Cloning and Expression of the Endo-1,3(4)-β-glucanase Gene from Paecilomyces sp. FLH30 and Characterization of the Recombinant Enzyme

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The cDNA encoding β-1,3(4)-glucanase, named PsBg16A, from Paecilomyces sp. FLH30 was cloned, sequenced, and over expressed in Pichia pastoris, with a yield of about 61,754 U/mL in a 5-L fermentor. PsBg16A has an open reading frame of 951 bp encoding 316 amino acids, and the deduced amino acid sequence of PsBg16A revealed that it belongs to glycoside hydrolase family 16. The purified recombinant PsBg16A had a pH optimum at 7.0 and a temperature optimum at 70°C, and randomly hydrolyzed barley β-glucan, lichenin, and laminarin, suggesting that it is a typical endo-1,3(4)-β-glucanase (EC 3.2.1.6) with broad substrate specificity for β-glucans.

Key words: cloning; expression; endo-β-1,3(4)-glucanase; Paecilomyces sp. FLH30; characterization

β-Glucans are polysaccharides comprising β-D-glucosylnes residues linked through β-1,3 and/or β-1,4 glycosidic bonds. They are particularly abundant in the endosperm cell walls of cereals such as barley, rye, rice, and wheat.1) Based on the type of glycosidic linkage, β-glucans can be grouped into four main categories: β-1,3-1,4-glucan (lichenan), β-1,4-glucan (cellulose), β-1,3-glucan (laminarin), and β-1,3(4)-glucan.2) Of the main cereal commodities, barley is the rough material used to make beer, in which β-1,3- and β-1,4-glucans account for about 70% w/w of the endosperm cell walls.3) Among these, β-1,3-1,4-glucan, in which the main chain contains heterogeneously linked β-1,3 and β-1,4 glucosidic bonds, is a major component of the cell wall of Poaceae, and is also found in lichens. It is commonly called lichenan.4)

β-Glucanases are important biotechnological aids in the brewing and animal feed-stuff industries. Four types of endo-acting enzymes, classified according to the types of glycosidic linkage they cleave, are capable of depolymerising 1,3-1,4-β-glucan: endo-1,3-1,4-β-glucanases (EC 3.2.1.73), endo-1,3(4)-β-glucanases (EC 3.2.1.6), endo-1,4-β-glucanases (EC 3.2.1.4), and to a lesser extent endo-1,3-β-glucanases (EC 3.2.1.39).2)

β-1,3-1,4-Glucanases (lichenan) strictly cleave the β-1,4-glycosidic linkage adjacent to a 3-O-substituted glucose residue in mixed linked β-glucans, but are inactive against β-1,4-glucans, but endo-1,3(4)-β-glucanase endohydrolis of (1→3)- or (1→4)-linkages in β-D-glucans when the glucose residue whose reducing group is involved in the linkage to be hydrolysed is itself replaced at C-3. Its substrates include lamarin, lichenin, and cereal α-glucans. The addition of exogenous β-1,3(4)-glucanases or β-1,3-1,4-glucanases can reduce the negative effects of barley β-glucan during mashing in the brewing industry and can improve β-glucan digestibility in poultry feed-stuffs.5,6)

Most reported microbial β-1,3-1,4-glucanases belong to glycoside hydrolase (GH) family 16 and are produced by bacteria, especially the genus Bacillus,7,8) but endo-1,3(4)-β-glucanase is found mainly in fungi such as Botryotinia fuckelianana,9) Phaffia rhodozyma,10) Rhizomucor miehei,11) Phanerochaete chrysosporium,12) Schizosaccharomyces pombe.13) Most β-1,3(4)-glucanases are not stable during the mashing and coating of feed pellets, but during the kilning process, high temperatures rapidly inactivate these endogenous enzymes,14,15) and the remaining unhydrolyzed non-starch polysaccharides can cause high viscosity and turbidity in brewer mash and thus decrease the filtration rate and extract yield, ultimately causing a flocculent precipitate in the finished beer.16) Ruminant animals digest cellulose depending on cellulolytic ruminal bacteria, but these bacteria cannot resist a low ruminal pH, and hence the rumen pH must be maintained at neutral. Hence, screening to obtain a β-glucanase with thermostability and optimal activity in the neutral pH range is of great importance to the brewing and animal feed-stuff industries.17)

