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ORIGINAL PAPER

Genetic and biochemical characterization of a protease-resistant mesophilic β-mannanase from *Streptomyces* sp. S27

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Abstract A β-mannanase gene, designated as *man5S27*, was cloned from *Streptomyces* sp. S27 using the colony polymerase chain reaction (PCR) method and expressed in *Escherichia coli* BL21 (DE3). The open reading frame consisted of 1,161 bp and encoded a 386-amino-acid polypeptide (Man5S27) with calculated molecular mass of 37.2 kDa. The encoded protein comprised a putative 38-residue signal peptide, a family 5 glycoside hydrolase domain, and a family 10 carbohydrate-binding module. Purified recombinant Man5S27 had high specific activity of 2,107 U mg^{-1} and showed optimal activity at pH 7.0 and 65°C. The enzyme remained stable at pH 5.0–9.0 and had good thermostability at 50°C. The K_m values for locust bean gum and konjac flour were 0.16 and 0.41 mg ml^{-1}, with V_{max} values of 3,739 and 1,653 μmol min^{-1} mg^{-1}, respectively. Divalent metal ions such as Mn^{2+}, Zn^{2+}, Ca^{2+}, Pb^{2+}, and Fe^{2+} enhanced the enzyme activity, but Ag^{+} and Hg^{2+} strongly inhibited the activity. Man5S27 also showed resistance to various neutral proteases (retaining >95% activity after proteolytic treatment for 2 h).

Keywords *Streptomyces* sp. S27 · β-Mannanase · Heterologous expression · High specific activity · Protease resistance

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Introduction

Hemicelluloses—the main constituents of plant cell walls—are linear or branched heteropolysaccharides [1]. According to the composition of the polymer backbone, hemicelluloses can be classified into xylans, mannans, arabinogalactans or arabinans [2]. Among them, mannans and heteromannans are the second most abundant hemicellulosic polysaccharides, consisting of a backbone of β-1,4-linked mannose or combination of glucose and mannose with side-chains of α-1,6-linked galactose residues [3]. In softwoods, galacto-glucomannan is one of the principal hemicelluloses, making up approximately 15–25% of total hemicellulose content, and glucomannan exists in hardwoods at lower levels (3–5%) [4]. The main enzyme for hydrolysis of mannans and heteromannans is β-mannanase (endo-1,4-β-D-mannanase, EC 3.2.1.78), which randomly cleaves the β-1,4-glycosidic linkages of the backbone [5].

Various types of organisms, including bacteria, fungi, plants, and mollusks, can produce β-mannanases [6–11]. To date, many β-mannanase genes have been cloned and expressed in heterologous systems [12]. Based on amino acid sequence similarity (http://www.cazy.org/), β-mannanases are grouped into glycoside hydrolase (GH) families 5, 6, and 113 [13], all of which belong to GH clan A [14, 15].

β-Mannanases have enjoyed widespread use in various industries, such as the food and feed industry, coffee extraction, oil drilling, the textile industry, and the bleaching of kraft pulps [16]. Studies on mannanases produced by *Streptomyces* have been carried out [17, 18], and so far only a few *Streptomyces* mannanase genes have been cloned and expressed [19]. In this study, we report the cloning and expression of a new β-mannanase gene from *Streptomyces* sp. S27. The purified recombinant enzyme shows high

stability at mesophilic and neutral to alkaline conditions and strong resistance to most metal ions and neutral proteases.

Materials and methods

Microorganism isolation

Soil sample was collected from the Flaming Mountain in the Turpan Basin of Xinjiang Uygur Autonomous Region, China. Selection medium containing (per liter) 5.5 g NaCl, 5.3 g K_2HPO_4, 3.0 g KH_2PO_4, 2.5 g $(NH_4)_2SO_4$, 0.5 g $CaCl_2$, 0.1 g $MgSO_4{\cdot}7H_2O$, 0.0075 g $FeSO_4{\cdot}7H_2O$, 0.0030 g $CoCl_2$, 0.0025 g $MnSO_4{\cdot}H_2O$, 0.0020 g $ZnSO_4$, and 6.0 g locust bean gum (LBG) (pH 7.0) was used to isolate mannanase-producing strains. Soil sample (about 5 g) was inoculated into 300 ml isolation medium and incubated at 42°C with agitation for 36 h. The suspension was serially diluted with sterile water, spread onto LBG agar plates (1.0 g l^{-1} LBG, 1% agar), and incubated at 30°C for 48 h. The plates were stained with 0.5% (w/v) Congo red solution for 20 min, and destained with 1 M NaCl solution for 20–25 min. Strains with clear zones around the colonies were defined as mannan-degrading microorganisms. Based on 16S rDNA sequence analysis, one strain, named as *Streptomyces* sp. S27, showing the highest mannanolytic activity, was selected for further analysis.

