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ORIGINAL PAPER

Purification and characterization of a thermostable endo-beta-1,4 mannanase from *Weissella viridescens* LB37 and its application in fruit juice clarification

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Abstract The isolation and characterization study of lactic acid bacteria was performed with a sample of fermented sausage, which was collected from a local market in Erzurum. As a result of sequencing analysis of 16S rRNA, this bacterium is found to be similar to *Weissella viridescens* (GenBank No: KM365459) at a ratio of 99 %. β-Mannanase was purified 188.07 times from *W. viridescens* by using ammonium sulfate precipitation, ion exchange chromatography and then employing gel filtration chromatography techniques. The purified enzyme has shown only a protein band at a level of 36.25 kDa with SDS-PAGE. The mannanase obtained after these purification steps has shown maximum activity at pH 5 and 40 °C, and the enzyme was determined to be quite stable within the range of pH 2–11 when temperature is higher than 50 °C. The activator or inhibitor effects of ions such as Cu^{2+}, Fe^{2+}, Ca^{2+}, Mg^{2+} and Zn^{2+} on activity of mannanase were also researched, and all metal ions except Ca^{2+} significantly affected enzyme activity in a positive direction. V_{max} and K_m values of mannanase for locust bean gum were 82.5 mg mannan/mL and 0.0178 mM. In addition, considering the biotechnological aspect, clarification of fruit juice was investigated. The purified enzyme was determined to be the most effective enzyme that can be used in clarifying the kiwi juice with a ratio of 117.21 %.

Keywords *Weissella viridescens* · Isolation · Molecular characterization · β-Mannanase · Clarification

Introduction

The endo-*β*-D-mannanase (EC 3.2.1.78, mannan endo-1.4-*β*-D-mannosidase) hydrolyzes the mannosidic linkages of mannans, galactomannans, glucomannans and galactoglucomannans [1]. Mannanase was characterized from many bacteria containing *Bacillus circulans* [2], *Vibrio* sp. strain MA-138 [3], *B. circulans* K-1 [4] and *Saccharophagus degradans* [5]. They have been isolated from fungi such as *Aspergillus niger* [6] and certain plant species like tomato [7].

Mannanases are used in paper and wood pulp industry and especially in food and feed industries. Since mannanase is effective in alkaline processes, it can be used effectively in the enzymatic bleaching processes of environmental problems [8, 9]. In addition, some studies have been reported that mannanase can be used to eliminate fruit juice turbidity [10].

In certain regions of the world, production of fermented sausages has become a tradition for a very long time. In the naturally fermented sausage microflora, lactic acid bacteria (LAB) play a significant role. These bacteria are Gram (+), durable to high acidic conditions, homo- and hetero-fermentative, in the form of rods or cocci, facultative anaerobic microorganisms, which do not create endospores. They

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are nonpathogenic except a few species and found in many foods such as meat and sea products (sausages, fish sauce, etc.) in particular, dairy products (yogurt, cheese, kefir, etc.), grain products (bread, broke, etc.), wine and vegetables (cabbage and pickles, etc.) naturally. They also play an important role in ensuring the maturation, production and durability of foods by adding them as a starter culture. They also ensure the safety and fermentation of meat by stabilizing them [11–15].

In this group, there are microorganisms belonging to species *Abiotrophia*, *Aerococcus*, *Alkalibacterium*, *Allofustis*, *Alloiococcus*, *Atopobacter*, *Atopococcus*, *Atopostipes*, *Bavariicoccus*, *Bifidobacterium*, *Carnobacterium*, *Catellicoccus*, *Desemzia*, *Dolosicoccus*, *Dolosigranulum*, *Enterococcus*, *Eremococcus*, *Facklamia*, *Fructobacillus*, *Globicatella*, *Granulicatella*, *Ignavigranum*, *Isobaculum*, *Lacticigenium*, *Lactobacillus*, *Lactococcus*, *Lactovum*, *Leuconostoc*, *Marinilactibacillus*, *Melissococcus*, *Oenococcus*, *Pediococcus*, *Pilibacter*, *Pisciglobus*, *Sporolactobacillus*, *Streptococcus*, *Tetragenococcus*, *Trichococcus*, *Vagococcus* and *Weissella* [16, 17].

Weissella was identified for the first time by Collins et al. [18]. These species were diagnosed by scientists isolating from different media. In these studies, it has been determined that especially *Weisellahalo tolerans*, *Weissella hellenica* and *W. viridescens* species can be found in meat and meat products [19].

