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Molecular Cloning and Sequencing of Alkalophilic *Cellulosimicrobium cellulans* CKMX1 Xylanase Gene Isolated from Mushroom Compost and Characterization of the Gene Product

Abhishek Walia[1*], Preeti Mehta[2], Shiwani Guleria[3], Anjali Chauhan[3] and C.K. Shirkot[3]
[1]Department of Microbiology; DAV University; Jalandhar, Punjab – India. [2]DBT-IOC Centre for Bioenergy Research, Indian Oil R&D Centre, Haryana, India. [3]Department of Basic Sciences (Microbiology Section); Dr. YS Parmar University of Horticulture and Forestry; Nauni, Solan – India

ABSTRACT

A xylanolytic bacterium was isolated from mushroom compost by using enrichment technique. Results from the metabolic fingerprinting, whole-cell fatty acids methyl ester analysis and 16S rDNA sequencing suggested the bacterium to be Cellulosimicrobium cellulans *CKMX1. Due to the xylanolytic activity of this bacterium, isolation and characterization of the xylanase gene were attempted. A distinct fragment of about 1671 bp was successfully amplified using PCR and cloned into* Escherichia coli *DH5α. A BLAST search confirmed that the DNA sequence from the amplified fragment was endo-1, 4-beta-xylanase, which was a member of glycoside hydrolase family 11. It showed 98% homology with* Cellulosimicrobium *sp. xylanase gene (Accession no. FJ859907.1) reported from the gut of Eisenia fetida in Korea.* In silico *physico-chemical characterization of amino acid sequence of xylanase showed an open reading frame encoding a 556 amino acid sequence with a molecular weight of 58 kDa and theoretical isolectric point (pI) of 4.46 was computed using Expasy's ProtParam server. Secondary and homology based 3D structure of xylanase was analysed using SOPMA and Swiss-Prot software.*

Key words: Cellulase free xylanase, *Cellulosimicrobium cellulans*, *E.coli*, Cloning, Gene

INTRODUCTION

Xylan is the major hemicellulosic constituent of hard and soft wood, and is the next most abundant renewable polysaccharide after cellulose. This complex heteropolysaccharide consists of a main chain of 1,4-β-D-xylose monomers and short chain branches consisting of O-acetyl, α-L-arabinofuranosyl and α-D-glucuronyl residues. Xylanases and associated debranching enzymes produced by a variety of microorganisms including bacteria, yeast and filamentous fungi, bring about the hydrolysis of hemicelluloses (Maheshwari et al. 2000). Xylanolytic enzymes are receiving increasing attention because of their potential application in pulp bleaching (Golugiri et al. 2012; Singh et al. 2013) and bioconversion of lignocelluloses into feedstocks and fuels (Kim et al. 2000). The xylan degrading system includes endo-1,4-xylanases (1,4-β-xylan xylanohydrolase; EC 3.2.1.8), which release long and short xylo-oligosaccharides, and other xylanases that attack only longer chains, and β-D-xylosidase (1,4-β-xylan xylohydrolase; EC 3.2.1.37), which remove D-xylose residues from short xylo-oligosaccharides (Saha 2003; Gomez et al. 2008).

[*]Author for correspondence: sunny_0999walia@yahoo.co.in

Recent advances in molecular biology and genetic engineering in the last two decades have opened up the areas of application of gene cloning and recombinant DNA technology for the construction of genetically modified microbial strains with selected enzyme machinery. To ensure the commercial utilization of hemicellulosic residues in the pulp and paper industries, the production of higher xylanase yields at low capital cost is required. In this respect, isolation and cloning of the xylanase gene represents an essential step in the engineering of the most efficient microorganisms (Hernandez et al. 2008; Deesukon et al. 2011). Several reports describe the production, purification, partial characterization, molecular cloning, sequencing and expression of the alkaline xylanase gene from alkalophilic *Bacillus* sp. strain C-125 in non-cellulase producing *E.coli* carrying a plasmid pCX311 (Liu et al. 2010; Verma and Satyanarayana, 2012; Driss et al. 2012). Such studies are essential to produce a more-efficient xylanase producer, which will allow improvement of paper quality (Walia et al. 2015). Furthermore, biochemical studies on xylanase-secreting and non-secreting microorganisms could lead to better understanding of the xylanase secretory process and the development of cloning strategies that would guarantee secretion of desired products. Various molecular and biotechnological aspects of xylanase producing microorganisms, such as the regulation of xylanase biosynthesis at the molecular level, and newer strategies, such as use of gene cloning, protein engineering, and site-directed mutagenesis for obtaining xylanase with novel properties, have been described in detail by Kulkarni et al.(2003).

