Abstract

Although the enzyme L-asparaginase (L-ASNase) from *Escherichia coli*, *Erwinia* and *Serratia* has been applied to treat certain lymphomas and leukemias, several medical complications such as severe immunological responses leading to hypersensitivity, anaphylaxis, etc. have limited its application. The researchers have documented that such impediments are due to the different biochemical and kinetic properties of L-ASNase, which are directly dependent on genetic variations in microbial strains. Therefore, there is a compelling need to explore novel L-ASNase producing microorganisms that would exhibit different serological properties while retaining similar and/or better therapeutic effects against cancer cells. Heretofore, L-ASNase producing bacterial strains from Bangladesh have never been isolated and characterized. Therefore, the main objective of this research was to isolate and characterize these strains from unexplored and ecologically different habitats that could lead to developing a potential therapeutic drug with fewer immunological responses and side effects over the existing drugs to treat cancer patients in near future. Since two L-ASNase producing bacterial strains were isolated from the soil of *Hatirjheel* lake in Dhaka at the initial stage of our project, molecular characterization and further studies were carried out for these two strains. It was revealed that they belonged to *Pseudomonas aeruginosa* and their DNA sequences were submitted to NCBI GenBank. The accession number OK446669 was obtained for the strain of *P. aeruginosa* EWUKR-1 and OL307081 for *P. aeruginosa* EWUKR-2. The specific activity of L-ASNase from EWUKR-2 (212.1 ± 14.8 U/mg protein) was significantly higher than that of EWUKR-1 (16.3 ± 0.8 U/mg protein) when they were grown in modified M9 media containing 0.5 g/l glucose at 37^0C for 24 hours. The experimental results revealed that both bacterial strains were extracellular L-ASNase producers. The enzyme from *P. aeruginosa* EWUKR-2 was partially purified using saturated ammonium sulfate followed by dialysis and concentrated using Vivaspin-20 centrifugal concentrator having MWCO of 30 kDa. The optimum temperature and pH of the partially purified enzyme were 37^0C and 7.5, respectively. The purification-fold after ammonium sulfate precipitation and yield of the concentrated enzyme were 2.8 and 101%, respectively. SDS-PAGE analysis revealed that the molecular weight of L-ASNase from *P. aeruginosa* EWUKR-2 was around 43 kDa. At the last stage of our research, additional nineteen L-ASNase producing bacterial strains were isolated and characterized from the soil samples of *Tejgaon* industrial area. Moreover, thirteen L-glutaminase (L-GLNase) producing bacterial strains, five L-GLNase producing fungal strains, thirty-seven protease producing bacterial strains, twelve lipase producing bacterial strains, twenty-four cellulase producing bacterial strains, twelve amylase producing bacterial strains and twenty-three pectinase producing bacterial strains were identified under this project. Some of these strains were characterized based on higher specific enzyme activities.

Keywords: Bacteria, Bangladesh, Cancer, Cellulase, L-asparaginase, L-glutaminase, Lipase, Pectinase, Protease, Soil, Therapeutic-drugs.