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Cell Surface Display of Cellulase Activity–Free Xylanase Enzyme on *Saccharomyces Cerevisiae* EBY100

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Abstract Cellulase-free xylanase has potential for its application in the selective removal of hemicellulose from kraft pulp to give good strength to paper. In this study, a gene (*xyn*) encoding cellulase activity–free xylanase enzyme (Xyn) was isolated from *Paenibacillus polymyxa* PPL-3. The *xyn* gene encoded a protein of 221 amino acids, and the purified Xyn was about 22.5 kDa measured by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. Moreover, the cellulase activity–free xylanase enzyme (Xyn) was displayed on the cell surface of *Saccharomyces cerevisiae* EBY100 using Aga2p as an anchor protein. Cell surface display of xylanase enzyme (Xyn) on *S. cerevisiae* EBY100 was confirmed by immunofluorescence microscopy. Optimum cell surface display of xylanase enzyme (Xyn) was observed at pH 7 and 40 °C. Therefore, cell surface–displayed xylanase enzyme (Xyn) can be used in the paper industry.

Keywords Cellulase-free xylanase · Yeast surface display · Kraft pulp · Biobleaching · *Paenibacillus polymyxa* PPL-3

Introduction

Xylan is the major constituent of hemicelluloses, a complex polysaccharide that is the most abundant biological carbon source in nature [1]. Xylanase produced by microorganisms has attracted a great deal of attention during the past few decades because of its potential biotechnological applications in various industries including the food, feed, fuel, textile, and paper and pulp industries and in waste treatment [2].

At present, bleaching process for kraft pulp uses large amounts of chlorine-based chemicals and sodium hydrosulfite, which are toxic, mutagenic, and persistent; they also

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cause numerous harmful disturbances in biological systems [1]. The use of eco-friendly technology is preferred in any industrial process; therefore, due to severe concern over the environmental hazards of toxic chemicals used in pulp and paper industries, alternative methods for bleaching of pulp have been suggested [3]. One of the most important large-scale biotechnological applications of recent years is the use of xylanases as bleaching agents in the pulp and paper industry, and treatment with xylanases facilitates the chemical extraction of lignin from pulp and leads to a significant reduction in the use of hazardous chemicals required for bleaching. For biobleaching applications, the candidate xylanase should be thermostable, alkali tolerant, and stable on kraft pulping, and its various properties, such as low molecular weight, net ionic properties, and specific action pattern must suit the pulping process requirements [4]. Moreover, to avoid damage to cellulose pulp, enzyme preparations should be free from cellulase activity [5]. Previously, genes encoding cellulase-free xylanase enzyme has been isolated from different bacterial strains such as thermoalkalophilic *Bacillus* sp. JB 99 [4], *Streptomyces* S27 [7], and *Streptomyces* sp. TN119 [8]. However, yeast strains displaying various active enzymes on the cell surface were developed by using genetic engineering techniques [6], but cellulase activity–free xylanase enzyme has not yet been surface displayed on yeast.

In this study, a gene (*xyn*) encoding cellulase activity–free xylanase had been isolated and characterized from *Paenibacillus polymyxa* PPL-3. Moreover, the xylanase enzyme (Xyn) was surface displayed on *Saccharomyces cerevisiae* EBY100 using Aga2p as an anchor protein for the potential use of the xylanase enzyme (Xyn).

Materials and Methods

Bacteria and Yeast Strains and Media

P. polymyxa strain PPL-3 was isolated from a paddy soil sample. *Escherichia coli* DH5α was used as a host for DNA manipulation and grown in Luria-Bertani (LB) medium. Recombinant *E. coli* DH5α was grown in LB medium with 50 μg/mL ampicillin. *S. cerevisiae* EBY100 was grown in YPD medium (1% yeast extract, 2% peptone, and 2% dextrose), and recombinant *S. cerevisiae* EBY100 was grown in synthetic dextrose (SD) medium without tryptophan (Clontech Laboratories Inc., Mountain View, CA).

