ABSTRACT

The present study deals with the enhanced production of enzyme alginate lyase by using a mutant strain of *Bacillus licheniformis*. The chemical mutagenesis was induced by using nitrous acid. Different NA concentrations (50-300 mM) and different exposure time (5-30 min) were investigated. Mutant auxotrophs of *B. licheniformis* were developed by growing the selected strains on NaCl-deficient medium. The final mutant NA-*aux*1 was able to produce 22.02±0.54 U/ml of alginate lyase which was significantly higher than the wild-type 9.46±0.41 U/ml. Electron microscopy revealed morphological characteristics of wild-type and mutant strains. The wild-type ISL-9 and mutant strain NA-*aux*1 were optimized for various parameters viz. medium volume (75 ml), medium pH (6.5), size of inoculum (1 ml) and time of incubation (84 h). A significant increase in alginate lyase activity was observed, when various factors including buffer pH (8.5), temperature (60°C), NaCl concentration (150 mM) were optimized. The enzyme activity was further stimulated by the inclusion of various metallic ions. The most notable finding was the addition of $CaSO_4$ which increased the enzyme activity significantly ($p \leq 0.05$). After the optimization of parameters for enzyme activity, the mutant NA-*aux*1 showed a 1.35-fold increase in the activity as compared to the wild-type which is highly significant (HS). The alginate oligosaccharides production ability of the alginate lyase was evaluated by the wild-type and mutant strains. Effect of substrate concentration (1 g/l), enzyme level (1.5 ml) and ionic strength of buffer (pH 8) was investigated. Under the optimized conditions, large number of reducing sugars were produced by the mutant strain NA-*aux*1 (69.25±1.12 mg/ml). The enzyme was partially purified with 60% ammonium sulphate precipitation followed by dialysis. The molecular weight of the enzyme from wild-type and mutant strain was found to be 35 kDa on SDS-PAGE.