Thus, the aim of this work was to isolate, clone and sequencing of xylanase gene and physico-chemical characterization of deduced amino acid sequence of xylanase gene by using Expasy's ProtParam server. Secondary and homology based 3D structure of xylanase was also analysed and compared with some other commercially important xylanases using significant improvement in protein secondary structure prediction by consensus prediction from multiple alignments (SOPMA) and Swiss-Prot software.

MATERIAL AND METHODS

Strain isolation

Alkalophilic *Cellulosimicrobium cellulans* CKMX1 was previously isolated from mushroom compost (Walia et al. 2013). Xylan degrading bacteria were isolated by the enrichment technique. The most predominant bacterial colonies capable of good growth on basal salt medium (BSM) with the following composition (g/L): Na_2HPO_4, 6.0; KH_2PO_4, 3.0; NaCl, 0.5; NH_4Cl, 1.0, 1 M $MgSO_4$ (2 mL) and 1 M $CaCl_2$ (0.1 mL) were picked and purified. The bacterial culture was grown and maintained in BSM, pH 8.0, containing 0.5% xylan. The bacterial culture was maintained in 30% glycerol at -20°C.

Identification of bacteria

The bacterium was identified using colony morphology observation, biochemical tests, whole-cell fatty acid methyl ester analysis and 16S rDNA sequencing.

Bacterial DNA extraction

Bacterial isolate was grown in nutrient broth at 35°C overnight and the cells were harvested by centrifugation at 5,000×g for 5 min. DNA was isolated from these cells by using Real genomic DNA extraction kit (Taiwan), which was suspended in 100 µL of elution buffer and quantified on 1% agarose gel. The total genomic DNA was kept at -20°C before use (Sambrook and Russel 2001).

PCR amplification of 16S rDNA and sequence determination

Species level identification of strain was conducted by 16S rDNA sequence comparison. PCR reaction was carried out in 20 µL reaction containing ~50ng of template DNA, 20 pmoles of each primer fC1 (5´-GCAAGTCGAGCGGACAGATGGGAGC-3´) and reverse primer rC2 (5´-AACTCTCGTGGTGTGACG GGCGGTG-3´), 0.2 mM dNTPs and 1 U Taq polymerase (Genei Bangalore) in 1x PCR buffer. Reaction were cycled 35 times as 94ºC for 30 s, 58ºC for 30 s, 72ºC for 1 min 30 s, followed by final extension at 72ºC for 10 min. The PCR products were analyzed on 1% agarose gel in 1x TAE buffer, run at 100 V for 1 h. Gels were stained with ethidium bromide and photographed. Amplified PCR products were eluted from the gel using gel extraction kit (Real genomic Hi Yield TM Gel/PCR DNA Extraction Kit), eluted fragment was then sequenced (Xcleris, India) using PCR primers.

Primers for cloning of xylanase gene

PCR Primers to amplify the xylanase gene were designed based on the endo-1,4-beta-xylanase gene sequences from different *Cellulosimicrobium* sp. DNA sequences of xylanases were obtained from GenBank (www.ncbi.nlm.nih.gov) and were aligned using ClustalW (Larkin et al. 2007) and CLC sequence viewer (www.clcbio.com). Conserved regions were detected.

i) Primers for cloning and sequencing of partial xylanase gene

Following set of primers were designed on the basis of gene sequences of earlier known xylanases submitted in NCBI database:

Primer set 1:

These primers were designed using known consensus sequences of xylanase:

Forward XylF: 5'-cgtcggcttcgcgctcgaccc-3' (21 mer)