Strains, medium, plasmids, and reagents

Escherichia coli JM109 and BL21 (DE3) cells were grown aerobically in Luria–Bertani (LB) broth or on LB agar plates at 37°C for recombinant plasmid amplification and gene expression, respectively. The plasmid pUC19 (TaKaRa, Japan) and the pGEM-T Easy vector (Promega, USA) were used for plasmid preparations and gene cloning, respectively. The pET-30a(+) vector for gene expression and enterokinase cleavage capture kit to remove the N-terminal fusion sequence of pET-30(+) were purchased from Novagen (USA). The DNA purification kit, restriction endonucleases, and *LA Taq* DNA polymerase with GC buffer were purchased from TaKaRa. All other reagents were of analytical grade.

Construction of the genomic library and gene cloning

To obtain the β-mannanase gene from strain S27, degenerate primers Man5SF (5′-TTCTCCATNCAYATGTA-3′) and Man5SR (5′-GTTGCCRCWCCANGACC-3′) (Y represents C or T, R represents A or G, W represents A or T, and N represents A, T, C or G) were designed based on the two conserved amino acid sequences (FSIHMY and WSWSGN) of GH 5 β-mannanases from *Streptomyces*. Touchdown polymerase chain reaction (PCR) was performed using *LA Taq* DNA polymerase with GC buffer as follows: 95°C for 5 min, followed by 30 cycles of 94°C for 30 s, 45–40°C (decreasing 0.5°C each cycle until 40°C) for 30 s, and 72°C for 30 s, and a final extension step at 72°C for 7 min. The PCR product was cloned into pGEM-T Easy vector for sequencing and BLAST analysis. Based on the known mannanase gene fragment sequence, a primer set (SF and SR) was designed and used to screen the genomic library of strain S27.

The genomic library of *Streptomyces* sp. S27 was constructed as described [20]. The genomic DNA of strain S27 was digested by *Sau*3AI. Fragments (5–10 kb) were cloned into the *Bam*HI site of pUC19, and transformed into *E. coli* JM109 to construct the genomic DNA library. For rapid screening, the genomic library was subdivided into 30 subpools, each containing 10 × 10 transformants. The first round of PCR screening was performed using the pooled DNA of each subpool as template. Once the objective subpool was determined, transformants in each row or column were pooled and used as the template for the second round of PCR screening. Using this approach, the positive clones were obtained, and the recombinant plasmid was isolated and sequenced.

Gene analysis

The nucleotide sequence was analyzed using the open reading frame (ORF) Finder tool (http://www.ncbi.nlm.nih.gov/gorf/gorf.html). The signal peptide was predicted by the SignalP 3.0 server (http://www.cbs.dtu.dk/services/SignalP/). DNA and protein sequence alignments were carried out using the blastn and blastp programs (http://www.ncbi.nlm.nih.gov/BLAST/), respectively. Multiple sequence alignments were performed with Clustal W [21]. Homology modeling and electrostatic analysis of the catalytic domain of *man5S27* was performed using Accelrys Discovery Studio software (DS 2.5, http://www.accelrys.com) with the GH 5 β-mannanase from *Thermomonospora fusca* (PDB entry 1BQC) as template [22].

Construction of expression plasmids

For the construction and expression of *man5S27* in *E. coli*, the following primers were used: Man5S27EF (5′-GGGGAATTCGCCACGGGCATCCGCGTCGGCAAC-3′) and Man5S27ER (5′-GGGAAGCTTTCACCCGTCGGCC GCGCTGCCGCG-3′) (*Eco*RI and *Hin*dIII site underlined, respectively). The PCR products were gel-purified, digested with *Eco*RI and *Hin*dIII, and cloned into the corresponding site of pET-30a(+). The recombinant plasmid pET-*man5S27* with a His-Tag at the N-terminus was transformed into *E. coli* BL21 (DE3) competent cells and confirmed by sequencing.

