Research Paper

Future of Food: Journal on Food, Agriculture and Society
11 (5) December 2023

Partial purification of tannase enzyme produced by *Bacillus licheniformis* isolated from local soils

RASHA AL HADDAD[1,*], MOHAMADKHAIR TAHLA[2] AND LINA AL AMIR[1]

[1]National Commission for Biotechnology, Damascus, Syria.
[2]Food Science Department, Faculty of Agriculture, Damascus University, Syria.

* Corresponding author: rassha-haddad@hotmail.com

Data of the article

First received : 15 November 2023 | Last revision received : 01 December 2023
Accepted : 20 December 2023| Published online : 28 December 2023
DOI : 10.17170/kobra-202307218419

Keywords

Tannase; *Bacillus licheniformis*; partial purification; ammonium sulphate; gel filtration.

Tannase is an enzyme that causes hydrolysis of a group of tannins (gallo-tannins) to gallic acid and glucose. This enzyme is of importance due to its numerous applications in many fields, such as in the food industry by enhancing tea and coffee flavour and improving the quality of fruit juices rich in tannins. Gallic acid is applied in the drug industry, and production of antioxidants is used in the oil industry. In this study, Tannase was produced by locally isolated *Bacillus licheniformis* using spent tea as substrate using submerged fermentation. The crude enzyme was extracted and centrifuged at 10,000 rpm for 10 minutes at 15˚C, and a partial purification was carried out using precipitation by ammonium sulphate 80%, then Sephadex G-75 gel filtration column (2.2×1.8 cm) with acetate buffered solution (0.2 M, pH 5.0) at a flow rate of 60 ml/h up to 3.16 fold with a specific activity reaching 0.931 IU/mg(unit of enzyme per mg). The molecular weight of the purified enzyme was determined to be 48 kDa using SDS-PAGE. Thus, the purification step is a step before applying the pure enzyme in the food industry.

1. Introduction

Tannase or Tannin Acyl Hydrolases (EC3.1.1.20), is a tannin-degrading enzyme that breaks down tannins from the gallo-tannins group into gallic acid (3,4,5-trihydroxybenzoic acid) and glucose (Van de Lagemaat and Pyle, 2005).

The importance of this enzyme is due to its wide applications in many industrial fields, especially food industry, such as the production of instant tea, iced tea and coffee flavourings (Lu *et al.*, 2009), and industrial clarification of beverages such as fruit juice, wine, and beer(Vaquero *et al.*, 2004; Aguilar *et al.*, 2007) and it is also used to reduce the anti-nutritional effects of poultry and animal feed along with food de-tanification (Xu, 2010). It is also used in industrial tannery effluent treatment (Orlita., 2004). One of the main commercial applications of tannase is in the hydrolysis of tannic acid into gallic acid (Chávez-González *et al.*, 2012) an intermediate precursor necessary for the synthesis of the antibiotic trimethoprim(Bajpai and Patil, 2008) and used in the production of propyl galate, mainly as an antioxidant in fats, oils, and beverages (Hota *et al.*, 2007).

Although tannase is present in plants, animals, and microorganisms, it is produced in substantial amounts

by the latter. Tannase is produced by bacteria, yeasts, and fungi such as Aspergillus niger, Penicillium sp, and Trichoderma sp, and bacteria, such as *LactoBacillus plantarum, Klebsiella pneumoniae, Enterococcus faecalis, Bacillus sphaericus, Bacillus cereus, Bacillus massieliensis* and *Bacillus licheniformis* (de Las Rivas.,2019; Kumar *et al*., 2015; Sharma and John, 2011; Mondal and Pati, 2000), where the microbial source of the enzyme is considered more stable than the other sources, (Chavez-Gonzalez *et al*., 2011).

A major factor that limits the application of tannase enzyme in industry is its high cost and the small number of studies carried out concerning the physical and chemical properties of this enzyme. Production of tannase in laboratories was accomplished by methods of small-scale fermentation using flasks and shaker incubators from which optimal conditions for production were determined. Production methods were developed to obtain a higher yield of enzymes at the lowest cost (Sharma *et al*., 2014). Pure tannic acid was previously used in tannase production as a sole carbon source, but it was of high cost and did not suit enzyme production at an economical level. The best solution was the use of crude tannins in tannase production (Selwal *et al*., 2011). Their main sources were agricultural wastes, forest wastes, and factory wastes (olive pomace, grape pomace, pomegranate peels, coffee pulp, tea residue, etc.), which are considered to be the best sources of tannin-rich substrates (Sharma *et al*., 2014; Bhoite and Murthy, 2015).

