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# Isolation and Screening of Tannase Producing Fungi

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**ABSTRACT**

Tannin acyl hydrolase (E.C.3.1.1.20) is usually called as tannase, which hydrolyses the ester and depside bonds of tannins and gallic acid, glucose and galloyl esters are the end products. The enzyme tannase had many applications in many industrial areas like food, pharmaceutical, chemical and beverages. The enzyme has potential uses in the treatment of tannery effluents and pre-treatment of tannin containing animal feed. With a long historical evidences and scientific publications, tannase is still considered as one of the costly industrial enzymes. In view of the growing demand, it is imperative to isolate high productive strains and develop economically feasible processes. The present study is concentrating on the isolation and screening methods of tannase enzyme from fungal source.

**Keywords**: Tannins, Tannin acyl hydrolase, *Aspergillus niger*, Plate assay

## INTRODUCTION

Tannins are natural occurring polyphenolic in nature having different molecular weights that are present in the plant cells [1]. They are differing from others in that they can precipitate proteins from solution. Tannins are categorised into two main groups based on the structure and properties. They have hydrolysed tannins and condensed tannins, and there is also an intermediate group that combines both the properties called Catechin tannins. Tannins are widely present in natural plants like tea, coffee, pomegranates, legumes, strawberries, sugarcane, nuts, some herbs and spices like cloves and cinnamon, alm kernel, *Phyllanthus emblica (*amla), monocots and other various species of plants or plant products which are used for human consumption [2]. Tannase is a Tannin Acyl Hydrolase (E.C. 3.1.1.20) is an inducible enzyme. Tannase (TAH) hydrolyse the ester and depside bonds in hydrolysable tannins such as tannic acid, methyl gallate, n-propyl gallate, ethyl gallate, and isoamyl gallate and finally releasing glucose and gallic acid as end products. Then the Gallic acid catalyzes the second step in the degradation of the polyphenol, tannic acid [3]. The enzyme tannase can be extracted from various sources such as microorganisms, plants and animals [4]. Tannase is extracted from fungi as they are fast growing and are elsewhere in the environment. Their environmental and genetic manipulation is easier. Fungal tannase is reported to be highly active over a wide range of pH and temperature than the other sources. Although tannase production by *Aspergillus niger* can occur in the absence of tannic acid, this fungus tolerates tannic acid concentrations as high as 20%(w/v), without having a deleterious effect on both the enzyme production and growth [5,6]. Tannase is an industrially important enzyme and has several applications in various industries such as foods, animal feeds, pharmaceutical, chemical and leather industries, and cosmetic, industry etc. [7-9]. In food industries the tannase enzyme is used as a clarifying agent in the preparation of instant tea [10-13],

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in the processing of coffee flavoured soft drinks [14-16] and as a debittering agent and clarifying and in fruit juices manufacture [17].

## MATERIALS AND METHODOLOGY

### Isolation and identification of fungi

Soil samples were collected from different locations of Bangalore University Campus. The collected soil samples used for isolation of fungi by serial dilution followed by plating on Czapek dox agar medium. Czapek dox agar medium containing the chemicals such as sucrose-30.0g; $NaNO_3$-2.0g; KCl-0.5g; $MgSO_4 \cdot H_2O$-0.5g; $K_2HPO_4$-1.0g; $FeSO_4 \cdot 7H_2O$-0.01g; Agar-15g; Distilled water-1000ml. Approximately 1g of soli sample is added to the sterile saline of 10ml and shake vigorously. Then, the soil particles are allowed for settling down and the supernatant is taken for the serial dilution. The serially diluted sample of 0.1ml is spread on the CZA plates and incubate for 3-5days at room temperature [18].

### Screening of tannase producing fungi

The screening of fungal strains which are capable of producing tannase enzyme is detected by using enrichment culture method in Czapek Dox minimal medium by adding tannic acid as the carbon source. The basal medium contains tannic acid-10g/L; $NaNO_3$ 3.0g; KCl-0.5g; $MgSO_4 \cdot H_2O$-0.5g; $K_2HPO_4$-1.0g; $FeSO_4 \cdot 7H_2O$-0.01g; agar-30g; Distilled water-1000ml. The tannic acid solution is sterilized separately by passing through a filter of cellulose nitrate membrane (25mm diameter, 0.45µm pore size, Whatman). The plates are incubated for 3-5 days at room temperature. The colonies are observed after incubation [19]. Microscopic characteristics of the fungal isolates are done by the wet mounting of the organisms. It is done by the stain called lactophenol cotton blue.

