Improved mannanase production from *Penicillium humicola* and application for hydrolysis property

Mona A. El-refai[a], Om Kalthoum H. Khattab[b], Siham A. Ismail[a], Amal M. Hashem[a], Amany A. Abo-Elnasr[b], Shaimaa A. Nour[a]

[a]Department of Chemistry of Natural and Microbial Products, Division of Pharmaceutical and Drug Industries, National Research Centre, Cairo [b]Department of Plant and Microbiology, Faculty of Science, Helwan University, Helwan, Egypt

Correspondence to Siham A. Ismail, Professor Assistant, Department of Chemistry of Natural and Microbial Products, Division of Pharmaceutical and Drug, Industries, National Research Centre, El-Behowth St, PO Box 12311, Dokki, Cairo, Egypt
Tel: +20 122 357 1676; fax: +20 233 370 931; e-mail: sihamabc188@gmail.com

Received 07 September 2014
Accepted 14 October 2014

Egyptian Pharmaceutical Journal
2014, 13:160–167

Objectives
The aim of this research is to produce β-mannanases from a new microbial source using wastes in their nutrition medium. The enzyme produced can be used for the production of galactomanno-oligosaccharides, which are very useful in the health and medical fields.

Materials and methods
Seven fungal strains and five bacterial strains were tested for the production of β-mannanases. Enzyme activity, protein content, and biomass production were determined in all the cultures produced using standard methods. Optimization studies to maximize enzyme production from the most potent microorganisms, including culture conditions and medium compositions, were also carried out. Preliminary studies for the production of galactomanno-oligosaccharides from locust bean gum using partially purified enzymes were also carried out and followed by thin-layer chromatography techniques and Somogyi methods.

Results and conclusion
The highest mannanase activities were produced by *Penicillium humicola* (8.8 U/ml) and *Penicillium* spp. v (7.75 U/ml) in shaking cultures after 10 days using gum locust bean as a carbon source. Among 13 carbon sources examined, coffee residue and ceratonia seeds were the best carbon sources (10.3 and 8.9 U/ml, respectively) for *P. humicola*, whereas the best nitrogen source was a mixture of peptone, urea, and ammonium sulfate for the same microorganism. The optimum temperature and pH for enzyme reaction was 55 and 5.5°C, respectively. The enzyme was thermostable and retained 80% of its activity after 1 h at 50°C. The highest reducing sugar of 8900 μg/ml was obtained from locust bean gum hydrolytes after 28 h.

Keywords:
bacterial strains, fungal strains, galactomanno-oligosaccharides, industrial wastes, β-mannanases

Egypt Pharm J 13:160–167

1687-4315

Introduction

Lignocellulose is a major component of plant cell walls and is mainly composed of lignin, cellulose, and hemicellulose. Mannans and heteromannans are a part of the hemicellulose fraction in plant cell walls. The structure of hemicellulose is a key component of many types of sugar such as xylans, mannans, heteromannans, galactans, and arabinans [1]. Mannans and heteromannans are distributed widely in hardwoods and softwoods, seeds of leguminous plants, and bean [2]. They can be divided into four types: unsubstituted (1, 4)-linked β-d mannans, galactomannans, glucomannans, and galactoglucomannans [3]. Mannans, whose main component is d-mannose, are important for several industries, including food, feed, and feed stocks. They can be broken down into simple sugars or oligosaccharides by a synergistic action of endomannanases (EC number 3.2.1.78, mannan endo-1, 4-β-mannosidase) and exo-acting β-mannosidase (EC number 3.2.1.25). The production of β-mannanase by microorganisms is more promising because of its low cost, high production rate, and easily controllable condition [4].

Various microorganisms can produce mannanases such as *Aspergillus awamori* [5], *Aspergillus oryzae* [6], *Penicillium oxalicum* [4], *Trichoderma harzianum* [7], *Rhodothermus marinus* [8], *Bacillus subtilis* [9], and *Streptomyces ipomoea* [10].

Many mannan-based carbon sources including locust bean gum, guar gum, and copra meal have been used to cultivate filamentous fungi [11–13]. Moreover, various inducers were used to improve mannanase production. Chaiongkarn *et al.* [14] isolated microorganisms from soil using copra meal as a carbon source for their growth.

Therefore, this study aimed to search for cheaper carbon and nitrogen sources for the production of mannanase and oligosaccharides.

Materials and methods

Materials

Locust bean gum was obtained from Sigma Chemicals (3050 Spruce St. St. Louis, MO 63103, USA) (a galactomannan from the seeds of *Ceratonia siliqua*, which consists of a straight-chain polymer of mannose with galactose branches on every fourth mannose). Coffee residue was bought from the local market (Giza, Egypt). All other chemicals used were of analytical grade.

