STRUCTURE - BASED ACTIVE SITE ENGINEERING OF *Pyrococcus furiosus* L-ASPARAGINASE FOR IMPROVING ITS EFFICACY

By

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DEDICATION

To my beloved parents and my brother and sister for being there whole heartedly and unconditional love... ...

CERTIFICATE

This is to certify that the thesis entitled **"Structure-based active site engineering of *Pyrococcus furiosus* L-asparaginase for improving its efficacy"** being submitted by **Mr. Saurabh Bansal** to the **Indian Institute of Technology Delhi**, for the award of the degree of **'Doctor of Philosophy'**, is a record of the bonafide research work carried out by him, which has been prepared under our supervision and guidance in conformity with the rules and regulations of the 'Indian Institute of Technology, Delhi'. The research reports and the results presented in this thesis have not been submitted for any degree or diploma in any other University or Institute.

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Saurabh Bansal

ABSTRACT

Thermophilic enzymes are characterized by their capacity to withstand extremes of physicochemical conditions and display optimum activity at high-temperatures. They are generally stable and such enzymes are always desirable for various industrial applications. In spite of being stable, the use of thermophile-derived enzymes for therapeutic purposes invites serious criticism. This is because they fail to act optimally at physiological conditions (temperature and pH) as opposed to mesophilic enzymes.

L-asparaginase which catalyzes L-asparagine into L-aspartic acid and ammonia is being used as chemotherapeutic agent in the treatment of leukemia since long. Besides this, the enzyme also has industrial importance as it inhibits the synthesis of acrylamide in baked and fried food products and can also be used to make diagnostic biosensors. Currently, available therapeutically useful L-asparaginases are derived from mesophilic sources like *Escherichia coli* and *Erwinia chrysanthemi*. These mesophile-derived enzymes are associated with many drawbacks, in particular, short half-life due to instability, and side-effects due to associated glutaminase activity. Therefore, in the present study, L-asparaginase from a hyperthermophile *Pyrococcus furiosus* (PfA) was selected for characterizing and evaluating the feasibility of this enzyme as a chemotherapeutic agent. This enzyme served as a model thermophilic enzyme for tuning their kinetic properties (activity, affinity) similar to their mesophilic counterparts for functioning at physiological conditions and also for structure-based identification of critical residues of thermophilic enzyme's active site which were selected for modification.

As stated earlier, although stable, thermophile-derived enzymes do not serve any therapeutic purpose owing to their optimum activity at higher temperature. To overcome

the problem, the present study is an attempt to combine thermophilic scaffold stability with mesophilic enzymatic activity. For this purpose, the PfA was cloned, expressed and purified, from *E. coli* host. Three active site mutants (two single and one double) of PfA were made through comparative structural and sequence analysis of mesophilic and thermophilic counterparts. A rational structural homology based approach was followed in this study. In addition to high substrate affinity and activity at physiological conditions as compared to WT, all the three PfA mutants were found to be stable and free from glutaminase activity (a desirable characteristic to reduce side effects). Most significantly, a strikingly high anti-proliferative activity of the mutants (in particular K274E and T53Q) on leukemic cell lines was observed. The anti-cancerous activity of PfA variants (WT and its three mutants) was more pronounced than that of commercially available asparaginase from *E. coli* (EcA II). From the data, it is proposed that the PfA and its mutants may serve as potential alternative anti-leukemic drug.

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