Original Research Article

Determination of tannin acyl hydrolase activity of *Streptomyces mirabilis* TBGS10 by measuring gallic acid using high-pressure liquid chromatography

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Abstract: Tannase is a hydrolytic enzyme, known as tannin acyl hydrolase, that acts on the ester bonds of hydrolyzable tannins to produce gallic acid. Tannase enzymes are obtained from various sources. Tannase from microbial sources, especially fungi, such as *Aspergillus* spp., has been used in the food, brewing, and pharmaceutical industries. Tannin acyl hydrolase activities of *Actinomycetes* are seldom reported, though they are known to produce a wide range of industrial enzymes. This communication deals with the tannase activity of a *Streptomyces* isolate from the shola forests of Munnar in the Western Ghats of Kerala, India. The isolate is identified as *S. mirabilis* TBGS10 based on morphological characters and the 16s rDNA homology. The isolate showed promising tannase activity in plate assays and under submerged and solid-state fermentation conditions. High-pressure liquid chromatography was used to determine the industrially important intermediate gallic acid produced through solid-state fermentation using cashew apple bagasse as the substrate. The extract was observed to have a gallic acid content of 142.624 g/mL with a retention time of 2.506 min. The tannase gene of the strain TBGS10 was PCR-amplified using specific primers designed based on similar *Streptomyces* sequences available in the NCBI-GenBank.

Keywords: *Streptomyces mirabilis*; tannase; HPLC; gallic acid production; solid-state fermentation

1. Introduction

Tannin acyl hydrolase, commonly known as tannase (TAH, EC. 3.1.1.20), is an alluring biocatalyst used to degrade tannin[1]. These versatile enzymes are widely used in the food industry, cosmetics, pharmaceuticals, tanneries, animal feeds, and environmental pollution control[2]. Tannase use is widespread in producing instant tea, fruit juices, beers, and acorn liquors[3]. Tannins are naturally occurring polyphenol compounds in many plants and foodstuffs, such as tea, fruits, nuts, etc. This enzyme catalyzes the hydrolysis of ester and depside bonds in complex tannins, gallotannins, ellagitannins, and gallic acid esters, and this reaction releases gallic acid and glucose as by-products. Gallic acid has a wide variety of applications, such as a developer in photography and printing inks, as a precursor molecule for the commercial production of an anti-microbial drug called trimethoprim, and as a food preservative. In addition, gallic acid possesses a wide range of biological activities, such as antioxidant, antibacterial, antiviral, and analgesic[4]. The tremendous biocatalytic potential of the tannase enzyme is mainly used in producing gallic acid, which has an industrial demand estimated at 8000 t per annum[5].

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The tannase enzyme is widely used in the food industry to produce instant tea and improve the taste and appearance of beverages such as fruit juices, beer, and wine. The bitterness and haze formation of fruit juices can be reduced by tannase application. De-tannification of feeds by this enzyme can improve the quality of animal feeds. Recently, lactic acid bacteria have been used to produce tannases using agricultural wastes rich in polyphenolic compounds as a source of nutrients and energy in submerged fermentation[6]. Utilizing these bioresources for tannase production has potential applications in the food industry[7]. For the cost-effective production of tannase by fermentation, the substrate is the most essential factor to be considered. Cashew apple bagasse is one of the most prevalent agricultural wastes in tropical regions and can be utilized as a cheaper substrate for tannase production.

Tannase is primarily produced from microorganisms through solid-state fermentation (SSF) or submerged fermentation (SmF). SSF is preferred over submerged fermentation because of its low cost, better yield, high enzymatic activity, easy operation, and lower water consumption[8]. Low-value agro-industrial wastes are widely used as the substrate for tannase production. Due to the easy downstream process and consistency in enzyme production, microorganisms are used for the industrial production of tannase more than other sources[9]. Tannase is a salient enzyme, but it has not been explored much yet due to a lack of adequate knowledge about the production medium, substrate utilization, and downstream processing. Identifying potential tannase-producing microorganisms and cost-effective substrates will reduce the cost of enzymes in the market. Among all other microbes, fungi are the most prominent producers of this enzyme, especially *Aspergillus* spp. and *Penicillium* spp.[10]. Besides fungi, many bacterial species have been reported to produce this enzyme, but tannase-producing actinomycetes have rarely been reported. The present study emphasized the tannase-producing *Streptomyces* isolated from the shola forests of Munnar, Kerala, India.

