Production of Tannase by Fungi Isolated from Different Soils

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Abstract: Tannases are enzymes that catalyze the production of gallic acid which is a versatile precursor of various chemicals used in food and pharmaceutical industries. This work is aimed at isolation and production of tannase from soil fungi. Moulds were isolated from soil samples that were collected from different sites in Ibadan Metropolis. Isolated fungi were screened on plate for tannase production. The best sets of fungi were selected to produce tannases under solid state fermentation using various substrates. Twenty (20) out of forty-two (42) isolated fungi were able to produce tannase. Isolates FR6, IAR15 and BG4 recorded highest zone of hydrolysis (20, 17 and 16 mm) on Tannic acid agar and were identified as *Aspergillus japonicus*, *Aspergillus tamarii* and *Neosartorya fumigata* respectively using their macroscopic and microscopic properties. Among different used substrates, highest production of tannase was observed when wheat bran (8.72 U/mL) was used as substrate which was followed by Moringa seed (7.90 U/mL). There was higher production of tannase by selected isolates when grown in used substrate (Wheat and Moringa seed) alone than when supplemented with tannic acid. Of all used carbon sources, Fructose and Maltose supported best production of tannase by the three fungi. $NaNO_3$ was the best nitrogen source among all the nitrogen sources used with the yield of 15.88 U/mL by *Aspergillus japonicus*. Optimum production of tannase was either recorded at pH 6.0 or 6.5 with selected isolates. *Aspergillus japonicus* had a considerable higher production than other two selected fungi. Best production of tannase is achieved with 1% of fructose as carbon source, 1% of $NaNO_3$ as nitrogen and Wheat bran as substrate at pH of 6.5.

Keywords: Tannase, *Aspergillus* species, *Neosartorya fumigata*, Moringa seed, Wheat bran.

INTRODUCTION

Enzymes are the crucial points of biotechnological processes being involved in various aspects of biochemical reactions. Tannase is an inducible enzyme that catalyses the breakdown of ester linkage in hydrolysable tannins such as tannic acid resulting in production of gallic acid and glucose [1]. Gallic acid is mostly used in pharmaceutical industry for production of anti-bacterial drug trimethoprim [2]. Tannase is also used in the production of acorn wine and instant tea [3]. Effluents of tanneries contains high amount of tannins, mainly polyphenols which are dangerous pollutants. These pollutants can be degraded by tannases [4].

Gallic acid (3,4,5-trihydroxyl benzoic acid), an organic substance occurring in many plants either as a free molecule or as part of tannic acid molecule is a useful product with wide application. It can be produced by microbial hydrolysis of tannic acids by tannases. Tannases are secreted by microorganisms such as *Aspergillus* species [5, 6, 7], *Penicillium* species [8] and *Klebsiella pneumoniae* [9] in either solid state or submerged fermentation. Advantages of tannase production by solid state culture compared with that produced by submerged culture have been reported [10].

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There is often a serious problem of disposing agricultural residues and wastes, which may cause serious environmental contamination and propagation of flies and rats if not correctly dealt with. In spite of the high demand and the diverse fields for tannase applications, the large-scale use of this is still limited at present by the shortage and high cost of its production. Hence, the need to reduce the cost of production using cheap and readily available agricultural waste become imperative. This work is aimed at isolating fungi with the ability to produce tannase and production of tannase using various agricultural wastes as substrate.

MATERIALS AND METHODS

Collection of Soil Samples

Soil samples were collected from different sites in Ibadan Metropolis. The sites are Botanical garden, University of Ibadan (7.3912° N; 3.9167° E); Research Farm, University of Ibadan (7.3912° N; 3.9167° E); Forestry Research Institute of Nigeria (FRIN), Ibadan (7.3911° N; 3.8582° E); National Institute of Horticulture and Research Training (NIHORT), Ibadan [7.3963° N; 3.8644° E]; and The Institute of Agriculture and Research Training (IAR&T), Moor Plantation [7.3872° N; 3.8355° E]. Soil samples were taken from 5-10 cm depth after the removal of surface soil debris. Randomly collected soil samples from each location were pooled together to form a composite sample and thoroughly mixed in sterile Ziploc bags and transported to the laboratory for further use.

