Biocatalysis and Agricultural Biotechnology 18 (2019) 101014

Contents lists available at ScienceDirect

Biocatalysis and Agricultural Biotechnology

journal homepage: www.elsevier.com/locate/bab

Talaromyces verruculosus tannase production, characterization and application in fruit juices detannification

Amitabh Aharwar, Dharmendra Kumar Parihar*

Department of Biotechnology, Guru Ghasidas Vishwavidyalaya, Bilaspur, 495009, Chhattisgarh, India

ARTICLE INFO

Keywords:
Tannase
Solid-state fermentation
Talaromyces verruculosus
Babul bark
Central composite design
Detannification

ABSTRACT

A potential tannase producing fungus S3-07 was isolated from the tannin-rich soil sample and screened as better tannase producer with 38 ± 3 mm diameter of hydrolysis zone formation. It was identified as *T. verruculosus* (accession number KX863699) on the basis of the internal transcribed spacer ribosomal DNA (ITS-rDNA) sequence analysis. Tannase was produced with 8.22 ± 0.35 Unit per gram dry substrate (U/gds) activity in the unoptimized condition through solid state fermentation (SSF) using Babul (Acacia nilotica) bark as a solid substrate. Central composite design (CCD) was used to maximize the production and achieved 3.91 fold increments (32.18 ± 1.31 U/gds). Ammonium sulfate precipitation with 70% saturation and DEAE fractionation obtained 13.289 specific activity, 47.489% yield and 6.896 purification fold. SDS-PAGE and zymographic analysis showed two bands of tannase with ~75 kDa and 58 kDa pH optima of tannase was measured at 8.00, whereas pH stability was recorded between 4.0 and 8.0. Tannase had temperature optima at 60 °C, however, stability was observed between 30 and 60 °C. In presence of ethanol, SDS, Mn^{2+} ions and gallic acid increased tannase activity, whereas ethyl acetate, tween 20, Ba^{2+} ion and urea inhibited maximum activity. Most of tannin reduction (93.27%) was measured in case of orange juice after 15 min incubation and 89.36% reduction in pomegranate juice after 30 min, whereas aonla juice was measured with 75.49% tannin reduction after 45 min incubation.

1. Introduction

Tannins are the secondary metabolites of plants (Govindarajan et al., 2016) moreover, they are the second most abundant group of plant phenolics after lignin and fourth most abundant biochemical compound after the cellulose and hemicelluloses (Aguilar et al., 2007). The phenolic compounds inhibit the growth of microbes, but some microbes are resistant against tannins (Bhat et al., 1998). The anti-nutritional effect of tannins was also observed because they bind to proteins and make the precipitate (Rodrıguez-Duran et al., 2011). Gallic acid (3, 4, 5-trihydroxy benzoic acid) is a product obtained after tannins degradation furthermore, it is used in the food industry, printing ink and photography (Bajpai and Patil, 2008). Gallic acid also has application in cancer for the treatment of leukemia and oral cancer and also has antimelanogenic activity. Tannase (E.C.3.1.1.20) degrades gallic acid ester and tannins that form glucose and gallic acid as final products (Lekha and Lonsane, 1997).

Tannase is produced by the microorganism which is the main source for industries (Yao et al., 2014). *Aspergillus* sp. and *Penicillium* sp. are major tannase producing fungi which have the ability to utilise hydrolysable and condensed tannin (Prigione et al., 2018). On other hand *Bacillus* sp. is the main tannase producing bacteria (Raghuwanshi et al., 2011). Tannase producing microorganisms have habitat in soil, water, wastes, pickle, beverage and feces (Jana et al., 2014). Tannase is industrially produced by submerged fermentation (SmF) and solid state fermentation (SSF) methods. However, SmF is usually preferred for bacterial and yeast tannase production whereas SSF for fungal tannase production. But at present, SSF is also being used for bacterial tannase production (Jana et al., 2013). SSF is significant in comparison to SmF because of the use of the naturally available low-cost substrate, extracellular tannase production moreover, less water and energy consumption (Chavez-Gonzalez et al., 2012; Bhoite and Murthy, 2015).

Optimization of the fermentation conditions for tannase production using one variable at a time is a time-consuming method because only one factor can be changed and others are remained constant moreover, the method does not explain the interaction among different factors (Raghuwanshi et al., 2011). While using statistical tools for the optimization of the fermentation condition is less time taking and inexpensive. These tools are being used to study the interaction among different nutritional and physiological factors, furthermore for the

* Corresponding author.
E-mail addresses: amitabh.aharwar@gmail.com (A. Aharwar), parihardkp@rediffmail.com (D.K. Parihar).

https://doi.org/10.1016/j.bcab.2019.01.052
Received 19 October 2018; Received in revised form 28 December 2018; Accepted 28 January 2019
Available online 29 January 2019
1878-8181/

screening of factors as well (Viswanath et al., 2016; Kumar et al., 2015).

Tannase is being applied for the processing and improvement of fruit juices, refreshing drinks, beer and wine moreover, it serves as debettering and clarifying agent (Lima et al., 2014). It stops the cream formation during the preparation of tea that enhances taste, clarity and antioxidant property (Chandini et al., 2013). Tannase has been used as a supplementary agent for animal feed improvement (Abdulla et al., 2016). It has application in the production of gallic acid and propyl gallate which are used in food industry as antibacterial and antioxidant agent respectively (Mahmoud et al., 2018; Fernandez-Lorente et al., 2011).

In this study, the potential fungal isolate was identified as *T. verruculosus*. This isolate was taken in SSF of *Acacia nilotica* bark for tannase production. CCD was used for the optimization of the variables to obtain maximum tannase production. Tannase was purified through ammonium sulfate precipitation followed by DEAE cellulose 52. Purified tannase was characterized and used in the fruit juices gallotannin reduction.

2. Materials and methods

2.1. Chemicals

All chemicals used in this study were of analytical grade and procured from Himedia bioscience, India.

2.2. Isolation of fungus

The sample was collected from the soil of vegetables degrading site from Bilaspur, Chhattisgarh, India. The sample was air dried for 24 h and stored at low temperature for further use. Isolation of fungus from the sample was performed in malt extract agar medium containing (gl^{-1}) malt extract 30.00, peptone 5.00 and agar 15.00 using serial dilution method. After 3 days incubation single, different colored and appearance of the fungal isolate was transferred to malt extract agar slant and maintained at optimized laboratory conditions.

2.3. Screening of fungal organisms

Screening of fungus was performed in tannic acid agar medium (pH 5.5) containing (gl^{-1}): tannic acid 10.00, $NaNO_3$ 6.00, KCl 0.5, $MgSO_4{\cdot}7H_2O$ 0.5, KH_2PO_4 1.5, $FeSO_4{\cdot}7H_2O$ 0.001 and agar 30.00 (Bradoo et al., 1996). Screening medium was prepared in the flask and autoclaved at 121.5 °C for 20 min. The medium plate was prepared then milky white color was appeared in medium plates due to tannic acid. The fungal isolates were point inoculated into screening medium plates and incubated at 27 °C for 3-days.

2.4. Identification of potential tannase producing fungal isolate

The potential fungal isolate was identified by morphological and molecular characteristics. For morphological characteristics, the fungal isolate was grown in malt extract agar medium. After a proper growth, the microscopic view was taken through a compound microscope (Zeiss compound microscope) and scanning electron microscope (FEI Nova NanoSEM 450). However, molecular ITS sequencing was performed by Chromous Biotech Pvt Ltd, Bangalore, India. Fungal DNA was isolated using a DNA isolation kit. PCR was performed for amplification of ITS region with ITS specific primer (forward primer 5′- TCMGTAGGTGADCCWBCGS - 3′ and reverse primer 5′- TCCTNCGYTKATKGVTADGH - 3′). Amplification reaction was done with 1 µl of 100 ng DNA, 400 ng forward primer, 400 ng reverse primer, 4 µl (10 mM each) dNTPs, 10 µl 10X chromTaq DNA polymerase assay buffer, 1 µl chromTaq DNA polymerase (3 U/µl) and X ml water in total 100 µl reaction volume. In the PCR, initial denaturation was performed at 95 °C for 5-min and further denaturation at 94 °C for 30 s, then annealing was done at 52 °C for 30 s followed by initial extension at 72 °C for 45 s and the final extension at 72 °C for 7 min. Total 35 cycles were performed for denaturation, annealing and initial extinction (but, not for initial denaturation and final extension). Obtained ITS sequence was aligned with similar sequences of other fungi using the BLASTN program of NCBI. Mega 6 software was used for the construction of the phylogenetic tree with 30 aligned sequences of fungi using Maximum likelihood analysis method and Kimura 2-parameter nucleotide substitution method.