Microorganisms

In this study, seven fungal strains, namely, *Aspergillus niger*, *Aspergillus repens*, *Penicillium corylophilum*, *Penicillium humicola*, *Penicillium* spp., *Penicillium* spp. v, and *Trichoderma viride*, and five bacterial strains, namely, *Bacillus circulans*, *Bacillus licheniformis*, *Bacillus megaterium*, *B. subtilis*, and *Geobacillus stearothermophilus* were obtained from the culture collection of the National Research Center, Dokki, Cairo, Egypt. All the fungal strains were maintained on potato dextrose agar and incubated at 30°C for 7 days before storage at 4°C with monthly subculturing. All the bacterial strains were maintained on nutrient agar for 48 h at 37°C and then stored at 4°C with monthly subculturing.

Culture media

For fungi

The basal medium for inoculum and enzyme production included: peptone (2 g/l); ammonium sulfate (1.5 g/l); urea (0.3 g/l); $MgSO_4 \cdot 0.7H_2O$ (0.5 g/l); K_2HPO_4 (10 g/l); and locust bean gum (10 g/l) [6]. The pH of the medium was adjusted at 5.3 before autoclaving. Each 250 ml Erlenmeyer flask contained 50 ml of the medium and was autoclaved for 15 min at 121°C. An inoculum culture was obtained by culturing the fungal strains in the above medium at 30°C for 48 h with shaking at 150 rpm. The culture flasks were inoculated by 10% of the inoculum and incubated at 30°C in a shaking incubator at 150 rpm for different periods. Thereafter, the fermented medium was centrifuged to separate the mycelium from the culture filtrate and the filtrate was used as the crude enzyme solution [6].

For bacteria

The basal medium for inoculum and enzyme production included beef extract (0.5 w/v %); peptone (1.0 w/v %); yeast extract (0.5 w/v %); $NaNO_3$ (0.5 w/v %); K_2HPO_4 (0.5 w/v %); $MgSO_4 \cdot 0.7H_2O$ (0.02 w/v %); and locust bean gum (2 w/v %). The pH of the medium was adjusted to 7.0 before autoclaving. The crude enzyme solution was obtained as mentioned above [15].

Estimation of biomass

The separated cells were washed with water and dried at 60°C until a constant weight was obtained.

Enzyme assay

An assay was performed by incubating 0.5 ml of appropriately diluted culture filtrate with 1 ml of 1% (w/v) locust bean gum (in 50 mmol/l sodium citrate buffer at pH 5.0) for 10 min at 50°C [6]. The reducing sugars produced were determined using the Nelson–Somogyi technique [16]. One unit of enzyme activity was defined as the amount of enzyme that released 1 μmol of mannose/ml/min.

Protein determination

This was carried out according to the method of Lowry *et al*. [17].

Determination of total sugars

Carbohydrate contents were determined using the phenol sulfuric acid method of Dubios *et al*. [18].

Optimization studies

The enzyme activity was tested at different incubation periods (ranged from 4-12 days) with different culture techniques (static and shaking) (at 150 rpm) culture techniques were tested. The effects of addition of various carbohydrates, and organic and inorganic nitrogen were evaluated in relation to enzyme yield. The experiments were conducted in triplicate, and the results are the average of these three independent trials.

Hydrolysis study

The ability of the produced enzyme to hydrolyze galactomannan polymer was tested in reaction mixture consisted of 2 ml enzyme solution (35 U/ml) and 2 ml 2% (w/v) locust bean gum (in 50 mmol/l citrate phosphate buffer pH 5.5) and carried out at 40°C for 28 h. The reactions were stopped by boiling for 10 min after 1, 2, 3, 4, 5, 6, 7, 8, 17, 20, 24, 26, and 28 h of incubation. The amounts of reducing sugar of hydrolysis products were estimated using Somogyi methods. Hence, the hydrolysis product patterns were determined by thin-layer chromatography using propanol : water (8.5 : 1.5 v/v) as the mobile phase. Detection was performed by spraying with phenol-sulfuric acid reagent as follows:

The chromate plates were sprayed with a solution containing 3 g phenol and 5 ml of concentrated sulfuric acid in 95 ml of ethyl alcohol and then heated for 10–15 min at 110°C [19].

Results and discussion

The results in Table 1 show that among the fungal cultures investigated, *P. humicola* and *Penicillium* spp. v were the most potent microorganisms and produced the highest mannanase activity (8.8 and 7.75 U/ml, respectively) after 10 days, so they selected for further studies. Table 1 also indicated that the mannanase activity of other cultures was ranged from 0.06 to 0.79 U/ml. No definite correlation was found between mycelial dry weight and mannanase activity of the culture filtrate. The protein content of the culture filtrate was not a criterion in the productivity of mannanase activity. All the bacterial tested were not promising (data not shown).