Purification and characterization of tannase have been attempted in numerous studies from both plant and microbial sources. The end products of the fermentation process usually contain some undesirable components, which are reduced to the greatest possible extent by applying several purification steps (Gayen and Ghosh, 2013; Dhiman et al 2023), and the enzyme must be concentrated to increase its specific effectiveness, so several methods have been used, such as precipitation with salts or solvents, followed by ion exchange chromatography methods or separation according to the principle of size. (Govindarajan et al .,2021)

The resulting tannase has been purified from different types of bacteria, such as *Bacillus* subtilis (Jana *et al*., 2013), *Bacillus* cereus (Mondal *et al*., 2001), Klebsiella pneumoniae (Sivashanmugam and Jayaraman, 2011), Lacto*Bacillus* plantarum (Iwamoto *et al*., 2008), Streptococcus gallolyticus (Jiménez *et al*., 2014) and Enterobacter cloacae (Govindarajana *et al*., 2021).

Several studies have indicated that the molecular weight of tannase from bacterial sources ranges between 40-90 KDa (Iwamoto *et al*., 2008; Sharma and John, 2011) and is mainly composed of one part. However, some studies have revealed that tannase produced by *Rhodococcus sp* and *L. plantarum* consists of two subunits (Nadaf and Ghosh., 2011; Beniwal *et al*., 2013), while the molecular weight of fungal tannase ranges between 50-320 KDa and often consists of two subunits (Böer *et al*., 2009).

This study aimed to purity of tannase and the molecular weight determination of the purified enzyme.

2. Materials and methods

2.1. Microorganism

Bacillus licheniformis was used in the current study. It was isolated from soil samples of an olive field in Lattakia, Syria, and named T13 in 2017. It was selected from among 30 isolates that produced tannase.
It was grown and maintained on nutrient agar (HiMedia, India) plates containing 0.5% tannic acid (Sigma Aldrich, China) filter sterilized as a substrate (it is rich in tannin) and sole source of carbon, plates were incubated at 30˚C for 48 hours (Brahmbhatt and Modi, 2015).

2.2. Tannase production

Bacterial tannase was produced from *Bacillus licheniformis* by submerged fermentation using spent tea as substrate.

The fermentation medium was prepared according to the method of Aftab et al (2016). The fermentation medium was sterilized in an autoclave after the addition of spent tea substrate with a concentration of 1.5% (w/v) and a pH of 5.0 and subsequently incubation was performed at 35°C for 48 hours with shaking at 150 rpm. Detailed information about enzyme production was presented in a recent work (Haddad *et al*.,2021)

ISSN-Internet 2197-411x OLCL 862804632

2.3. Determination of Tannase Activity

The fermentation product was centrifuged at 10,000 rpm for 10 minutes at 15˚C, then it was filtered, and an estimation of tannase activity was assayed (Brahmbhatt and Modi, 2015).

Tannase was assayed by the method based on chromogen formation between gallic acid and rhodanine (2-thio-4-ketothiazolidine). An enzyme sample (0.25 ml) of culture filtrate and a substrate solution of methyl gallate 0.05M (0.25 ml) were pre-incubated at 30˚C for 5 minutes, then 300 µL of 0.667% methanolic rhodanine (Sigma Aldrich, Germany) was added to the mixture and incubated for 5 minutes at 30˚C. This was followed by the addition of 200 µL of KOH (0.5M). The mixture was then diluted to 5 ml by adding distilled water. After incubation for another 10 minutes at room temperature, absorbance at 520 nm was measured by a spectrophotometer. One unit of tannase activity is defined as the amount of enzyme required to release 1 µmol of gallic acid per minute under standard assay conditions (Sharma *et al.*, 2000; Fang *et al.*, 2019).

