Copyright © 2019 University of Bucharest
Printed in Romania. All rights reserved
ISSN print: 1224-5984
ISSN online: 2248-3942

Rom Biotechnol Lett. 2019; 24(4): 586-594
doi: 10.25083/rbl/24.4/586.594

Received for publication, May, 25, 2017
Accepted, February, 25, 2018

Original paper

Tannase activity by *Lactobacillus brevis* strains isolated from fermented food

MERIH KIVANÇ[1*] ONUR TEMEL[2]

[1]Anadolu University Faculty of Sciences Department of Biology, Eskisehir, Turkey
[2]Anadolu University Graduate School of Sciences Biotechnology Department, Eskisehir, Turkey

Abstract In this study, 17 L. brevis strains obtained from fermented products in previous studies were purified and these selected isolates were identified by the DuPont Qualicon RiboPrinter® system. Spectrophotometric and visual reading method indicated that isolate *L. brevis* A6X showed the highest activity. It was observed that pyrogallol product with the decarboxylase activity of gallic acid was composed of tannic acid using the thin layer chromatography. The proximate molecular weights of the enzyme produced by the selected isolate of *L. brevis* were determined by the SDS-PAGE method, and also the effect of different temperature, pH, substrate concentration, the concentration of tannic acid and various minerals on activity were all investigated. As a result of the test, it was found that the optimum temperature was 37°C, pH 5.0, methyl gallate concentration used as the substrate 7 mM and tannic acid concentration 1.75 mM. While enzyme activity increased in the presence of Ca^+, Zn^+ and K^+ ions, it did not show any changes in the presence of surfactant (Tween 80), inhibitor (DMSO), denaturing agent (urea). In addition, in presence of Mg^+, Hg^+ and Zn^+ ions caused the tannase activity to decrease.

Keywords Tannase, lactic acid bacteria, *Lactobacillus brevis*, methyl gallate, pyrogallol.

To cite this article: KIVANÇ M, TEMEL O. Tannase activity by *Lactobacillus brevis* strains isolated from fermented food. *Rom Biotechnol Lett*. 2019; 24(4): 586-594. DOI: 10.25083/rbl/24.4/586.594

*Corresponding author: MERIH KIVANÇ, Anadolu University Faculty of Sciences Department of Biology, 26470, Eskisehir, Turkey
E-mail: mkivanc@anadolu.edu.tr

Introduction

Vegetable tannins are water-soluble polyphenols in a variety of plants including food and animal feed. Tannins have the ability to bind with proteins to form indigestible complexes and they, thus, are not easily degraded by plant-eating animals. They are thought of dietary undesirable because they prevent the digestive enzymes and the impact makes use of vitamins and minerals and thereby it may result in adverse health effects (CHUNG & al [1]; BHAT & al [2]; RODRIGUEZ & al [3]).

Tannase (tannin acyl hydrolase) catalyses the galloyl ester bonds of being tannic acid the most typical hydrolysable tannins to glucose and gallic acid which operate in the synthesis of propyl gallate, so that their protein-binding properties are prevented (RODRIGUEZ & al [3]; AGUILAR and GUTIERREZ-SANCHEZ [4]). Tannase is extensively utilized for the preparation of wine, coffee-flavoured soft drinks, clarification of beer and fruit juices, and detannification of foods. Gallic acid is utilized in the food sector in the synthesis of antibacterial drugs and pharmaceutical industries and foodstuffs preservatives, respectively, pyrogallol and gallates. However, propyl gallate is a very great importance food antioxidant (SHARMA and GUPTA [5]: KWON & al [6]; BELUR & al [7]).

Plant, animal, and microbial sources are known as tannase manufacturer; it is widely obtained from a diverse classification of microorganism. Recently, OSAWA *&* al [8] have reported isolated lactobacilli species including *Lactobacillus plantarum*, *L. paraplantarum* and *L. pentosus.* Lactobacilli can play a crucial role in the production of many fermented foods, which are presented as potential probiotics (AGUILAR *& al* [9]; RODRIGUEZ & al [3]; OSAWA & al [8], MATEI & al [10]). As far as is known, there is no information regarding the tannase activity by *L. brevis.* Therefore, in this paper, we demonstrated the tannase production capability and reported the degradation of tannic acid by *L. brevis.* It is the bacteria' ability to ferment carbohydrates that the food producers make use of which eventually helps desirable foods to be created. The fact that *L. brevis* was as a crucial member of the probiotic microorganisms was recognized only recently and these microorganisms actively promoted the health of the human gut. *L brevis* is probiotics that show anti-cancer activities in addition containing anti-inflammatory properties. (IYER and VERSALOVİC [11]). While *L. brevis* provides natural protection for foods, especially for fruit juice products, when it is used in fruit juice that contains grains like pomegranate juice, these products that have sour tastes due to the grains can be improved into more favourable products and eventually the consumers can be presented healthier products. The lactic acid bacteria have not been used much in studies of tannase enzymes. However, with the ecological advantages it possesses, it constitutes a potential in this area (OSAWA & al [8]). Therefore, tannase activity abilities of the isolates of *L. brevis* isolated from fermented vegetables were investigated for the first time in the present study, and attempts were exerted to determine the culture conditions for tannase production.

