Food Chemistry 234 (2017) 68–75

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

# Biochemical properties and application of a novel β-1,3-1,4-glucanase from *Paenibacillus barengoltzii*

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ARTICLE INFO

*Article history:*
Received 13 December 2016
Received in revised form 25 March 2017
Accepted 25 April 2017
Available online 27 April 2017

*Keywords:*
β-1,3-1,4-Glucanase
*Paenibacillus barengoltzii*
Gene cloning
Characterization
β-Gluco-oligosaccharides
Brewing

ABSTRACT

A novel endo-β-1,3-1,4-glucanase gene (*PbBglu16A*) was cloned from *Paenibacillus barengoltzii* and heterogeneously expressed in *Escherichia coli*. The recombinant β-1,3-1,4-glucanase (PbBglu16A) was purified to homogeneity with a recovery yield of 78.6% and a specific activity of 431.8 U $mg^{-1}$. The molecular mass of PbBglu16A was estimated to be 44.0 kDa by SDS-PAGE. The optimal pH and temperature of PbBglu16A were 6.0 and 55 °C, respectively. The enzyme was stable within pH 3.5–9.0 and up to 55 °C. PbBglu16A exhibited high substrate specificity towards barley β-glucan, oat β-glucan and lichenin. PbBglu16A showed an endo-type cleavage pattern and hydrolyzed endogenous enzyme-deactivated oat bran into β-gluco-oligosaccharides with a yield of 7.0%, which mainly consisted of trioligosaccharide and tetraoligosaccharide. Further, PbBglu16A could promote mashing with a reduced filtration time (14.0%) and viscosity (3.4%). Thus, PbBglu16A might be a promising candidate for the production of β-gluco-oligosaccharides and in brewing industry.



## 1. Introduction

β-Glucans are non-starch linear polysaccharides that commonly exist in the endosperm of cereals, including barley, oats, rye, rice, wheat, and sorghum. They may often have adverse effects on cereal-grain-based industry, for example, resulting in higher viscosity of barley β-glucans during the mashing process in the brewing industry (Furtado et al., 2011). β-Glucans are also known to increase digesta viscosity leading to a low efficiency of nutrient digestion and absorption in poultry, while contributing to reduced feed passage rate (Smits & Annison, 1996).

β-Glucanases, which catalyze the hydrolysis of β-glucan, include four types: β-1,3-1,4-glucanase (lichenase, EC 3.2.1.73), β-1,4-glucanase (cellulase, EC 3.2.1.4), β-1,3-glucanase (laminarinase, EC 3.2.1.39), and β-1,3(4)-glucanase (EC 3.2.1.6) (Luo et al., 2010). β-1,3-1,4-Glucanases exhibit a strict substrate specificity for cleavage of β-1,4 glycosidic bonds in 3-*O*-substituted glucopyranose units to yield products mainly consisting of 3-*O*-β-cellobiosyl-D-glucose and 3-*O*-β-cellotriosyl-D-glucose (Sun et al., 2012). They usually exist in both plants and microorganisms. The plant β-1,3-1,4-glucanases belong to the glycosyl hydrolase (GH) family 17, while microbial β-1,3-1,4-glucanases are classified as members of GH family 16. As an important biotechnological aid in brewing and feedstuff industries, many 1,3-1,4-β-glucanases have been isolated and characterized from a number of microorganisms, in which most of them are from bacteria, including *Bacillus* species, such as *B. subtilis* (Tang, He, Chen, Zhang, & Ali, 2004), *B. amyloliquefaciens* (Niu, Zhu, Wang, & Li, 2014), *B. licheniformis* (Chaari et al., 2012), *B. macerans* (Borriss, Buettner, & Maentsaelae, 1990) and *B. tequilensis* (Wang, Niu, Liu, Chen, & Li, 2014). Several fungal β-1,3-1,4-glucanases have also been reported from *Paecilomyces thermophila* (Yang, Yan, Jiang, Fan, & Wang, 2008), *Rhizomucor miehei* (Tang et al., 2012), and *Penicillium occitanis* (Chaari et al., 2014). Furthermore, many β-1,3-1,4-glucanase genes have been cloned from microorganisms, such as, *B. subtilis* (Masilamani, Sharma, Muthuvel, & Natarajan, 2013), *B. amyloliquefaciens* (Sun et al., 2012), *B. circulans* (Kim, Kim, Ko, Youn, & Lee, 2004), *B. licheniformis* (Teng et al., 2007), *Clostridium thermocellum* (Ribeiro et al., 2012), *Penicillium pinophilum* (Chen et al., 2012), *Paecilomyces thermophila* (Hua, Yan, Jiang, Li, & Katrolia, 2010), *Orpinomyces* sp. (Comlekcioglu, Ozkose, Akyol, Yazdic, & Ekinci, 2011), and *Bispora* sp (Luo et al., 2010).

