



**International Journal of Biological  
&  
Pharmaceutical Research**  
Journal homepage: [www.ijbpr.com](http://www.ijbpr.com)

**IJBPR**

## OPTIMIZATION OF CULTURAL CONDITIONS FOR TANNASE PRODUCTION IN SUBMERGED FERMENTATION BY *ASPERGILLUS NIGER* AVM-1

**Amitisinh V. Mangrola\*, Hitesh V. Patel, Chaitanyasinh J. Chudasama, Chintan N. Vavadia,  
Hardik Shah**

Department of Biochemistry, Shri Alpesh N. Patel Post Graduate Institute, Anand-388 001, Gujarat, India.

### ABSTRACT

Tannase (E.C. 3.1.1.20), is an inducible extra-cellular enzyme produced by a number of microbes, animals and plants. The present study explores the natural ability of fungi to utilize tannin as a source of carbon. Tea waste dumped soil samples were used for isolation of tannase producing fungi. In this study, *Aspergillus niger* AVM-1 was isolated from the tea waste dumped soil and was exploited for tannase production under submerged fermentation. Optimization parameters such as incubation period, tannic acid (inducer) concentration, pH and temperature were investigated for maximum tannase production. The optimum conditions were found to be incubation period (96 hrs), tannic acid (2 % w/v), pH (5.5) and temperature (30°C). The DNA damage protecting activity of product obtained after tannic acid hydrolysis was assessed bringing about the DNA damage with H<sub>2</sub>O<sub>2</sub> + UV exposure in absence of the hydrolyzed products of tannic acid. The experiment was carried out by using Agarose gel electrophoresis to analyze DNA damage in presence of H<sub>2</sub>O<sub>2</sub> + UV. However, there was no damage of DNA by H<sub>2</sub>O<sub>2</sub> + UV exposure in presence of the hydrolysis products. This organism may have a great potential in the tannin degradation which could be of great commercial value.

**Key Words:** Tea waste dump soil, Screening, *Aspergillus niger* AVM-1, Cultural conditions, Antioxidant property.

### INTRODUCTION

Tannins are water-soluble polyphenols naturally occurred as secondary metabolites in higher plant. It has been considered as the fourth abundant constituents after cellulose, hemicelluloses and lignin (Rana and Bhat, 2005). They are often considered nutritionally undesirable because they form complexes with protein, starch and digestive enzymes and cause a reduction in nutritional value of food. Moreover, tannin found in industrial effluent water may also cause environmental problems. Therefore, attempts are made to optimize the production of tannase enzyme from microbial source. Tannic acid hydrolysis can

be carried out by acid or alkali or microbial tannase enzyme. The enzymatic hydrolysis has advantage over than the other methods because it is less energy intensive and less polluting (Hadi *et al.*, 1994). Tannase hydrolyses hydrolysable tannins and catalyses the hydrolysis of ester bonds in tannic acid releasing glucose and gallic acid (Mahapatra *et al.*, 2005). The most important source to obtain the enzyme is by microbial way because the produced enzymes are more stable than similar ones obtained from other sources. Microorganisms can produce tannase in high quantities in a constant way. A variety of microorganisms including bacteria, yeast and filamentous fungi have been reported to produce tannase, of which the most potent producers are fungi. Many fungal species have been reported to produce tannase including *A. niger*, *A. oryzae*, *A. aureus*, *A. flavus*, *A. foetidus*, *A. japonicas*,

Corresponding Author

**Amitisinh V. Mangrola**  
Email: [avmang@gmail.com](mailto:avmang@gmail.com)

*P. chrysogenum* and *R. oryzae* (Belur and Mugeraya, 2011). Submerged fermentation (SmF) is mostly preferred because the sterilization and process-control methods are easier in this system (Lekha and Lonsane, 1994). Most of the commercial applications of tannase are in the manufacturing of instant tea. Tannase is also used as a clarifying agent in some fruit juices and in cold drinks with coffee flavor. In the medical industry, tannase is used in the production of gallic acid, a substrate for the chemical synthesis of trimethoprim, propyl gallate, dyes and inks. The present study deals with the isolation and screening of tannase producing fungi from tea waste dump soil samples. Attempts were made to optimize the cultural conditions for maximum tannase production by the conventional ‘one-factor-at-a-time’ methodology. Moreover, application of enzyme hydrolytic products for evaluation of its antioxidant property was investigated in this study.