Materials and Methods

Materials

L. brevis strains isolated from the fermented products was provided by the Department of Biology, Faculty of Science of Anadolu University. The *L. brevis* strains stock culture was maintained in MRS agar slants. The *L. brevis* strains were preserved within Ependorf tubes of 20% glycerol at -85°C.

Automated ribotyping

The *L. brevis* strains whose biochemical and physical properties were determined were identified through automatic ribotyping. On the basis of the system lies comparing the size of the band that occurred as a result of 16S rRNA being cut by the EcoRI enzyme and its execution on the gel with the marker used, and defining them on the basis of the most appropriate similarity to bacteria in the database. The identification procedures were performed through the kit according to administrator's instructions.

Ribotyping patterns were finally compared by Ribotyping analysis software through the databases of similar code patterns so as to identify the genus and species.

Tannase and gallate decarboxylase activity determination

In order to determine the tannase activity of lactic acid bacteria, firstly the cultures were planted into the MRS broth, and were incubated in the oven 37°C for 24 hours. The culture over the MRS agar was suspended within 1 ml of substrate medium (pH 5, 33 mmol/l NaH_2PO_4, and containing 20 mmol/l methyl gallate) in the form of $9x10^8$ cells/ml. The tubes were incubated at 37°C for 24 hours. After incubation, the tubes were alkalized by adding an equal amount of saturated $NaHCO_3$ solution (pH 8.6) and were left at room temperature for 1 hour. At the end of this period, the tubes constituting brown and green colours were evaluated as tannase positive.

In order to determine the decarboxylase gallate activity, 50 µl were taken from the overnight culture and transferred into 10 ml of MRS broth containing 10 mM gallic acid and the tubes were incubated at 37°C for 3 days. After incubation, by being mixed in equal amounts with

saturated $NaHCO_3$ solution, it was left in the oven at 37ºC for 1 hour. The change of medium's colour from brown to a clear yellow was evaluated as positive in terms of decarboxylase activity (OSAWA and WALSH [12]).

Screening of tannase-producing bacteria by plate assay

The cultures showing tannase activity were incubated at 37ºC for 24 hours after being transferred into the tubes containing 5 ml MRS broth medium. After incubation, 10 µl was taken each culture and inoculated into the Brain Heart Infusion Agar (BHI) with supplemented 2% tannic acid and 0.5% yeast extract. Inoculated petri dishes were incubated at 37°C for 72 hours. After incubation, the formation of a clear zone around the colonies was regarded as positive in terms of tannase activity (NISHITANI and OSAWA [13]).

Determination of the tannase activity of *L. brevis* strains by spectrophotometer

Tannase enzyme activity of lactic acid bacteria were analysed according to the method proposed by NISHITANI and OSAWA [14].

Detection of tannic acid degradation by thin layer chromatography

Tannic acid, gallic acid and pyrogallol in the culture media, according to KWON & al [6] were analysed by the TLC method. The prepared 5 ml of MRS Broth was centrifuged at 8000 g 20 minutes after the isolates planted in the medium were revived. After that, the cells were suspended in the 2 ml of 33 mM ammonium acetate ($CH_3CO_2NH_4$) buffer (pH 5.6) containing 1% of tannic acid. After being incubated at 37°C for 24 hours under aerobic conditions, this reaction mixture was centrifuged (8000 g x 20 min.) and a supernatant was obtained. The supernatant was spotted onto the thin layer. Glass plates were kept at room temperature for 30-45 minutes. By placing the TLC plaque on the tank, formic acid-acetonitrile-toluene was conducted (1:40:20). And then, spraying was conducted onto the plate by the iodine solution (0.5 g iodine in 100 ml containing 95% ethanol). The plate was heated at 110°C for 10 minutes and the reaction products were observed (KWON & al [6]).

Effect of culture conditions on the activity of tannase

After the A6X, ES2 and KT2 strains showing the highest tannase activity were developed separately in the medium and were centrifuged at 8000 g for 20 minutes, their supernatant parts were used in the tests. Different substrate concentrations were prepared in phosphate buffer at different pH values and at different incubation temperatures and the cultural conditions in which the highest tannase activity was achieved were determined.