In this study, firstly, it has been aimed to purify mannanase enzyme from *W. viridescens* LB37 and characterize this enzyme. And the final step was clarification of some fruit juices with purified mannanase. For this purpose, the ammonium sulfate precipitation was performed on *W. viridescens* LB37 bacteria, which was isolated and diagnosed from fermented sausage. DEAE-Sephadex and Sephacryl S-200 chromatography techniques were used for purification in the manner of ion exchange and gel filtration. Physicochemical parameters (optimum pH and temperature) and kinetic parameters (V_{max} and K_m) of purified enzyme were also determined. Resistance of purified enzyme to some ions was investigated as well. In addition, the effects of both crude and purified enzyme were determined in clarification of some fruit juices such as grape, peach, orange, pomegranate, kiwi and apple.

Materials and methods

Isolation of LAB

The sausage sample was bought from a local market in Erzurum. In total, 25 g of sample was added into 225 mL of sterile saline peptone water and the solution was homogenized (Laboratory Blender Stomacher 400, Seward Medical, London, UK) for 1 min. Afterward, decimal solutions were prepared and sample dilutions with an amount of 0.1 mL were spread onto de Man Rogosa Sharpe Agar (MRS) plates before being incubated for 24 h at 30 °C under anaerobic conditions (anaerobic generating Kit, Merck) [13].

Identification of test strain

A total of five different colonies were picked and subcultured to obtain pure culture. One of these colonies was randomly chosen to purify the culture, and this colony was spread on a MRS Petri dish. It was stored in 15 % glycerol and MRS broth at −86 °C to be used in future studies. Test strain was Gram-stained and examined by light microscopy to determine its morphology. It was preliminarily identified in accordance with the phenotypic properties such as temperature range for growth (15–45 °C), carbon dioxide production from glucose, the growing ability in MRS broth with sodium chloride content of 2.0–10 %, and it was tested for catalase production. Total DNA of the test strain (LB 37) was isolated in accordance with the description of Adiguzel [20], and sugar metabolism ways of the isolate was identified by API 50 CHL test strip (Bio-Mérieux, France).

16S rRNA and bacterial systematic region were amplified with UNI16S-L (5′-ATTCTAGAGTTTGATCATGGCTCA-3′) and UNI16S-R (5′-ATGGTACCGTGTGACGGGCGGTGTGTA-3′) primers and then cloned into pGEM-T Easy cloning vector (Promega, UK). As a result of cloning, the positive plasmids were isolated and sequence analysis was performed by Macrogen (Holland). 16S rRNA gene sequences were compared with bacterial sequences in the GenBank, and GenBank accession number with similarity ratios was obtained [14].

Mannanase purification from *W. viridescens* LB37

After filtration processes performed with Whatman filter paper, 0–20, 20–40, 40–60, 60–80 and 80–100 % of ammonium sulfate precipitation procedure were performed on homogenate, respectively. The precipitate was dissolved in 20 mM sodium citrate (pH 6.0) buffer and then dialyzed against the same buffer.

Column chromatography

The enzyme solution was applied to DEAE-Sephadex ion exchange column (2.5 × 30) which was pre-equilibrated with 20 mM sodium citrate (pH: 6.0) buffer after precipitation and dialysis. The column was washed with the buffer and 3 mL of enzyme fractions which were attached to the column collected at 3 mL/min of flow speed by generating

1 M NaCl gradient. The elution absorbance was spectrophotometrically measured at 280 nm (PG Instrument T80 Double Beam UV–Vis spectrophotometer).

Gel filtration

The active fractions from chromatography steps were concentrated by lyophilization, and then, undesired content was removed by dialysis. This solution has been applied to the Sephacryl S-200 (120 cm × 1 cm) column, which was pre-equilibrated with 20 mM phosphate buffer (pH 5.0), and mannanase was dissolved in the same buffer with 0.5 M NaCl. In all fractions, absorbance was controlled in accordance with Warburg's method and activity assay was performed by using locust bean gum as substrate. Active fractions were combined and stored at 4 °C after gel filtration for further use.

Protein content assay

The protein contents in all enzyme solutions were determined by Bradford [21] method that has commonly used bovine serum albumin (BSA).

Mannanase activity assay

Locust bean gum was used as a substrate in all mannanase activity measurements. After hydrolyzing locust bean gum with mannanase, it has been allowed to create a colorful complex with DNSA (3.5-dinitrosalicylic acid) and activity was measured the absorbance at 540 nm. Distilled water was used as blank instead of the enzyme. Amount of enzyme producing one micromole of mannose per minute under the experimental conditions was determined as one unit of mannanase activity [22, 23].