2.4. Determination of protein concentration

The protein concentration was determined according to the method of (Bradford, 1976). The assay depends on the observation of an acidic solution of Coomassie Brilliant Blue G-250 at a wavelength of 595 nm. The µg of protein was estimated using the µg standard of bovine serum albumin (BSA) with concentrations between 0.1 and 1 µg/ml

For the Coomassie Brilliant Blue G-250 reagent, 100 mg of Coomassie Brilliant Blue G-250 was Dissolved in 50 ml of 95% ethanol then 100 ml of 85% (w/v) phosphoric acid was added to the solution above. the volume was completed to 1000 ml by distilled water, filtered through Whatman No.1 filter papers, and stored in a dark and clean bottle at 4°C.

200µl of Coomassie Brilliant Blue G-250 Reagent was added to 800 µl of enzyme solution left at room temperature for 15 min then the absorbance was Measured at 595 nm.

2.5. Partial purification of tannase

The purification of tannase from microbial sources generally involves sequential chromatography techniques, mainly, ion exchange, gel filtration, and HPLC (Chaitanyakumar and Anbalagan, 2016; Goncalves *et al.*,2011). In these steps, a considerable amount of enzyme is lost due to autolysis and some remain physically adsorbed on the matrix. To overcome these constraints, two-steps purification systems have been employed.

2.5.1. Precipitation with ammonium sulphate

The enzyme was precipitated by adding solid ammonium sulphate to the enzyme extract with continuous slow stirring at a temperature of 4 °C until reaching a saturation point of 80%. The precipitated protein was separated by centrifugation at 9000 rpm and re-suspended in a minimum amount of acetate buffer (0.2 M, pH 5.0), and it was kept at 4 °C until use (Jana *et al.*, 2013).

2.5.2. Gel-filtration using Sephadex G-75 gel

The Sephadex G-75 gel (Sigma, USA) was suspended using an acetate buffer (0.2 M, pH 5.0). The gel was degassed with a vacuum pump using a Buechner flask, then it was gently poured into a (2.2×1.8 cm) glass column, and it was washed with acetate buffered solution (0.2 M, pH 5.0) three times. the 10 ml of the solution containing the enzyme from the previous step was loaded onto the Sephadex G-75 column. The protein fractions (5 ml) were eluted at a flow rate of 60 ml/h. Fractions showing enzyme activity were collected with a volume of 1 ml and pooled in small tubes absorbance measurements were performed at λ = 280 nm, and contents of the tubes with the highest absorbance were collected to determine tannase activity and to estimate the protein concentration fractions corresponding to high tannase activity were pooled and stored at -20°C.

2.6 SDS-PAGE and determination of the molecular weight of tannase

The Prpbable Molecular mass of The purified tannase was determined by sodium dodecyl sulphate- polyacrylamide gel electrophoresis (SDS-PAGE) according to the method of Laemmli's (Laemmli., 1970)

using standard marker proteins. Electrophoresis was done on 12% gel and the separated protein band was detected by Coomassie blue staining. The stacking gel consisted of 4% polyacrylamide.

The loading buffer containing SDS (2% w/v), β-mercaptoethanol (5% v/v), glycerol (1% v/v), and bromophenol blue (1% w/v) was mixed with protein concentrate samples (4:1 ratio). The resulting solution was heated to 95 ◦C for 5 min in a temperature-controlled water bath to promote protein denaturation. The operating conditions were 120 V and 400 mA for two hours. The gels were stained with Coomassie Brilliant Blue R-250. The standard marker pre-stained protein ladder (molecular weight ranging from 10 to 250 kDa) was used to identify the sample by its molecular weight.

3. Results and discussion

3.1 Partial purification of tannase produced by *Bacillus licheniformis*

The purification steps of extracellular tannase from *Bacillus licheniformis* were carried out according to (Jana *et al*., 2013) as follows:

3.1.1 Preparation of the crude enzyme

Tannase was produced by *Bacillus licheniformis* using spent tea as a substrate. 100 ml of crude enzyme was used to perform the purification steps.

3.1.2 Precipitation by ammonium sulphate 80%:

The tannase purified by ammonium sulphate 80% had a 1.88 fold yield increase and 14.95% recovery with the specific activity of 0.554U/mg Table (1).

These findings were consistent with the previous studies that recommend subjecting the enzyme extract of tannase to precipitation with ammonium sulphate because of its importance in the partial purification of the enzyme (Jana *et al*., 2013) precipitation with ammonium sulphate increased yield 1.25 fold.