Optimum temperature and pH for tannase activity

The 50 µl of the cell-free extracts obtained from the *L. brevis* strain, by being added to the NaH_2PO_4 33 mM (pH 5.0) buffer prepared as 5 ml containing 5 mM methyl gallate, was incubated at different temperatures for 24 hours (4, 20, 25, 30, 37, 45, 55). Taking on 100 µl from the mixture incubated, an equal volume of saturated $NaHCO_3$ (pH 8.6) solution was added. This mixture then was incubated for 2 hours at different temperatures. Subsequently, this mixture was vortexed and centrifuged (8000 g x 20 minutes). After centrifugation, it was evaluated by being pipetted into 96 welled Elisa petri and being evaluated at 450 nm.

Optimum pH for tannase activity

In order to determine the optimum pH of the tannase activity, 50 µl from cell-free filtrate of *L. brevis* strains, the prepared 5 ml of 33 mM NaH_2PO_4 containing 5 mM methyl gallate were added and mixed to the buffer at different pH (3, 4, 5, 6, 7, 8, 9) and was incubated at 37ºC for 24 hours; after incubation, by taking 100 µl from each mixture separately and mixing them with equivalent saturated $NaHCO_3$, they were incubated at 37ºC for 24 hours. They were evaluated at 450nm at the spectrophotometer.

Effect of tannic acid concentration

The 50 µl of extract (supernatant) obtained from the isolates was added into 5 ml different concentration of tannic acid (0.438, 0.875, 1.750, 3.500, 7.000 mM) phosphate buffer (33 mM, pH 5.0) and was incubated at 37ºC for 24 hours. After incubation, 100 µl of this mixture was taken and added into equal volume of saturated $NaHCO_3$ solution (pH 8.6) was added; after incubation at 37ºC for 2 hours again, this mixture was vortexed and centrifuged (8000 g x 20 minutes). Subsequently, 100 µl of this mixture was taken and transferred into 96 well petri dish and evaluated at 450 nm at the spectrophotometer.

Effect of different additives in tannase activity

Different metal ions such as magnesium chloride ($MgCl_2$), potassium chloride (KCl), zinc chloride ($ZnCl_2$), mercury-II chloride ($HgCl_2$), calcium chloride ($CaCl_2$), Tween 80 (surfactant), urea (denaturing), chelation, ethylene diamine tetra acetic acid (EDTA) and inhibitor dimethyl sulfoxide (DMSO) were separately prepared in 5 ml of phosphate buffer and were thawed in 1 mm (33 mM, pH 5.0). Subsequently, the supernatant of *L. brevis* stains prepared of 50 µl were added into 5 ml mixture and were incubated 37ºC for 24 hours were added to the mixture and at 37ºC for 24 hours. After incubation, the mixture was taken into different tubes and added the same volume of

$NaHCO_3$ (pH 8.6) solution and were incubated at 37ºC for 2 hours. After incubation, it was vortexed and centrifuged (8000 g x 20 minutes). After centrifugation, 100 µl was pipetted and transferred into 96 welled petri in the form of four series and evaluated at 450 nm wave length.

Molecular mass determination by SDS-PAGE

The molecular size of the tannase enzyme was investigated according to the SDS-PAGE method by RODRIGUEZ & al [9]. The separation gel with 12.5% acrylamide/bisacrylamide concentrations 1000 u and 1,2 U concentrations were compared with the tannase enzyme (Sigma 42395). Dimensional analysis of the protein profile obtained was carried out by the broad-range protein marker (Sigma M4038).

Determination protein content with Bradford method

The solution whose protein concentration is unknown was treated by the Bradford reagent, the absorbance of the solution prepared against the blind was read at room temperature in a spectrophotometer. Standard protein solutions prepared at different concentrations (BSA) were also treated with reagent and their absorbance values were measured, and using the measurement results, a concentration graph against absorbent was drawn. The protein amount of this solution whose concentration was unknown was calculated from this graph. 5 µl standard protein solution was transferred into a tube with 250 µl Bradford solution. In order for the colour to become stable, it was distributed to the petri with multiple wells and was left at room temperature for 20 minutes. Subsequently, the plate was evaluated at the spectrophotometer at 595 nm and the amount of protein was calculated.

Results

Automated ribotyping of *L. brevis strains*

L. brevis strains isolated from the fermented food are given in Table 1. RiboPrinter® Microbial Characterization System (DuPont Qualicon) results are shown in the Figure 1. A total of 17 strains of the *L. brevis* clustered into 6 Ribogroup pattern sets. Similarity between these 6 ribogroups ranged from 0.79 to 0.98. *Eco*RI ribotyping differentiated the isolates into 6 distinct ribotypes (Fig. 1). The 6 distinct ribotypes belonged to 7 different DUP-IDs.

