

ABSTRACT

The world's demand for proteases reaches 60%, but only a few work optimally under extreme conditions such as hypersaline, high temperature, and extreme pH. Therefore, exploration of bacteria that produce stable proteases in extreme environments is needed. Bacillus clausii JIG-0%B, a halophilic bacterium from Madura salt ponds, can live in 0-20% NaCl salinity. This study aims to obtain a pure partial protease fraction from Bacillus clausii JIG-0%B and characterize its resistance to high salinity (halostable). The research phase included screening of protease potential, protease production, partial purification (ammonium sulfate fractionation and dialysis), protease activity test using the Kunitz method, and determination of protein content using the Lowry method. Protease characterization includes determination of optimal pH, temperature, and NaCl concentration. Protease stability was tested at various NaCl concentrations. The results showed that the optimal medium for protease production by Bacillus clausii JIG-0%B was modified halophilic medium with casein at 5% NaCl. After 96 hours incubation, pure partial protease with specific activity of 654.737 U/mg was obtained. Optimal protease activity occurred at pH 7, temperature 50°C, and 10% NaCl. Protease activity remained stable at 2.5%-15% NaCl concentration, indicating the adaptation of protease to various salinity conditions so that it can be widely applied. Enzyme kinetics showed a KM value of 0.164 mg/mL substrate and Vmax of 13.182 $\mu\text{mol/mL}\cdot\text{min}$, indicating high affinity to casein substrate. The thermodynamics of the protease showed inactivation constant (k_i) = 0.0031 min^{-1} , half-life ($t_{1/2}$) = 223.548 min, and free energy (ΔG_i) = +105.84 kJ/mol, indicating high stability. This protease activity will decrease due to the chelation of the metal cofactor by EDTA. Halostable protease from Bacillus clausii JIG-0%B has great potential to be applied in various fields such as soy sauce industry, leather tanning, and bioremediation in high salinity.

Keywords: Halophilic bacteria, Bacillus clausii, halostable protease, high salinity, Madura salt pond.