

## Some Extreme Halophilic Microorganisms Produce Extracellular Proteases with Long Lasting Tolerance to Ethanol Exposition

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**Abstract :** Extremophiles constitute a potentially valuable source of proteases for the development of biotechnological processes; however, the number of available studies in the literature is limited compared to mesophilic counterparts. Therefore, in this study, Peruvian halophilic microorganisms were characterized to select suitable proteolytic strains that produce active proteases under exigent conditions. Proteolysis was screened using the streak plate method with gelatin or skim milk as substrates. After that, proteolytic microorganisms were selected for phenotypic characterization and screened by a semi-quantitative proteolytic test using a modified method of diffusion agar. Finally, proteolysis was evaluated using partially purified extracts by ice-cold ethanol precipitation and dialysis. All analyses were carried out over a wide range of NaCl concentrations, pH, temperature and substrates. Of a total of 60 strains, 21 proteolytic strains were selected, of these 19 were extreme halophiles and 2 were moderates. Most proteolytic strains demonstrated differences in their biochemical patterns, particularly in sugar fermentation. A total of 14 microorganisms produced extracellular proteases, 13 were neutral, and one was alkaline showing activity up to pH 9.0. Proteases hydrolyzed gelatin as the most specific substrate. In general, catalytic activity was efficient under a wide range of NaCl (1 to 4 M NaCl), temperature (37 to 55 °C) and after an ethanol exposition performed at -20 °C for 24 hours. In conclusion, this study reported 14 candidates extremely halophiles producing extracellular proteases capable of being stable and active on a wide range of NaCl, temperature and even long lasting ethanol exposition.

**Keywords :** biotechnological processes, ethanol exposition, extracellular proteases, extremophiles

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