The thermophilic fungus Paecilomyces sp. FLH30, isolated from soil samples in Mt. Guan in Henan Province, grows well from 40 to 50°C and produces extracellular β-1,3(4)-glucanase, and the crude enzyme has an optimal temperature of 70°C. Here we report the molecular cloning, overexpression, and biochemical properties of PsBg16A, a family 16 endo-β-glucanase from Paecilomyces sp. FLH30. The amount of protein secreted by P. pastoris reached a concentration of up to 7.2 g L−1 in a 7-L fermentor culture. The recombinant β-1,3(4)-glucanase (PsBg16A) had a temperature optimum at about 70°C, and had high catalytic activity on both β-1,3-1,4-glucan and laminarin. These biochemical properties render it potentially suited for use in brewing and in oligosaccharide industrial applications.

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Materials and Methods

Strains, vectors, media, and chemicals. For isolation of RNA, Paecilomyces sp. FLH30 was grown at 45°C in a medium for 84 h. The medium of culture contained (g L−1): laminarin, 10; barley β-glucan, 10; tryptone, 20; yeast extract, 10; KH2PO4, 0.5; MgSO4·7H2O, 0.2; CaCl2, 0.2; and FeSO4·7H2O, 0.01. A P. pastoris expression kit was purchased from Invitrogen (San Diego, CA). Eukaryochis coli strain MJ109 (Strategene, Santa Clara, CA) was used for propagation of plasmids, and P. pastoris GS115 (Invitrogen, Carlsbad, CA) was used for protein expression. Plasmids pMD18-T simple (Takara, Ohtsu, Japan) and pPIC9K (Invitrogen, San Diego, CA) were used as cloning and expression vectors respectively.

Barley β-glucan, lichenan, laminarin, cellulose, Avicel, carboxymethylcellulose (CMC-Na), birchwood xylan, glucose, cellobiose, cellulotriose, and cellulotetraose were purchased from Sigma Chemical (St. Louis, MO). Restriction endonucleases and T4 DNA ligase were from New England Biolabs (Ipswich, MA). DNA polymerase Pfu was from Promega (Madison, WI). All other chemicals used were analytical grade reagents unless otherwise stated.

Cloning of the full length β-1,3(4)-glucanase cDNA. For isolation of genomic DNA and total RNA, fungal mycelia were collected by centrifugation (5,000 × g, 10 min) and washed twice with water at 4°C. The mycelia were frozen and ground to a fine powder in liquid nitrogen. A Fungal DNA Midi Kit (Omega Bio-Tek, Norcross, GA) was used for extracting DNA, the total RNA was isolated with TriZol reagent (Invitrogen, Carlsbad, CA), and mRNAs were purified using a Oligotex mRNA Midi Kit (Qiagen, Hilden, Germany). Genomic DNA of Paecilomyces sp. FLH30 was used as template for subsequent polymerase chain reaction (PCR) amplification. To clone the β-1,3(4)-glucanase gene, degenerate primers, PbBg16A-COD1 (CGGCAGGATCGATCAGTGAGGGTGTTTGTG) and PbBg16A-COD2 (GCGGCTGTCGGCGCGCAGAATGNTCRTCTCG, H = A/T/C, R = A/G, = A/T/C/G), were designed based on the conserved sequences (GEIDIEG and DTITFCGDWA) of known β-1,3(4)-glucanase of 10 filamentous fungi from the subphylum Ascomycota using the CODEHOP algorithm (Rose et al., 2003). The PCR conditions were as follows: 4 min at 95°C, followed by 10 cycles of 95°C for 30 s, 61–55°C for 30 s, and 72°C for 1 min, followed by 20 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 7 min. PCR products of the correct size was purified and ligated to pMD18-T simple vector for sequencing. The full-length cDNA sequence of β-1,3(4)-glucanase was obtained by 5′ and 3′ rapid amplification of cDNA ends (RACE) using a SMART RACE cDNA Amplification Kit (Clontech, Palo Alto, CA) following the manufacturer’s instructions, using specific primers PbBg16A-SP1 (5′-CGCGCTGTCGGCGCGCAGAATGNTCRTCTCG-3′) and PbBg16A-R1 (5′-GGTTGAGAGGATTTG-3′) for RACE, and PbBg16A-SP2 (5′-GGCCAGTTCGGCGCGGCTCGGTCG-3′) for 3′ RACE. The PCR product obtained was purified, cloned, and sequenced. The β-1,3(4)-glucanase cDNA sequences from Paecilomyces sp. FLH30 were deposited in the GenBank nucleotide sequence database under accession no. HQ825092.