### Plate Assay

The plates with filled with 0.01M $FeCl_3$ which reacts with metabolites and forms the brown colour [20]. This results with the clear zone around the colonies and size of the clear zone is measured.

## RESULT

Fifteen potent fungal strains were designated out of fifteen isolates isolated from soil samples collected at varying environmental stress conditions. They were isolated from in and around Bengaluru, Karnataka, India. The pattern of distribution of isolates is given in Table 1. Four different kind of eco-stressed soil samples were used for the isolation of tannase producers. The isolated fungi are growing in the tannic acid containing medium. They consume tannic acid as their carbon source and grow in plate after incubation. Among 15 isolates only two are able to producing the colony in the tannic acid medium. That means they are able to produce tannase enzyme and utilize it (Table 2).

**Table 1: Fungal isolates from different regions of soil samples**

| | | |
|---|---|---|
| **1** | Coconut tree | 06 |
| **2** | Near park soil | 03 |
| **3** | Near Bangalore university campus | 04 |
| **4** | Near Gandhi bhavan | 02 |



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**Table 2: Screening of fungal isolates for tannin hydrolysis**

| | | |
|---|---|---|
| **1** | *Aspergillus* spp. | 20 |
| **2** | *Fusarium* spp. | 15 |

Wet mounting of the tannase producers (isolates of Figure 1 and 2) with lactophenol cotton blue revealed that the isolates of tannase producer belonged to *Aspergillus* spp. and *Fusarium* spp. (Figure 3 and 4). Tannase producers are subjected to the 0.01M $FeCl_3$ and leads to the formation of zone around the colonies. Based on the diameter of the zone, efficiency of the tannase enzyme is predicted. In this study *Aspergillus* spp. produces large zone than *Fusarium* spp.

**Figure 1: Fungal isolate 1 in tannin acid medium**

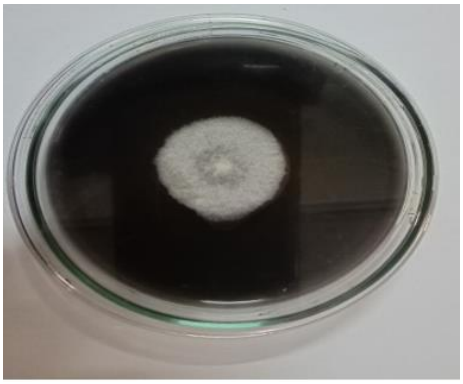

**Figure 2: Fungal isolate 2 in tannin acid medium**

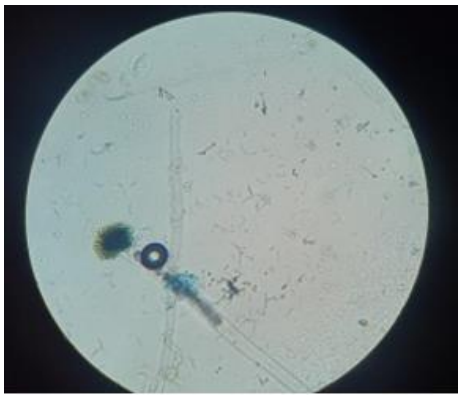

**Figure 3: Microscopic observation of *Aspergillus* spp. after lacto phenol cotton blue staining. 10X**



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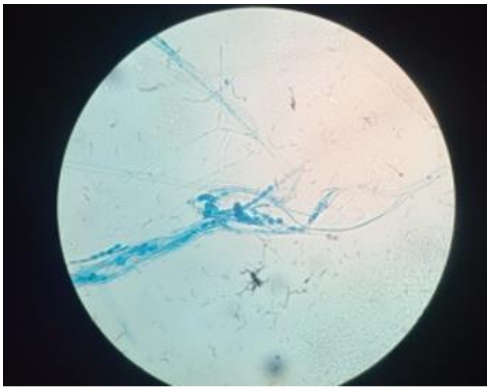

**Figure 4: Microscopic observation of *Fusarium* spp. after lacto phenol cotton blue staining. 10X**

## DISCUSSION

Thus, in the present study, various fungal tannase producers were isolated from the various soil samples. They are screened on the basis of efficiency of tannin production and morphological characters are studied by microscopic observation. The potential isolate identified as the *Aspergillus spp.* and it will be used for the tannase enzyme production and purification which is used in food and pharmaceutical industries as well as in the treatment of tannery effluent.

