ARTICLE

Production and characterization of manno-oligosaccharides from hydrolysis of mannan substrates by recombinant beta-mannanase from *Bacillus licheniformis* (Weigmann) Chester DSM 13

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ABSTRACT

Mannooligosaccharides (MOS) is a prebiotic produced by the hydrolysis of mannans and promotes gut health. One route in producing MOS is via enzymatic method, which employs microbial enzyme (e.g., *β*-mannanase) for mannan hydrolysis. In this work, we investigated the stability and propensity of *β*-mannanase from *Bacillus licheniformis* (*Bl*ManB) overexpressed in *Lactiplantibacillus plantarum* WCSF1 towards *β*-MOS production using several mannan sources. We also determined the fermentation conditions for optimal enzyme production in the food-grade system. Maximal crude enzyme yield was obtained (17.3 ± 1.2 kU per liter of fermentation broth with specific activity of 194 U/mg protein) by maintaining the pH at 6–6.5 at fermentation. Crude *Bl*ManB is stable at storage conditions of -20°C and 4°C. The crude overexpressed enzyme was also stable and active at conversion conditions (30 °C and 37 °C) in the presence of 2 mM dithiothreitol (DTT) . Analysis by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) indicated that the crude enzyme produced

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known linear MOS of 235.88 mg/g using locust bean gum, 121.63 mg/g using copra meal, 71.71 mg/g using guar gum, 21.33 mg/g using spent coffee ground, and 86.76 mg/g using konjac glucomannan. The study has also shown that crude *Bl*ManB overexpressed in *L. plantarum* WCSF1 could be efficiently used to produce food-grade MOS.

INTRODUCTION

In the recent consensus established by the international standard, prebiotics is defined as "a substrate that is selectively utilized by host microorganisms conferring a health benefit" (Gibson et al. 2017). It is exclusively consumed by the host microorganisms that promote a healthy microbiota and thereby holistically improves the health and immunity of the host (Bindels et al. 2015; Gibson et al. 2017). Among the carbohydrate-based prebiotics, manno-oligosaccharides (MOS) has attracted significant research interest owing to its ability to stimulate growth of probiotic bacteria in *in vitro* fecal fermentation (Gibson et al. 2017; Lam and Cheung 2019; Prayoonthien et al. 2019). In addition, previous reports show its potential to modulate immune response as well as confer physiological benefits to companion and farmed animals (Cuong 2013; Lourenço et al. 2015; Pawar et al. 2017). Several recent works have also illustrated that synthesized MOS can serve as a dietary supplement to substitute animal synthetic antibiotics and vaccines (Chee et al. 2010; Kumar et al. 2015), thereby augmenting the efforts in sustaining food safety and sustainability.

There are generally three routes to produce MOS – physical, chemical, and enzymatic methods. Hydrothermal treatment is a commonly employed physical method that uses fixed-volume reactors designed to maintain specific temperature and, sometimes, pressure (Smith et al. 2013). Chemical methods utilize detergent, acid, or base as hydrolysis agents (Jana et al. 2021). An alternative to these physical and chemical methods for MOS production is the enzymatic method, which employs microbial enzyme for mannan hydrolysis. All these routes produce MOS by degrading mannans. Mannans are complex polysaccharides integrated in hemicelluloses in plant tissues associated with cellulose and lignin (Moreira and Filho 2008; Yamabhai et al. 2016), and constituent of glycoproteins in yeast cell walls (Yamabhai et al. 2016) and algae (Chauhan et al. 2012). Their linear backbone constitute of β-1,4-linked mannopyranosyl residues or a mix of mannopyranosyl and glucopyranosyl residues, and may contain α-1,6-linked galactose substituents or acetyl groups (Scheller and Ulvskov 2010). Mannans are found in agricultural products and wastes such as locust bean gum, konjac, copra meal, spent coffee ground and guar gum.

These hydrolytic methods have been implemented to meet the demand for MOS, while being cost-efficient. The use of microbial enzymes for processing substrates, like mannans, has been favored by industries because chemical methods may alter the product during the process and requires neutralization or acid/base removal to recover the product (Jana et al. 2021); whereas these enzymes catalyze reactions in an economical and environmentally safe manner (Nigam 2013). β-One of the enzymes utilized for MOS production is mannanase (EC 3.2.1.78). It is an endo-acting enzyme that produces β-(1,4)-linked MOS, or β-MOS, by random cleavage of (1→4)-β-D-mannosidic linkages in mannans through double displacement mechanism (McCarter and Stephen Withers 1994).

