Sixteen thermophilic/thermotolerant strains isolated from composting soils included in the present study were identified on the basis of morphological and molecular characteristics. The molecular characterization was based on Amplified rDNA Restriction Analyses (ARDRA) of the 18S rDNA as well as sequence analysis of ITS1-5.8S-ITS2 and D1/D2 hyper-variable region of 26S rDNA. The dendrogram constructed by combined cluster analysis of restriction pattern obtained by MboI, Hinfl and Rsal revealed three distinct clades with members of mucorales, i.e., Rhizomucor pusillus and Mucor indicus forming an out group. The other clade was characterized with Malbranchea flava showing an early divergence, while, Myceliophthora sp. and Corynascus sepedonium showing high similarity coefficient were clubbed together. The phylogenetic trees constructed on the basis gene sequences of ITS1-5.8S-ITS2 and D1/D2 hyper-variable region of 26S rDNA, showed similar topology. However, ITS gene tree was more robust as more nodes within ITS tree received higher measure of support from bootstrapping. Thermophilic fungal isolates, i.e., Thermomyces lanuginosus, M. flava and Melanocarpus albomyces, producing alkaline active xylanases showed an earlier phylogenetic divergence as compared to other isolates included in the study. When cultured on rice straw containing solidified medium M. flava, T. lanuginosus and Myceliophthora sp., produced 3475, 9556 and 633.7 (U/g DW substrate) xylanase, respectively. The bleaching of Decker pulp with the xylanases from different fungal strains included in the study was carried out. Treatment of the pulp with M. flava xylanase resulted in maximal release of chromophores (237, 254 and 280 nm) as well as the increased brightness of resultant hand sheets was found to be 2.04% ISO units.

M. flava which showed distinct phylogenetic origin and a good source of alkaline active xylanase was taken up for further optimization of culture conditions for improving the production of hemicellulases by classical one variable at a time approach and response surface methodology based on Box-Behnken design of experiments. It was found that optimal enzyme activities, i.e., 16,802, 9.7, 58, 3.25 (units/g DW substrate) of xylanase, β-xylosidase, acetyl esterase and α-L-arabinofuranosidase, respectively, were produced on sorghum straw based solidified culture medium. Using combination of one factor at a time and Response Surface Methodology (RSM) optimization approaches, 2.26, 3.12, 6.02 and 3.26 folds increase in the levels of xylanase, β-xylosidase, acetyl esterase and α-L-arabinofuranosidase, activities, respectively, when compared to those under unoptimized conditions were achieved. The culture
under optimal process conditions produced, 8 functionally distinct xylanases, in addition 5, 10 and 6, β-xylosidase, acetyl esterase and α-L-arabinofuranosidase isoforms, respectively, as revealed by zymogram developed against native PAGE were observed. The partial secretome of *M. flava* was characterized using peptide mass fingerprinting (LC/MS/MS) and the protein spots were identified as xylanases (GH-11) and cellobioaldodehydrogenase, cellobiohydrolase constituting the components of cell wall degrading enzymes.

The regulation of xylanases and acetyl esterases was studied in presence of various complex carbohydrates, monosaccharides, disaccharides, alcohols and glycerol as carbon sources under shake flask cultures. Oatspelt xylan as a carbon source resulted in induction of eight electrophoretically distinct xylanases out of which three were found to be constitutive in nature. However, maximal expression of seven esterase isoforms was observed in presence of rice straw.

Two xylanases, MFX I and MFX II, from *M. flava* MTCC 4889 with molecular masses of 25.2 and 30 kDa and pIs of 4.5 and 3.7, respectively were purified to homogeneity. The xylanases were optimally active at pH 9.0 and at 60°C, exhibited a half-life of 4 h at 60°C, and showed distinct mode of action and product profiles with birchwood, oat spelt, and larchwood xylan, as well as wheat and rye arabinoxylan. The xylanases were most active on larchwood xylan with *K*<sub>m</sub> values of 1.25 and 3.7 mg/ml. *K*<sub>cat</sub>/*K*<sub>m</sub> values suggested that the xylanases preferentially hydrolyzed rye arabinoxylan. The biochemical characterization as well as liquid chromatography/mass spectrometry (LC-MS/MS) analysis of tryptic digests of MFX I and MFX II revealed similarity with known fungal xylanases belonging to the GH11 and GH10 glycosyl hydrolase families, respectively.

An acetyl esterase with a molecular mass and pI of 42 kDa and 3.0, respectively was purified to homogeneity. The purified esterase was optimally active at 50°C and at pH 6.0 and appreciably stable at 60°C (pH 6.0 and 7.0) for 4h. At 70°C, ~ 80% of activity was still recoverable after 4 h of incubation. The activity of purified esterase was positively modulated by all monovalent, divalent metal ions as well as SDS, PMSF, DTT and β-mercaptoethanol. The purified esterase was most active against pNP acetate and it could also recognise pNP butyrate but showed very low activity against pNP ferulate. The estimated *K*<sub>m</sub>, *V*<sub>max</sub> and *k*<sub>cat</sub> values for the purified esterase with pNP acetate as a substrate were 7.42 mM, 2.78 μmol min<sup>-1</sup>mg protein<sup>-1</sup> and 233.52 min<sup>-1</sup>, respectively. The purified esterase on the basis of characterization was putatively classified as carbohydrate esterase (CE-4).