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# Productionof Tannase Enzyme by *Penicilliumduclauxii,* Screened and Isolated from Soil.

**Suman Lata[1]*, and Km. Poonam Rani[2]*.**

[1]Department of Biotechnology, Meerut Institute of Engineering and Technology,Meerut, Uttar Pradesh, India.
[2]Student of Master of Technology in Biotechnology Department of Biotechnology Meerut Institute of Engineering and Technology, Meerut Uttar Pradesh, India.

**ABSTRACT**

Tannase are enzymes that may be used in different industrial sectors as, for example,food and pharmaceutical.They are obtained mainly from microorganisms, as filamentous fungi. However, the diversity of fungi stays poorly explored for tannase production. Most of the research was focused on fungal tannase, as tannin was earlier considered as bacteriostatic. After the discovery of bacterial tannase in 1983, several studies on bacterial tannase were published. Despite the long history and numerous publications, tannase is still considered as one of the costly industrial enzymes. This is due to less titer and long fermentation time of the processes. In view of the growing demand, it is imperative to isolate high productive strains and develop economically feasible processes. A total of 19samples collected from the tannin-rich soil. The isolated cultures were screened for their tannase producing capability by observing the zone of hydrolysis on tannic acid agar plates. Among thefungal strains selected as tannase producers,The isolate S4RD4 showed largest zone of clearance of 30 mm (diameter) on tannic acid agar plate. Hence, it was selected for further study and identified as *Penicillumduclauxii*NFCCI,PUNE,INDIA .To enhance the production level of the enzyme different culture conditions were optimized and observed that optimum temperature and pH for tannase production was 30$^{o}$C and 5.5 respectively. Maximum growth and enzyme production was recorded after 96 hrs of incubation period in the medium(B-modified synthetic medium) containing 1% tannic acid. Malt extract (2%) with $NaNO_3$(0.2%) was found to the best nitrogen source and sucrose found to be a best carbon source for tannase enzyme production. Among the additives, metal ions$Cu^{2+}$,$Mg^{2+}$, $Mn^{2+}$, $CO^{2+}$, $K^{+}$ $Ca^{2+}$, $Fe^{3+}$ affected enzyme production. Tween-20,Tween-40, Tween-60, Tween-80,Triton X-100 and SDS detergents tested, these detergents inhibited the production of tannase. The optimization of culture conditions enhanced the production level of tannase (33.41 U/ml) by (1.36) fold. This study reviews the microbial sources, isolation and screening methods, modes of production, substrates and media, temperature and pH of fermentation, duration of fermentation and location of tannase enzyme.

**Keywords:**Tannaseenzyme, Gallic acid, Tannic acid, Identification, Tannase Assay.

***Corresponding author***

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## INTRODUCTION

The enzyme tannase (E.C. 3.1.1.20) also known as tannin acyl hydrolase, is a hydrolytic enzyme that acts on tannin. It catalyses the hydrolysis of bonds present in the molecules of hydrolysable tannins and gallic acid esters producing gallic acid and glucose[17].Tannase is an inducible enzyme produced by variety of microorganisms such as fungi, bacteria and yeast [1].Microorganisms have been the most important source for the production of industrial enzymes due to their biochemical diversity and their technical and economic advantages [4].

Tannins are naturally occurring plant phenolics compounds that have wide ranging effects on animals and microbes.They are polyphenolic secondary metabolites of plants which form hydrogen bonds in solutions, resulting in the formation of tannin-protein complexes. Tannins are present in large number of feed and forages. The formation of complexes of tannins with nutrients, such as carbohydrates, proteins and minerals, has negative effects on their utilization. High concentrations of tannins depress voluntary feed intake and digestive efficiency [3]. The nutrient value of tanniferous feed may be enhanced by various detannification procedures viz., physical, chemical and biological. In biological treatment, various tannase producing microbial strains have been tried for reduction of tannin content and nutritive enhancement of treated material[64].
In this respect, tannase find potential applications in feed, food and beverage industry. Tannase is used as clarifying agent in some wines, juices of fruits and refreshing drinks with coffee flavor [5,12].The use of tannase helps in overcoming the problem of undesirable turbidity in these drinks which poses the quality problem. Enzymatic treatment of fruit juices reduces bitterness, haze and sediment formation, hence are acclaimed for health benefits and industrial use. Tannase is also being used for production of instant tea preparations. The enzyme has potential uses in treatment of tannery effluents and pretreatment of tannin containing animal feed [2, 54].