Recent reports have demonstrated that the hydrolysate of oat β-glucan by lichenase can enhance the growth of health-promoting

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http://dx.doi.org/10.1016/j.foodchem.2017.04.162
0308-8146/

probiotic strains (Jaskari et al., 1998). Also, it has slow aging, improves nutrition absorption rate, and offers antihypercholesterolemic effects (Wang et al., 2014). The oligoglucans also can be used as functional food additives for selective growth of human beneficial intestinal microflora (bifido bacteria and lactobacilli) (Barreteau, Delattre, & Michaud, 2006). The lichenan hydrolysate prepared by *Penicillium occitanis* lichenase has showed a high antioxidative capacity and exhibited a high antimicrobial activity against *Escherichia coli*, *Bacillus subtilis*, *Klebsiella pneumonia* and *Salmonella typhimurium* (Chaari, Belghith-Fendri, & Ellouz-Chaabouni, 2015). Additionally, β-1,3-1,4-glucanases have been reported to have various applications in brewing industry to reduce brewer mash viscosity and turbidity, increase filtration rate, enhance the yield and produce high-quality brewers malt (Chaari et al., 2014). Thus, it is highly desirable to develop novel β-1,3-1,4-glucanases with good biochemical properties.

As a genus of facultative anaerobic bacteria, *Paenibacillus* sp. is widely distributed in nature. Several β-1,3-1,4-glucanase genes have been cloned from this genus, including *Paenibacillus* sp. S09 (Cheng, Xu, Wang, Wang, & Zhang, 2014), *Paenibacillus* sp. X4 (Na et al., 2015), *Paenibacillus* sp. F-40 (Yang et al., 2007), and *Paenibacillus barcinonensis* BP-23 (Alexandra Cerda, Valeria Valenzuela, Diaz, & Javier Pastor, 2016). A thermophilic marine bacterium *Paenibacillus barengoltzii* CAU904 has been isolated from the South China Sea (Fu et al., 2014). In this study, a novel β-1,3-1,4-glucanase from *P. barengoltzii* CAU904 was gene cloned, expressed and biochemically characterized. The purified enzyme was further investigated for its potential for the production of β-gluco-oligosaccharides and in brewing industry. To the best of our knowledge, this is the first report on β-1,3-1,4-glucanase from *P. barengoltzii.*

## 2. Materials and methods

### 2.1. Materials

β-Glucan (from barley), β-glucan (from oat), lichenin, laminarin, pullulan, xylan (from birchwood), soluble starch, *p*NP-β-xylopyranoside, *p*NP-β-galactopyranoside, and *p*NP-β-glucopyranoside were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Chelating Sepharose Ni-iminodiacetic acid (Ni-IDA) resins were obtained from GE Healthcare (Piscataway, NJ, USA). Silica gel plate was the product of E. Merck Co. (Darmstadt, Germany). All other chemicals were of analytical grade.

### 2.2. Strains and media

*P. barengoltzii* CAU904 has been deposited in the China General Microbiological Culture Collection Center (CGMCC) under accession number CGMCC9530. The strain was maintained on Lysogeny Broth (LB) plate and stored at 4 °C until use.

### 2.3. Gene cloning and sequence analysis of a β-1,3-1,4-glucanase

A β-1,3-1,4-glucanase gene (*PbBglu16A*) was amplified using the genomic DNA of *P. barengoltzii* CAU904 as the template by polymerase chain reaction (PCR) with primer PbBglu16AF (5′-ATGATGC TAAAACGCGGTAA-3′) and PbBglu16AR (5′-AATGTTTACCTTCGTAAA CATCC-3′). The PCR was performed according to the following procedure: pre-denaturation at 94°C for 5 min; 35 cycles of 94°C for 30 s, 54°C for 30 s, and 72°C for 80 s; and a final extension of 10 min. The PCR products were purified, ligated into the pMD19-T vector and sequenced.