## MATERIALS AND METHODS

### Sample collection

Soil samples were collected from different tea waste dump sites of Anand and Kheda district regions, Gujarat, India. The samples were collected using sterile spatula and stored in sterile plastic bags and were transported to the laboratory for further investigations.

### Screening media

Tannic acid agar medium (TAA) was used for the isolation of tannase producing fungi and for the primary screening of fungal isolates (Pinto *et al.*, 2001). The ingredients of TAA medium containing (g/l): NaNO<sub>3</sub>, 3.0; KH<sub>2</sub>PO<sub>4</sub>, 1.0; MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.5; KCl, 0.5; FeSO<sub>4</sub>·7H<sub>2</sub>O, 0.01; Tannic acid, 10; Agar, 30; pH 4.5. The medium was sterilized at 121° C for 15 min. The solution of tannic acid was sterilized separately by filtering through 0.22 µm cellulose acetate filter and then added to the medium. The modified Czapek-Dox’s minimal medium was used for secondary screening of fungi (Bradoo *et al.*, 1996). The ingredients of Czapek-Dox’s minimal medium containing (g/l): Tannic acid, 10; NaNO<sub>3</sub>, 6; KCl, 0.52; MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.52; KH<sub>2</sub>PO<sub>4</sub>, 1.52; FeSO<sub>4</sub>·7H<sub>2</sub>O, 0.01; ZnSO<sub>4</sub>·7H<sub>2</sub>O, 0.01; Cu (NO<sub>3</sub>)<sub>2</sub>·3H<sub>2</sub>O, 0.01; pH 4.5. Sterilization and adjustment of pH were carried out as mentioned in preparation of TAA medium.

### Isolation of Tannase producing fungal isolates

The soil samples were enriched in a Tannin acid broth with pH 4.5. The broth was incubated at 30°C on rotary shaker (150 rpm) for 72 hrs. After enrichment, the samples were plated on TAA plates and incubated at 30°C for 96 hrs. Fungi form zone of clearance around its colonies due to the hydrolysis of tannin were selected and purified (Murugan *et al.*, 2007). The isolated colonies were designated as AVM-1 to AVM-4 and were exploited for primary screening.

### Screening and selection of tannase producing fungal isolates

Primary screening was carried out using TAA plates as described by Bradoo *et al.*, (1996). The plates were point inoculated with the isolates and incubated at 30° C for 96 hrs. The diameters of zone of clearance around the fungal colonies were measured after 96 hrs of incubation. Fungal isolates, which showed highest tannase activity in the primary screening, were subjected to secondary screening using submerged fermentation technique (Batra and Saxena, 2005).

### Production of Tannase in submerged fermentation (SmF)

Submerged fermentation (SmF) was carried out in 250ml of Erlenmeyer flasks containing 100ml of sterile modified Czapek-Dox’s minimal medium containing 1% tannic acid. The medium was inoculated with 1% inoculum (O.D<sub>660nm</sub>=1.0 containing approximately 10<sup>7</sup> spores/ml) of each of potent tannase producing fungal isolates viz., AVM-1 and AVM-2 and incubated for 96 hrs at 30°C in rotary shaker (150 rpm). After incubation, cultured broth was centrifuged at 10,000 rpm for 10 min at 4°C. The supernatant served as crude enzyme source was used for enzyme assay.