The K_m and V_{max} values of purified enzyme (Michaelis constants) were determined by measuring the activities with 0.3 mM locust bean gum. These constant values were obtained by analyzing the data in accordance with the Lineweaver–Burk plot.

Optimum pH

Some buffers in different pHs were used to determine the optimum pH for purified mannanase from *W. viridescens* LB37. For pH values between 3–9 and 2–5, acetate buffers were prepared; however, phosphate buffers were used for pH 6–8, Tris-HCl buffers for pH 8–9 and carbonate buffers for pH 9–11, respectively.

Optimum temperature

Optimum temperature of purified mannanase was determined at different temperatures ranging from 20 to 90 °C.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

Subunit assay of purified mannanase was obtained using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and the gel obtained was photographed [24].

Molecular weight determination by gel filtration

Sephadex G-100 column (3.0 × 50 cm) was used in order to specify the molecular weight of the mannanase obtained from *W. viridescens* LB37. Column was equilibrated with solution of 0.05 M Na_2HPO_4 and 1 mM dithioerythritol (pH 7.0) buffers. First, protein marker (bovine serum albumin, 66 kDa; pepsin, 34 kDa; β-lactoglobulin and lysozyme, 14 kDa; egg ovalbumin, 45 kDa; trypsinogen, 24 kDa) was passed through the column and it was eluted using the same buffer. Then, the purified mannanase solution was passed through the column and eluted as the same conditions in gel filtration. The difference between purified enzyme and protein marker gave the molecular weight of purified enzyme [25].

Effects of metal ions on enzyme activity

The inhibitor and/or activator effects of some ions like Cu^{2+}, Fe^{2+}, Ca^{2+}, Mg^{2+} and Zn^{2+} on the mannanase activity purified from *W. viridescens* LB37 were investigated. The concentrations of effectors were 1 mM and 5 mM, and all measurements were repeated three times.

Fruit juice clarification

Grape, orange, pomegranate, kiwi, peach and apple fruits were carefully washed, dried and homogenized to prepare the fruit juice. First, 2 mL enzyme solution with 17.6 ± 1.6 EU/mL was added into 10 g of each fruit homogenates (equal volume of distilled water was added instead of enzyme for control) and the same experiment was performed with also 2 mL of crude extract. Fruit homogenates were treated at a natural pH value for 4 h at 50 °C, and a certain amount of fruit juice was filtered through filter paper for 15 min before measuring the volume [10]. The degree of clarification in fruit juice was measured by determining the increased amount of fruit juice by β-mannanase enzyme activity along with release of water stored in the fibrous structure of the fruit.

Statistical analysis

All measurements were repeated three times in a row in order to determine the mannanase activity that was given as mean ± standard. All of the statistical analyses were performed using the SPSS 15.0 software (SPSS Inc, Chicago,

Table 1 The purification process of purified β-mannanase enzyme from *Weissella viridescens* LB37

Enzyme fraction	Volume (mL)	Activity EU/mL	Total activity (EU)	Yield (%)	Protein (mg/mL)	Specific activity (EU/mg protein)	Purification fold	Test and *p*
Crude extract	30	45.19 ± 0.08	1.36×10^3	100	132.40 ± 0.04	0.34	–	*F*: 3.790
$(NH_4)_2SO_4$	25	33.70 ± 0.17	8.43×10^2	61.9	7.60 ± 0.08	4.43	13.03	$p = 0.003$*
DEAE-Sephadex	25	23.60 ± 0.03	5.90×10^2	43.4	2.03 ± 0.01	11.63	34.2	
Sephacryl S-200	25	11.51 ± 0.06	2.79×10^2	20.5	0.18 ± 0.01	63.94	188.07	

The sample size for all experiments was three

*Mean value followed by different letters differs significantly ($p < 0.05$)

IL, USA), and 'one-way' or 'two-way' ANOVA was used to compare the significant differences of all studied variables. In Tukey's test, significant differences were calculated within 95 % confidence intervals and differences which have *p* values less than 0.05 were considered as statistically significant.

Results and discussion

Characterization of LAB

In this study, conventional and molecular techniques were used for the identification of test isolate. According to conventional methods, test isolate was positive in terms of gas production by irregular rods, Gram positive and glucose utilizing. However, endospore and catalase were found to be negative. The optimum bacterial growth was obtained at a temperature between 15 and 37 °C with 5.5–6.5 pH and 2–6.0 % salt concentration. Since the analysis was performed using API 50 CHL test system, the test strain was determined as *W. viridescens*. These results were consistent with the data obtained from the literature [26]. In other respects, according to one of the molecular techniques 16S rRNA sequencing, the test isolate contained about 1452 nucleotides. According to another blast study conducted by using the database included in the GenBank, the sequence of this isolate resembled *W. viridescens* LB37 (GenBank number: KM365459) with a similarity of 99 %. Results have shown similar values (≥98 %), which were obtained by Kulwichit et al. [27] and Han et al. [28], who have verified that the similarity of 16S rRNA gene sequences of *W. viridescens*-type strains was higher than 98 %, in terms of 16S rRNA gene sequences of *W. viridescens* isolate.

