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Isolation of β-mannanase from *Cocos nucifera* Linn haustorium and its application in the depolymerization of β-(1,4)-linked D-mannans

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Abstract

β-Mannanase was extracted from coconut (*Cocos nucifera* Linn) haustorium and purified through ammonium sulfate precipitation and sepharose 6B–lectin affinity chromatography. Coconut β-mannanase is an acidic protein with a pI of 3.75. The molecular mass of coconut β-mannanase (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) was found to be 44 kDa and was confirmed by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. The optimum temperature and pH for enzyme activity was 70°C and 5.2. The enzyme was used for the preparation of neutraceutical dietary supplement from galactomannans of guar gum and tender coconut kernel having a β-(1,4)-linked D-mannose backbone. Depolymerized guar gum has 92% of oligosaccharides with a degree of polymerization of 3 and 7. Tender coconut kernel has a degree of polymerization of 9–39 oligosaccharides along with disaccharides and trisaccharides. Hence this mannanase will be useful to depolymerize β-(1,4)-linked D-mannose polysaccharides from most plant sources to produce prebiotics in a cost-effective technique.

Keywords: *Galactomannan, β-mannanase, haustorium, Cocos nucifera Linn, prebiotic*

Introduction

β-Mannanase (endo1, 4-β-D-mannan mannohydrolase, EC 3.2.1.78) hydrolyzes the 1,4-β-D-mannopyranosyl linkage backbone of mannans, glucomannans, galactomannans and galactoglucomannans to produce mannanoligosaccharides. Mannanoligosaccharides are second-generation prebiotics (Gibson and Roberfroid 1995). Mannanoligosaccharides are non-digestable oligosaccharides possessing important physiological properties to behave as dietary fiber and prebiotics. Prebiotic oligosaccharides are also advantageous as they are selectively utilized by *Bifidobacteria*, decrease enteric pathogenic bacteria, regulate immunoreactions, and improve the integrity of intestinal mucosa, thereby increasing the human health level and the worth of a breeding animal (Wong and Saddler 1993; Gomes and Steiner 1998; Kobayashi et al. 1987; Dhawan and Kaur 2007; Moreira and Filho 2008).

Endo β-mannanases catalyze the cleavage of the internal β-1,4 mannopyranosyl linkages of the mannan polymer randomly to mannanoligosaccharides. Mannanases

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are also useful for the enzymatic analysis of polysaccharide structures (McCleary and Matheson 1986). β-Mannanase occurs widely in microorganisms possessing different characteristics in terms of molecular weight, isoelectric focusing (pI), optimum pH and temperature, so forth (Reese and Shibata 1965; Araujo and Ward 1990; Hossain et al. 1996; Gomes and Steiner 1998). The microbial mannanases from *Streptomyces* sp. (Takahashi et al. 1984), *Bacillus subtilis* (Mendoza et al. 1994; Zakaria et al. 1998), *Sclerotium* (*Athelia*) *rolfsii* (Sachslehner and Haltrich 1999), *Bacillus stearothermophilus* (Zhang et al. 2000), *Aspergillus awamori* (Kurakake and Komaki 2001) and *Trichoderma harzianum* (Ferreira and Filho 2004) have been purified and characterized. A few β-mannanases have been purified and characterized from higher organisms such as blue mussels (*Mytilus edulis*) and snails (*Helix pomatia*) (Yamaura and Matsumoto 1993; Yamaura et al. 1996; McCleary 1988). Mannans are an important component of the hemicellulose family, and can be classified into four subfamilies: linear mannan, glucomannan, galactomannan and galactoglucomannan. Galactomannans are the reserve polysaccharides in endosperm of plants in the *leguminoseae* family. Guar gum (*Cyamopsis tetragonolobus*) has a linear back bone of (1→4)-diequatorially linked β-D-mannose residues, some of which carry single-sugar side chains of α-L-galactose attached by (1→6) linkages. The molar ratio of mannose to galactose ratio is ~1.6. Coconut meal contains 26% mannans, 61% galactomannans and 13% cellulose. Even though there are studies on the endo manannase gene mapping of plants such as arabidopsis, rice, poplar and tomato, there is no report on the characterization of endo β-mannanase isolated from coconut haustorium and its use in the production of prebiotics. Previous studies in our laboratory have shown that coconut haustorium is a rich source of lipase, β-mannanase and α-galactosidase. In the present study we have purified and characterized a thermo-stable β-mannanase from coconut haustorium to make the prebiotic mannanoligosaccharides from guar gum and coconut kernal.