Eight strains had the same pattern as the type strain and similarity level of 98-88%. Four isolates had the same pattern as the type strain DUP 5010. Other strains merged at the similarity level of 97-79% with the type strain.

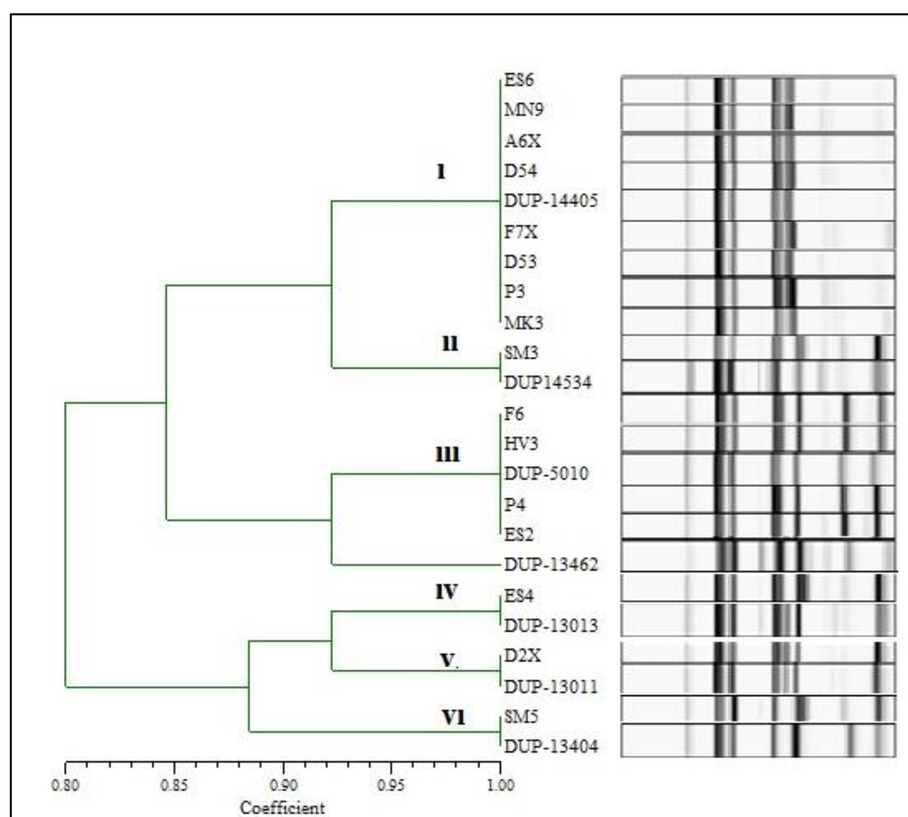

Figure 1. Cluster analysis of the *L. brevis* isolates from fermented food. Dendrogram based on UPGMA cluster analysis.

Table 1. Tannase and Gallate decarboxylase activities of *L. brevis* isolates that were isolated from fermented foods.

Isolates	Fermented Products	Tannase	Gallate decarboxylase	Colony count (CFU/ml)	Specific tannase activity U/10^7 CFU	U/mg Protein
MN9	Pickled mushroom (*Agaricus bisporus* (J.E. Lange) Imbach	++	+	2,8x10^7	0,83	2,93
F-7X	Pickled green bean (*Phaseolus vulgaris* L.)	++	-	3,2x10^7	0,80	6,20
D2X	Pickled tomato (*Solanum lycopersicum L.)*	+	+	2,9x10^7	0,71	2,35
P3	Picled beetroots (*Beta vulgaris* L.)	+	+	2,6x10^7	0,69	1,97
P4	Picled beetroots (*Beta vulgaris* L.)	+	+	2,5x10^7	0,56	1,92
ES2	Sucuk (Fermented meat products)	+	+++	3,2x10^7	0,62	1,90
DS4	Sucuk (Fermented meat products)	+	+	2,4x10^7	0,66	1,47
HV3	Pickled carrot (*Daucus carota L)*	++	+	1,8x10^7	0,71	1,86
ES4	Sucuk (Fermented meat products)	+	+	3,3x10^7	0,67	2,93
SM3	Bread dough (Dough)	+	-	2,4x10^7	0,52	1,76
DS3	Sucuk (Fermented meat products)	+	-	4,6x10^7	0,66	1,98
ES6	Sucuk (Fermented meat products)	+++	++	2,9x10^7	1,14	5,57
A6X	Picled cucurbita*(Cucurbita pepo L)*	++	+++	1,2x10^7	1,17	2,98
SM5	Bread dough (Dough)	+	-	2,5x107	0,77	3,49
MK3	Kefir (Fermented milk products)	++	-	5,0x107	0,80	4,35
F6	Pickled green bean (*Phaseolus vulgaris* L.)	++	-	2,4x107	0,84	5,95
KT2	Pickled cucurbita(*Cucurbita pepo L.)*	+++	+	1,7x107	1,09	7,06

The sequence analysis method was very good at identifying the organisms by species.