Cloning of a Gene (*xyn*) Encoding Cellulase Activity–Free Xylanase Enzyme

A gene (*xyn*) encoding cellulase activity–free xylanase enzyme (Xyn) was isolated from the genomic DNA of *P. polymyxa* PPL-3 by polymerase chain reaction (PCR) amplification on the basis of a reference sequence of *Paenibacillus* sp. JDR-2 complete genome sequence (accession number: NC_012914, region 5342147–5342782 bp). The sense primer 5′-ATGTTTAAGTTGAAAAAGAAGGT-3′ and antisense primer 5′-TTACCACACCGT TACGTTCG-3′ were used with Super-Therm DNA polymerase (JMR, Sidcup, Kent, UK), 1.5 mM $MgCl_2$, 2 mM dNTP in a final volume of 50 μL for 30 cycles (denaturation at 95 °C for 30 s, annealing at 51 °C for 30 s, and extension at 72 °C for 1 min, followed by final incubation at 72 °C for 10 min). The anticipated product of 635 bp was isolated after agarose gel electrophoresis of the PCR product using a gel extraction kit (NucleoGen, Seoul, Korea). Amplified fragments were cloned into the pGEM-T easy vector (Promega, Fitchburg, WI) and transformed into *E. coli* DH5α. Standard procedures for restriction endonuclease digestions, agarose gel electrophoresis, purification of DNA from agarose gels, DNA ligation,

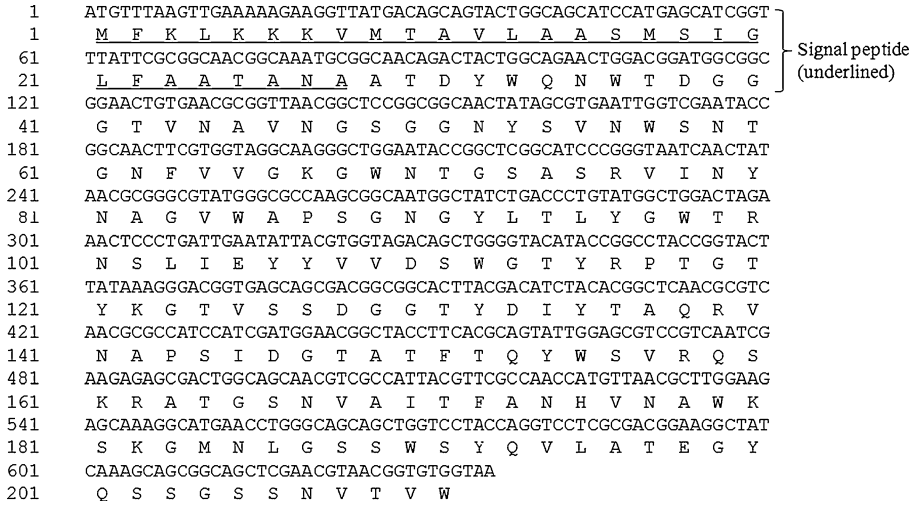

Fig. 1 Nucleotide sequences and deduced amino acid sequences of a gene (*xyn*) encoding cellulase activity–free xylanase (Xyn) enzyme of *P. polymyxa* PPL-1

and other cloning-related techniques were followed [9]. The nucleotide sequence is available in the GenBank database of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/) under the accession number GU584876 for the 16 S rDNA and HM769760 for *xyn* of *P. polymyxa* PPL-3. Moreover, multiple alignment analysis had been conducted using the DNAMAN analysis system.

Plate Assay for Cellulase-Free Xylanase Activity

To observe the cellulase-free xylanase activity of Xyn prior to yeast cell surface, agar diffusion method was used for the detection of cellulose and xylanase enzyme activity of Xyn enzyme according to Jung and Kim [10]. The *E. coli* DH5α harboring *xyn* gene was inoculated onto cellulase activity assay plate [LB plate with 0.5% (wt/vol) of carboxyl methyl cellulose, 0.5% Congo Red] and xylanase activity assay plate [LB plate with 0.5% (wt/vol) of oat spelt xylan] for the detection of cellulase and xylanase activity, respectively.

Expression, Purification, and Characterization of Xylanase Enzyme

For high expression of the xylanase enzyme, the PCR product generated with the sense primer 5′-TTAA GCT AGC GCA ACA GAC TAC TGG CAG-3′ (with *Nhe*I restriction site, underlined nucleotides) and antisense primer 5′-AATT GGA TCC CCA CAC CGT TAC GTT CGA-3′ (with *Bam*HI restriction site, underlined nucleotides) was cloned into

Fig. 2 Multiple sequence alignment of other bacterial xylanases. Multiple alignment was obtained using the ▶ DNAMAN analysis system. Consensus sequences have been mentioned bellow the alignments using the xylanase enzyme sequences of *P. polymyxa* PPL-3 (GU584876), *Paenibacillus* sp. JDR-2 (YP_003013364), *Paenibacillus* sp. HY8 (ABD66557), *Paenibacillus* sp. DG-22 (ABI96991), *Aeromonas punctata* (BAA06837), *Bacillus amyloliquefaciens* FZB42 (YP_001422939), *Paenibacillus* sp. W-61 (BAE93061), *Clostridium* sp. ISDg (YP_001559210), *Paenibacillus* sp. D14 (ZP_04852431), and *P. polymyxa* 1.794 (DQ100298)