Expression and purification of the recombinant Man5S27

The positive transformant harboring pET-*man5S27* was grown overnight at 37°C in LB medium supplemented with 50 μg ml^{-1} kanamycin. The culture was then transferred into fresh LB medium (1:100 dilution) containing kanamycin and grown aerobically at 37°C to A_{600} of ~0.6. Isopropyl-β-D-1-thiogalactopyranoside (IPTG) was then added to final concentration of 0.8 mM for enzyme induction.

After induction at 30°C for 8 h, cells were harvested by centrifugation at 12,000 × *g* for 5 min at 4°C, and then resuspended in sonication buffer A (20 mM Na_2HPO_4/0.1 M citric acid, pH 6.0) at 5 ml per gram wet weight. The cell suspension was sonicated on ice, and cell debris was removed by centrifugation. The concentrated supernatant (crude enzyme, 5 ml) was applied to a 1-ml Ni–NTA chelating column (Qiagen, Germany) that had been previously equilibrated with buffer B (20 mM Tris–HCl, 500 mM NaCl, pH 7.6). The protein was eluted using a step gradient of imidazole (0, 20, 40, 60, 80, 100, 200, and 300 mM) in buffer B containing 500 mM NaCl and 10% glycerol. The fractions with enzyme activity were concentrated and stored at 4°C before characterization.

The homogeneity of the purified enzyme was evaluated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE), using a 12% running gel [23]. The protein was visualized by staining with Coomassie brilliant blue R-250. Protein concentration was determined according to the Bradford method [24], using bovine serum albumin as the standard. To remove the upstream 43-residue N-terminal fusion sequence of pET-30a(+) containing the enterokinase recognition sequence DDDDK, recombinant Man5S27 was incubated with enterokinase at room temperature for 16 h, and the truncated protein was recovered by spin-filtration.

Enzyme assay and biochemical characterization

β-Mannanase activity was determined using the 3,5-dinitrosalicylic acid (DNS) method as described [25]. The standard reaction system was composed of 0.1 ml appropriately diluted enzyme and 0.9 ml 0.1 M McIlvaine buffer (pH 7.0) containing 0.5% (w/v) LBG. After incubation at 65°C for 10 min, the reaction was stopped by addition of 1.5 ml DNS reagent, boiled in a water bath for 5 min, and cooled to room temperature. Absorbance at 540 nm was measured. One unit of β-mannanase activity was defined as the amount of enzyme required to release 1 μmol of reducing sugar from the substrate per minute.

The optimal pH of purified recombinant Man5S27 for β-mannanase activity was determined at 50°C using the following buffers: 0.1 M McIlvaine buffer (pH 4.0–8.0) and 0.1 M glycine-NaOH buffer (pH 9.0–12.0). pH stability was determined by pre-incubating the purified recombinant enzyme at 37°C for 1 h at pH 4.0–11.0 in the indicated buffers without substrate and then measuring residual enzyme activity under standard conditions (pH 7.0, 65°C, 10 min).

The temperature optimum of purified recombinant Man5S27 was determined at different temperatures (30–80°C) according to the enzyme assay procedure mentioned above. Thermostability was monitored by pre-incubating the enzyme in 0.1 M McIlvaine buffer (pH 7.0) at 50°C or 60°C for 2, 5, 10, 20, 30, and 60 min without substrate. Residual enzyme activity in each case was then assayed under standard assay conditions.

The effects of various metal ions and chemical reagents on recombinant enzyme activity were determined in 0.1 M McIlvaine buffer (pH 7.0) containing 5 mM NaCl, KCl, $AgNO_3$, $CaCl_2$, $CoCl_2$, $NiSO_4$, $CuSO_4$, $MgSO_4$, $MnSO_4$, $ZnSO_4$, $Pb(CH_3COO)_2$, $HgCl_2$, $FeSO_4$, $CrCl_3$, ethylenediamine tetraacetic acid (EDTA), SDS or β-mercaptoethanol. The assay system without any additive was used as control.