Table 1 Survey of some fungal strains for the production of extracellular β-mannanase in shaking cultures

Fungal strain	Incubation period (days)	Dry weight (g/flask)	Protein (mg/ml)	E activity[a] (U/ml)
Aspergillus niger	4	0.37	0.75	0.64
	7	0.35	1.46	0.72
	10	0.33	1.31	0.41
	12	0.32	1.41	0.30
Aspergillus repens	4	0.36	0.24	0.14
	7	0.36	0.45	0.06
	10	0.32	0.23	0.00
	12	ND	ND	ND
Penicillium corylophilum	4	0.37	0.35	0.68
	7	0.30	1.52	0.79
	10	0.23	1.44	0.37
	12	0.23	1.53	0.40
Penicillium humicola	4	0.32	1.28	4.30
	7	0.26	1.33	7.20
	10	0.27	1.44	8.80
	12	0.23	1.72	8.26
Penicillium spp.	4	0.34	1.42	0.59
	7	0.25	1.51	0.62
	10	0.21	1.46	0.79
	12	ND	ND	ND
Penicillium spp. v	4	0.35	0.81	5.21
	7	0.38	1.30	6.30
	10	0.28	1.26	7.75
	12	0.25	1.47	7.04
Trichoderma viride	4	0.40	1.33	0.21
	7	0.30	1.39	0.21
	10	0.26	1.55	0.11
	12	ND	ND	ND

[a]β-mannanase activity (U/ml).

Time course of mannanase production

The time course of mannanase production by the static culture technique (in an incubator) or shaking culture (on a rotary shaker at 150 rpm) was studied for *P. humicola* and *Penicillium* spp. v. The results presented in Table 2 show that mannanase was maximally produced after 10 days of fermentation by the shaking technique (8.8 U/ml for *P. humicola* and 7.75 U/ml for *Penicillium* spp. v). Blibech *et al*. [20] found that the maximum mannanase yield (8 U/ml) by *Penicillium accitanis* was observed on the sixth day of incubation. Generally, the highest level of β-mannanase produced from fungal strains was obtained after 3–11 days as reported by other authors [21,22].

Effect of different carbon sources

Agroindustrial byproducts are available in large amounts and they have been used for the production of several enzymes [23,24]. Several types of agroindustrial byproducts were evaluated as substrates for mannanase production in comparison with locust bean gum (control) (Table 3). *P. humicola* and *Penicillium* spp. v were grown, with significant differences in the raw materials. In case of *P. humicola* sesame waste was enhanced the mycelial

Table 2 Biosynthesis of β-mannanase using shaking and static culture techniques at different incubation periods

Fungal strain	Time course (day)	Shaking culture			Static culture		
		Dry weight (g/flask)	Protein (mg/ml)	E activity[a] (U/ml)	Dry weight (g/flask)	Protein (mg/ml)	E activity[a] (U/ml)
Penicillium humicola	5	0.35	1.28	6.50	0.32	1.66	1.37
	6	0.33	1.33	7.00	0.37	1.69	1.33
	7	0.32	1.33	6.97	0.38	1.60	2.73
	8	0.30	1.61	8.31	0.35	1.62	1.98
	9	0.30	1.27	8.63	0.36	1.60	1.19
	10	0.27	1.40	8.81	0.40	1.22	1.62
	11	0.25	1.35	8.33	0.41	1.32	2.56
	12	0.24	1.72	8.12	0.38	1.37	2.44
Penicillium spp. v	5	0.28	1.60	6.10	0.32	2.14	2.05
	6	0.29	1.97	6.13	0.34	2.05	2.90
	7	0.32	1.99	6.30	0.32	3.12	4.25
	8	0.31	1.98	7.13	0.30	2.11	3.38
	9	0.32	2.06	7.10	0.32	1.84	3.10
	10	0.30	2.32	7.75	0.31	2.02	2.50
	11	0.30	2.34	7.74	0.28	1.91	2.19
	12	0.28	2.35	7.04	0.26	2.30	1.68

[a]β-mannanase activity (U/ml).

Table 3 Effect of different carbon sources on the production of *Penicillium humicola* and *Penicillium* spp. v extracellular β-mannanase

Carbon source	*Penicillium humicola*			*Penicillium* spp. v		
	Dry eight (g/flask)	Protein (mg/ml)	E activity[a] (U/ml)	Dry weight (g/flask)	Protein (mg/ml)	E activity[a] (U/ml)
Gum locust bean[b]	0.26	1.40	8.80	0.28	2.00	7.75
Black seeds waste	1.37	7.30	0.20	1.40	8.34	0.43
Ceratonia seeds	0.28	2.83	8.87	0.70	6.79	8.39
Coffee residue	1.86	7.38	10.30	3.00	5.00	5.00
Corn cobs	1.30	3.39	0.18	1.23	2.82	0.23
DOM	2.13	3.18	1.14	1.89	3.20	0.60
Erucasativa waste	1.77	4.49	0.23	0.36	4.57	0.15
Defatted palm kernel wastes	0.49	2.30	2.01	3.53	3.05	3.21
Potato peels	0.65	3.74	0.45	1.11	4.41	0.15
Rice bran	0.99	3.39	1.06	1.26	4.02	1.39
Sesame waste	2.34	6.34	0.51	1.36	11.48	0.00
Wheat bran	0.79	2.77	1.38	0.92	3.22	0.29
Wheat embryo waste	0.75	3.37	0.18	1.07	5.85	0.28
Wheat husk	1.64	3.20	1.49	1.43	3.57	0.40

[a]β-mannanase activity (U/ml); [b]Control.