Reverse XylR: 5'-cggtgatgcgcacgtccacgcc-3' (22 mer)

ii) Primers for cloning and sequencing of complete xylanase gene

Primer set 1:

These primers were designed from the closest match of known partial sequence of xylanase CKMX1:

Forward XylCompF: 5'-atgaccaggaccatctggagacgacc-3' (26 mer)

Reverse XylCompR: 5'-tcaggcgacctcgcaggcggcaccgtcg-3' (28 mer)

The above primers were custom synthesized and supplied in lyophilized form by ITC Promega. Primers were regenerated by suspending in autoclaved double distilled water to make 1.0 mL of 1 pM primer before use.

Xylanase gene isolation

PCR was performed using the genomic DNA isolated from the bacterium as a template. PCR reaction were cycled 30 times as denaturation was 94ºC for 45 s, annealing was 45ºC for 1 min and extension was 72ºC for 2 min followed by final extension at 72ºC for 10 min.

Cloning and transformation

Purified PCR products (amplicons) was ligated into pGEM-T easy cloning vector as per manufacturer's instructions (pGMET- clone kit, Promega) and transformed into *E. coli* DH5α competent cells using the heatshock method. Successful transformants were selected using blue/white screening. Plasmids were extracted using a plasmid extraction kit (Axygen Prep™ Plasmid Mini prep Kit), according to manufacturer's instruction from the culture with positive colony PCR results verified by restriction digest, and was sent for sequencing by the commercial sequencing facility (Xcleris lab).

Plate assay for screening recombinants

The recombinants containing desired PCR product inserts were screened for the expression of xylanase activity by the Congo-red assay described by Teather and Wood (1982). The recombinant *E. coli* clones containing the recombinants were overlaid with 0.5% oat-spelt xylan, dissolved in solid LB and then incubated at 37ºC overnight. Then, the plates were stained with 1% Congo-red for 30 min and destained with 1 M NaCl. Positive clones were identified by a zone of clearance around xylanase-expressing clones truncated.

Sequencing and phylogenetic analysis of xylanase gene of *C. cellulans* CKMX1

The sequence was aligned with corresponding sequences of 16S rRNA from the database using BLAST from the website http://www.ncbi.nlm.nih.gov/blast (Altschul et al.1997). Multiple alignments were generated by the MULTALIN program from the website: http://prodes.toulouse.inra.fr/multialin/multialin.html (Corpet 1998). The phylogenetic trees were constructed after truncating the sequences to the length of shortest sequence in a given alignment data by neighbor-joining algorithm using PHYLIP Package (Felsenstein et al. 1993). The stability among clades of a phylogenetic tree was assessed by taking 1000 replicates of the data set and was analyzed using the programs SEQBOOT, DNADIST, NEIGHBOR and CONSENSE of the PHYLIP package. Tree was viewed with the help of TreeView from the website http://taxonomy.zoology.gla.ac.uk/rod/treeview.html (Page 1996).

Physico-chemical characterization of amino acid sequence of xylanase

Amino acid sequence of xylanase gene of *C. cellulans* CKMX1 was deduced by using xylanase gene sequence of strain CKMX1 and translates into protein sequence by expasy protein software. Theoretical isoelectric point (pI), molecular weight, total number of positive and negative

residues, instability index (Guruprasad et al. 1990), aliphatic index (Ikai 1980) were computed using the Expasy's ProtParam server (Gasteiger et al. 2005) (http://us.expasy.org/tools/protparam.html). Amino acid composition of the protein sequences could reveal their nature; hence, amino acid composition was also computed.

Secondary structure prediction

SOPMA was employed for calculating the secondary structural features of the selected target protein sequences considered for this study (Geourjon and Deleage 1995).

Homology-based structural model of xylanase

Homology-based structural model of xylanase from *C. cellulans* CKMX1 was built using SWISS-MODEL server (http://swissmodel.expasy.org/). Exo-beta-1, 4-glucanase from *Cellulomonas fimi* (PDB ID: 3CUG, 69.16% sequence identity) was used as template to build the structural model of xylanase. The model was visualised using PyMOL (Delano 2002) The PyMOL Molecular Graphics System (2002) found online (http://pymol.org)].