Due to its perceive importance for food and feed production, several researchers have isolated β-mannanases from *Bifidobacterium* (Kulcinskaja et al. 2013), *Bacillus* (Wongsiridetchai et al. 2018), *Streptomyces* (Ariandi et al. 2015), and *Aspergillus* (von Freiesleben et al. 2016) to name a few; and illustrated overexpression of β-mannanase in bacterial food grade expression systems (Nguyen et al. 2019; Song et al. 2017). Moreover, enzymes derived from recombinant sources are more competitive in terms of cost and quality than those derived from native sources (Bucke and Chaplin 1990). Specifically, Sak-ubol et al. (2016) demonstrated that β-mannanase from *Bacillus licheniformis* (*Bl*ManB) was successfully produced in *Lactiplantibacillus plantarum* WCSF1. While there are several enzyme sources used to produce MOS, to our knowledge, *Bl*ManB from crude extract of *L. plantarum* WCSF1 had not been intensively studied towards MOS production and substrate specificity, i.e., combination of enzyme–substrate that would facilitate optimum hydrolysis.

In this work, we assessed the stability and propensity of *Bl*ManB overexpressed in *Lactiplantibacillus plantarum* WCSF1 towards β-MOS production using several mannan sources. We utilized β-mannanase in crude cell extracts of *L. plantarum*, as a safe and cost-effective way for enzyme production. Reaction conditions for optimum β-mannanase production was determined, crude enzyme stability was assessed, and β-MOS formed from enzymatic hydrolysis were analyzed.

MATERIALS AND METHODS

Chemicals and Bacterial Strains

All chemicals utilized were pure and of analytical grade unless otherwise stated. Low viscosity locust bean gum (LBG) and guar gum (GG) were obtained from Sigma (Missouri, USA), while konjac glucomannan (KG) was obtained from Megazyme (Wicklow, Ireland). Spent coffee ground (SCG) obtained from residue of hot water extraction of ground coffee was dried at 55 °C until decrease in weight is <0.001 g. Copra meal was obtained from residual waste of oil extraction from coconut kernels, dried at 55 °C, and defatted by submerging in hexane overnight. Mannose (M1) standards was obtained from Sigma (Missouri, USA), while oligosaccharide standards, mannobiose (M2), mannotriose (M3), mannotetraose (M4), mannopentaose (M5), mannohexaose (M6), 6^1-α-D-galactopyranosyl-mannobiose (GM2), 6^1-α-D-galactopyranosyl-mannotriose (GM3), and 6^3,6^4-α-D-galactopyranosyl-mannopentaose (GGM5), were obtained from Megazyme (Wicklow, Ireland). The recombinant *Lactiplantibacillus plantarum* WCFS1 strain harboring the expression plasmid that contains β-mannanase from *Bacillus licheniformis* (*Bl*ManB) was from the culture collection of Food Biotechnology Laboratory, BOKU Vienna, Austria. The plasmid lacks a signal peptide and contains erythromycin resistance gene as selection marker.

Fermentation Conditions for β-Mannanase Production

Recombinant *L. plantarum* WCFS1 containing *Bl*ManB was cultivated as described by Sak-ubol et al. (2016) with some modifications and periodic pH adjustment between 6.0 and 6.5 using sterile 3.0 M sodium hydroxide. An aliquot of actively growing culture was transferred in 1 L MRS with 5 µg/mL erythromycin (MRSE), such that its starting optical density at 600 nm (OD_{600}) was 0.1, and then incubated at 37 °C. At OD_{600} of 0.3, IP-673 (25 ng/mL) was added in the culture to induce expression of β-mannanase (Eijsink et al. 1996). Cell turbidity was monitored at OD_{600}, and cells were harvested at several time points for monitoring. Cells were separated from the culture supernatant through successive centrifugation (4000 rpm, 4 °C, 15 min; Centrifuge 5804, Eppendorf, USA) and decantation. Harvested cells were washed twice using phosphate-buffered saline (PBS, pH 7.4), added with lysis buffer (0.020 M Tris-HCl, 0.150 M NaCl, pH 8.0), and ruptured by sonication (Vibra-Cell Sonicator, USA) while on ice at 70% power, 15% cycle (two

times for 2 min, with 2 min rest in between). Cell extracts were centrifuged (13000 rpm, 4 °C, 20 min; Thermo Scientific, USA) and decanted. Beta-mannanase activity in the crude extracts were determined using standard assay reaction.