3.1.3 Gel-filtration using Sephadex G-75 gel

The second step was performed using a Sephadex G-75 gel column as described above, its results are shown in Figure (1) where the absorbance of each fraction measured at λ =280 nm is presented together with the obtained enzyme activity. It is reasonable that fractions with the highest enzyme activity values contain with a high probability the purified enzyme, from this figure it can be concluded that they lay between fractions 58 and68

Tannase purified by gel filtration was a 3.16 fold yield increase and 12.4% recovery with the specific activity of 0.931U/mg as mentioned in Table (1). This table shows a summary of the purification steps and results of tannase produced by *Bacillus licheniformis*. It clearly showed that enzyme purification leads to an increase in the specific enzyme activity and a decrease in the total protein concentration. The enzyme was purified up to approximately 3.16 fold. This result seems to be reasonable in comparison with literature data regarding the purification of tannase produced by *Bacillus* strains. Several purification steps including Precipitation by ammonium sulphate and size exclusion chromatography.

Jebur (2020) was able to purify the tannase produced

Table 1. Purification steps of tannase from *Bacillus* licheniformis.

Purification steps	volume [ml]	activity [U/ml]	Total activity [U]	Protein concentration [mg/ml]	Specific activity [U/mg]	purification fold	Enzyme yield [%]
Crude extract	100	0.315	31.5	1.07	0.294	1	100
Precipitation with ammonium sulphate	10	0.471	4.71	0.85	0.554	1.88	14.95
Sephadex G-75 Gel	10	0.391	3.91	0.42	0.931	3.16	12.4

ISSN-Internet 2197-411x OLCL 862804632

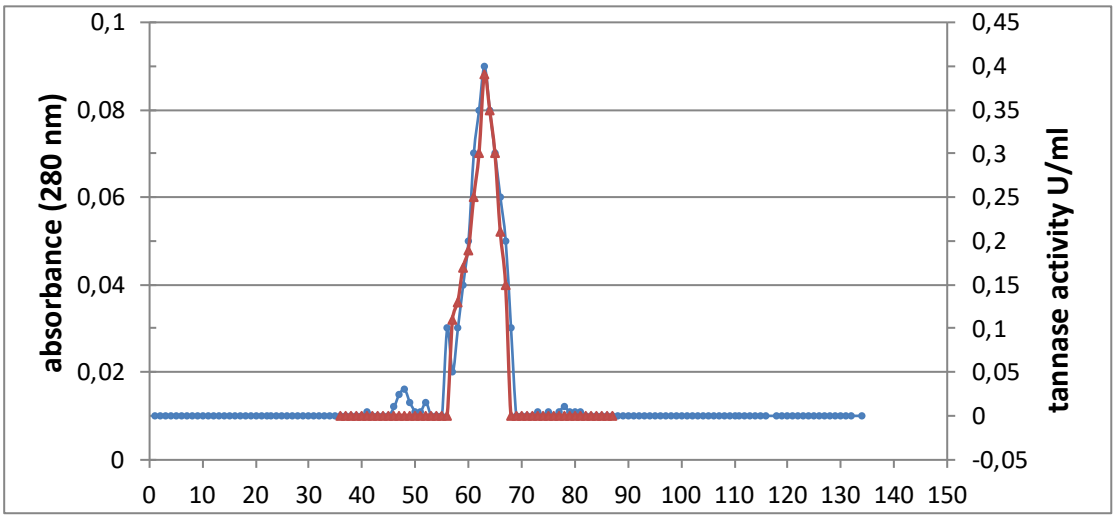

Figure 1. Purification of tannase using Sephadex G-75 column acetate buffered solution (0.2 M, pH 5.0) at a flow rate of 60 ml/h. Column size was (2.2×1.8 cm).

from *Bacillus licheniformis* HJ2020 MT1927.1 after purification by several steps, represented by precipitation with ammonium salts, then by DEAE-cellulose ion exchange column, and then by Sephadex G-100 chromatography column, and the enzymatic yield was 25.39%, and the 5.24 fold. Moreover, Jana *et al.* (2013) managed to purify the tannase produced by *Bacillus* subtilis PAB2 using precipitation with ammonium salts, followed by the use of Sephadex G-75 chromatography column, and the enzyme yield was 24.18% and 5.04 fold. Sharma and John (2011) also purified the tannase produced from Enterobacter sp, and the enzyme yield was 162% and 7.1 fold.