One of the major application of tannase is the production of gallic acid. Gallic acid is used for the manufacture of an anti-malarial drug Trimethoprim. Gallic acid is a substrate for the chemical and enzymatic synthesis of propyl gallate, used as anti-oxidants in fats and oils.Applications of tannase include removal chill haze formation of beer and wines and detannification of poultry feed to improve the feed efficiency[2].

It is present as intracellular or extracellular enzyme produced by several micro-organisms and plant cells. Tannase has been isolated from number of micro-organisms like fungi, bacteria and yeast. Many fungal species have been reported to produce tannase, including *Aspergillus aculeatus, A. aureus*, *A. flavus*, *A. foetidus, A. japonicas, A. niger, A. oryzaeAureobasidium pullulans*, *Fusarium solani F. subglutinans*, *Paecilomycesvariotii, Penicilliumatramentosum; P.chrysogenum*, *P. variable,* and *R. oryzae*as reviewed by Belur andMugeraya.and Chavez-Gonzalez. The vast majority produce in submerged cultures,while *Aspergillus* and *Penicillium*are the most active microorganisms capable of producingtannase through both submerged and solid state fermentations [19, 10]. The fungal species *Aspergillus* and *Penicillium*are the most active micro-organisms capable of producing tannase through submerged and solid state fermentation.

In the last decade, there have been a number of efforts to improve the production, recovery, and purification processes of the enzyme. These efforts include the looking for new sources for tannase enzyme production (5,6], the development of novel fermentation systems [7,8], the optimization of culture conditions [9,10], the production of the enzyme by recombinant microorganism [11,12], the design of efficient protocols for tannase recovery and purification [11] Technological advances on tannase processing must be supported by basic investigation. Physicochemical properties of several tannase have been characterized [12,13],there have been a special interest in the description of tannase and tannase gene structure [14].

With the rapid advancement in the field of genetic and protein engineering enzymes have found their way into many new industrial processes. Enzymes offers biological alternative to the chemical processes at industrial scale. Biological methods are an important mechanism of organic chemical removal in the natural systems [3].

In view of the growing demand, it is important to isolate high productive strains and develop economically feasible processes. With this view studies on isolation, screening and production of tannase from *Penicillumduclauxii*, NFCCI, PUNE,INDIAby submerged fermentation technique was carried out.

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## MATERIAL AND METHODS

### Micro-Organism

### Fungal strain

The tannase producing fungal strain used in the present investigation was isolated from soil sample collected from Ghaziabad district (UP).The culture was maintained on malt extract medium which consist of malt extract (2%), K2HPO**4** (0.1%), NH4Cl (2%) and Agar (2%). For enzyme production, this medium was supplemented with 1% tannic acid. The culture was maintained on malt extract agar slant at 4ºC and subcultured twice in a month**.** The strain was identified as *Penicilliumduclauxii*by NFCCI, Pune, INDIA.

### Collection of soil samples

Nineteen different samples were collected from various locations such asfrom Meerut, Garh, Delhi, Ghaziabad, by digging the soil up to 5-6 inches deep fromthe soil surface. Samples were collected (in sterile polythene bags and stored at $4^{o}$Cuntil use) from different litter sites such as soil containing decaying material, soil fromagricultural field, soil receiving kitchen wastes, soil receiving tea wastes, soil receivingdomestic wastes, garden soil etc.

### Serial dilution technique

In the present investigation, for isolation of tannase producing microorganisms, the soil sample was plated by serial dilution method (Figure2).Ten gram of soil was suspended in 90 ml of sterile distilled water and shaken well. The sample was serially diluted through a series of dilution to obtain a final concentration of $10^{-5}$. From each dilution, 200 µl was pipette out and spread on the surface of sterile solid agar plate supplemented with 1% tannic acid.

### Sprinkling method

A new method for soil sample plating has been used, in which 1 to 2 milligram (mg) of soil sample was simply sprinkled over the tannic acid solid agar plates (supplemented with 1% tannic acid).The plates were incubated at 30ºC for 96 hrs. After, different fungal colonies appeared on the plates. These fungal colonies were isolated and purified. They were screened for tannase producing capability qualitatively.

