Production, Purification and Characterization of a Novel Thermostable Alpha-Amylase from *Caldimonas manganoxidans*

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Amylases play a major role as hydrolytic enzymes in starch-based industries. It is desirable for enzymes used in industry to have thermo-tolerant properties to withstand temperatures encountered in industrial processes. Thermophiles are naturally endowed with thermo-stable enzymes which are suited for specialized industrial applications. In the present study we attempted to screen and isolate a thermophilic bacterium that produced a novel thermostable alpha-amylase and to perform purification and characterization processes of the enzyme. Nelum-wewa hot water springs in Sewanapitiya, Polonnaruwa has one of the highest recorded temperatures of a water body in Sri Lanka. Water and soil samples were collected, under sterile conditions, from four distinct sampling sites and were transported to the laboratory in a cold box (0°C). Water temperature and pH were recorded to be 52°C and pH 7 respectively. Samples collected were inoculated on to culture agar plates and broth containing 0.5% (w/v) peptone and 0.2% (w/v) yeast extract, supplemented with a salt solution. Soluble starch (1% w/v) was added to induce the production of alpha amylase. Cultivation of bacteria was done at 50°C under high agitation in shaker water bath and the pH was maintained at 6.9 during the culture. Isolation of bacteria was done by streak plate and dilution plate methods. Amylase producing bacteria were identified by the clearance zones produced on starch agar plates visualized with iodine solution. The bacteria with the highest amylase activity was identified by morphological and biochemical tests and 16s rRNA analysis as *Caldimonas manganoxidans NMS1*. Alpha amylase activity was assayed by the method described by Bernfeld (1955) and the maximum supernatant alpha amylase activity of 56 U/ml was obtained on incubation at 50°C for 20 hours. The extracellular amylase enzyme was purified by ammonium sulphate fractionation and DEAE ion exchange chromatography. The specific activity of the purified enzyme was observed to be 2143U/mg with 21-fold purification and 57% of amylase activity retention. Polyacrylamide gel electrophoresis showed a single band of protein indicating that the enzyme was purified to homogeneity. The purified enzyme had the highest activity at 50°C and was stable up to 60°C. The enzyme showed a pH range of 6 to 9 with maximum amylase activity observed at pH 6.9 and was stable within the pH values of 6 to 9. The Km and Vmax values calculated from Lineweaver-Burk plot were 1.7mg/ml and 217µmol/min/mg of protein respectively. The optimum production of extracellular α-amylase was shown in a media comprising of 10% soya powder and 1% soluble starch solution. The enzyme showed a relative enhancement of activity with calcium ions (Ca^{2+}) and manganese ions (Mn^{2+}).

Keywords: amylase, *Caldimonas manganoxidans NMS,* ion exchange chromatography