2. Materials and methods

2.1. Screening for tannase production

Soil samples were collected from different sites of the shola forest in Munnar, Kerala, India. Actinomycetes were isolated from a soil sample via serial dilution and the spread plate method using an ISP2 medium. The isolated cultures were screened for tannin acyl hydrolase activity on starch casein agar (SCA) media containing 1% tannic acid as a substrate. The culture was incubated at 28 ± 2 ℃ for five days[11]. An induced inoculum was prepared using spore suspension (1×10^8 spores/mL) of cultures grown for five days on starch casein agar slants with 1% tannic acid and inoculated into 200 mL of a starch casein broth (SCB) containing 1% tannic acid for the evaluation of tannase production under submerged batch fermentation. The cultures in flasks were incubated for seven days at 28 ± 2 ℃ with constant agitation at 120 rpm. Samples of 1 mL were withdrawn at regular intervals of 24 h, and the cell-free supernatant was obtained via centrifugation at 10,000 rpm for 15 min at 4 ℃. The extracellular tannase activity was determined using the rhodamine spectrophotometric method[12].

2.2. Identification of bacterial isolate

The isolated strain TBGS10 was identified based on cellular morphology, spore orientation, cultural characters, biochemical analysis, and 16S rDNA homology analysis. Colony characters and carbohydrate utilization were carried out on the ISP media: ISP1, ISP2, ISP3, ISP4, ISP5, ISP6, YEME, oatmeal agar, and peptone-yeast-iron agar[13]. The spore orientation of the strain was analyzed using scanning electron microscopy. The images were recorded using a Carl Zeiss EVO 18 scanning electron microscope at 4600 times magnification with an accelerating voltage of 5 kV. Spore chain morphology was examined using the inclined cover slip culture method[14] and observed under a Nikon Optiphot inverted phase contrast microscope. The

16S rDNA gene was amplified with universal primers 8-27F and 1495R for molecular identification using genomic DNA as a template. Genomic DNA was extracted using the phenol-chloroform method described by Murray and Thomson in 1980[15]. The amplified genes were purified and sequenced. The sequence similarity was searched in NCBI with the BLAST search program. Similar sequences were retrieved from Genbank and aligned using Clustal W. The maximum likelihood method was used to analyze the phylogenetic relationships based on the Poisson correction model[16] using the MEGA6 software[17]. The reliability of a particular clade in the phylogenetic tree was determined using bootstrap analysis with 1000 replicates.

2.3. Amplification of the tannase gene

Tannase gene sequences from *Streptomyces* spp. were retrieved from the NCBI database; specific primers were picked from conserved regions and synthesized by outsourcing to Integrated DNA Technologies. Four primers were designed to amplify the tannase gene (**Table 1**). PCR was performed using a Bio-Rad S1000™ thermal cycler with the following protocol: 98 ℃ for 1 min, 35 cycles of 98 ℃ for 10 s, 60 ℃ for 30 s, and 72 ℃ for 90 s, and a final extension of 72 ℃ for 10 min. The amplified product was resolved in 1% EtBr-agarose gel. The PCR product was purified using a Macherey-Nagel NucleoSpin PCR clean-up kit according to manufacturer instructions and sequenced using the same PCR primers following Sanger sequencing protocols on an ABI Prism DNA analyzer. The nucleotide sequences were translated using the ExPASy translate tool (https://web.expasy.org/translate/). The phylogenic relations were analyzed as described.

Table 1. List of forward and reverse primers designed with Primer3 software.

Primer	Sequence
STANF1	5′-ACACCTCCTTCTTCGATGGG-3′
STANR1	5′-GTAACTGCACAGGTCGCG-3′
STANF2	5′-ATGGGAAAGCAATGAGACGG-3′
STANR2	5′-CGGCTGTAGGTGATCTTCGA-3′
STANF3	5′-GGAAAGCAATGAGACGGCCT-3′
STANR3	5′-CTGGGTGGGGATGTACTGG-3′

2.4. Solid-state fermentation of cashew apple bagasse for tannase production

Dried and powdered cashew apple bagasse of 5 g was placed in 250-mL Erlenmeyer flasks and moistened with a mineral salt solution (0.5% w/v NH_4Cl, 0.1% w/v $MgSO_4.7H_2O$, and 0.1% w/v $NaNO_3$) at an initial pH of 5. The substrate in the flask was sterilized using an autoclave at 121 ℃ for 20 min and then cooled to room temperature. The seed inoculum of *S. mirabilis* TBGS10 (a spore suspension of 1×10^7 spores/mL) was added to the substrate and incubated at 30 ℃ for five days.

2.5. Detection of tannase using high-pressure liquid chromatography

The efficiency of *S. mirabilis* TBGS10 in gallic acid production from cashew apple bagasse was analyzed using high-pressure liquid chromatography (HPLC). The sample for HPLC analysis was prepared according to El Sohafy et al[18]. The fermented cashew apple bagasse was filtered to separate the mycelia, boiled for 5 min, and treated with 5 mL of 25% HCl in a water bath for 25 min. The mixture was extracted using n-butanol, dried under reduced pressure, and dissolved in methanol[18]. A standard Sigma-Aldrich gallic acid solution was prepared in methanol at 1 mg/mL[19]. Gallic acid was quantified using a Shimadzu HPLC system attached to a Prominence SPD-M20A diode array UV/VIS detector. Data acquisition and instrumental control were performed using the Shimadzu LabSolution version 5.73 software. The compounds were separated on a general-purpose Shimadzu C-18 column (250 × 4.6 mm, 5μm particle size). The mobile phase comprised 70:30

water and 1 mL/min (Spectrochem, India, HPLC grade). The flow rate was 1 mL/min, and the injection volume was 10 µL. The pump consisted of a Shimadzu CBM-20A LC-6AD system interface and a high-pressure adjustable volume dynamic mixer. The analysis was performed at room temperature (25 °C), and the compound was detected at 280 nm. The samples for analysis were separately filtered using a 0.22 µm Millipore filter before injection. At the same retention time as that for the standard gallic acid, the peak area of the samples was measured.