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Isolation of Fungi

Each composite soil sample was serially-diluted and 1 mL of appropriate dilution was inoculated into sterilized Potato Dextrose Agar supplemented with Streptomycin (300 mg/L) using pour plate technique [11]. Distinct colonies were sub-cultured severally to obtain pure culture. Isolated fungi were identified using macroscopic and microscopic characteristics [12].

Primary Screening and Selection Process of Isolates for Tannase Production

Primary screening was carried out using Tannic Acid Agar (TAA) as described by Pinto *et al*. [13]. TAA was compounded with Tannic acid (10g/L) and included in a medium of the following composition $NaNO_3$ (3g/L), KCl 0.5g/L; $MgSO_4.7H_2O$ (0.5g/L); KH_2PO_4 (1.0g/L); $FeSO_4.7H_2O$ (0.01g/L) and Agar-agar (30g/L) [13]. The plates were point inoculated with mycelial strands of the isolates and incubated (MRC Incubator, Model BOD-150) at 28 ±2°C for 96 hours. The diameters of zone of clearance around the fungal colonies due to breakdown of tannic acid to gallic acid and glucose were measured after 96 hours of incubation. Fungal isolates with high tannase activity were subjected to secondary screening.

Production of Tannase in Solid State Fermentation

The medium as used in Primary screening was inoculated with 1.0 mL of spore suspension (2 x 10^8CFU/mL) and incubated at 28 ±2°C for 120 hours. Crude tannase was extracted by addition of 40mL of distilled water, mixed well and filtered using Whatman filter paper No. 1. The filtrate was centrifuged at 10,000rpm for 15 minutes with cold centrifuge (MRC centrifuge, Thomas Scientific), the supernatant layer was separated (crude enzyme) and tannase activity and protein concentration were estimated [14].

Influence of Production Parameters

Effect of Carbon Source on Tannase Activity

One percent (1%, w/v) concentration of the different carbon sources (Glucose, Fructose, Maltose, Lactose and Sucrose) was included in each case as sole carbon source in a Basal medium as defined under Primary screening. Each Basal medium was autoclaved at 121 °C for 15 minutes. One milliliter of fungal inoculum was added to 40 mL of sterile basal medium, incubated at 28 ±2°C for 120 hours and tannase activity was estimated [14].

Effect of Nitrogen Source on Tannase Activity

Basal medium with 1% (w/v) different nitrogen sources (Peptone, Urea, NH_4SO_4, NH_4Cl and $NaNO_3$) was compounded separately autoclaved 121 °C for 15 minutes. 40mL of each sterile basal medium was inoculated with 1.0 mL of fungal inoculum, incubated at 28 ±2°C for 120 hours and tannase activity was determined [14]

Effect of pH on Tannase Activity

The different production media were prepared at different pH (4.5, 5.0, 5.5, 6.0 and 6.5) using Hydrochloric acid (1M HCl) and Sodium hydroxide (1M NaOH). Each production medium was sterilized 121 °C for 15 minutes. Each medium was inoculated with 1.0ml of standardized inoculum which was incubated at 28 ±2 °C for 120 hrs and assayed for enzymatic activity [14].

Effect of Substrates on Tannase Activity

Ten grams of differently processed agricultural substrates (Wheat bran, corn bran, palmkernel cake, moringa seed and groundnut shell) were air dried, milled using Qlink blender (Model No. QBL 1861A) separately to obtain homogenized powders (with 90% passing through 300nm pore sieve) and utilized as substrates for enzyme production under solid state fermentation. 1.0mL of the fungal inoculum was added to 40ml sterile production medium, incubated at 28 ±2°C for 120 hours and assayed for enzymatic activity [14]

Effect of Tannic Acid on Tannase Activity

Tannic acid (0.5%,w/v) was added to the production medium and was sterilized. This was inoculated and incubated at 28±2 °C for 120 hours.

Enzyme Extraction and Assay

Tannases were extracted by obtaining cell free supernatant (crude tannase) from each fungal isolate by cold centrifugation (MRC centrifuge) at 10,000rpm for 15 minutes. The assay was carried out using the method of Pinto *et al*. [15] and absorbance was measured using UV-visible spectrophotometer (Jenway, 6405UV/Vis England) at 310nm wavelength and the absorbance recorded.

Statistical Analysis

The experimental data was analyzed using Analysis of Variance to determine the Means. The level of

significance was set at P≤0.05. The data were analyzed using SPSS version 23.

