ABSTRACT

An extracellular DNase free RNase was produced by submerged fermentation. Four bacterial strains i.e. *Bacillus megaterium* NRRL 3712, *Bacillus licheniformis* NRRL 1001, *Bacillus thuringenesis* NRRL 798 and *Pseudomonas putida* NRRL 922 were screened for the production of RNase by plate and shake flask method. Among them, *Bacillus megaterium* NRRL 3712 was selected and further used for optimization. Different nutritional i.e. carbon and nitrogen and cultural conditions like pH, temperature, volume of medium and incubation time were optimized for the enhanced production of RNase. Different carbon sources such as glucose, maltose, starch, glycerol and sucrose and nitrogen sources i.e. tryptone, yeast extract and peptone were evaluated for the increased production of RNase. The maximum RNase activity (510 U/ml) was achieved in minimal salt medium containing 1.5% glucose and 0.5% yeast extract at 30°C, pH 7 for 48 h at 200 rpm in 500 ml conical flask. The RNase was partially purified by acetone precipitation and ammonium sulphate precipitation. RNase was purified up to 6.6-fold with an overall yield of 44.5%. The specific activity of RNase was 825 U/mg. The molecular mass of partially purified RNase was 45 kDa. The enzyme showed optimum activity at pH 7 and temperature 40°C. RNase was stable between pH 5-7 and at temperature up to 45°C. The RNase was inhibited by Ca^{+2}, Mg^{+2}, Cu^{+2}, Hg^{+2}, Mo^{+6}, Fe^{+2}, Ni^{+2}. The RNase activity was significantly increased by EDTA. The V_{max} and K_m were 555.6 U/ml and 71.67 µg/ml.