RESULTS

Isolation and identification

Xylanolytic bacteria was previously isolated from the enriched culture on basal salt media (BSM) containing xylan as the sole carbon source and identified as *C. cellulans* CKMX1 according to morphological, biochemical, whole-cell fatty acid methyl ester analysis and 16S rDNA sequence (Walia et al. 2013; 2014).

Phylogenetic analysis of isolate CKMX1 according to 16S rDNA sequence

Universal primers were used successfully to amplify 16S rDNA from bacterial isolate CKMX1, yielding an amplicon of the expected size, i.e., ~1136 bp. The sequence of 16S rDNA from CKMX1 was then analyzed using BLASTn analysis (http://www.ncbi.nlm.nih.gov/blast) and was found to have 97% homology with several *C. cellulans* strains reported earlier. The 16S rDNA sequence of CKMX1 was also compared with the corresponding sequences of eight different *Cellulosimicrobium* sp. reported earlier. Sequence analysis revealed that CKMX1 belonged to *C. cellulans* strain CKMX1 as it showed maximum homology (97%) with *C. cellulans* strain AMP-11 (Accession no. HM104377).

To trace out the evolutionary patterns of the test isolate and to determine the relationship with other selected sequences at NCBI, a phylogenetic tree was also constructed using the neighbour-joining (J) method of mathematical averages among 16S rDNA sequence of CKMX1 and the corresponding sequence of eight different *Cellulosimicrobium* sp. strain CKMX1 was united with quite high statistical support by the bootstrap estimates for 1,000 replications. The resulting phylogenetic tree also verified CKMX1 as *C. cellulans* as it clustered closely with *C. cellulans* with high (80%) boot strap value. The 16S rDNA sequence of the strain has been deposited in the GenBank database under accession number JN135476.

Detection and isolation of xylanase gene from genomic DNA of *C. cellulans* CKMX1 using specific primer based PCR method

Xylanase gene specific primers were designed for the isolation of partial xylanase i.e., XylF and XylR, as mentioned in the materials and methods section, were used for the detection and amplification of partial xylanase gene from *C. cellulans* CKMX1. PCR reaction resulted in the amplification of ~564 bp fragment as expected for partial xylanase gene. The amplified fragment was then excised from the gel and was used for cloning into pGEM-T easy vector.

DNA sequencing and cloning of PCR product into pGEM-T easy vector system

The purified PCR product was ligated to pGEM-t easy vector and finally transformed to competent *E. coli* (DH5α) cells. Positive clone containing insert was identified using colony PCR. Plasmid was then isolated from the selected positive colony and was sequenced. Sequence of 564 bp corresponding to partial xylanase gene of CKMX1 was obtained. The partial xylanase gene sequence of the strain was deposited in the GenBank database under accession number HF546135.

Amplificantion and sequencing of complete xylanase gene from *C. cellulans* CKMX1

After obtaining the partial sequence of xylanase gene from *C. cellulans* CKMX1, primers were designed as described in the material and methods section based on the known partial sequence of xylanase CKMX1. An expected amplification

product of ~1671 bp was obtained in PCR amplification using designed primers (Fig. 1).

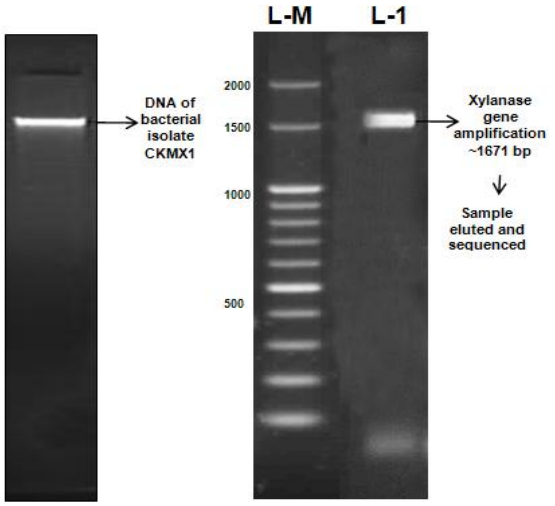

Figure 1 - Agarose gel electrophoresis showing the isolation of DNA and amplification of complete xylanase gene from *C. cellulans* CKMX1. Lane M: 100 bp Marker, Lane 1: Showing ~1671 bp amplification.