Materials and methods

Materials

p-Nitro-phenyl-β-D manopyranoside (PNPM), β-mannanase from *H. pomatia*, *p*-nitrophenyl-α-D-galactopyranoside (PNPG), α-galactosidase from green coffee bean, bovine serum albumin, locust bean gum, guar gum, sepharose 6B, and molecular weight markers were purchased from Sigma-Aldrich (St Louis, MO, USA). Ampholytes for Rotofor were from Bio-Rad (Trivandrum, Kerala, India). Lectin was isolated and purified from locally available Jack fruit seed. Coconut haustorium was procured from the laboratory plantation. All the other reagents used were of analytical grade.

Enzyme assay and protein determinations

The specific β-mannanase activity of haustorium was assayed with PNPM as a substrate. PNPM (1 mM) was incubated with an appropriate amount of enzyme sample in 0.1 M acetate buffer (pH 5) at 40°C for 10 min. The reaction was stopped by adding 0.5 ml of 1 M Na_2CO_3 and the absorbance of the released *p*-nitro-phenol was determined at 400 nm using a spectrophotometer. One unit of β-mannanase was defined as the amount of enzyme needed to produce 1 μmol/min *p*-nitro-phenol per minute (Kurakake and Komaki 2001). The α-galactosidase activity of coconut

haustorium was assayed using PNPG as the substrate. The assay mixture had a final concentration of 43 mM citrate, 5.3 mM *o*-nitro-phenyl α-D-galactoside and approximately 0.015–0.03 units α-galactosidase. One unit of α-galactosidase will hydrolyze 1 μmol/min *o*-nitro-phenyl α-D-galactoside to *o*-nitro-phenol and D-galactose per minute at pH 4 at 25°C (Borooah et al. 1961). The general glycosidase enzyme assay was carried out with 0.5% locust bean gum (galactomannan) in citrate phosphate buffer (50 mM, pH 5.2) with 100 μl mannanase, incubated at 50 ± 0.5°C for 30 min and the reducing sugar released was determined by the dinitrosalicylic acid method (Stalbrand et al. 1993) at 540 nm. A standard curve was made with different concentrations of D-mannose. One unit of the general mannanase activity is defined as the micromoles of mannose released per minute per milliliter of the enzyme under the experimental conditions.

The concentration of the protein was determined using the Bio-Rad protein assay according to the method of Bradford (1976), using bovine serum albumin as standard. The concentration of protein was monitored by observing the absorbance at 595 nm.

Extraction and purification of β-mannanase. All of the purification steps were performed at 4°C unless stated otherwise. The crude extracellular and intracellular mannanase was obtained from coconut haustorium by macerating the haustorium into a pulp with 0.05 M phosphate buffer (pH 7.0) and sand, and filtering the mashed pulp through cheesecloth. The filtrate was centrifuged at 2,655 × *g* for 10 min at 4°C. The crude supernatant was subjected to 20–90% ammonium sulfate saturation. The precipitated protein was collected by centrifugation (2,655 × *g*) and dissolved in 0.05 M phosphate (pH 7) buffer. Further purification was done by affinity chromatography using a glass column of 30 cm height and 2.1 cm inner diameter filled with sepharose 6B beads (diameter 45–165 μm) loaded with jack lectin. The column was loaded with 8.5 ml dialyzed enzyme sample (specific activity, 39.86 mg/ml) and equilibrated with phosphate saline buffer, pH 7.4. The void volume of the column was 3.72 ml. The bound mannanase was selectively eluted with D-galactose (5 to 100 mM) in the same buffer at a flow rate of 3 ml/min. The active fractions were combined and dialyzed against the same buffer and the dialysate was used in further experiments as the purified mannanase.