2.5. Production of tannase

2.5.1. Substrate and inoculum preparation

Babul bark was used as a solid substrate for tannase production, which is collected from the Babul tree on the campus of Guru Ghasidas Vishwavidyalaya, Bilaspur, India. Other solid substrates given in Table S1 were collected from the market of Bilaspur and used for tannase production. The substrates were dried at 45 °C for 24 h in the hot air oven. The inoculum was prepared aseptically by the addition of sterile distilled water containing 0.1% tween 80 into the sporulated fungal culture.

2.5.2. Estimation of tannin

Solid substrates were dried in hot air oven at 45 °C for 24 h. Tannin estimation of solid substrates was performed by Hagerman and Butler (1978).

2.5.3. Solid state fermentation

In 250 ml Erlenmeyer flask, 10 g of the substrate were taken and moistened with 15 ml of mineral salt solution (pH 7.0) containing 2% NH_4NO_3, 1% KH_2PO_4, 1% $MgSO_4.7H_2O$ and 1% KCl. After the autoclaving, 2 ml of 10^7 spores/ml inoculum of spore suspension was inoculated into the solid substrate and incubated at 27 °C for 120 h of incubation.

2.5.4. Enzyme extraction

The fermented substrate was extracted adding 100 ml of 0.05 M Tris-HCl buffer (pH 8.0) to the flask and placed it in a rotary shaker at 100 rpm for 30 min at low temperature. Whatman no. 1 filter paper was used for the crude extract filtration and the filtrate was centrifuged at 6000 rpm for 10 min. The obtained supernatant (used as a crude enzyme) was stored at low temperature for further study.

2.6. Enzyme assay

Enzyme assay was performed as by Mondal et al. (2001) based on the absorbance and colorimeter method. One unit of enzyme activity is defined as the hydrolysis of 1 mM substrate (tannic acid) in 1 min under assay condition.

2.7. Statistical optimization of tannase production

2.7.1. Plackett-Burman design

It is a factorial type design has been used for the screening of the most important factors. There are seven factors have been taken which are temperature (°C), pH, initial moisture level (%), spore concentration, incubation period (h), tannic acid and NH_4NO_3. Total 13 runs were generated in which 12 runs with low (−1) and high (+1) levels of factors, whereas 1 run with central or middle (0) level of factors (Table 1).

2.7.2. Central composite design

Design expert 9 software was used for the optimization of factors using Response surface methodology (Central composite design) for tannase production. Every factor (variable) had five different levels -α, −1, 0, +1 and +α (α = 1.68) which represent lower, low, middle, high and higher level respectively (Table S2). CCD generated 20

Table 1
Plackett-Burman design in response of enzyme activity.

Run	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6	Factor 7	Response	
	A:Temperature (°C)	B:pH	C: Initial moisture level (%)	D:Spores concentration (power of 10)	E: Tannic acid (%)	F:Incubation period (h)	G: Ammonium nitrate (%)	R:Enzyme activity (U/gds)	
								Actual	Predicted
1	17	8	64.28	8	0.5	48	0.1	1.27 ± 0.11	1.14
2	17	4	37	4	0.5	48	0.1	3.06 ± 0.22	2.85
3	42	8	64.28	4	0.5	48	2.5	1.13 ± 0.09	1.74
4	17	4	64.28	4	5	168	0.1	8.98 ± 0.43	9.01
5	42	4	37	4	5	48	2.5	4.08 ± 0.26	3.95
6	42	4	64.28	8	0.5	168	2.5	2.01 ± 0.12	1.22
7	17	8	37	8	5	48	2.5	4.33 ± 0.17	3.88
8	42	4	64.28	8	5	48	0.1	1.39 ± 0.14	1.70
9	42	8	37	4	0.5	168	0.1	0.49 ± 0.03	0.22
10	17	8	64.28	4	5	168	2.5	9.57 ± 0.47	9.54
11	29.5	6	50.64	6	2.75	108	1.3	3.13 ± 0.26	3.13
12	17	4	37	8	0.5	168	2.5	2.07 ± 0.11	2.86
13	42	8	37	8	5	168	0.1	0.44 ± 0.04	0.71

experimental run plan of significant factors were performed on the response of tannase production.

2.8. Purification of tannase

2.8.1. Ammonium sulfate precipitation

Ammonium sulfate was added slowly into the crude extract by continuous stirring to obtain the up to 70% saturation and left it overnight at 4 °C for precipitation. Centrifugation was performed at 8000 rpm for 15 min at 4 °C for the collection of precipitate which was dissolved in 0.1 M Tris-HCl buffer (pH 8.0). Dialysis was performed against the same buffer for 24 h at 4 °C by changing the buffer 3 times for the removal of ammonium sulfate.

2.8.2. Ion exchange chromatography

DEAE cellulose 52 (Himedia) was taken in 0.1 M Tris-HCl buffer (pH 8.0) and leave for 3–4 h, then glass column was filled with DEAE cellulose and equilibrated with Tris-HCl buffer (pH 8.0) overnight. Then partially purified (dialyzed) enzyme was loaded in the column and left for 2 h. Enzyme elution was performed adding 0.3 M NaCl. Fractions were taken out with 1 ml/min flow rate and analyzed for protein estimation and enzyme assay. Fractions with the highest activity was stored at low temperature and used for the further experiment. Protein estimation of the samples was performed by Lowry et al. (1951) method.

2.8.3. SDS-PAGE and zymography

SDS-PAGE was performed to estimate the molecular weight and purity of tannase (Laemmli, 1970). Protein samples (crude enzyme, partially purified and purified enzyme) were resolved in the 12% resolving gel at 90 V for 3 h at 4 °C. After run, the gel was cut into two parts, first part having protein marker, crude, partially purified and purified enzyme was stained with Coomassie brilliant dye while the second part of gel containing purified enzyme was proceeded for zymography (Prajapati et al., 2017). Gel was left in 50 ml of 0.5% tannic acid solution for 30 min at low temperature then left it for 5 min at room temperature. After incubation the gel was placed in 0.5% quinine hydrochloride solution for 3–4 h to visualize the tannase activity on the gel (Aoki et al., 1979).

2.9. Characterization of tannase

2.9.1. Temperature optima and stability

Optima was recorded with different temperatures ranging from 30 to 90 °C under standard assay condition. Whereas thermal stability of the enzyme was also measured at the time interval of every 30 min for 4 h with different temperature (30, 40, 50, 60, 70, 80 and 90 °C) and the residual activity was observed.

2.9.2. pH optima and stability

Optimum pH was analyzed by incubating the enzyme at different pH ranging 3.0–10.0 using buffers likes glycine HCl buffer (pH 3), Acetate buffer (pH 4–5), Phosphate buffer (6–7), Tris HCl buffer (pH 8–9) and Glycine NaOH buffer (pH 10). However, pH stability was checked at room temperature with different pH in the time interval of every 30 min, then residual activity was measured.

2.9.3. Effect of solvents

Solvents such as methanol, ethanol, isopropanol, acetone, chloroform, ethyl acetate, hexane, glycerol, acetonitrile, liquid ammonia, formaldehyde, and dimethyl sulfoxide (DMSO) in 1% concentration were used in the reaction mixture to see their effect on enzyme activity. Residual activity was recorded with control (100%) having no solvents.