growth (2.34 g) but the enzyme activity was very low (0.51U/ml), whereas rice bran, hyphaene (dom), wheat bran, wheat husk, and defatted palm kernel wastes reduced the activity to about 12–23% of the control. It seems that defatted palm kernel waste improved the growth of *Penicillium* spp. v (3.53 g/flask), but the enzyme activity was low as 3.21 U/ml (Table 3). From all the agricultural waste used, ceratonia seeds promoted the biosynthesis of β-mannanase for *Penicillium* spp. v and yielded 8.39 U/ml. It is clear that both coffee residue and ceratonia seeds were the best inducers for mannanase synthesis by *P. humicola* (10.3 and 8.87 U/ml, respectively). Coffee residue increased the activity by 1.2-fold compared with locust bean gum. Chantorn *et al*. [4] found that Robusta coffee residue appeared to be a more efficient carbon source for *Penicillium oxalium* KUB-SN2-1 mannanase. The large variation in the mannanase activity by using different wastes in the nutrition medium may be due to the different in the nature of cellulose, or hemicellulose and/or the presence of some other components in these materials (activators or inhibitors) and variation in substrate accessibility. On comparison with other fungal producer and wastes (Table 4), it was observed that our results were higher than those reported by other mannanase producers.

Therefore, coffee residue and ceratonia seeds were used for *P. humicola* and ceratonia seeds waste for *Penicillium* spp. v as a sole carbon sources in the subsequent experiments (replacing locust bean gum).

Table 4 Comparison of mannanase activities obtained by several fungi reported by other authors with our results

Organism	Carbon source	E activity[a] (U/ml)	References
Aspergillus niger gr	Copra meal	1.966	[25]
A. flavus	Copra meal	1.325	[25]
A. niger	Palm kernel cake	2.48	[26]
Sclerotium rolfsii	Palm kernel cake	3.16	[26]
Trichoderma harzanum	Wheat bran	8.20	[7]
Thermomyces lanuginosus	Corn cobs (coarse)	0.3	[27]
Schizophyllum commune	Corn cobs (coarse)	3.24	[27]
S. commune	Wheat bran	0.9	[27]
S. commune	Wheat straw	7.7	[27]
Penicillium occitanis	Acacia seed	7.9	[27]
P. humicola	Coffee residue	10.3	This study
P. humicola	Ceratonia seeds	8.87	This study

[a]β-mannanase activity (U/ml).

Effect of nitrogen source

The mechanisms that govern the formation of extracellular enzymes are influenced by the availability of precursors for protein synthesis. Furthermore, the nitrogen source can significantly affect the pH of the medium during the course of fermentation [28]. The effect of various inorganic and organic nitrogen sources on mannanase synthesis was studied. This was done by eliminating $(NH_4)_2SO_4$, peptone, and urea from the original culture medium and using (on equivalent *N*-basis) ammonium sulfate; baker's yeast; peptone; sodium nitrate; urea; or yeast extract. The results in Table 5 indicated that for *P. humicola*, the control media or peptone or yeast extract appeared to be the best nitrogen sources that enabled the production of the highest mannanase activity using coffee residue as a sole carbon source (10.3, 10.0, and 9.5 U/ml, respectively) and yielding 8.87, 8.8, and 8.5 U/ml, respectively, when using ceratonia seeds as a carbon source (Table 5). Also, the effect of different nitrogen sources using *Penicillium* spp. v showed that the mixture of ammonium sulfate, peptone, and urea (control) produced the highest

Table 5 Effect of different nitrogen sources with coffee waste and ceratonia waste on the production of *Penicillium humicola* extracellular β-mannanase

Nitrogen source	Coffee waste			Ceratonia waste		
	Dry weight (g/flask)	Protein (mg/ml)	E activity[a] (U/ml)	Dry weight (g/flask)	Protein (mg/ml)	E activity[a] (U/ml)
Control[b]	1.86	7.20	10.30	0.28	2.83	8.87
Baker's yeast	1.91	3.62	9.11	0.37	2.25	5.79
$NaNO_3$	1.91	4.44	6.41	0.33	2.97	4.67
NH_4SO_4	1.89	3.68	4.15	0.25	1.14	4.50
Peptone	1.85	6.90	10.00	0.29	3.66	8.80
Urea	2.10	5.00	3.53	0.34	3.06	5.86
Yeast extract	1.86	6.80	9.50	0.33	3.20	8.50

[a]β-mannanase activity (U/ml); [b]Control nitrogen source: ammonium sulfate (0.15)+peptone (0.2)+urea (0.03).

mannanase activity (8.4 U/ml), followed by the use of only yeast extract as a nitrogen source (7.4 U/ml), whereas inhibition occurred with the other nitrogen sources (Table 6). Our results are in agreement with those obtained previously by Hashem *et al.* [6]. Some investigators reported that fungi produce more enzymes on the addition of complex organic nitrogen sources [28,29]. Naganagouda *et al.* [25] found that the best nitrogen source was the combined form of yeast extract and ammonium nitrate for *Aspergillus* spp.; however, Youssef *et al.* [30] reported that ammonium chloride was the best preferable nitrogen source for *A. niger*.