Isoelectric focusing

Isoelectric focusing was performed using a mini-Rotofor (Bio-Rad Laboratories). Purified coconut haustorium extracts at 4°C were dialyzed against phosphate buffer of pH 7 for 30 min, along with 2% ampholyte (pH 5–8; Bio-Rad) and is focused at 4°C for 5 h at 12 W. The fractions were harvested and the isoelectric point of the enzyme was determined.

Polyacrylamide gel electrophoresis

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed in a 10% w/v polyacrylamide gel by the method of Laemmli (1970). Protein bands were visualized by Coomassie brilliant blue R-250 staining. A molecular weight marker for SDS-PAGE ranging from 14.3 to 97.4 kDa (Genie, India) was used.

Matrix-assisted laser desorption ionization time-of-flight mass spectrometry of β-mannanase and galactomannans

Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS, Japan) (Shimadzu; Biotech Axima CFR plus, Japan) was performed to confirm the molecular mass of the mannanase enzyme. The matrix used is sinapinic acid in the linear mode. The molecular mass and degree of polymerization (DP) of the depolymerized galactomannan samples was done with 2,5-dihydroxy benzoic acid matrix in the linear mode.

Circular dichroism spectroscopy

The circular dichroism spectrum of -manannase was carried out in a spectropolarimeter (Jasco J-810, USA) scanned from 190 to 300 nm under a nitrogen atmosphere. The data were analyzed by K_2D_2 software (Perez-Iratxeta and Andrade Navarro, Bio Med Central Ltd, 2008).

Effect of pH and temperature on β-mannanase activity

The optimum pH of mannanase activity was determined using citrate phosphate buffer at pH 3–7 under standard assay conditions. The effect of temperature on the mannanase activity was determined by incubating the purified enzyme with the substrate at temperatures ranging from 30 to 80°C in 0.1 M citrate phosphate buffer of pH 5.2.

Kinetic parameters

The Michaelis–Menten constant (K_m) and the maximum reaction velocity (V_{max}) for mannanase was determined by varying the substrate concentration, keeping all the other parameters constant. The enzyme kinetics was determined using an enzyme kinetics software (Sigma plot, version 7.01, 2001, Richmond, California, USA), and K_m and V_{max} were calculated from the Hanes Woolf plot [S] versus [S] / [V]. From these values, the catalytic constant for the conversion of substrate to product (k_{cat}) and the catalytic efficiency ratio were calculated (Cornish-Bowden 2004).

Depolymerization of β-1,4-D-galactomannans

β-1,4-Galactomannan from guar gum and tender coconut kernel 1% (w/v) were suspended in 0.1 M citrate phosphate buffer of pH 5. 2. After the addition of purified mannanase, the solution was incubated at 30 ± 2°C for 24 h and 72 h. The solution was then passed first through 'hyflow' supercel and then through 0.45 μm and 0.22 μm Millipore ultrafiltration membranes. The supernatant was applied to MALDI TOF-MS analysis.

Results and discussion

Aqueous extract from coconut haustorium contains lipases, α-galactosidase as well as β-mannanase. The activity of lipase was 90.5 olive oil units/ml and that of

α-galactosidase using the specific substrate PNPG was 0.1374 units. The β-mannanase enzyme was very difficult to separate from the abundant acidic lipases and α-galactosidase by the classic purification methods such as ion exchange chromatography and gel permeation chromatography.

Purification of mannanase enzyme by sepharose–lectin affinity chromatography

The results of the ammonium sulfate precipitation and affinity chromatography purification of β-mannanase from the coconut haustorium are summarized in Table I. The β-mannanase was purified 693-fold with a specific activity of 0.534 units/mg protein. The enzyme activity was assayed using PNPM as the specific substrate. Affinity chromatography using lectin (specifically binds to D-galactose and D-mannose) was successful in separating β-mannanase from α-galactosidase and lipases. The lipase was excluded from the column along with the void volume. The β-mannanase was eluted with a D-galactose gradient of 5 to 10 mM and the α-galactosidase was eluted by a higher molarity (40 mM) of D-galactose.

