Abstract

The aim of the current study was the isolation of urokinase producing bacteria from soil, fermented food and sea water samples from different areas of Pakistan. This resulted in the isolation of thirty five urokinase producing bacterial isolates which were further subjected to secondary screening. Maximum enzyme activity 52.6±0.03FU/ml/min was obtained from the bacterial isolate that was identified as *Enterococcus gallinarum* by 18s RNA genome sequencing. Different cultural and physical parameters such as incubation time, temperature, pH, carbon and nitrogen source, inoculum size and trace elements were optimized. Maximum production 81.3±0.02 FU/ml/min was recorded at 96hrs of incubation, 37°C temperature, 7 pH with sucrose as carbon and soya flour as nitrogen source, $CaCl_2$ as trace element when M-5 fermentation medium was inoculated with 3% inoculum size. Enzyme was partially purified using 80% ammonium sulphate precipitation and molecular weight of purified urokinase was determined as 35kDa. Kinetic parameters such as K_m and V_{max} were obtained as 1.38mg/ml and 184.72± 0.02FU/ml/min, respectively. Thermodynamic analysis of purified enzyme was also conducted and parameters such as activation energy (Ea), enthalpy of activation (ΔH) and change in entropy were determined as -37.64KJ/mol, 40.85KJ/mol and - 168 KJ/mol, respectively. The purified enzyme was also successfully applied for the disintegration of blood clot in *In vitro* studies.