

CHARACTERIZATION OF ANTIFUNGAL METABOLITES PRODUCED BY *BACILLUS ATROPHAEUS*

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Biological control can be defined as the use of living organisms or their metabolites to reduce the population density of other pest organisms, contributing to the decrease in the use of chemical pesticides. The microorganism ceparium of Microbiología ambiental del INQUISAL has a bacterium of the *Bacillus* genus isolated from bat guano, which in previous works showed chitinolytic activity and capacity to inhibit phytopathogenic fungi.

The objective of this work was to characterize the nature of the metabolites involved in the antifungal activity present in the cell-free supernatant (CFS) of *Bacillus atrophaeus* A14 with antifungal activity.

B. atrophaeus strain A 14 was cultured in Standard Nutrient (SN) during 72 h. CFS was obtained by centrifugation at 4 ° C for 15 min at 10,000xg. For the characterization of the metabolites of interest, different tests were carried out: i) extraction with *n*-butanol saturated in water; obtaining two fractions: a butanolic and an aqueous fraction; ii) acid precipitation, obtaining two fractions: one soluble in acid and the other insoluble in acid, with subsequent dissolution in methanol; iii) precipitation with ethanol and subsequent dissolution in water. The antifungal activity of each of the fractions was determined by diffusion in agar on Petri dishes containing the potato-glucose agar, using *Colletotrichum acutatum*, (a causal agent of anthracnose), as a reporter fungus of the antifungal activity in several crops. The plates were cultured for 72 h at 30 ° C. The activity was visualized by the absence of fungi growth around the well with fraction under study. In parallel, the following controls were used: *n*-butanol; methanol; ethanol and acidic water (pH 1.0). None of the controls presented antifungal activity indicating that the activity obtained by the fractions were due to metabolites present in the CFS of *B. atrophaeus* sp. A14.

The antifungal activity was found mainly in the butanolic fraction and in the acid precipitate dissolved in methanol. Subsequently, the fractions that presented antifungal activity were used in the bioautography assay on silica plates. *C. acutatum* spores were used as reporter fungus. After incubating the plate at 72 h and 30 °C, the inhibitory effect of the metabolites was observed and the R_f was estimated. Parallel these fractions were analyzed by means of ¹H-NMR.

From the results of this work, it is concluded that the metabolites with antifungal activity of *B. atrophaeus* A14 CFS belong to the lipopeptide family due to their solubility in butanol and methanol, and insolubility with HCl. The results obtained by ¹H-NMR and bioautography agree with those reported in the bibliography for Fengycin. Fengycin is a cyclic lipopeptide that can adopt and mimic secondary structures (loops and even vesicular) with surfactant nature, conferring selective antimicrobial activities.

It is planned to deepen these studies in the future and apply them to the control of diseases caused by fungi in the context of an integrated pest management strategy.