Isoelectric focusing

Isoelectric focusing analysis gave an isoelectric point of 3.75 for the coconut haustorium β-mannanase, and hence is an acidic protein. It is reported that many bacterial as well as fungal -manannases have acidic pI values (Viikari et al. 1993). β-Mannanase is an acidic protein in *H. pomatia* (snail) and also *S.* (*Athelia*) *rolfsii*, which has a pI of 2.75 (Sachslehne and Haltrich 1999), whereas the enzyme from *M. edulis* is basic having a pI of 7.8.

Polyacrylamide gel electrophoresis

The purified-manannases were analyzed electrophoretically under denaturing conditions. Only one protein band was detected under the staining condition specified. The analysis revealed a single band on the SDS-PAGE that corresponds to a molecular weight of 44 kDa. This is similar to that of the earlier reports for β-mannanase obtained from *M. edulis* (39 kDa) (Xu et al. 2002), from *H. pomatia* (37–42 kDa) (McCleary 1988; Yamaura and Matsumoto 1993; Yamaura et al. 1996), and from *S.* (*Athelia*) *rolfsii* (46.5 kDa) (Sachslehner and Haltrich 1999). The results from SDS-PAGE are confirmed by MALDI TOF-MS, and are similar to the reported values of 40.0 kDa for *Bacillus* sp. (Ooi and Kikuchi 1995), 39.2 kDa for *M. edulis* (Xu et al. 2002), 39.0 for *B. subtilis* KU-1 (Zakaria et al. 1998) and 37 kDa for *H. pomatia* (McCleary 1988).

Table I. Summary of β-mannanase purification from coconut haustorium.

Purification step	Total activity[a] (units)	Protein[b] (mg ml)	Specific activity (units/mg)	Recovery (%)
Crude (total glycosidase activity)	33.228	0.1762	9.429	–
90% ammonium sulfate precipitation	61.452	39.86	0.0770	0.82
Affinity separation	0.1425	0.0178	0.5337	693.12

[a]Activity was measured in 0.1 M acetate buffer (pH 5) at 40°C using 1 mM PNPM as substrate. [b]The protein was measured using the method of Bradford (1976), using bovine serum albumin as the standard.

Circular dichroism spectroscopy

The far-UV region (190–240 cm^{-1}) in the circular dichroism spectrum normally shows the secondary structure of -manannase, and the chromophore is the peptide bond. The circular dichroism spectrum of -manannase was plotted using K_2D_2 software. The result shows that about 3.25% -helix and 49.36% -sheets were found in the -manannase. From the near-UV region (240–400 nm), the tertiary structure can be determined and the chromophores are phenylalanine, tyrosine and tryptophan. The enzyme was found to be a typical –2 type of globular protein.

Enzyme properties

The optimal pH for β-mannanase activity was 5.2 (Figure 1). Figure 1 shows the decrease in activity of mannanase at higher pH values of 6 and 7. β-Mannanase from haustorium can be used in slightly acidic conditions to depolymerize galactomannans. Several mannanases of microbial sources as well as from higher organisms such as mollusca reported in the literature had molecular masses similar to that of mannanase from haustorium; however, the catalytic properties are obviously different. The optimal pH for mannanase (5.2) is similar to the enzyme from *M. edulis* (Xu et al. 2002), fungal β-mannanase, with pH between 3 and 5.5 (Stalbrand et al. 1993; Christgau et al. 1994; Ademark et al. 1998; Viikari et al. 1993) and from *B. subtilis* WY34 pH 6 (Jiang et al. 2006). The optimal temperature for β-mannanase activity in the present investigation was 70°C (Figure 2). The coconut mannanase is a thermophilic enzyme and shows a higher activity at 65–75°C compared with 65–70°C from *B. subtilis* WY34 (Jiang et al. 2006).

