J Food Sci Technol (August 2015) 52(8):5292–5298
DOI 10.1007/s13197-014-1609-y

ORIGINAL ARTICLE

Purification and characterization of β -mannanase from *Bacillus pumilus* (M27) and its applications in some fruit juices

Ahmet Adiguzel · Hayrunnisa Nadaroglu · Gulsah Adiguzel

Revised: 8 September 2014 / Accepted: 13 October 2014 / Published online: 1 November 2014

Abstract Thermo alkaline mannanase was purified from the bacteria of *Bacillus pumilus* (M27) using the techniques of ammonium sulphate precipitation, DEAE-Sephadex ion exchange chromatography and Sephacryl S200 gel filtration chromatography with 111-fold and 36 % yield. It was determined that the enzyme had 2 sub-units including 35 kDa and 55 kDa in gel filtration chromatography and SDS-PAGE electrophoresis systems. The optimum pH and temperature was determined as 8 and 60 °C, respectively. It was also noticed that the enzyme did not lose its activity at a wide interval such as pH 3–11 and at high temperatures such as 90 °C. Additionally, the effects of some metal ions on the mannanase enzyme activity. Moreover, the clarifying efficiency of purified mannanase enzyme with some fruit juices such as orange, apricot, grape and apple was also investigated. Enzymatic treatment was carried out with 1 mL L^{-1} of purified mannanase for 1 h at 60 °C. It was determined that the highest pure enzyme was efficient upon clarifying the apple juice at 154 % rate.

Keywords Mannanase · *Bacillus pumilus* (M27) · Purification · Characterization · Clarification

A. Adiguzel
Faculty of Science, Department of Molecular Biology and Genetic, Ataturk University, 25240 Erzurum, Turkey

H. Nadaroglu (✉)
Department of Food Technology, Erzurum Vocational Training School, Ataturk University, 25240 Erzurum, Turkey
e-mail: hnisa25@atauni.edu.tr

G. Adiguzel (✉)
Department of Food Hygiene and Technology, Faculty of Veterinary, Ataturk University, 25240 Erzurum, Turkey
e-mail: gulsah@atauni.edu.tr

Introduction

Commonly found in nature, the mannanase (EC 3.2.1.78) is an extracellular enzyme which is located within the structures of mannans and heteromannans (galactomannan, glucomannan and galactoglucomannan) - part of the hemicellulose fraction of the trees with soft and hard tissues, locust bean seeds, beans and other leguminous plants- and which hydrolyzes the β-1,4-D-mannosidase bonds (Tenkanen et al. 1997).

The mannanases are produced by animals, plants and microorganisms. The mannanase is purified and characterized from some types of fungi such as *Agaricus bisporus*, *Aspergillus tamarii*, *A. aculeatus*, *A. awamori* (Civas et al. 1984; Christgau et al. 1994; Kurakake and Komaki 2001; Setati et al. 2001; Tang et al. 2001), from some types of bacteria such as *Bacillus agaradhaerens*, *B.* AM001, *B. stearothermophilus*, *B. subtilis*, *Clostridium thermocellum*, *Flavobacterium* spp., *Paenibacillus curdlanolyticus*, *Dictyoglomus thermophilum* (Akino et al. 1989; Talbot and Sygusch 1990; Mendoza et al. 1994; Zakaria et al. 1998; Gibbs et al. 1999; Halstead et al. 1999; Bettiol and Showell 2002; Pason et al. 2006) and from some other organisms such as *Lycopersicones culentum* (plant), *Gastropoda pulmonata* (snail), *Pomacea insular* (snail), *Littorina brevicula* (mollusk) (Filichkin et al. 2009; Charrier and Rouland 2001; Yamamura et al. 1996). The microbial β-mannanases are commonly used in improving the quality of food, softening of pulps in paper and pulp industry, reducing the viscosity of coffee extracts, oil extraction and detergent industries (Dhawan and Kaur 2007).

Although *Bacilllus* spp. is soil-borne microorganisms, it is a major contamination agent in dairy food-products including meat, spices, cereals, baby food, rice, and vegetables. Due to their ability to form spores, these microorganisms continue their vital functions in the process of preparation of many foods (milk and fruit juice pasteurization, packaging, etc.). A few strain belonging to this genus (*B. licheniformis*,

B. subtilis, *B. thuringiensis* and *B. pumilus*) are commonly found in meat and meat products and traditional European sausages, but making food-borne disease incidence is low (Drobniewski 1993; Matarante et al. 2004; Yamanaka et al. 2007). The many species within the genus *Bacillus* are potential to secrete some extracellular enzymes such as high amylase, proteases, xylanases and cellulases. So, they play an important role in many biotechnological processes (Demir et al. 2011; Nadaroglu et al. 2010; Nadaroglu and Demir 2012).