### Identification and preservation of potent fungal isolate

The fungal isolates were examined microscopically after staining with lactophenol cotton blue stain. Simplified key was used for identification of isolates to the level of genera (Barnett and Hunter, 1987). Moreover, morphological identification of potent isolate AVM-1 was also confirmed at Agharkar Research Institute, Pune, India. The fungal isolates were grown on PDA slants supplemented with 0.01% tannic acid and maintained at 4°C (working cultures). Stock cultures were maintained in 20% glycerol. The subculturing was performed every three weeks to assure its viability.

### Tannase assay

Tannase activity was assayed by the spectrophotometric method of methanolic rhodanine (Sharma *et al.*, 2000). Accordingly, 0.25ml of the cell free extract and 0.3 ml of methanolic rhodanine (0.66% w/v) solution was incubated for 5 min at 30°C and 0.2ml of 0.5 M potassium hydroxide was added. The samples were diluted with 4.0ml of distilled water and incubated at 30°C for 10 min. The enzyme activity was calculated from the change in the optical density value at 520nm. One unit of tannase activity is defined as the amount of enzyme required to liberate one micromole of gallic acid per minute under defined assay conditions.

### Optimization of culture conditions for tannase production

Optimization of culture conditions were studied by maintaining all factors constant except the one being studied. All parameters were carried out in 250ml

Erlenmeyer flask containing 100ml of minimal medium and were run in triplicates. Effect of incubation period was studied by inoculating 1.0ml of inoculum containing  $10^7$  spores/ml in 100 ml of minimal medium and incubated at 150 rpm for 144 hrs at 30°C. For enzyme extraction, culture medium was centrifuged at 10,000 rpm for 15 minutes and supernatant was used as extracellular enzyme source. Effect of tannic acid concentration on tannase production was evaluated by varying its concentration from 1 to 5% (w/v) in 100 ml of minimal medium. Effect of pH was studied by cultivating the isolate in different pH values (pH 4-7) of the minimal medium. Effect of temperature on enzyme production was investigated by incubating the organism at various temperatures ranging from 25 to 50°C. The activity of tannase was determined after an incubation of 96 hrs at 30°C.

#### **Determination of hydrolytic products of tannic acid by TLC**

The hydrolytic products of the tannic acid by action of crude tannase were characterized by TLC. Fermented broth, gallic acid and glucose standards were applied on silica gel plate (Silica gel 60 F<sub>254</sub>, E. Merck Ltd., Germany). The chromatogram was placed in ethyl acetate, chloroform and formic acid (4:4:1) solvent system. After drying, the chromatogram was developed by spraying a solution of FeCl<sub>3</sub> and then placed in oven at 105°C for 5 min. Identification of hydrolytic products was studied by comparing it with the standards (Naidu *et al.*, 2008).

#### **Evaluation of antioxidant property of enzyme hydrolytic product (gallic acid)**

In present investigation, antioxidant property of enzyme hydrolytic products was evaluated (Russo *et al.*, 2000; Skandran *et al.*, 2009). The  $\lambda$  phage DNA was used as test DNA in experiment. Untreated  $\lambda$  phage DNA was used as a control during the run of gel electrophoresis along with UV and H<sub>2</sub>O<sub>2</sub> treated sample of DNA.

#### **Statistical analysis**

The data were analyzed by one way ANOVA with the help of SPSS. All the data were presented as a mean value with its standard deviation. Error bars in the figures indicate the standard deviation values of the triplicates. P<0.05 was considered as statistically significant.

## **RESULTS AND DISCUSSION**

#### **Isolation and identification of fungal isolates**

In present study, a total of 7 soil samples were collected from different tea waste sites of Anand and Kheda regions and samples were screened for the isolation of fungi. A total of 4 fungi were isolated which were identified according to their macro and micro morphology. The obtained result shows that isolates belong to the genera of *Aspergillus* and *Penicillium* (Table 1).