PCR product was then cloned to pGEM-T easy vector system and transformed to competent *E. coli* (DH5α) cells. Clones containing insert was then identified using colony PCR. Plasmid was then isolated from selected positive colony and was sequenced. Sequence of 1671 bp corresponding to complete xylanase gene of CKMX1 was obtained.

Sequence analysis of complete xylanase gene of *C. cellulans* CKMX1

Sequence of complete xylanase gene of *C. cellulans* CKMX1 was analysed with corresponding sequences of nineteen different sequences of xylanase gene from *Cellulosimicrobium* sp. reported earlier. Sequence analysis revealed that xylanase gene of *C. cellulans* CKMX1 showed maximum homology (98%) with xylanase gene of *Cellulosimicrobium* sp. (Accession no. FJ859907.1) reported from the gut of *Eisenia fetida* in Korea, followed by 75.6% homology with *Streptomyces rameus* strain L2001 (Accession no. KC011007).

C. cellulans CKMX1 was united with quite high statistical support by the bootstrap method and value inferred higher than 40% are only presented in Figure 2. Phylogenetic tree based on complete xylanase sequences (Fig. 2) validated that the xylanase gene of *C. cellulans* CKMX1 clustered closely with xylanase gene of other reported isolates of *Cellulosimicrobium* sp. with high boot strap value (100%). The complete xylanase gene sequence of the strain has been deposited in the GenBank database under accession number HG425182. Multiple sequence alignment of xylanase gene of strain CKMX1 with closely related strain HY-13 was done by ClustalW alignment software. Nucleotide sequence of CKMX1 was then used to deduce the amino acid sequence of the xylanase protein using expasy protein translation tool (http://us.expasy.org/tools.html). Amino acid sequence corresponding to xylanase protein from CKMX1 was also aligned with the corresponding amino acid sequence from *C. Cellulans* strain HY-13 reported from the gut of *E. fetida* in Korea.

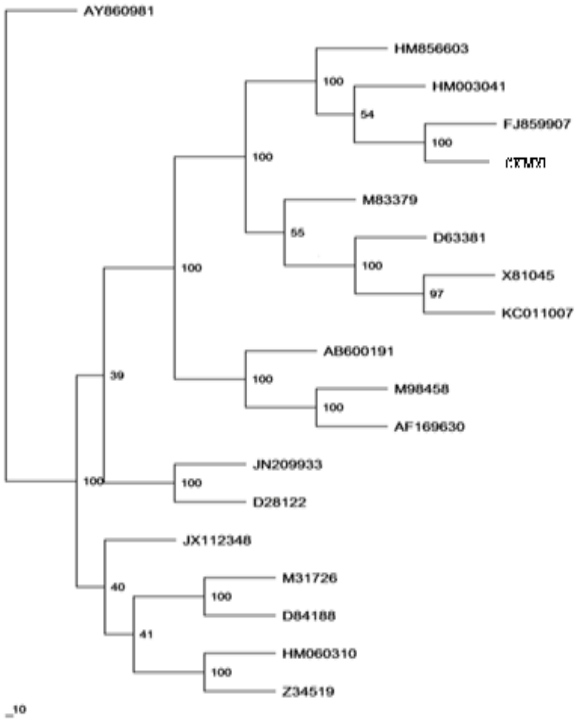

Figure 2 - Neighbour-joining phylogenetic tree based on complete xylanase gene sequence data of *C. cellulans* CKMX1 and related strains. Isolate used in the study is in boldface.

Physico-chemical characterization of amino acid sequence of xylanase

Theoretical isoelectric point (pI), molecular weight, total number of positive and negative residues, instability index, and aliphatic index were computed using the Expasy's ProtParam server (http://us.expasy.org/tools/protparam.html).

Amino acid composition of the protein sequences could reveal their nature; hence, amino acid composition was also computed (Table 1).