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AAD10834        .MFKLNKKVFALVSVIALGFSSLFTSTAHAA.TDYWQNWT    38
ABD66557        miiKfsKKVmtLVlaasmsFg.LFatTsnAA.TDYWQNWT    38
YP_003013364    miiKfsKKVmtLVlaasmsFg.LFatTsnAA.TDYWQNWT    38
ABI96991        .MFKrsKKmltvilaasmsFg.vFaaTssAA.TDYWQNWT    37
YP_001422939    .MFKfgKKlmtvVlaasmsFg.vFaaTssAA.TDYWQNWT    37
ZP_04852431     ..mKLkKKmltLlltasmsFg.LFgaTssAA.TDYWQyWT    36
GU584876        .MFKLkKKVmtaVlaasmsig.LFaaTAnAA.TDYWQNWT    37
BAA06837        .MiKskKKfltvciaalmsFS.LFaaTsnAA.TDYWQyWT    37
DQ100298        ..clslKrmltvVlaasmsFg.vFatTssAA.TDYWQNWT    36
BAE93061        .MFKfkKKflvgltaafmsiS.mFsaTAsAAgTDYWQNWT    38
Consensus             k                 f  t  aa tdywq wt

AAD10834        DGGGTVNATNGSGGNYSVNWTNGGNFVVGKGWGTGNASRV    78
ABD66557        DGGGTVNAvNGSGGNYSVtWqNtGNFVVGKGWnTGspnRt    78
YP_003013364    DGGGTVNAvNGSGGNYSVtWqNtGNFVVGKGWnTGspnRt    78
ABI96991        DGGGTVNAvNGSGGNYSVtWqNtGNFVVGKGWnvGspnRt    77
YP_001422939    DGGGTVNAvNGSGGNYSVsWqNtGNFVVGKGWtyGtpnRV    77
ZP_04852431     DGGGTVNAvNGSGGNYSVtWqNsGNFVVGKGWsvGspnRt    76
GU584876        DGGGTVNAvNGSGGNYSVNWsNtGNFVVGKGWnTGsASRV    77
BAA06837        DGGGTVNATNGSGGNYSVtWsNvGNFVVGKGWGTGsptRt    77
DQ100298        DGGGTVNAvNGSGGNYSVtWkdtGNFVVGKGWtTGspnRt    76
BAE93061        DGGGTVNAvNGSGGNYSVNWsNtGNFVVGKGWtTGsAfRt    76
Consensus       dgggtvna ngsggnysv w   gnfvvgkgw  g   r

AAD10834        VNYNAGVFSPSGNGYLTFYGWTRNSLIEYYVVDSWGTYRP   118
ABD66557        iNYNAGVwaPSGNGYLTlYGWTRNSLIEYYVVDSWGTYRP   118
YP_003013364    iNYNAGVwaPSGNGYLTlYGWTRNSLIEYYVVDSWGTYRP   118
ABI96991        iNYNAGVwSPSGNGYLTlYGWTRNaLIEYYVVDSWGTYRP   117
YP_001422939    VNYNAGVFaPSGNGYLTFYGWTRNaLIEYYVVDSWGTYRP   117
ZP_04852431     iNYNAGiwePSGNGYLTlYGWTRNSLIEYYVVDSWGTYRP   116
GU584876        iNYNAGVwaPSGNGYLTlYGWTRNSLIEYYVVDSWGTYRP   117
BAA06837        VNYNAGVwaPSGNGYLTlYGWTRNSLIEYYVVDSWGTYRP   117
DQ100298        iNYNAGVwaPSGNGYLalYGWTRNSLIEYYVVDSWGTYRP   116
BAE93061        iNYNAGVwaPnGNGYLTlYGWTRapLIEYYVVDSWGTYRP   118
Consensus        nynag   p gngyl  ygwtr  licyyvvdswgtyrp