Furthermore, the results of morphological identification of Agharkar Research Institute, Pune revealed that potent isolate AVM-1 was belongs to *Aspergillus niger*. Several fungal genera have been reported to produce tannase such as *Aspergilli* (Batra and Saxena, 2005; Pinto *et al.*, 2001;), *Penicilli* (Murugan *et al.*, 2007).

#### **Primary screening of tannase producers**

The selection of promising fungal isolates for secondary screening was carried out using primary screening method. The obtained results showed that the sum of colony and zone of clearance diameters vary among fungal isolates which was ranged from 12 to 35 mm (Table 2). The largest sum of colonies and zone of clearance (35 mm) was recorded with isolate AVM-1 followed by AVM-2 (29 mm) (Fig. 1), therefore they were selected as the potent tannase producers and were subjected to secondary screening.

#### **Secondary screening of selected fungal isolates**

Two selected fungal cultures from the primary screening step were subjected to secondary screening for tannase production. Isolate AVM-1 exhibited the highest tannase activity (3.18 U/ml) followed by isolate AVM-2 (2.5 U/ml). Murugan *et al.*, (2007) reported 4.7 times more extracellular tannase (16.8 U/ml) than intracellular tannase by *Aspergillus niger*.

#### **Effect of incubation time on tannase production**

The samples were drawn from minimal medium at regular time intervals of 24 hrs upto 144 hrs. The obtained results showed that the maximum tannase activity (3.18 U/ml) was achieved at 96 hrs of incubation (Fig. 2). This might be the fungi would have entered into its exponential phase. After 96 hrs of incubation, the enzyme activity was found to decrease considerably with further increase in the incubation time. Gautam *et al.*, (2002) have reported that the reason for the initial increase in tannase activity and followed by a decrease may be due to the inhibition or denaturation of the enzyme. Lekha and Lonsane (1997) and Sabu *et al.*, (2005a) reported maximum extra-cellular tannase production by *A. niger* in 96 hrs of incubation.

#### **Effect of tannic acid concentration on tannase production**

In present work, maximum tannase activity (4.5 U/ml) was achieved at 2% tannic acid concentration, thereafter enzyme activity was declined gradually when tannic acid concentration exceeded 2% (Fig. 3). Banerjee *et al.*, (2007) stated that the repression of enzyme of activity might be due to formation of insoluble complexes with membrane protein of the organism thereby both growth and enzyme production may be inhibited. Our findings are in good agreement with the reported work of Banerjee *et al.*, (2001) which showed maximum

extracellular tannase production in liquid submerged fermentation containing 2% tannic acid.

#### Effect of pH on tannase production

The Hydrogen ion concentration showed profound effect on the biological activities of the organism. The obtained result showed maximum enzyme activity (7.0 U/ml) at pH 5.5. By increasing the pH value above 5.5, there was a drastically decrease in enzyme activity (Fig. 4). These might be due to the preference of fungal cultures for acidic medium. The reason behind decrease in enzyme activity is the fluctuation in pH which might affects the protein structure. The results were agreed well with the findings of Sabu *et al.*, (2005b), they reported optimum pH of 5.5 for tannase production by *Aspergillus niger* ATCC 16620.

#### Effect of Temperature on tannase production

Temperature plays an important role in microbial growth and enzyme activity. The obtained results showed maximum tannase activity (7.5 U/ml) at temperature of 30°C. Above 30°C, tannase activity was decreased gradually (Fig. 5). This might be due to denaturation of some of the heat sensitive biochemical products produced during fermentation (Kar and Banerjee, 2000) whereas temperatures below 30°C, enzyme activity was lowered, this might be due to freezing of the protoplasmic membrane at lower temperature which causes inactivation of solute transport systems in the cells (Papagianni, 2004). Our obtained results are in good accordance with the reported work of Lal and Gardner, (2012) i.e., optimum temperature of 30°C for tannase production by *A. niger*.