3.2- Protein separation using SDS-PAGE

The molecular weight of the purified enzyme by gel filtration method (Sephadex-G-75 column) was analysed using SDS-PAGE Figure (2) lane (A) represents SDS-PAGE results for the standard molecular weight markers. The analysis revealed a single band with a molecular mass of 48 kDa after purification with Sephadex G-75 gel (lane B) and many bands in lane (C) of crude enzyme one of them being 48 kDa.

The molecular weight of tannase varies depending on the organism. According to Yao *et al.*, (2014) the molecular weight of the tannase enzyme was discovered to be between 50-320 kDa and is naturally monomeric. It has also been observed that tannase comprises two or more subunits. In contrast, Aharwar & Parihar (2018) indicated that bacterial tannase has a low MW ranging from 31 to 90 kDa, but fungal tannase has a large MW ranging from 45 to 310 kDa. A molecular weight of 52 kDa was reported for tannase produced from *Bacillus* subtilis PAB2 (Jana *et al.*, 2013), 46.5 kDa for tannase produced from K. pneumoniae (Iwamoto *et al.*, 2008), 50 kDa for tannase produced Lacto*Bacillus* plantarum (Sivashanmugam and Jayaraman, 2011).

4. Conclusions

Tannase enzymes produced by *Bacillus licheniformis* through submerged fermentation using spent tea as substrates were purified up to 3.16 fold and had a specific activity reaching 0.931 IU/mg. Purified enzyme had a molecular weight of 48 kDa. Similar low molecular weight values have been reported for tannase produced by other *Bacillus* spp. Further investigations may demonstrate the applications of this finding.

Acknowledgments

This research was performed in the laboratories of the National Commission for Biotechnology, Damascus, Syria, which is gratefully acknowledged.

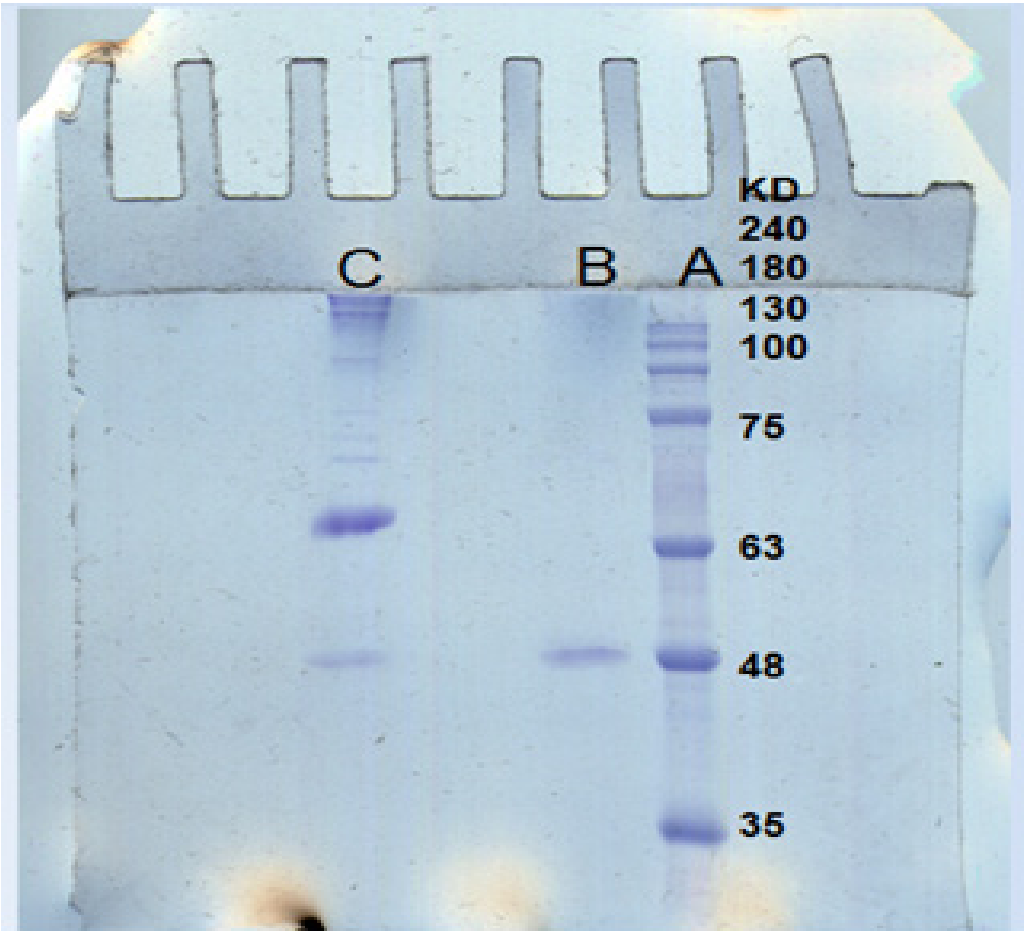

Figure 2. SDS-PAGE of crude and purified tannase protein. Lane (A) markers protein (lane B) purified tannase protein (C) crude enzyme protein