2.9.4. Effect of surfactants

Surfactants like sodium dodecyl sulfate (SDS), tween 20, tween 80 and Triton 100 were used in reaction mixture in 1% concentration. Residual activity was analyzed with control having no surfactants in the reaction mixture.

2.9.5. Effect of metal ions

Different metal salts such as $Al_2(SO_4)_3$, $BaCl_2$, $CaCl_2$, $CdCl_2$, $CoCl_2$, $CuCl_2$, $FeCl_3$, $FeSO_4$, $HgCl_2$, $LiSO_4$, $MgSO_4$, $MnCl_2$, $NiSO_4$, $SnCl_2$, $ZnSO_4$, NaCl and KCl in 1 mM concentration were used in reaction mixture to measure the effect of metal ions on enzyme activity. However, residual activity was observed with control (100%) without adding metal ions in the reaction mixture.

2.9.6. Effect of additives

Sodium azide, sodium deoxycholate, phenylmethylsulfonyl fluoride (PMSF), gallic acid, $NaNO_3$, NH_4Cl, NH_4NO_3, $(NH_4)_2SO_4$, urea and thiourea in 1 mM concentration, whereas 1% concentration of 2-mercaptoethanol was used in the reaction mixture. Residual activity was measured with control (100%) without additives in reaction mixture.

2.10. Application in detannification of fruit juices

Aonla, pomegranate and orange fruits were peeled and the juice was extracted pressing in the cheesecloth after crushing in the blender. For tannin removal, 10 ml of each fruit juice was treated with 1 ml of purified enzymes at 37 °C at 100 rpm. At the interval of every 5 min of incubation, 1 ml of each fruit juice was taken out for residual tannin

Table 2
Experimental result of tannase production through central composite design.

Run	A:Tannic acid (%)	B:Incubation period (h)	C:Moisture (%)	Response Enzyme activity (u/gds)	
				Actual	Predicted
1	5.28	108	54	32.18 ± 1.31	31.39
2	5.28	108	54	31.53 ± 1.43	31.39
3	8.1	144	64	27.07 ± 1.07	27.77
4	8.1	72	44	15.81 ± 0.64	16.23
5	5.28	47.46	54	16.00 ± 0.57	16.02
6	5.28	108	70.82	19.00 ± 0.94	18.69
7	5.28	108	54	30.97 ± 1.37	31.39
8	5.28	108	54	30.39 ± 1.29	31.39
9	5.28	108	37.18	16.04 ± 0.83	15.90
10	5.28	168.54	54	22.64 ± 1.14	22.21
11	0.52	108	54	14.56 ± 0.438	15.01
12	5.28	108	54	31.95 ± 1.26	31.39
13	10.03	108	54	25.69 ± 1.14	24.82
14	5.28	108	54	31.26 ± 1.29	31.39
15	2.45	72	44	17.01 ± 0.91	16.59
16	8.1	72	64	18.16 ± 0.73	18.39
17	2.45	72	64	13.51 ± 0.48	13.46
18	8.1	144	44	20.99 ± 0.97	21.32
19	2.45	144	64	15.86 ± 0.67	15.74
20	2.45	144	44	14.53 ± 0.73	14.58

estimation.

3. Results and discussion

3.1. Isolation, screening and identification of the fungal isolate

Isolates were picked up and screened in tannic acid agar medium with 1% tannic acid by the appearance of the hydrolysis zone of tannic acid, which was due to tannase production around the fungal growth in screening medium. Fungal isolate S3-07 was observed as potential tannase producer with the hydrolysis zone of 38 ± 3 mm diameter appeared in 3-day growth shown in Fig. S1 (B). In morphological characteristics, isolate S3-07 had mycelia with white, yellow color, bivercilliate conidiophore, flask-shaped phialides, globose conidia (with green color), plane surface colony and dense sporulation shown in Fig. S1 (A), (C) and (D). Molecular characterization was done by PCR reaction, agarose gel electrophoresis and sequencing reaction with ITS-rDNA sequence. Isolated genomic DNA and the PCR amplified product of ITS region of the fungal isolate were analyzed with a single band in 1% agarose gel electrophoresis presented in Fig. S2 (A). PCR product was measured with 557 base pairs sequence which was used in a BLASTN program of NCBI for the alignment. A maximum similarity of 100% was obtained with *Talaromyces verruculosus* isolate ATT281. Obtained sequence was submitted to GenBank database then accession number KX863699 was received. Then, a phylogenetic tree was prepared using Mega 6 software in which maximum likelihood method (a statistical method), bootstrap method (phylogeny test) and kimura-2 parameter model (Substitution Model) were selected to draw phylogenetic tree which is presented in Fig. S2 (B).

3.2. Tannase production

Tannin was estimated in different substrates and among different substrates, Babul (Keekar) bark was observed with highest tannin content 121.3 ± 4.2 mg/g dry substrate (gds), whereas minimum tannin content 1.19 ± 0.11 mg/g ds was observed in rice bran. Tannase production through SSF was performed with these substrates then maximum activity (8.22 ± 0.35 U/gds) was observed with Babul bark, while minimum activity (2.14 ± 0.26 U/gds) measured with rice bran. A list of substrates with their tannin content and tannase production (enzyme activity) is given in Table S1.

3.3. Statistical optimization of tannase production

3.3.1. Plackett-Burman design

Total 13 experimental runs plan was obtained through design expert software which is given in Table 1 which was performed in the response of enzyme activity to screen the significant variables. Variation in enzyme activity was observed in the results of experimental runs from 0.44 ± 0.04 to 9.57 ± 0.47 u/gds. Temperature (^{o}C), pH and spore concentration had a negative effect on tannase production, whereas initial moisture level, tannic acid, incubation time and NH_4NO_3 have a positive effect on tannase production.

Enzyme activity was explained in following equation (1) in terms of coded factors:

$$Y = 3.23–1.65\ A - 0.36\ B + 0.82\ C - 1.32\ D + 1.56\ E + 0.69\ F + 0.63\ G \ldots \ldots \quad (1)$$

Y is the response (enzyme activity U/gds), A-Temperature (^{o}C), B-pH, C- Initial moisture level (%), D-Spore concentration (power of 10), E-Tannic acid (%), F-Incubation time (h) and G-NH_4NO_3 (%). This equation (1) (in terms of coded factors) can be used to make predictions about the response for given levels of each factor. High and low levels of the factors are coded as +1 and −1 respectively. The coded equation is used for the identification of the relative impact of the factors by comparing the factor coefficients.F-value of the model was 34.04 which implies that the model is significant. Model terms are significant if the values of "Prob > F″ less than 0.05. In this case, A, C, D, E, F, G was significant model terms, whereas B was a non-significant model term. The difference between "Predicted R^2" by 0.8227 and "Adjusted R^2″ of 0.9507 was less than 0.2. Signal to noise ratio is measured by "Adequate Precision", whereas a ratio greater than 4 is desirable for the model. In this study, this ratio was 18.095 which indicates an adequate signal and however this model can be used to navigate the design space.