#### Determination of hydrolytic products of tannic acid (gallic acid) by TLC

In present study, tannic acid was hydrolyzed with crude tannase enzyme and characterized its hydrolytic product (gallic acid) by TLC. Thin layer chromatogram clearly showed that the  $R_f$  value of sample (b) (0.69) was similar with the  $R_f$  value of standard gallic acid (a) (0.69) which indicates the presence of gallic acid in the sample (Fig. 6). The production of gallic acid by these organisms might be due to the hydrolysis of tannic acid by crude tannase enzyme.

#### Evaluation of antioxidant property of enzyme hydrolytic product (Gallic acid)

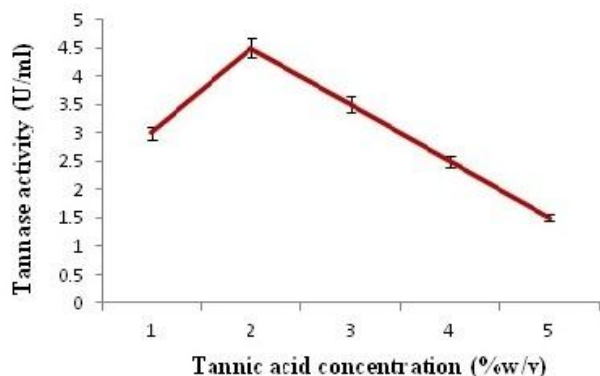
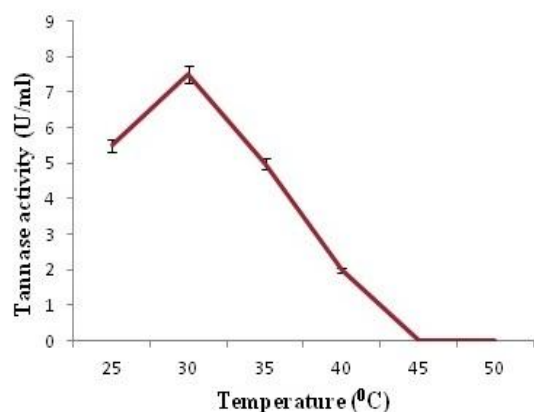
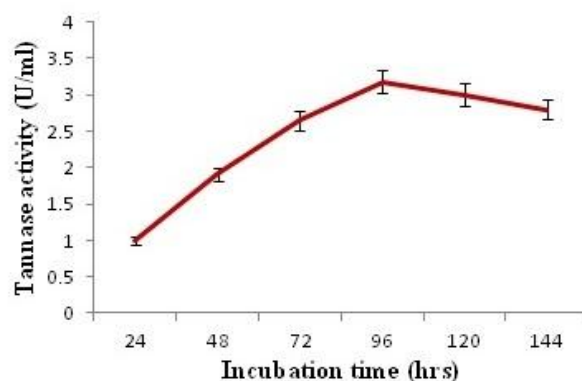
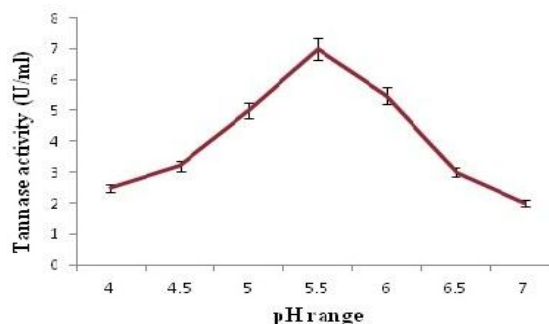
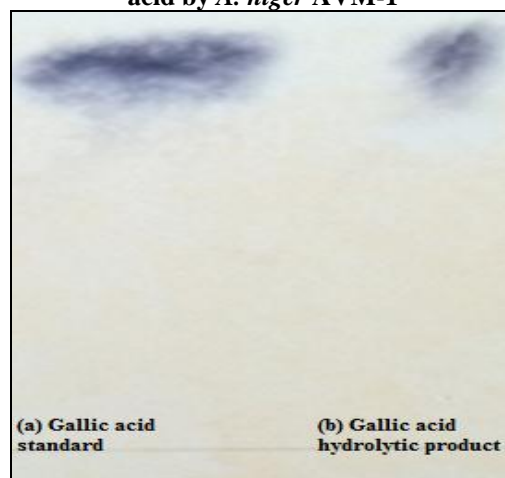
The antioxidant properties of tannic acid degradation product were checked by using DNA damage assay. The obtained results shows that the DNA damage was observed in first sample (a) where the DNA was treated with 100 mM  $H_2O_2$  and UV radiation. Sample (b) which was unexposed control  $\lambda$  phage DNA, shows no damage. Sample (c), which was treated DNA with 100 mM  $H_2O_2$  and 100  $\mu$ L of cell free supernatant containing gallic acid shows similar band as in control (Fig. 7). The reason for protection of DNA damage against  $H_2O_2$  and UV radiation was due to presence of gallic acid compound which possess antioxidant activity. There are a report which states an application of tannase for production of gallic acid and employed as antioxidants in foods, cosmetics, hair products, adhesives, and lubricant industry (Yu and Li, 2006). Min-Jer and Chinshuh, (2008) reported enzymatic modification by tannase which increased the antioxidant activity of green tea.

