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ORIGINAL RESEARCH PAPER

Identification, heterologous expression, and characterisation of β-1,3-xylanase *Bc*Xyn26B from human gut bacterium *Bacteroides cellulosilyticus* WH2

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Abstract The cell walls of red and green algae contain β-1,3-xylan, which is hydrolysed by the endo-type enzyme β-1,3-xylanase. Notably, only marine-bacteria-derived β-1,3-xylanases have been functionally characterised to date. In this study, we characterised the enzymatic properties of a potential β-1,3-xylanase (*Bc*Xyn26B) derived from the human gut bacterium, *Bacteroides cellulosilyticus* WH2. The codon optimized *Bc*Xyn26B gene was synthesised and expressed in *Escherichia coli* BL21(DE3). The recombinant protein was purified by a two-step purification process using Ni-affinity chromatography followed by anion exchange chromatography, and its enzymatic properties were characterised. The recombinant *Bc*Xyn26B exhibited specific hydrolytic activity against β-1,3-xylan and released various β-1,3-xylooligosaccharides, with β-1,3-xylobiose as the primary product. The optimum reaction temperature was 50 °C, higher than that for other enzymes derived from marine bacteria. This study represents the first report on the identification, heterologous expression, and characterisation of β-1,3-xylanase from human gut microbes. Notably, the substrate specificity of *Bc*Xyn26B indicates that human gut *Bacteroides* species possess an unknown β-1,3-xylan utilisation system.

Keywords *Bacteroides cellulosilyticus* · β-1,3-Xylan · β-1,3-Xylanase · Human gut bacterium · Macroalgae · Seaweed

Introduction

The human gut microbiota comprises diverse bacteria that utilise various polysaccharides. These microbes are crucial for our symbiotic relationship, and their composition is altered by diet to maintain host homeostasis by regulating the metabolic patterns of the substances produced by the gut microbiota (Ge et al. 2021). Therefore, understanding the interactions among diet, gut bacteria, and the host is crucial for understanding their biological functions. *Bacteroides,* the dominant bacterial group present in the human gut, utilises polysaccharides from both plant and host sources (Koropatkin et al. 2012). These bacteria are rich in genes encoding carbohydrate-active enzymes (CAZymes), which are organised into polysaccharide utilisation loci (PULs) and distributed throughout the genome (Xu et al. 2007). This PUL system enables nutrient acquisition by *Bacteroidetes* and contributes

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to establishing a microbial ecosystem. Among the human gut microbiota, *Bacteroides cellulosilyticus* exhibits the most extensive carbohydrate-degrading mechanism. *B. cellulosilyticus* WH2 is a gram-negative anaerobic bacterium isolated from the human gut (Robert et al. 2007). Notably, its whole genome has been sequenced, and the presence of numerous CAZymes and PULs indicates its proficiency in metabolising polysaccharides with diverse structures and polymerisation degrees (McNulty et al. 2013).

Seaweed, a non-digestible dietary component, has garnered significant attention as a valuable food resource. Consuming seaweed-derived polysaccharides reduces the risk of obesity (Tucker & Thomas 2009), type II diabetes (Meyer et al. 2000), breast cancer (Park et al. 2009), and coronary heart disease (Streppel et al. 2008). Although enzymes required to degrade red algae are derived from marine bacteria (Araki et al. 1999; Aoki & Kamei 2006; Shan et al. 2014), human gut bacteria also exhibit genes encoding CAZymes that hydrolyse marine polysaccharides (O'Sullivan et al., 2010, Jin et al. 2021). Additionally, the genes encoding CAZymes, which are involved in agarose degradation, are transferred from the marine bacterium *Zobellia galactanivorans* to the human gut bacterium *Bacteroides plebeius*, which was isolated from Japanese gut microflora (Hehemann et al. 2010). Therefore, the potential of gut bacteria to utilise seaweed polysaccharides has garnered research attention.

β-1,3-Xylan is a polysaccharide that is present in the cell walls of red and green algae such as *Porphyra*, *Bangia*, *Caulerupa*, *Bryopsis*, and *Udotea* species (Iriki et al. 1960), but not in any terrestrial plant species. β-1,3-xylanases (1,3-β-D-xylan xylanohydrolase; EC 3.2.1.32) are enzymes that play a crucial role in hydrolysing the β-1,3-glycosidic linkages of β-1,3-xylan, resulting in the production of short-chain β-1,3-xylooligosaccharides. This enzyme offers several beneficial applications, such as analysing the structural makeup of algal cell walls (Kiyohara et al. 2006), preparing protoplasts from red algae (Araki et al. 1994), and transforming algal biomass (Umemoto et al. 2012). To date, a relatively small number of β-1,3-xylanases have been identified and characterised, including those from *Vibrio* sp. strain XY-214 (Araki et al. 2000), *Alcaligenes* sp. strain XY-234 (Okazaki et al. 2002), *Vibrio* sp. strain AX-4 (Kiyohara et al. 2005), *Pseudomonas* sp. strain ND137 (Aoki & Kamei 2006), *Thermotoga neapolitana* strain DSM 4359 (Okazaki et al. 2013), *Psychroflexus torquis* ATCC 700755 (Kudou et al. 2015), *Flammeovirga pacifica* strain WPAGA1 (Cai et al. 2018; Yi et al. 2020), and *Brevundimonas vesicularis* (Liang et al. 2015). These β-1,3-xylanases are derived from marine bacteria. However, no β-1,3-xylanases have been identified in human gut bacteria.