Purification and characterization of mannanase from *W. viridescens* LB37

Mannanase produced as extracellularly from *W. viridescens* LB37 bacteria was purified in three steps: ammonium sulfate precipitation, DEAE-Sephadex and Sephacryl S-200 chromatography. The results obtained are illustrated in Table 1.

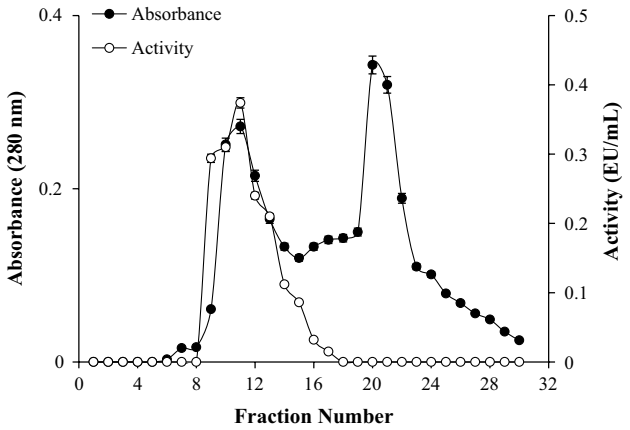


Fig. 1 Purification of mannanase by ion exchange chromatography using DEAE-Sephadex

In the first step, the highest precipitation ratio of purified mannanase was at a range of 60–80 % and the mannanase collapsed the ammonium sulfate saturation was in the range from 0 to 100 %, respectively. In the first purification step, the production yield was 49.6 % with 13.03-purification-fold (Table 1). In the second step, the dialyzed enzyme was applied to DEAE-Sephadex column and purified 34.2-fold with a purification yield of 43.4 %. In the last step, fractions from DEAE-Sephadex were lyophilized and applied to Sephacryl S-200 column. The enzyme was purified from column and 188.07-fold and yield of 20.5 %, and specific activity was calculated as 63.94 EU/mg protein (Table 1). The mannanase was purified from *W. viridescens* LB37 by ion exchange chromatography and gel filtration, and the purification profiles of these processes are given in Figs. 1 and 2, respectively. According to the results of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), purified mannanase consists of a single subunit

as 36.25 kDa (Fig. 3). Sephadex G-100 gel filtration was applied, and molecular weight was calculated as 36.45 kDa from the graph (Fig. 4).

In another study, the β-mannanase gene was amplified and cloned from *Aspergillus aculeatus* to *Aspergillus oryzae*. The molecular weight of the recombinant enzyme was 45 kDa [29]; however, it was found to be 40 kDa for *A. niger* [30].

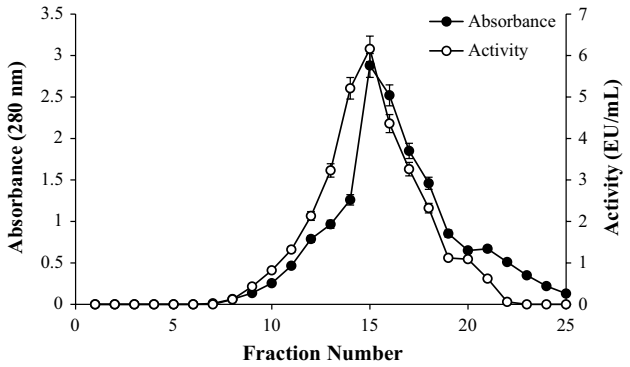


Fig. 2 Elution profile of protein and mannanase activity on Sephacryl S-200

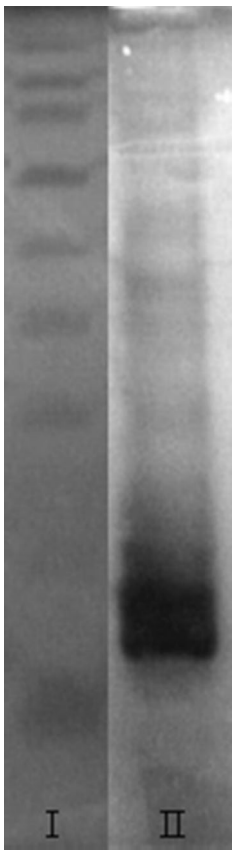


Fig. 3 SDS-PAGE pattern of mannanase [standard protein marker (I); purified mannanase from *W. viridescens* LB37 (II)]

