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Purification and characterization of thermostable glucose oxidase from *Talaromyces funiculosus* AMS1 isolated from Nelumwewa hot water springs

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The industrial uses of glucose oxidase are in glucose biosensors and food and beverage industries. *Talaromyces funiculosus* AMS1 isolated from Nelumwewa hot water springs in the Polonnaruwa district has been shown to produce glucose oxidase. The main focus of this study was to purify and characterize the enzyme glucose oxidase from *Talaromyces funiculosus* AMS1. The isolated fungus was inoculated on agar slants consisted of peptone, glucose, and agar,at a pH of 5.6±0.2, and was incubated at 30^{o}C for 14 days until sporulation. Spores were washed with sterile water and pooled. Spore suspension was used as the inoculum for submerged fermentation and it was done in a medium consisted of $NaNO_3$, KH_2PO_4, $MgSO_4.7H_2O$, $FeSO_4.7H_2O$, $CaCO_3$, peptone, and glucose ata final pH of 5.6±0.2.To determine the effect of temperature on enzyme production, submerged fermentation was carried out at different incubation temperatures (25^{o}C, 30^{o}C, 35^{o}C and 40^{o}C) on a rotary shaker, using fermentation medium and spore suspension in a 19:1 ratio. The highest glucose oxidase production was obtained at 30^{o}C which was 3.3 U/ml after incubation for 88 hours. 428U/mg specific activity with 12 fold purification and 24% yield was obtained after purification by $(NH_4)_2SO_4$ fractionation, dialysis and DEAE Sephadex A-25 anion exchange chromatography. Homogeneity of the purified enzyme was determined by polyacrylamide gel electrophoresis in which single band was observed at the R^f value of 0.29. The optimum temperature and pH for the enzyme activity were 40^{o}C and 5.1 respectively. 90.8% of its original activity was retained after 60 minutes of incubation at 40^{o}C.The K_m and V_{max} values for β-D-glucose were 6.91 mM and 0.74 µmol/min/mg, respectively.The effect of salts on glucose oxidase activity was investigated by incubating the enzyme in 0.1mM salt solutions and It was observed that the enzyme was completely inhibited by Fe^{2+}, Hg^{2+} and Ag^{+}.A decrease in enzyme activity was obtained by Na^{+}, Mg^{2+}, Ca^{2+}, Co^{2+}, Zn^{2+}, Cd^{2+}, and Cu^{2+}. The purified enzyme was shown to have a good stability at 40^{o}C and further studies are in progress to investigate the potential industrial applications.

Keywords: Glucose oxidase, *Talaromyces funiculosus* AMS1, DEAE Sephadex A-25

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