In this study, we identified a potential β-1,3-xylanase gene in the genome of *B. cellulosilyticus* WH2, a human gut bacterium. This potential β-1,3-xylanase *Bc*Xyn26B (recombinant protein of the *Bc*xyn26B gene) has not yet been biochemically characterised. We further aimed to analyse the enzymatic properties of *Bc*Xyn26B. The present investigation constitutes the first biochemical examination of β-1,3-xylanase derived from human intestinal bacteria. Our findings indicated that *Bc*Xyn26B is an endo-type β-1,3-xylanase with a higher catalytic efficiency than previously identified β-1,3-xylanases from marine bacteria.

Materials and methods

Sequence analysis

Amino acid sequence homology queries were searched in the Basic Local Alignment Search Tool (BLAST) from the National Center for Biotechnology Information (Altschul et al. 1990). Sequence alignment and protein signal peptide prediction were performed using MUSCLE (Edgar 2004) SignalP 5.0 (Petersen et al. 2011) software, respectively.

Materials

β-1,3-Xylan from the green alga *Caulerpa racemosa* var. *laete-virens* was prepared using the method described by Iriki et al. (Iriki et al. 1960). β-1,3-xylo-oligosaccharides were obtained through the partial hydrolysis of β-1,3-xylan (1 g) using 2 mL of 1N trifluoroacetic acid (TFA) at 70 °C for 1.5 h. Following neutralisation to pH 7.0, the hydrolysis products were separated into β-1,3-xylo-oligosaccharides with various degrees of polymerisation using thin-layer chromatography (TLC). All other chemicals utilised were of the highest quality commercially available.

Bacterial strains and plasmids

The host strains employed in this study were *E. coli* DH5α and BL21 (DE3) (Nippon Gene, Tokyo, Japan) for cloning and recombinant protein production, respectively. The expression vector used was pET-22b (+) (Merck Millipore, Darmstadt, Germany).

Protein expression and purification

The *Bc*Xyn26B gene was chemically synthesised by Integrated DNA Technologies (Coralville, IA, USA) with codon optimisation for the *E. coli* expression system (Fig. S1) based on its amino acid sequence (GenBank accession number: ALJ61530). The gene was amplified from chemically synthesised DNA fragments via PCR, with primers specifically designed to avoid potential signal peptide sequences. The length of the amplified gene was 1,026 base pairs, which encoded amino acids 20 to 360. The primer sequences were: BcellWH2_04313_F_NdeI 5′-AAAACATATGTGTGAAAACAAAGAACATGA TACACCGG-3′ and BcellWH2_04313_R 5′-AAA ACTCGAGCTGCGGCAGTTTGCTCCAATCCA GTTCTGCTGCATGAATATAACC-3′.

The recombinant His-tagged protein was expressed by ligating the PCR products into the *Xho*I and *Nde*I sites of the pET-22b (+) vector (Merck Millipore) using the DNA Ligation High Ver.2 (TOYOBO, Tokyo, Japan) following the manufacturer's instructions. *E. coli* BL21 (DE3) cells with the expression plasmid were cultured in 250 mL of 2×YT medium supplemented with 100 µg/mL ampicillin at 37 °C with shaking (180 rpm). Once the culture reached the mid-log growth phase (optical density at 600 nm of 0.6), it was cooled to 20 °C before induction with isopropyl-β-D-thiogalactopyranoside at a final concentration of 1 mM. After 24 h of incubation at 20 °C, the cells were harvested through centrifugation (8,000×*g*, 10 min, 4 °C), suspended in lysis buffer, and mixed with a protease inhibitor. The suspension was sonicated for 10 min, mixed with DNase I (10 units/µL), and incubated at 4 °C for 10 min. Centrifugation (12,900×*g*, 10 min, 4 °C) was performed twice. Following centrifugation, the supernatant was filtered using a 0.45-µm filter. The filtrate was purified on a 5 mL Bio-Scale Mini Nuvia IMAC Ni-Charged column (Bio-Rad Laboratories, Hercules, CA, USA) using a Profinia protein purification system (Bio-Rad Laboratories), following the manufacturer's instructions. The enzyme solution was dialysed against a 25 mM Tris–HCl buffer (pH 7.5) and further purified by anion-exchange chromatography. Enzyme purification was performed on an ÄKTA start chromatography system (Cytiva, Marlborough, MA, USA) with a 5 mL Bio-Scale Mini Macro-Prep High Q Cartridge column (Bio-Rad Laboratories). Elution was performed using a linear gradient of buffer ranging from 0 to 500 mM NaCl and 25 mM Tris–HCl (pH 7.5). The fractions containing β−1,3-xylanase were combined and subjected to dialysis in a buffer solution comprising 25 mM Tris–HCl (pH 7.5). To determine the homogeneity of the protein, sodium dodecyl–sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) was employed (Laemmli 1970).

