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Title: Cloning, Expression and Characterization of Thermostable Enzymes: Ligase, Lyase and Protease

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Abstract: Organisms known to thrive in extreme conditions particularly high temperatures have enzymes or proteins with high structure, kinetic and thermal stability. They are more efficient than mesophiles in performing their activity at high temperature. Owing to these qualities inherent in them they are studied for their structure, function, stability, biophysical aspects and their potential applications in industries. In the present thesis, we are reporting the cloning, expression, purification and characterization of three proteins: Arginosuccinate lyase (ASL) and DNA ligase from *Pyrococcus furiosus* and Carboxy Terminal Protease from *Thermotoga maritima*. All the three proteins under study were found to be highly thermostable. Various biophysical tools namely, circular dichroism, gel filtration, dynamic light scattering and differential scanning calorimetry, etc have been used to characterize the proteins. Also, we attempted to crystallize ASL. Carboxy Terminal Protease (CTP) from *Thermotoga maritima* was cloned and protein expression attempts were made but we were not able to express it in the *E. coli* strains tried. We studied and understood various properties and nature of some thermophilic proteins.


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