3.3.2. Central composite design (CCD)

Optimization was performed with Babul (Keekar) bark because it had the highest tannin content among different substrates and also had maximum tannase production. The CCD was used (response surface methodology) for the optimization of variables such as tannic acid, initial moisture level and incubation period for maximum tannase production. However, other factors were taken with temperature 29.5 °C, pH 6.5, spore concentration 10^7 and ammonium nitrate 2.05%. Results of 20 experimental runs in the response to enzyme activity are

Table 3
Analysis of variance for Response Surface Quadratic model table.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	945.07	9	105.01	238.04	< 0.0001	significant
A-Tannic acid	116.18	1	116.18	263.36	< 0.0001	
B-Incubation period	46.30	1	46.30	104.97	< 0.0001	
C-Moisture	9.37	1	9.37	21.24	0.0010	
AB	25.21	1	25.21	57.15	< 0.0001	
AC	14.00	1	14.00	31.75	0.0002	
BC	9.17	1	9.17	20.78	0.0010	
A^2	237.10	1	237.10	537.47	< 0.0001	
B^2	271.49	1	271.49	615.44	< 0.0001	
C^2	358.02	1	358.02	811.59	< 0.0001	
Residual	4.41	10	0.44			
Lack of Fit	2.26	5	0.45	1.05	0.4786	not significant
Pure Error	2.15	5	0.43			
Cor Total	949.48	19				

given in Table 2, in which the variation of enzyme activity was observed from 13.51 ± 0.48 (minimum) to 32.18 ± 1.31 u/gds (maximum). In this design, polynomial analysis and quadratic model were used for enzyme activity measurement. An equation is given below in terms of coded factors for the prediction of tannase activity.

$$Y = 31.39 + 2.92A + 1.84\,B + 0.83C + 1.78AB + 1.32AC + 1.07BC - 4.06A^2 - 4.34B^2 - 4.98C^2$$

Y represents enzyme activity and A, B and C represent tannic acid, incubation period and initial moisture level respectively. This equation can be used for the predictions of response (enzyme activity) for given levels of each factor. The factors with high levels are coded +1, whereas −1 for low levels. By comparison of the factor coefficients, the coded equation can be applied for the identification of the relative impact of factors.

This Model has 238.04 F-value which indicates that the model is significant (Table 3). There is only a 0.01% (p-value Prob > F = < 0.00001) chance that an F-value this large could occur due to noise. Model terms are significant if the value of "Prob > F" less than 0.05, whereas values greater than 0.1 indicates that the model terms are not significant. A, B, C, AB, AC, BC, A^2, B^2 and C^2 are significant model terms which is presented in Table 3. The Lack of Fit F-value of 1.05 indicates that Lack of Fit is not significant relative to the pure error. There is a 47.86% chance that a "Lack of Fit F-value" this large could occur due to noise. This model is fitted because of non-significant lack of fit which is essential for the model.

The correlation coefficient (R^2) of 0.995 indicates that 99.5% of the variation in enzyme activity could be explained by the independent variable and the model as well (Table 4). The difference of Predicted R^2 (0.978) and Adjusted R^2 (0.991) is less than 0.2 which is good for the model. Adequate Precision measures the signal to noise ratio, which is greater than 4 is desirable, however the ratio of 38.175 implies an adequate signal here.

Maximum enzyme activity was predicted 31.39 U/gds in CCD model whereas, obtained actual value was 32.18 ± 1.31 U/gds at 5.28% tannic acid, 108 h incubation time and 54% initial moisture level.

Table 4
Statistical analysis of CCD.

Model terms	Values
Std. Dev.	0.66
Mean	22.25
C.V. %	2.98
PRESS	20.49
R^2	0.995
Adjusted R^2	0.991
Predicted R^2	0.978
Adequate Precision	38.175

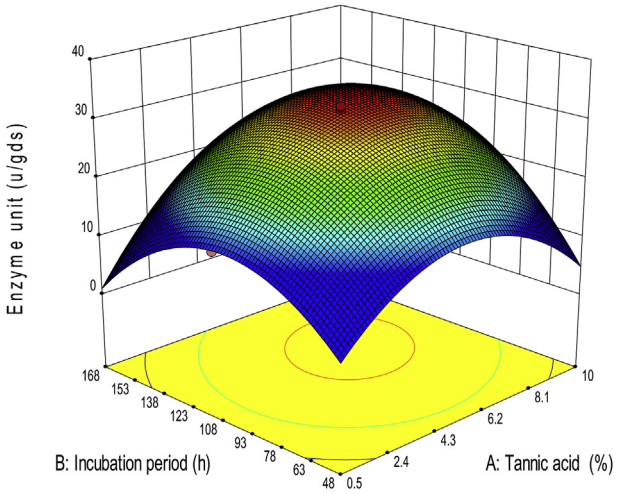

Fig. 1. Response surface curve is showing interaction between tannic acid and incubation period.

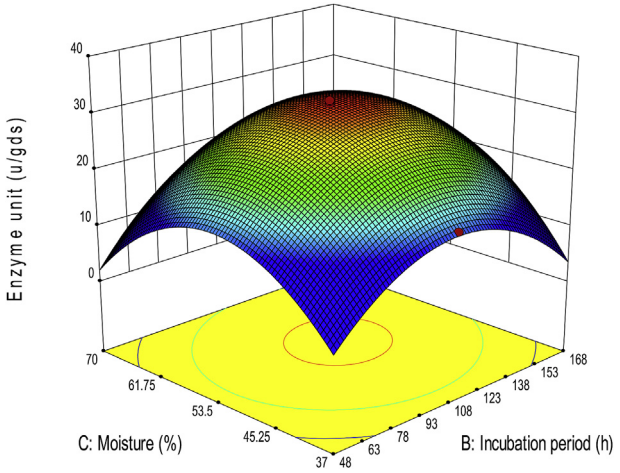

Fig. 2. Response surface curve is showing interaction between incubation period and initial moisture level.

Response surface curves is demonstrated in Figs. 1–3, are showing the interaction between significant factors.

Tannic acid used as a carbon source however, it induced tannase production. In this study, maximum tannase activity was observed at 5.28% tannic acid concentration. Madeira Jr. et al. (2011) observed maximum tannase activity at 6% tannic acid concentration with castor bean residues by *Paecilomyces variotii,* whereas Battestin and Macedo

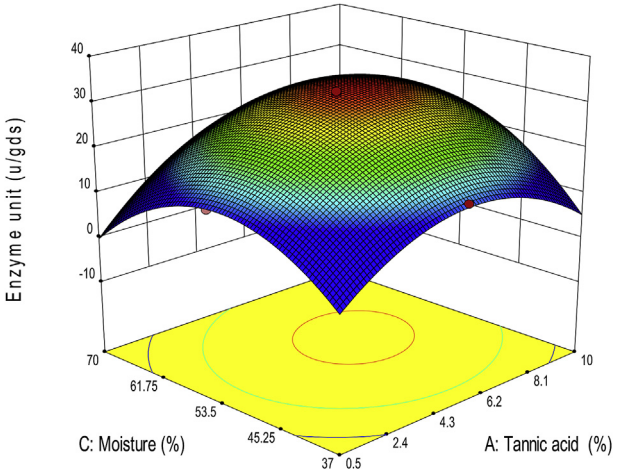

Fig. 3. Response surface curve is showing interaction between tannic acid and initial moisture level.

(2007) reported maximum tannase production (1.3–1.5 U/ml) at 12% tannic acid concentration with solid substrate coffee husk and wheat bran by *Paecilomyces variotii*.

Moisture plays a very important role in SSF. However, air and oxygen supply for the micro-organism decreases as moisture level increases, therefore it inhibits the growth of microbes and enzyme production as well (Kumar et al., 2007). However, when moisture has lower and higher level it decreases the degradation of organic matters of solid substrates (Mandal and Ghosh, 2013). Tannase production has been observed optimum at 40–90% moisture content (Aharwar and Parihar, 2018). In our study, the initial moisture level was observed optimum at 54%, while, Sharma et al. (2014) observed maximum tannase production at 60% moisture, moreover Xiao et al. (2015) found the optimum moisture level at 62.5%. Incubation time was observed optimum at 108 h and above this optima, tannase production was decreased, this may be due to the decrease of nutrient which also leads the decrease in the growth of fungus or may be feedback inhibition. Liu et al. (2018) observed the 118 U/ml tannase activity of *A. niger* at 168 h incubation period.