RESULTS

A total of 42 fungal isolates were obtained from five soil samples with the highest (15) in Botanical Garden (BG) Soil which correspond to highest number (7) of tannase-producing fungi (Table **1**). The least fungal isolates (4) obtained from Institute and Research Farm soil also correspond to the least number (2) of tannase-producing fungi. Fungal count from the five soil samples ranged from 1.2 x 10^7 to 6.2 x 10^7CFU /g.

Table **2** shows the diameter of zones of hydrolysis of tannase-producing fungi in which the highest zone of hydrolysis (20 mm) was recorded by isolate FR6, followed by isolate IAR15 (17 mm) and least tannase activity (3 mm) was observed in isolate BG1 and FR8. Three fungal isolates (FR6, IAR15 and BG4) were selected for further studies based on the size of the hydrolysis zone.

The three fungal isolates (FR6, IAR15 and BG4) used for further studies were identified as *Aspergillus japonicus*, *Aspergillus tamarii* and *Neosartorya fumigata* respectively in accordance with their macroscopic and microscopic characteristics.

The effect of different agro waste on tannase activity of the isolates as shown in Table **3** revealed the highest tannase production by *Neosartorya fumigata* BG4 which was recorded with wheat bran (8.72 U/mL), followed by Moringa seed (7.46 U/mL) and least in Palm kernel cake (6.50U/mL). *Aspergillus japonicus* FR6 displayed highest tannase production with Moringa seed (7.90 U/mL) as substrate, followed by corn bran (7.02 U/mL) and the least activity was with groundnut shell (6.62 U/mL). The highest and the least tannase activity by *Aspergillus tamarii* IAR15 with different agro wastes were observed in Moringa seed (7.20 U/mL) and Corn bran (6.48 U/mL) respectively. Wheat bran and Moringa seed were used as substrates for further studies based on their tannase activities. Statistical analysis revealed that the tannase activity among different isolates were significantly different (P>0.05) within each substrate except with Moringa seed and Palmkernel cake.

Effect of tannic acid on tannase activity on best two substrates was examined as shown in Table **4**. In isolate BG4, higher tannase activity was observed without the addition of tannic acid to wheat bran and Moringa seed when compared with wheat bran and Moringa seed supplemented separately with tannic acid. Isolates FR6 and IAR15 had the highest activity of 7.90 U/mL and 7.20 U/mL respectively with Moringa seed only. For the three isolates, higher activities for both substrates were observed without the addition of tannic acid (0.5%) with the exception of wheat bran inoculated with isolate IAR15. Statistical analysis revealed that tannase activity of isolates FR6 and IAR15 on substrates (Wheat bran and Moringa seed) supplemented with tannic acid showed no significantly difference.

Effect of different carbon sources on production of tannase by selected fungi was studied on fermentation media supplemented separately with Moringa seed and Wheat bran (Table **5**). *Neosartorya fumigata* BG4 recorded highest production of tannase with sucrose and lactose as the source of carbon in media separately supplemented with Moringa seed (7.58 U/mL) and Wheat bran (8.56 U/mL) respectively. Least

Table 1: Count of Tannase-Producing Fungi from Different Soil Samples

Sample Codes	Fungal Count (x 10^7cfu/g)	Number of isolates	Number of tannase-producing fungi
BG	6.2	15	7
IRF	1.2	4	2
FR	3.9	10	4
NHR	2.3	5	3
IAR	2.8	8	4
Total		42	20

KEY:
BG – Botanical Garden Soil.
IRF – Institute and Research Farm Soil.
FR – Forestry Research Institute of Nigeria Soil.
NHR – National Institute of Horticulture and Research Training Soil.
IAR – Institute of Agriculture and Research Training (Moor Plantation) Soil.

Table 2: Primary Screening of Fungal Isolates for Tannase Production

Locations	Isolate codes	Diameter of zone of hydrolysis (mm)
IRF	IRF1	4.0
	IRF2	5.0
BG	BG1	3.0
	BG2	8.0
	BG3	6.0
	BG4	16.0
	BG5	9.0
	BG6	5.0
	BG7	13.0
FR	FR6	20.0
	FR7	5.0
	FR8	3.0
	FR9	8.0
IAR	IAR2	10.0
	IAR7	12.0
	IAR9	9.0
	IAR15	17.0
NHR	NHR1	8.0
	NHR2	9.0
	NHR3	7.0

Values are Means of duplicate reading KEY: As in Table **1**.