### Qualitative screening of tannaseproducing fungi

The tannase producing fungal isolates were screened by observing zone ofhydrolysis around colonies. The isolates producing zone around its growth on maltextract medium agar plates (supplemented with 1% tannic acid) were selected as tannest producers. FeC13 testing was used for the purpose of screening the potent tannaseproducer fungi [29]. FeC13 reacts with tannic acid present in themedium and produces deep brown colour. The solid agar tannic acid plates withisolated fungal colonies (point inoculated and incubated for 72 hrs) were floodedwith FeC13 solution. The positive fungal strain hydrolyzed the tannic acid present inthe surrounding medium and shown a zone of clearance around the colony. [37].

### Quantitative estimation of tannase

### Tannase production

The selected fungal isolate was used for extracellular tannase production inmodified malt extract liquid medium. The Erlenmeyer flask containing medium was inoculated with discs (8 mm diameter) of 48 hr grown fungal culture. Theflask was incubated at 30ºC for 3-5 days. After incubation, the fermentation broth was filtered through Whatman filter paper (No.1) [36]. The tannase activity from the culturesupernatant (crude enzyme) was determined by performing tannase assay.

**Tannase assay**

Tannase was assayed following the method of Mondal*et al.* (2006) using tannicacid (1%) as substrate, prepared in acetate buffer (0.2 M, pH 5.5). The reaction mixturewas prepared by the addition of 0.5 ml substrate with 0.1 ml of crude enzyme andincubated at 40oC for 20 min. The enzymatic reaction was stopped by adding 3 mlBovine serum albumin (BSA) (l mg/ml). The tubes were centrifuged at 5000 x g for 10min. The precipitate was dissolved in 2ml SDS-triethanolamine solution followed by theaddition of 0.5 ml FeCl3 reagent. The contents were kept for 15 min. for stabilizing thecolour formed and the absorbance was measured at 530 nm against the blank. One unit oftannase activity can be defined as the amount of enzyme which is able to hydrolyze 1Mof substrate tannic acid in 1 min under assay conditions.

**Optimization of culture conditions for tannase production**

The following culture conditions were optimized to increase the maximumproduction of tannase from *Penicilliumduclaucxii*.

**Effect of different medium on enzyme production**

To obtain maximum tannase production, different production medium (pH 5.5)such as medium A (Synthetic medium for tannase production), B (Synthetic medium fortannase production), C (CzapekDox‟s medium), D (Modified Malt extract Medium) weretested. The composition of these medium was in g/L. The flasks of different mediumwere inoculated with 72 hrs grown culture of *Penicilliumduclauxii*and incubated at 30ºCfor 5 days and growth as well as tannase activity was determined.

**Effect of incubation period on enzyme production**

To evaluate the effect of different incubation period on tannase production, theincubation period of the medium range was varied from 24 hrs to 120 hrs. To study theoptimal incubation period for maximum tannase production, the flasks containingproduction medium B (pH 5.5) were inoculated at $30^0$C.

**Effect of pH on enzyme production**

The effect of initial pH of production medium for tannase production was studied.Flask containing production medium B was adjusted at various pH ranges from 4.5, 5.0,5.5, 6.0, 6.5 by 1N HCl and 1N NaOH solution and were inoculated at $30^0$C and assayedfor tannase activity as described earlier.

**Effect of temperature on enzyme production**

To study the effect of different temperatures on tannase production, the flaskscontaining medium kept at temperature range was varied from 30-50°C. To study theeffect of incubation temperature for maximum tannase production, the flasks with theproduction medium were inoculated at various temperatures such as 30, 35, 40, 45 ,50 55,50$^0$C for 72 hrs. The general procedure mentioned earlier was followed for tannase assay.

**Effect of carbon source on enzyme production**

To study the effect of different carbon source on tannase production from*penicilliumduclauxii*, the medium was supplemented with different sugar (maltose,sucrose, mannose, dextrose, lactose) with concentration 1.0 % (w/v). The productionmedium (medium B) with 1% tannic acid was kept as control. These flasks wereinoculated with fungal culture and incubated at 30oC for 96 hrs and tannase activity wasestimated.

**Effect of nitrogen source on enzyme production**

To study the different nitrogen sources on tannase production with variousorganic such as yeast extract, malt extract, beef extract, peptone and inorganicnitrogenous sources such as NaNO3, NH4Cl, KNO3, NH4NO3 and these flask withmedium B were inoculated with *penicilliumduclauxii*and incubated at 30o C for 3 daysand tannase activity was estimated.