Maximum tannase production was reported by Kumar et al. (2015) at pH 5.2, temperature 34.97 °C, incubation time 91.34 h and agitation speed 103.34 rpm in SmF through *Klebsiella pneumoniae*. However, Bhoite and Murthy (2015) observed 115.99 U/gds tannase production by *Penicillium verrucosum* at pH 5, moisture level 50% and fermentation time 96 h through SSF of Coffee pulp. Sharma et al. (2014) used tea residues and produced 1.86 U/gds tannase by *A. niger* at 30 °C, 96 h, 60% w/v moisture content. Whereas, Lima et al. (2014) used Barbados cherry, as a solid substrate and observed 41.64 U/ml tannase production after 72 h of fermentation of *Penicillium montanense* with 3.5% tannic acid and 70% moisture. Wang et al. (2013) obtained 62 U/gds tannase production from *A. niger* using tea stalks as a solid substrate at 28 °C, 96 h and Moisture level with 1:1.8 ratio (w/v). Moreover, Wu et al., (2018) measured 245 U/gds tannase activity from *Aspergillus tubingensis* CICC 2651 using tea stalks with tannin (7.49%), glucose (8.11%), $(NH_4)_2SO_4$ (9.26%), and yeast extract (2.25%). Tannase production was observed 301.7 U/gds (3.02 fold increase) with cashew testa, K_2HPO_4 (3.40 mM), sodium chloride (0.47 mM) and temperature (32–35 °C) using *A. niger* after optimization through response surface methodology (Viswanath et al., 2016). Varadharajan et al. (2017) obtained maximum activity 139.22 IU/ml through submerged fermentation by *A. oryzae* at 1.99% substrate concentration, 4.89 pH, 34.91 °C temperature, and 70.65 h incubation time.

3.4. Purification

Tannase is purified through ammonium sulfate precipitation, dialysis and DEAE cellulose 52. Protein content and enzyme activity was checked in each step, whereas purification detail is given in Table 5. Specific activity, yield and purification fold were 3.64, 65.78% and 1.89 in dialysed sample respectively, whereas 13.29, 47.49% and 6.9 in DEAE cellulose purified sample respectively. Beniwal et al. (2013) obtained 3.22 specific activity, 33.1% yield and 8.47 fold purification after final purification by DEAE cellulose. However, Gayen and Ghosh (2013) measured 22.48 specific activity, 11.77% yield and 19.89 fold purification. After purification through Sephadex G200 Gel filtration, Abdel-Naby et al. (2016) obtained specific activity 182.8 U/mg protein, 15.8 purification fold and 36.7% yield. Likewise, Mahmoud et al. (2018) obtained specific activity 1026.12, yield 64.6% and purification fold 24.21 through Sephadex G-200 after ammonium sulfate precipitation.

Purity of tannase was checked in SDS-PAGE and result was shown in Fig. 4. Purified tannase have bands ~75 kDa and 58 kDa which were observed by Zymography. While Abdel-Naby et al. (2016) measured 108 kDa molecular weight of purified tannase of *Aspergillus oryzae*. Likewise, Mizuno et al. (2014) analyzed 45–85 kDa molecular mass of purified tannase. *Aspergillus phoenicis* tannase has 218 kDa molecular weight containing two subunits of 120 and 93 kDa (Riul et al., 2013). Mahmoud et al. (2018) observed 65 kDa mass of purified tannase of *Kluyveromyces marxianus*.

3.5. Characterization of tannase

The optimum temperature of tannase was observed at 60 °C, whereas the stability of tannase was observed between the ranges of 30–60 °C presented in Fig. 5(A) and (B). Tannase stability was recorded at 50 °C with 50.37% residual activity after 4 h of incubation, whereas 49.94% was at 60 °C after 3 h of incubation. However, it had 31.03% residual activity after 2 h of incubation at 70 °C. But major activity decrease was observed at 80 °C and 90 °C with 53.18 and 35.29% residual activity after 30 min of incubation. Whereas tannase was more stable at 30 °C and 40 °C with 91.17 and 80.07% relative activity up to 4 h of incubation.

Madeira Jr. et al. (2015) measured temperature optima at 70 °C and stability was observed at a range of 20–60 °C. Temperature optima and activity range of tannase from *Aspergillus phoenicis* was observed at 60 °C and stability up to 70 °C by Riul et al. (2013). However, Beniwal et al. (2013) obtained temperature optima of *Enterobacter cloacae* tannase at 50 °C and stability up to 60 °C. Tannase from *Aspergillus oryzae* was measured with the optima of 40 °C moreover, it was active upto 40 °C (Mizuno et al., 2014). Lopes et al. (2018) obtained the optimum temperature of *Saccharomyces cerevisiae* tannase at 30 °C and its stable activity range was 30–60 °C.

Optimum pH was observed at pH 8.0, whereas pH stability of tannase was observed in the range of 4–8 pH which are illustrated in

Table 5
Purification of tannase.

Sample	Protein mg/ml	Total activity	Total protein	Specific activity	Yield (%)	Purification Fold
Crude	16.7	32180	16700	1.927	100	1
Dialysed	5.81	21169	5810	3.644	65.783	1.89
DEAE Cellulose 52	1.15	15282	1150	13.289	47.489	6.896

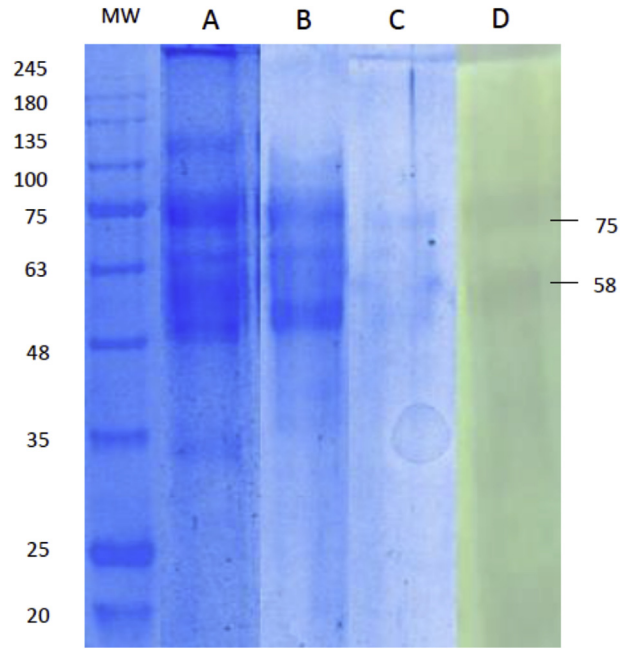

Fig. 4. SDS-PAGE and zymographic analysis of *T. verruculosus* tannase. **(MW)** Molecular weight marker **(A)** Crude enzyme **(B)** Partially purified tannase **(C)** Purified tannase **(D)** Zymography of purified tannase.

Fig. 6(A) and (B). Tannase had much stability at pH 7 and pH 8, with residual activity 92.63% and 80.64% after 4 h of incubation respectively, whereas strong inhibition was recorded at pH 3.0 and 10.0 with residual activity 54.61% and 43.69% after 90 min of incubation respectively. Roy et al. (2018) observed pH optima of *Streptomyces* sp. tannase at 6.0, while stability was measured at the range of 5.5–6.5. Lopes et al. (2018) found the optimum pH of tannase from *Saccharomyces cerevisiae* at 6.0 and stability at 3.0–9.0 pH. *Kluyveromyces marxianus* tannase had two pH optima of 4.5 and 8.5 whereas stability was observed between 4.0 and 5.5 pH (Mahmoud et al., 2018). Tannase from *Paecilomyces variotii* had pH optima at 5.0 and stability at 4.0–6.5 (Madeira Jr. et al., 2015). Jimenez et al. (2014) used recombinant tannase and analyzed pH optima at 6.0–8.0, while stability at the range of 4.0–9.0. Lima et al. (2014) observed the pH optima at 9.0 and also the stability over the wide range of pH. Abdel-Naby et al. (2016) observed optima of *Aspergillus oryzae* tannase at 5.5, whereas stability was between 4.5 and 7.5 pH.