Sequence analysis. Nucleotide and deduced amino acid sequences were analyzed with the ExPASy Proteomics Tools (http://www.expasy.org/tools/). Database homology searches of the nucleotide sequences obtained were carried out using BLAST in GenBank at the NCBI. The amino acid sequences were aligned using the ClustalX2 program (http://www.clustal.org/download/current/). The signal peptide was analyzed by Signal P 3.0 server (http://www.cbs.dtu.dk/services/SignalP/). A search analysis of conserved domain and signature sequences was carried out using ScanProsite (http://www.expasy.org/tools/ScanProsite/). N-glycosylation sites were predicted using NetNGlyc1.0 (http://www.cbs.dtu.dk/services/NetNGlyc/).

Recombinant expression of PbBg16A in P. pastoris. To construct the expression vector in P. pastoris, a gene fragment containing PbBg16A without the signal peptide coding sequence was amplified by PCR using primers PbBg16AASNABIF (5′-AAGCCTTACATGTCGCGGCCTCTACGGTACG-3′, SmB1 site underlined) and PbBg16AASNARIR (5′-TCTCGAAGGTCAGTTAGGCCGGGTAGACCGT TTAGGGAG-3′, AvrII site underlined). PCR amplification was carried out using DNA polymerase Pfu at an annealing temperature of 65°C. The purified PCR product was cloned into vector pPIC9K between the SnaB1 and AvrII sites in in-frame fusion with the α-factor signal peptide. The recombinant plasmid was linearized with restriction endonuclease SacI, followed by electroporation of P. pastoris GS115-competent cells. In vivo screening of multiple inserts (His+ transformants) and selecting pPIC9K transformants was done according to the manual of the Multi-copy Pichia Expression Kit (catalog no. K1750-01, Invitrogen). Recombinant expression and fed-batch fermentation (high cell-density fermentation) were performed following the Pichia Fermentation Process Guidelines (version B, 053002).

Enzyme assay and protein determination. All enzyme assays, unless otherwise stated, were carried out at 70°C for 10 min in 50 mM MES (2-(N-morpholino) ethane sulfonic acid) buffer at pH 7.0, a final substrate concentration of 1.0% w/v. The hydrolysis of barley β-glucan was quantified by measuring the reducing sugars released by the dinitrosalicyclic acid (DNS) method of Miller. The absorbance of the reaction mixture was determined at 540 nm. One unit of enzyme activity was defined as the amount of enzyme required to release 1 μmol of reducing sugars per mg under the above assay conditions. Protein concentrations were measured by the Lowry method with bovine serum albumin (BSA) as the standard. Specific activity was expressed as units per mg of protein.