AAD10834        TGTLKGTVSSDGGTYDIYTSTRTNAPSIDGTQTFQQYWSV   158
ABD66557        TGThKGTVtSDGGTYDIYTtmRyNAPSIDGTQ.tfaqywS   157
YP_003013364    TGThKGTVtSDGGTYDIYTtmRyNAPSIDGTQ.tfaqywS   157
ABI96991        TGTfKGTVnSDGGTYDIYTtmRyNAPSIDGTQ.tfaqywS   156
YP_001422939    TGTyKGTVnSDGGTYDIYTtmRyNAPSIDGTQ.tfpqywS   156
ZP_04852431     TGThKGTVnSDGGTYDIYTtmRyNAPSIDGTQ.tfQqfwS   155
GU584876        TGTyKGTVSSDGGTYDIYTaqRvNAPSIDGTa.tftqywS   156
BAA06837        TGTyKGTVtSDGGTYDIYTtmRyNAPSIDGTQ.tfpqywS   156
DQ100298        TGTyKGTVtSDGGTYDIYTtmRydAPSIeGqkTtr1qywS   156
BAE93061        TGTyKGTVkSDGGTYDIYTtTRyNAPSIDGdnTtftqywS   158
Consensus       tgt kgtv sdggtydiyt  r  apsi g

AAD10834        RQSKRATGSNVAITFSNHVNAWKSKGMNLGSSWAYQALCV   198
ABD66557        vrqskratgvnssitfsnhvnawaskgmnlgSswsyqvla   197
YP_003013364    vrqskratgvnssitfsnhvnawaskgmnlgSswsyqvla   197
ABI96991        vrqskratgvnsaiafsnhvnawaskgmnlgSswsyqvla   196
YP_001422939    vrqskrptgvnstitfsnhvnawpskgmylgmswsyqvma   196
ZP_04852431     vrqskrptgsnvsitfsnhvnawrskgmnlgSswsyqvla   195
GU584876        vrqskratgsnvaitfanhvnawkskgmnlgSswsyqvla   196
BAA06837        vrqskrptgsnvsitfsnhvnawrnagmnlgSswayqvla   196
DQ100298        vrqtkrptggnstitfsnhvnawaskgmhlgnwsyqvla   196
BAE93061        vrqskrptgsnAaitfsnhvnawkshgmnlgSnwayqvla   198
Consensus

AAD10834        EGYQSSGSANVIVW.                            212
ABD66557        tegyqSsgssnvtvw                            212
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expression vector pET-28a (Novagen, Darmstadt, Germany), resulting in the addition of a C-terminal $(His)_6$ tag. *E. coli* BL21 (DE3) carrying pET-28a/*xyn* was grown at 37 °C to mid-log phase in LB medium containing 50 μg/mL kanamycin. Expression was then induced by adding isopropylthiogalactoside to a final concentration of 0.5 mM, and further growth was continued for 5 h. The cells were harvested by centrifugation (6,000×*g*, 10 min) and washed twice with 10 mM Tris–HCl buffer (pH 7). The cells were resuspended in the same buffer and stored at −20 °C [11]. The frozen cells were mixed with 50 mM Tris–HCl buffer (pH 7.5) containing 1 mg of bovine DNase I (Sigma, St. Louis, MO, USA) and incubated at 37 °C for 30 min. Triton X-100 was added to the suspension to attain a final concentration of 2.5% (vol/vol). The supernatant was collected and stored at 4 °C. The solubilized recombinant Xyn with His-tag was applied on a HisTrap kit (Amersham Pharmacia Biotechnology, Uplala SE-751 84, Sweden). Purification of expressed His_6-tagged protein was carried out accordingly as previously described by Guo et al. [12], and protein was eluted with 100 mM imidazole with 0.1% Triton X-100. The purified protein sample was analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE).

After electrophoresis, separated proteins on the gel were transferred electrophoretically to polyvinylidene fluoride membranes (Millipore, Billerica, MA) for Western blot analysis. Immunodetection was achieved using a His-probe (H-15) antibody (catalog no. sc-803; Santa Cruz Biotechnology, Inc., Santa Cruz, CA) as the primary monoclonal antibody and goat anti-rabbit IgG as the secondary antibody. Color development was performed in 100 mL of Tris-borate (TB) buffer (50 mM Tris–HCl, pH 7.6) containing 50 mg of 3,3′-diaminobenzidine and 40 μL of 30% H_2O_2.

Protein concentrations were determined according to Bradford method [13]. The effect of pH (3–11) and temperature (20–90 °C) on xylanase activity was examined with purified xylanase enzyme by using 1% xylan (Sigma, X0627) as substrate, and the amounts of the released reducing sugars were determined by the dinitrosalicylic acid method [14]. Blank samples were used to correct the nonenzymatic release of reducing sugars.