Conflict of interest

The authors declare that there is no conflict of interest.

References

Aftab, M. N., Mukhtar, H., & Ul-Haq, I. (2016). Production and Characterization of Tannase from a Newly Isolated *Bacillus* subtilis. Pakistan Journal of Botany, 48(3), 1263-1271.

Aguilar, C. N., Rodr´ıguez, R., Gutierrez-Sanchez, G., Augur, C., Favela-Torres, E., Prado-Barrag´an, L. A., Ram´ırez-Coronel, A. Y., & Contreras-Esquivel, J. C. (2007). Microbial Tannases: Advances and Perspectives. Applied Microbiology and Biotechnology, 76(1), 47-59. doi: 10.1007/s00253-007-1000-2

Aharwar, A., & Parihar, D. K. (2018). Tannases: Production, properties, applications. Biocatalysis and Agricultural Biotechnology, 15, 322–334. doi: 10.1016/j.bcab.2018.07.005

Bajpai, B., & Patil, S. (2008). A New Approach to Microbial Production of Gallic Acid. Brazilian Journal of Microbiology, 39(4), 708-711. doi: 10.1590/S1517-838220080004000021

Beniwal, V., Kumar, A., Goel, G., & Chhokar, V. (2013). A Novel Low Molecular Weight Acido-Thermophilic Tannase from Enterobacter cloacae MTCC 9125. Biocatalysis and Agricultural Biotechnology, 2(2), 132-137. doi: 10.1016/j.bcab.2013.03.002

Bhoite, R. N., & Murthy, P. S. (2015) Biodegradation of coffee pulp tannin by Penicillium verrucosum for production of tannase, statistical optimization and its application. Food and Bioproducts Processing, 94, 727–35. doi: 10.1016/j.fbp.2014.10.007

Böer, E., Bode, R., Mock, H.-P., Piontek, M., & Kunze, G. (2009). Atan1p-an extracellular tannase from

ISSN-Internet 2197-411x OLCL 862804632

the Dimorphic Yeast Arxula adeninivorans: Molecular cloning of the Atan1 gene and characterization of the recombinant enzyme. Yeast, 26(6), 323-337. doi: 10.1002/yea.1669

Bradford, M. M. (1976). A Rapid and Sensitive Method for the Quantization of Microgram 596 Quantities of Protein Utilizing the Principle of Protein-Dye Binding. Analytical Biochemistry, 72, 248-254. doi: 10.1006/abio.1976.9999

Brahmbhatt, D., & Modi, H. A. (2015). Comparative studies on methods of tannase assay. International Journal for Research in Applied Science and Engineering Technology, 3(III), 715–20.

Chaitanyakumar, A., & Anbalagan, M. (2016). Expression, purification, and immobilization of 768 tannase from Staphylococcus lugdunensis
MTCC 3614. Applied Microbiology and Biotechnology Express, 6, 89. doi: 10.1186/s13568-016-0261-5

Chavez-Gonzalez, M., Rodr´ıguez-Duran, L. V., Balagurusami, N., Prado-Barrag´an, A., Rodr´ıguez, R., Contreras, J. C., & Aguilar, C. N. (2011). Biotechnological Advances and Challenges of Tannase: An Overview. Food and Bioprocess Technology, 5, 445-459. doi: 10.1007/s11947-011-0608-5

Chavez-Gonzalez, M. L., Contreras Esquivel, J. C., Prado-Barragan, L. A., Rodriguez, R., Aguilera-Carbo, A. F., & Aguilar, C. N. (2012). Microbial and Enzymatic Hydrolysis of Tannic Acid: Influence of Substrate Chemical Quality. Chemical Papers, 66, 171-177. doi: 10.2478/s11696-011-0112-4

de-Las-Rivas, B., Rodríguez, H., Anguita, J., & Muñoz, R. (2019). Bacterial tannases: Classification and biochemical properties. Applied Microbiology and Biotechnology, 103(2), 603–623. doi: 10.1007/s00253-018-9519-y

Dhiman, S., Mukherjee, G., Kumar, A., & Majumdar, R. S. (2022). Purification, characterization and application study of bacterial tannase for optimization of gallic acid synthesis from fruit waste. Journal of Scientific & Industrial Research, 81, 1029–1036.