Purification and electrophoretic analysis of the recombinant PbBg16A. To purify the recombinant PbBg16A, the crude supernatant (20 mL) was concentrated to 2.0 mL by ultrafiltration using a 10-kDa membrane (Millipore), and then the sample was diluted to 20 mL with 20 mM Tris–HCl buffer (pH 8.5) and re-concentrated two times, and 2.0 mL of the protein solution was loaded onto a Q-Sepharose Fast Flow column (10 × 1.0 cm) pre-equilibrated with 20 mM Tris–HCl buffer (pH 8.5). The bound proteins were eluted with a gradient of 150–300 mM NaCl at a flow rate of 1.0 mL min−1. Fractions having enzyme activity were pooled and concentrated to 1.0 mL by ultrafiltration using a 10-kDa membrane. The concentrated solution was applied to a Sephacryl S-100HR column (1 × 1.0 cm) equilibrated with 20 mM phosphate buffer (pH 7.2), the proteins were eluted at a flow rate of 0.3 mL min−1, and the fractions with β-1,3(4)-glucanase activity were pooled. Homogeneity was checked by SDS–PAGE, performed using 12.5% w/v acrylamide in gels, as described by Laemmli. Protein bands were visualized by Coomassie Brilliant Blue R-250 staining. A β-1,3(4)-glucanase zymogram was made under conditions described by Yang.

Biochemical characterization of PbBg16A. The optimum pH was determined at 70°C in four different buffers (50 mM) in a pH range of 2.5 to 11.0: citrate buffer between 2.5 and 5.5; MES buffer between pH 5.0 and 7.0; phosphate buffer between pH 6.0 and 8.5; and glycine-NaOH buffer between pH 8.5 and 11.0. To determine enzyme stability at different pH values, the enzyme was pre-incubated with the above buffers for 30 min at 50°C. After cooling of the treated enzyme samples on ice for 30 min, the residual activity of the samples was measured by standard assay. The optimal temperature was determined at various temperatures (30–100°C) in 50 mM MES buffer pH 7.0. To determine thermostability, the enzyme was pre-incubated for 30 min at the above temperatures by assaying the residual enzyme activity under standard conditions.

To investigate the effects of various metal ions and chemical reagents on recombinant PbBg16A activity, enzymatic activity was measured in MES buffer pH 7.0 containing 4 mM each of Na+, K+, Ca2+, Li+, Co2+, Cu2+, Ni2+, Fe3+, Mn2+, Zn2+, Pb2+, Ag+, Hg2+, SDS, EDTA, and β-mercaptoethanol at 70°C. Reactions were carried out in the absence of any chemicals were used as controls. Substrate specificity was determined using polysaccharides. β-1,3(4)-Glucanase activity on the polysaccharides was determined using 1.0% w/v of substrate in 50 mM MES buffer (pH 7.0) at 70°C for 10 min and by measuring reducing sugars by the DNS method. One enzyme units was defined as the amount that produces 1 μmol of reducing sugar per min.

The kinetic parameters, Km and Vmax, were determined in 50 mM MES buffer (pH 7.0) containing 0.1–10.0 mg/mL of barley β-glucan,
lichenan, and laminarin after incubation with purified recombinant PsBg16A at pH 7.0 at 70°C for 2 min. The data were plotted according to the Lineweaver–Burk method by SigmaPlot Enzyme Kinetics Module.

For enzymatic hydrolysis of β-glucan, barley β-glucan (1% w/v), lichenan (1% w/v), and laminarin (1% w/v) in 1.0 mL of 50 mM MES buffer (pH 7.0) was incubated with 10 units of enzyme at 50°C. Aliquots were withdrawn during hydrolysis, and samples were boiled for 5 min to terminate the reaction, and spotted on silica gel plates 60F254 (Merck, Darmstadt, Germany). The plates were developed with 2 runs of the butanol-acetic acid-water (2:1:1, v/v) solvent system, followed by heating for several min at 130°C in an oven, and then spraying of the plates with a methanol-sulfuric acid mixture (95:5, v/v). A mixture of cellobiose consisting of glucose (G1), cellobiose (G2), cellotriose (G3), cellotetraose (G4), a mixture (95:5, v/v) was used as standard.