Enzyme assay

The activity of β-1,3-xylanase was measured by quantifying the liberation of reducing sugars from β-1,3-xylan in a reaction mixture containing 0.5% (w/v) β-1,3-xylan, an appropriate amount of enzyme, and 50 mM 2-morpholinoethanesulfonic acid (MES)-NaOH buffer (pH 6.0). The mixture was incubated at 40 °C for 10 min, and the amount of reducing sugar produced was measured using the Somogyi–Nelson method (Somogyi 1952), with D-xylose serving as a standard. The amount of enzyme that releases 1 µmol of D-xylose per minute under the previously mentioned conditions is considered one unit of enzyme activity.

*Bc*Xyn26B characterisation

The optimal pH of *Bc*Xyn26B was determined by measuring its activity in 80 mM Britton–Robinson buffer (pH 3–10) using β-1,3-xylan as a substrate using the method described above. To determine the optimal temperature for *Bc*Xyn26B, activity was measured in the range of 4–60 °C. The activity of enzymes was evaluated by adding 10 mM Ca^{2+}, Mg^{2+}, Mn^{2+}, Ni^{2+}, Ag^{+}, Cu^{2+}, Zn^{2+}, Fe^{2+}, ethylenediaminetetraacetic acid (EDTA), N-bromosuccinimide (NBS), or 1 mM dithiothreitol (DTT) to the reaction mixture to determine the effect of metal ions and chemicals on enzyme activity. The substrate specificity of the enzyme for polysaccharides was

evaluated using β-1,4-xylan, β-1,4-mannan, laminarin (β-1,3-glucan with β-1,6-linkage), carboxymethyl cellulose (CMC), Avicel (microcrystalline cellulose) and starch. These substrates were used at a final concentration of 0.5% (w/v). The digestion products obtained were analysed using TLC.

TLC

The analysis of digestive products from various polysaccharides and β-1,3-xylooligosaccharides was performed using TLC on silica gel 60 plates, which were developed in a solvent system consisting of n-butanol and acetic acid (2:1, v/v) and included D-xylose and β-1,3-xylooligosaccharides as standards. The TLC plate was sprayed with diphenylamine/aniline/phosphate reagent (Bailey and Bourne 1960) and heated at 95 °C for 20 min to visualise the digestion products.

Results

Molecular phylogenetic analysis of β−1,3-xylanase

Genes encoding proteins with primary structural sequence homology to the β-1,3-xylanase *Pt*Xyn26A (Kudou et al. 2015) from *P. torquis* were observed in the genomes of four human gut bacterial species of the genus *Bacteroides* (*B. cellulosilyticus, B. intestinalis, B. gallinarum,* and *B. timonensis*) (Fig. 1). We primarily focused on *B. cellulosilyticus*, whose

