Chapter 6: Conclusions and Future prospects

Function based metagenomic study for the production of cellulase enzyme lead to following conclusions:

1. A rapid and simple method was developed for the isolation of metagenomic DNA (mgDNA) from goat rumen digesta using the CTAB based lysis and PEG to remove tannins followed by isopropanol precipitation. The yield of mgDNA was 240.6 µg/g rumen digesta with the purity of 1.75 and 0.9 with respect to protein and tannins, respectively. The isolated mgDNA was suitable for the restriction digestion and PCR amplification.
2. The mgDNA library was constructed in pUC19 cloning vector with the insert size of 0.5-2.0 kb from the goat rumen mgDNA. The library was assessed by digesting the recombinant pUC19 plasmid DNAs from randomly selected *E. coli* DH5α transformants with *Kpn*I. The shift in the recombinant pUC19 DNA bands confirmed the presence of insert in all the selected transformants.
3. The functional metagenomic approach for the cellulase coding gene from the rumen mgDNA revealed a clone 'T3' showing cellulolytic activity after screening in CMC (1%) agar medium. The sequencing data revealed the size of cellulolytic DNA insert to be 1,501 bp containing an ORF of 1,122 bp encoding a cellulase enzyme. The same was named as 'CelT3' and the nucleotide sequence of the cellulase coding gene was deposited in GenBank of NCBI data library under the accession number KX226390.
4. IPTG at 0.5 mM was found to be optimum for the expression of CelT3 protein. The purification of CelT3 protein using Ni-NTA affinity chromatography revealed a single protein band of approximately ~45 kDa size.
5. The nucleotide sequence of CelT3 on translation to the protein sequence showed encoding of 373 amino acids. The BLASTp analysis of CelT3 amino acid sequence revealed 96% sequence homology with the cellulase enzyme produced by the uncultured bacterium (accession no. AFJ05146).
6. The multiple sequence alignment of the CelT3 cellulase showed possession of a glycosyl hydrolase family 5 (GH5) catalytic domain.

7. Homology modelling for protein structure prediction for CelT3 revealed that the CelT3 cellulase consisted of 21 coils, 11 β-strands and 10 α-helices.
8. Ramachandran plot for the predicted model of CelT3 cellulase consisted of 96.6%, 2.8% and 0.6% amino acid residues in favoured, allowed and outlier regions, respectively. The result is significant as >96% percentage of residues is in the favoured region postulating that the protein is stable.
9. The highest CelT3 specific activity (17.74 U mg^{-1}) was obtained when CMC (1%) was used as the substrate. The relative activity decreased significantly when Avicel, filter paper and Laminarin were used as the substrate.
10. The enzyme kinetics of CelT3 cellulase revealed V_{max} and K_m values to be 222.22 U mg^{-1} and 0.35 mg ml^{-1}, respectively. The purified protein exhibited a specific activity of 17.74 U mg^{-1} with 4.42 purification fold.
11. The optimum temperature and pH for the maximum enzyme activity of CelT3 was found to be 45°C and 6.0, respectively. The maximum cellulase activity was observed in the presence of Ca^{2+} and Fe^{2+} ions. Ni^{2+} mildly inhibited the activity whereas Mg^{2+}, Co^{2+}, Zn^{2+} and Cu^{2+} significantly inhibited the CelT3 activity.

Future prospects

The CelT3 harbouring transformed *E. coli* BL21(DE3) need to be cultured in large scale for the production and extraction of the endoglucanase cellulase.

The extracted cellulase may be used in the related industries with due assessment.