3. Results

3.1. Screening for crude tannase production

A total of 105 actinomycetes were isolated from the soil sample using the spread plate method on the ISP2 medium. On screening for the tannase enzyme, the formation of a clear zone around the colony of the isolate TBGS10 on starch casein agar plates with 1% tannic acid indicated positive tannase production due to the hydrolysis of tannic acid (**Figure 1**). Incubation time plays a vital role in the production of extracellular tannase. The time course observation of tannase production revealed that the strain TBGS10 produced the maximum amount of tannase at 48 h of incubation (7.26 U/mL), where one unit is defined as the quantity of enzyme required to liberate one µM of gallic acid /min under specified conditions (**Figure 2**). The maximum enzyme production was observed during the exponential growth phase of the organism.

Figure 1. SCA plate showing zone of hydrolysis around the colony.

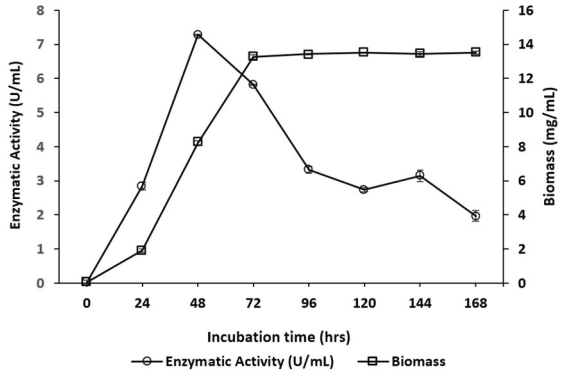

Figure 2. Extracellular tannase production by isolate TBGS10 under submerged batch fermentation.

The cultures in the flasks were incubated at 28 ± 2 °C for seven days with constant agitation at 120 rpm, and 1 mL of the sample was withdrawn at regular intervals of 24 h and assayed for tannase activity.

3.2. Identification of actinomycete isolate

The micro-morphological observation revealed that the organism was a gram-positive, spore-bearing actinobacteria. The morphological and biochemical characteristics of strain TBGS10 are shown in **Table 2**. Spore chain morphology was observed using the inclined-cover-slip culture method under the inverted phase

contrast microscope and shown in **Figure 3A**. The scanning electron microscopy image of the spore chain was in spirals, with more than 20 spores in a chain, and the spore surface was smooth (**Figure 3B**). In most of the ISP media, the aerial mass color appeared greyish-to-greyish brown (**Figure 3C**) and the reverse-side colony color was greyish yellow, while the oatmeal agar showed dark brown colonies. The isolate produced melanoid pigments in peptone-yeast-iron agar. The isolate utilized carbohydrates, such as D-glucose, L-arabinose, D-fructose, meso-inositol, D-mannitol, and sucrose, but only a trace of growth was observed in cellulose, L-rhamnose, and D-xylose. The isolate produced melanoid pigments in peptone-yeast-iron agar. The 16s rDNA sequencing resulted in 1417 bp of nucleotides and was deposited in GenBank (Accession No. OK445685.1). A BLAST similarity search showed 99.15% to 99.88% homology with different *Streptomyces mirabilis* strains. The phylogenetic tree constructed using the maximum likelihood method and the Tamura-Nei model via the MEGA6 software is illustrated in **Figure 4**. Based on the polyphasic approach, the isolate was identified as *Streptomyces mirabilis*.

Table 2. Morphological and biochemical characters of isolate TBGS10.

Morphological characteristic	
Colonies	Grey rough colonies
Aerial mass color	Greyish-to-greyish brown
Reverse colony color	Greyish-yellow
Melanin pigments	Positive
Spore chain	Spirals with more than 20 spores
Spore surface morphology	Smooth
Biochemical characteristic	
Gram staining	Gram-positive
Carbohydrate utilization	
D-glucose	+++
L-arabinose	+++
D-fructose	++
Meso-inositol	+++
D-mannitol	+++
Sucrose	+++
Cellulose	+
L-rhamnose	+
D-xylose	+