β-Mannanase obtained from *P. humicola* (10.8 U/ml) was higher than that obtained from *Penicillium* spp. v; therefore, *P. humicola* was chosen for further work and the properties of its crude enzyme were studied.

Some properties of the crude *Penicillium humicola* β-mannanase

Effect of pH value of the reaction mixture

P. humicola mannanase showed optimum activity at pH 5.5 (Fig. 1). In general, optimum pH for the activity of most fungal mannanases was in the acidic range [3,6,31,32].

Effect of temperature of the reaction mixture

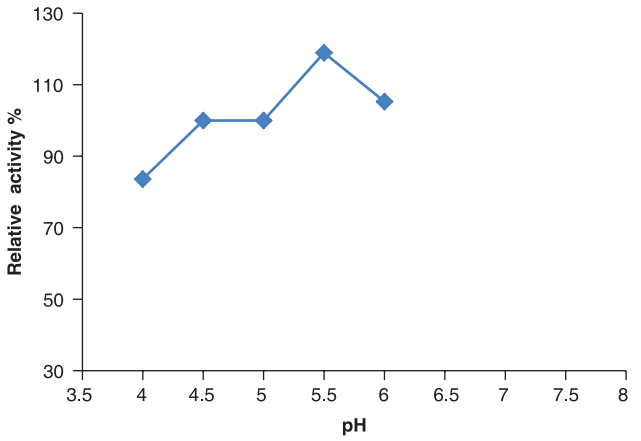
P. humicola mannanase had an optimum temperature for activity at 50°C. It was still active at a higher temperature and retained 89 and 83% of its activity at 55–60°C, respectively (Fig. 2). There are several commercial advantages in carrying out enzymatic reactions at a higher temperature [33]. Enzymatic digestion at a high temperature (60–65°C) may reduce microbial contamination of the material being processed. In addition, higher temperatures increase the rate of substrate digestion and increase the solubility of the polymeric substrates such as carbohydrates, rendering them more amenable to enzymatic attack [34].

Table 6 Effect of different nitrogen sources with ceratonia waste on the production of *Penicillium* spp. v extracellular β-mannanase

Nitrogen source	Dry weight (g/flask)	Protein (mg/ml)	E activity[a] (U/ml)
Control[b]	0.70	6.79	8.39
Baker's yeast	1.38	6.04	0.23
$NaNO_3$	1.14	6.55	0.13
NH_4SO_4	1.00	5.02	0.12
Peptone	1.26	5.30	0.03
Urea	1.29	6.62	0.42
Yeast extract	1.34	6.33	7.43

[a]β-mannanase activity (U/ml); [b]Control nitrogen source: ammonium sulfate (0.15)+peptone (0.2)+urea (0.03).

Figure 1



Effect of pH of the reaction mixture on the crude *Penicillium humicola* β-mannanase. Control: at pH 5.0 represent 100%.

pH stability

The pH stability of the tested enzyme was investigated after preincubation of the crude enzyme in buffer with different pHs (4.5–6) for different periods (15–60 min). The crude enzyme showed good stability within a broad pH range of 4.5–6 for 60 min. The enzyme retained about 81 and 82% of its original activity after 60 min of incubation at pH 5 and 5.5, respectively. Also, it retained 80% of its activity after 60 min of exposure at pH 6.0 (Fig. 3).

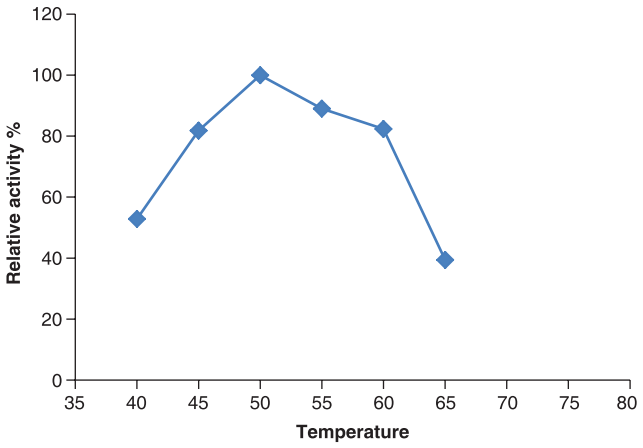
Thermal stability

The enzyme solution was incubated in the absence of its substrate at 40, 45, 50, and 55°C in a water bath and the residual activities at different periods up to 1 h were determined under the optimum conditions (pH 5.5, 50°C). The enzyme retained 98.4% of its activity after 60 min at 40°C and 95% of its activity at 45°C. After heating the enzyme at 50°C up to 15 min, most of the activity was retained (96%) and still retained 79.8% of its activity after 60 min (Fig. 4). The enzyme was still active after 15 and 30 min of incubation at 55°C and yielded 86 and 56.4% activity, respectively. The most adverse effect on the crude enzyme was after 1 h (36.9%) at 55 and 60°C.