In this study, β-1,4-mannanase enzyme produced extracellularly from the *B. pumilus* (M27) bacteria that was isolated from fermented sausage was purified, and characterized; its utility in the fruit juice clarification industry was investigated.

Materials and methods

Characterization of test bacteria

The sausage sample which was taken from a retail shop in Erzurum was aseptically brought to the laboratory. A 25 g sample was added to 225-mL peptone water (Merck, 7228), and homogenized in a stomacher (Laboratory Blender Stomacher 400, Seward Medical, London, UK) at room temperature for 3 min. The homogenate was ten times diluted in 0.85 % NaCl (Merck, 6404). 100 μL sample from dilution tubes were plated on tryptic soy agar (TSA) plates and incubated at 30 °C during 24–48 h. Different colonies were selected and analysed for Gram staining, cell morphology, oxidase and catalase reaction, presence of endospore, pH and temperature necessities, and the effect of NaCl concentration on the growth of isolates (Matos et al. 2006; Adiguzel and Atasever, 2009). Sugar fermentation patterns of test isolates were determined using the API 50CHB-test strips (bioMérieux, France). Based on Bergey's manual of systematic bacteriology, M27 was identified as *Bacillus pumilus* (Holt and Noel 1984).

At the end of the study, test strain (M27) was cultured on TSA medium and harvested with a scraper for total DNA extraction. Scrape cells were washed twice in 1 mL of STE buffer (50 mM Tris, 150 mM NaCl, 50 mM Na_2-EDTA) and then it was resuspended in STE (500 μL) to lysate at 75 °C, 30 min. The lysate was administered twice in CTAB/NaCl mixture and, DNA was extracted using phenol: chloroform: isoamyl alcohol (25:24:1) and precipitated in isopropanol as described by Adiguzel (2008).

Oligonucleotide primers were specifically developed to anneal the conserved positions in the 3′ and 5′ regions of the bacterial 16S rRNA genes; those were used to proliferate the 16S rRNA gene from purified genomic DNA. Whereas the forward primer UNI16S-L (5′-ATTCTAGAGTTTGATCAT GGCTCA-3′) corresponded to the positions from 11 to 26 of *Escherichia coli* 16S rRNA, and the reverse primer UNI16S-R (5′-ATGGTACCGTGTGACGGGCGGTGTGTA-3′) corresponded to all positions from 1,411 to 1,393 of *E. coli* 16S rRNA (Adiguzel et al. 2011). As mentioned by Beffa et al. (1996), the condition of PCR reaction was actualized, and, therefore, the PCR product was cloned to the pGEM-T vector system (Promega, UK).

The 16S rRNA gene sequence was created using the Applied Biosystems model 373A DNA sequencer after performing PCR amplification and cloning of the 16S rRNA gene of our strain (Macrogen, Korea). Then, BLAST search (Altschul et al. 1990) was used to compare this sequence with the one obtained from GenBank (Adiguzel et al. 2009). The related database was benefited for obtaining the 16S rRNA gene sequence of the species that were considered to be like the most closely our strain.

Purification of mannanase from *B. pumilus* (M27)

The purification process included 3 steps as ammonium sulfate precipitation, DEAE-cellulose and Sephacryl S200 gel filtration chromatography; and the extracellularly-produced mannanase enzyme was purified from the bacteria called *B. pumilus* (M27).

After filtering the crude enzyme extract, the crude enzyme extract was centrifuged at 5,000 rpm for 15 min. Right after this process, the enzyme homogenate ammonium sulfate was precipitated at intervals between 0 and 20 %, 20 and 40 %, 40 and 60 %, 60 and 80 %, and 80 and 100 %, respectively. Ammonia sulphate was used for the saturation of the obtained supernatant up to 60–80 %. After dissolving the precipitate in 20 mM sodium citrate (pH: 6.0), the precipitate was placed in a dialysis bag and dialyzed by stirred against the same buffer for 4 h at 4 °C.

Anion exchange chromatography

After the dialysis of the suspension had been performed through ammonium sulfate precipitation, 20 mM sodium citrate (pH: 6.0) and the previously equilibrated DEAE-cellulose ion exchange column (2.5×30) were realized. Until the eluate protein detection failed, the column was washed with the same buffer. Afterwards, the proteins attached to the column were eluted by applying NaCl gradient from 0 up to 1 M. The fractions were collected at a 3 mL/min flow rate as 3 mL. Absorbance of protein elution was measured at 280 nm spectrophotometrically. Locust bean gum substrate was used to measure the relevant activity in terms of fractions; subsequently, the active fractions were combined allowing to be kept at 4 °C.