To determine its resistance to different neutral proteases, purified recombinant Man5S27 (100 μg ml^{-1}) was incubated with trypsin (pH 7.6, 25°C), α-chymotrypsin (pH 7.8, 25°C), collagenase (pH 7.4, 37°C), subtilisin A (pH 7.4, 37°C), and proteinase K (pH 7.5, 37°C) at a ratio of 1:10 (protease:Man5S27, w/w), respectively. After 1 or 2 h incubation, residual activity was measured under standard assay conditions. For the control sample, the recombinant enzyme was incubated under the same conditions in the absence of protease.

Substrate specificity and kinetic parameters

The substrate specificity of Man5S27 for various substrates was determined after incubation at 65°C for 10 min in McIlvaine buffer (0.1 M, pH 7.0) containing one of the following substrates (0.5%, w/v): LBG (mannose/galactose ~4:1), guar gum (mannose/galactose ~2:1), konjac flour (mannose/glucose ~1.6:1), barley β-glucan, oat spelt xylan, and carboxymethyl cellulose-sodium (CMC-Na). Reactions were terminated by adding 1.5 ml DNS. The amount of released reducing sugar was estimated as described above.

K_m and V_{max} values for the purified recombinant Man5S27 were determined in McIlvaine buffer (0.1 M, pH 7.0) containing 0.125–5.0 mg ml^{-1} LBG or konjac flour as substrate at 65°C. Data were determined from a Lineweaver–Burk plot using the nonlinear regression computer program GraFit. All assays were performed in triplicate.

Analysis of the hydrolysis products

To analyze the hydrolysis products, 0.5% (w/v) LBG was hydrolyzed by purified recombinant Man5S27 at 60°C for

12 h. The hydrolysis products were assayed by high-performance anion exchange chromatography (HPAEC) with a Dionex model 2500 (Dionex, USA).

Nucleotide sequence accession number

The nucleotide sequence for the *Streptomyces* sp. S27 β-mannanase gene (*man5S27*) was deposited in the GenBank database under accession number HM062521.

Results

Cloning and sequence analysis of *man5S27*

A 201-bp DNA fragment was amplified from the genomic DNA of strain S27 using degenerate primers Man5SF and Man5SR. The PCR product was cloned into pGEM-T Easy vector, sequenced, and showed 82% identity to the putative β-1,4-mannanase from *Streptomyces* sp. s6-204. Based on the partially identified mannanase gene sequence, primers SF (5′-CTACAACACCGCTTCCGAGGTCCAG-3′) and SR (5′-GTAGCCCACCCGGAGCGACTG-3′) were designed and used to screen the genomic library of strain S27. After two-round screening, one positive clone (PS1811) containing the target gene—*man5S27*—was exactly determined among the 3,000 transformants. The complete open reading frame (ORF) consisted of 1,161 bp with 68.3% G+C content, starting with ATG and terminating with a TGA codon, and encoded a 386-residue polypeptide with calculated mass of 37.2 kDa. SignalP analysis revealed the existence of an N-terminal signal peptide at amino acid residues 1–38.

The deduced amino acid sequence of Man5S27 was compared with other GH 5 β-mannanase sequences available in GenBank. The highest identity was 76% with the putative β-1,4-mannanase from *Streptomyces* sp. s6-204 (GenBank accession no. ABY90130), followed by the characterized β-1,4-mannanase from *Streptomyces lividans* 66 (71% identity; GenBank accession no. AAA26710). Based on sequence analysis, the mature protein consists of two domains: a family 5 catalytic domain and a family 10 carbohydrate-binding module (CBM). Between these two functional domains there is a glycine-rich sequence. Two putative catalytic glutamate residues in Man5S27, Glu166 and Glu261, were found to be highly conserved among GH 5 members [22]. The homology-modeled structure of Man5S27 displays the classical eightfold (α/β)-barrel in which the catalytic acid–base and nucleophile glutamates are at the end of β-strand 4 and 7, respectively (data not shown). Analysis of solvent-exposed amino acids revealed a high frequency of positively charged residues on the surface (Fig. 1).

