Chapter 3

Thermophilic Enzymes
Enzymes exhibit exquisite catalytic power unmatched by conventional catalysts. Many applications of enzymes range from serving as catalysts for chemical synthesis for finding use in diagnostic testing, foods and pharmaceuticals. However, on several instances naturally occurring enzymes are not well suited for industrial application. Problems include instability and low catalytic activity on non natural substrates and non-performance at elevated temperature. Engineering an enzyme by modifying the gene sequences so as to get a desired structural domain is a plausible strategy.

The first step in engineering an enzyme is to create molecular diversity starting from a target gene or family of related genes. This diversity can be introduced by creating mutations and/or by recombination. The gene products are sorted out by screening or selection and those encoding improved products can be returned for further application.

In contrast to natural evolution, enzyme engineering has a defined goal, and the key processes mutation, recombination and selection or screening can be controlled carefully by the researcher. Useful reasonably sized gene libraries can be created by multiple mutation in a particular region across a limited number of positions. (Olson and Saccar 1988).

Protein engineers are becoming increasingly aware that many protein structures are not confined to smaller number of amino acids but are affected by residues far from active sites. Computer application in
protein engineering can help one in searching for variants resulting from one to several amino acid substitution libraries depending on length.

Most random mutagenesis methods create mutations in single base i.e. point mutation. The degeneracy of the genetic code provides access to only about six amino acid substitutes instead of nineteen, thus significantly reducing the potency of diversity. A library of recombined genes may provide an excellent starting point for creating novel function. This type of library can be generated by DNA shuffling (Stemmer 1994) and staggered extension process (Zaho et al 1998).

Of the several strategies, of getting a desired thermophilic enzyme of desired quality as stated above, have several advantages as well as several limitations. One needs to proceed from the very limited amount of information regarding the final product and then predicting the function of a protein in which mutation is incorporated. This is an evolving field of research.

Considering the problem associated with designing a thermophilic enzyme, researchers have focused their attention to thermophilic microorganisms. One of the thermophilic enzyme that have come into vogue is enzyme DNA polymerase from thermophilic bacteria Thermus aquaticus. The enzyme requires special mention as polymerase chain reaction is one of those techniques that has revolutionised the whole field of molecular biology.
The release of noxious gases like carbon monoxide, oxides of nitrogen etc. has resulted in global warming has contributed to increase in atmospheric temperature and melting of polar ice caps. The increased incidence of atmospheric temperature will force many of the plants to survive with decreased capability to metabolise the available resources like carbon dioxide, nutrients and water. This in turn will have far reaching consequences on the flora of planet earth. To compensate for this awesome, situation plants have to be equipped with heat stable enzymes. Genetic manipulation of the flora to evolve facultatively thermophilic organisms is a good strategy.

The screening of cyanobacteria for metal tolerance focused our research further on thermophilic cyanobacterium *Mastigocladus laminosus*. Thermophilic enzymes responsible for metabolism at elevated temperature from the cyanobacterium *Mastigocladus laminosus* can find a solution to global warming. We have conducted experiments on this cyanobacterium especially on the enzymes of the nitrogenase pathway *viz.*, glutamine synthetase, nitrate reductase. Both these enzymes were found to be heat stable indicating that there exist some differences in amino acid sequence, of the enzyme, compared to mesophilic counterpart, which confers thermostability. The results rejects the feasibility of the role of heat shock proteins in mediating thermostability of *Mastigocladus laminosus*.

Ribulose bi phosphate carboxylase of thermophilic origin was not characterised till date. Based on the observations of nitrate reductase and glutamine synthetase our results point to the existence
of thermostable ribulose biphosphate carboxylase in *Mastigocladus laminosus*.

Nitrogenase from *Mastigocladus laminosus* showed comparatively decreased affinity towards its substrate nitrogen. However the results of the present study point to the fact that thermophilic cyanobacteria is a good candidate for exploitation as biofertiliser under heavy metal stressed mesophilic conditions. The results on nitrogenase under heavy metal stress are discussed in the following chapter. The enzyme glutamine synthetase and nitrate reductase showed maximum specific activity in the temperature range 45-60°C and remained constant at this temperature range. Nitrate reductase was assayed as nitrite transport rate activity. The results of this enzyme under heavy metal stress are encompassed in detail in the following chapter. The maximum growth rate of the organism was also noted in the temperature range 45-60°C. However the organism can tolerate temperature up to 75°C. Glutamine synthetase from the organism showed a specific activity of 0.2μg gamma glutamyl hydroxamate/mg protein/minute in the temperature range 45-60°C.