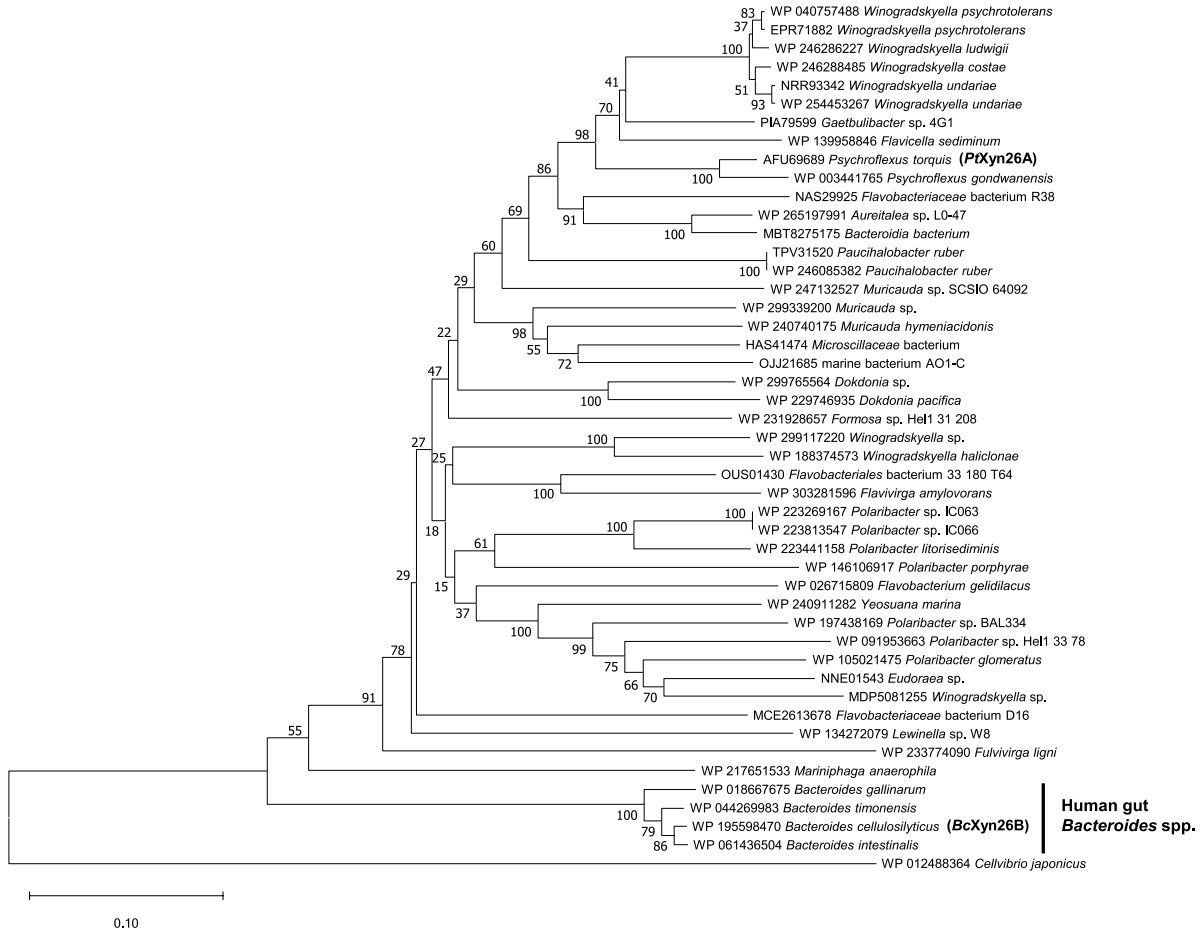

Fig. 1 Molecular phylogenetic analysis of *Pt*Xyn26A and homologous proteins. The tree is constructed with complete or near-complete sequences, aligned with ClustalW, and created using the neighbour-joining method. The bar indicates the relative sequence distance

genome has already been sequenced, and named the potential β-1,3-xylanase from *B. cellulosilyticus* *Bc*Xyn26B.

Sequence analysis

The *Bc*Xyn26B gene comprises 1,083 nucleotides and encodes a protein containing 360 amino acids with a predicted molecular weight of 41,160. The N-terminal amino acid sequence of *Bc*Xyn26B exhibits a potential signal peptide, with the predicted cleavage site situated between positions 16 (Ser) and 17 (Cys) (Fig. S2). This signal peptide indicated the presence of a lipoprotein signal. Therefore, removing the signal peptide yielded a mature protein of 344 amino acids with a molecular weight of 39,297 and an isoelectric point of 4.73. The *Bc*Xyn26B-gene-encoded amino acid sequence was compared with previously reported β-1,3-xylanases (Fig. 2). *Bc*Xyn26B comprises only a catalytic domain and is homologous

Fig. 2 Amino acid sequence alignment of different β-1,3-xylanases. The sequences are identified as follows: *Bc*Xyn26B, *Bacteroides cellulosilyticus* β-1,3-Xylanase; *Pt*Xyn26A, *Psychroflexus torquis* ATCC 700755 β-1,3-Xylanase; *Tn*Xyn26A, *Thermotoga neapolitana* DSM 4359 β-1,3-Xylanase; *Alc*TxyA, *Alcaligenes* sp. XY-234 β-1,3-Xylanase; *Pse*AxnB, *Pseudomonas* sp. ND137 β-1,3-Xylanase; *Fp*Xyl512, *Flammeovirga pacifica* WPAGA1 β-1,3-Xylanase; *Pv*XylII, *Pseudomonas vesicularis* MA103 β-1,3-Xylanase; *Fp*Xyl88, *Flammeovirga pacifica* WPAGA1 β-1,3-Xylanase; *Vib*TxyA, *Vibrio* sp. XY-214 β-1,3-Xylanase; and *Vib*Xyl4, *Vibrio* sp. AX-4 β-1,3-Xylanase. The signal peptide sequence of *Bc*Xyn26B predicted using SignalP-5.0 is surrounded. Grey boxes indicate amino acids that are conserved in all sequences. Putative catalytic residues (Glu-166 and Glu-255 in *Bc*Xyn26B) are indicated by black arrowheads. The numbers at the start and end of the respective lines indicate the amino acid numbers from Met-1

to the β-1,3-xylanases classified into the carbohydrate-hydrolysing enzyme family 26 (GH26). The

