

TITLE: BIOPROSPECTION OF PROTEASE PRODUCING BACTERIAL COLONIES VIA *EX-SITU* SOIL TREATMENT WITH SKIMMED MILK AND SOY EXTRACT

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ABSTRACT:

Microbial protease have several applications such as leather industries, food, pharmaceutical, cosmetics and cleaning. The soils are a source of isolation of protease producing bacteria. There are no studies involving Amapá soils and protease producing bacteria. This study aimed to select protease producing bacterial colonies from floodplain soil. The soil sample was collected from a single point at the Environmental Protection Area of the Curiaú River, Macapá, Amapá. Then, it was divided in five soil samples, place into disinfected pet bottles and treated every two weeks for six months by the addition of 20 grams of soy extract powder and skim milk powder and in solution by dissolving 20 grams of powder in 100ml of distilled water. A soil sample was maintained untreated. The soil bacteria was cultivated for two weeks with pH 7 at 37°C in nutrient broth with 1% of skim milk or soy extract. After this period the cultures were subjected to a serial dilution and plated in triplicate on nutrient agar containing 1% skim milk and bromocresol green at pH 7 and incubated at 37°C. Colonies with protease activity were identified by the formation of clearing halo around of the colonies. The efficient of enzyme is determined for the enzyme Activity Index (AI), which is calculated by the sum of the diameter of the halo plus diameter of the colony divided by the diameter of the colony. The protease producing colonies were identified after 48 hours of incubation through the clearing halo around the colonies even under different concentrations of skim milk (0.5% and 0.1%; 1%). The enzyme activity from the colonies was confirmed on plates containing 1% of skimmed milk when compared to those with 0% of skimmed milk, and 16 out 27 (59,25%) of the colonies presented protease activity. Approximately 31,7% of the colonies were from protein powder source and 22% from protein solution source. None of the tested colonies from untreated soil showed protease activity. The analysis of the AI showed that the samples with protein powder source presented the best AI, with the highest value of 1.91 after 72 hours of incubation and the lowest of 1.07 after 48 hours. The study demonstrated that by using soy extract powder or in solution and skimmed milk powder or in solution together with the *ex-situ* samples of floodplain soil placed into pet bottles induced the appearing of protease producing bacterial colonies.

Keywords: Curiaú. Bromocresol Green. Enzyme Activity Index. Protease,