Results

Cloning of the full length β-1,3(4)-glucanase cDNA and sequence analysis

Using degenerate PCR and TAIL-PCR techniques, a 1,214-bp fragment containing an open reading frame of 951 bp was cloned from Paecilomyces sp. FLH30. The full-length ORF of PsBg16A coded for a peptide of 317 amino acids with a theoretical molecular mass of 34.2 kDa, and pl 4.97. SignalP analysis indicated the presence of an N-terminal signal peptide at residues 1–18. Motif Scan indicated that PsBg16A has a catalytic domain belonging to glycosyl hydrolase (GH) family 16. No potential N-glycosylation sites (Asn-Xaa-Thr/Ser-Zaa, where Zaa is not Pro) were found.

A phylogenetic tree comprising other fungal and bacteria β-1,3(4)-glucanases using ClustalX and MEGA 4.0 was constructed (Fig. 1). The results indicated that PsBg16A has high homology with other fungal GH family 1–18. Motif Scan indicated that PsBg16A has a catalytic domain belonging to glycosyl hydrolase (GH) family

Biochemical properties of the purified recombinant PsBg16A

The purified recombinant PsBg16A exhibited optimal activity at about pH 7.0 at 70°C (Fig. 4a). Following incubation at 50°C for 30 min without substrate, the enzyme retained greater than 80% activity at pH 4.5–10.0 (Fig. 4b). Enzyme activity was optimal at 70°C (Fig. 4c). After incubation at 65°C for 30 min, the enzyme retained more than 90% of its initial activity (Fig. 4d).

The effects of various cations and compounds (4 mM) on PsBg16A activity indicated that enzymatic activity was enhanced a little or not affected by the presence of Na+, K+, Li+, SDS, DTT, and β-mercaptoethanol individually. Fe3+, Zn2+, Hg2+, and EDTA strongly inhibited the enzyme activity of PsBg16A. Partial inhibition (<80%) was observed in the presence of certain metal ions, including Ca2+, Ni2+, Ag+. Co2+ enhanced the enzymatic activity by 3.32-fold.

Expression and purification of PsBg16A in P. pastoris

Recombinant PsBg16A was successfully expressed in P. pastoris GS115 and secreted into the medium. The transformants were further screened for possible multiple inserts with YPD plates containing various concentrations of G418, a strain having highest β-1,3(4)-glucanase activity, which reached 1,850 U mL−1 in a shake flask culture after induction with methanol for 72 h. SDS–PAGE revealed that recombinant PsBg16A was the major protein in the culture supernatant.

The strain that showed the highest β-1,3(4)-glucanase activity in the shake flask culture was subjected to high-cell-density fermentation. Before the induction phase, no β-1,3(4)-glucanase activity was detected in the culture supernatant. After methanol induction, the cell weight and the β-1,3(4)-glucanase activity in the supernatant increased substantially, reaching a maximum of 61,754 ± 1,640 U mL−1 after 96h of induction. The maximal concentration of secreted proteins was 8.4 g L−1.

The recombinant PsBg16A was purified by ion exchange and gel filtration chromatography. The purified enzyme showed a single band with an apparent molecular mass of 36 kDa on SDS–PAGE, a little higher than the calculated molecular weight of the protein (34.2 kDa, Fig. 3). The specific activity of the purified PsBg16A was 8,649 U mg after 1.31-fold purification, with a final activity yield of 65.2%.

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barley \(\beta\)-glucan (100\%), followed by lichenan (68.7\%) and laminarin (22.3\%). CMC-Na, Avicel, and birch-wood xylan were not degraded by PsBg16A, suggesting that it is a typical \(\beta\)-1,3(4)-glucanase (EC 3.2.1.6).