The Open Reading Frame (ORF) was analyzed at NCBI ORF finder (http://www.ncbi.nlm.nih.gov/gorf/orfig.cgi/). Homology searches were performed by BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The conserved domains were analyzed by InterPro (http://www.ebi.ac.uk/interpro/scan.html). The molecular weight and isoelectric point were predicted using ProtParam tool (http://web.expasy.org/protparam/) at ExPASy (www.expasy.org). Multiple alignments and signal peptide analysis were performed by Clustal Omega (http://www.ebi.ac.uk/Tools/msa/clustalo/) and SignalP4.1 (http://www.cbs.dtu.dk/services/SignalP/), respectively.

### 2.4. Expression and purification of the recombinant β-1,3-1,4-Glucanase (PbBglu16A)

The coding region of β-1,3-1,4-glucanase gene (*PbBglu16A*) without signal peptide was amplified by PCR using two primers PbBglu16ANcoF (5′-TGACTCCATGGGCGCTCCCAACTGGCAGTTG-3′) and PbBglu16ANotR (5′-TGACTGCGGCCGCGTTTACCTTCGTAAACATCCACTT-3′). The PCR product was purified by gel extraction kit (Biomed, Beijing), digested with two restriction enzymes *Nco*I and *Not*I, and then ligated into the pET28-a(+) vector, and transformed into *E. coli* DH5α. The positive transformants were incubated at 37 °C in LB medium containing 50 μg/mL kanamycin, until $OD_{600}$ reached 0.6–0.8. Subsequently, the recombinant enzyme was induced by i sopropyl-β-D-1-thiogalactopyranoside (IPTG) at a final concentration of 1 mM at 30 °C for 6 h. The cells were harvested by centrifugation at 8000*g* for 10 min followed by re-suspension in 20 mM Tris-HCl buffer (pH 8.0) containing 300 mM NaCl and 20 mM imidazole. After sonication, the solution was centrifuged at 12,000*g* for 10 min at 4 °C and the supernatant was used as crude enzyme solution.

The crude enzyme solution was loaded on Ni-IDA column pre-equilibrated by buffer A (50 mM Tris-HCl buffer pH 8.0 containing 300 mM NaCl and 20 mM imidazole). The column was sequentially eluted by buffer B (50 mM Tris-HCl buffer pH 8.0 containing 300 mM NaCl and 50 mM imidazole) and buffer C (50 mM Tris-HCl buffer pH 8.0 containing 300 mM NaCl and 200 mM imidazole). The fraction eluted by buffer C was collected and further concentrated using a Pellicon Cassette concentrator (Millipore) with a 10 kDa cutoff membrane. The purity of recombinant enzyme was analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The protein concentration was determined using Lowry method with bovine serum albumin as a standard (Lowry, Rosebrough, Farr, & Randall, 1951).

### 2.5. Enzyme assay

Activity of 1,3-1,4-β-glucanase was determined using 1% (w/v) barley β-glucan as a substrate according to the previously reported method (Yang et al., 2008). Briefly, 150 μL properly diluted enzyme solution was added to 50 μL 1% (w/v) barley β-glucan dissolved in 50 mM citrate buffer (pH 6.0). The mixture was incubated at 55 °C for 10 min. The reaction was then terminated by adding 200 μL dinitrosalicylic acid (DNS) reagent containing 1 M NaOH. The content of reducing sugar was determined by DNS method. One unit of β-1,3-1,4-glucanase activity was defined as the amount of enzyme required to produce 1 μmol glucose-equivalent reducing sugars per minute under the above assay conditions.

### 2.6. pH and temperature profiles

The optimal pH of PbBglu16A was determined using various 50 mM buffers, viz., citrate buffer (pH 3.0–6.0); 2-(morpholino) ethanesulfonic acid buffer (MES, pH 5.5–7.0); 3-(N-Morpholino) propanesulfonic acid buffer (MOPS, pH 6.0–8.5); Tris-HCl buffer (pH 7.0–9.0); and glycine-NaOH buffer (pH 8.5–10.5). For pH sta-