Screening of tannase-producing bacteria by plate assay

All the *L. brevis* strains showed positive tannase activity. Except SM3, F7X, SM5, F6 and MK3, gallate decarboxylase activity was also observed in the other isolates (Table 1). Emergence of a clear zone around the colonies on the BHI agar confirmed the findings.

Determination of the tannase activity of *L. brevis* by spectrophotometer

Tannase specific activity of the isolates in optimum conditions was calculated of 1 unit tannase as the hydroxylation of 1 µmol tannic acid per minute and illustrated in Table 1. *L. brevis* KT2, *L. brevis* ES6, *L. brevis* A6X demonstrated high level activity. *L. brevis* demonstrated activity between the 0.52-1,17 U/ml values. It was found that the lowest activity value was *L. brevis* SM3 and 0,52 U/ml (Table 1).

Detection of tannic acid degradation by thin layer chromatography method

It was observed that the isolates tested by thin layer chromatography generated ES4, P3, ES2, A6X, DS4, D2X, P4, ES6, and KT2, MN9 and gallotanens gallic acid and pirogallol; but, other isolates only generated gallic acid (Figure 2).

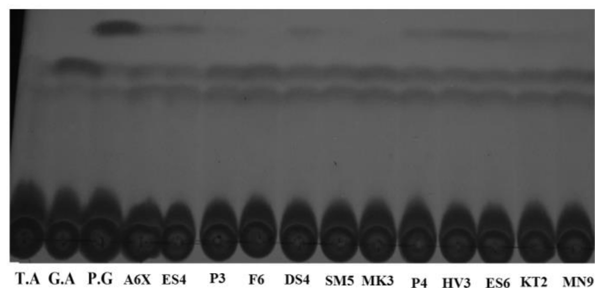

Figure 2. Demonstration of reaction products by thin layer chromatography.
PG: pyrogallol, **GA:** gallic acid, **TA:** tannic acid.

SDS-PAGE implementation

It was found that the molecular weight of the enzyme found in the cell-free filtrates of the selected 3 strains of *L. brevis* was nearly 55 kDA (Figure 3).

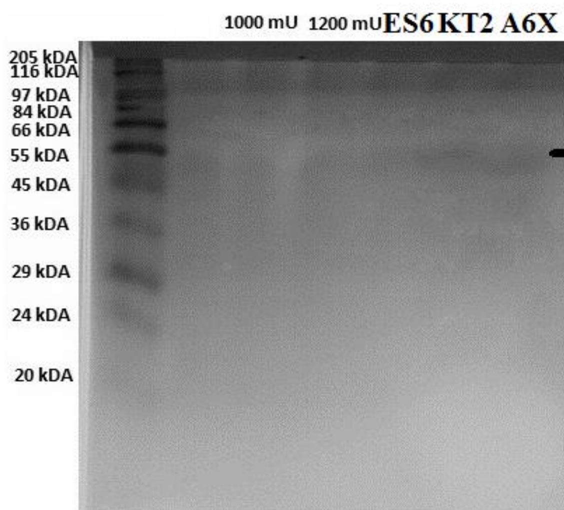

Figure 3. SDS-PAGE analysis showed result of the molecular weights of maximum activity tannase isolates.

Effect of tannase activity on the culture conditions

The effect of temperature in 3 isolates with the highest tannase activity on the enzyme activity is illustrated in Figure 4. The maximum tannase activity of the isolates were obtained at 37ºC. (Figure 4). It was found that at 37ºC, *L. brevis* was A6X 1,10 U/ml, *L. brevis* ES6 1,04 U/ml and for *L. brevis* KT2, it was 1,03 U/ml.

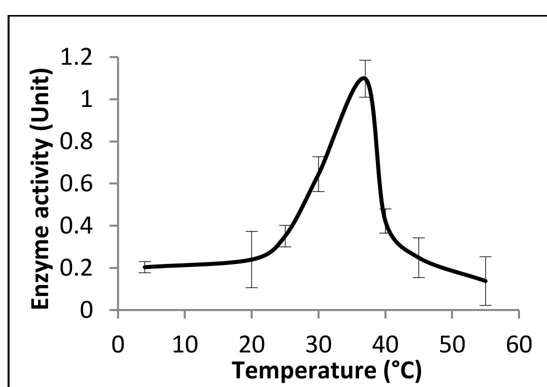

Figure 4. Optimum temperature for tannase activity *L. brevis* A6X.