## CONCLUSION

By concluding the current study, *Aspergillus niger,* a local isolate was found to be able to secrete tannase on the enrichment with minimal media. However, the isolation, purification and characterization of the enzyme is necessary to determine the commercial production and application in the industries.

## REFERENCES


1. Bhat TK, Singh B, Sharma OP. Microbial degradation of tannins–a current perspective. Biodegradation. 1998 Sep 1;9(5):343-57.
2. Boadi DK, Neufeld RJ. Encapsulation of tannase for the hydrolysis of tea tannins. Enzyme and Microbial Technology. 2001 May 7;28(7-8):590-5.
3. Ganga PS, PS G. EFFECT OF ENVIRONMENTAL FACTORS ON THE PRODUCTION OF FUNGAL TANNASE. II.
4. Paz JE, Guyot S, Herrera RR, Sánchez GG, Esquivel JC, Castañeda GS, Aguilar CN. Alternativas Actuales para el Manejo Sustentable de los Residuos de la Industria del Café e del Café en México Current Alternatives for Sustainable Management of Coffee Industry By Current Alternatives for Sustainable Management of Coffee Industry By-Products Products in Mexico. Revista Científica de la Universidad Autónoma de Coahuila. 2013;5(10).
5. Chowdhury SP, Khanna S, Verma SC, Tripathi AK. Molecular diversity of tannic acid degrading bacteria isolated from tannery soil. Journal of applied microbiology. 2004 Dec;97(6):1210-9.
6. Saxena S, Saxena RK. Statistical optimization of tannase production from Penicillium variable using fruits (chebulic myrobalan) of Terminalia chebula. Biotechnology and applied biochemistry. 2004 Feb;39(1):99-106.
7. Takino Y, inventor; Coca Cola Co, assignee. Enzymatic solubilization of tea cream. United States patent US 3,959,497. 1976 May 25.
8. Yu X, Li Y, Wang C, Wu D. Immobilization of Aspergillus niger tannase by microencapsulation and its kinetic characteristics. Biotechnology and applied biochemistry. 2004 Oct;40(2):151-5.
9. Agbo F, Spradlin JE, inventors; Mondelez International Inc, assignee. Enzymatic clarification of tea extracts. United States patent US 5,445,836. 1995 Aug 29.
10. Aguilar CN, Gutiérrez-Sánchez G. Sources, properties, applications and potential uses of tannin acyl hydrolase. Food Science and Technology International. 2001 Oct;7(5):373-82.




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11. Lekha PK, Lonsane BK. Comparative titres, location and properties of tannin acyl hydrolase produced by Aspergillus niger PKL 104 in solid-state, liquid surface an submerged fermentations. Process Biochemistry. 1994 Jan 1;29(6):497-503.
12. Belur PD, Mugeraya G. Microbial production of tannase: state of the art. Research Journal of Microbiology. 2011 Jan 1;6(1):25-40.
13. Lu MJ, Chu SC, Yan L, Chen C. Effect of tannase treatment on protein–tannin aggregation and sensory attributes of green tea infusion. LWT-Food Science and Technology. 2009 Jan 1;42(1):338-42.
14. Noguchi N, Ohashi T, Shiratori T, Narui K, Hagiwara T, Ko M, Watanabe K, Miyahara T, Taira S, Moriyasu F, Sasatsu M. Association of tannase-producing Staphylococcus lugdunensis with colon cancer and characterization of a novel tannase gene. Journal of gastroenterology. 2007 May 1;42(5):346-51.
15. Lal D, Gardner JJ. Production, characterization and purification of tannase from Aspergillus niger. European Journal of Experimental Biology. 2012;2(5):1430-8.
16. Guo LH, Yang SK. Study on gallic acid preparation by using immobilized tannase from Aspergillus niger. Sheng wu gong cheng xue bao= Chinese journal of biotechnology. 2000 Sep;16(5):614.
17. Rout S, Banerjee R. Production of tannase under mSSF and its application in fruit juice debittering.
18. Aneja KR. Experiments in microbiology, plant pathology and biotechnology. New Age International; 2007.
19. Lu MJ, Chen C. Enzymatic modification by tannase increases the antioxidant activity of green tea. Food Research International. 2008 Jan 1;41(2):130-7.
20. Aguilar CN, Rodríguez R, Gutiérrez-Sánchez G, Augur C, Favela-Torres E, Prado-Barragan LA, Ramírez-Coronel A, Contreras-Esquivel JC. Microbial tannases: advances and perspectives. Applied microbiology and biotechnology. 2007 Aug 1;76(1):47-59.




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