ISOLATION AND CHARACTERIZATION OF AN ANTIBIOTIC PRODUCED BY *Streptomyces* K-155

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Streptomyces, secondary metabolites, antibiotics

Introduction. *Streptomyces* is a genus of filamentous Gram-positive bacteria well known as the main producers of bioactive secondary metabolites like insecticides, antitumoral, anti-hypertensives, immunosuppressants and specially antibiotics[1]. In our laboratory we isolated a strain from soil and refer to it as K-155. An rDNA 16S analysis identified it as *Streptomyces* and a comparison made using the NCBI BLAST tool closely related K-155 with *S. thermocarboxydus* and *S. aureus*. During the polyphasic characterization of K-155, antibiotic activity against Gram-positive bacteria and yeast was found. The relevance of this work relies on the fact that neither *S. thermocarboxydus* nor *S. aureus* are reported as antibiotic producers, so the antibiotic may be new. Therefore, the aim of this work is to determine the species of K-155 and identify the antibiotic(s) produced.

Methods. A maximum likelihood phylogenetic tree was made using the 16S rDNA sequences from the RDP[(2)] database and MUSCLE program in order to identify K-155 species. For the antibiotics production different culture media were tested and after selecting the most adequate, the supernatant was extracted with different solvents like hexane, dicloromethane, ethyl acetate, butanol and methanol to determine in which one the compounds were soluble. The solvent extract was fractioned by column chromatography and further purified by HPLC. Properties like thermal and pH stability were determined using the supernatant, the extract and the fractions from the column chromatography. Structural analyses were made using MS, IR, UV and NMR analysis..

Results. Phylogenetic tree (data not shown) indicates that K-155 may belong to *S. thermocarboxydus* group. However, K-155 does not cluster with any of the closest reported strains, suggesting that K-155 might be a novel species. In order to accurately determine the species of K-155, analysis of other housekeeping genes, must be done. For production of the bioactive compounds, YMG was found to be the best culture media. The best solvent to extract the antibiotics from supernatant was methanol, because it maintained the activity against Gram-positive bacteria and yeast (Fig.1). The methanol extract was then fractioned by column chromatography with a mixture of acetonitrile:water. Active fractions were purified by HPLC on a Nucleodur $C_{18\ pyramid}$ using triethylammonium acetate buffer as mobile phase (Fig. 2). Active peak was collected and lyophilized and the purified compound was used to perform IR, MS and NMR analysis.

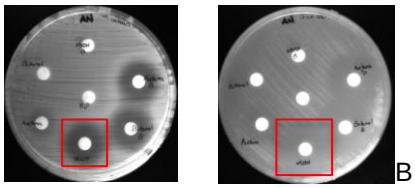
A B

Fig.1. Antibiosis assay against *M. luteus* (A) and *S. cerevisiae* (B). Red frame indicates the extract in methanol.

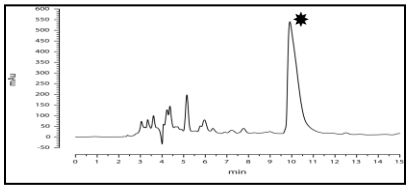

Fig.2 Chromatogram of the antibiotic purification. Mobile phase: TEAA 100%. The active peak is marked with an asterisk.

After analyzing the structural spectra, some signals were found, allowing us to propose some functional groups like amino, carbonyl, heteroatoms, and OH among others. The mass spectra indicated an m/z of 600. Max UV absorption was found below 200 nm (~190 nm) indicating the absence of conjugated double bonds. The active fractions were stable in neutral or basic conditions and up to 121°C for 20 minutes. Inhibitory concentration 50 was determined using an active fraction from the column chromatography and the purified compound obtained after the purification with HPLC. The results are shown in Table 1. The fraction 20 shows a higher inhibitory effect than the purified compound because that fraction still has remains from the buffer.

Table 1. Inhibitory Concentration 50 of the purified compound and the fraction 20 from the column chromatography.

Fraction	IC_{50} (µg/mL) against *M. luteus*	IC_{50} (µg/mL) against *S. cerevisiae*
Purified compound	276.1	489.3
Fraction 20	35.81	357.0

Conclusions. K-155 strain seems to be a novel species producing a polar, thermo stable, neutral antibiotic, with a molecular weight of 600 amu. The characteristics suggest that the antibiotic belongs to the group of siderophores or macrolides with a deoxyaminosugar moiety.

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References

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