Table 3: Effect of Substrates on Tannase Activity (U/mL)

Isolates	Substrates				
	Wheat Bran	Corn Bran	Groundnut Shell	Moringa seed	Palm kernel cake
Neosartorya fumigata BG4	8.72±0.02^{c}	6.94±0.02^{b}	6.72±0.02^{c}	7.46±0.02^{a}	6.50±0.20^{a}
Aspergillus japonicus FR6	6.96±0.02^{b}	7.02±0.02^{c}	6.62±0.02^{b}	7.90±0.20^{b}	6.64±0.02^{a}
Aspergillus tamarii IAR15	6.70±0.20^{a}	6.48±0.02^{a}	6.54±0.02^{a}	7.20±0.20^{a}	6.92±0.02^{b}

Mean values in the same column followed with different superscripts are statistically and significantly different at P<0.05.

Table 4: Effect of Tannic Acid on Tannase Activity (U/mL)

Isolates	Substrate with and without tannic acid			
	Wheat Bran without tannic acid	Wheat Bran + 0.5% tannic acid	Moringa seed without tannic acid	Moringa seed + 0.5% tannic acid
Neosartorya fumigata BG4	8.72±0.02^{c}	7.24±0.02^{b}	7.46±0.02^{a}	6.94±0.02^{b}
Aspergillus japonicus FR6	6.96±0.02^{b}	6.84±0.02^{a}	7.90±0.20^{b}	6.40±0.20^{a}
Aspergillus tamarii IAR15	6.70±0.20^{a}	7.00±0.20^{a}	7.20±0.20^{a}	6.52±0.02^{a}

Means values in the same column followed with different superscriptsare significantly different at P<0.05.

production of tannase by *Neosartorya fumigata* BG4 with Moringa seed and Wheat bran was recorded when fructose (7.16 U/mL) and glucose (7.60 U/ml) was used as source of carbon respectively. *Aspergillus* spp. (FR6 and IAR15) in Moringa seed substrates recorded the highest tannase production with maltose as source of

Table 5: Effect of Carbon Sources on Tannase Activity (U/mL)

Substrates	Isolates	Carbon Source/Tannase Activity (U/mL)				
		Glucose	Fructose	Maltose	Lactose	Sucrose
Moringa seed	*Neosartorya fumigata* BG4	7.40±0.20^{b}	7.16±0.02^{a}	7.44±0.02^{a}	7.34±0.0^{a}	7.58±0.02^{b}
	Aspergillus japonicus FR6	7.02±0.02^{a}	7.44±0.02^{b}	7.76±0.02^{a}	7.32±0.0^{a}	7.48±0.02^{b}
	Aspergillus tamarii IAR15	7.26±0.02^{b}	7.36±0.02^{b}	7.48±0.02^{a}	7.46±0.0^{a}	7.14±0.02^{a}
Wheat bran	*Neosartorya fumigata* BG4	7.60±0.02^{a}	7.64±0.02^{b}	7.60±0.02^{a}	8.56±0.02^{c}	8.08±0.02^{b}
	Aspergillus japonicus FR6	7.60±0.20^{b}	9.32±0.02^{c}	8.24±0.02^{b}	7.68±0.02^{a}	7.66±0.02^{b}
	Aspergillus tamarii IAR15	9.16±0.02^{c}	7.60±0.20^{b}	9.06±0.02^{c}	8.20±0.20^{b}	8.24±0.02^{c}

Mean values in the same column followed with different lower case letters are statistically and significantly different at P<0.05.

carbon but the two fungi had least production with different carbon sources (Glucose – 7.02 U/mL for *A. japonicus* and Sucrose – 7.14 U/mL for *A. tamarii*). *Aspergillus japonicus* has the highest production of tannase with fructose (9.32 U/mL) as the source of carbon in medium with wheat bran substrate; this was followed with maltose (8.24 U/mL) and the least was observed in glucose (7.60 U/mL). Highest tannase activity was obtained with glucose as source of carbon when *Aspergillus tamarii* IAR15 was grown on wheat bran. Tannase activity by *Aspergillus tamarri* IAR15 on wheat bran substrate ranged from 7.60 U/mL (Fructose) to 9.16 U/mL (glucose). Statistical analysis revealed that the carbon sources have significant effect on tannase production among the selected isolates on both substrates.

