PREFACE

Acute lymphoblastic leukemia is a type of cancer of WBC, where excess multiplication of immature and malignant cells in bone marrow occurs and was reported to be most common types of cancer in children. Various treatment methods followed for the improvement of cure rate of acute lymphoblastic leukaemia include chemotherapy, radiation therapy, use of steroids, intensive medical treatments like bone marrow and stem cell transplantation. Among all the treatments, chemotherapy is mostly preferred where 80% of cure rate is observed in children and 30% in adults with acute lymphoblastic leukaemia. Wide range of drugs are used in combination protocols of chemotherapy like methotrexate, thioguanine, prednisolone, vincristine, dexamethasone, daunorubicin, mercaptopurine and L-Asparaginase. Even though many drugs are available in the market, efficacy remains doubtful at later stages of disease. Also, side effects such as thrombosis, pancreatitis, immunosuppression and other minor problems remain major reasons for their limited usage.

Bacterial L-Asparaginases are one such kind having high therapeutic value and so are extensively used in the treatment of leukaemia particularly in acute lymphoblastic leukaemia. Two types of enzymes are mainly present namely type I and type II. Type I enzymes are expressed in the cytoplasm whereas type II are expressed under anaerobic conditions in the periplasm of the membrane. As type II display high specific activity, these are widely used as agents of chemotherapeutics for treating acute lymphoblastic leukaemia. The enzyme L-Asparaginase catalyzes the conversion of L-Asparagine or L-Glutamine into their respective acids (L-Aspartic acid and L-Glutamic acid) and ammonia. Malignant blast cells have reduced expression of the enzyme L-Asparagine synthetase and so they are unable to synthesize ample amounts of L-Asparagine for their
rapid growth. So, they depend on L-Asparagine for their protein biosynthesis that is present in the circulation. Normal cells are able to synthesize the necessary amino acids for their growth and metabolism by themselves. When enzyme L-Asparaginase is injected into circulation, depletion of substrate needed by tumour cells occurs, thus leading to the impairment of protein synthesis followed by cell cycle arrest and finally leading to death.

L-Asparaginase is present in different sources such as Bacillus, Serratia, Xanthomonas, Aerobacter, Proteus, Vibrio, Psuedomonas and Aspergillus. L-Asparaginase sourced from Escherichia coli and Erwinia species were found to possess very good anti-tumour properties particularly against acute lymphoblastic leukaemia. But it was well reported that, therapy with L-Asparaginase is accompanied by serious side effects like hypersensitive reactions, tissue toxicity, immunosuppression and also other complications like acute pancreatitis, liver damage and thromboembolism. By the approach of PEGylation of enzyme, the properties of enhanced half life, decreased toxicity, enhanced solubility and stability of the drug along with reduced immunogenicity are attained.

The major reasons for side effects of this enzyme drug are mainly due to its intrinsic glutaminase side activity. Amino acid glutamine is the major transporter of nitrogen in the blood and also donates amino group in many biological reactions. Prolonged depletion of levels of glutamine in the body leads to various side effects. So enzymatic drug used for the treatment should have high catalytic activity, low $K_m$ and should prefer substrate L-Asparagine rather than L-Glutamine. As the presence of partial glutaminase side activity was reported for the enzyme which is the reason for many of the side effects, glutaminase-free enzyme is essential for obtaining promising results in
clinical studies. So, as enzyme sourced from *Pectobacterium carotovorum* meet this criteria and so it was selected for the present work.

Keeping the potentiality of the drug in consideration for treating acute lymphoblastic leukaemia, the present work has been carried out to come up with more information related to optimization of medium components for production of glutaminase free L- Asparaginase for better treatment and *in silico* analysis and characterization studies related to enzyme and finally creating mutations by site directed mutagenesis at the amino acid level to obtain an enzyme with desired properties.