Hydrolysis property

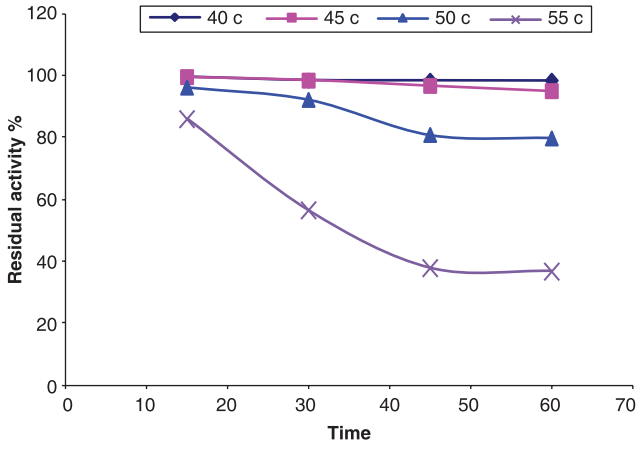
The time course of hydrolysis of locust bean gum by crude extracellular *P. humicola* β-mannanase was studied. The amount of reducing sugars released is shown in Table 7. The highest reducing sugar (8900 μmol/ml) was achieved after 28 h, followed by 26 h (8870 μmol/ml). In addition, various sizes of oligosaccharides detected by thin-layer chromatography were obtained from the hydrolysis locust bean gum as shown in Fig. 5a and b. Similarly, mannanase from *P. oxalicum* KUB-SNS-1 hydrolyzed copra mannan into mannotriose and mannabiose [4]. Hence, some fungal mannanases hydrolyze mannotetraose to mannotriose and mannabiose through a transglycosylation reaction [35]. The authors showed that *P. humicola* mannanase efficiently hydrolyzed locust bean gum into galactomanno-oligosaccharides. Mannanase produced from *P. humicola* had interesting properties and could be applied in a prebiotic preparation. However, the degradation products and the most suitable conditions to obtain higher quantities of oligosaccharides still need further investigation.

Figure 2



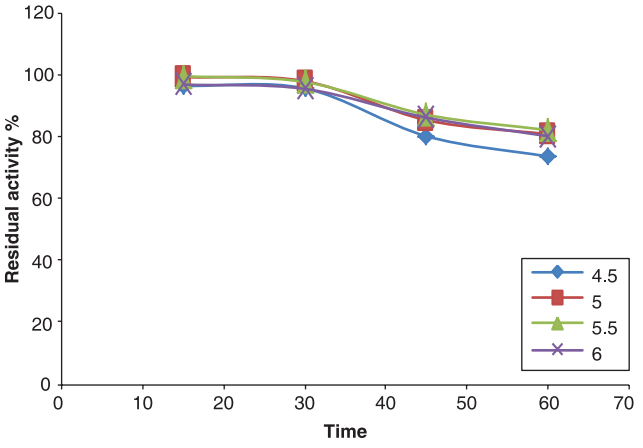
Effect of temperature of the reaction on the crude *Penicillium humicola* β-mannanase. Control: at pH 5.5 and temperature 50°C represent 100%.

Figure 4



Thermal stability of the crude *Penicillium humicola* β-mannanase.