**Effect of additives (metals ions and detergents)**

The effect of different additives on tannase production in the production mediumB was studied by adding different salts such as MgCl2, KCl, CuSO4, FeCl3, CoCl2 anddetergent such as Tween-20, Tween-40, Triton X-100 and SDS inoculated with *penicillium duclauxii* incubated at 30oC. After incubation, filtered the fermentation brothand tannase activity in culture supernatant was determined by performing tannase assay.

**Tannase production under optimized culture conditions**

The medium optimized at this stage for tannase production by from *Penicillium duclauxii* was termed as Tannase Production Medium and was used in further studies.The composition or production medium and optimized conditions for tannase production by *Penicillum duclauxii* were also studied.

## RESULTS AND DISCUSSION

**Isolation and screening of tannase producing fungal strains**

**Isolation of tannase producing fungal strains**

In the present study, isolation of fungal tannase producers was carried out fromnineteen different soil samples. Total eighty six fungal strains were isolated on tannic acid agar plates by usingserial dilution technique. On thebasis of clear zone formation around the growth of fungal culture, fifty nine fungal isolatesshowing tannase activity on solid plate (Table1). Out of fifty nine, only nine fungalisolates were selected as potent tannase producers on the basis of diameter of zone ofclearance (Table 1). Further, these cultures were purified and maintained on malt extractagar medium.The fungal species of genus *Aspergillus* and *Penicillium*are the most activemicro-organisms capable of producing tannase through submerged and solid statefermentation [19]. There are few reports on tannase productionfrom *Rhizopus*sp. [31,24] and *Fusarium* sp. [23,28]. However,*Trichoderma* sp. is the leastexplored fungi for tannase production and characterization. *Trichoderma* sp. has beenreported as tannase producers [23, 28].

**Qualitative screening of tannaseproducing fungi**

The selection of promising fungal isolates for tannase production was carried outusing screening method.The isolates producing zone around itsgrowth on malt extract medium agar plates (supplemented with 1% tannic acid) wereselected as tannase producers. FeC13 testing was used for screening the potent tannaseproducer fungi. Selection of the strains was done on the basis of the degree ofzone of clearance around colonies which was due to the tannase activity in the medium.Culture exhibiting zone of clearance after removal of the FeC13 were confirmed astannase producers. The results obtained from this study showed that zone of clearanceproduced by different fungal isolates vary from 9 mm to 30 mm (Table 1). The isolateS4RD4 showed largest zone of clearance of 30 mm (diameter) on tannic acid agar plate(Fig. 2). The isolate S4RD4 produced tannase activity of (24.68 U/ml). It wasidentified as *Penicilliumduclauxii*by National Fungal Culture Collection of India(NFCCI), PUNE, INDIA.

**Optimization of culture conditions for maximum tannaseproduction**

Tostudy, the optimization of culture conditions for maximum tannase production wascarried out using 'one-factor-at-a-time' approach, by changing one control variable at atime while holding the rest parameters constant. The different parameters optimized wereas follows:

**Screening of different medium for tannase production**

Four production media such as, Synthetic medium A, Synthetic medium B,medium C (CzapekDox's medium), medium D (Modified Malt extract Medium) wastested in an attempt to improve the tannase production from *Penicilliumduclauxii*. Amongthe different medium tested, growth as well as enzyme production (24.39 U/ml) wasfound to be maximum in medium B (Synthetic medium supplemented with 1% tannicacid), followed with medium A and C (Fig.3). The minimum enzymeproduction was observed in medium

D.Several fungal species belonging to genera*Aspergillus* and *Penicilliumsuch* as *PenicilliumCharlesi*, *Penicillium variable,Aspergillus fumigatus, Aspergillus versicolor, Aspergillus flavus, Aspergilluscaespitosum, PenicilliumCrustosum,*and*Penicilliumrestrictum*which exhibits tannaseproducing activity on synthetic medium containing 1% tannic acid [23].Maximum tannase production from *Paecilomycesvariotii*and *Aspergillusfoetidus*using synthetic medium were reported by [57, 50]. Many researchers used malt extract broth (supplemented with 1% tannic acid) for maximum tannase production from fungi [41].