β-Mannanase Activity Assay

Mannanase activity in the crude extract was analyzed by DNS assay as previously described (Sak-Ubol et al. 2016). A 100 µL of an appropriately diluted crude enzyme was reacted with 900 µL 0.5% LBG solution at 800 rpm for 5 min, and then heated at 99°C for another 5 min to stop the reaction. DNS method was then performed to account for the reducing sugar released in the reaction mixture using mannose as standard. Exactly 100 µL DNS reagent and 100 µL of the incubated mixture were mixed, heated at 99°C for 10 min, cooled in ice for 5 min, and diluted to 1 mL using distilled water. The absorbance of the total mixture was measured at 540 nm. Measurements were done in triplicates, and RSD is always ≤5%. A unit (U) of β-mannanase activity is equal to the amount of enzyme that produces 1 µmol of mannose per minute under the experimental conditions. Volumetric activity is expressed as enzyme units per volume of the fermentation broth (U/mL fer), and specific activity is expressed as enzyme units per mass of protein (U/mg protein).

Protein Concentration Determination

Protein content was determined using Bradford method (Bradford 1976) with bovine serum albumin as standard. An appropriately diluted crude enzyme of 15 µL volume was incubated in the dark with 600 mL Bradford reagent, and its absorbance was read at 595 nm. Protein concentration and specific activity (U/mg protein) were determined. Measurements were done in triplicates, and RSD is always ≤5%.

Gel Electrophoresis

The molecular mass of overexpressed *Bl*ManB was determined using the SDS-PAGE. Laemmli buffer was mixed with approximately 1 mg protein/mL of the crude enzyme and allowed to react at 90 °C for 5 min. Bio-safe Coomassie (Bio-Rad, California, USA) was used to visualize the protein bands by staining the gel for four hours. Protein mass was determined by parallel comparison with a molecular weight ladder (Bio-Rad Precision Plus™ Protein Standard).

Evaluation on the Influence of Temperature and Protease Inhibitor on Crude Enzyme Stability

The influence of protease inhibitors and glycerol on the stability of crude β-mannanase at different temperatures was studied based on the method of Songsiriritthigul et al. (2010) with some modifications. The protease inhibitors used was 2.0 mM dithiothreitol (DTT). Crude enzyme was incubated at various temperatures (-20 °C, 4 °C, 30 °C, 37 °C, and 50 °C) and at certain time intervals (t), enzyme activity was measured using standard assay. Residual activity is calculated as A_t/A_0 (where A_t is activity at t, and A_0 is the initial activity at $t = 0$). Inactivation constant k_{in} is determined by plotting $ln(A_t/A_0)$ against time t; and half-life is calculated using $t_{½} = ln\ 2/k_{in}$ (Polizzi et al. 2007).

Production of MOS from Mannan Substrates

A reaction mixture containing 0.5% mannan substrate (LBG, CM, GG, SCG, or KG) in citrate buffer (pH 6.0) and 2 mM DTT was reacted with 5 U/mL enzyme at 37 °C with mixing at 200 rpm for 24 h. Enzyme activity was then determined, and MOS products were analyzed using thin layer chromatography (TLC) and high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD). Measurements were done in duplicates.

Quantification of MOS using HPAEC-PAD

Quantitative analysis of the enzymatically synthesized MOS was performed using the procedure of Intaratrakul et al. (2017). MOS products were properly diluted and analyzed using HPAEC-PAD on a Dionex DX-500 system (California, USA) with CarboPac PA1 column (250 mm x 4 mm). Eluent used consisted of 150 mM sodium hydroxide. Isocratic elution was done for 40 min at 0.5 mL/min flow rate at 30 °C. Another solvent was used for column cleanup and ran in between samples which consisted of 0.5 M sodium acetate trihydrate dissolved in 150 mM sodium hydroxide. Carbohydrate components and their corresponding amounts were cross-referenced to known MOS standards.