Organic solvents react with enzyme's hydrophobic region then the result is protein precipitation. Acetone, ethanol, glycerol, methanol and propanol increased tannase activity whereas, acetic acid, acetonitrile, chloroform, DMSO, ethyl acetate, formaldehyde and hexane inhibited tannase activity (Table 6). Ethanol was observed with maximum increase (136.8% residual activity) in enzyme activity, whereas ethyl acetate with maximum decrease (69.35% residual activity). Jana et al. (2013) observed the same result with glycerol, isopropanol, ethanol and methanol as activator, whereas Goncalves et al. (2011) measured the effect of the organic solvents such as acetone, butanol and glycerol, as an inhibitor, moreover isopropanol, acetonitrile and ethanol as an activator. However, methanol, DMSO, isoamyl alcohol and butanol were observed as an inhibitor by Chaitanyakumar and Anbalagan (2016), whereas hexane, toluene and benzene were as the activator.

Surfactants increase the solubility of enzymes and substrates in the organic solvent that can increase or decrease the enzyme activity (Samiey et al., 2014). SDS increased tannase activity (residual activity 140.68%) maximum, whereas tween 80, tween 20 and Triton 100 inhibit tannase activity (residual activity 24.5, 23.24 and 26.4% respectively). Likewise, Beniwal et al. (2013) observed tween 20, tween 80, Triton X-100 and SDS as the inhibitor. Surfactants inhibit the enzyme activity by reducing the hydrophobic interactions. On another hand, SDS, tween 80 and Triton X-100 were found as an activator (Goncalves et al., 2011; Qiu et al., 2011).

Metal ion plays important role in the catalytic activity of tannase furthermore, it is required as a cofactor of enzyme (Jana et al., 2014). Cd^{2+}, Fe^{3+}, Mg^{2+}, Mn^{2+}, Sn^{2+}, Zn^{2+}, Na^{+} and K^{+} ions served as an activator, whereas, Ba^{2+}, Ca^{2+}, Co^{2+}, Cu^{2+}, Fe^{2+}, Hg^{2+}, Li^{2+} and Ni^{2+} ions were observed as inhibitor (Table 7). Maximum increase was observed in the presence of Mn^{2+} ion with 116.48% residual activity whereas, maximum inhibition was recorded with Ba^{2+} ion with 75.52% residual activity. Abdel-Naby et al. (2016) found that Ca^{2+} and Mg^{2+} increased tannase activity whereas Hg^{2+}, Fe^{2+}, and Cu^{2+} decreased it. Moreover, *Staphylococcus lugdunensis* tannase was inhibited by Zn^{2+}, Fe^{2+}, Fe^{3+} and Mn^{2+}, while Na^{+} increased the activity (Chaitanyakumar and Anbalagan, 2016). Mahmoud et al. (2018) observed the inhibitory effect of Na^{+}, K^{+}, Mg^{2+}, Ba^{2+}, Hg^{2+}, $Co^{2+}$and Zn^{2+}, moreover Mn^{2+} Ca^{2+}, Cd^{2+} Cu^{2+}, Li^{+} and Fe^{3+} were observed as the activator.

Among different additives, NH_4Cl, NH_4NO_3, $(NH_4)_2SO_4$, gallic acid and sodium azide increased tannase activity. Maximum 109.13% residual activity was measured with gallic acid. However, urea and thiourea strongly decreased tannase activity with 34.39% and 44.13% residual activity, whereas EDTA, phenylmethylsulfonyl fluoride (PMSF), sodium deoxycholate and $NaNO_3$ slightly inhibited tannase activity. A list of the effect of different additives on enzyme activity is given in Table 8. Sodium azide, EDTA, PMSF, β-mercaptoethanol, formaldehyde and urea were recorded as the inhibitor by Jana et al. (2013); Yao et al. (2013); Qiu et al. (2011). However, Kar et al. (2003) used $(NH_4)_2SO_4$, NH_4NO_3, and NH_4Cl that increased tannase activity. Moreover, gallic acid (Beniwal et al., 2013) and Sodium choleate (Sharma et al., 2008) were recorded as an activator.

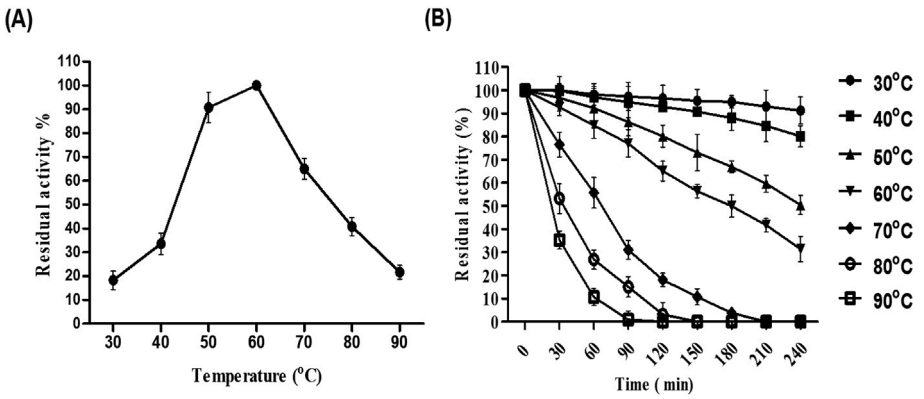

Fig. 5. (A) Temperature optima **(B)** Temperature stability.

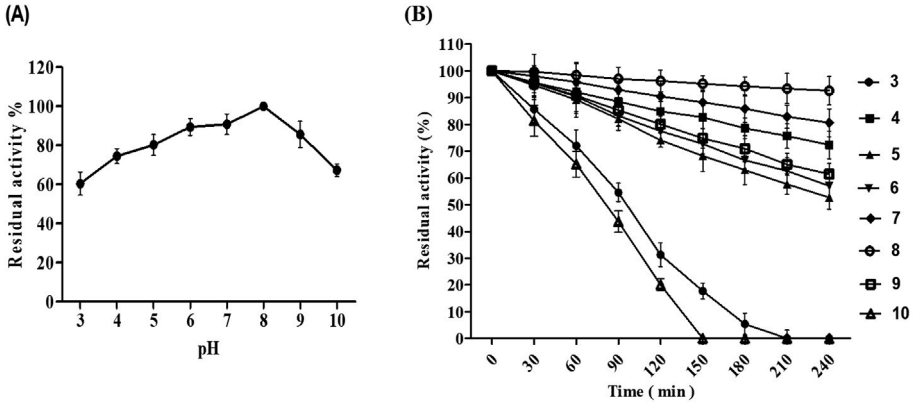

Fig. 6. (A) pH optima **(B)** pH stability.

Table 6
Effect of organic solvent on tannase activity.

S.No.	Solvent	Residual activity (%)
1	Control	100.0
2	Acetic acid	77.14 ± 3.6
3	Acetone	120.32 ± 4.2
4	Acetonitrile	72.36 ± 2.7
5	Ammonia liquid	100.92 ± 2.6
6	Chloroform	69.76 ± 3.2
7	DMSO	94.4 ± 4.4
8	Ethanol	136.8 ± 4.8
9	Ethyl Acetate	69.35 ± 3.7
10	Formaldehyde	72.23 ± 2.5
11	Glycerol	129.35 ± 3.9
12	Hexane	72.86 ± 3.1
13	Methanol	134.4 ± 4.4
14	Propanol	131.38 ± 3.6

Table 7
Effect of metal ions on tannase activity.