Gel filtration chromatography

The active fractions provided from the anion exchange column were combined, dialyzed, desalted, and concentrated,

respectively with the anamicon membrane concentrator at 10-kDa cutoffs. By performing the aforementioned method, an enzyme solution was obtained, and this solution was administered to the Sephacryl S-200 column (120 cm×1 cm) pre-equilibrated with 20 mM phosphate buffer (pH: 5.0) which included 0.5 M NaCl. The salt concentration was adjusted using a gradient mixer. Subsequently, the enzyme was obtained with the same buffer. Afterwards, as mentioned above, the fractions had been analyzed, the active fractions were combined, concentrated; and they were kept at 4 °C to be used later.

All these steps performed for the purification of the mannanase enzyme were presented in Table 1. In the first step, mannanase enzyme's ammonium sulfate saturation was determined to be varying between 0 and 90 % ranges. The range of 60–80 % included the highest rate for the precipitation of the mannanase enzyme. So the mannanase enzyme was purified 33 times more with 74.5 % efficiency, and this was applied to DEAE-cellulose ion exchange column. In the second step, the enzyme peaked at DEAE-cellulose ion exchange column, and it was purified 69 times more with 31.5 % efficiency. In the third and the last step, the concentrated enzyme fraction was obtained from the ion exchange column, and was applied to the Sephacryl S200 column. Just a single peak was achieved with 110.6-fold purification. Besides, 36 % efficiency and 22 EU/mg protein specific activity was obtained as shown in Table 1.

Protein concentration

Bradford's method (Bradford 1976) that uses bovine serum albumin (BSA) in pre-determined standards was used to establish the protein concentration spectrophotometrically (absorbance at 280 nm). Briefly, 0.1 mL sample was added to 4.9 mL Comassie Brilliant blue solution (0.1 %, *w*/*v*) containing phosphoric acid (0.1 %, (*v*/*v*) and mixed. Then, it was allowed to stand at room temperature for 10 min and the absorbance was measured at 595 nm against blind sample which was formed by using pure water instead of enzyme.

Determination of mannanase activity

Locust bear gum substrate was used to determine the mannanase activity (Miller 1959). A 0.1 mL enzyme solution on the locust bean gum substrate dissolved within 0.9 mL 20 mM sodium citrate buffer (pH:6.0) was added to create the mixture of the reaction. After fulfilling this process, the mixture was incubated at 50 °C, and then through adding an average of DNS (3,5-dinitrosalisilic acid), the mannanase activity was specified monitoring the increase of absorbance at 540 nm (Bailey et al. 1992).

Locust bean gum as a substrate to determine Km and Vmax values was administered to identify the mannanase activity by taking measurements at 540 nm. The blank solution which was prepared by adding the enzyme-including buffer instead of the enzyme solution was used as a sample (Miller 1959).

SDS polyacrylamide gel electrophoresis

The sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was done, using a 10–30 % discontinued electrophoresis method developed by Laemmli (1970). After administering 20 μg protein to each sample, the electrophoresis process was performed in a gel casting apparatus (Bio-RAD) execution buffer (0.25 M Tris, 1.92 M Glycine, 1 % SDS (pH: 8.3) at 4 °C.

The gel was dyed with 0.1 % Coomassie Brilliant Blue R-250 in 50 % methanol, 10 % acetic acid, and 40 % distilled water for 1.5 h. Subsequently, the gel was washed with 50 % methanol, 10 % acetic acid and 40 % distilled water until obtaining its surface as limpid. And after this process, the electrophoretic pattern was photographed

Molecular weight determination by gel filtration

Column (3×70 cm) of Sephadex G100 was used to determine the molecular weight of the mannanase enzyme. Performing 0.05 M Na_2HPO_4, 1 mM dithioerythritol, pH: 7.0 buffer, the column was equilibrated until reaching zero absorbance.