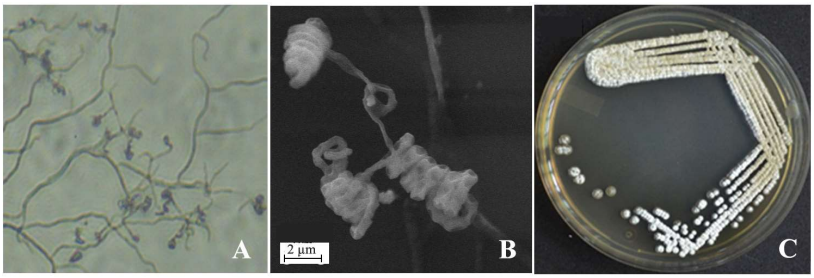

Figure 3. Microscopic and cultural characteristics of *Streptomyces mirabilis* TBGS10: **(A)** microphotograph showing spore chain morphology of *S. mirabilis* TBGS10, **(B)** scanning electron microscopy showing spore surface morphology, and **(C)** growth (7 days) on ISP medium.

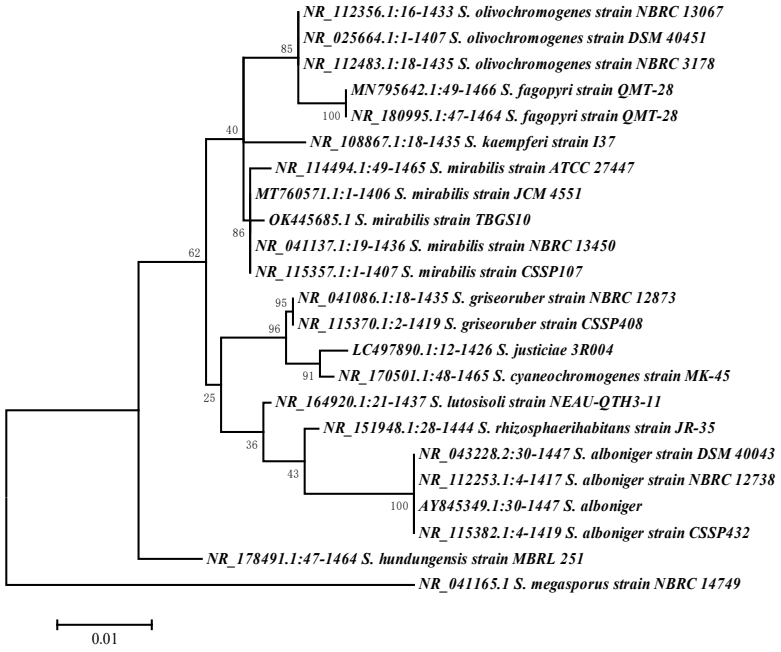

Figure 4. Phylogenetic tree based on 16S rRNA gene sequences, showing relationships of isolate TBGS10 (constructed on 30 August 2023).

The evolutionary history was inferred using the maximum likelihood method and the Tamura-Nei model[20]. The tree with the highest log likelihood (−3049.3097) is shown. The percentage of trees where the associated taxa cluster together is displayed next to the branches. The initial trees for the heuristic search were obtained automatically by applying the Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the maximum composite likelihood (MCL) approach and selecting the topology with a superior log likelihood value. The tree was drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 23 nucleotide sequences. The tree was rooted with *S. megasporus* strain NBRC 14749 (NR_041165.1). All positions containing gaps and missing data were eliminated. There were a total of 1392 positions in the final dataset. Evolutionary analyses were conducted in MEGA6[17].

3.3. Amplification and identification of tannase gene

Primers STANF1 5′-ATGAGACGACTTCTGACTGTCCT-3′ and STANR1 5′-TCAGTGCCGGGACGGGG-3′ gave better amplification in the PCR reaction, and the amplicon was sequenced. The resulting nucleotide sequences (1404 bp) were translated (**Figure 5**), and a homology search was performed. *Streptomyces mirabilis* TBGS10 tannase showed high similarities of 99.78% with the tannase/feruloyl esterase family alpha/beta hydrolase of *S. mirabilis* strain NBC_01472 (GenBank: MCX4615223.1) and 99.56% with that of *Streptomyces* sp. S1D4-20 (GenBank: QDN54811.1). These tannases also clustered with *S. mirabilis* strain TBGS10 in the phylogenetic tree constructed using the maximum likelihood method (**Figure 6**).

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>S. mirabilis TBGS10 Tannase
MRRPLTVLAAGVPLAAAVYLPTASAEPENGSASASFACSAWSVKAPAGTEVESVTAVRQAGGTIIGTGALGGS
VSGVPAYCEVTVTLTHPGDDDHAEVRTWLPVSGWNGRFQAIGGAAYLAGDNGVGLGTAVKNGYAAVSTDAGVG
DALDTDWALNSNGQVNTALLKNFASRSQHEAAVVGKEVVDGVYGKRPAYSYFNGCSTGGRQGYMEAQRYPDDY
DGILANAPAVNWDEFEVATLWPQVVMNNEKTYPSKCEFDAFTNAAVKACDSLDGVKDGLVNDLSRCDFDPRTL
IGTKVVCDGKELTITAADAAVVRKIWDGPRTASGKKLWSGVPVGADLSALAGLTEPDADGNVAGAPFPVPAAW
VKLWVAKDPSLDISKITYSRFTQLFKQSQAEYDKVIGTDDPDLSGFRRSGGKLLTWHGLADQYIPTQGTVQYR
ERVEREMGGAKRVDDFYRLFL
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Figure 5. Translated amino-acid sequences of *Streptomyces mirabilis* TBGS10 tannase.