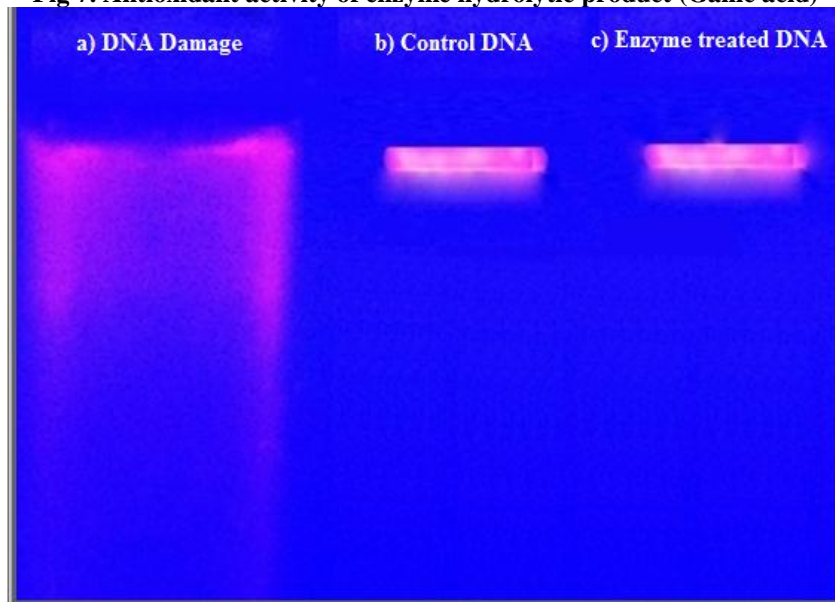
**Table 1. Macroscopic and microscopic characteristics of fungal isolates**

No. of isolates	Characteristics used in identification to genera level			Characteristics used in identification to species level		Scientific name of isolate
	Hyphae	Conidiophores	Conidia	Microscopic features	Macroscopic features	
AVM-1	Septate	Non-septate, seen metulae & phialides radiating from entire vesicle surface.	Spherical conidia, joining together in a one chain.	Smooth conidiophores	Color changes from white to yellow during growth	<i>Aspergillus niger</i>
AVM-2	Septate	Non-septate	Spherical conidia	Smooth conidiophores	Color changes from white to green	<i>Aspergillus oryzae</i>
AVM-3	Septate	Non-septate, terminating in a globose swelling	Spherical conidia with chain structure.	Rough conidiophores, sclerotia are present	colonies are yellow-green and reverse goldish to red brown to yellow	<i>Aspergillus flavus</i>
AVM-4	Septate	septate, bearing a clusters of branches, phialides born on cylinder branches	Single celled spherical conidia remaining together in one chain	-	-	<i>Penicillium</i> spp.