Table 1 The purification process of mannanase from *B. pumilus* (M27)

Enzyme fraction	Volume	Activity	Total activity		Protein	Specific activity	Purification
	mL	EU/mL	EU	%	(mg/mL)	EU/mg	Fold
Crude extract	50	25.7±0.22	1.29×10^2	100	128.8±1.18	0.199	–
$(NH_4)_2SO_4$	48	20.1±1.11	9.65×10^2	74.8	3.1±0.38	6.5	32.7
DEAE-sephadex	20	15.2±0.32	3.04×10^2	31.5	1.1±0.3	13.82	69.45
Sephacryl S 200	20	5.5±2.5	1.1×10^2	36.2	0. 25±0.44	22.0	110.6

One EU of β-mannanase was defined as the amount of enzyme required to release 1 μmole of mannose reducing sugar equivalents per min under the defined assay conditions

Before eluting from the column with the same buffer, the standard protein solution (bovine serum albumin, 66 kDa; egg ovalbumin, 45 kDa; pepsin, 34 kDa; trypsinogen, 24 kDa; ß-lactoglobulin and lysozyme, 14 kDa) was administered to the column. After applying the standard protein solution, the pure mannanase enzyme was performed to the column, and the column was eluted under the same conditions, as well. The flow-rate of the column was measured as 20 mL/h. We finally compared the elution volume with standard proteins (Whitaker 1963).

Studies on certain in vitro chemicals and metal ions

The effect of the Mn^{2+}, Co^{2+}, Cu^{2+}, Zn^{2+}, Fe^{2+}, Ca^{2+} and Ni^{2+} metal ions were investigated in differently inhibitory concentrations on the purified mannanase enzyme. Each inhibitor solution was prepared at 1 and 5 mM in the reaction mixture of cuvette, and each solution was added in a cuvette containing 0.5 mL enzyme. Its total volume was adjusted to 3 ml with buffer solution. A control assay of the enzyme activity was done without inhibitors and resulting in activity was taken as 100 %. For each concentration, whole metal ions were tested in three replications. The effect of each agent was determined by measuring the enzyme activity using the locust bean gum as a substrate.

Clarification of some fruit juice

The fruits collected at local markets such as peach, grape, apple, apricot and orange were used for preparing the fruit juice. First of all, the fruits were washed elaborately; then they were kept in air to dry, and they were homogenized. Into the 10 g fruit sample homogenate, 2 mL enzyme solution was added (adding 2 mL distilled water within a controlled environment). Using 2 mL crude homogenate, the same process was re-operated. At a natural pH value, the fruit homogenates were processed with enzyme for 1 h at 60 °C. The juice at a specific amount was filtered through a paper filter for 12 min, and volume measurement of the fruit juice was carried out before calculating the juice extract. A control was prepared without added enzyme for all the assays (Demir et al. 2011; Nadaroglu et al. 2010). Clarified juice yield (%) was calculated as (volume of clear juice * 100) / (volume of sample).

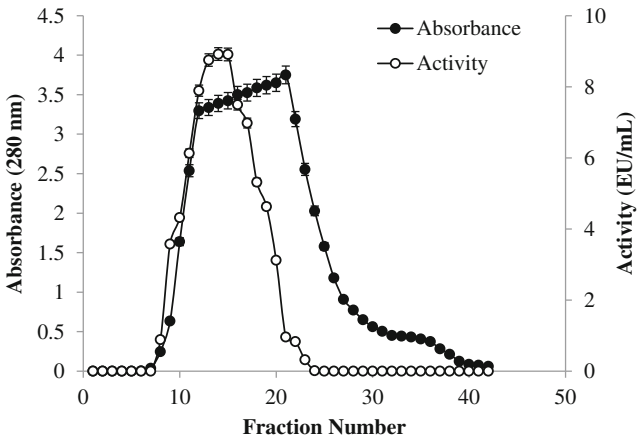

Fig. 1 Purification of mannanase by ion exchange chromatography using DEAE-sephadex

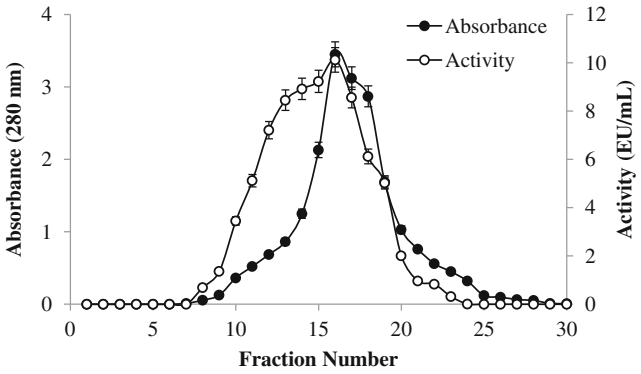

Fig. 2 Elution profile of protein and mannanase activity on Sephacryl S200

Statistical analysis

For determining the samples' mannanase activities, all tests were performed in three replications. Average±standard errors of all obtained data were defined. The average standard errors of the data were expressed. SPSS version 10.0 software (SPSS Inc., Chicago, IL., USA) was used for the statistical analysis; and Tukey test was performed for determining the significant differences at 95 % confidence interval ($p<0.05$).