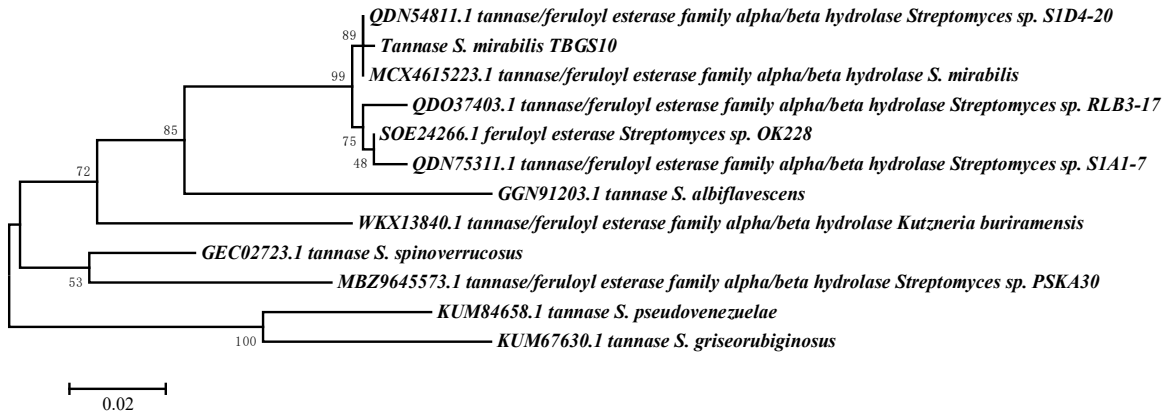
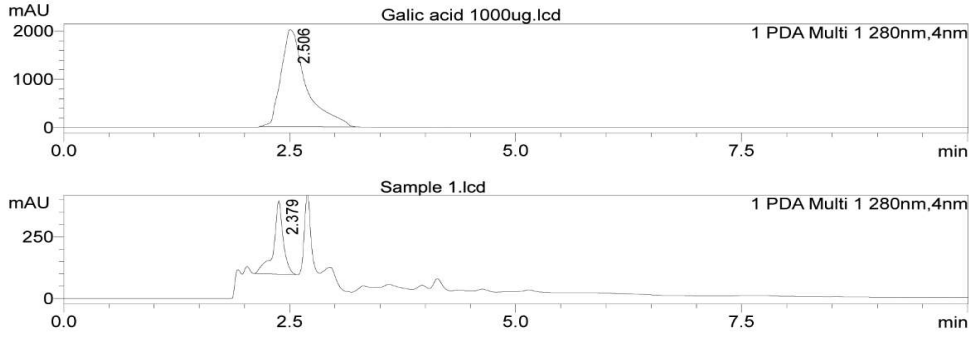


Figure 6. Maximum likelihood tree showing phylogenetic amino acid sequences of different tannases similar to *S. mirabilis* TBGS10 (constructed on 30 August 2023).

The evolutionary history was inferred using the maximum likelihood method based on the Poisson correction model[16]. The tree with the highest log likelihood (−2690.2172) is shown. The percentage of trees where the associated taxa cluster together is displayed next to the branches. The initial trees for the heuristic search were obtained automatically by applying the Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using a JTT model and then selecting the topology with a superior log likelihood value. The tree was drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 12 amino acid sequences. All positions containing gaps and missing data were eliminated. There were a total of 447 positions in the final dataset. Evolutionary analyses were conducted in MEGA6[17].

3.4. Detection of tannase using high-pressure liquid chromatography

The HPLC analysis showed a good separation under the conditions described, and well-resolved peaks were observed in the standard gallic acid (GA). The ethanol extracts of the fermented cashew apple bagasse were analyzed and compared with the standard. It was found that the extract contained GA of 142.624 µg/mL (retention time of 2.506 min) compared with 1000 µg/mL in the standard (retention time of 2.506 min). The chromatogram of GA is shown in **Figure 7**. The results confirmed the efficiency of the *S. mirabilis* TBGS10 culture in producing gallic acid from dried cashew apple bagasse.