**Table 2. Primary screening of tannase producers on tannic acid agar plates**

Fungal isolates	The sum of colony and zone of clearance diameters (mm) $\pm$ SD
AVM-1	$35 \pm 1.2$
AVM-2	$29 \pm 2.0$
AVM-3	$17 \pm 0.7$
AVM-4	$12 \pm 0.5$

**Fig 1. Zone of clearance on MMT agar plate****Fig 3. Effect of tannic acid concentration on tannase activity of *Aspergillus niger* AVM-1.****Fig 5. Effect of temperature on tannase activity of *Aspergillus niger* AVM-1****Fig 2. Effect of Incubation time on tannase activity of *Aspergillus niger* AVM-1.****Fig 4. Effect of pH on tannase activity of *Aspergillus niger* AVM-1****Fig 6. TLC analysis of conversion of tannic acid to gallic acid by *A. niger* AVM-1**

**Fig 7. Antioxidant activity of enzyme hydrolytic product (Gallic acid)**

## CONCLUSION

The present study shows the successful optimization of culture conditions for the maximum tannase production. Optimized cultural conditions gave maximum tannase activity (7.5 U/ml). Based on the results obtained, we conclude that the overall enzyme activity is too good and may be considered for large scale production. The fungal isolate AVM-1 was able to grow on 2% tannic acid at acidic pH 5.5 under submerged fermentation, this confer to this isolate remarkable potential for its application in bioremediation of tannery effluent water. Moreover this is the first study reporting on evaluation of

antioxidant property of gallic acid produced by *Aspergillus* spp. which protects DNA damage against  $H_2O_2$  and UV radiation. Antioxidant property of gallic acid could be used to remove tannins from various substances like fodder for sheep and goats, and thus improve the health of these animals.

## ACKNOWLEDGEMENT

The authors are thankful to Dr. Rajesh Patel, Department of Life Sciences, H.N.G.U, Patan, Gujarat, India for his moral support during tannin biodegradation study.

## REFERENCES

- Banerjee D, Mondal KC and Pati BR. Tannase production by *Aspergillus aculeatus* DBF9 through solid-state fermentation. *Acta Microbiologica et Immunologica Hungarica*. 2007; 54(2): 159-166.
- Banerjee D, Mondal KC and Pati BR. Production and characterization of extracellular and intracellular tannase from newly isolated *Aspergillus aculeatus* DBF 9. *Journal of Basic Microbiology*. 2001; 41: 313-318.
- Barnett HL and Hunter BB. Illustrated genera of imperfect fungi. 4<sup>th</sup> ed. Prentice Hall, Inc. New Jersey. 1987: 35-39, 92, 94,130.
- Batra A and Saxena RK. Potential tannase producers from the genera *Aspergillus* and *Penicillium*. *Process Biochemistry*. 2005; 40: 1553-1557.
- Belur PD and Mugeraya G. Microbial production of tannase: State of the art. *Research Journal of Microbiology*. 2011; 6: 25-40.
- Bradoo S, Gupta R and Saxena RK. Screening for extracellular tannase producing fungi: Development of a rapid and simple plate assay. *Journal of General and Applied Microbiology*. 1996; 42: 325-329.
- Gautam P, Sabu A, Pandey A, Szakacs G and Soccol CR. Microbial production of extra-cellular phytase using polystyrene as inert solid support. *Bioresource Technology*. 2002; 83 (3): 229-233.
- Hadi TA, Banerjee R and Bhattacharyya BC. Optimization of tannase biosynthesis by a newly isolated *Rhizopus oryzae*. *Bioprocess Engineering*. 1994; 11: 239-243.
- Kar B and Banerjee R. Biosynthesis of tannin acyl hydrolase from tannin rich forest residue under different fermentation conditions. *Journal of Industrial Microbiology and Biotechnology*. 2000; 25: 29-38.
- Lal D and Gardner JJ. Production, characterization and purification of tannase from *Aspergillus niger*. *European Journal of Experimental Biology*. 2012; 2: 1430-1438.