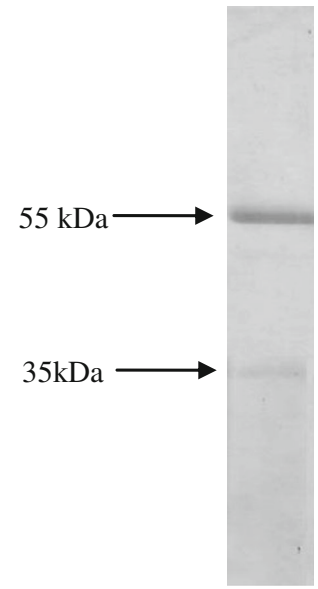

Fig. 3 SDS-PAGE electrophoretic pattern of mannanase [purified mannanase enzyme from *B. pumilus* (M27)]

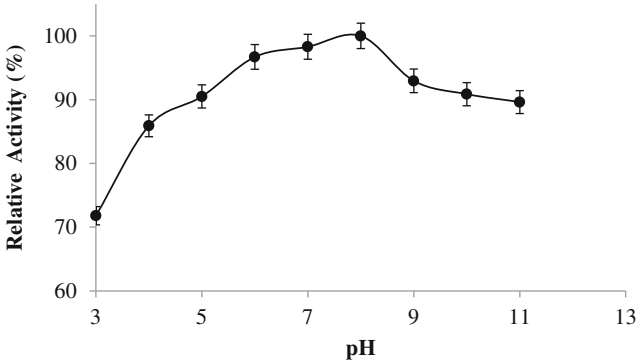

Fig. 4 The effect of pH on the activity of purified mannanase from *B. pumilus* (M27)

Results and discussion

Isolation of test strain

The test strains isolated in this study was identified by utilizing various conventional methods. The results obtained suggested that the gram-positive, aerobic, motile, catalase, oxidase and endospore positive rod and that it was reproduced well at 30–45 °C at pH 5.0–7.0 and at 2–6 % salt concentrations. Later on, in consequence of the analyses performed by utilizing the API 50CHB test system, this isolate was identified to be the *Bacillus pumilus* (data not shown). These results obtained were determined to comply with the literature data (Bottone and Peluso 2003; Parvathi et al. 2009; Liu et al. 2013).

16S rRNA gene sequence analysis

The test isolate was determined to be containing nearly 1,452 nucleotides (nt) at the end of the 16S rRNA sequence analysis. Then, it was also determined as a result of the blast study conducted through the GenBank database that this isolate resembled *B. pumilus* (Genbank Number; KF895383) at a rate of 99 % which indicated a strong resemblance.

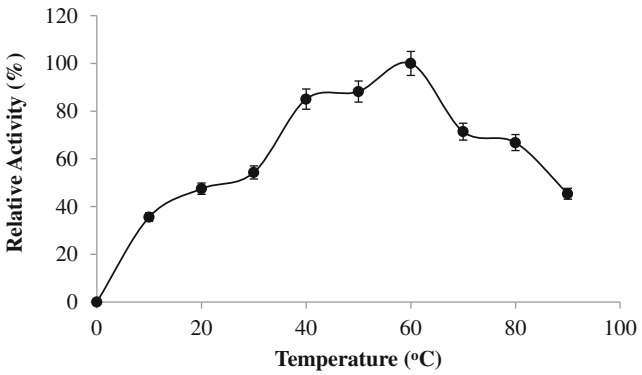

Fig. 5 The effect of temperature on the activity of purified mannanase from *B. pumilus* (M27)

Table 2 Effect of some metal ions on the mannanase activity from *B. pumilus* (M27)

Chemical compounds	Concentration (mM)	Relative activity (%)	Concentration (mM)	Relative activity (%)
None	–	100±0.0	–	100±0.0
Ca^{2+}	1	140.0±0.1	5	140.2±0.6
Mn^{2+}	1	288.6±1.3	5	328.7±1.01
Co^{2+}	1	130.4±0.5	5	218.6±0.2
Zn^{2+}	1	272.2±2.3	5	315.7±2.4
Cu^{2+}	1	100.2±0.2	5	228.2±0.6
Fe^{2+}	1	343.2±1.6	5	344.1±0.3
Ni^{2+}	1	100.2±2.0	5	123.7±1.8

Each value represents the mean of 3 replicates experiments±standard deviation

As confirmed by Antara et al. (2002) and Mandal et al. (2008), similarity of 16S rRNA gene sequences to *B. pumilus* type strains was higher than 98 %, and in line with this, we also had a similar value (≥98 %) to the one determined by them in terms of the 16S rRNA gene sequences of *B. pumilus* isolates.