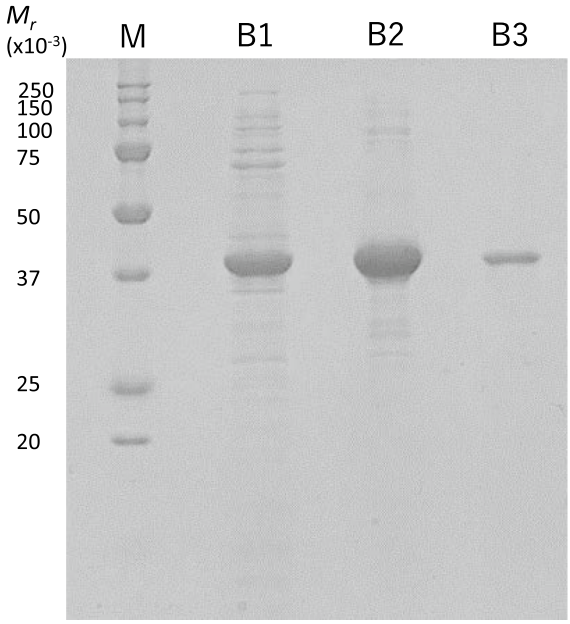

Fig. 3 Sodium dodecyl-sulfate–polyacrylamide gel electrophoresis (analysis of *Bc*Xyn26B purification). Lanes M: protein marker; B1: crude extract of *Escherichia coli* BL21 (DE3); B2: protein after purification using Ni-affinity chromatography; and B3: protein after purification using anion change chromatography

*Bc*Xyn26B protein sequence features two conserved putative catalytic residues, Glu-166 and Glu-255 (Fig. 2, black arrowheads), corresponding to Glu-138 and Glu-234, respectively, in the catalytic module of β-1,3-xylanase from *Vibrio* AX-4. These residues serve as an acid/base catalyst and a nucleophile, respectively (Kiyohara et al. 2005). Furthermore, these two Glu residues were observed to be conserved in all β-1,3-xylanases characterised.

Expression and purification

The *E. coli*-produced recombinant *Bc*Xyn26B was extracted and purified to homogeneity by a two-step purification process using Ni-affinity chromatography followed by anion exchange chromatography. The purified enzyme migrated as a single band on SDS–PAGE and its molecular weight was estimated to be approximately 40,000 (Fig. 3, lane B3).

Substrate specificity

The substrate specificity of *Bc*Xyn26B was assessed using various polysaccharides (Fig. 4) and pNP-β-xylosides. The enzyme hydrolysed β-1,3-xylan but not β-1,4-xylan, avicel, CMC, laminarin, β-1,4-mannan, starch, or pNP-β-xylosides under the conditions outlined in "Materials and Methods."

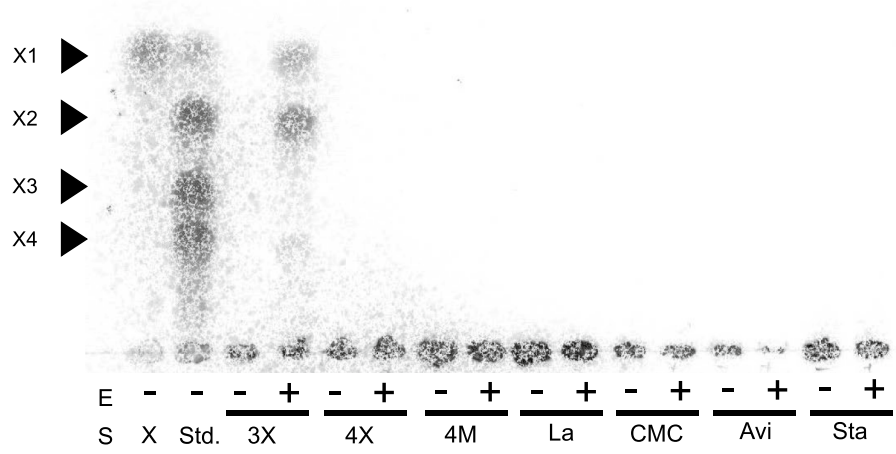

Fig. 4 Thin-layer chromatography of the hydrolysis products obtained from various polysaccharides using purified *Bc*Xyn26B. *Bc*Xyn26B (1.41 µg/mL) was incubated with various substrates (0.5% w/v) in 50 mM 2-(N-morpholino)ethanesulfonic acid (MES) buffer (pH 6.0) at 40 °C for 16 h. Subsequently, the hydrolysates were separated on a Silica Gel 60 TLC plate. X: xylose; Std.: D-xylose, β-1,3-xylobiose, β-1,3-xylotriose, and β-1,3-xylotetraose; 3X: β-1,3-xylan; 4X: β-1,4-xylan; 4 M: β-1,4-mannan; La: laminarin (β-1,3-glucan); CMC: carboxymethycellulose; Avi: avicel (cellulose); Sta: starch; and X1 to X4: xylose to β-1,3-xylotetraose

Consequently, *Bc*Xyn26B was considered to be a β-1,3-xylanase (EC 3.2.1.32).