Construction of Plasmids, Transformation into Yeast, and Activity Assay

The gene fragment encoding cellulase activity–free xylanase enzyme was amplified from total DNA of *P. polymyxa* strain PPL-3 using the sense primer 5′-TTAAGCTAGCG-CAACA GACTACTGGCAG-3′ (with *Nhe*I restriction site, underlined nucleotides) and antisense primer 5′-AATTGGATCCCCACACCGTTACGTTCGA-3′ (with *Bam*HI restriction site, underlined nucleotides). The PCR amplification protocol consisted of a 5-min denaturation at 95 °C, followed by 30 cycles of denaturation at 95 °C for 1 min, annealing at 52 °C for 1 min, extension at 72 °C for 1 min, and a final hold for an extra 10 min at 72 °C. The amplified fragment was digested by *Nhe*I and *Bam*HI, and introduced into the *Nhe*I and *Bam*HI sites of plasmid pCTCON. The resulting plasmids were pCTCON-*xyn*. The constructed pCTCON-*xyn* plasmid was used to transform *E. coli* DH5α and screened in LB medium containing 50 μg/mL ampicillin. Finally, the pCTCON-*xyn* plasmid was transformed into the yeast *S. cerevisiae* EBY100. The recombinant yeasts were screened in SD medium without tryptophan.

For the expression of the fusion proteins, recombinant *S. cerevisiae* EBY100 harboring the pCTCON-*xyn* plasmid were precultivated in SD medium at 30 °C overnight, harvested by centrifugation (at 5,000 rpm for 10 min) and then resuspended in SD medium and cultivated at 30 °C. The cells were then harvested by centrifugation (at 5,000 rpm for 10 min) and again resuspended in 50 mM Tris/HCl (pH 7.4) buffer for activity assay.

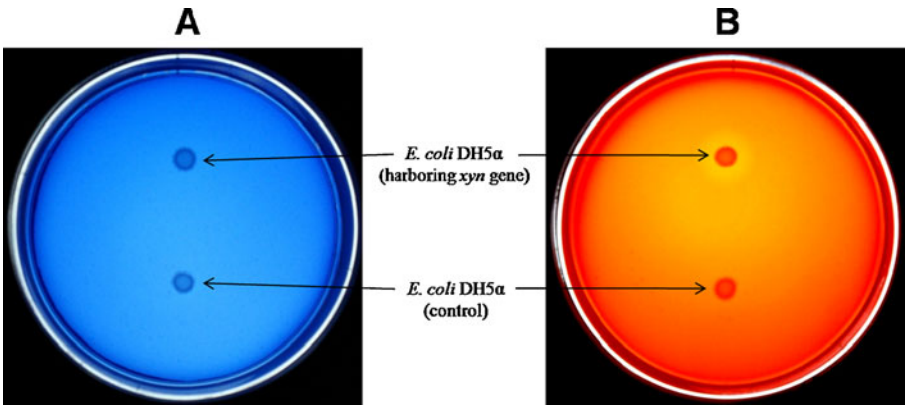

Fig. 3 Cellulase-free xylanase activity assay of *E. coli* DH5α harboring *xyn* gene. a *E. coli* DH5α harboring *xyn* gene produced yellow halo zone surrounded by red background due to xylanase activity on the xylanase activity assay plate (LB plate with 0.5% of oat spelt xylan), and b *E. coli* DH5α harboring *xyn* gene did not produce any halo zone on cellulase activity assay plate (LB plate with 0.5% of carboxyl methyl cellulose)

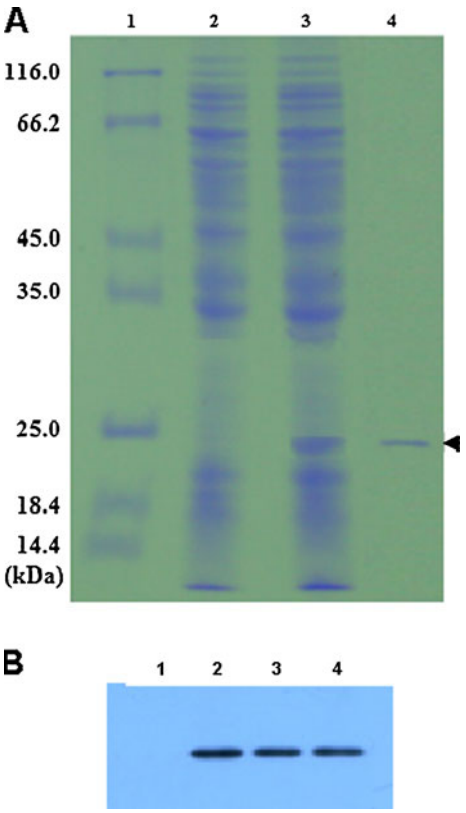

Fig. 4 **a** Electrophoretic analysis of cellulase activity–free xylanase (Xyn) enzyme. Separation was performed on a 12.5% (wt/vol) SDS-PAGE. The gel was stained with Coomassie blue R-250. Lane 1: molecular weight markers; lane 2: crude extract from *E. coli* BL21 (DE3) containing pET-28a(+)/*xyn*; lane 3: crude extract from isopropylthiogalactoside-induced *E. coli* BL21 (DE3) containing pET-28a(+)/*xyn*; lane 4: purified xylanase enzyme from the His-Trap kit (Amersham). **b** Western blot analysis of Xyn enzyme. Lane 1 for control (*E. coli* BL21 containing pET-28a and lanes 2 to 4 for *E. coli* BL21 (DE3) containing pET-28a(+)/*xyn* (three replications)

