

TITLE: PARTIAL CHARACTERIZATION OF PROTEASE-PRODUCING BACTERIA ISOLATED FROM PRESERVED SOIL IN THE CERRADO BIOME.

AUTHORS: ALVES, B. A., FERREIRA-MACHADO, A. B; PAIVA, A. D.

INSTITUTION: Departamento de Microbiologia, Imunologia e Parasitologia/ Instituto de Ciências Biológicas e Naturais/ UFTM

ABSTRACT:

Proteases are a valuable group of hydrolytic enzymes with a fundamental role in the physiological and metabolic regulation of all organisms. Microorganisms are able to produce different proteolytic enzymes that are very attractive for industrial purposes. This study describes the isolation and partial characterization of protease-producing bacteria from soil samples collected from preserved environments in the Cerrado biome, in Uberaba/MG. Soil samples were collected in preserved environments and cultured in skim milk agar medium, at 37°C, under aerobic and microaerophilic conditions. The Enzymatic Index (EI), expressed by the relationship between the hydrolysis zone diameter and the colony diameter, were determined for the protease-producing bacteria, and molecular identification of the strains was performed by 16S rRNA gene sequencing. The protease production was analyzed along the growth of the producer cells and the proteolytic activity of the crude extracts was determined. The proteases were characterized regarding the effect of pH, temperature and the presence of metal ions during reaction. Proteolytic activity was confirmed in 32 bacterial isolates, being 23 strains isolated in aerobiosis. The Enzymatic Index (EI) varied from 1.1 to 3.5. Protease-producing isolates showing $EI \geq 1.5$ (n=15) were selected for protease quantification. Seven isolates showed higher production of extracellular proteases after 48h of growth ($p < 0.05$). The strains selected as the best protease producers, AB-4 (184.444 U/mL), AP-2 (188.611 U/mL), AP-10 (201.111 U/mL), AE-2 (211.944 U/mL) and MB-3 (260.555 U/mL), were identified as *Bacillus toyonensis*, *Bacillus licheniformis*, *Bacillus siamensis*, *Bacillus licheniformis* and *Bacillus thuringiensis*, respectively. *B. licheniformis* AE-2, *B. thuringiensis* MB-3 and *B. licheniformis* AP-2 showed high hydrolytic activity in all the pH values evaluated (2 to 12). *B. siamensis* AP-10, *B. licheniformis* AE-2, *B. toyonensis* AB-4 and *B. licheniformis* AP-2 produced proteases with higher catalytic activity in all evaluated temperatures (10 to 80°C). The proteolytic activity of the five isolates was not influenced by the presence of metal ions. This study was the first report of protease-producing bacteria isolated from Cerrado biome located in Uberaba/MG and provided insights on the bacterial community in the Cerrado soils, implying that this ecosystem may be a reservoir for novel and diverse enzymes, with potential biotechnological application.

Keywords: bioprospection, *Bacillus*, protease, biotechnology

Development Agency: CNPq, Fapemig.