SUMMARY

Cellulose is the most abundant polymer on earth, and many microorganisms have evolved strategies to utilize cellulose as energy source. The increased demands of renewable sources of fuels compel us to think about new enzymes which can degrade the cellulose. The number of microbes on earth is more than the stars in the universe (Curtis and Sloan, 2004). The excellent capabilities of microbes to get evolved fast and become genetically and physiologically stable allow them to survive under extreme conditions. The hidden enzymatic treasure of microbial communities can be explored using culture-dependent and culture-independent (metagenomics) approaches.

In the present study we explored the new needful cellulases (Endogluconase and β-glucosidase) using the above approaches. The cellulases were screened from different sites (soil, water and sediments) using plate based screening methods or functional screening. The soil is the most complex habitat and has huge diversity of microbes and it has been reported that just one gram of soil has 10 billion microbes of different species (Rosselló-Mora and Amann, 2001). Only 1% of microbes are cultured using traditional culture-dependent approach so to explore the hidden diversity both approaches were used.

The first objective of the study was the collection of samples from different environmental niches, followed by prepration of fosmid metagenomic libraries and screening of these libraries for cellulases. Samples were collected from forest, mangrove and hot springs. The probability of getting a novel cellulase is more from forest and mangroves because these sites are rich in lignocellulosic biomass. The hot springs are potentially great source of thermophillic enzymes. Therefore, these sites were keenly explored for isolation of cellulase positive thermophiles.

Five fosmid metagenomic libraries were prepared and screened for cellulyotic enzymes. From these libraries we got 47 putative positive clones which were sent for sequencing to find out novel cellulases-encoding ORFs. After sequencing, the clones were analyzed using sequence based screening method to get novel ORF (positive hits). As a result, we got two novels ORFs which encoded endoglucanase and β-glucosidase from mangrove and hot spring libraries respectively. Amino

acid similarities show that the β-glucosidase-encoding ORF fell into the glycosyl hydrolase family 3 (GH3), and the endoglucanase-encoding fell into the glycosyl hydrolase family 16 (GH16). The novel ORF encoding β-glucosidase recovered from the metagenomic clones was cloned and overexpressed in heterologous *E. coli* expression system to check whether it was bioactive or not. The enzymatic activity was checked on substrate plates.

In the second objective we collected samples from the hot springs from Chumatang and Puga valley and thermophiles were isolated using culture- based approach. We got 110 total thermophiles from these sites. We got 8 β-glucosidase positive thermophiles after functional screening. Out of these 8, one microorganism was novel and belongs to the genus *Geobacillus*. The whole genome of this novel strain was sequenced and analyzed to find out β-glucosidase encoding genes.

In the third objective of the study, the yield of novel endoglucanase Cel5R recovered from metagenomic library was enhanced using high cell density fermentation. The yield of endoglucanase was increased 40-folds in comparison to shake flask which is the highest reported yield in *E. coli* till date. The effect of complex nitrogen source on yield of enzyme is also studied. The enzyme has the activity in the pH range of 5.0-6.0. The enzyme is halotolrent and halostable, hence a good candidate for biofuel industries. The enzyme retain 100% activity in 3M salt even after 30 days of incubation and it has activity in the presence of various organic solvents like acetone, acetonitrile, benzene, Propanol, ethanol, ethyleneglycol, toluene, DMSO and toluene. It is active in the presence of various surfactants like Tween-20, Tween-80, Triton X-100, SDS and Sarkosyl as well.

In crux, from the present study we successfully obtained fosmid metagenomic libraries and novel cellulase encoding ORFs. The novel cellulase producing strain of *Geobacillus* was isolated from thermophiles. The yield of endoglucanase was increased to very high levels using high cell density fermentation which help in bringing down the cost of enzyme and make the process economically suitable for industrial applications.