$NaNO_3$ was the best nitrogen source for the production of tannase in both selected substrates (Moringa seed and Wheat bran) for BG4 (*Neosartorya fumigata*) and FR6 (*Aspergillus japonicus*) (Table **6**). In Moringa seed-containing substrate, tannase production with different nitrogen sources by BG4 (*Neosartorya fumigata*), FR6 (*Aspergillus japonicus*) and IAR15 (*Aspergillus tamarii*) ranged from 7.66 -11.20 U/ml, 7.80 – 10.80 U/ml and 7.80 – 9.14 U/ml respectively. In Wheat bran substrate, 7.56 – 13.76 U/ml, 7.86 – 15.88 U/ml and 9.02 – 10.66 U/ml tannase activity was observed in BG4 (*Neosartorya fumigata*), FR6 (*Aspergillus japonicus*) and IAR15 (*Aspergillus tamarii*) respectively with different nitrogen sources. There was significant different in the production of tannase by selected isolates with used substrates in each of the nitrogen sources.

Highest production of tannase by *Neosartorya fumigata* BG4 was recorded at pH 6.0 in both selected substrates – Moringa seed (9.54 U/ml) and Wheat bran (8.66 U/ml) and decreased thereafter (Table **7**). Production of tannase by *Aspergillus japonicus* FR6 in both selected substrates (Moringa seed and Wheat bran) increased with increase in pH and ranged from 7.42 to 10.48 U/ml and 7.60 to 9.68 U/ml respectively. Highest production of tannase by *Aspergillus tamarii* IAR15 was obtained at pH 6.5 in both selected substrates – Moringa seed (9.80 U/ml) and Wheat bran (10.32 U/ml). The tannase production by the 3 isolates are significantly different from one another at different pH.

DISCUSSION

The ability of the fungi isolated from different soil samples to produce tannase may be attributed to the

Table 6: Effect of Nitrogen Sources on Tannase Activity (U/mL)

Substrates	Isolates	Nitrogen Source/Tannase Activity (U/mL)				
		NH_4Cl	Peptone	Urea	NH_4SO_4	$NaNO_3$
Moringa seed	*Neosartorya fumigata* BG4	7.94±0.02^{a}	9.38±0.02^{c}	7.66±0.02^{a}	7.70±0.02^{a}	11.2±0.02^{b}
	Aspergillus japonicus FR6	8.28±0.02^{b}	8.60±0.02^{a}	7.80±0.02^{b}	8.50±0.02^{b}	10.8±0.02^{b}
	Aspergillus tamarii IAR15	9.14±0.02^{c}	8.76±0.02^{b}	8.78±0.02^{c}	7.80±0.02^{a}	8.16±0.02^{a}
Wheat bran	*Neosartorya fumigata* BG4	7.56±0.02^{a}	7.84±0.02^{a}	8.12±0.02^{a}	9.80±0.02^{b}	13.76±0.02^{c}
	Aspergillus japonicus FR6	9.68±0.02^{c}	8.36±0.02^{b}	8.38±0.02^{b}	7.86±0.02^{a}	15.88±0.02^{c}
	*Aspergillus tamarii*IAR15	9.02±0.02^{b}	9.70±0.02^{a}	9.80±0.02^{c}	10.66±0.02^{c}	9.86±0.02^{a}

Mean values in the same column followed with different lower case letters are statistically significantly different at P<0.05.

Table 7: Effect of pH on Tannase Activity (U/mL)

Substrates	Isolates	pH/Tannase Activity (U/mL)				
		4.5	5.0	5.5	6.0	6.5
Moringa seed	*Neosartorya fumigata* BG4	7.88±0.02^{b}	8.68±0.02^{c}	9.28±0.02^{c}	9.54±0.02^{c}	7.52±0.02^{a}
	Aspergillus japonicus FR6	7.42±0.02^{a}	7.62±0.02^{a}	7.74±0.02^{a}	9.34±0.02^{b}	10.48±0.02^{c}
	Aspergillus tamarii IAR15	7.62±0.02^{a}	7.64±0.02^{a}	8.80±0.02^{b}	8.90±0.02^{a}	9.80±0.02^{b}
Wheat bran	*Neosartorya fumigata* BG4	7.57±0.03^{a}	7.54±0.02^{a}	8.42±0.02^{b}	8.66±0.02^{a}	7.64±0.02^{a}
	Aspergillus japonicus FR6	7.60±0.02^{a}	8.12±0.02^{b}	8.16±0.02^{ab}	9.14±0.02^{b}	9.68±0.02^{b}
	*Aspergillus tamarii*IAR15	8.74±0.02^{c}	9.22±0.02^{c}	9.20±0.02^{c}	9.16±0.02^{b}	10.32±0.02^{c}