Purification and characterization of mannanase from *B. pumilus* (M 27)

In Figs. 1 and 2, it was presented the elution profiles of the mannanase enzyme purified from the bacterium *B. pumilus* (M27) benefiting from both anion-exchange chromatography and gel filtration chromatography.

As could be seen in Fig. 3, the mannanase enzyme was specified to be including 2 sub-units as 35 kDa and 55 kDa at the end of the sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Sephadex G-100 gel filtration chromatography was used to measure the molecular weight of

Table 3 Production of some fruit juice by using mannanase from *B. pumilus* (M27)

Fruit (5 g)		Control	Mannanase	Crude extract
Orange	Volume	15.0±0.11	16.5±1.3	16±0.2
	Yield (%)	–	110.1±0.6	106.7±0.7
Apricots	Volume	9.5±0.2	11.0±2.4	11.5±0.12
	Yield (%)	–	115.8±0.21	121.1±0.32
Grape	Volume	11.5±1.01	13.5±0.16	12.0±4.1
	Yield (%)	–	117.4±1.45	104.3±1.21
Apple	Volume	11.0±2.11	17.0±1.23	17.0±0.46
	Yield (%)	–	154.5±1.11	154.5±0.45
Peach	Volume	6.5±0.44	7.5±0.01	14.0±0.4
	Yield (%)	–	115.4±0.15	215.4±1.2

the enzyme's active form; the graphics of log MA-Kav was drawn and the same result was obtained.

As reported in a study that was carried out before, the beta-mannanase gene of *Aspergillus aculeatus* was propagated and was placed on *A. oryzae*. The molecular weight of the obtained recombinant enzyme was determined as 45 kDa (Christgau et al. 1994), and the molecular weight of the mannanase enzyme obtained from *A. niger* was determined as 40 kDa (Ademark et al. 1998).

The studies were carried out in 7 different environments varying from pH 2 to pH 9 for specifying the optimum pH of the mannanase enzyme purified and produced from *B. pumilus* (M27). Acetate buffers were used from pH 2 to 5, phosphate buffers were used from pH 6 to 8, and Tris /HCl buffers were used from pH 8 to 9. Locust bean gum substrate was performed for measuring the activity, and the obtained results were benefited for calculating the activity. The graphics of activity - pH were drawn, and the results were all presented in Fig. 4. It was determined that the optimum pH value of the purified mannanase enzyme produced from *B. pumilus* (M27) for the locust bean gum substrate was 5.0.

Optimum temperature

For the locust bean gum substrate, the activity was measured at degrees varying between 20 and 90 °C keeping for 5 min at each temperature for determining the optimum pH value of the purified mannanase enzyme. As it was presented in Fig. 5, the mannanase enzyme was fairly active between 30 and 80 °C, and this indicated the maximum activity at 60 °C. Similarly, it was also determined that the highest pH value of beta-mannanase enzymes of *A. aculeatus* and *A. nigers* was 5.0 and the highest operating temperature was determined as 60–70 °C (Christgau et al. 1994; Ademark et al. 1998).

The results of V_{max} and K_m values

Several studies have been carried out related to V_{max} and K_m values of the mannanase enzyme using the 0.3 mM locust bean gum. V_{max} was determined as 78.74 mol/min mg and K_m was determined as 0.592 mM for a pure mannanase enzyme.

The effects of some metal ions on the activity of the mannanase enzyme purified from *B. pumilus* (M 27)

In this study, the effects of the Mn^{2+}, Co^{2+}, Cu^{2+}, Zn^{2+}, Fe^{2+}, $Ca^{2+}$and Ni^{2+} metal ions upon the activity of the mannanase enzyme purified from *B. pumilus* (M 27) was investigated. In Table 2, the inhibition effects obtained in the study and the activity values versus concentration were presented; moreover, as could be understood from Table 2, whole metal ions enhanced the activity of purified mannanase enzyme. On the other hand, Mn^{2+} and Zn^{2+} metal ions had the highest activation, and those enhanced the mannanase activity up to 295.9 and 279.4 %, respectively. These results improved that all the metal ions could not inhibit the mannanase enzyme purified from *B. pumilus* (M 27), but they at least activated. And because mannanase enzymes had such features, they were regarded to be fairly convenient for the industrial use of mannanase.

Production juice using raw fruit material (especially the fruit with high-mannanase content) without any enzymatic treatments requires a highly complicated process. The use of enzymes increases the juice output, but it also reduces its viscosity. In Table 3, the experiments results obtained from yielding fruit juice from apple, orange, peach, grapes and apricots were presented. On the other hand, it should also be taken into consideration that the most significant advantage of producing fruit juice by enzymatic treatment was compared with the control.