S.No.	Metal salt	Residual activity (%)
1	Control	100
2	$BaCl_2$	75.52 ± 3.4
3	$CaCl_2$	98.23 ± 4.2
4	$CoCl_2$	89.33 ± 5.1
5	$CdCl_2$	104.15 ± 2.7
6	$CuCl_2$	91.96 ± 3.5
7	$FeCl_3$	108.37 ± 4.4
8	$FeSO_4$	97.43 ± 3.1
9	$HgCl_2$	89.71 ± 5.3
10	$LiSO_4$	79.32 ± 3.6
11	$MgSO_4$	109.16 ± 2.8
12	$MnCl_2$	116.48 ± 4.9
13	$NiSO_4$	90.17 ± 5.4
14	$SnCl_2$	105.57 ± 3.3
15	$ZnSO_4$	109.66 ± 4.1
16	NaCl	105.38 ± 3.9
17	KCl	104.0 ± 4.6

3.6. Detannification of fruit juices

Fruit juices are beneficial for health because of their antioxidant property and nutrient richness as well. Presence of tannin content in fruit juices makes them haze, bitter, astringent and also form sediment during the storage period, however, these obstacles can be removed by detannification. Fruit juices mostly contain gallotannin however in this study, estimated tannin content was 1.635 mg/ml, 0.759 mg/ml, and 0.281 mg/ml in aonla, pomegranate and orange juices respectively. 10 ml of fruit juices was treated with 1 ml of purified enzyme (504 U/ml). The tannin reduction was 75.52% in the Aonla juice (after 45 min of incubation) and 89.29% in pomegranate juice (after 30 min of incubation) whereas 93.27% of tannin was degraded in orange juice (after 15 min of incubation). Sharma et al. (2014) observed detannification in guava juice using different volumes (0.5%–1.0% and 2.0%) of the partially purified enzyme (2.97U/mL) and measured the reduction of tannin 40.59, 53.69, and 59.23% respectively after 60 min of incubation. Whereas, Bhoite and Murthy (2015) treated aonla and pomegranate juice with the partially purified enzyme (303U/mg) and analyzed 27% and 37% tannin reduction respectively after 120 min of incubation. In other hand, Rout and Banerjee (2006) observed 25% tannin reduction in 10 ml of pomegranate juice by adding one ml of the enzyme (35.6U/mL) after 120 min of incubation whereas 49% reduction was measured using gelatin with the enzyme (1:1). Srivastava and Kar (2009) used immobilized tannase (15 beads containing 36.6 U enzyme) with 68% tannin removal in aonla/myrobalan juice (15 ml) after 180 min of incubation. Lima et al. (2014) used 1 ml of tannase (41.64 U/ml) for the clarification of 10 ml grape juices and observed 46% of tannin removal after 120 min of incubation.

Table 8
Effect of additives.

S.No.	Additive	Residual activity (%)
1	Control	100.0
2	NH_4Cl	102.16 ± 2.6
3	NH_4NO_3	107.19 ± 3.2
4	$(NH_4)_2SO_4$	104.12 ± 3.7
5	EDTA	98.41 ± 2.8
6	Gallic acid	109.13 ± 4.3
7	2-mercaptoethanol	99.87 ± 3.5
8	PMSF	94.14 ± 5.3
9	Sodium Azide	102.77 ± 2.4
10	Sodium Deoxycholate	92.62 ± 3.3
11	$NaNO_3$	96.98 ± 2.5
12	Thiourea	44.13 ± 3.11
13	Urea	34.39 ± 2.9

4. Conclusion

In this study, *T. verruculosus* tannase production was optimized in SSF using Babul bark as substrate. This is the first report on *T. verruculosus* tannase whereas Babul bark was also a good substrate for tannase production. The significant tannin reduction was observed in the juices of aonla, pomegranate and orange.

Author contribution

Author AA did all the experiment work and Author DKP guided for the experiment, did the writing work, analyzed all the data and arranged the manuscript in publishable format.

Conflicts of interest

The authors have stated that they have no conflict of interest for publication.

Acknowledgement

Author (AA) is very grateful to Rajiv Gandhi National Fellowship (201415-RGNF-2014-15-SC-MAD-75034), University Grant Commission, New Delhi, India for providing financial support as junior research fellowship. The authors are also grateful to the Dr. Harisingh Gour University, Sagar (M.P.) for providing Scanning Electron Microscope facilities.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2019.01.052.

References

Abdel-Naby, M.A., El-Tanash, A.B., Sherief, A.D.A., 2016. Structural characterization, catalytic, kinetic and thermodynamic properties of *Aspergillus oryzae* tannase. Int. J. Biol. Macromol. 92, 803–811.

Abdulla, J.M., Rose, S.P., Mackenzie, A.M., Ivanova, S.G., Staykova, G.P., Pirgozliev, V.R., 2016. Nutritional value of raw and micronised field beans (*Vicia faba* L. var. minor) with and without enzyme supplementation containing tannase for growing chickens. Arch. Anim. Nutr. 70, 350–363.

Aguilar, C.N., Rodriguez, R., Gutierrez-Sanchez, G., Augur, C., Favela-Torres, E., Prado-Barragan, L.A., Ramirez-Coronel, A., Contreras-Esquivel, J.C., 2007. Microbial tannases: advances and perspectives. Appl. Microbiol. Biotechnol. 76, 47–59.

Aharwar, A., Parihar, D.K., 2018. Tannases: production, properties, applications. Biocatal. Agric. Biotechnol. 15, 322–334.

Aoki, K., Tanaka, T., Shinke, R., Nishira, H., 1979. Detection of tannase in polyacrylamide gels. J. Chromatogr. 170, 446–448.

Bajpai, B., Patil, S., 2008. A new approach to microbial production of gallic acid. Braz. J. Microbiol. 39, 708–711.

Battestin, V., Macedo, G.A., 2007. Tannase production by *Paecilomyces variotii*. Bioresour. Technol. 98, 1832–1837.

Beniwal, V., Kumar, A., Goel, G., Chhokar, V., 2013. A novel low molecular weight acido-thermophilic tannase from *Enterobacter cloacae* MTCC 9125. Biocatal. Agric. Biotechnol. 2, 132–137.

Bhat, T.K., Singh, B., Sharma, O.P., 1998. Microbial degradation of tannins – a current perspective. Biodegradation 9, 343–357.

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 Bioprod. Process. 94, 727–735.

Bradoo, S., Gupta, R., Saxena, R.K., 1996. Screening of extracellular tannase producing fungi: development of a rapid and simple plate assay. J. Gen. Appl. Microbiol. 42, 325–329.

Chaitanyakumar, A., Anbalagan, M., 2016. Expression, purification and immobilization of tannase from *Staphylococcus lugdunensis* MTCC 3614. Amb. Express 6, 89.

Chandini, S.K., Subramanian, R., Rao, L.J., 2013. Application of enzymes in the production of RTD black tea beverages: a review. Crit. Rev. Food Sci. Nutr. 53, 180–197.

Chavez-Gonzalez, M., Rodriguez-Duran, L.V., Balagurusamy, N., Prado-Barragan, A., Rodriguez, R., Contreras, J.C., Aguilar, C.N., 2012. Biotechnological advances and challenges of tannase: an overview. Food Bioprocess Technol. 5, 445–459.

Fernandez-Lorente, G., Bolivar, J.M., Rocha-Martin, J., Curiel, J.A., Munoz, R., Rivas, B., Carrascosa, A.V., Guisan, J.M., 2011. Synthesis of propyl gallate by transesterification of tannic acid in aqueous media catalysed by immobilised derivatives of tannase from *Lactobacillus plantarum*. Food Chem. 128, 214–217.

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 *Penicillium notatum* NCIM 923. BioMed Res. Int. https://doi.org/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. J. Mol. Catal. B Enzym. 71, 29–35.

Govindarajan, R.K., Revathi, S., Rameshkumar, N., Krishnan, M., Kayalvizhi, N., 2016. Microbial tannase: current perspectives and biotechnological advances. Biocatal. Agric. Biotechnol. 6, 168–175.

Hagerman, A.E., Butler, L.G., 1978. Protein precipitation method for the quantitative determination of tannins. J. Agric. Food Chem. 26, 809–812.

Jana, A., Halder, S.K., Banerjee, A., Paul, T., Pati, B.R., Mondal, K.C., Mohapatra, P.K.D., 2014. Biosynthesis, structural architecture and biotechnological potential of bacterial tannase: a molecular advancement. Bioresour. Technol. 157, 327–340.

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, cytosafe tannase from *Bacillus subtilis* PAB2. Biochem. Eng. J. 77, 161–170.