Of all the isolates tested, the highest activity was obtained in pH 5. *L. brevis* A6X had 99,4% activity, *L. brevis* ES6 98,6%, and *L. brevis* KT2 95,04% (Figure 5).

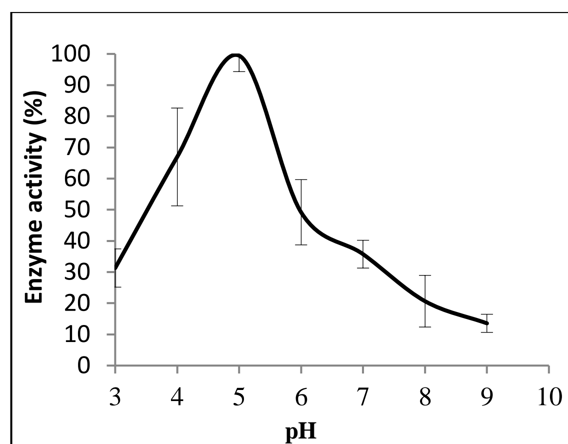

Figure. 5 Demonstrated of effect pH on tannase activity *L. brevis* A6X.

For the investigation of the effect of substrate concentration on the tannase activity, different concentrations of methyl gallate were analysed as the substrate and it was found that maximum enzyme activity for all the isolates tested was 7 mM methyl gallate. Further increase in substrate concentration caused the tannase activity to decrease (Figure 6). It was found that *L. brevis* KT2 was 1,05 U/ml, *L. brevis* ES6 1,11 U/ml, and *L. brevis* A6X 1,14 U/ml.

With all the isolates tested, highest activity was observed in 1.75 mM tannic acid. In 1,75 mM tannic acid concentrations, the following values of activities were obtained; *L. brevis* KT2 was 1,06 U/ml, and *L. brevis* A6X 1,17 U/ml l (Figure 7).

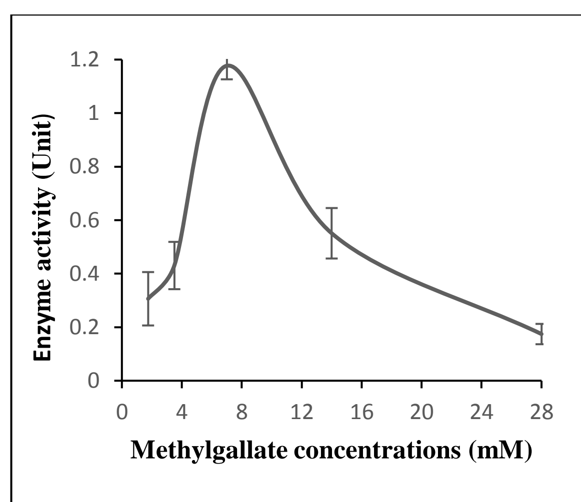

Figure. 6. Demonstrated of effect substrate (methylgallate) concentration on tannase activity *L. brevis* A6X.

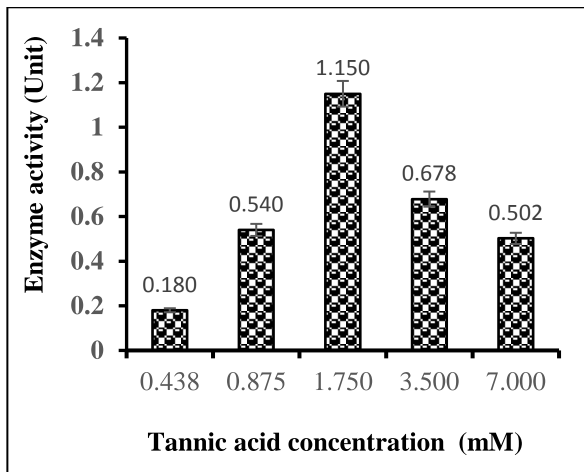

Figure 7. Demonstrated optimum tannic acid concentration as the used substrate on tannase activity *L. brevis* A6X

Effect of different additives in tannase activity

While enzyme activity decreased in the presence of Hg^{+2} and Mg^{+2} ions, it was observed that Ca^{+2} and Zn^{+2} lactic acid bacteria did not have any impact on the tannase activity, and some increase was observed in K^{+} tannase activity. Furthermore, it was found that agents such as some surfactant (Tween 80), chelate (EDTA), inhibitor (DMSO) and a denaturing agent (Urea) did not have any effect on the tannase activity (Table 2).

