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**Purification and functional characterization of thermostable alpha amylase from
Caldimonas manganoxidans MS1 isolated from Maha Oya hot water spring in
Sri Lanka**

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We have previously isolated the thermophilic bacteria *Caldimonas manganoxidans* MS1 from Maha Oya hot water spring in Ampara District of Sri Lanka and biochemical properties were characterized and identified by 16S rRNA gene sequencing. In this study we have purified the α -amylase from *Caldimonas manganoxidans* MS1 to homogeneity, which was confirmed by polyacrylamide gel electrophoresis. Functional properties were characterized to determine its suitability for the industrial applications. Enzyme production was optimized by varying the culture conditions. The maximum production of 31 U/mL was obtained with a medium containing 10% soybean flour, 1% soluble potato starch, 0.5% NaCl enriched with 0.5 g/L KCl, 0.5 g/L $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.04 g/L MnSO_4 , 0.3 g/L FeSO_4 , 0.87 g/L K_2HPO_4 , and 0.022 g/L CaCl_2 , after 18 hours of incubation at 50°C and pH 6.9 with a maximum cell count of 1.8×10^7 . The precipitate obtained at 40% ammonium sulphate saturation showed an activity of 25 U/mL with a recovery of 74% at 1.2 fold purification after dialysis. The purified enzyme obtained after DEAE-Sephadex A-25 ion exchange chromatography showed an activity of 19 U/mL and a specific activity of 467 U/mg at 18 fold purification with a recovery of 49%. A single band in polyacrylamide gel electrophoresis indicated homogeneous purification. The purified enzyme showed a maximum activity at 50°C and pH 6.9. The enzyme was stable from 10-50°C and pH 6-8. The enzyme activity was enhanced in the presence of Ca^{2+} , Mg^{2+} , Mn^{2+} , Fe^{2+} , Co^{2+} , Zn^{2+} and Na^+ while Fe^{3+} , Cu^{2+} , AsO_4^{3-} , Cd^{2+} , Hg^{2+} , EDTA, SDS and Clorox showed inhibition of enzyme activity. The enzyme retained 83% and 88% of its activity in presence of SDS and TritonX-100 respectively. This suggested possible application of the enzyme in detergent industry. From the kinetic studies, the obtained K_m and V_{max} values from the Lineweaver-Burke plot were 1.33 mg/mL and 33 $\mu\text{mol}/\text{min}/\text{mL}$ respectively.

Keywords: α -Amylase, *Caldimonas manganoxidans* MS1, DEAE-Sephadex A-25, Polyacrylamide gel electrophoresis.

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