Jimenez, N., Barcenilla, J.M., de Felipe, F.L., de las Rivas, B., Munoz, R., 2014. Characterization of a bacterial tannase from *Streptococcus gallolyticus* UCN34 suitable for tannin biodegradation. Appl. Microbiol. Biotechnol. 98, 6329–6337.

Kar, B., Banerjee, R., Bhattacharyya, B.C., 2003. Effect of additives on the behavioural properties of tannin acyl hydrolase. Process Biochem. 38, 1285–1293.

Kumar, M., Rana, S., Beniwal, V., Salar, R.K., 2015. Optimization of tannase production by a novel *Klebsiella pneumoniae* KP715242 using central composite design. Biotechnol. Rep. 7, 128–134.

Kumar, R., Sharma, J., Singh, R., 2007. Production of tannase from *Aspergillus ruber* under solid-state fermentation using jamun (*Syzygium cumini*) leaves. Microbiol. Res. 162, 384–390.

Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227, 680–685.

Lekha, P.K., Lonsane, B.K., 1997. Production and application of tannin acyl hydrolase: state of the art. Adv. Appl. Microbiol. 44, 215–260.

Lima, J.S.D., Cruz, R., Fonseca, J.C., Medeiros, E.V.D., Maciel, M.D.H.C., Moreira, K.A., Motta, C.M.D.S., 2014. 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.). Scientific World J. https://doi.org/10.1155/2014/182025.

Liu, F., Wang, B., Ye, Y., Pan, L., 2018. High level expression and characterization of tannase tan7 using *Aspergillus niger* SH-2 with low-background endogenous secretory proteins as the host. Protein Expr. Purif. 144, 71–75.

Lopes, L.M.D.M., Batista, L.H.C., Gouveia, M.J., Leite, T.C.C., Mello, M.R.F.D., Assis, S.A.D., Sena, A.R.D., 2018. Kinetic and thermodynamic parameters, and partial characterization of the crude extract of tannase produced by *Saccharomyces cerevisiae* CCMB 520. Nat. Prod. Res. 32, 1068–1075.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193, 265–275.

Madeira Jr., J.V., Ferreira, L.R., Macedo, J.A., Macedo, G.A., 2015. Efficient tannase production using Brazilian citrus residues and potential application for orange juice valorization. Biocatal. Agric. Biotechnol. 4, 91–97.

Madeira Jr., J.V., Macedo, J.A., Macedo, G.A., 2011. Detoxification of castor bean residues and the simultaneous production of tannase and phytase by solid-state fermentation using *Paecilomyces variotii*. Bioresour. Technol. 102, 7343–7348.

Mahmoud, A.E., Fathy, S.A., Rashad, M.M., Ezz, M.K., Mohammed, A.T., 2018. Purification and characterization of a novel tannase produced by *Kluyveromyces marxianus* using olive pomace as solid support, and its promising role in gallic acid production. Int. J. Biol. Macromol. 107, 2342–2350.

Mandal, S., Ghosh, K., 2013. Optimization of tannase production and improvement of nutritional quality of two potential low-priced plant feedstuffs under solid state fermentation by *Pichia kudriavzevii* isolated from fish gut. Food Biotechnol. 27, 86–103.

Mizuno, T., Shiono, Y., Koseki, T., 2014. Biochemical characterization of *Aspergillus oryzae* native tannase and the recombinant enzyme expressed in *Pichia pastoris*. J. Biosci. Bioeng. 118, 392–395.

Mondal, K.C., Banerjee, D., Jana, M., Pati, B.R., 2001. Colorimetric assay method for determination of the tannin acyl hydrolase (EC 3.1.1.20) activity. Anal. Biochem. 295, 168–171.

Prajapati, B.P., Suryawanshi, R.K., Agrawal, S., Ghosh, M., Kango, N., 2017. Characterization of cellulase from *Aspergillus tubingensis* NKBP-55 for generation of fermentable sugars from agricultural residues. Bioresour. Technol. 250, 733–740.

Prigione, V., Spina, F., Tigini, V., Giovando, S., Varese, G.C., 2018. Biotransformation of industrial tannins by filamentous fungi. Appl. Microbiol. Biotechnol. 102, 10361–10375.

Qiu, Y., Niu, H., Huang, W., He, Y., Wu, X.H., 2011. Properties and secondary structure of tannase from *Penicillium herquei*. Biotechnol. Bioproc. Eng. 16, 858–866.

Raghuwanshi, S., Dutt, K., Gupta, P., Misra, S., Saxena, R.K., 2011. *Bacillus sphaericus*: the highest bacterial tannase producer with potential for gallic acid synthesis. J. Biosci. Bioeng. 111, 635–640.

Riul, A.J., Goncalves, H.B., Jorge, J.A., Guimarae, L.H.S., 2013. Characterization of a glucose- and solvent-tolerant extracellular tannase from *Aspergillus phoenicis*. J. Mol. Catal. B Enzym. 85–86, 126–133.

Rodrıguez-Duran, L.V., Valdivia-Urdiales, B., Contreras-Esquivel, J.C., Rodrıguez-Herrera, R., Aguilar, C.N., 2011. Novel strategies for upstream and downstream processing of tannin acyl hydrolase. Enzym. Res. https://doi.org/10.4061/2011/823619.

Rout, S., Banerjee, R., 2006. Production of tannase under mSSF and its application in fruit juice debittering. Indian J. Biotechnol. 5, 346–350.

Roy, S., Parvin, R., Ghosh, S., Bhattacharya, S., Maity, S., Banerjee, D., 2018. Occurrence of a novel tannase (tan BLP) in endophytic *Streptomyces* sp. AL1L from the leaf of *Ailanthus excelsa* Roxb. 3. Biotech 8, 33.

Samiey, B., Cheng, C.-H., Wu, J., 2014. Effects of surfactants on the rate of chemical reactions. J. Chem. https://doi.org/10.1155/2014/908476.

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. Prep. Biochem. Biotechnol. 44, 281–290.

Sharma, S., Agarwal, L., Saxena, R.K., 2008. Purification, immobilization and characterization of tannase from *Penicillium variable*. Bioresour. Technol. 99, 2544–2551.

Srivastava, A., Kar, R., 2009. Characterization and application of tannase produced by *Aspergillus niger* ITCC 6514.07 on Pomegranate Rind. Braz. J. Microbiol. 40, 782–789.

Varadharajan, V., Vadivel, S.S., Ramaswamy, A., Sundharamurthy, V., Chandrasekar, P., 2017. Modeling and verification of process parameters for the production of tannase by *Aspergillus oryzae* under submerged fermentation using agro-wastes. Biotechnol. Appl. Biochem. 64, 100–109.

Viswanath, V., Leo, V.V., Prabha, S.S., Potty, V.P., Jisha, M.S., 2016. Optimized production of tannase from Cashew Testa using *Aspergillus niger* MTCC 5898. Food

Biotechnol. 30, 249–262.
Wang, F., Ni, H., Cai, H.N., Xiao, A.F., 2013. Tea stalks – a novel agro-residue for the production of tannase under solid state fermentation by *Aspergillus niger* JMU-TS528. Ann. Microbiol. 63, 897–904.
Wu, C., Zhang, F., Li, L., Jiang, Z., Ni, H., Xiao, A., 2018. Novel optimization strategy for tannase production through a modified solid-state fermentation system. Biotechnol. Biofuels 11, 92.
Xiao, A., Huang, Y., Ni, H., Cai, H., Yang, Q., 2015. Statistical optimization for tannase production by *Aspergillus tubingensis* in solid-state fermentation using tea stalks. Electron. J. Biotechnol. 18, 143–147.
Yao, J., Chen, Q.L., Shen, A.X., Cao, W., Liu, Y.H., 2013. A novel feruloyl esterase from a soil metagenomics library with tannase activity. J. Mol. Catal. B Enzym. 95, 55–61.
Yao, J., Guo, G.S., Ren, G.H., Liu, Y.H., 2014. Production, characterization and applications of tannase. J. Mol. Catal. B Enzym. 101, 137–147.