The hydrolysis pattern of β-1,3-xylan incubated with purified *Bc*Xyn26B was analysed using TLC. Notably, β-1,3-xylobiose (X2) and β-1,3-xylotriose (X3) were identified as primary products during the early incubation period (Fig. 5). However, the release of D-xylose (X1), β-1,3-xylotetraose (X4), and oligosaccharides with over four xylose units was deferred.

When *Bc*Xyn26B hydrolysed β-1,3-xylotriose (X3), it produced β-1,3-xylobiose (X2) and D-xylose (X1). Similarly, the hydrolysis of β-1,3-xylotetraose (X4) by the enzyme yielded the same products: β-1,3-xylobiose (X2) and D-xylose (X1). However, the enzyme showed no catalytic activity towards β-1,3-xylobiose (X2) (Fig. 6).

Biochemical characterisation

We further examined the effects of pH and temperature on the activity of the purified *Bc*Xyn26B. The enzyme exhibited optimal activity at pH 6.0 and was stable across a wide pH range (6.0–10.0) following incubation at 4 °C for 24 h (Fig. 7). The optimal temperature for *Bc*Xyn26B activity was 50 °C during a 10-min incubation, with over 80% of the initial activity sustained at 50 °C for 24 h (Fig. 8).

Notably, 80–100% of the enzyme activity was inhibited on treatment with Mn^{2+}, Ag^{+}, Cu^{2+}, Zn^{2+}, Fe^{2+}, EDTA, DTT, and N-bromosuccinimide, which is a modification reagent for tryptophan residues in proteins. Similarly, treatment with Ni^{2+} resulted in a 30% inhibition. In contrast, Ca^{2+} and Mg^{2+} exhibited no significant effects on the enzyme activity (Table S1).

The kinetic parameters of *Bc*Xyn26B for β-1,3-xylan were determined by fitting the experimental

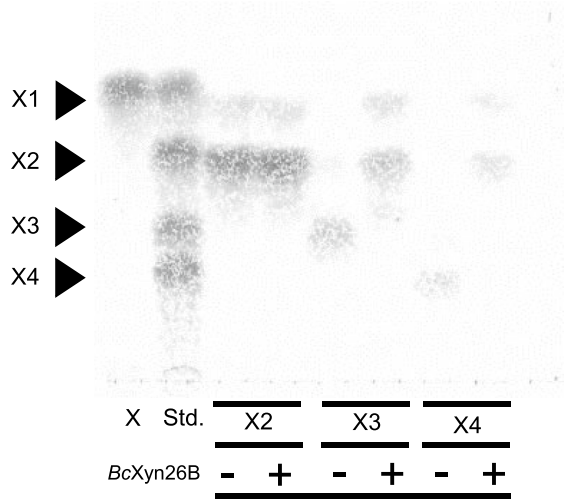

Fig. 6 Hydrolysis products of β-1,3-xylo-oligosaccharides using purified *Bc*Xyn26B. *Bc*Xyn26B (16.2 μg/mL) was incubated with various substrates in 50 mM 2-(N-morpholino) ethanesulfonic acid (MES) buffer (pH 6.5) at 30 °C for 16 h. Subsequently, the hydrolysates were separated on a Silica Gel 60 TLC plate. X: xylose; Std.: D-xylose, β-1,3-xylobiose, β-1,3-xylotriose, and β-1,3-xylotetraose; and X1 to X4: xylose to β-1,3-xylotetraose