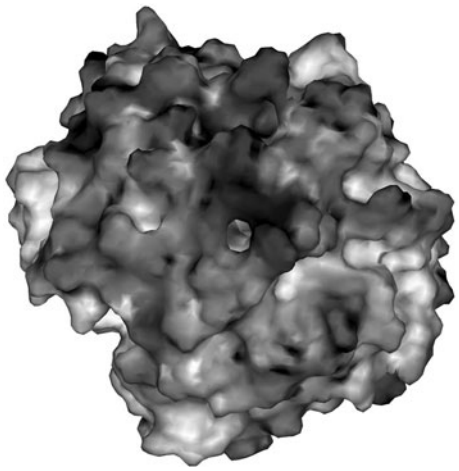

Fig. 1 Charge distribution on the surface of the homology-modeled structure of Man5S27. Negatively and positively charged surfaces are *white* and *black*, respectively

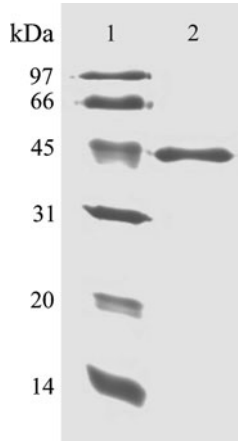

Fig. 2 SDS–PAGE analysis of the purification of recombinant Man5S27 expressed in *E. coli* BL21 (DE3). *Lane 1*, standard protein molecular mass marker; *lane 2*, purified recombinant Man5S27 following Ni–NTA chelate chromatography

Expression and purification of the recombinant enzyme

The recombinant Man5S27 containing an N-terminal His-tag was cloned into the vector pET-30a(+) and then transformed into *E. coli* BL21 (DE3). After induction with IPTG, the crude enzyme activity in the cell lysate was 26.78 U ml^{-1}.

The recombinant enzyme in the lysate was purified to electrophoretic homogeneity by using single-step affinity chromatography on Ni–NTA column. SDS–PAGE analysis revealed the purified enzyme as a single band with molecular mass ~43.0 kDa (Fig. 2). Purified recombinant Man5S27 exhibited specific activity of 2,107 U mg^{-1} after 21.5-fold purification. After digestion with enterokinase, 43-amino-acid residues were removed from the N-terminus of recombinant Man5S27, and the truncated protein showed one band of ~38.0 kDa, which was essentially identical to the calculated value (data not shown).

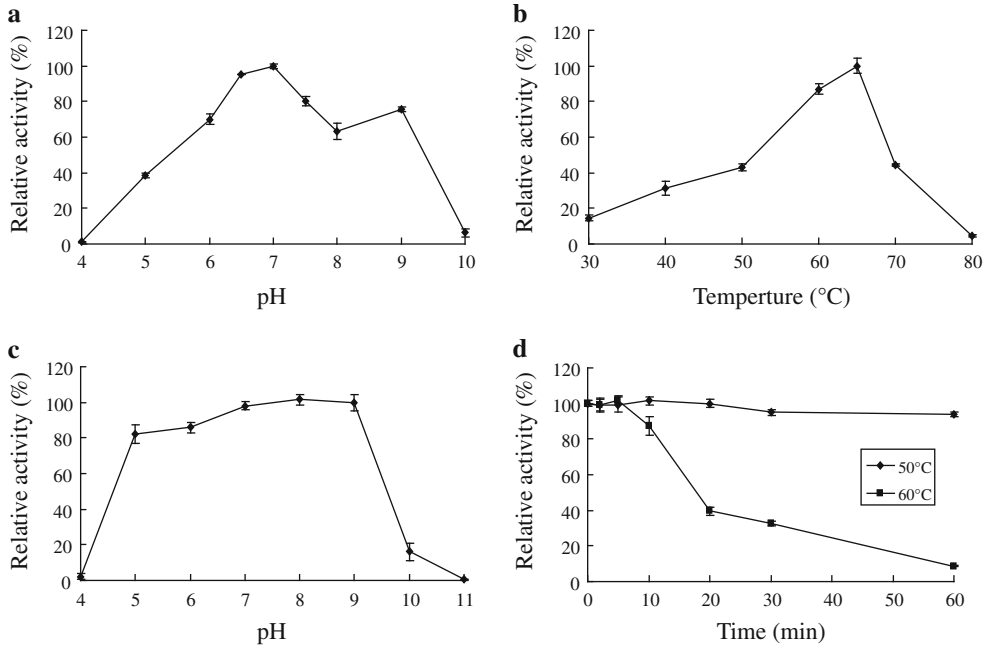

Fig. 3 Characterization of purified recombinant Man5S27. **a** Effect of pH on β-mannanase activity. The activity assay was performed at 50°C in buffers with pH ranging from 4.0 to 10.0. **b** Effect of temperature on β-mannanase activity measured in 0.1 M McIlvaine buffer (pH 7.0). **c** Effect of pH on stability of β-mannanase activity. After incubating purified recombinant Man5S27 at 37°C for 1 h in buffers with pH ranging from 4.0 to 11.0, activity was measured in 0.1 M McIlvaine buffer (pH 7.0) at 65°C. **d** Thermostability of purified recombinant Man5S27. The enzyme was pre-incubated at 50°C or 60°C in 0.1 M McIlvaine buffer (pH 7.0), and aliquots were removed at specific time points for measurement of residual activity at 65°C. Each value in the panels represents the mean of triplicates with standard deviation

