

LIST OF FIGURES

Figure No.	Figure captions	Page No.
Fig 1.1	Schematic representation of construction of metagenomic libraries from environmental samples	2
Fig. 1.2	Industrial applications of metagenomics	4
Fig. 3.1	pUC19 cloning system	34
Fig. 3.2	pET28a(+) expression system	44
Fig. 3.3	Strategy for regulating the expression of genes cloned into a pET vector	46
Fig 4.1	mgDNA from goat rumen digesta	54
Fig 4.2	mgDNA extracted from goat rumen digesta using different methods	56
Fig 4.3	Bar graph plots on the comparative analysis of five different DNA extraction methods (P1–P5)	56
Fig 4.4	PCR amplification of 16S rRNA gene	58
Fig 4.5	Restriction digestion of mgDNA isolated by P5 method	58
Fig 4.6	mgDNA partially digested with <i>Bam</i> HI	59
Fig 4.7	pUC19 plasmid DNA	60
Fig 4.8	<i>Bam</i> HI-digested pUC19 plasmid DNA on 0.8% agarose gel	61
Fig 4.9	Recombinant pUC19 plasmid DNA from randomly selected <i>E. coli</i> DH5 α recombinant colonies	62
Fig 4.10	Linearization of recombinant pUC19 plasmid DNA from randomly selected <i>E. coli</i> DH5 α recombinant colonies using <i>Kpn</i> I	62
Fig 4.11	Screening of mgDNA library for cellulolytic clones on CMC agar	63
Fig 4.12	Recombinant pUC19 plasmid DNA from the cellulolytic T3 clone	64
Fig 4.13	<i>Bam</i> HI-catalysed digestion of recombinant pUC19 plasmid containing celT3 cellulolytic clone	65
Fig 4.14	DNA sequence of the CelT3 gene	65

Fig 4.15	PCR amplification of CelT3 gene	66
Fig 4.16	pET28a(+) plasmid DNA and its digestion with <i>Nde</i> I and <i>Hind</i> III	67
Fig 4.17	Double digestion of CelT3 gene with <i>Nde</i> I and <i>Hind</i> III	67
Fig 4.18	Transformation of <i>E. coli</i> DH5 α cells by pET28CT3	68
Fig 4.19	pET28a(+) plasmid DNA from <i>E. coli</i> DH5 α cells	69
Fig 4.20	Recombinant pET28a(+) DNA digestion with <i>Nde</i> I and <i>Hind</i> III	69
Fig 4.21	Cell free extract of recombinant clone [“pET28a(+) with CelT3” in <i>E. coli</i> BL21(DE3)] showing cellulolytic activity on CMC agar plate	70
Fig 4.22	SDS-PAGE of recombinant protein CelT3	71
Fig 4.23	Protein sequence of cloned cellulase gene	71
Fig 4.24	Phylogenetic tree of CelT3 protein	73
Fig 4.25	Multiple sequence alignment of CelT3 with GH5 cellulases	74
Fig 4.26	Ribbon representation of three dimensional structure of CelT3 cellulase	75
Fig 4.27	Ramachandran plot for validation of CelT3 structure prediction	75
Fig 4.28	Effects of pH on the activity of CelT3	77
Fig 4.29	Effect of temperature on the activity of CelT3	78
Fig 4.30	Effect of metal ions on the activity of CelT3	79
Fig 4.31	Effect of substrate concentration on the initial velocity of CelT3 cellulase catalyzed reaction	79
Fig 4.32	Determination of K_m and V_{max} of the CelT3 recombinant cellulase using Lineweaver-Burk plot	80