**Production of Tannase Time course of tannase production**

To determine the optimum incubation timerequired for maximum tannase production from *Penicilliumduclauxii,* the flask wasinoculated with fungal culture and incubated at 30°C. Samples were withdrawn atregular intervals of 24 hrs for estimation of tannase activity.The results shown that thetannase activity was initially detected at 48 hr. and increasing with the time. Maximumgrowth and yield of tannase (27.12 U/ml) was obtained after 96 hrs of incubation (Fig. 4). Decreased enzyme yieldon prolonged incubation could also be due to reduced nutrient level of medium. It hasbeen reported that tannase activity decreased after reaching maximum level, due toinhibition of enzyme or degradation of enzyme [64).Similar observations have been reported for tannase enzyme production by manyinvestigators [28,8,29,14].There are various reports to study different incubation period for maximalextracellular production of tannase production by fungi. maximum extra-cellular tannase production by *Aspergillus niger andRhizopusoryzae*at 96 hrs and 120 hrs of incubation respectively[12].Maximum production of extracellular tannase found in *A. aculaetus*after 72 hrs[27].However, maximum tannase production from *Trichoderma viride*was reported at 48 hrs[33].

**Effect of pH of the culture medium**

The production of tannase enzyme from fungi strongly depends on theextracellular pH because culture pH strongly influences many enzymatic processes andtransport of various components across the cell membranes which in turn support the cellgrowth and product production (45).the pH of medium was adjusted at different pH range (4.5-6.5).Maximumtannase production (29.86 U/ml) from *Penicilliumduclauxii*was observed at pH 5.5(Figure 5).The growth and enzyme production was however, considerable atpH 5.5-6.0. Further increase in pH of production medium, tannase enzyme productionwas found to decrease at pH 6.5. There are reports describing of the optimum pH as 5.5for tannase production [27,13,14,33,23,19).Furthermore, the optimal initial medium pH for tannase production by submergedfermentation has also been reported in acidic range of 4.0-4.5 [1,24].Tannase production by *Aspergillus niger andAspergillus oryzae*at pH 4.5 respectively, while some researchers reported maximumtannase production at pH 5.0 from *Aspergillus oryzae*(33,8,44].The initial pH of medium 6.0 has also been reported for tannase production fromfungus such as *Aspergillus awamori, Aspergillus japonicus,Asergillusniger*respectively[23]. Some fungus alsoproduced tannase at pH ranges from 6.0-6.5 [51,54].

**Effect of incubation temperature**

The effect of incubation temperature on tannase production from *Penicilliumduclauxii*was studied in the temperature range of $30^0$C-$50^0$C. The optimum incubationtemperature for tannase enzyme production (29.87 U/ml) from fungus was observed at$30^0$C (Fig.6). Further rise in temperature, decreased the production of tannase and theminimum tannase activity was observed at temperature 40ºC.The fermentation temperature for optimum production oftannase is mostly reported to be 30°C [34,38,8,45,33,44].Some investigators also mentioned tannase production from fungi at temperature35°C-40°C [45,24,35]. Furthermore researcher also focused on tannase production from thermophilicfungi at temperature 50ºC-60ºC [45,62,27].

**Effect of substrate concentration**

To determine the optimum concentration of substrate for production of tannase, theflasks of production medium containing different concentrations of tannic acid rangingfrom 0.5 - 2.0 % were investigated. The maximum tannase activity of 30.42 U/ml wasobserved at 1.0% concentration of tannic acid in the production medium (Fig.7).Tannase activity was affected by concentration of substrate in the medium. Maximumtannase activity was observed at a concentration of 1% (w/v) tannic acid used as inducer.Similar observations have been reported that tannase production from *Aspergillusochraceus*[1) and *Aspergillus*

*niger*[12].It was also observed that 2% tannic acid was suitable for tannase production, maximum extracellular tannase and gallic acid produced after36 hrs in liquid submerged fermentation containing 2% tannic acid[27].

