Agrobacterium tumefaciens*-mediated random insertional mutagenesis

Fungi are considered an important source of bioactive compounds applied in the food, pharmaceutical, and agrochemistry industries. Intriguingly, only 10% of the estimated 1.5 million species of fungi have been described, and only a few of these species have been investigated for the production of bioactive metabolites. Among these, the endophytic fungi are a rich source of novel compounds. One important issue in microbial screening programs is how to improve the chances of finding new metabolites. An approach that should be considered is the determination of metabolic and genetic diversity within species. Previous studies have shown that *E. nigrum* is a common sugarcane endophyte, and an extensive genetic variation can be found among isolates from a single location and host. This fungus is used as biocontrol agent and commercial production of fluorescent compounds with biotechnological application. However, little is known regarding the genes related to the synthesis of bioactive metabolites in *Epicoccum.* In this context, the objectives of this study were: 1) to evaluate the *in vitro* antimicrobial activity of endophytic isolates of *Epicoccum* spp. against microbial pathogens; 2) to compare the physiological diversity to genetic variation assessed by AFLP markers, analysis of the ITS1-5.8S-ITS2 region, and polyketide synthase genes, 3) to characterize an *Agrobacterium*-mediated insertional library, to identification of genes involved in antimicrobial pathways. The results of three different methods showed that antimicrobial activity might be underestimated depending on method. Regardless of which method was used, approximately 30% of *Epicoccum* strains inhibited the pathogenic microorganisms. This demonstrates the importance of evaluate different strains in screening programs to guarantee the maximum information on the metabolic diversity of a species. A wide range in antimicrobial activity was observed, and it was correlated with the genetic variability assessed by AFLP markers, ITS, and PKS genes. A library of 832 insertional transformants was screened for a lack in antimicrobial activity. 128 transformants were not able inhibit all the pathogens tested. The T-DNA flank region sequencing revealed that from 41 mutants that produced amplification products by TAIL-PCR, 73.17% had insertion of the vector sequences adjacent to the edges of the binary T-DNA. For the 11 remaining mutants it was possible to identify with high similarity hypothetical proteins from fungi, some of them with unknown function, and other domains involved in gene expression regulation, transport, energetic metabolism and other enzymatic functions. The analysis of the organic extract by bioautography revealed the lack of antimicrobial activity of some of the mutants in comparison to the extract of the wild-type strain. The large number of mutants characterized in this work, in association with chemical investigation represents a promising approach for the molecular study of the biosynthesis of several compounds with complex structures produced by this species.

Financial Support: CNPq and Fapesp.