ID#1 Compound Name: GALLIC ACID

Data File Name	Sample Name	Sample ID	Ret. Time	Area	Height	Conc.
Galic acid 500ug.lcd	GALIC ACID	500ug	2.576	17809060	752008	500.000
Galic acid 1000ug.lcd	GALIC ACID	1000ug	2.506	39624901	2019664	1000.000
gallic acid sample 1.lcd	Gallic acid	Sample 1	2.379	2216161	298435	142.624
Average			2.487	19883374	1023369	547.541
%RSD			4.023	94.503	87.175	78.653
Maximum			2.576	39624901	2019664	1000.000
Minimum			2.379	2216161	298435	142.624
Standard Deviation			0.100	18790437	892124	430.660

Figure 7. HPLC analysis of standard gallic acid and ethanol extracts of fermented cashew apple bagasse.

4. Discussion

Tannins are polyphenolic secondary metabolites synthesized by plants, where they play a crucial role in plant growth regulation and defense against insect, microbe, and mite attacks[21]. However, tannase delays soil humus formation because it precipitates soil enzymes[22]. It is also an anti-nutritional factor that slows animal food digestibility[23]. Tannase is an enzyme belonging to the esterase family that catalyzes the hydrolysis of the galloyl ester bond of tannins and produces gallic acid as a significant by-product. GA is widely used in food to synthesize food preservatives and in the pharmaceutical industry to synthesize antibacterial drugs[24].

Microbes are the most crucial source of tannase because they consistently produce stable enzymes. Fungi, such as *Penicillium*[25] and *Aspergillus*[26], are proven potential sources of tannase. Most screening programs for microorganisms with tannase activity have been restricted mainly to fungi for years. The search for new and different tannase-degrading organisms has increased. Actinomycetes are a well-known source of enzyme production. Recently, the possibility of obtaining high yields of commercially valuable tannase from actinomycetes has attracted researchers' attention; however, few investigations have been reported. In the present study, *Streptomyces mirabilis* TBGS10 isolated from the shola forests of Munnar is a potent source of the tannase enzyme. The maximum enzyme production at the exponential growth phase is highly advantageous. The earlier report of tannase production by an endophytic *Streptomyces* sp. AL1L was 6.96 U/mL at 48 h of incubation[21], almost similar to this study's results. While *Streptomyces* sp. SKA1 produced 8.50 U/mL tannase on the fifth day of incubation[9], *Streptomyces* sp. AT13 produced 18.12 U/mL on the fifth day under optimized conditions using statistical experimental designs[27]. Tannase is an inducible enzyme; the characteristics of tannase may vary depending on the source of isolation, species, and substrate used for its production. Post-translational modifications are limited to bacterial tannase, but glycosylation occurs in fungi and yeast. The characteristics of tannase produced by microorganisms vary depending on cultural circumstances and glycosylation patterns[28].

Waste products from the agro-industrial sector can be used as a biosynthesis medium for various medicinal and industrially applicable enzymes[29]. Selecting suitable biomass for the production of the tannase enzyme is critical. In the scenario of Kerala, the cashew agroindustry has a significant role in the local economy. Most cashew apples deteriorate in the ground, and the beverage industry discards 40% (w/w) of the cashew apple bagasse as the end product[30], an inexpensive substrate source for solid-state fermentation. The selected agro-waste was supplemented with and without various mineral sources for enzyme production. Only a negligible amount of enzyme was produced in the experiment without the supplement. Adding minerals provides additional moisture content to a solid medium, which is also essential in controlling enzyme production. The effect of ions and co-factors on tannase activity has been reported previously. The divalent metal ions enhance the tannase activity of *Rhizopus oryzae,* as reported by Kar et al.[31] in 2003.

Gallic acid is an industrially important intermediate produced by the hydrolysis of tannic acid by tannase[32]. Previous reports showed that *Aspergillus* spp. has been widely utilized to hydrolyze tannic acid into gallic acid[33]. Bacteria, such as *Klebsiella pneumoniae* and *Corynebacterium* sp., are well documented as producers of gallic acid from the crude extract of tara-gallotannin[34]. This study determined tannase production through solid-state fermentation using a selective and indirect method, which was HPLC[35]. Tannic acid was used as the substrate to inject into HPLC, and it was found to contain 142.624 µg/mL of GA. The hydrolyzed GA from tannic acid must equal the GA corresponding to the tannase activity.

The tannase gene of *S. mirabilis* TBGS10 shows similarity with the tannase/feruloyl esterase family alpha beta hydrolase of other strains of *Streptomyces.* Genomic data of the tannase gene of *Streptomyces* is rarely reported, and there has been heed for it. The insight of molecular analysis of the tannase gene opens up a

gateway to molecular cloning and large-scale production of tannase. It could help direct tannase application and serve as the underpinning theory for gene modification.

5. Conclusion

The present study's results indicate that *Streptomyces mirabilis* TBGS10 is a potent source of the tannase enzyme. Under optimal culture conditions, the strain produced the enzyme substantially. Determining tannase by measuring the intermediate gallic acid was straightforward and successful. Purifying the tannase enzyme using existing technology is tedious and energy-consuming; hence, the enzyme has not yet been fully explored. Cashew apple bagasse is a suitable and cheaply available substrate for producing tannase enzymes through solid-state fermentation.

