TITLE: OSMAC: An efficient strategy for the production of bioactive secondary metabolites.

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ABSTRACT

Microorganisms are an inexhaustible and important source of bioactive secondary metabolites with different modes of action. Actinobacteria, have been shown to be efficient for performing the biosynthesis of a wide variety of bioactive secondary metabolites, especially antibiotics. Although secondary metabolites are not essential to microbial growth, they are essential for the survival and maintenance of these organisms in the environment in which they are inserted. Several strategies have been applied for the activation of cryptic microbial pathways, such as OSMAC, heterologous and homologous expression, co-culture and ribosomal engineering. In preliminary studies in our research group, showed that the actinobacteria *Streptomyces sp.* CAAT 1-54 showed pronounced antimicrobial activity for different Gram positive and negative bacteria. Based on studies of isolation and structural characterization, the antimicrobial activity was attributed to the presence of the substances Lysolipin I and Lysolipin X, antibiotics of the class of the polyketides. However, when the actinobacteria *Streptomyces sp.* CAAT 1-54 was grown in solid rice medium, LC-DAD-MS analyzes indicated the presence of a new secondary metabolite with molecular mass 357 u, which had not been observed in liquid fermentations (PD, ISP-2 and GYE). This crude extract was subjected to preparative LC-UV fractionation, where the molecular mass 357 u compound was isolated. Analysis by LC-MS/MS and 1D and 2D NMR allowed the characterization of the compound, which was assigned to the antibiotic BE-13793C, belonging to the class of staurosporines. The isolated compound showed antimicrobial activity in the disk-diffusion assays against the bacteria *Streptococcus uberis*, *Staphylococcus aureus* ATCC 25.923, *Pseudomonas aeroginosa* ATCC 27.853 and *Escherichia coli* K12. The results obtained in this work confirm the importance in the OSMAC methodology in the activation of cryptic pathways for the production of bioactive compounds.

Keywords: Actinobacteria, bioactive secondary metabolites, antimicrobial, LC-MS/MS.

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