The hydrolysis products of barley \(\beta\)-glucan and lichenan by recombinant PsBg16A were analyzed by thin-layer chromatography (TLC). TLC analysis showed that PsBg16A hydrolyzed barley \(\beta\)-glucan, laminarin, and lichenan to produce glucose and oligosaccharides with different DPs (DP2–DP4) as the predominant products (Fig. 5). The results indicate that PsBg16A can effectively hydrolyze \(\beta\)-1,4 bonds and some \(\beta\)-1,3 linkages in \(\beta\)-glucan, suggesting that it is a typical endo-\(\beta\)-1,3(4)-glucanase.

Discussion

In this study, a GH family 16 endo-\(\beta\)-1,3(4)-glucanase gene from Paecilomyces sp. FLH30 was cloned and successfully expressed in P. pastoris. The GH family 16 proteins were classified into five subgroups, nonspecific endo-1,3(4)-\(\beta\)-glucanases, \(\beta\)-1,3-galactanases, \(\beta\)-1,4-galactanases/carragenases, xyloglucan transglycosylases, and licheninases, based on multiple sequence alignment, phylogenetic analysis, and substrate specificity. Web-based software is available to identify the subgroup of GH family 16 protein from the amino acid sequence (http://www.ghdb.uni-stuttgart.de). The putative catalytic residues in PsBg16A, Glu114, and Glu119 are highly conserved among GH family 16 members. The alignment with other endo-\(\beta\)-glucanases is shown in Fig. 2. In most fungal endo-1,3(4)-\(\beta\)-glucanases, the motif GEIDIIE is conserved, but in most bacterial \(\beta\)-1,3-1,4-glucanases, the motif DEIDIE is conserved. The deduced amino acid sequence of PsBg16A exhibited highest identity, of 83%, with a GH family 16 endo-1,3-1,4-\(\beta\)-glucanase (ADK55597.1) from Paecilomyces thermophile. It is similar to ones reported for other endo-1,3(4)-\(\beta\)-glucanases, including those from Talaromyces emersonii CBS 814.70 and Phanerochaete chrysosporium. Substrate specificity studies indicate that the enzyme is an endo-1,3(4)-\(\beta\)-glucanase (EC 3.2.1.6). This family of enzymes catalyze the endohydrolysis of 1,3- or 1,4-linkages in \(\beta\)-d-glucans when the glucose residue whose reducing group is involved in the linkage to be hydrolyzed is itself replaced at C-3. The reduced activity observed for lichenan, which, like the preferred substrate (\(\beta\)-glucan), contains \(\beta\)-1,3 and \(\beta\)-1,4 linkages, indicates that the number and distribution of linkages probably influences...
activity, similarly to the endo-1,3(4)-β-glucanase from *Rhizomucor miehei*. Both endoglucanases have a β-sandwich structure formed by two large anti-parallel β-sheets assembled face to face, very similar to that of *Bacillus* β-glucanases but distinct from the β-sandwich folds specific to family 11 xylanases. Therefore the nonspecific characteristic might be ascribed to the special structure of these enzymes. From another point of view, substrates xylan, barley β-glucan, lichenan, and cellulose have similar structures and are probably able to fit the same active center of the enzyme, i.e., *T. reesei* endoglucanase I and *Humicola insolens* endoglucanase I, which can hydrolyze not only β-glucan and cellulose but also laminarin and oat-spelt xylan. The substrate specificity of PsBg16A is different from other GH family 16 non-specific endo-1,3(4)-β-glucanases which do not hydrolyze CMC-Na, Avicel, or Birchwood xylan.

### Table 1. Substrate Specificity and Kinetic Parameters of Purified Recombinant PsBg16A

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Main linkage (monomer)</th>
<th>Specific activity (U mg⁻¹)</th>
<th>$K_m$ (mg mL⁻¹)</th>
<th>$V_{max}$ (μmol min⁻¹ mg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barley β-glucan</td>
<td>1,3-1,4-β-(glucose)</td>
<td>8,649</td>
<td>2.87</td>
<td>7,482</td>
</tr>
<tr>
<td>Lichenan</td>
<td>1,3-1,4-β-(glucose)</td>
<td>5,946</td>
<td>4.26</td>
<td>3,641</td>
</tr>
<tr>
<td>Laminarin</td>
<td>1,3-β-(glucose)</td>
<td>1,929</td>
<td>9.22</td>
<td>1,768</td>
</tr>
</tbody>
</table>