RESULTS AND DISCUSSION

Overexpression of *Bl*ManB in *L. plantarum*

The β-mannanase from *Bacillus licheniformis* (*Bl*ManB) overexpressed in *Lactiplantibacillus plantarum* WCSF1 yielded 20 kU/L volumetric activity in the cell extract as demonstrated by Sak-Ubol et al. (2016) in their study. This food grade system was used for enzyme production, and its crude extract was used for MOS production. Production of *Bl*ManB was induced using IP-673, a 19-residue peptide pheromone (Eijsink et al. 1996). SDS-PAGE of the crude extract showed a band at 41 kDa, which agrees with that of *Bl*ManB in the aforementioned study.

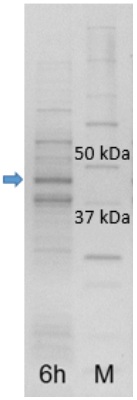

Figure 1: SDS-PAGE of the crude cell extract after 6 h fermentation containing *Bl*ManB overexpressed in *L. plantarum*; M indicates protein standard

The pH of the cultivation medium has influenced *Bl*ManB production. Fermentation time for maximal *Bl*ManB production without pH adjustments is at 6 h, at mid-log stage (Figure 2-A, entire growth curve not shown). In another setup where the pH of the fermentation medium was periodically adjusted between 6.0 and 6.5, which is within the reported optimum pH of *Bl*ManB (pH 6.0-7.0) (Songsiriritthigul et al. 2010), the optimum fermentation time for enzyme production at these conditions is at 6 h to 8 h, at mid-log to late-log stage (Figure 2-B). It also shows that in the fermentations with controlled pH, peak specific and peak volumetric activities were 1.1-fold and 2.9-fold higher, respectively, compared with uncontrolled pH fermentations, as supported by an increase in cell density (OD_{600}). This suggests that pH control improves the stability of *Bl*ManB and its production. Note that it is during the log phase of lactobacilli growth that lactic acid, acetic acid, ethanol, and other acidic by-products of glycolysis, phosphoketolase pathway, and sugar transport are produced (Hogg 2013; Koistinen et al. 2007; Sedewitz et al. 1984). A study by Intaratrakul et al. (2017) showed that β-mannanase from *B. circulans* (Man6.7) overexpressed in *L. plantarum* increased its intracellular enzyme activity by 1.7-fold when pH was controlled at its optimum pH during cultivation (Intaratrakul et

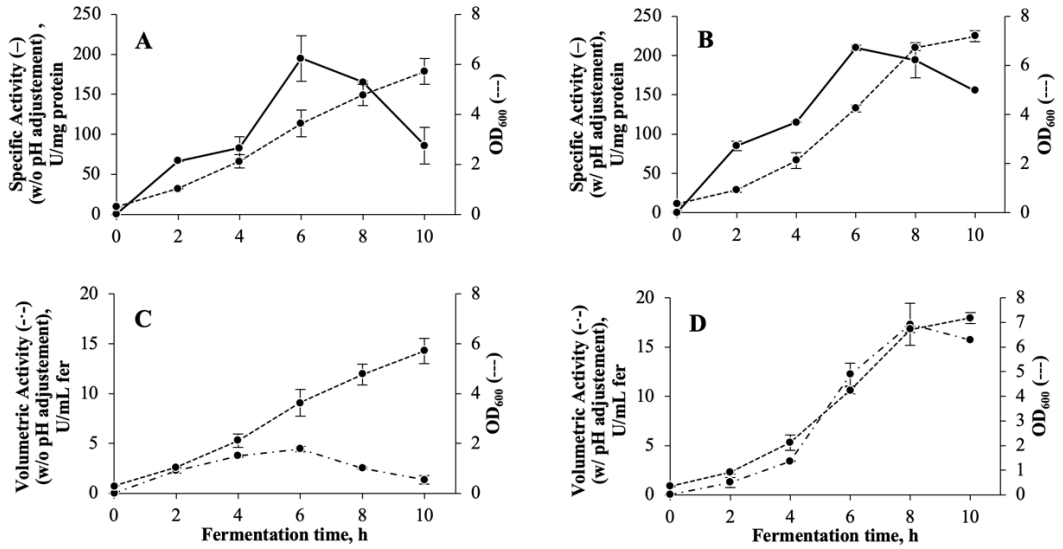

Figure 2: Effect of fermentation pH on bacterial cell density (OD_{600}, ---), specific activity (–), and volumetric activity (-·-) of *Bl*ManB overexpressed in *L. plantarum*. A, C fermentation without pH adjustments; B, D fermentation with pH adjustments