Author contributions

Conceptualization, SS (Shiburaj Sugathan) and SA; methodology, SS (Shiburaj Sugathan); software, SKM; validation, SS (Shiburaj Sugathan), SKM, and SS (Sajna Salim); formal analysis, SKM; investigation, SS (Shiburaj Sugathan) and SKM; resources, SS (Shiburaj Sugathan); data curation, SKM; writing—original draft preparation, SS (Sajna Salim); writing—review and editing, SS (Shiburaj Sugathan); visualization, SS (Shiburaj Sugathan); supervision, SS (Shiburaj Sugathan); project administration, SS (Shiburaj Sugathan); funding acquisition, SS (Shiburaj Sugathan) and SA. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors affirm that they have no known financial or interpersonal conflicts that would have appeared to impact the research presented in this study.

References

1. Chávez-González M, Rodríguez-Durán LV, Balagurusamy N. Biotechnological advances and challenges of tannase: An overview. *Food Bioprocess Technology* 2012; 5(2): 445–459. doi: 10.1007/s11947-011-0608-5
2. Wu C, Zhang F, Li L, et al. Novel optimization strategy for tannase production through a modified solid-state fermentation system. *Biotechnology for Biofuels and Bioproducts* 2018; 11: 92. doi: 10.1186/s13068-018-1093-0
3. Jana A, Halder SK, Ghosh K, et al. Tannase immobilization by chitin-alginate based adsorption entrapment technique and its exploitation in fruit juice clarification. *Food Bioprocess Technology* 2015; 8: 2319–2329. doi: 10.1007/s11947-015-1586-9
4. Beniwal V, Kumar A, Sharma J, Chhokar V. Recent advances in industrial application of tannases: A review. *Recent Patents on Biotechnology* 2013; 7(3): 228–233. doi: 10.2174/18722083113076660013
5. Dhiman S, Mukherjee G, Singh AK. Recent trends and advancements in microbial tannase-catalyzed biotransformation of tannins: A review. *International Microbiology* 2018; 21(4): 175–195. doi: 10.1007/s10123-018-0027-9
6. Farha AK, Yang QQ, Kim G, et al. Tannins as an alternative to antibiotics. *Food Bioscience* 2020; 38: 100751. doi: 10.1016/j.fbio.2020.100751
7. García Méndez MG, Morales Martínez TK, Ascacio Valdés JA, et al. Application of lactic acid bacteria in fermentation processes to obtain tannases using agro-industrial wastes. *Fermentation* 2021; 7(2): 48. doi: 10.3390/fermentation7020048
8. Barrios-González J. Solid-state fermentation: Physiology of solid medium, its molecular basis and applications. *Process Biochemistry* 2012; 47(2): 175–185. doi: 10.1016/j.procbio.2011.11.016