Fang, X., Du, M., Liu, T., Fang, Q., Liao, Z., Zhong, Q., Chen, J., Meng, X., Zhou, S., & Wang, J. (2019). Changes in the biotransformation of green tea catechins induced by different carbon and nitrogen sources in Aspergillus niger RAF106. Frontiers in Microbiology, 10, 2521. doi: 10.3389/fmicb.2019.02521

Gayen, S., & Ghosh, U. (2013). Purification and Characterization of Tannin Acyl Hydrolase Produced by Mixed Solid State Fermentation of Wheat Bran and Marigold Flower by Penicilliumnotatum NCIM 923. BioMed Research International, 2013, 1-6. doi: 10.1155/2013/596380

Goncalves, H. B., Riul, A. J., Terenzi, H. F., Jorge, J. A., & Guimaraes, L. H. S. (2011). Extracellular tannase from Emericella nidulans showing hypertolerance to temperature and organic solvents, Journal of Molecular Catalysis B: Enzymatic, 71(1-2), 29–35. doi: 10.1016/j.molcatb.2011.03.005

Govindarajan, R. K., Mathivanan, K., Khanongnuch, C., Srinivasan, R., Unban, K., Deepak, A. C., Al-Farraj, D. A., Alarjani, K. M., & Al-Qahtany, F. S. (2021). Tannin acyl-hydrolase production by *Bacillus* subtilis KMS2-2: Purification, characterization, and cytotoxicity studies. Journal of King Saud University - Science, 33(3), 101359. doi: 10.1016/j.jksus.2021.101359

Al-Haddad, R., Tahla, M. K., & Al-Amir, L. (2021). Improvement of Tannase Production from *Bacillus* Bacteria by Submerged Fermentation of Spent Tea. The Scientific Journal of King Faisal University: Basic and Applied Sciences, 22(1), 65-69. doi: 10.37575/b/agr/0041

Hota, S. K., Dutta, J. R., & Banerjee, R. (2007). Immobilization of Tannase from Rhizopusoryzae and its efficiency to produce Gallic acid from tannin rich agro residues. Indian Journal of Biotechnology, 6, 200-204.

Iwamoto, K., Tsurutab, H., Nishitainia, Y., & Osawa, R. (2008). Identification and Cloningof a Gene Encoding Tannase (tannin acyl hydrolase) from Lacto-*Bacillus* plantarum ATCC 14917(T). Systematic and Applied Microbiology, 31(4), 269-277. doi: 10.1016/j.syapm.2008.05.004

Jana, A., Maity, C., Halder, S. K., Das, A., Pati, B. R.,

Mondal, K. C., & Mohapatra, P. K. D. (2013). Structural Characterization of Thermostable, Solvent Tolerant, Cytosafetannase from *Bacillus* subtilis PAB2. Biochemical Engineering Journal, 77, 161-170. doi: 10.1016/j.bej.2013.06.002

Jebur, H. A. (2020). Optimization of Production and Partial Purification of Tannase from Local Isolate of *Bacillus licheniformis* HJ2020 MT14171.1. Plant Archives, 20(2), 2963-2968.

Jiménez, N., Barcenilla, J. M., Felipe, F. L. d., Rivas, B. D. L., & Muñoz, R. (2014). Characterization of a Bacterial Tannase from Streptococcus gallolyticus UCN34 Suitable for Tannin Biodegradation. Applied Microbiology and Biotechnology, 98(14), 6329-6337. doi: 10.1007/s00253-014-5603-0

Kumar, M., Rana, S., Beniwal, V., & Salar, R. K. (2015). Optimization of Tannase Production by a Novel klebsiella pneumoniae KP715242 Using Central Composite Design. Biotechnology Reports, 7, 128-134. doi: 10.1016/j.btre.2015.06.002