Table 1: Half-life (t½) of activity of *β*-mannanase in crude cell extract at different storage and incubation temperatures, and protease inhibitors

TEMPERATURE	HALF-LIFE		
	Crude enzyme	Crude enzyme +33% Glycerol	Crude enzyme +2 mM DTT
-20 °C	62 d	143 d	ND
4 °C	30 d	ND	ND
30 °C	35 h	ND	164 h
37 °C	5.2 h	ND	146 h
50 °C	0.64 h	ND	0.79 h

Legend: ND – not determined

al. 2017), inferring that pH control is essential in producing target enzymes in an expression system of lactic acid bacteria.

The highest specific activity (210 U/mg) and volumetric activity (17 U per mL of culture) were obtained with pH-controlled fermentation. The food grade system has demonstrated high production of the target protein. Volumetric activity is normally used to validate a fermentation process (Illanes 2011).

Crude Enzyme Stability

The stability of recombinant *Bl*ManB in crude cell extract was examined at storage temperatures (i.e., 4 °C and -20 °C) by monitoring residual *β*-mannanase activity. Table 1 shows that half-life of the recombinant *Bl*ManB in crude cell extract was higher when stored at -20 °C than at 4 °C, and a substantial improvement in enzyme stability was observed when glycerol was added showing that *Bl*ManB was stable at these storage conditions.

Initial investigations of crude enzyme stability at incubation temperatures (i.e., 30 °C, 37 °C, 50 °C) showed rapid loss in enzymatic activity. It might be due to the presence of proteases in the crude cell extract. Dithiothreitol (2 mM DTT) was added to enzyme storage buffer and the half-lives of *Bl*ManB in the crude cell extract at 30 °C and 37 °C significantly increased (Table 1). DTT notably protects the enzyme from activity loss through the oxidation of sulfhydryl groups. DTT is also known to disrupt protein and peptide bonds by reducing disulfide groups that prevent interaction and bond formation between cysteine residues of the proteins (Lundblad and MacDonald 2010), hence it might inhibit the activity of certain proteases in the crude extract. As the results the stability of *Bl*ManB in the crude extract was improved in the presence of DTT (2 mM).

The half-life of the crude enzyme, either with or without the presence of DTT, drastically decrease at 50 °C. A previous study of this *Bl*ManB reported that the purified enzyme has an approximate half-life of 72 h (Songsiriritthigul et al. 2010). It might be due to the sensitivity of DTT to high temperature, hence its reducing power was lost rapidly at 50°C.

MOS Production and Characterization from Mannan Hydrolysis

MOS conversions from commercial mannan sources (i.e., LBG, KG, GG) and agricultural by-products (i.e., CM, SCG) was analyzed to determine heterogeneity of MOS conversion products. Pure LBG was used as the standard mannan substrate. Each substrate (0.5% in total mixture) was added with citrate buffer pH 6.0, supplemented with 2 mM DTT to sustain enzyme stability, and subjected to enzymatic hydrolysis using 5 U/mL crude *Bl*ManB at 37 ºC. Although crude *Bl*ManB in the presence of DTT (2 mM) was most stable at 30 ºC, the crude enzyme was also stable 37 ºC (Table 1). Hence, enzymatic mannan hydrolysis was performed at 37 ºC as the conversion would be faster at higher temperature. Interestingly, stability studies showed that the crude enzyme sustained more than 90% of its activity (data not shown) under these conditions after 24 h of the conversion.

Quantitation of the hydrolyzed products from LBG, CM, GG, SCG and KG by HPAEC analysis confirmed the presence of several MOS with authentic standards (Figure 3) and their amounts are listed in Table 2. It shows that mannan hydrolysis using crude *Bl*ManB has produced M2, M3, and M4 (DP 2-4). Several studies have shown that MOS of DP 2-4 has effectively improved the growth of probiotics and limits the proliferation of pathogens (Bissaro et al. 2015, Sharma et al. 2017) probably by decreasing pH as short chain fatty acids from probiotics accumulated during MOS fermentation via a pathway mediated by glycolytic enzymes (Srivastava and Kapoor 2017) and/or by blocking pathogenic bacteria to attach to the gut mucosa (Spring et al. 2000). Crude *Bl*ManB demonstrated successful MOS production from mannan substrates by enzymatic hydrolysis based on the diversity of the oligosaccharide produced.

