SUMMARY

Cellulose, hemicellulose and starch are among the most abundant polymers on earth. In order to utilize these polysaccharides, microbes have evolved different modifications. Cellulose utilization by microbes is one of the major carbon utilization pathways. As the demand of industrially important enzymes has increased over the years so, there is necessity to unravel new enzymes having properties suitable for industrial processes. Microbes are the store house of these industrially important enzymes. It has already been estimated that the number of microbes on earth is more than the stars in the universe (Curtis and Sloan, 2004). Microbes exhibit ubiquitous habitat ranging from normal to extreme conditions. The incredible talent of microbes to get evolved and become genetically and physiologically stable allows them to survive under different conditions. The hidden enzymatic repertoire of microbial communities can be explored using two different approaches viz culture-dependent and culture-independent.

In the present study, we have used both the approaches to explore high diversity of microbes and the large repertoire of industrially important enzymes. Amylases, cellulases and hemicellulases were screened from different sites (soil, water and sediments). Among all the samples, soil has the highest diversity of species as it has already been estimated that one gram of soil can harbor up to 10 billion microorganisms of possibly thousands of different species (Rosselló-Mora and Amann, 2001). However, only 1% of soil microbial population is culturable and has been seen on plates. Therefore, in order to explore the hidden diversity of microbes, combinatorial approaches were used.

The first objective of the study involved the collection of samples from different environmental niches, isolation of thermophilic culturable isolates and preparation of fosmid metagenomic libraries. Sampling was done from diverse habitats such as forest, mangrove and hot springs. The sampling from forest and mangrove soil rich in lignocellulosic biomass increases the probability of getting novel cellulases and hemicellulases. The abundance of thermophilic

microorganism in hot springs offers a variety of thermostable industrially important enzymes. Therefore, these (forest soil, mangrove soil and hot springs) sampling sites were selected for the isolation and screening of thermostable industrially important hydrolytic enzymes.

Total six fosmid libraries were prepared from collected samples and screened for cellulolytic, hemicellulolytic enzymes. As a result of screening, 43 putative positive clones were obtained and analyzed for redundancy check. Sequencing analysis of positive clones was done to isolate any novel ORF if present in the clones. Among the clones sent for sequencing based on the functional screening, no clone was found to have a novel ORF. Therefore in addition to function based screening, all the other ORFs of the sequenced clones were also analyzed using sequence based screening to get a positive hit (novel ORF) if present. Consequently, one novel xylosidase-encoding ORF (*xyl43p*) was obtained from Palampur forest soil metagenomic library which was further analyzed using sequence based methods. Amino acid sequence analysis of xyl43p revealed that it belongs to GH43 family and has low homology and similarity to the already known sequences in NCBI database. Domain based prediction servers were used to predict the conserved residues, domains function and evolutionary relationships of xyl43p protein. xyl43p has a single domain encoding xylosidase. Homology modeling of xy143p protein using SWISS suggested that it has tertiary structure similar to enzymes from GH43 family. However, complete sequence could not be modeled due to its high novelty and less similarity. The novel ORF obtained from the metagenomic library was further pursued for cloning, expression and characterization in the heterologous expression system. xyl43p was overexpressed in *E. coli* expression host to check the activity. However, we did not observe any activity in xyl43p protein after functional analysis on substrate plates.

Besides, thermophilic culturable isolates obtained from hot spring sites were screened for starch hydrolyzing enzymes. Total 19 isolates were found to be positive for amylase and 11 for pullulanase. All the thermophilic isolates

were found to belong to the genus *Geobacillus* as confirmed by 16S rRNA sequence analysis. Out of all the positive thermophilic isolates, amylase of one isolate from the Manikaran hot spring was used for further purification and characterization. The purified α-amylase was thermostable with a temperature optimum at 80°C and pH optima at 6.0. The enzyme retained more than 90% of the residual activity even after 48 h of incubation with buffers of pH 5.0, 6.0, 7.0. The thermostability of α-amylase was significantly increased when incubated in the presence of substrate. Interestingly, enzymatic activity was not found to be increased in the presence of any divalent metal ion. Most important property of the enzyme viz. its ability to hydrolyze raw starches, increases its potential to be used as an industrial candidate for biofuel production when used with other commercially available enzymes.

In crux, the main aim of the study was to explore industrially important hydrolytic enzymes. After exploring the culturable and unculturable "sequence space" from soil and other ecosystems, we have successfully purified a thermostable α-amylase with unmistakable industrially important properties suggesting its potential for commercial exploitation. Besides, other thermostable enzymes with promising industrially important properties were also obtained from the screening of culturable isolates.