Discussion

Seventeen *L. brevis* strain, one of the fermented foods was isolated. It was defined by automatic ribotyping. *L. brevis* strains were screened in terms of tannase activity. It was found that tannase activity of all *L. brevis* strains were positive. Except for the *L. brevis* SM3, F7X, SM5, F6 and MK3 strains, it was observed that gallate decarboxylase activity in *L. brevis* strains was positive. Tannase activity in the *L. brevis* strains was investigated for the first time by OSAWA & al [8]. However, the researchers reported that *L. brevis* did not have tannase activity. In our study, it was found that *L. brevis* A6X strain was a potential strain for tannase production and gallate decarboxylase activity. In a similar study, it was reported for the first time that *L. plantarum, L. paraplantarum* and *L. pentosus* had tannase activities (OSAWA & al [8]).

KWON & al [6] examined the tannase activity of lactic acid bacteria isolated from Kimchi and reported that *L. plantarum* and *Lactobacillus pentosus* had high tannase activity.

L. brevis A6X was observed to have high gallate decarboxylase activity. Similarly, OSAWA & al [8] reported that lactic acid bacteria had the gallate decarboxylase activity. Furthermore, they also reported that *Lactobacillus gasseri* did not show any tannase activity, but had gallate decarboxylase activity.

L. brevis strains showed visual and spectrophotometric tannase activity. Similarly, in order to show tannase activity in the *L. plantarum* strains, spectrophotometric and visual method was used by the researchers (OSAWA & al [8]; AYED and HAMDI [15]). The isolates with high tannase activity produced similar results in visual evaluation as well. It was observed that visual reading method as defined by OSAWA and WALSH [12] was a simple but useful method in the search of tannase bacterial enzymes.

Activity of culture conditions of *L. brevis* A6X on the tannase activity was investigated. The maximum tannase activity of *L. brevis* A6X at 37ºC was obtained as 1.10 U/ml. The increase in temperature caused a reduction in tannase activity. RODRIGUEZ & al [3] found that optimum room temperature for enzyme activity was 30ºC. It was also reported that maximum enzyme activity was 6.26 U/ml. similar optimum temperature values were also reported for fungal tannase (AGUILAR & al [16]). However, in *Bacillus cereus* it was found that optimum temperature was 40ºC (MONDAL & al [17]).

The highest tannase activity in the *L. brevis* A6X strain was obtained at pH 5. Similar results were also

Table 2. Effect of different compounds on *L. brevis* strains tannase activity

Isolate	Tannase activity (U/ml)									
	Control	$MgCl_2$	KCl	$CaCl_2$	$ZnCl_2$	$HgCl_2$	Tween 80	Urea	EDTA	DMSO
A6X	1,17	1,04	1,22	1,20	1,19	0,40	1,17	1,17	1,15	1,17
KT2	1,09	0,91	1,13	1,10	1,10	0,34	1,11	1,08	1,08	1,06
ES6	1,14	0,96	1,19	1,15	1,14	0,39	1,15	1,15	1,13	1,15

reported by (RODRIGUEZ & al [3]). However, it was reported that *L. plantarum* for tannase activity was optimum pH 6 (AYED and HAMDI, [12]).

It was found that optimum substrate concentration was 7 mM methyl gallate. Increase in substrate concentration reduced the tannase activity. It was found that 7 mM methyl gallate was 1.14 U/ml together with *L. brevis* A6X. The highest activity was obtained in the 1.75 mM tannic acid concentration. RODRIGUEZ & al [3] reported that the highest enzyme activity was obtained in 6.25 mM methyl gallate.

The presence of Mg^{2+} and Hg^{+2} ions led to the decrease enzyme activity. Zn, on the other hand, did not have any impact. Low concentration of metal ions can act as a cofactor for the enzyme. Catalytic activity of enzymes increases. However, higher concentrations, on the other hand, decrease the enzyme activity. Similarly, it was reported that tannase enzyme of L. plantarum was inhibited by the Mg^{+2} and Hg^{+2} ions (RODRIGUEZ & al, [3]). Hg ions caused inhibition. It was reported that *Aspergillus niger* was inhibited by the Mg^{+2} ions together with the tannase enzyme obtained (SABU & al [18]). Inhibitory effect of heavy metal ions was reported by VALEER and (ULMER [19]). K^{+} and Ca^{+2} ions, on the other hand, caused partial increase in the enzyme activity. RODRIGUEZ & al [3], on the other hand, reported that the enzyme obtained by the *L.plantarum* was not affected by the K^{+} and Ca^{+2} ions. Tween 80, DMSO and urea did not have any effect on the *L. brevis* A6X tannase activity. However, EDTA was partly affected. It was reported that substances such as tween 80, DMSO, EDTA and urea did not have any effect on *L. plantarum* tannase enzyme activity.