Immunofluorescence Microscopy

Immunostaining was performed according to the method of Liu et al. [15]. Mouse polyclonal antibody against HA tag was used as the primary antibody at a dilution rate of 1:100. Cells and the antibody were incubated on ice for 30 min. After the cells had been washed with phosphate-buffered saline, the second antibody, fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG was diluted at 1:200 and reacted with the cells on ice for 1 h. After washing with phosphate-buffered saline, the image of cells was observed with a fluorescence microscope. Moreover, to calculate the surface displayed ratio of the enzyme on the cell surface, we observed 100 cells with three replications.

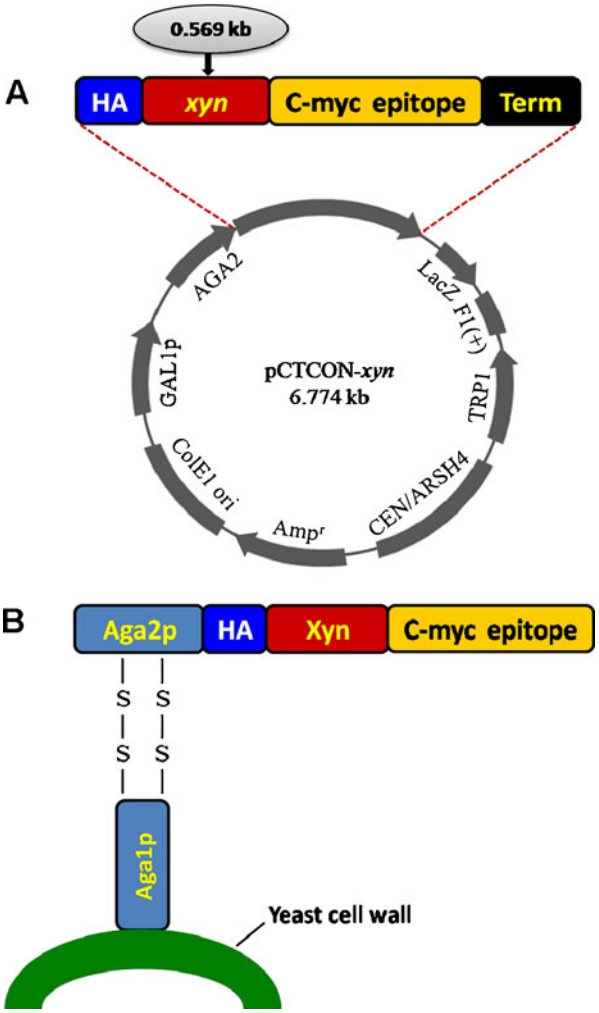

Fig. 5 Schematic design of a yeast surface display system for *xyn* gene of *P. polymyxa* PPL-1. **a** Vector construction of pCTCON-*xyn* for yeast transformation. **b** Yeast cell surface display mechanism

Results and Discussion

Cloning, Sequencing, and Phylogenetic Analysis of *xyn* Gene

A xylanase gene (*xyn*) was cloned in pGEM-T easy vector (Promega). After sequencing, a total of 635-bp nucleotide sequences were found in the open reading frame of *xyn* gene (Fig. 1). Moreover, we constructed a phylogenetic tree of the xylanase enzyme by the DNAMAN analysis system (Fig. 2). The phylogenetic tree demonstrates that the gene encoding xylanase enzyme of *P. polymyxa* PPL-3 was the highest to the gene encoding xylanase enzyme of *P. polymyxa* JDR-2 (accession number: YP_003013364.1). Similarly, few researchers also isolated genes encoding cellulase-free xylanase enzymes such as Shrinivas et al. [4] from *Bacillus* sp. JB 99, Li et al. [7] from *Streptomyces* S27, and Zhou et al. [8] from *Streptomyces* sp. TN119.

