



INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI  
SHORT ABSTRACT OF THESIS

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**SHORT ABSTRACT**

Research on extremophilic proteins has seen immense growth in the past decade. Modern mechanistic approaches to engineer such proteins *in vitro* will have impact in industrial biotechnology economy. Developing *a priori* knowledge about the mechanism behind protein extreme-stability by understanding genomic and proteomic attributes is the foundation for protein engineering. The present thesis entitled “Exploring molecular adaptations of extremophilic proteins: a platform for protein engineering” is an effort in this direction. Protein stability can be affected at three hierarchies of protein organization – DNA level, protein sequence and structure level. Can these factors be exploited for protein engineering to fill the caveat of attaining pre-determined mutations? The further objectives of the thesis were proposed to fill the existing lacuna.

Initially, a curated database on Extreme-stable Protein Database (ExProtDB) was created. The novelty of the database lies in the fact that it gives consolidated and comprehensive information of all types of extremophiles and extremophilic proteins. Then, the rationale behind the specific codon usage patterns in extremophiles was deciphered. Relative abundance analysis, 1-9 scale ranking, nucleotide compositions, attribute weighting and machine learning algorithms were employed to arrive at findings. Codon usage patterns exhibited harmony among different extremophiles and has been detailed. However, the codon attribute preferences and their selectivity of extremophiles varied in comparison to non-extremophiles. Although codon study is being partially deciphered in thermophiles, this is probably the first report of codon preferences pertaining to all extremophile classes. Further, the attributes contributing to extreme-stability of proteins was analysed. An attempt was also made to generate ranking

model for prepredicting desirable mutants. The positively contributing attributes have been further exploited to design mutations in a mesophilic (non-extremophilic) protein in such a way to increase the content of these attributes to endorse the prediction of generated models. Also, a pertinent account of *in silico* prediction of plausible mutations and their validation in *Bacillus subtilis* lipase (a chosen mesophilic enzyme) by substituting the preferred protein attributes to attain extreme-stability of proteins was done. Six different types of extreme-stable mutants were generated and validated further. *In silico* validation of mutant was done by AHP generated ranking models, various mutation prediction servers (HotSpot Wizard, I-Mutant2, Cupsat, iPTREE-STAB, WET-STAB and ERIS web servers), Substrate Docking (Autodock 4.2 version), Ramachandran analysis (PROCHECK) and Contact Map analysis (CMView 1.1.1). This was followed by *in vitro* validation of predicted mutations. Experimentation was employed for *in vitro* cloning, mutagenesis, expression, purification and characterization of lipases. The content of secondary structure enhanced extreme-stability (with increased  $T_m$  value) which was confirmed through FT-IR (ATR mode) and Differential Scanning Fluorimetry (Thermal shift assay). The mutants obtained were further employed for detergent formulation applications. To further increase the enzyme stability engineered lipases were immobilized on synthesized ZnO nanoparticles as it is economical, biocompatible and environmental friendly. The synthesized ZnO nanoparticles and lipase immobilization was confirmed using UV-Vis, FT-IR, Raman spectroscopy, DLS (for particle size), powder XRD, TEM and FESEM analysis. For selection of better washing conditions, the washing process was done at different temperatures and time intervals using different concentrations of detergent and lipase activities. Results also showed that the maximum removal of oil and grease stains were up to 87% and 82%, respectively. The thesis concludes with summarizing the findings. It also states the future scopes and upcoming challenges.

Conclusively, this work reveals a promising perspective of using low-cost nanoparticle conjugated biocatalysts which was produced through rationalized protein engineering approach and was instrumented for detergent formulation to overcome the drawbacks of the chemical counterparts.