MOS products formed from LBG conversion are M2, M3, M4, M6, GGM5, and 4 other detectable sugars of DP 2-6 (Figure 3-A, Table 2). The spectrum of the MOS produced by *Bl*ManB

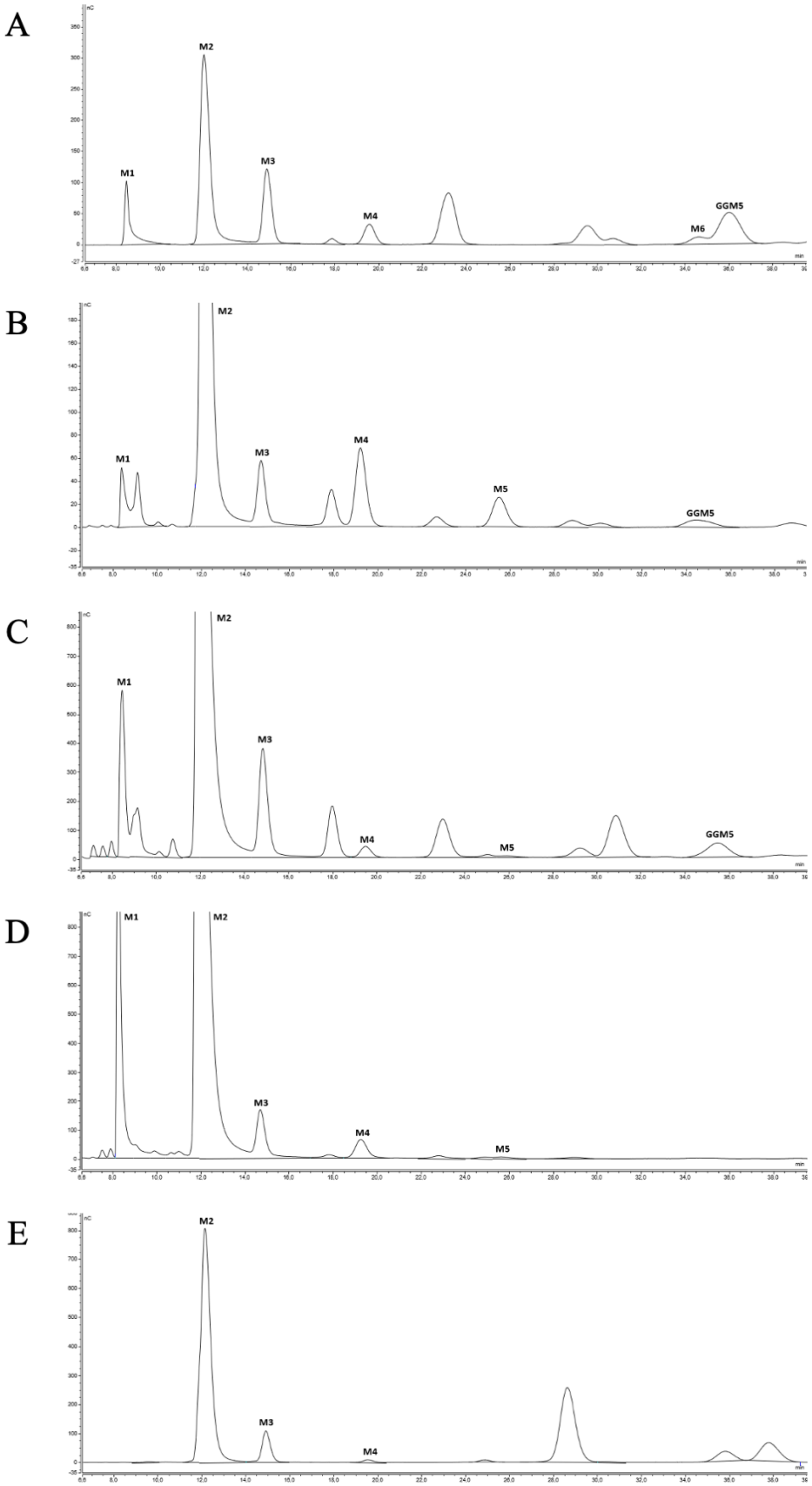

Figure 3: HPAEC-PAD chromatogram of MOS formed from enzymatic hydrolysis of (A) LBG, (B) CM, (C) GG, (D) SCG, and (E) KG, catalyzed by recombinant *Bl*ManB. Some peaks remain unidentified.