Maximum percentage composition of alanine was 15.1%, followed by threonine 11.2%, glycine 10.4%, valine 7.9% and so on. The amino acid composition of xylanase polypeptide showed that it contained 63 negatively charged residues (Asp+Glu) and 37 positively charged residues (Arg+Lys). The xylanases from different microorganisms showed significant similarity when theoretical protein parameters were compared (Table 2). The pI values of all protein sequences were in the range of 4.32-9.57, indicating that all considered xylanase sequences were acidic, except *C. flavigena* XIB (9.57), *C. flavigena* DSM 20109 (8.94), *Cellvibrio gilvus* ATCC 13127 (8.70) and *Bacillus subtilis* (9.18), *Pedobacter saltans* DSM 12145 (9.16). The xylanase of *C. cellulans* CKMX1 had the highest pI value of 4.66, which showed the xylanase sequence was acidic.

Amino acid		No.	Percentage composition (%)
Ala	(A)	84	15.1
Arg	(R)	28	5.0
Asn	(N)	12	2.2
Asp	(D)	42	7.6
Cys	(C)	5	0.9
Gln	(Q)	23	4.1
Glu	(E)	21	3.8
Gly	(G)	58	10.4
His	(H)	10	1.8
Ile	(I)	18	3.2
Leu	(L)	34	6.1
Lys	(K)	9	1.6
Met	(M)	4	0.7
Phe	(F)	20	3.6
Pro	(P)	24	4.3
Ser	(S)	29	5.2
Thr	(T)	62	11.2
Trp	(W)	15	2.7
Tyr	(Y)	14	2.5
Val	(V)	44	7.9
Pyl	(O)	0	0.0
Sec	(U)	0	0.0

Table 1 - Deduced amino acid composition of xylanase of *C. cellulans* CKMX1.

Table 2 - Theoretical protein parameters of few xylanases calculated using bioinformatics tools.

Organism	Molecular weight (Da)	Amino acid No.	*p*I	No. of charged residues	Total no. of atoms	Instability index	Aliphatic index
Cellulosimicrobium cellulans CKMX1	58489.6	556	4.66	100	8085	22.89	74.53
Cellulosimicrobium sp. HY-13	58296.3	556	4.59	101	8052	25.17	75.23
Cellulosimicrobium sp. HY-12	42925.1	395	5.59	77	5984	38.99	77.65
Cellulomonas flavigena	26314.5	261	5.21	23	3506	26.50	34.71
Cellulomonas flavigena XIB	35110.1	332	9.57	31	4773	31.17	47.59
Cellulomonas flavigena DSM 20109	50310.5	472	8.94	62	6886	33.65	56.14
Cellulomonas fimi ATCC 484	141665.2	1350	4.32	260	19715	27.78	85.79
Cellvibrio gilvus ATCC 13127	49781.9	469	8.70	66	6831	27.32	60.43
Bacillus sp. A	45294.3	396	4.65	106	6267	32.69	78.31
Bacillus subtilis	22409.4	206	9.18	24	3056	16.14	52.52
Geobacillus stearothermophilus	70596.5	620	4.96	149	9814	37.08	77.23
Paenibacillus polymyxa E681	67509.2	631	5.95	87	9346	17.43	73.76
Pedobacter saltans DSM 12145	33580.5	306	9.16	57	4745	37.78	95.33
Granulicella mallensis MP5ACTX8	34016.8	314	6.63	56	4795	42.29	90.38

Instability index showed that all the considered sequences were classified as stable with values ranging from 16.14 to 38.99, except *Granulicella mallensis* MP5ACTX8 (42.29). The xylanase sequence of *C. cellulans* CKMX1 showed instability index (22.89) as a value >40 indicated a stable protein. Aliphatic index for the xylanase sequences ranged from 34.71-95.33. The very high aliphatic index of all xylanase sequences indicated that these xylanases could be stable for a wide

temperature range, including the xylanase sequence of *C. cellulans* CKMX1, which showed 74.53 aliphatic index.