Fig. 4. Characterization of Purified Recombinant PsBg16A.

a. The influence of pH on β-1,3(4)-β-glucanase activity was determined at 70°C using 50 mM of various buffers: citrate, MES, phosphate, glycine-NaOH; b. Influence of pH on the stability of β-1,3(4)-β-glucanase activity. After incubation at 50°C for 30 min in buffers ranging from pH 2.0 to 11.0, the purified β-1,3(4)-β-glucanase activity of the recombinant PsBg16A was assayed in 50 mM MES buffer (pH 7.0) at 70°C. c. The optimal temperature was measured at various temperatures in 50 mM MES buffer (pH 7.0). d. Thermostability of PsBg16A. The enzyme was pre-incubated at various temperatures in MES buffer (pH 7.0), and aliquots were removed at specific time points to measure the residual activity at 70°C at pH 7.0. Values represent mean ± SD of triplicate samples.

Fig. 5. Thin-Layer Chromatography of Hydrolytic Products of 1% w/v Barley β-Glucan (a), 1% w/v Lichenan (b), 1% w/v Laminarin (c) and with 10 U PsBg16A in 50 mM MES Buffer, pH 7.0, at 50°C and at Intervals of up to 8 h. Standards are G1–G5 (lane Gn).
P. pastoris has been found to be an excellent host for the production of heterologous β-glucanases, including GH family 16β-1,3-1,4-glucanases from certain bacteria. In this study, the improved yield and enzyme activity may be attributed mainly to multiple integrated copies of PsBg16A. The presence of multiple integrated copies of a desired expression cassette has been reported to be an important factor in increasing foreign protein production in P. pastoris.12,31,32 In the present study, high-level extracellular production (61,754 U mL\(^{-1}\)) of PsBg16A was achieved and the highest concentration of secreted proteins (8.4 g L\(^{-1}\)) was reached after incubation of transformed P. pastoris cells with methanol for 4 d.

PsBg16A was cloned from an thermophilic fungus. It encodes a protein that displayed optimal activity at pH 7.0 at 70 °C. The pH optimum is different from most β-1,3(4)-glucanases, with pH optima around acidic pH, 4.0–6.5.12,33 The optimal temperature is higher than those for most reported fungi β-1,3(4)-glucanases, which range from 40 °C to 60 °C.12,33,34 The 3D structure of Paecilomyces sp. FLH30 β-glucanase was predicted using the SWISS-MODEL server (http://swissmodel.expasy.org/) in the automated mode, based on P. chrysosporium laminarinase 16A (PDB ID, 2w39) as the closest template. The theoretical results indicated that PsBg16A has a topology consisting mainly of two eight-stranded antiparallel β-sheets arranged in a jellyroll β-sandwich and 13 short helices. Just like laminarinase 16A of P. chrysosporium,35 the extensive loops are stabilized by four disulfide bridges, at positions Cys95–Cys273, Cys137–Cys233, Cys153–Cys176, and Cys250–Cys270. PsBg16A has no potential N-glycosylation sites, and its thermal stability possibly contributed to these internal disulfide bridges. PsBg16A has three acidic amino acid residues (E114, D116, and E119) and three neutral amino acids (I115, I117, and I118) at the active site. We assume that neutral pH is conducive of acid/base catalysis. Glycoside hydrolases have been classed into 125 families based on amino acid sequence homology (http://www.cazy.org/). β-1,3(4)-Glucanases are generally divided into GH families 16 and 81. Most reported bacterial β-1,3(4)-glucanases are classified into GH family 81 and have limited activity on β-glucan or lichenan, but also laminarin. This characteristic makes it more suitable than conventional glucanases for the hydrolysis of glucans in animal feed, food, and beer wort, because these industries require the combined activities of different types of glucanases.

References