Plate Assay for Xylanase Activity

Agar diffusion method was used for the detection of cellulose and xylanase activity of Xyn enzyme (Fig. 3). The *E. coli* DH5α harboring *xyn* gene was inoculated on cellulase activity assay plate [LB plate with 0.5% (wt/vol) of carboxyl methylcellulose] and xylanase activity

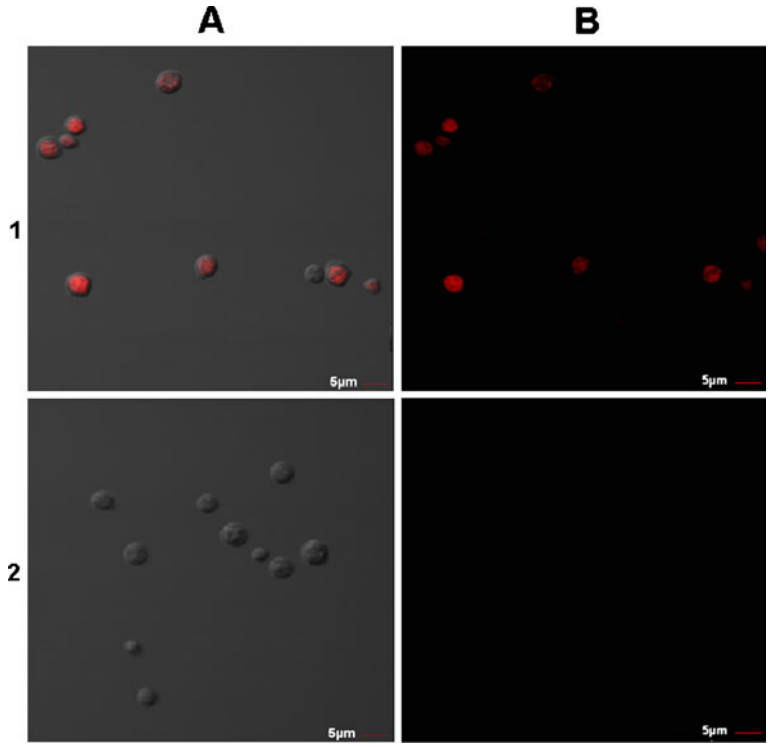

Fig. 6 Immunofluorescence labeling of xylanase (Xyn) enzyme on the cell surface of *S. cerevisiae* EBY100. **a** *S. cerevisiae* EBY100 harboring pCTCON-*xyn* were immunologically labeled with the anti-HA antibody as the primary antibody and FITC-conjugated anti-IgG as the secondary antibody, whereas **b** the control cells (*S. cerevisiae* EBY100 harboring pCTCON) did not show florescent at all: (1) bright-field picture and (2) dark-field picture

assay plate [LB plate with 0.5% (wt/vol) oat spelt xylan] for the detection of cellulase, and xylanase activity, respectively. The *E. coli* DH5α harboring *xyn* gene showed characteristic yellow halo zone surrounded by red background due to xylanase on the xylanase activity assay plate. On the other hand, the *E. coli* DH5α harboring *xyn* gene did not show any activity on the cellulase activity assay plate. *E. coli* DH5α was used as control. This result suggests that the xylanase (Xyn) enzyme do not have cellulase enzyme activity. The Xyn enzymes come out from *E. coli* DH5α harboring *xyn* gene and hydrolyze xylan remaining on the LB media surrounding *E. coli* DH5α colony.

Characterization of Xylanase Enzyme

The calculated molecular weight of xylanase (Xyn) was 22.678 kDa by DNAMAN software. The purified xylanase (Xyn) enzyme was confirmed to be about 22.7 kDa by SDS-PAGE (Fig. 4), which corresponds well with the estimated molecular mass of 22.5 kDa for the 221 amino acids. PSORT (Compute p*I*/Mw tool; http://www.expasy.org/tools/pi_tool.html) revealed a potential signal sequence of 28 amino acids, and the calculated p*I* of Xyn was