Secondary structure prediction

Significant improvement in protein secondary structure prediction by consensus prediction from multiple alignments (SOPMA) was employed for calculating the secondary structural features of the selected target protein sequences considered for this study. The secondary structure indicates whether a given amino acid lies in a helix, strand or coil. Secondary structure features as predicted using SOPMA are represented in Table 3. The results revealed that random coils dominated among secondary structure elements, followed by alpha helix, extended strand and beta turn in *C. cellulans* CKMX1.

Table 3 - Calculated secondary structure elements of *C. cellulans* CKMX1.

Secondary structure	*C. cellulans* CKMX1 (%)
Alpha helix (Hh)	27.70
3_{10} helix (Gg)	0.00
Pi helix (Ii)	0.00
Beta bridge (Bb)	0.00
Extended strand (Ee)	19.24
Beta turn (Tt)	7.19
Bend region (Ss)	0.00
Random coil (Cc)	45.86
Ambigous states	0.00
Other states	0.00

Homology based 3D structure prediction

The molecular model of the endo-1,4-β xylanase is described as consisting of an alpha helix and several beta pleated sheets with a compact structure and is shown in Figure 3.

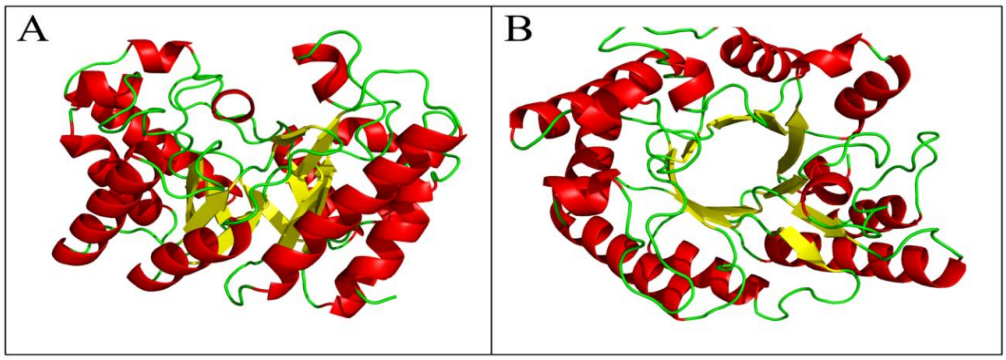

Figure 3 - Homology based 3D structure of xylanase proteins by using Swiss-Prot model A) Side view B) Top view.

DISCUSSION

Several recombinant xylanases from bacteria, yeast and fungi have been cloned and expressed in *E. coli* by construction of genomic libraries, followed by expression cloning. In this study, a highly xylanolytic *C.cellulans* CKMX1 was isolated from mushroom compost. *Cellulomonas* sp. is known to produce and secrete a variety of hydrolytic enzymes, and thus can utilize various complex carbohydrates in their natural habitats such as soil. Xylanases are produced in various amounts by bacteria and filamentous fungi to degrade xylan as a source of energy and have potential application in a wide range of industrial processes (Kulkarni et al. 2003; Keshwani and Cheng 2009). Xylanases encoding genes from *Cellulomonas* species have been cloned and sequenced (Bhalero et al. 1990). Multiple xylanases may be produced by the organisms to enhance the utilization of xylan (Wong et al. 1988).

In the present study, *C. cellulans* CKMX1 had unique ability to produce cellulase-free xylanase (Walia et al. 2012; Walia et al. 2013 and 2014). The results also showed the xylan hydrolysis by endoxylanases (1,4-β-D-xylan xylanohydrolase E.C.3.2.1.8). Therefore, *C. cellulans* CKMX1 isolated from mushroom compost was selected for further identification of xylanse encoding gene responsible for xylanase production. The PCR results obtained showed that the *C. cellulans* CKMX1 exhibited a band of 1671 bp, corresponding to a fragment of xylanase encoding