Means values in the same column followed different lower case letters are statistically significantly different at P<0.05.

fact that soil is a rich source for potential enzyme-producing organisms [16, 17]. The isolated tannase-producing fungi were able to hydrolyse tannic acid which proved that they have ability to produce tannase [18,19]. The fungal isolates with highest tannase activity were identified as *Neosartorya fumigata* BG4, *Aspergillus japonicus* FR6 and *Aspergillus tamarii* IAR15, a result similar to the report of Souza *et al*. [20] and Aracri *et al*. [21] who reported *Aspergillus* species to be good tannase-producing fungi.

Among the different agricultural substrates used in solid state fermentation, high yield of tannase was obtained using wheat bran, followed by Moringa seeds after 5 days of incubation. This observation suggests that these substrates could act as inducer for tannase production. In addition, the high enzyme activity with these substrates may be due to the presence of nutrients required by the fungus for metabolism and subsequent growth. Wheat bran is a suitable medium for growth of microorganisms and production of different metabolites, since it contains the essential nutritional factors such as carbohydrates, protein, minerals for metabolic activity. Hence it serves as a better carbon and nitrogen source, thus it is used for microbial growth and production of many kinds of products especially enzymes [19, 22].

The addition of 0.5% tannic acid to different substrates for the production of tannase had no significant effect. The maximum tannase activity was recorded in *Neosartorya fumigata* using wheat bran only (without tannic acid) as substrates. Tannic acid could be having inhibitory effect on the growth of the *Neosartorya fumigata* which was similar to previous result using wheat bran and tamarind seed as substrate [23].

Preference for carbon sources have been demonstrated by fungi in fermentation processes even other than production of enzymes, tannins which are widely distributed indifferent part of plants such as bark, heartwood, grasses, seed and flowers are not exactly the same [24]. Fructose with wheat bran favoured the production of tannase most among the carbon sources used and this is in contrast with the report of Reddy and Reddy [7] that sucrose is the best carbon sources which favoured maximum tannase production. Selwal *et al*. [25] reported that an additional carbon source promotes initial growth of the biomass, since tannic acid is harder to metabolize that simple sugars. Maltose with Moringa seed favoured the production of tannase most among the carbon sources used and this agrees with the work of Roushdy *et al*. [6].

The best nitrogen source for maximum tannase production (15.88 U/ml) by the fungal isolates was $NaNO_3$. Nitrogen is an essential nutrient in amino acid synthesis. They have regulatory effects on enzyme synthesis. Tannase or any other enzyme production depends mainly on the availability of nitrogen sources in the medium which has been reported by some researchers [7,21].

The effect of pH on enzyme activity depends on the nature of amino acids at the active site, which undergoes protonation and deprotonation [26]. The effect of pH was examined and the result showed that maximum tannase yield by *Aspergillus japonicus* FR6 and *Aspergillus tamarii* IAR15 was observed at pH 6.5. This observation agrees with the work of Amit *et al*. [27] who reported maximum tannase by *Aspergillus* species at pH 6.5. *Neosartorya fumigata* BG4 showed maximum tannase yield at pH 6.0 which is in line with the report of some researchers [21, 28, 29].

The optimum parameters for best tannase producers studied were combined to produce improved tannase in terms of activity. After the optimized

production a considerable increase in activity was noticed in the tannase produced by the three isolates. This may be due to combined optimum factors that improved the folding, the ionic angular alignment of the tannase molecules. Various scientific reports are in support of this finding [1, 30, 31].

CONCLUSION

Neosartorya fumigata BG4, *Aspergillus japonicus* FR6 and *Aspergillus tamarii* IAR15 are tannase-producing fungi. Wheat bran and Moringa seed are good substrates for tannase production especially when they are not supplemented with tannic acid. Higher tannase production was obtained in Fructose and Maltose when compared to other carbon sources. $NaNO_3$ gave optimum production of tannase. Highest production was either obtained at pH 6 or 6.5. Fructose (1%) as carbon source, 1 % $NaNO_3$ as nitrogen source, with wheat bran as substrate at pH 6.5 gave best production of tannase.

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