As reviewed in the literature, fruit juice purification process was performed using the mannanase enzyme. The results obtained in our study were compatible with the ones in the literature (Zhao et al. 2010).

Conclusions

The mannanase was firstly purified and characterized from Bacillus pumilus (M27) This research was focused on the food industry. To sum up, the findings obtained from the mannanase enzyme's catalytic activities purified from *B. pumilus* (M 27) were very high, and those were resistant to metal ions and highly stable against the temperature and different pHs. Besides, those were efficient upon the clarification of fruit juice; therefore, those could be regarded as commonly applicable.

References

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

Adiguzel G. (2008) Phenotypic and genotypic characterization of lactic acid bacteria isolated from Turkish dry fermente sausage PhD Thesis, Ataturk University, Graduate School of Health Sciences, Erzurum, Turkey, 110

Adiguzel G, Atasever M (2009) Phenotypic and genotypic characterization of lactic acid bacteria isolated from Turkish dry fermented sausage. Rom Biotech Lett 14(1):4130–4138

Adiguzel A, Inan K, Sahin F, Arasoglu T, Gulluce M, Belduz AO, Baris O (2011) Molecular diversity of thermophilic bacteria isolated from pasinler hot spring (Erzurum, Turkey). Turk J Biol 35:267–274

Adiguzel A, Ozkan H, Baris O, Inan K, Gulluce M (2009) Identification and characterization of thermophilic bacteria isolated from hot springs in Turkey. J Microbiol Meth 79:321–328

Akino T, Kato C, Horikoshi K (1989) Two *Bacillus* beta-mannanases having different COOH termini are produced in *Escherichia coli* carrying pMAH5. Appl Environ Microbiol 55(12):3178–3183

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410

Antara NS, Sujaya IN, Yokota A, Asano K, Aryanta WR, Tomita F (2002) Identification and succession of lacticacid bacteria during fermentation of 'urutan', a Balinese indigenous fermented sausage. World J Microbiol Biotech 18:255–262

Bailey MJ, Biely P, Poutanen K (1992) Interlaboratory testing methods for assay of xylanase activity. J Biotech 23:257–270

Beffa T, Blanc M, Lyon PF, Vogt G, Marchiani M, Fischer JL, Aragno M (1996) Isolation of *Thermus* strains from hot composts (60 to 80 °C). Appl Environ Microbiol 62:1723–1727

Bettiol JP, Showell MS (2002) US Patent 6376445

Bottone EJ, Peluso R (2003) Production by *Bacillus pumilus* (MSH) of an antifungal compound that is active against Mucoraceae and *Aspergillus* species: preliminary. J Med Microbiol 52:69–74

Bradford MM (1976) Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Chem 2:248–253

Charrier M, Rouland C (2001) Mannan-degrading enzymes purified from the crop of the brown garden snail helix aspersa müller (*Gastropoda pulmonata*). J Exp Zool 290(2):125–135

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 l33 (5):917–925

Civas A, Eberhard R, Le Dizet P, Petek F (1984) Glycosidases induced in *Aspergillus tamarii* Secreted alpha-D-galactosidase and beta-D-mannanase. Biochem J 219:857–863

Demir N, Nadaroglu H, Tasgin E, Adıgüzel A, Gulluce M (2011) Purification and characterization of a pectin lyase produced by *Geobacillus stearothermophilus* (Ah22) and fruit juice application. Ann Microbiol 61(4):939–946

Dhawan S, Kaur J (2007) Microbial mannanases: an overview of production and applications. Crit Rev Biotech 27(4):197–216

Drobniewski FA (1993) *Bacillus cereus* and related species. Clin Microbiol Rev 6:324–338

Filichkin SA, Priest HD, Givan SA, Shen R, Bryant DW, Fox SE, Wong WK, Mockler TC (2009) Genome-wide mapping of alternative splicing in *Arabidopsis thaliana*. Genome Res 20(1):45–58

Gibbs MD, Reeves RA, Sunna A, Bergquist L (1999) Sequencing and expression of a β-Mannnase gene from the extreme thermophilic *Dictyoglomus thermophilum* Rt46B1, and characteristics of the recombinant enzyme. Curr Microbiol 39:351–357

Halstead JR, Vercoe PE, Gilbert HJ, Davidson K, Hazlewood GP (1999) A family mannanase produced by *Clostridium thermocellum* as a component of the cellulosome contains a domain which is conserved in mannanases from anaerobic fungi. Microbiol 145:3101–3108