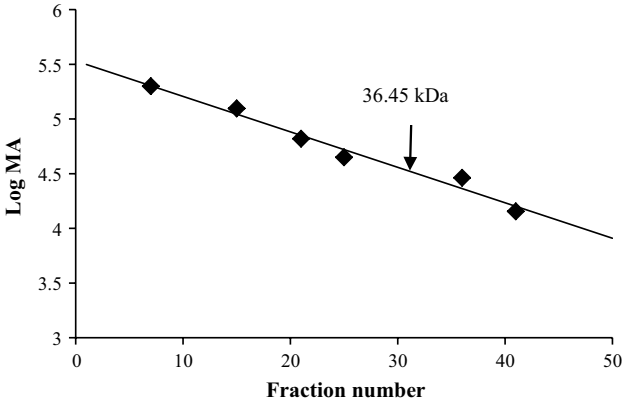


Fig. 4 Gel filtration graph of purified β-mannanase enzyme from *W. viridescens* LB37. Standard protein: myosin (200 kDa), β-galactosidase (125 kDa), bovine serum albumin (SSA) (66 kDa), ovalbumin (45 kDa), carbonic anhydrase (29 kDa), lysozyme (14.3 kDa)

Optimum pH

Activity measurements with locust bean gum substrate within range of pH 3.0–11.0 were taken, and the results obtained are given in Fig. 5 as relative activity −pH. The optimum pH value of mannanase obtained from *W. viridescens* LB37 bacteria was found as 6.0.

Optimum temperature

The optimum temperature of purified enzyme obtained from *W. viridescens* LB37 was determined between 20 and 90 °C by performing activity measurements at every temperature values after waiting 5 min. It has been determined that purified enzyme was highly active between 30 and 70 °C and showed maximum activity at 40 °C and the results are shown in Fig. 6 as relative activity–temperature.

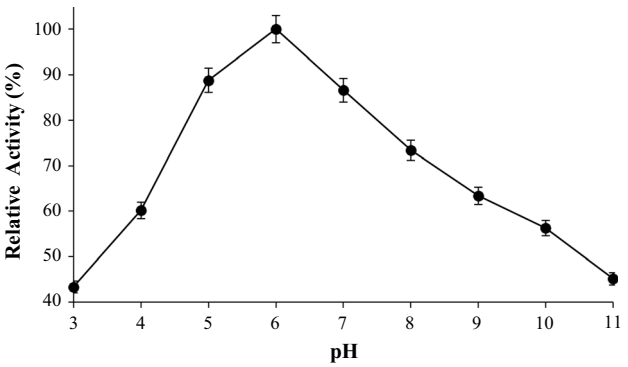


Fig. 5 Effect of pH on the activity of the purified mannanase

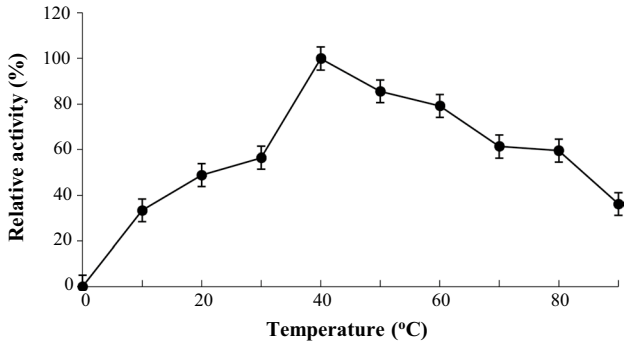


Fig. 6 Effect of temperatures on the activity of purified mannanase

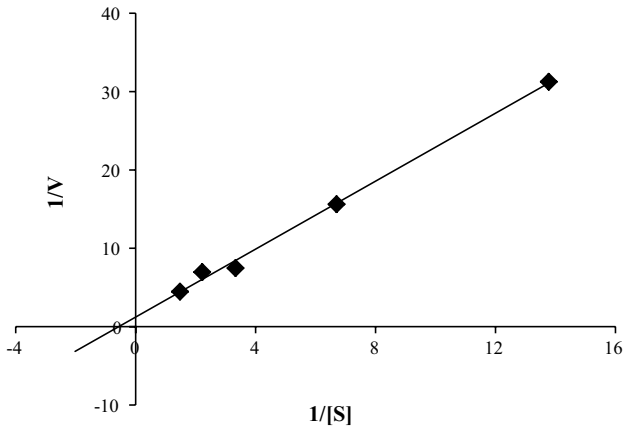


Fig. 7 Lineweaver–Burk graph of β-mannanase enzyme from *W. viridescens* LB37 for locust bean gum substrate

The results of V_{max} and K_m values

V_{max} and K_m values of mannanase for 0.3 mM of locust bean gum were found as 82.5 mg mannan/mL and 0.0178 mM, respectively (Fig. 7).