- Lekha PK, and Lonsane BK. Production and application of tannin acyl hydrolase: State of art. *Advances in Applied Microbiology*. 1997; 44: 215-260.
- Lekha PK and Lonsane BK. Comparative titers, location and properties of tannin acyl hydrolase produced by *Aspergillus niger* PKL104 in solid state, liquid surface and submerged fermentation. *Process Biochemistry*. 1994; 29: 497.
- Mahapatra K, Nanda RK, Bag SS, Banerjee R, Pandey A, Szakacs G. Purification, characterization and some studies on secondary structure of tannase from *Aspergillus awamori nakazawa*. *Process Biochemistry*. 2005; 40: 3251-3254.
- Min-Jer L and Chinshuh C. Enzymatic modification by tannase increases the antioxidant activity of green tea. *Food Research International*. 2008; 41: 130-137.
- Murugan K, Saravanababu, S, Arunachalam M. Screening of tannin acyl hydrolase (E.C.3.1.1.20) producing tannery effluent fungal isolates using simple agar plate and SmF process. *Bioresource Technology*. 2007; 98: 946-949.
- Naidu RB, Saisubramanian N, Selvakumar D, Janardhanan S and Puvanakrishnan R. Partial purification of tannase from *Aspergillus foetidus* by aqueous two phase extraction and its characterization. *Current Trends in Biotechnology and Pharmacy*. 2008; 2: 201-207.
- Papagianni M. Fungal morphology and metabolite production in submerged mycelial processes. *Biotechnology Advances*. 2004; 22: 189-259.
- Pinto GAS, Leite SGF, Terzi SC and Couri S. Selection of tannase-producing *Aspergillus niger* strains. *Brazilian Journal of Microbiology*. 2001; 32: 24-26.
- Rana NK and Bhat TK. Effect of fermentation system on the production and properties of tannase of *Aspergillus niger* van Tieghem MTCC 2425. *Journal of General and Applied Microbiology*. 2005; 51: 203-212.
- Russo A, Acquaviva R, Campisi A, Sorrenti V, Di-Giacomo C, Virgata G, Barcellona ML and Vanella A. Bioflavonoidas antiradicals, antioxidants and DNA cleavage protectors. *Cell Biology and Toxicology*. 2000; 16: 91-98.
- Sabu A, Pandey A, Daud MJ and Szakacs G. Tamarind seed powder and palm kernel cake: two novel agro residues for the production of tannase under solid state fermentation by *Aspergillus niger* ATCC 16620. *Bioresource Technology*. 2005a; 96: 1223-1228.
- Sabu A, Kiran GS and Pandey A. Purification and characterization of tannin acyl hydrolase from *A. niger* ATCC 16620. *Food Technology and Biotechnology*. 2005b; 43: 133-138.
- Sharma S, Bhat TK and Dawra RK. A spectrophotometric method for assay of tannase using rhodanine. *Analytical Biochemistry*. 2000; 279: 85-89.
- Skandrani I, Bouhlel I, Limem I, Boubaker J, Bhouri W, Bhouri W, Neffati A, Sghaier MB, Kilani S, Ghedira K and Ghedira-Chekir L. *Moricandida arvensis* extract protect against DNA damage, Mutagenesis in bacteria system and scavenge the superoxide anion. *Toxicology in Vitro*. 2009; 23: 166-175.
- Yu XW and Li YQ. Kinetics and thermodynamics of synthesis of propyl gallate by mycelium -bound tannase from *Aspergillus niger* in organic solvent. *Journal of Molecular Catalysis B: Enzymatic*. 2006; 40: 44-50.