SUMMARY
Five different cellulolytic bacteria were isolated from three phytophagous insects. The cellulolytic bacteria, *Enterobacter cloacae* (isolated from termite, *Heterotermas indicola*), *Pseudomonas aeurginosa* and *Klebsiella pneumoniae* (isolated from *Oryctes rhinoceros*, coconut beetle) and *Pseudomonas fluorescence* and *Proteus mirabilis* (isolated from *Bombyx mori*, mulberry silkworm). The bacterial strains were characterized through standard biochemical tests and identified through 16S rRNA typing. The cellulolytic ability of the newly isolated strains was studied by standard cellulase assay. All the isolated cellulolytic bacteria were found to utilize both amorphous and crystalline forms of cellulose. The 16S rRNA sequences of isolated bacteria were deposited in NCBI with the following accession numbers: FJ799063 (*E. cloacae*), GU213909 (*P. aeurginosa*), HQ231794 (*K. pneumoniae*), HQ231795 (*P. fluorescence*) and HQ231796 (*P. mirabilis*).

Cellulase gene from each isolated bacterium was cloned through shot gun cloning method. The genomic DNA of each cellulolytic bacterium was isolated, purified and digested with restriction enzyme *BamHI*. The restricted genomic DNA was ligated with *BamHI* digested pET20 b(+) vector DNA using T<sub>4</sub> DNA ligase and transformed in to *E. coli* and the transformants were screened and selected by their total cellulase enzyme activity. The cellulase gene containing inserts in pET20b(+) plasmid were studied by restriction analysis. Highest cellulase enzyme activity showing cellulase gene clone, one from each cellulolytic bacterium was selected and studied further. The DNA insert in these selected clones were sequenced and the sequences were analysed and deposited in the NCBI with the following accession numbers: pET- cel-Ec(GQ368735-*E. cloacae*), pET- cel- Pa (GQ872426-*P. aeurginosa*), pET- cel -Kp (HM235918-*K. pneumoniae*), pET- cel -Pf (HM235919 - *P. fluorescence*) and pET -cel -Pm (HM235922 - *P. mirabilis*).
Recombinant \textit{Z. mobilis} strains were developed by subcloning the cellulase gene inserts from each pET cel clones in pKT230, a stable plasmid vector replicating in \textit{Z. mobilis}. Thus five different cellulase gene containing constructs in pKT230 vector were developed and named as follows: \textit{pKT cel Ec}, \textit{pKT-cel-Pa}, \textit{pKT-cel-Kp}, \textit{pKT-cel-Pf} and \textit{pKT-cel-Pm}. These plasmids were introduced into \textit{Z. mobilis} by CaCl\textsubscript{2} competent cell transformation. The transformants of \textit{Z. mobilis} harbouring the above recombinant plasmids were screened and selected further by standard cellulase enzyme assays. The intracellular localization of the expressed cellulase enzyme in the transformants was also determined. The molecular weights of cellulase proteins expressed from the recombinant \textit{Z. mobilis} strains were determined by Zymogram analysis. The molecular masses of the cellulase enzyme expressed from clones, \textit{pKT cel Ec}, \textit{pKT cel Pf}, \textit{pKT cel Pa}, \textit{pKT cel Kp} and \textit{pKT cell Pm} were 50, 40, 75, 42 and 41 kDa respectively. The molecular weights of the cellulase enzyme were in accordance with the deduced amino acid sequence of the cloned cellulase genes.

Lignocellulosic substrates such as, rice straw, sugarcane bagasse and coripith were pretreated following physical, chemical and biological methods. Pretreatment with NaOH (4 and 6\%) released a maximum of 78\% and 80\% cellulose in both sugarcane baggase and rice straw whereas HCl (3\%) released 70\% cellulose in coir pith. The pretreated substrates were analyzed by FTIR. The pretreated sugarcane bagasse, rice straw and coripith along with carboxymethyl cellulose were used as substrates for cellulosic ethanol production.

The recombinant \textit{Z. mobilis} strains developed with bacterial cellulase genes were able to utilize glucose, CMC, pretreated bagasse, straw and coripith under anaerobic conditions. The wild type \textit{Z. mobilis} and \textit{Z. mobilis} transformed with pKT230 plasmid produced 12\% (V/V) ethanol using yeast extract supplemented with glucose as a substrate and no ethanol production
was found when CMC, pretreated bagasse, straw and coir pith were used as substrates. But the recombinant *Z. mobilis* (harbouring *pKT-cel-Ec*) produced ethanol 12% (using glucose), 5.5% (using CMC), 4% (using 4% NaOH pretreated bagasse), 3.5% (using 6% NaOH pretreated rice straw) and 3% (using 3% HCl pretreated coirpith) in 72 hr. The recombinant *Z. mobilis* strains could be improved further by simultaneous expression of additional cellulase genes and the strains could be used for industrial level cellulosic ethanol production.