The effects of ions on the activity of the mannanase from *W. viridescens* LB37

The activator and/or inhibitor effects of some ions such as Cu^{2+}, Fe^{2+}, Ca^{2+}, Mg^{2+} and Zn^{2+} on enzyme activity were investigated in order to show the resistance of the mannanase purified from *W. viridescens* LB37 against metal ions. Inhibitor/activator results of ions tested in 1 and 5 mM concentrations are shown in Table 2. As a result, Ca^{2+} ions found to be inhibiting the enzyme, while other ions increased the activity of the purified mannanase. However, Fe^{2+} and Mg^{2+} metal ions activated the enzyme at a ratio of 366.0 % and 358.42 % in 5 mM of concentration, respectively. Since almost all metal ions could activate the enzyme, it would be suitable to be used as enzymes for industrial purposes.

Some fruit juice obtained by using purified mannanase from *W. viridescens* LB37

The elimination of high viscosity and turbidity in juice production requires high cost. The use of mannanase in fruit juice production helps mannans to be hydrolyzed; thus, viscosity of the medium decreases; and since release of the water held within the same structure occurs, the amount of fruit juice also increases. In Table 3, the results of the experiments regarding yield of fruit juices obtained from orange, pomegranate, kiwi, grape, peach and apple are given. In addition, the great advantage of using enzymes in the fruit juice production was also presented by giving a comparison with control. The mannanase purified from *W. viridescens* LB37 was effective in the peach juice production with a yield of 117.21 %.

Purified mannanase obtained from *W. viridescens* LB37 was compared with other mannanases (from fungi and other bacteria) comparing several kinetic parameters (Table 4). It was shown that fungal mannanases had lower optimal pH values than bacterial mannanases. Also, all

Table 2 The effect of some chemical compounds on β-mannanase activity

Chemical compounds	Concentration (mM)	Relative activity (%)	Concentration (mM)	Relative activity (%)	Test and *p*
None	–	100 ± 0.0	–	100 ± 0.0	$F = 6.428$
Cu^{2+}	1	180.33 ± 0.10	5	338.34 ± 0.17	$^*p = 0.000$
Fe^{2+}	1	210.24 ± 0.01	5	366.0 ± 5.20	
Ca^{2+}	1	62.32 ± 0.17	5	34.36 ± 0.15	
Mg^{2+}	1	364.04 ± 0.11	5	358.42 ± 0.20	
Zn^{2+}	1	108.42 ± 0.13	5	160.14 ± 0.13	

All metal ions were used as chloride salt ($CuCl_2$, $FeCl_2$, $CaCl_2$, $MgCl_2$, $ZnCl_2$)

The sample size for all experiments was three

*Mean value followed by different letters differs significantly ($p < 0.05$)

Table 3 The effect of β-mannanase treatment on the yields of juice volumes

Fruit (5 g)	Control	Homogenate	β-Mannanase
Grape			
Volume	8.04 ± 0.12	8.4 ± 0.02	9.06 ± 1.05
Yield (%)	–	104.5	112.7
Peach			
Volume	7.8 ± 1.14	8.0 ± 2.45	8.5 ± 2.1
Yield (%)	–	102.6	108.97
Orange			
Volume	9.0 ± 0.8	9.5 ± 3.4	9.7 ± 2.0
Yield (%)	–	105.6	107.8
Pomegranate			
Volume	10.0 ± 0.58	10.5 ± 0.02	11.0 ± 0.03
Yield (%)	–	105.0	110.0
Kiwi			
Volume	8.02 ± 1.1	8.5 ± 0.56	9.4 ± 0.35
Yield (%)	–	105.98	117.21
Apple			
Volume	8.06 ± 2.04	8.5 ± 0.01	8.8 ± 0.18
Yield (%)	–	105.5	109.2

Table 4 Comparison of the purified β-mannanase of *W. viridescens* LB37 properties with β-mannanases other β-mannanase

Phylum	Species of origin (name)	Optimum pH	Optimum temperature (°C)	Specific activity (U/mg)	References
Fungi	*Aspergillus sulphureus*	2.4	50	366	Chen et al. [34]
	Sclerotium rolfsii	2.9	72	380	Gubitz et al. [33]
	Penicillium wortmannin	6.0	60	n.a	Reese and Shibata [31]
Bacteria	*Lactobacillus plantarum* (M24)	8.0	50	82.5	Nadaroglu et al. [14]
	Bacillus sp.	8.0	60	22.0	Akino et al. [32]
	Bacillus pumilus (M27)	9.0	60	470	Adiguzel et al. [10]
	Weissella viridescens LB37	5.0	40	60.53	In this research

mannanases were thermostable. In the literature, there have been several studies indicating that fruit juice clarification process could be performed by using the mannanase [10, 14, 31–34].