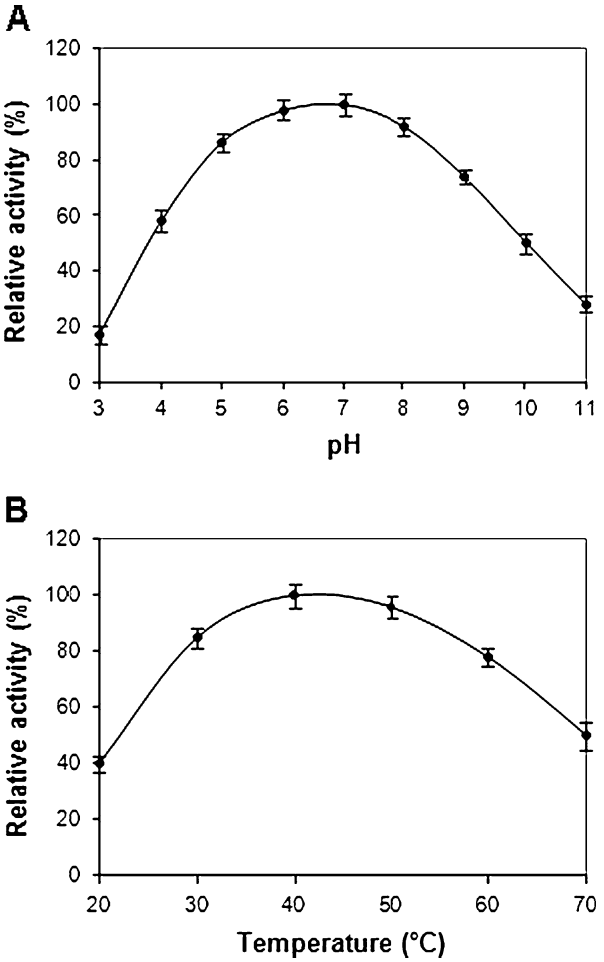

Fig. 7 Effects of pH and temperature on the relative activity of *S. cerevisiae* cell surface–displayed xylanase (Xyn) enzyme. **a** Effect of pH on xylanase activity was assayed at 37 °C. **b** Effect of temperature on xylanase activity was assayed at pH 7. *Values* indicate the means of three replications, and *vertical bar* shows standard deviation

9.55. The molecular weight of Xyn (22.66 kDa) was low, which is almost similar with that of xylanase from *Bacillus* sp. JB 99 [7], *Bacillus* sp. K-1 [16], *Bacillus* sp. TAR-1 [17], and *Geobacillus thermoleovorans* [18]. The low apparent molecular weight with basic p*I* of xylanase could also be advantageous for pulp treatment application; as such, enzymes would have greater access to the xylan component of the wood matrix [19, 20]. Moreover, expression of Xyn enzyme was confirmed by Western blot analysis.

Confirmation of Successful Display of Active Xylanase

The plasmids for surface display of xylanase (Xyn) enzyme were constructed as shown in Fig. 5. In pCTCON-*xyn*, the xyn gene (569 bp without TAA stop codon) was fused to the gene encoding N-terminal HA epitope and C-terminal C-myc epitope and was expressed under the control of the GAL1 promoter. The HA peptide at the N-terminal of Xyn was used for immunofluorescent detection. *S. cerevisiae* EBY100 cells expressing the HA–Xyn–C-myc fusion protein became fluorescent due to the binding of an anti-HA tag antibody followed by binding of an FITC-conjugated secondary antibody, whereas the control cells were not fluorescent in immunofluorescence microscopy (Fig. 6), which indicated that the Xyn was successfully displayed on the cell surface of *S. cerevisiae* EBY100 harboring pCTCON-*xyn*. Similarly, Liu et al. [15] observed surface display of lipase enzyme (Lip2) on *S. cerevisiae* by the immunofluorescence microscopy. In addition, the surface displayed ratio of the Xyn on the cell surface was 93% (data not shown).

Characterization of Cell Surface–Displayed Xylanase Enzyme

The effect of pH and temperature on the activity of yeast cell surface–displayed xylanase (Xyn) enzyme was studied by using xylan (0.5%) as a substrate (Sigma, X0627). The effect of pH was determined at 37 °C in various buffers ranging from pH 3 to 11. Optimum activity was observed at pH 7. The temperature dependence of xylanase activity toward xylan was determined by measuring activity at various temperatures ranging from 20 to 70 °C at pH 7. Optimum activity was observed at 40 °C (Fig. 7). The high temperature and pH optima as well as cellulase-free nature of such enzymes facilitate their usage for application in paper and pulp factory, since these very features are prime requisite of the industrial pulping process [21].

Conclusion

In this study, a gene encoding cellulase activity–free xylanase enzyme was isolated from *P. polymyxa* PPL-3. The *xyn* gene encoded a protein of 221 amino acids, and the purified Xyn protein was about 22.7 kDa by SDS-PAGE. The xylanase enzyme (Xyn) was displayed on the surface of *S. cerevisiae* EBY100 using Aga2p as an anchor protein and confirmed by immunofluorescence microscopy. Optimum activity of Xyn was observed at 55 °C and pH 7. Further studies need to be carried out for the potential industrial use of *S. cerevisiae* cell surface-displayed xylanase (Xyn) enzyme.

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