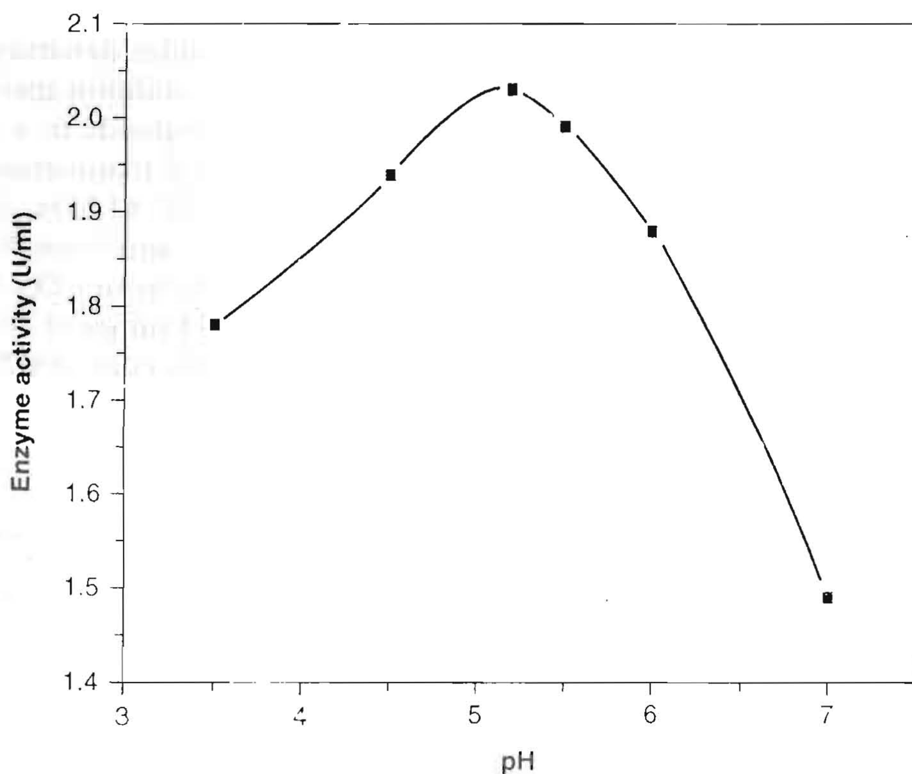

Figure 1. Effect of pH on β-mannanase activity.

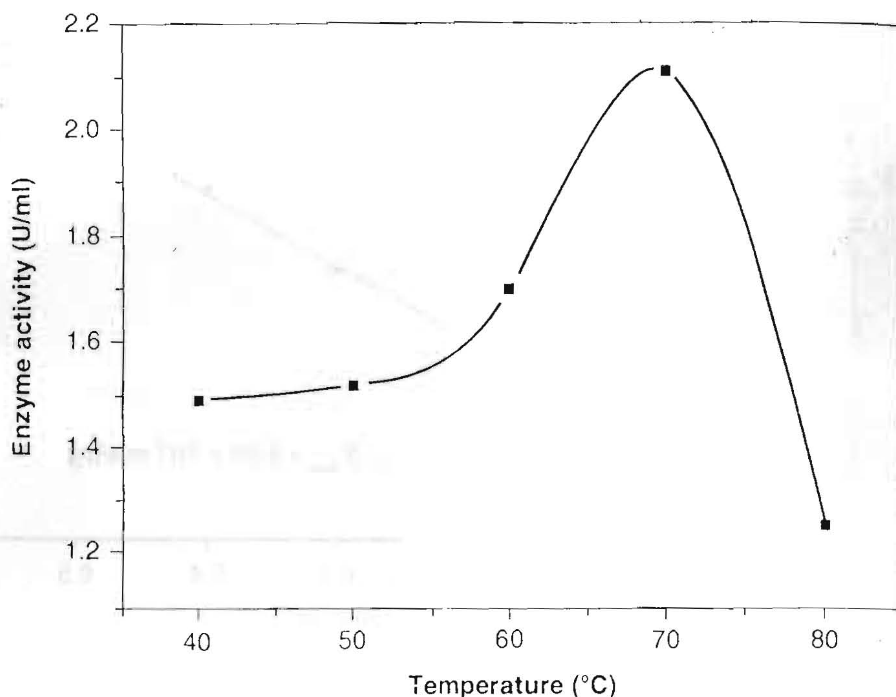

Figure 2. Effect of temperature on β-mannanase activity.