Conclusion

Lactic acid bacteria are microorganisms that are quite abundant and mostly nonpathogenic. Our analyses indicated that the *W. viridescens* LB37 could be considered as an important candidate for the production of a mannanase, extracellularly. Purification of mannanase from *W. viridescens* LB37 was yielded as 188.07-fold. The enzyme is thermotolerant with enzymatic activity at acidic pH levels. In addition, the enzyme showed resilient stability in the presence of metal ions. Finally, they have been effective in the clarification of different fruit juices such as orange, pomegranate, kiwi, grape, peach and apple; thus, β-mannanase purified from *W. viridescens* LB37 could be used for industrial fruit juice productions, especially in problematic processes such as turbidity and viscosity.

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Compliance with ethical standards

Conflict of interest None.

Compliance with ethics requirements The authors, Gulsah Adiguzel, Zeynep Sonmez, Ahmet Adiguzel and Hayrunnisa Nadaroglu, hereby confirm that this manuscript is performed according to and follows the COPE guidelines and has not already been published nor is it under consideration for publication elsewhere. This article does not contain any studies with human or animal subjects.

References

1. Chandra MRS, Lee YS, Park IH, Zhou Y, Kim KK, Choi YL (2011) Isolation, purification and characterization of a thermostable β-mannanase from *Paenibacillus* sp. DZ3. J Korean Soc Appl Biol Chem 54(3):325–331
2. Yanan L, Yang P, Meng K, Wang Y, Luo H, Wu N, Fan Y, Yao B (2008) Gene cloning, expression, and characterization of a novel β-mannanase from *Bacillus circulans* CGMCC 1416. J Microbiol Biotechnol 18:160–166
3. Tanaka M, Umemoto Y, Okamura H, Nakano D, Tamaru Y, Araki T (2009) Cloning and characterization of a *β*-1,4-mannanase 5C possessing a family 27 carbohydrate-binding module from a marine bacterium, *Vibrio* sp. strain MA-138. Biosci Biotechnol Biochem 73:109–116
4. Yoshida S, Sako Y, Uchida A (1998) Cloning, sequence analysis, and expression in *Escherichia coli* of a gene coding for an enzyme from *Bacillus circulans* K-1 that degrades guar gum. Biosci Biotechnol Biochem 62:514–520