gene (Fig. 1). The nucleotide sequence of 1671 bp amplicon shared 98% homology to xylanase gene of *Cellulosimicrobium* sp. HY-13 endo-beta-1,4-xylanase (xylK-1) gene, (Accession no. FJ859907), thus confirming the isolation of xylanase gene from *C. cellulans* CKMX1 (Fig. 1). A positive clone, indicating that the clone contained an insert of 1671 bp in size with a 58% G+C content, in conformity with the high G+C levels was found in nucleotide sequences from other *Cellulosimicrobium* sp., e.g., endo-beta-1, 4-xylanase gene from *Cellulosimicrobium* sp. HY-13 (Kim et al. 2009). The conserved domain search of the xylanases gene across the *Cellulosimicrobium* sp. and other bacterial species indicates the significance of enzyme in xylan hydrolysis. The mature protein consisted of amino acids 556 with calculated molecular weight of 58 KDa. To-date, many xylanases genes have been cloned from different microorganisms, including *Actiomadura* sp. S14 (Thayat et al. 2011), *Paenibacillus* sp. 12-11 (Zhao et al. 2011) and *Streptomyces* sp. S27 (Li et al. 2009). However, this study was to clone a xylanase gene from *C. cellulans* CKMX1 and a very little information was available regarding cellulase-free xylanases from *C. cellulans* CKMX1. Further, the work regarding hyperxylanolytic production could be done *in vivo*. Amino acid composition determines the fundamental properties of the enzyme (Arora et al. 2009). The amino acid composition of xylanase sequences is represented in Table 1. The pI values of all protein sequences were in the range of 4.32-9.57, indicating that all considered xylanase sequences were acidic except *C. flavigena* XIB (9.57), *C. flavigena* DSM 20109 (8.94), *Cellvibrio gilvus* ATCC 13127 (8.70), *B. subtilis* (9.18) and *Pedobacter saltans* DSM 12145 (9.16). The xylanase enzyme of *C. cellulans* CKMX1 had the pI value of 4.66 which showed that the xylanase sequence was acidic (Arora et al. 2009). The calculated isoelectric point (pI) will be useful because at pI, solubility is least and mobility in an electro focusing system is zero. The instability index, which gives clue about the stability of a protein *in vitro,* can be calculated using Expasy's ProtParam server (Geourjon and Deleage 1995). All the considered sequences were classified as stable with values ranging from 16.14 to 38.99, except *Granulicella mallensis* MP5ACTX8 (42.29). The xylanase sequence of *C. cellulans* CKMX1 (22.89) indicated a stable protein (Table 2).

The aliphatic index (AI), which is defined as the relative volume of a protein occupied by aliphatic side chains, is regarded as a positive factor for the increase of thermal stability of globular proteins. It can be calculated by using equation, i.e., Aliphatic index= X(Ala) + a*X(Val) + b*X(Leu)) + b*X(Ile) and Expasy's ProtParam server (Gasteiger et al. 2005). Aliphatic index for the xylanase sequences ranged from 34.71-95.33 (Table 2). The very high aliphatic index of all xylanase sequences indicated that these xylanases could be stable for a wide temperature range, including the xylanase sequence of *C. cellulans* CKMX1, which showed 74.53 aliphatic index (Arora et al. 2009). The secondary structure indicates whether a given amino acid lies in a helix, strand or coil. Secondary structure features as predicted using SOPMA are represented in Table 3. The results revealed that random coils dominated among the secondary structure elements, followed by alpha helix, extended strand and beta turn. The molecular model of the endo-1,4- β xylanase is described as consisting of an alpha helix and several beta pleated sheets with a compact structure and is shown in Figure 3.

This is the first report describing cellulase-free xylanase by *C. cellulans* CKMX1 isolated from mushroom compost. Coupled with the small compact structure of the enzyme allowed it to degrade the xylan without damaging the cellulose fibres in the cellulose-hemicellulose matrix. This enzyme was also used in pulp and paper industry and showed positive results.

CONCLUSION

An endo-1,4-beta-xylanase gene from actinomycetes was isolated and characterized. The bacterium was identified as *C. cellulans* CKMX1 based on the morphological, biochemical characterisation, whole cell fatty acid methyl ester analysis and 16S rDNA sequence. The xylanase gene of this bacterium, however, showed higher similarity to *Cellulosimicrobium* sp. deposited in the NCBI database.

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