Kinetic parameters

The K_m, V_{max} and K_{cat} values of β-mannanase were found to be 2.563×10^{-3} mol/ml, 7.908×10^{-6} mol/sec/g, and 1.797×10^{-9} mol/sec/mol enzyme with locust bean gum as the substrate. For the specific substrate PNPM, the K_m, V_{max} and K_{cat} values of enzyme were 9.114×10^{-8} mol/ml, 6.98×10^{-2} mol/sec/g, and 1.58×10^{-6} mol/sec/mol (Figure 3). These data are compared with the mannanase from *M. edulis*, which has a reported K_m value of 3.95 mg/ml as locust bean gum substrate (Xu et al. 2002), and mannanase from *Aspergillus niger*, which has a K_m value of 0.30 mM using PNPM as substrate (Ademark et al. 1999). When the substrate concentration was plotted against the ratio of the substrate concentration to the rate of the reaction (Hanes–Woolf plot), the primary plot was obtained and K_m and V_{max} values were obtained automatically from the secondary plot values using Sigma plot software (version 7.01, 2001). The Hanes–Woolf equation can be represented as:

$$\frac{[S]}{V} = \frac{1}{V_{max}}[S] + \frac{K_m}{V_{max}}$$

where [S] is the substrate concentration, V_{max} is the maximal velocity and K_m is the binding affinity of substrate at $(1/2)V_{max}$ (Michaelis constant). The lower the K_m value, the higher the affinity between the enzyme and the substrate. The Hanes–Woolf plot avoids both the misleading impression of the experimental error and the uneven distribution of the points by a Lineweaver–Burk plot and the angular distortion of the errors of the Eadie–Hofstee plot (Cornish-Bowden 2004).

Depolymerization of β-1,4-D-galactomannan

Both guar gum and tender coconut kernel were depolymerized by the purified β-mannanase at pH 5.2 to produce di, tri and oligogalactomannans. A reaction mixture

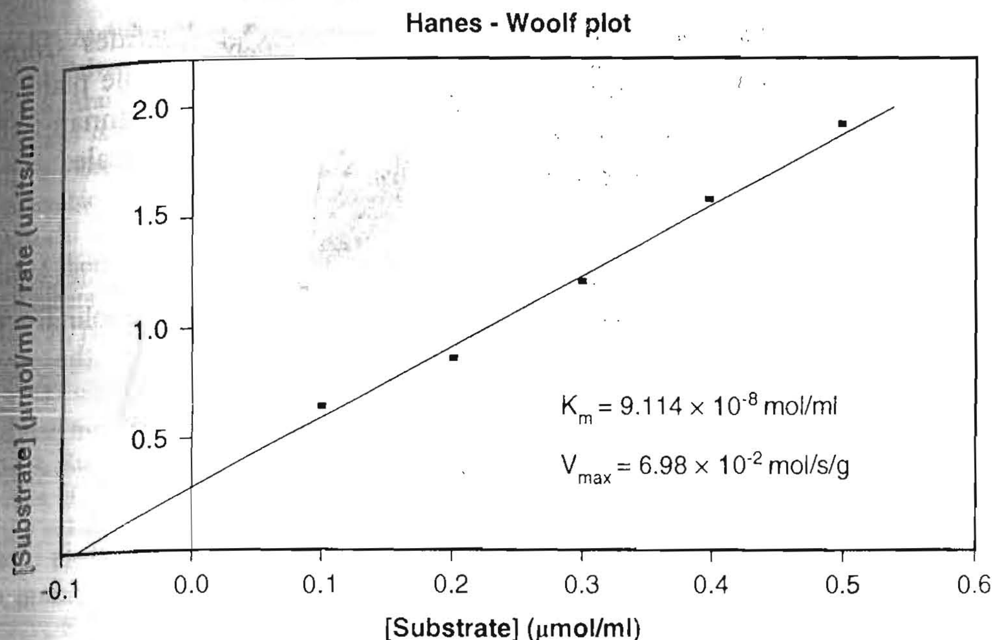

Figure 3. Kinetics of β-mannanase from coconut haustorium using PNPM as substrate.

