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Partial purification and sugarcane bagasse induction of extracellular thermostable Amylase by *Bacillus* sp. under submerged fermentation

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Abstract

Enzyme with relatively low stability shows an industrial incompetence which creates an urgent need of potential enzymes with high stability in a cost effective way. Thus, thermostable enzymes derived from microorganism are the alternative resources for convenient industrial application. Our present study was aimed for production of thermostable amylase with wide range of pH and temperature stability, and induction of over production of the enzyme by using sugarcane bagasse as the sole carbon source. The strain was an isolate from railway track of Cuttack railway station, Odisha, India. Morphologically the strain was identified as *Bacillus* species by gram staining, and a potential amylase producer in normal Luria Bertani (LB) broth at 60°C. The optimum extracellular amylase production observed was 0.085 U/ml after 6 hours at 60°C under submerged culture condition. Extracellular amylase was precipitated through ammonium salt fractionation, and partially purified by Sephadex G-50 column chromatography. The partially purified protein under SDS-PAGE was found to be resolved in to three distinct bands of molecular weights approximately 108.69 kDa, 78.12 kDa and 65.63 kDa. The amylase enzyme showed wide range of temperature stability till 90°C and pH stability from pH 5 to 8, respectively. However, the optimum stability of amylase was found at 90°C in pH 8. The induction study has been carried out with abundantly available agro-industrial waste product sugarcane bagasse for over production of amylase in four different concentrations (0.5%, 1.0%, 2.0%, and 5.0%). The enzyme production was enhanced with increase in concentration of sugarcane bagasse as compared to the control LB medium. The highest amylase production observed was 0.190 U/ml with 5.0% inducible substrate after 10 hours of submerged culture condition at 60°C. Thus, the strain is found to be a potential producer of extracellular amylase and further studies are needed for the optimization of increased production and induction of other possible enzymes by different inducible substrates.

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