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Isolation of a geobacillus species from a hot water spring in Sri Lanka and characterization of alpha amylase enzyme

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Thermostable α – amylase producing *Geobacillus* sp. NMS 2 was isolated from soil samples of a hot water spring in Nelumwewa. In Nelumwewa, which is situated in Sevanapitiya in the Polonnaruwa District, the highest temperature observed was 61 °C and pH was 4.8. Both water and soil samples were collected under sterile conditions. Samples were incubated in nutrient broth (pH 6.8) containing 1% (w/v) soluble starch as carbon source and a salt solution containing 0.05% KCl, 0.05% MgSO₄ · 7H₂O, 0.04% MnSO₄, 0.087% K₂HPO₄, and 0.0022% CaCl₂. Water and soil sample were incubated at 50 °C and 70 °C in an orbital shaker at 150 rpm, but only soil samples showed high optical density at 70 °C. A soil sample was selected for further studies and bacteria were isolated by streak plate method and dilution plate method. The bacterial isolate which had the highest α – amylase activity was identified by morphology, biochemical tests and 16S rRNA gene sequencing. The results showed that the isolated bacteria belonged to the genus *Geobacillus* and was named as *Geobacillus* sp. NMS 2. This bacterium was cultivated at 37 °C, 50 °C, 60 °C and 70 °C and maximum enzyme production was found at 50 °C with a supernatant activity of 43 U/ml after 12 hours incubation. At 37 °C maximum activity was 19.2 U/ml after 16 hours incubation. At 70 °C maximum activity was 13 U/ml after 16 hours incubation. The enzyme was purified by ammonium sulphate fractionation and DEAE chromatography. In polyacrylamide gel electrophoresis only one protein band was observed showing that the enzyme was homogeneous. The specific activity of the purified enzyme was 420 U/mg with a folds purification of 2.8. From the Lineweaver – Bruke plot, the K_m and V_{max} values of *Geobacillus* sp. NMS 2 α – amylase were calculated as 3.4 mg/ml and 460 $\mu\text{mol}/\text{min}^{-1}/\text{mg}^{-1}$ respectively. The enzyme was stable over a broad temperature range from 10 to 60 °C with optimum activity at 50 °C. *Geobacillus* sp. NMS 2 α -amylase showed optimum activity at pH 6.9 and it was stable over the pH range of 6.9 to 9. α - Amylase from *Geobacillus* sp. NMS 2 is stable at 60 °C. The thermostability at 60 °C and pH stability from 6 – 9 makes this enzyme suitable to be used in the detergent industry.

Keywords: Thermostable, α – amylase, purification, characterization, *Geobacillus* sp. NMS 2

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