5. Hutcheson SW, Zhang H, Suvorov M (2011) Carbohydrase systems of *Saccharophagus degradans* degrading marine complex polysaccharides. Mar Drugs 9:645–665
6. Naganagouda K, Salimath PV, Mulimani VH (2009) Purification and characterization of endo-β-1,4-mannanase from *Aspergillus niger* gr for application in food processing industry. J Microbiol Biotechnol 19:1184–1190
7. Prakash R, Johston SL, Boldingh HL, Redgwell RJ, Atkinson RG, Melton LD, Brummell DA, Schröder R (2012) Mannans in tomato fruit are not depolymerized during ripening despite the presence of endo-β-D-mannanase. J Plant Physiol 169:1125–1133
8. Cuevas M, Lee JK, Spradling AC (1996) α-Spectrin is required for germline cell division and differentiation in the Drosophila ovary. Development 122:3959–3968
9. Chauhan PS, Puri N, Sharma P, Gupta N (2012) Mannanases: microbial sources, production, properties and potential biotechnological applications. Appl Microbiol Biotechnol 93:1817–1830
10. Adiguzel A, Nadaroglu H, Adiguzel G (2015) Purification and characterization of β-mannanase from *Bacillus pumilus* (M27) and its applications in some fruit juices. J Food Sci Technol 52:5292–5298
11. Stiles ME, Holzapfel WH (1997) Lactic acid bacteria of foods and their current taxonomy. Int J Food Microbiol 36:1–29
12. Leroy F, De Vuyst L (2004) Lactic acid bacteria as functional starter cultures for the food fermentation industry. Trends Food Sci Technol 15:67–78
13. Adiguzel G, Atasever M (2009) Phenotypic and genotypic characterization of lactic acid bacteria isolated from Turkish dry fermented sausage. Romanian Biotechnol Lett 14(1):4130–4138
14. Nadaroglu H, Adiguzel A, Adiguzel G (2015) Purification and characterisation of β-mannanase from *Lactobacillus plantarum* (M24) and its applications in some fruit juices. Int J Food Sci Technol 50:1158–1165
15. Tanguler H (2010) Identification of predominant lactic acid bacteria isolated from salgam beverage and improvement of its production technique. Ph.D. Thesis, Cukurova University, Institute of Natural and Applied Sciences, Adana, Turkey, p 393
16. Felis GE, Salvetti E, Torriani S (2015) Systematics of lactic acid bacteria: current statues, chap 2. In: Mozi F, Raya RR, Vignolo GM (eds) Biotechnology of lactic acid bacteria: novel applications. Wiley Blackwell Publisher, Hoboken
17. Manivasagan P, Venkatesan J, Kim SK (2014) Seafood processing by-products: trends and applications, chap 16. Springer, New York, pp 341–354. doi:10.1007/978-1-4614-9590-1_16
18. Collins MD, Samelis J, Metaxopoulos J, Wallbanks S (1993) Taxonomic studies on some leuconostoc-like organisms from fermented sausages: description of a new genus *Weissella* for the *Leuconostoc paramesenteroides* group of species. J Appl Bacteriol 75:595–603
19. Björkroth KJ, Schillinger U, Geisen R, Weiss N, Hoste B, Holzapfel WH, Korkeala HJ, Vandamme P (2002) Taxonomic study of *Weissella confusa* and description of *Weissella cibaria* sp. nov., detected in food and clinical samples. Int J Syst Evol Microbiol 52(1):141–148
20. Adiguzel G (2008) Phenotypic and genotypic characterization of lactic acid bacteria isolated from Turkish dry fermented sausage. Ph.D. Thesis, Ataturk University, Graduate School of Health Sciences, Erzurum, Turkey, p 110
21. Bradford MM (1976) Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Chem 72:248–253
22. Miller GL (1959) Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem 31:426–428
23. Bailey MJ, Biely P, Poutanen K (1992) Interlaboratory testing methods for assay of xylanase activity. J Biotechnol 23:257–270
24. Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage. Nature 22:680–685
25. Whitaker JR (1963) Determination of molecular weight of proteins by gel filtration on sephadex. Anal Chem 35:1950–1953
26. Liu JY, Li AH, Ji C, Yang WM (2009) First description of a novel *Weissella* species as an opportunistic pathogen for rainbow trout *Oncorhynchus mykiss* (Walbaum) in China. Vet Microbiol 136:314–320
27. Kulwichit W, Nilgate S, Chatsuwan T, Krajiw S, Unhasuta C, Chongthaleong A (2007) Accuracies of *Leuconostoc* phenotypic identification: comparison of API systems and conventional phenotypic assays. BMC Infect Dis 2(7):69
28. Han SK, Hong H, Kwak HL, Kim ES, Kimi MJ, Shrivastav A, Oh MH, Kim HY (2014) Identification of lactic acid bacteria in pork meat and pork meat products using SDS-PAGE, 16S rRNA Gene sequencing and MALDI-TOF Mass spectrometry. J Food Safety 34:224–232
29. Christgau S, Kauppinen S, Vind J, Kofod LV, Dalbøge H (1994) Expression cloning, purification and characterization of a beta-1,4-mannanase from *Aspergillus aculeatus*. Biochem Mol Biol Int 33(5):917–925
30. Ademark P, Varga A, Medve J, Harjunpää V, Drakenberg T, Tjerneld F, Stålbrand H (1998) Softwood hemicellulose-degrading enzymes from *Aspergillus niger*: purification and properties of a beta-mannanase. J Biotech 63:199–210
31. Reese ET, Shibata Y (1965) β-Mannanases of fungi. Can J Microbiol 11:167–183
32. Akino T, Nakamura N, Horikoshi K (1988) Characterization of three beta-mannanases of an alkalophilic *Bacillus* sp. Agric Biol Chem 52:773–779
33. Gubitz GM, Hayn M, Sommerauer M (1996) Mannan-degrading enzymes from *Sclerotium rolfsii*: characterization and synergism of two endo beta-mannanases and a beta-mannosidase. Bioresour Technol 58(2):127–135
34. Chen X, Cao Y, Ding Y, Lu W, Li D (2007) Cloning, functional expression and characterization of *Aspergillus sulphureus* β-mannanase in *Pichia pastoris*. J Biotechnol 128:452–461