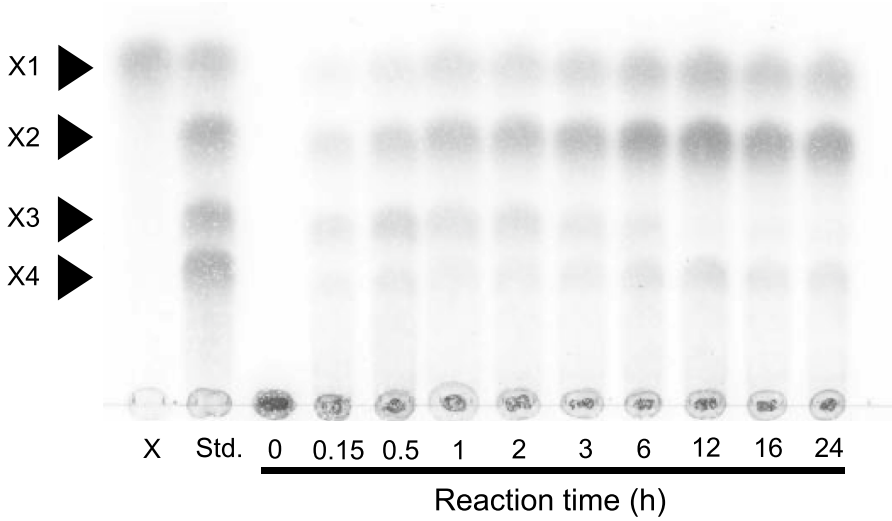

Fig. 5 Hydrolysis products of β-1,3-xylan using purified *Bc*Xyn26B. *Bc*Xyn26B (1.41 μg/mL) was incubated with β-1,3-xylan (0.5% w/v) in 50 mM 2-(N-morpholino)ethanesulfonic acid (MES) buffer (pH 6.0) at 40 °C for the indicated periods. Subsequently, the hydrolysates were separated on a Silica Gel 60 TLC plate. X: xylose; Std.: D-xylose, β-1,3-xylobiose, β-1,3-xylotriose, and β-1,3-xylotetraose; and X1 to X4: xylose to β-1,3-xylotetraose

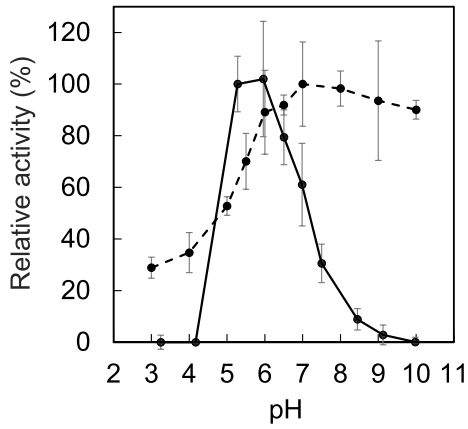

Fig. 7 Optimal pH for activity and stability of the purified *Bc*Xyn26B. The continuous and broken lines represent *Bc*Xyn26B activity profiles at pH ranging from 3–10 and pH stability profiles, respectively

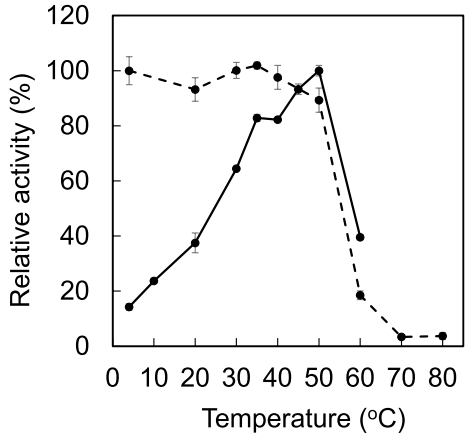

Fig. 8 Optimal temperature for activity and thermal stability of the purified *Bc*Xyn26B. The continuous and broken lines represent *Bc*Xyn26B activity profiles at various temperatures and thermal stability profiles, respectively

data to the Michaelis–Menten equation using Excel Solver (Microsoft) through nonlinear least-squares fitting with various concentrations of β-1,3-xylan as the substrate. The K_m value of *Bc*Xyn26B for β-1,3-xylan was 5.04 mg/mL, whereas k_{cat} was 61.33 s^{-1}. The kinetic parameters of *Bc*Xyn26B were evaluated compared with those of β-1,3-xylanase derived from the mesophilic marine bacterium *Alcaligenes* sp. strain XY-234 (*Alc*TxyA) (Okazaki et al. 2002). The k_{cat} value for *Bc*Xyn26B compared with that of β-1,3-xylan was 4.34 times greater than that of *Alc*TxyA, amounting to 61.33 s^{-1} and 14.12 s^{-1}, respectively. Moreover, the overall catalytic efficiency (k_{cat}/K_m) of *Bc*Xyn26B for β-1,3-xylan was 4.19 times higher than that of *Alc*TxyA.

Discussion

Bacteroides spp. constitute 25% of the anaerobic organisms and are the primary human gut bacteria, playing a crucial role in fibre utilisation (Wexler 2007). This species has developed a highly effective and specialised method for degrading carbohydrates by creating closely linked and strictly controlled co-expressed gene clusters known as PULs (El Kaoutari et al. 2013). Among the commensal gut bacteria, the strictly anaerobic cellulolytic *B. cellulosilyticus* WH2 exhibits the largest carbohydrate degradation mechanism. Its genome is anticipated to encode over 373 glycoside hydrolases (GHs), distributed across 21 GH families and often located within PULs (McNulty et al. 2013). This mechanism enables *B. cellulosilyticus* to utilise persistent polysaccharides efficiently, potentially contributing to its dominance in the human gut (Ali-Ahmad et al. 2017).

In this study, we identified a potential β-1,3-xylanase (*Bc*Xyn26B) in the *B. cellulosilyticus* WH2 genome. The *Bc*Xyn26B gene consists of 1,083 bp and encodes 360 amino acid residues. The enzyme possesses a signal peptide, and its mature protein consists of 344 amino acid residues with a molecular weight of 39,297. Additionally, it possesses a lipoprotein signal peptide, suggesting that *Bc*Xyn26B is anchored to the membrane through lipid modification of the N-terminal cysteine residues of the mature protein (Cys17). The signal peptide is cleaved following transportation to the membrane through the general secretion pathway (Pugsley 1993). The present study marks the first report of a β-1,3-xylanase expressed as a lipoprotein. The catalytic domain of *Bc*Xyn26B (Cys17–Gln360) is homologous to the β-1,3-xylanase of GH26. Family 26 GH belongs to the superfamily clan GH-A and is characterised by a conserved $(\beta/\alpha)_8$-barrel architecture consisting of eight repeating β-strands and α-helices. Two catalytic Glu residues are conserved at the ends of β-strands 4 and 7 of GH-A enzymes. In this study, a BLAST query indicated that two potential catalytic Glu residues were conserved in the catalytic module of *Bc*Xyn26B

(Glu-166 and Glu-255). Additionally, the codon-optimised DNA of the mature protein region of *Bc*Xyn26B was chemically synthesised, and the recombinant enzyme was obtained using the *E. coli* protein expression system. Notably, the recombinant enzyme, *Bc*Xyn26B, specifically hydrolysed β-1,3-xylan when it was reacted with various polysaccharides. Moreover, substrate specificity analysis demonstrated that *Bc*Xyn26B hydrolysed β-1,3-xylan and produced β-1,3-xylo-oligosaccharides with various polymerisation degrees. Notably, *Bc*Xyn26B degraded tetrasaccharides into disaccharides and monosaccharides when it was reacted with β-1,3-xylooligosaccharides. Therefore, *Bc*Xyn26B was identified as an endo-type β-1,3-xylanase (1,3-β-D-xylan xylanohydrolase; EC 3.2.1.32). *Bc*Xyn26B demonstrated optimal activity at 50 °C and pH 6.0. The elevated optimum reaction temperature of *Bc*Xyn26B, compared to other β-1,3-xylanases derived from marine bacteria, may be attributed to adaptation to the human gut environment, which consistently maintains a temperature of approximately 36–37 °C, which is higher than that of the marine environment. The presence of NBS inhibited the enzyme activity, indicating the involvement of active-site-based tryptophan residues in substrate stacking. Moreover, DTT inhibited enzyme activity, indicating the presence of a disulfide bond in the conformation of this enzyme, which helps maintain the conformation of the substrate-binding site. In addition, the k_{cat} value for β-1,3-xylan was 61.33 s^{-1} at 50 °C and pH 6.0, 4.3 times higher than that of mesophilic β-1,3-xylanase from a marine bacterium, with a k_{cat} value of 14.12 s^{-1} at 37 °C and pH 7.0.

To our knowledge, this is the first study to identify and characterise β-1,3-xylanase from a human gut bacterium. β-1,3-xylan cannot be degraded in the small intestine. However, these results indicate that when β-1,3-xylan reaches the large intestine, it is degraded and utilised by *B. cellulosilyticus*. Therefore, consuming seaweeds containing β-1,3-xylan can alter the intestinal microflora, affecting overall human health. *Bacteroides* are considered beneficial bacteria owing to the significant association between intestinal *Bacteroides* and obesity and diabetes (Qiu et al. 2019) and studies demonstrating weight loss through dietary interventions that increase *Bacteroides* abundance. Therefore, β-1,3-xylan may serve as a prebiotic to increase the number of beneficial *Bacteroides* species in the human gut. Additionally, the β-1,3-xylanase gene was observed only in *Bacteroides*, indicating a possible horizontal gene transfer from marine bacteria. *Bacteroides* have likely adapted to the integration into PULs of enzymes that use niche marine polysaccharides, such as β-1,3-xylan. This adaptation serves as a strategy to thrive in a harsh gut environment, where the primary nutrient source is persistent polysaccharides consumed by the host. Further progress in research on human health and seaweed-based diets will help develop novel prebiotic products from seaweed, benefiting various industries, such as food, cosmetics, and pharmaceuticals.

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Author contributions SH contributed to the design and implementation of the research. SH and FO analysed the results and wrote the manuscript. FO conceived and supervised the original project.

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Data availability The authors declare that the data supporting the findings of this study are available within the paper and its Supplementary Information files. Should raw data files be needed in another format, they are available from the corresponding author upon reasonable request.

Declarations

Competing Interests The authors declare that they have no affiliations with or involvement in any organisation or entity with any financial interest in the subject matter or materials discussed in this manuscript.

Ethical approval This article contains no studies with human participants or animals performed by any of the authors.

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