Abstract

Superior lignocellulolytic microbes are required for efficient bioconversion of plant biomass. This study aimed to isolate and characterize cellulose degrading microbes and screen efficient cellulase encoding genes from forest litter and compost samples collected from North-East India. Top five bacteria and eight fungi were evaluated for their lignocellulolytic capabilities. Selected bacterial strains were tested for filter paper and rice straw degradation ability. The bacterium, *Pseudomonas resinovorans* strain MNP-60 treated filter paper was degraded faster compared to reference strain, *Cellulomonas cellulans* MTCC 23. The strain MNP-60 had higher Mn peroxidase, lignin peroxidase, total cellulase, endoglucanase and β-glucosidase activities compared to the reference strain, *C. cellulans* MTCC 23. Fourier Transform Infrared (FTIR) based study indicated the efficiency of the bacteria in degradation of lignin and cellulose of rice straw. Similarly, selected fungal strains were tested for rice straw degradation ability. The fungus *Fusarium equiseti* strain TWRF-10 had higher endoglucanase, exoglucanase, and endoxylanase activities, whereas *Penicillium simplicissimum* strain TRF-27 had higher laccase and Mn peroxidase activities. Scanning electron spectroscopic (SEM) and FTIR analysis indicated changes in the surface morphology and chemical components in the fungi treated rice straw, respectively. X-ray diffraction (XRD) based study indicated the strain TRF-27 caused a 65% reduction in cellulose crystallinity. Environmental metagenomes are the potential source to retrieve genes for efficient cellulases. A total of four fosmid libraries were constructed using the metagenomic DNA extracted from compost samples and were screened for cellulolytic activity. The metagenomic DNA insert from a promising cellulase positive clone (pFOS-C1) was sequenced. An ORF (*ghc1*) encodes a protein of 376 amino acids with a molecular mass of 40.63 kDa had very weak homology (31%) with glycosyl hydrolase (GH) family protein.
The protein, pET28a-GHC1 exhibited strong activity on carboxymethyl cellulose (CMC) at pH 5.0 and 40°C. Molecular dynamics and docking based study indicated the interaction of the residues, Ser$^{162}$, Ser$^{229}$, Ala$^{228}$, Val$^{230}$ and Gly$^{289}$ with the CMC substrate in the active site. The specific activity of the pET28a-GHC1 was 17 fold higher than the reference commercial cellulase indicating its potential for industrial applications.