Figure 3

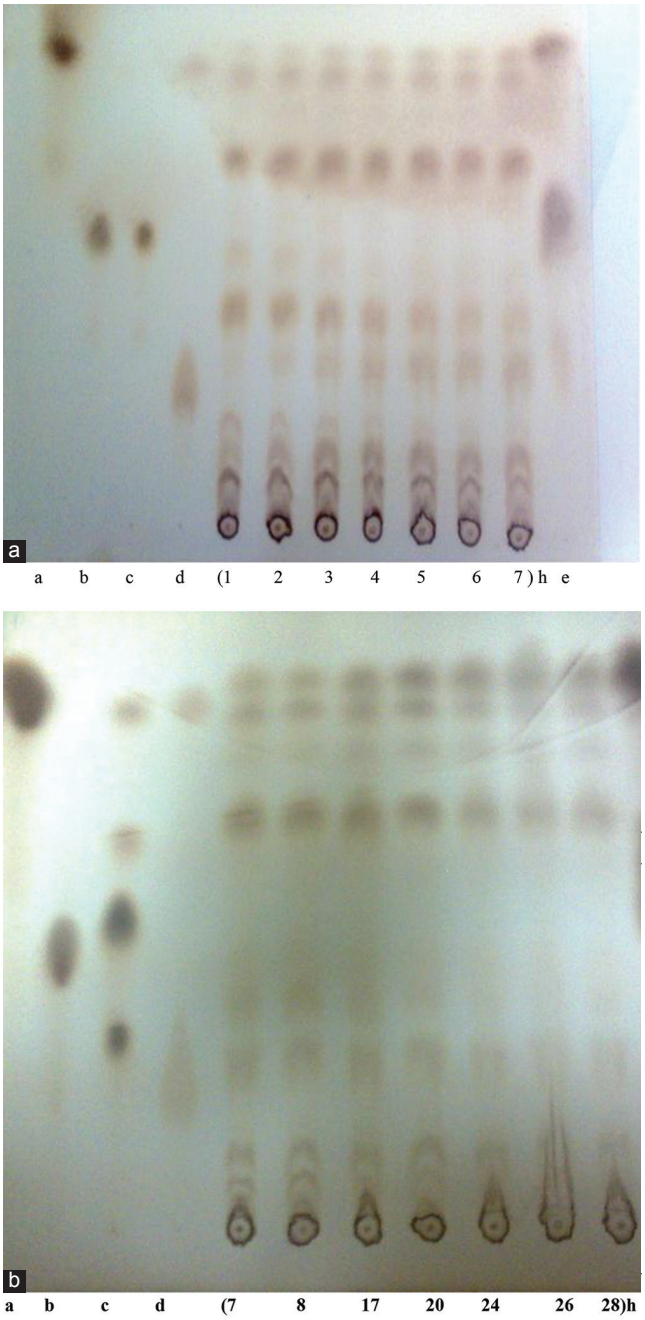


pH stability of the crude *Penicillium humicola* β-mannanase.

Table 7 Hydrolysis of locust bean gum by *Penicillium humicola* extracellular β-mannanase at different time intervals

Time of hydrolysis (h)	Reducing sugar (%)
1	22.78
2	28.50
3	38.50
4h***	43.40
5h	47.20
6h	50.40
7h	53.20
8h	57.00
17h	73.00
20h	75.60
24h	83.60
26h	88.70
28h	89.00

Figure 5



(a) Locust bean gum degradation by extracellular β-mannanases at various periods of 1, 2, 3, 4, 5, 6, and 7 h. Lan a: mannose, lan b: melibiose, lan c: raffinose, lan d: stachyose, lan 1: 1 h, lan 2: 2 h, lan 3: 3 h, lan 4: 4 h, lan 5: 5 h, lan 6: 6 h, lan 7: 7 h, lan e: mannose+melibiose +raffinose+stachyose. (b) Locust bean gum degradation by extracellular β-mannanases at various periods of 7, 8, 17, 20, 24, 26, and 28 h. Lan a: mannose, lan b: melibiose, lan c: raffinose, lan d :stachyose.

Conclusion

This study focuses on the optimization of culture parameters for the maximal production of crude extracellular mannanase from *P. humicola* using agricultural waste as a carbon source. The modified medium with an initial pH of 5.5 at 30°C produced the highest mannanase activity of 10.3 U/ml after 10 days. Finally, the enzyme tested was found to have the ability to hydrolyze galactomannan and produced galactomanno-oligosaccharides, which can be used in food and feed industries.

Acknowledgements

The authors are grateful to National Research Center for supporting this research study.

Conflicts of interest

There are no conflicts of interest.

References

1 Zyl WH, Rose SH, Trollope K, Gorgens JF. Fungal β-mannanases: mannan hydrolysis, heterologous production and biotechnological applications. Process Biochem 2010; 45:1203–1213.

2 Dhawan S, Kaur J. Microbial mannanases: an overview of production and applications. Crit Rev Biotechnol 2007; 27:197–216.

3 Kote NV, Patil AGG, Mulimani VH. Optimization of the production of thermostable endo-β-1, 4 mannanase from a newly isolated *Aspergillus niger gr* and *Aspergillus flavus gr*. Appl Biochem Biotechnol 2009; 152:213–223.

4 Chantorn, ST, Buengsrisawat K, Pokaseam A, Sombat T, Dangpram P, Jantawon K, Nitisinprasert S. Optimization of extracellular mannanase production from *Penicillium oxalicum* KUB-SN2-1 and application for hydrolysis property. Songklanakarin J Sci Technol 2013; 35:17–22.

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

6 Hashem AM, Ismail AMS, El-Refai MA, Abdel-Fattah AF. Production and properties of β-mannanase by free and immobilized cells of *Aspergillus oryzae* NRRL 3488. Cytobios 2001; 105:115–130.

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

8 Politiz O, Krash M, Thomsen KK, Borriss R. A highly thermostable endo-(1,4)-β-mannanase from the marine bacterium *Rhodothermus marinus*. Appl Microbiol Biotechnol 2000; 53:715–721.

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

10 Monitel, MD, Rodriguez J, Perez-Leblic MI, Hernandez M, Arias ME, Copa-Patino JL. Screening of mannanases in *Actinomycetes* and their potential application in the biobleaching of pine kraft pulps. App Microbiol Biotechnol 1999; 52:240–245.

11 Ademark P, Varga A, Medve JHarjunpa¨a¨ V, Drakenberg T, Tjerneld F, Sta¨lbrand H. Softwood hemicellulose-degrading enzymes from *Aspergillus niger*: purification and properties of a β-mannanase. J Biotechnol 1998; 63:199–210.

12 McCutchen CM, Duffaud GD, Leduc P, Peterson ARH, Tayal A, Khan SA, Kelly RM. Characterization of extremely thermostable enzymatic breaker (α -1,6-galactosidase and-β 1,4 mannanase) from the hyper thermophilic *Eubacterium Thermotogane apolitana* 5068 for hydrolysis of guar gum. Biotechnol Bioeng 1996; 52:332–339.

