

## 1.9 Summary

Bacteria are ubiquitous and the most abundant organisms on Earth. Bacterial cells present in the extreme environments are well adapted because of structural features and enzyme systems, which work efficiently in the conditions they exist. In this thesis, thermophilic bacterial diversity of hot springs present in parts of Himalayan ranges in states of Sikkim, Meghalaya and Himachal Pradesh, India is described. This work was started with the aim of isolation of thermophilic restriction endonucleases with novel specificities or better properties than the existing enzymes. The thesis is divided into five chapters. Chapter I deals with the existing literature on the aspects of bacterial diversity, methods of its classification and potential for production of beneficial products for human use. The second part of the review discusses important features of the enzymes of Type II Restriction-Modification system and emphasizes current status of related research. In chapter II, isolation of thermophilic bacteria from the hot springs and characterization of a novel bacterial species, *Caldimonas meghalayensis* AK31<sup>T</sup>, by polyphasic taxonomic approach is described. The isolated bacteria were screened for the production of Type II restriction endonucleases. One of the bacterial isolates, *Tepidimonas fonticaldi* PL17 containing Type II restriction endonuclease was selected for the restriction endonuclease enzyme studies and was characterized through polyphasic taxonomy and genome sequenced, described in chapter III. Chapter IV deals with the purification, characterization of the restriction endonuclease TfoI from *T. fonticaldi* PL17 and identification of the restriction-modification gene cluster responsible for the production of TfoI. TfoI methyltransferase (M.TfoI) was cloned and over-expressed in *E. coli* DH5 $\alpha$  and its methylation activity was confirmed, as discussed in chapter V. The M.TfoI is classified to be  $\beta$ -class according to the conserved motifs present in the protein sequence. Through homology modelling supported by the available literature, key residues important for catalysis of methyltransferase activity are predicted. The presence of the studied RM system in diverse bacteria from varied habitats prompted us to study the phylogeny of the methyltransferases of the system which suggests propagation of the RM system by horizontal gene transfer in different hosts.