L-asparaginase being an antineoplastic agent, is used for the treatment of acute lymphoblastic leukemia (ALL). Different microbial sources have been reported with L-asparaginase activity but the only commercially available L-asparaginase which is used in chemotherapy of ALL is from *E.coli* and *Erwinia chrysanthemi*. Researchers throughout the world are in search of a newer microbial source for L-asparaginase, as *E.coli* and *Erwinia chrysanthemi* L-asparaginases cause severe side effects. So there is a need for a L-asparaginase enzyme with an activity higher or equal or almost similar to the commercially available L-asparaginases with lesser side effects. Apart from being used in pharmaceutical application, L-asparaginase is also used in the food industry for the pretreatment of starch based foods for preventing the formation of acrylamide, a potential carcinogen and neurotoxin.

In the present study, actinomycetes being least explored microorganisms and good source of L-asparaginase were isolated and screened for high L-asparaginase production. The isolate 5 showed highest L-asparaginase activity which was later identified by 16 S rDNA gene sequencing as *Streptomyces* sp. ABR2. This isolate was subjected to optimization of L-asparaginase production. Under optimized conditions L-asparaginase activity of *Streptomyces* sp. was found to be 18.8 IU/ml. L-asparaginase gene from *Streptomyces* sp. was amplified using L-asparaginase gene specific primers. The amplified product was confirmed by sequencing and BLAST analysis. Repeated screening for actinomycetes with significant L-asparaginase activity did not yield a potential candidate to be studied further.

An industrially important bacterial strain, *Bacillus licheniformis* was obtained from Microbial type Culture Collection, IMTECH, Chandigarh, India. *B. licheniformis*
was subjected to optimization of L-asparaginase production in submerged fermentation for different parameters like pH, inoculum size, effect of carbon and nitrogen sources. Optimization of the media for L-asparaginase production led to an increase in the activity from 40 IU/ml to 71.11 IU/ml.

Genomic DNA from *B. licheniformis* was isolated using conventional methods and used for amplification of L-asparaginase genes using gene specific primer pairs. The amplified products were then confirmed by sequencing and BLAST analysis, which showed similarity to the L-asparaginase genes of *Bacillus licheniformis* in NCBI database. Both the genes coding for ansA1 and ansA3 from *B. licheniformis* were cloned and overexpressed in *E.coli* BL21 cells. The recombinant proteins were purified by one step process using affinity chromatography and then were characterized for various biochemical parameters. SDS-PAGE analysis revealed that both the recombinant proteins are monomers of ~37 kDa. Recombinant ansA1 enzyme was found to be highly unstable and showed very low activity when compared to recombinant ansA3 (rBliAIII) enzyme while recombinant ansA3 enzyme was catalytically active and stable. Recombinant ansA3 enzyme showed optimum activity of 407.65 IU/mg at 37°C and pH 8.

Further characterization of recombinant ansA3 enzyme showed that it has higher substrate specificity for L-asparagine with negligible glutaminase activity. The kinetic parameters for hydrolysis of L-asparagine were calculated i.e. Km was 0.671 x 10^{-3}M, V_{max} was 36.60 µmol min^{-1}, k_{cat} was 36.8 s^{-1} and k_{cat}/K_{m} was 5.4 x 10^{4} M^{-1} s^{-1}.

Computational structure was generated with the help of ITASSER for recombinant ansA3 from *B. licheniformis* and was used to superimpose on *E.coli* and
Erwinia, which indicated that B. licheniformis L-ASNase is structurally identical with overall structure of both E.coli and Erwinia L-ASNases. With the help of sequence homology studies of B. licheniformis L-ASNase to E.coli and Erwinia L-ASNases, active and binding sites for rBliAIII were determined.

Enzyme engineering is currently a promising tool to modify or to improve enzymes with desired properties. Therefore, recombinant ansA3 enzyme was modified using rational design to enhance its catalytic properties. The mutant enzymes thus produced i.e G238N, E232A and Q112H showed loss of activity while the D103V mutant enzyme showed higher activity as compared to the recombinant native ansA3 (rBliAIII). The D103V mutant enzyme in thermal inactivation studies showed that though the activity decreased rapidly at higher temperatures, the mutant enzyme had significant activity at higher temperatures upto 100°C. Moreover, D103V mutant enzyme had upto 3 fold increased half life when compared with the native recombinant ansA3 enzyme. These studies suggested that replacement of charged and acidic amino acid to hydrophobic amino acid at 103 position increased the physical properties of L-asparagenase. It is also proving that aspartic acid is a rate limiting factor for L-asparaginase. Also, charge carried by aspartic acid may have unwanted interaction with other residues and created the limited opening of substrate binding pocket. Against aspartic acid at 103, non interacting hydrophobic valine may have created the substrate site with a preference to Asparagine which increased the catalytic efficiency of the mutant enzyme. Due to these enhanced properties, the D103V mutant enzyme has a potential to be used in pharmaceutical industry as well as in food industry.
List of Publications