Characterization of purified recombinant Man5S27

The purified recombinant Man5S27 showed optimal activity at pH 7.0, and over 70% of maximal activity was remained in the pH range 6.0–9.0 (Fig. 3a). More than 80% of the enzyme activity was retained after incubation at pH 5.0–9.0 and 37°C for 1 h (Fig. 3c). The optimal temperature for Man5S27 was 65°C (Fig. 3b). In the thermostability assay, Man5S27 remained quite stable at 50°C after incubation at pH 7.0 for 1 h. Under the same conditions, Man5S27 lost almost all of its activity after incubation at 60°C for 1 h (Fig. 3d).

The effects of different metal ions and chemical reagents on the enzyme activity of the recombinant Man5S27 was tested (data not shown). Addition of most metal ions and EDTA at concentration of 5 mM had no or little effect on the activity of Man5S27. The activity was almost completely inhibited by Hg^{2+} and Ag^{+}. On the other hand, the activity of Man5S27 was significantly enhanced in the presence of 5 mM Ca^{2+}, Zn^{2+}, Mn^{2+}, Pb^{2+}, Fe^{2+} or β-mercaptoethanol.

Purified recombinant Man5S27 was strongly resistant to all tested neutral proteases. After treatment with trypsin, α-chymotrypsin, collagenase, subtilisin A, and proteinase K for 1 h, the recombinant enzyme retained almost 100% activity, respectively. When treated for 2 h, more than 95% of the activity remained.

Table 1 Substrate specificity of purified recombinant Man5S27

Substrate	Substrate specificity (U mg^{-1})	Relative activity[a] (%)
Locust bean gum	2,107 ± 182	100.00 ± 8.66
Konjac flour	1,312 ± 110	62.25 ± 5.22
Guar gum	73.54 ± 11.57	3.49 ± 0.55
Oat spelt xylan	ND[b]	ND
Barley β-glucan	ND	ND
CMC-Na	ND	ND

[a] Values represent mean ± standard deviation (SD, $n = 3$) relative to untreated control samples

[b] Not detectable

Substrate specificity and kinetic parameters

The hydrolytic activity of Man5S27 towards various substrates was determined (Table 1). The highest activity was observed with LBG (100%), followed by konjac flour (62.25%) and guar gum (3.49%). No activity was detected in the presence of barley β-glucan, oat spelt xylan or CMC-Na.

K_m and V_{max} values of Man5S27 using LBG as substrate were 0.16 mg ml^{-1} and 3,739 μmol min^{-1} mg^{-1}, respectively. With konjac flour as substrate, the K_m and V_{max}

values were 0.41 mg ml^{-1} and 1,653 μmol min^{-1} mg^{-1}, respectively.

Analysis of hydrolysis product

After hydrolysis of LBG, the enzyme was removed from the reaction mixture by precipitation, followed by ultrafiltration with a 10-kDa cutoff membrane. The hydrolysis products were analyzed by HPAEC with oligosaccharide standards. The test system without enzyme was used as control. The main products of LBG were 3.23% mannose, 0.74% mannobiose, 22.14% mannotriose, 2.21% mannotetraose, 6.89% mannopentaose, and 64.79% other mannan oligosaccharides.

Discussion

Streptomyces have a complex lifecycle and capacity to secrete a wide range of enzymes active against the main lignocellulose constituents. Several β-mannanases of *Streptomyces* spp. have been purified and characterized [17–19]. In this study, a new GH 5 β-mannanase gene was cloned from *Streptomyces* sp. S27. Sequence analysis showed that Man5S27 contained four regions: an N-terminal leader sequence, a GH 5 domain, a short glycine-rich linker, and a C-terminal CBM 10 domain. The catalytic domain of Man5S27 demonstrated some typical structural characteristics of GH 5 β-mannanases, such as a catalytic center composed of Asn, Glu, and Pro (NEP) and eight strictly conserved residues, Arg88, His124, Asn165, Glu166, His232, Tyr234, Glu261, and Trp290 [26].