containing 1% (w/v) guar gum and showing an initial high viscosity (1,075 centipoises) was gradually liquefied during enzymatic hydrolysis at 30 ± 2°C; after about 10 h, the viscosity reduces completely (0 centipoises). Similarly, 2 g tender coconut gel as such in phosphate buffer was hydrolyzed at 30 ± 2°C for 72 h. The distribution of oligosaccharides was analyzed by MALDI TOF-MS. Oligosaccharide of DP 21-37 and DP 32 is predominating in the original guar gum, while the depolymerized guar gum gave oligomannans of DP 3 and DP 7. The unhydrolyzed tender coconut gel contained oligoglucans of DP 4–97, whereas the hydrolyzed ones gave 50% of mannan disaccharides and trisaccharides along with oligomers of DP 9–39. The fungal mannanase as well as mannanase from *M. edulis* gives mannobiose and mannotriose on hydrolysis (Xu et al. 2002). This oligomannans can be used as prebiotic without any costly downstream processing. Prebiotics are being made available in almost every product imaginable and, as they become increasingly popular, more and more uses of them are going to be adopted by the industry. So far, such uses include kefir, yogurt and other dairy drinks, sports products, functional waters, nutrition bars, weight-loss products, soymilk, infant foods and formulas, green foods, prebiotic supplements, mineral supplements, medical foods, as well as pet foods and animal feeds.

Conclusion

In the carbohydrate family, the indigestible oligosaccharides are of particular interest and may, in the next decade, be one of the most fascinating functional food ingredients. Prebiotics are principally oligosaccharides, as they stimulate the growth of *Bifidobacteria* in the intestine, and they may have anticarcinogenic, antimicrobial, hypolipidemic and glucose-modulating activities. Coconut haustorium was found to be a good source of β-mannanase. In this work, β-mannanase was isolated and purified from coconut haustorium, which is an acidic globular protein of molecular mass 44 kDa with an optimum pH of 5.2, temperature of 70°C and a K_m value of 9.114×10^{-8} mol/ml enzyme using PNPM as substrate. This endo enzyme is capable of randomly

depolymerizing β-(1,4)-linked D-mannose containing polysaccharides from plant sources. This is the first report of a cheaper, eco-friendly and versatile plant enzyme that can be used to produce mannanoligosaccharides from galactomannan-rich plant materials, such as guar gum and tender coconut kernel, on a large scale.

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Declaration of interest

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References

Ademark P, Varga A, Medve J, Harjunpaa V, Drakenberg T, Tjerneld F, Stalbrand H. 1998. Soft-wood hemicellulose-degrading enzymes from *Aspergillus niger*: Purification and properties of a β-mannanase. J Biotechnol 63:199–210.

Ademark P, Lundqvist J, Hagglund P, Tenkanen M, Torto N, Tjerneld F, Stalbrand H. 1999. Hydrolytic properties of a β-mannosidase purified from *Aspergillus niger*. J Biotechnol 75:281–289.

Araujo A, Ward OP. 1990. Hemicellulose of *Bacillus species*: Preliminary comparative studies on production and properties of mannanases and galactanases. J Appl Bacteriol 68:253–261.

Borooah J, Leaback DH, Walker PG. 1961. Enzymatic assay of α Galactosidase. Biochem J 78:106–110.

Bradford MM. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Anal Biochem 72:248–254.

Christgau S, Kauppinen S, Vind, J, Kofod LV, Dalboge H. 1994. Expression cloning, purification and characterization of a β-1,4-mannanase from *Aspergillus aculeatus*. Biochem Mol Biol Int 33:917–925.

Cornish-Bowden A. 2004. Fundamentals of enzyme kinetics. 3rd ed. London: Portland Press.

Dhawan S, Kaur J. 2007. Microbial mannanase: An over view of production and application. Crit Rev Biotechnol 27(4):197–216.

Ferreira HM, Filho EXF. 2004. Purification and characterization of a β-mannanase from *Trichoderma harzianum* strain T4. Carbohydr Polym 57:23–29.

Gibson GR, Roberfroid MB. 1995. Dietary modulation of the human colonic microflora: Introducing the concepts of prebiotics. J Nutr 125:1401–1412.

Gomes J, Steiner W. 1998. Production of a high activity of an extremely thermostable β-mannanase by the thermophilic eubacterium *Rhodothermus marinus*, grown on locust bean gum. Biotechnol Lett 20:729–733.