13 Oda Y, Komaki T, Tonomura K. Production of extracellular β-mannanase and β-mannosidase by *Enterococcus casseliflavus* FL2121 isolated from decayed Konjak. Food Microbiol 1993; 10:353–358.

14 Chaiongkarn A, Titapoka S, Keawsompong S, Nitisinprasert S. *β-Mannanase producing fungal strain KUB-SN2-1Proceedings of ABIC agricultural biotechnology for better living and a clean environmental*. Bangkok, Thailand: Queen Sirikit National Convention Center; 2009. 69.

15 Dan Z, Wenxiang P, Gang S, Hongzhi L, Xue L, Jingping G. Optimization of mannanase production by *Bacillus sp.* HDYM-05 through factorial method and response surface methodology. Afr J Microbiol Res. 2012; 6:176–182.

16 Smogi M. Notes on sugar determination. J Biol Chem 1952; 195:19–23.

17 Lowry OH, Rosebrough NH, Farr AL, Randall R. Protein measurement with the Folin phenol reagent. J Biol Chem 1951; 193:265–275.

18 Dubios M, Gills KA, Hamilton JK, Rebers PA, Smith F. Colorimetric method for determination of sugars and related substances. Anal Chem 1956; 28:350–356.

19 Adachi, S. A rapid method for the assay of lactulose. Anal Biochem 1965; 12:137–142.

20 Blibech M, Ghorbe RE, Chaari F, Dammak I, Bhiri F, Neifar M, Chaabouni SE. Improved mannanase production from *Penicillium occitanis* by fed-batch fermentation using acacia seeds. ISRN Microbiol 2011; 2011:1–5.

21 Bhoria P, Singh G, Hoondal GS. Optimization of mannanase production from *Streptomyces sp.* PG-08-03 in submerged fermentation. Bioresources 2009; 4:1130–1138.

22 Mohamad SN, Ramanan, RN, Mohamad R, Ariff AB. Improved mannan degrading enzymes production by *Aspergillus niger* through medium optimization. New Biotechnol 2011; 28:146–152.

23 Howard RL, Abotsi E, Jansen Van Rensburg EL, Howard S. Lignocellulose biotechnology: issues of bioconversion and enzyme production. Afr J Biotechnol 2003; 2:602–619.

24 Mabrouk MEM, El Ahwany AMDEI. Production of β-mannanase by *Bacillus amyloliquefaciens* 10A1 cultured on potato peels. Afr J Biotechnol 2008; 7:1123–1128.

25 Naganagouda K, Salimath PV, Mulimani VH. Purification and characterization of endo-β-1,4 mannanase from *Aspergillus niger* gr for application in food processing industry. J Microbiol Biotechnol 2009; 19:1184–1190.

26 Abd-Aziz S, Ab-Razak NA, Musa MH, Hassan MA. Production of mannan-degrading enzymes from *Aspergillus niger* and *Sclerotium rolfsii* using palm kernel cake as carbon source. Res J Environ Sci 2009; 3:251–256.

27 Gübitz GM, Hayn M, Urbanz G, Steiner W. Purification and properties of an acidic β-mannanase from *Sclerotium rolfsii.* J Biotechnol 1996; 45:165–172.

28 Lin TC, Chen C. Enhanced mannanase production by submerged culture of *Aspergillus niger* NCH-189 using defatted copra based media. Process Biochem 2004; 39:1103–1110.

29 Rajoka MI, Akhtar MW, Hanif A, Khalid AM. World J Microbiol Biotechnol 2006; 22:991–998.

30 Youssef SA, El-Naggar MY, El-Assar SA, Beltagy EA. Optimization of cultural conditions for β-mannanase production by a local *Aspergillus niger* isolate. Int J Agri Biol 2006; 8:539–545.

31 Blibech M, Ghorbel RE, Fakhfakh I, Ntarima P, Piens K, Bacha AB Chaabouni SEPurification and characterization of a low molecular weight of β-mannanases from *Penicillium occitanis* Pol6Appl Biochem Biotechnol 2010; 160:1227–1240.

32 Adesina CF, Oluboyede AO, Agunbiade SO, Aderibigbe OB, Kolade OH, Oluwole ME. Production and characterization of fungal extracellular β-mannanase. Am J Res Commun 2013; 1:2325–4076.

33 Ward OP, Moo-Young M. Enzymatic degradation of cell wall and related plant polysaccharides. Crit Rev Biotechnol 1989; 8:237–274.

34 Brock TD. *Introduction, an overview. Thermophiles: general, molecular and applied microbiology.* New York: John Wiley & Sons Inc.; 1986. 1–16.

35 Puchart V, Katapodis P, Biely P, Kremnicky L, Christakopoulos P, Vrsanska M, *et al.* Production of xylanases, mannanases, and pectinases by the thermophilic fungus *Thermomyces lanuginosus.* Enzyme Microbiol Technol 1999; 24:355–361.