9. Aharwar A, Parihar DK. Tannases: Production, properties, applications. *Biocatalysis and Agricultural Biotechnology* 2018; 15: 322–334. doi: 10.1016/j.bcab.2018.07.005
10. Nishitani Y, Osawa R. A novel colorimetric method to quantify tannase activity of viable bacteria. *Journal of Microbiological Methods* 2003; 54(2): 281–284. doi: 10.1016/S0167-7012(03)00063-0
11. Girdhari SN, Peshwe SA. Screening of agro-residues for the production of microbial tannase. *International Journal of Advanced Research* 2017; 5: 625–631. doi: 10.21474/IJAR01/2790
12. Sharma S, Bhat TK, Dawra RK. A spectrophotometric method for assay of tannase using rhodanine. *Analytical Biochemistry* 2000; 279(1): 85–89. doi: 10.1006/abio.1999.4405
13. Shirling EB, Gottlieb D. Methods for characterization of Streptomyces species. *International Journal of Systematic Bacteriology* 1966; 16(3): 313–340. doi: 10.1099/00207713-16-3-313
14. Chakrabarti T. *Detection of Polyene Class of Antifungal Antibiotics. Actinomycetes-Isolation, Screening, Identification and Gene Cloning in Streptomyces. Laboratory Manual*. Institute of Microbial Technology (IMTECH); 1998.
15. Murray MG, Thompson WF. Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Research* 1980; 8(19): 4321–4326. doi: 10.1093/nar/8.19.4321
16. Zuckerkandl E, Pauling L. Evolutionary divergence and convergence in proteins. In: Bryson V, Vogel HJ (editors). *Evolving Genes and Proteins*. Academic Press; 1965. pp. 97–166.
17. Tamura K, Stecher G, Peterson D, et al. MEGA6: Molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution* 2013; 30(12): 2725–2729. doi: 10.1093/molbev/mst197
18. El Sohafy SM, Metwalli AM, Harraz FM, Omar AA. Quantification of flavonoids of *Psidium guajava* L. preparations by Planar Chromatography (HPTLC). *Pharmacognosy Magazine* 2009; 4(17): 61–66.
19. Samee W, Vorarat S. Simultaneous determination of gallic acid, catechin, rutin, ellagic acid, and quercetin in flower extracts of *Michelia alba*, *Caesalpinia pulcherrima*, and *Nelumbo nucifera* by HPLC. *Thai Pharmaceutical and Health Science Journal* 2007; 2: 131–137.
20. Tamura K, Nei M. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution* 1993; 10(3): 512–526. doi: 10.1093/oxfordjournals.molbev.a040023
21. Roy S, Parvin R, Ghosh S, et al. Occurrence of a novel tannase (*tan* B_{LP}) in endophytic *Streptomyces* sp. AL1L from the leaf of *Ailanthus excelsa* Roxb. *3 Biotech* 2018; 8: 33. doi: 10.1007/s13205-017-1055-4
22. Mueller-Harvey I, Parkes RJ. Measurement of volatile fatty acids in pore water from marine sediments by HPLC. *Estuarine, Coastal and Shelf Science* 1987; 25(5): 567–579. doi: 10.1016/0272-7714(87)90115-6
23. Kumar SS, Sreekumar R, Sabu A. Tannase and its applications in food processing. In: Parameswaran B, Varjani S, Raveendran S (editors). *Green Bio-processes. Energy, Environment, and Sustainability*. Springer; 2019. pp. 357–381. doi: 10.1007/978-981-13-3263-0_19
24. Sharma S, Gupta MN. Synthesis of antioxidant propyl gallate using tannase from *Aspergillus niger* van Tiegham in nonaqueous media. *Bioorganic & Medicinal Chemistry Letters* 2003; 13(3): 395–397. doi: 10.1016/S0960-894X(02)00977-0
25. de Lima JS, Cruz R, Fonseca JC, et al. Production, characterization of tannase from *Penicillium montanense* URM 6286 under SSF using agroindustrial wastes, and application in the clarification of grape juice (*Vitis vinifera* L.). *The Scientific World Journal* 2014; 2014: 182025. doi: 10.1155/2014/182025
26. Neethu RS, Pradeep NS. Isolation and characterization of potential tannase-producing fungi from mangroves and tanneries. *Indian Journal of Applied Microbiology* 2018; 21(3): 1–13.
27. Tripathi AD, Lakshmi B. Statistical optimization of extracellular tannase production by *Streptomyces sp*. AT 13 using response surface methodology and Plackett-Burmen design. *Bioscience Biotechnology Research Communications* 2018; 11(4): 691–698. doi: 10.21786/bbrc/11.4/21
28. Böer E, Bode R, Mock HP, et al. Atan1p—An extracellular tannase from the dimorphic yeast *Arxula adeninivorans*: Molecular cloning of the *ATAN1* gene and characterization of the recombinant enzyme. *Yeast* 2009; 26(6): 323–337. doi: 10.1002/yea.1669
29. Lekshmi R, Nisha SA, Vasan PT, Kaleeswaran B. A comprehensive review on tannase: Microbes associated production of tannase exploiting tannin rich agro-industrial wastes with special reference to its potential environmental and industrial applications. *Environmental Research* 2021; 201: 111625. doi: 10.1016/j.envres.2021.111625
30. Podrigues THS, Dantas MAA, Pinto GAS, Gonçalves LRB. Tannase production by solid state fermentation of cashew apple bagasse. In: Mielenz JR, Klasson KT, Adney WS, McMillan JD (editors). *Applied Biochemistry and Biotecnology. ABAB Symposium*. Humana Press; 2007. pp. 675–688. doi: 10.1007/978-1-60327-181-3_55
31. Kar B, Banerjee R, Bhattacharyya BC. Effect of additives on the behavioural properties of tannin acyl hydrolase. *Process Biochemistry* 2003; 38(9): 1285–1293. doi: 10.1016/S0032-9592(02)00329-1
32. Pinto GAS, Leite SGF, Terzi SC, Couri S. Selection of tannase-producing *Aspergillus niger* strains. *Brazilian Journal of Microbiology* 2001; 32: 24–26. doi: 10.1590/S1517-83822001000100006

33. Seth M, Chand S. Biosynthesis of tannase and hydrolysis of tannins to gallic acid by *Aspergillus awamori*—Optimisation of process parameters. *Process Biochemistry* 2000; 36(1–2): 39–44. doi: 10.1016/S0032-9592(00)00179-5
34. Deschamps AM, Lebeault JM. Production of gallic acid from tara tannin by bacterial strains. *Biotechnology. Letters* 1984; 6: 237–242. doi: 10.1007/BF00140043
35. Nalan Yılmaz S, Erdoğan Ç, Merih K, Muzaffer T. A method for the determination of tannase activity based on gallic acid measurement by high-performance liquid chromatography (HPLC). *African Journal of Microbiology Research* 2011; 5(2): 158–163. doi: 10.5897/AJMR10.548