Hossain MZ, Abe J, Hizukuri S. 1996. Multiple forms of β-mannanase from Bacillus sp.KK01. Enzyme Microb Technol 18:95–98.

Jiang Z, Wei Y, Li D, Li L, Chai P, Kusakabe. 2006. High-level production, purification and characterization of a thermostable β-mannanase from the newly isolated *Bacillus subtilis* WY34. Carbohydr Polym 66:88–96.

Kobayashi Y, Echigen R, Mada M, Mutai M. 1987. Effects of hydrolysates of konjac mannan and soyabean oligosaccharides on intestinal flora in man and rats. In: Mitsuoka T, editor. Intestinal flora and food factors. Tokyo: Gakkai Shuppan Centre. pp 79–97.

Kurakake M, Komaki T. 2001. Production of β-mannanase and β-mannosidase from *Aspergillus awamori* K4 and their properties. Curr Microbiol 42:377–380.

Laemmli UK. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 277:680–685.

McCleary BV. 1988. β-D-mannanase. Methods Enzymol 160:596–614.

McCleary BV, Matheson NK. 1986. Enzymatic analysis of polysaccharide structure. In: Tipson RS, Horton D, editors. Advances in carbohydrate chemistry and biochemistry. Academic Press; London. pp 147–276.
Mendoza NS, Arai M, Kawaguchi T, Yoshida T, Joson LM. 1994. Purification and properties of mannanase from *Bacillus subtilis*. World J Microbiol Biotechnol 10:551–555.
Moreira LR, Filho EX. 2008. An overview of mannan structure and mannan-degrading enzyme systems. Appl Microbiol Biotechnol 79:165–178.
Ooi T, kikuchi D. 1995. Purification and some properties of β-mannanase from Bacillus sp. World J Microbiol Biotechnol 11:310–314.
Reese T, Shibata Y. 1965. β-mannanase of fungi. Can J Microbiol 11:167–183.
Sachslehner A, Haltrich D. 1999. Purification and some properties of a thermostable acidic endo-β-1,4-D-mannanase from *Sclerotium (Athelia) rolfsii*. FEMS Microbiol Lett 177:47–55.
Stalbrand H, Siika-Aho M, Tenkanen M, Viikari L. 1993. Purification and characterization of two mannanase from *Trichoderma ressei*. J Biotechnol 29:229–242.
Takahashi R, Kusakabe I, Kobayashi H, Murakami K, Maekawa A, Suzuki T, . 1984. Purification and some properties of mannanase from *Streptomyces species*. Agric Biol Chem 48:2189–2195.
Viikari L, Tenkanen M, Buchert J, Ratto M, Bailey M, Siika-aho M, Linko M. 1993. Hemicellulases for industrial applications. In: Saddler JN, editor. Bioconversion of forest and agricultural plant residues. Wallingford: CAB International. pp 131–182.
Wong KKY, Saddler JN. 1993. Applications of hemicellulases in the food, feed, and pulp and paper industries. In: Coughlan MP, Hazlewood GP, editors. Hemicellulose and hemicellulases. London: Portland. pp 127–143.
Xu B, Hagglund P, Stalbrand H, Janson J. 2002. Endo-β-1,4-mannanase from blue mussel, *Mytilus edulis*: Purification, characterization, and mode of action J Biotechnol 92:267–277..
Yamaura I, Matsumoto T. 1993. Purification and some properties of endo-1,4-β-D-mannanase from a mud snail, *Pomacea insularus* (de Ordigny). Biosci Biotechnol Biochem 57:1316-1319.
Yamaura I, Nozaki Y, Matsumoto T, Kato T. 1996. Purification and some properties of endo-1, 4-β-D-mannanase from a marine mollusk, *Littorina brevicula*. Biosci Biotechnol Biochem 60:674–676.
Zakaria MM, Yamamoto S, Yagi T. 1998. Purification and characterization of an endo-1,4-β-mannanase from *Bacillus subtilis* KU-1. FEMS Microbiol Lett 158:25–31.
Zhang J, He ZM, Hu K. 2000. Purification and characterization of β-mannanase from Bacillus *licheniformis* for industrial use. Biol Lett 22.1375–1378.