Conclusion

As far as is known, no information is available demonstrating tannase activity with *L. brevis*. In this study, it was shown that isolates of *L. brevis* had tannase activity. It was found that optimum conditions for the tannase enzyme produced by *L. brevis* A6X tannaz were; temperature 30°C, pH 5,7 mM methyl gallate and 7 mM tannic acid. Although the tannase activity of the *L. brevis* strains is lower than some tannase activity values given in the literature, *L. brevis* strains have the potential to reduce the undesirable effects of tannins in the beverage, food, cosmetics and pharmaceutical industries. Therefore, *L. brevis* A6X can successfully be used with fermented vegetables and beverages. However, there is a need for further studies for the usability of it especially in the fruit juice sector.

Acknowledgments

This work was financially supported by the Anadolu University of Scientific Research Project Program.

References

1. K.T. CHUNG, C.-I. WEI, M.G. JOHNSON. Are tannins a double-edged sword in biology and health? *Trends in Food Science and Technology,* 9:168 (1998).
2. T.K. BHAT, B. SINGH, O.P. SHARMA. Microbial degradation of tannins – A current perspective. *Biodegradation,* 9: 343 (1998).
3. H. RODRÍGUEZ, B. DE LAS RIVAS, C. GÓMEZ-CORDOVÉS, R. MUÑOZ. Characterization of tannase activity in cell-free extracts of *Lactobacillus plantarum* CECT 748^{T}. *International Journal of Food Microbiology,* 121: 92 (2008).
4. C.N. AGUILAR, G. GUARREZ-SANCHEZ. Sources, properties, applications and potential uses of tannin acyl hydrolase. *Food Science and Technology International,* 7: 373 (2001).
5. S. SHARMA, M.N. GUPTA. Synthesis of antioxidant propyl gallate using tannase from *Aspergillus niger* van Teighem in nonaqueous media. *Bioorganic and Medicinal Chemistry Letters,* 13: 395 (2003).
6. T. KWON, S. SHIM, J.H. LEE. Characterization of lactobacilli with tannase activity isolated from Kimchi. *Food Sci. Biotechol*., 17: 1322 (2008).
7. P.D. BELUR AND G. MUGERAYA. Microbial production of tannase: state of the art. *Research Journal of Microbiology,* 6: 25 (2011).
8. R. OSAWA, K. KUROISO, S. GOTO, A. SHIMIZU. Isolation of tannin-degrading lactobacilli from human and fermented foods. *Applied and Environmental Microbiology,* 66: 3093 (2000).
9. C.N. AGUILAR, R.RODRIGUEZ, G. GUTIERREZ-SANCHEZ, C. AUGUR, AND E. FAVELA-TORRES. Microbial tannases: Advances and perspectives. *Applied Microbiol. Biotechnol.,* 76: 47 (2007).
10. B. MATEI, J. SALZAT, CF DIGUȚĂ, C.P. CORNEA, G. LUȚĂ, E.R. UTOIU, F. MATEI. Lactic acid bacteria strains isolated from Kombucha with potential probiotic effect. Rom Biotechnol Lett 23(3) (2018).
11. C.IYER AND J. VERSALOVİC. Lactic acid bacteria: probiotics with anti-cancer activities. In; Ljungh A, Wadstrom T (editors) Lactobacillus molecular biology: from genomics to probiotics. Caister Academic Press. P. 153 (2009).

12. R. OSAWA, T.P. WALSH. Visual reading method for detection of bacterial tannase. *Appl. Environ. Microbiol.*, 59: 1251 (1993).
13. Y. NISHITANI, R. OSAWA. Deceptive halo formation by tannase – defective bacteria on tannin-treated plate media. *Microbes Environ.*, 20: 117 (2005).
14. Y. NISHITANI, R. OSAWA. A novel colorimetric method to quantify tannase activity of viable bacteria. *Journal of Microbiological Methods,* 54: 281 (2003).
15. L. AYED, M. HAMDI. Culture conditions of tannase production by *Lactobacillus plantarum. Biotechnology Letters,* 24: 1763 (2002).
16. C.N AGUILAR, C. AUGUR, E. FAVELA, G. VINIEGRA-GONZÁLEZ. Induction and repression patterns of fungal tannase in solid-state and submerged cultures. *Process Biochemistry,* 36: 565 (2001).
17. K. C. MONDAL, D. BANERJEE, R. BANERJEE, B. R. PATI. Production and Characterization of tannase from *Bacillus cereus* KBR9. *Journal of General and Applied Microbiology,* 47: 263 (2001).
18. A. SABU, G.S. KIRAN, A. PANDEY. Purification and characterization of tannin acyl hydrolase from *Aspergillus niger* ATCC 16620. *Food Technology and Biotechnology* , 43: 133 (2005).
19. B.L.VALEER, D.D. ULMER. Biochemical effects of mercury, cadmium and lead. *Annual Review in Biochemistry,* 41: 91 (1972).