Laemmli, U. K. (1970). Cleavage of Structural Proteins During the Assembly of the Head of Bacteriophage T4. Nature, 227, 680-685. doi: 10.1038/227680a0

Lu, M.-J., Chu, S.-C., Yan, L., & Chen, C. (2009). Effect of Tannase Treatment on Protein-Tannin Aggregation and Sensory Attributes of Green Tea Infusion. LWT - Food Science and Technology, 42(1), 338-342. doi: 10.1016/j.lwt.2008.05.015

Mondal, K. C., & Pati, B. R. (2000). Studies on the Extracellular Tannase from Newly Isolated *Bacillus licheniformis* KBR6. Journal of Basic Microbiology, 40(4), 223-232. doi: 10.1002/1521-4028(200008)40:4<223::AID-JOBM223>3.0.CO;2-L

Mondal, K. C., Banerjee, D., Banerjee, R., & Pati, B. R. (2001). Production and Characterization of Tannase from *Bacillus* cereus KBR9. Journal of General and Applied Microbiology, 47(5), 263-267. doi: 10.2323/jgam.47.263

Nadaf, N. H., & Ghosh, J. S. (2011). Production, Purification and Characterization of Tannase from *Rhodococcus* NCIM 2891. Current Research Journal of Biological Sciences, 3(3), 246-253.

Orlita, A. (2004). Microbial Biodeterioration of Leather and its Control: A Review. International Biodeterioration and Biodegradation, 53(3), 157-163. doi: 10.1016/S0964-8305(03)00089-1

Selwal, M.K., Yadav, A., Selwal, K.K., Aggarwal, N.K., Gupta, R. and Gautam, S. K. (2011). Tannase production by Penicillium atramentosum kM under SSF and its application in wine clarification and tea cream solubilization. Brazilian Journal of Microbiology, 42(1), 374–387. doi: 10.1590/S1517-83822011000100047

Sharma, K. P, & John, P. J. (2011). Purification and Characterization of Tannase and Tannase Gene from Enterobacter sp. Process Biochemistry, 46(1), 240-244. doi: 10.1016/j.procbio.2010.08.016

Sharma, N. K., Beniwal, V., Kumar, N., Kumar, S., Pathera, A. K., & Ray, A. (2014). Production of Tannase Under Solid-State Fermentation and its Application in Detannification of Guava Juice. Preparative Biochemistry & Biotechnology, 44(3), 281-290. doi: 10.1080/10826068.2013.812566

Sharma, S., Bhat, T. K., & Dawra, R. K. (2000). A spectrophotometric Method for Assay of Tannase Using Rhodanine. Analytical Biochemistry, 279(1), 85-89. doi: 10.1006/abio.1999.4405

Sivashanmugam, K., & Jayaraman, G. (2011). Production and Partial Purification of Extra-Cellular Tannase by Klebsiella Pneumoniae MTCC 7162 Isolated from Tannery Effluent. African Journal of Biotechnology, 10(8), 1364-1374. doi: 10.5897/AJB10.1209

Van-de-Lagemaat, J., & Pyle, D. L. (2005). Modelling the Uptake and Growth Kinetics of Penicillium glabrumin A tannic acid-containing solid-state fermentation for tannase production. Process Biochemistry, 40(5), 1773-1782. doi: 10.1016/j.procbio.2004.06.044

Vaquero, I., Marcobal, Á., & Muñoz, R. (2004). Tannase activity by lactic acid bacteria isolated from grape must and wine. International Journal of Food Microbiology, 96(2), 199-204. doi: 10.1016/j.ijfoodmicro.2004.04.004

ISSN-Internet 2197-411x OLCL 862804632

Xu, F. (2010). Methods of Reducing the Inhibitory Effect of Tannin of the Enzymatic Hydrolysis of Cellulosic Material. Novozymes, Inc. Retrieved from https://patentscope.wipo.int/search/ru/detail.jsf;jsessionid=A1AB02F3C13EBB04B47BE2256F1DC117.wapp2nA?docId=US42833831&_cid=P20-KR8W99-40018-1

Yao, J., Guo, G. S., Ren, G. H., & Liu, Y. H. (2014). Production, characterization and applications of tannase. Journal of Molecular Catalysis B: Enzymatic, 101, 137–147. doi: 10.1016/j.molcatb.2013.11.018

ISSN-Internet 2197-411x OLCL 862804632