β-Mannanases from different sources showed different properties. The pH profile of Man5S27 has a major peak at pH 7.0 with a shoulder at pH 9.0. The reason might be attributed to the effect of salts present in the buffers used [27]. Different from the acidic pH optima (pH 1.5–5.0) of fungal β-mannanases, the neutral pH optimum of Man5S27 was similar to that of β-mannanases from *Streptomyces* spp. and *Bacillus* spp. (pH 6.0–7.6) [16]. When enzymes perform catalysis at high pH, their acid/base residues have to maintain a higher pK_a value [28]. The mechanism underlying the neutral properties of Man5S27 might be ascribed to the pK_a values of its catalytic residues, which are 7.4 (Glu166) and 5.2 (Glu261), respectively. Also, Man5S27 has high pH stability at neutral and alkaline conditions (pH 5.0–9.0). When compared with acid-stable β-mannanases, more positively charged residues were found on the surface, probably contributing to the pH stability of Man5S27 (Fig. 1) [29]. To confirm this hypothesis, subsequent site-specific mutants and crystallographic studies are in progress.

Other characteristic features of Man5S27 were also determined. The specific activity of purified Man5S27 was 2,107 U mg^{-1}, which was higher than that of Man3 from *Streptomyces ipomoea* CECT 3341 (56 U mg^{-1}) [18], of β-mannanase from *Bacillus subtilis* 5H [30], and of ManA from *S. lividans* 66 (876 U mg^{-1}) [19], but lower than that of β-mannanases from *Bacillus circulans* CGMCC1554 (4,839 U mg^{-1}) [31] and *Bacillus subtilis* WY34 (8,302 U mg^{-1}) [32]. The K_m value of Man5S27 for the substrate LBG (0.16 mg ml^{-1}) is relatively lower than that of known bacterial and fungal β-mannanases (Table 2) [12]. Therefore, Man5S27 has better affinity for substrates and higher catalytic efficiency. After treatment with various proteases for 2 h, Man5S27 retained more than 95% activity, giving it potential application in combination with proteases in the textile industry. Like Man3 from *S. ipomoea* CECT 3341, Man5S27 showed enhanced activity in the presence of Mn^{2+}, a general adverse factor on the activity of most β-mannanases [18]. The enzyme activity is also increased by Ca^{2+} and Zn^{2+}, but it is

Table 2 Physical–chemical properties of β-mannanases from *Streptomyces* spp. and *Bacillus* spp.

Microbial source	Enzyme	Optimum temperature (°C)	Optimum pH	K_m[a] (mg ml^{-1})	Specific activity (U mg^{-1})	Reference
Streptomyces sp. S27	Man5S27[b]	65	7.0	0.16	2,107	This study
Streptomyces ipomoea CECT 3341	Man3[c]	55	7.5	3.4	56	[18]
Streptomyces lividans IAF36	Mannanase[b]	58	6.7	0.77	876	[19]
Streptomyces galbus NR	Mannanase[c]	40	6.5	NR[d]	NR	[33]
Bacillus subtilis 5H	Mannanase[c]	55	7.0	NR	1,900	[30]
Bacillus circulans CGMCC1554	Mannanase[b]	60	7.6	NR	4,839	[31]
Bacillus subtilis WY34	Mannanase[c]	65	6.0	7.6	8,302	[32]

[a] K_m values determined using locust bean gum as substrate

[b] Recombinant enzyme

[c] Purified enzyme

[d] Not reported

significantly inhibited by Hg^{2+} and Ag^{+}, which is similar to other β-mannanases.

In summary, compared with the β-mannanases from *Streptomyces* spp. and *Bacillus* spp. that have application potential in the pulp and paper industry, Man5S27 has many favorable characteristics, such as high stability under alkaline mesophilic conditions, high specific activity, high catalytic efficiency towards LBG, no cellulase activity, and strong resistance to most metal ions and neutral proteases. These superior properties make Man5S27 a good candidate for basic research and various industrial applications, especially in the detergent or pulp and paper industry.

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