ABSTRACT

Xylan, the second most abundant plant polysaccharide comprising a backbone of β-1,4 linked xylopyranosyl residues. It is a hetero polysaccharide that contains repeating group of acetyl, 4-O-methyl-D-glucuronosyl and α-arabinofuranosyl residues linked to the backbone and has binding properties mediated by covalent and non covalent interactions with lignin, cellulose and other polymers. Xylanases are glycoside hydrolases group of enzymes that depolymerize the glycoside linkages in the heteroxylan backbone. The complete depolymerization of xylan requires the interaction of a number of the main-chain and side-chain cleaving enzyme activities, of which endoxylanase (E.C.3.2.1.8), exoxylanase (E.C.3.2.1.37) and β-xylosidase play vital role. These enzymes are of great importance in several industries such as in the paper pulp bleaching industries, animal feed industries, etc. Production cost of xylanase can be reduced by using highly abundant, low cost and easily available xylan rich agro residues as raw material. In India, an agriculture based country, these agro residues are generated throughout the year.

In the present study, two potent xylanase producing microorganisms were isolated from garden soil. They were identified as *Penicillium citrinum* xym2 and *Bacillus subtlis* xym4, on the basis of phylogenetic analysis of 28S rDNA and 16S rDNA sequences, respectively. Both the isolates were employed for production of xylanases under submerged fermentation of various agro residues and both of them showed a significantly higher xylanase yield on wheat bran as compared to that of expensive birchwood xylan. Optimization of cultural conditions and media parameters for both the isolates through one factor at a time (OFAT) and response surface methodology (RSM) approach considerably increased the production of xylanases by them. Consequently, xylanase production by *P. citrinum* xym2 was enhanced from 712 IU/ml (unoptimized) to 1853 IU/ml (OFAT) to 2834 IU/ml (RSM), whereas the enzyme production by *B. subtilis* xym4 was enhanced from 981.5 IU/ml (unoptimized) to 2100 IU/ml (OFAT) to 11800 IU/ml (RSM). *P. citrinum* xym2 also produced 1492 IU/ml of carboxymethyl cellulose, on the other hand, *B. subtilis* xym4 produced significant amount of β-xylosidase and FPase with marginal cellulase activity. Xylanase enzyme cocktail obtained from the *Penicillium* (XEC^P) and *Bacillus* (XEC^B) were further employed in saccharification of wheat bran for liberation of reducing sugar. Reducing sugar released by XEC^B was further optimized through Plackett-Burman (PB) design and RSM. Using these statistical approaches resulted in release of 11.5 mg/l of sugar. The sugar rich broths obtained after XEC^P and XEC^B treatments were fermented to

xylitol by *E. coli* xyl6 with xylitol concentration of 3.2g/l and 4.5 g/l, respectively. *B. subtilis* xym4 and *E. coli* xyl6 were used in a simultaneous saccharification and fermentation (SSF) experiment under various conditions. SSF resulted in markedly higher amount of xylitol production (98.4g/l) under condition4 where *E. coli* xyl6 added to the fermentation medium 24 h after the inoculation of *B. subtilis* xym4.