**Effect of carbon source**

Tannase enzyme production depends on the availability of carbon sources in themedium and carbon sources have been shown to regulatory effects on tannase enzymesynthesis [23). To investigate the effect of carbon source on tannaseproduction from *Penicilliumduclauxii*was studied (Fig.8).The flasks containingproduction medium of different carbon sources such as sucrose, maltose, mannose,dextrose, and lactose were inoculated with fungal spores and incubated for 96 hrs at30ºC. The maximum tannase enzyme production wasobserved with sucrose (33.41U/ml) followed by maltose (19.44 U/ml) and then withmannose (18.87 U/ml). These results are in agreement with those reported for tannaseproduction from different microorganisms earlier such as *Aspergillus oryzae*[9,38,55] and *Penicilliumatramentosum*[36].Sucrose on degradation will act as a better carbon source for tannase productionfrom *Aspergillus terreus*[40,27].

**Effect of different nitrogen sources**

Tannase production depends on the availability of nitrogen sources in themedium. It has been shown to have regulatory effects on enzyme synthesis [23]. The effect of nitrogen source on tannase production from *Penicillumduclauxii*mwas studied by supplementing the production medium with various organic (2%) such asyeast extract, malt extract, beef extract, peptone and inorganic (0.2%) nitrogenoussources such as NaNO3, NH4Cl, KNO3, NH4NO3. (Fig.9) However, enzymeproduction in the presence of sodium nitrate (36.67 U/ml) followed ammonium chloride(24.32 U/ml).Similar results were reported by [27]. Also investigated the effect of different nitrogen sources on tannin degradation by *Aspergillus awamori*and found that nitrogen sources supportedtannase production better [54].Among the inorganic sources sodium nitrate was found to be best[61] obtained results for tannase production and concluded that microorganismsnecessitate high level of nitrogen in order to produce enzymes.the effect of supplementation of differentinorganic and organic nitrogen sources on tannase production. The organic nitrogensources such as yeast extract, and malt extract gave considerable enzyme production [63].

**Effect of additives(metals ions and detergents)**

The effect of additives (metals ions and detergents) on tannase enzyme production from *Penicilliumduclauxii*was studied by adding different salts (0.2% w/v) and detergents (0.1%v/v) in the production medium B. After incubation, samples were withdrawn for analysis and tannase activity was determined. The metal ions $Ca^{+2}$, $Na^{+}$, $Mg^{+2}$, $Mn^{+2}$ and $Zn^{+2}$ did not affect enzyme production.The maximum tannase enzyme activity was observed in presence of $Mg^{2+}$ (30.09 U/ml), followed by$K^{+}$ (29.89 U/ml). However, metal ions like $Cu^{+2}$, $Co^{+2}$, $Fe^{+3}$ and detergents (Tween-20, Tween-40, Tween-60, Tween-80 and SDS) inhibited tannase enzyme activity (Fig.10 and 11).On the other hand, detergent such as Triton X-100 did not inhibit the tannase enzyme production (29.23 U/ml).Kar*et al*. (2003) reported highest tannase production by *Aspergillusfoetidus*and *Rhizopusoryzae*in the presence of $Mg^{+2}$ in the medium. $CaCl_2$ to be the most suitable for tannase production by *Aspergillusjaponicus* as compared to the other used mineral salts (NaCl, KCl, $CuSO_4$, $MnSO_4$, $FeSO_4$, $ZnSO_4$ and $CdSO_4$) [1]. also studied the effect of different mineral salts on tannase production and found $CaCl_2$ enhanced both tannase production and tannin degradation by the fungal strains[57,58].The effect of some metal ions and common chemicals on tannase activity and observed that the enzyme was inhibited by all metal ions tested except $Mg^{2+}$ [1,8].The results indicated that tannase production was stimulated on addition of combination of $Mg^{+2}$, $Ca^{+2}$, $Mn^{+2}$ and $Zn^{+2}$ in the fermentation media. On the hand, metal ions such as $Ba^{2+}$, $Co^{2+}$ and $Fe^{2+}$ inhibited tannase biosynthesis from *Aspergillus niger*. [29] reported that tannase production from *Aspergillus niger*,the enzyme showed good stability in presence of detergents such as Tween20, 80, Triton X-100 and SDS).

**Table 1: Isolation and screening of tannase producing fungal cultures**

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| Sample No. | Sample collected | Total no. of Tannase producers | Code of tannase isolates | Zone of clearance (mm) |
|---|---|---|---|---|
| **S1** | Mohiddinpur, Meerut (soil from root of jamun tree) | 4 | S1V1 (brown)<br>S1V2 (white)<br>S1v3 (light green)<br>S1v4v (muddy yellow) | 9<br>17<br>7<br>6 |
| **S2** | