ABSTRACT

Bacillus genus comprises variety of species that are diverse, commercially useful and widely distributed in nature. They produce large number of enzymes that plays an important role in medical, pharmaceutical, textile, leather, paper and food industry. A one most viable example is uricase or urate oxidase enzyme that plays a key role in medical field as a therapeutic drug. This enzyme is absent in higher primates and can largely be produced from Bacillus spp. The bacteria was recovered from soil of 5 different locations in Lahore. Different biochemical tests were performed for its confirmation. A broth was prepared for the uricase enzyme production from bacillus spp. in which different chemicals were introduced like KH2PO4 (0.2%), MgSO4.7H2O (0.1%), K2HPO4 (0.2%), NaCl (0.01%), CaCl2 (0.01%), and uric acid (0.5%). Bacillus spp. produces extracellular enzyme that is why the enzyme was assayed from extracellular medium (17.87 µmol/mL/min). Nutritional and physical parameters were optimized for maximum production of uricase. Best results were obtained when 0.5% uric acid used as an inducer, sucrose as a carbon source, peptone as a nitrogen source at pH 8 and media was incubated at temperature 37°C for 36 hours. Ammonium sulphate precipitation was used for partial purification of uricase enzyme resulted in 4.67 fold purification with enhancement of specific activity of 301.5 µmol/mL/min. The molecular weight of uricase is 35 kDa determined by SDS poly-acrylamide gel electrophoresis. Uricase showed maximum activity at 37°C and pH 8. Kinetic characterization of uricase revealed uric acid as the highly specific substrate for enzyme with Km value of 0.052 mg/mL and Vmax of purified uricase was 27.7 µmol/mL/min.