Holt JG, Noel RK (1984) Bergey's Manual of Systematic Bacteriology, Volume 1–4. Lippincott Williams & Wilkins, New York

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. Nat 22:680–685

Liu Y, Lai Q, Dong C, Sun F, Wang L, Li G, Shao Z (2013) Phylogenetic diversity of the *Bacillus pumilus* group and the marine ecotype revealed by multilocus sequence analysis. Plos One 8(11):1–11. doi:10.1371/journal.pone0080097

Mandal V, Sen SK, Mandal NC (2008) Optimized culture conditions for bacteriocin production by *Pediococcus acidilactici* LAB 5 and its characterization. Indian J Biochem Biotech 45(2):106–110

Matarante A, Baruzzi F, Cocconcelli PS, Morea M (2004) Genotyping and toxigenic potential of *Bacillus subtilis* and *Bacillus pumilus* strains occurring in industrial and artisanal cured sausages. Appl Environ Microbiol 70(3):5168–5176

Matos JST, Jensen BB, Barreto SFHA, Hojberg O (2006) Spoilage and pathogenic bacteria ısolated from two types of Portuguese dry smoked sausages after shelf-life period in modified atmosphere package. Cienc Tecnol Aliment 5(3):165–174

Mendoza NS, Arai M, Kawaguchi T, Cubol FS, Panerio EG, Yoshida T (1994) Isolation of mannanutilizing bacteria and the culture conditions for mannanase production. World J Microbiol Biotechnol 10: 51–54

Miller GL (1959) Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem 31:426–428

Nadaroglu H, Demir N (2012) Purification and characterization of a novel serine protease compositain from compositae (*Scorzonera hispanica L*). Eur Food Res Technol 234(6):945–953

Nadaroglu H, Taskin E, Adiguzel A, Gulluce M, Demir N (2010) Production of a novel pectin lyase from bacillus pumilus (P9), purification and characterization and fruit juice application. Rom Biotech Lett 15(2):5167–5176

Parvathi A, Krishna K, Jose J, Joseph N, Nair S (2009) Biochemical and molecular characterization of *Bacillus pumilus* isolated from coastal environment in cochin, India. Braz J Microbiol 40:269–275

Pason P, Kyu KL, Ratanakhanokchai K (2006) *Paenibacillus curdlanolyticus* strain B-6 xylanolytic-cellulolytic enzyme system that degrades insoluble polysaccharides. Appl Environ Microbiol 72:2483–2490B

Setati ME, Ademark P, Van Zyl WH, Hahn-Hagerdal B, Stålbrand H (2001) Expression of the *Aspergillus aculeatus* Endo-b-1,4-mannanase Encoding Gene (*man1*) in *Saccharomyces cerevisiae* and characterization of the recombinant enzyme. Protein Exp Purif 21:105–114

Talbot G, Sygusch J (1990) Purification and characterization of thermostable beta- mannanase and alpha-galactosidase from *Bacillus stearothermophilus*. Appl Environ Microbiol 56(11):3505–3510

Tang CM, Waterman LD, Smith MH, Thurston CF (2001) The cel4 gene of *Agaricus bisporus* encodes a beta-mannanase. Appl Environ Microbiol 67(5):2298–2303

Tenkanen M, Makkonen M, Perttula M, Viikari L, Teleman A (1997) Action of *Trichoderma reesei* mannanase on galactoglucomannan in pine kraft pulp. J Biotechnol 57:191–204

Whitaker JR (1963) Determination of molecular weight of proteins by gel filtration on sephadex. Anal Chem 35:1950–1953

Yamamura I, Nozaki Y, Matsumoto T, Kato T (1996) Purification and some properties of endo-β-1,4 mannanase from a marine mollusk *Littorina brevicula*. Biosci Biotechnol Biochem 60:674–676

Yamanaka H, Moriyoshi K, Ohmoto T, Ohe T, Sakai K (2007) Degradation of bisphenol a by *Bacillus pumilus* isolated from kimchi, a traditionally fermented food. Appl Biochem Biotech 136:39–51

Zakaria MM, Ashiuchi M, Yamamoto S, Yagi T (1998) Optimization for β-mannanase production of a psychrophilic bacterium, *Flavobacterium* sp. Biosci Biotechnol Biochem 62:655–660

Zhao J, Shi P, Luo H, Yang P, Zhao H, Bai Y, Huang H, Wang H, Yao B (2010) An acidophilic and acid-stable β-mannanase from *Phialophora* sp P13 with high-mannan hydrolysis activity under simulated gastric conditions. J Agr Food Chem 58:3184–3190