was similar to those isolated from unidentified *Bacillus* species (Kim et al. 2018), *B. subtilis* YH12 (Liu et al. 2015), and *Bacillus pumilis* (Zang et al. 2015), whose MOS products ranged from DP 2-7. Products from CM and GG hydrolysates are M2, M3, M4, M5, GGM5, and few unidentified sugars of DP 2-6. Hydrolysis product profiles of CM and GG (Figure 3-B and C, respectively) are similar with LBG (Figure 3-A) because all three are galactomannans. The diversity of MOS fragments

Table 1: Product analysis of oligosaccharides released from LBG, CM, GG, SCG, and KG by recombinant *Bl*ManB after 24 h conversion

SUBSTRATE	SUGAR CONCENTRATION, mg/g[a]							
	M1	M2	M3	M4	M5	M6	GGM5	Total MOS[b]
Locust bean gum	10.77 ± 0.11	131.08 ± 0.42	32.14 ± 0.89	18.28 ± 0.21	LD	10.89 ± 0.07	43.49 ± 0.86	235.88
Copra meal	3.53 ± 0.07	18.67 ± 0.46	16.39 ± 1.50	24.76 ± 1.61	11.27 ± 1.86	ND	50.54 ± 7.29	121.63
Guar gum	8.33 ± 0.69	15.67 ± 0.53	11.61 ± 0.80	1.41 ± 0.02	0.21 ± 0.00	ND	42.81 ± 0.39	71.71
Spent coffee ground	16.90 ± 0.08	14.86 ± 0.09	3.37 ± 0.06	2.56 ± 0.15	0.54 ± 0.00	ND	ND	21.33
Konjac glucomannan	0.75 ± 0.43	39.31 ± 12.25	42.84 ± 5.92	4.61 ± 0.43	ND	ND	ND	86.76

[a] ND = none detected, LD = low detection
[b] Total MOS did not include M1 and unidentified oligosaccharides
Mean ± standard deviation of three readings

Table 2: Reducing sugar released by recombinant *Bl*ManB in mannan substrates after 24 h conversion

SUBSTRATE	REDUCING SUGAR, µmol/g substrate
Locust bean gum	2617
Copra meal	335
Guar gum	498
Spent coffee ground	196
Konjac glucomannan	3738

obtained in CM hydrolysates were similar to the MOS produced using *β*-mannanases isolated from *Streptomyces* sp. BF3.1 (Ariandi et al. 2015), and *Bacillus subtilis* WY34 (Jiang et al. 2006); and that of GG hydrolysates were the same to those obtained using *β*-mannanase isolated from *Bifidobacterium adolescentis* (Kulcinskaja et al. 2013) and *Aspergillus nidulans* (von Freiesleben et al. 2016), and *β*-mannanase (RmMan5A) expressed in *Pichia pastoris* (Li et al. 2017).

Most of the identified fragments were linear MOS products, because it is likely that binding on the mannan backbone was done preferentially along the unsubstituted mannopyranosyl groups. Studies of structural properties of *β*-mannanases show that endo-acting glycoside hydrolases possess binding subsites, which interact noncovalently to the ligand for structural recognition (Suganuma et al. 1996). Lower concentrations of M4 and/or M5 fragments indicated that the binding site of the *Bl*ManB may contain ample subsites to bind with mannopyranosyl residues of at least four mannose monomer units and hydrolyze the ligand (mannan) in the middle (Bissaro et al. 2015). This is feasible for GH26 *β*-mannanases, like *Bl*ManB used in this study, because they contain prolonged loops in its binding subsites that allows the enzyme to bind with polymeric substrates of varying DP (Sharma et al. 2017). Meanwhile, the observed high concentrations of GGM5 suggested non-degradation of GGM5 due to presence of α-galactopyranosyl branches (Sharma et al. 2017). Subjecting the galactomannans to an aqueous system may have weaken intramolecular H-bonding and partially loosen the helical structure but are insufficient for efficient enzyme interaction.

The discrepancies in the MOS products of LBG, CM, and GG hydrolysates quantified in Figure 3 may be attributed to the degree of galactose substitution in these galactomannans. LBG have been reported to contain mannose to galactose ratio of 4:1 (Picout et al. 2002); CM used in this study has an approximate ratio of 7:1; and GG has 2:1 (Duffaud et al. 1997). Presence of more galactopyranosyl substituents provide more hydrogen interaction on the main chain that resist structural changes (Moreira and Filho 2008), resulting to the variations in MOS products from the different hydrolysates.

Even though SCG is a lowly substituted galactomannan, with approximately 10:1 mannose to galactose ratio (Navarini et al. 1999), MOS conversion products in its hydrolysate were notably low (Table 2) because it has other components in it. SCG contains up to 51% polysaccharide of the dry weight with 21.2% to 39.3% mannan (Jooste et al. 2013; Mussatto et al. 2011; Nunes et al. 2005); hence, SCG has lower mannan content than the other substrates in this study. Its other components such as protein and lignin (Mussatto et al. 2011) could have led to poor enzyme–substrate interaction. Beta-mannanases isolated from other bacteria sources (i.e., *Bacillus* sp. GA2(1) (Wongsiridetchai et al. 2018) and *Bacillus* sp. E-BMABS (Yoo et al. 2015)) have similar SCG hydrolysate product profile with this study.

KG hydrolysate has 86.76 mg/g of identified MOS mainly from M2 and M3, and small amounts of M4 (Table 2). Chromatogram of KG hydrolysate (Figure 3-E) showed presence of these MOS, as well as unidentified peaks of significant amounts. Identification and quantification of these oligosaccharides will further increase the MOS reported. In fact, results from Table 3 suggests that KG is susceptible to *Bl*ManB hydrolysis. Because Table 3 shows the reducing sugar released, it also accounts for the unidentified oligosaccharides. Generally, result shows that crude *Bl*ManB has high preference to soluble and linear mannans. *Bl*ManB has the highest propensity in KG degradation, suggesting ease of hydrolysis along the glucomannan backbone. Glycosyl residues along the glucomannan backbone provide noncovalent bonds to water and other polysaccharides, and the conformation of glucomannan chains is an extended two-fold screw axis, making the hydrogen interaction of hydroxyl groups between C-6 and C-2 atoms improbable (Northcote, 1972). These structural features suggest that these loose chains weaken packing and organization, and that the backbone is already exposed for enzyme hydrolysis. Another reason for preferential hydrolytic activity in KG is probably because of its linear conformation. *Bl*ManB from previous studies also showed high activity to linear mannan and glucomannan substrates (Songsiriritthigul et al. 2010; Yamabhai et al. 2011).

Between the two pure galactomannan sources (i.e., LBG and GG), *Bl*ManB has preference to LBG over GG as shown in Table 3. GG has more galactopyranosyl substituents than LBG (Duffaud et al. 1997; Picout et al. 2002). Low propensity to CM and SCG degradation is mainly due to the presence of other components, hereby decreasing mannan content and impeding effective enzyme–substrate interaction.

This study used crude recombinant *β*-mannanase from cell exudates for mannan hydrolysis instead of a purified enzyme. It has the potential for the development of more economical and less laborious (Geiger et al. 2016) processes for MOS production since the enzyme cost could be reduced and enzyme purification could be avoided. Crude enzyme obtained from lactic acid expression system is recognized as safe, hence crude *Bl*ManB could be efficiently used for the production of MOS, a valuable ingredient in food and feed.

CONCLUSION

We have determined the fermentation conditions for high production of *Bl*ManB overexpressed in *L. plantarum* WCSF1. Crude *Bl*ManB was found to be stable and active at conversion conditions. We have also demonstrated that enzymatic hydrolysis of various mannan substrates produces known linear MOS: 235.88 mg/g using locust bean gum, 121.63 mg/g using copra meal, 71.71 mg/g using guar gum, 21.33 mg/g using spent coffee ground and 86.76 mg/g using konjac glucomannan. The study has also shown that crude *Bl*ManB overexpressed in *L. plantarum* WCSF1 could be efficiently used to produce food-grade MOS.

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CONFLICT OF INTEREST

All authors have no conflict of interest to disclose.

CONTRIBUTIONS OF INDIVIDUAL AUTHORS

All authors contributed to in the conception of the study. THN, SLBA, and DH supervised and directed the study. LSGA performed the analyses and processed the data. LSGA and AAY analyzed the data. All authors provided their expertise to the final version of the manuscript.

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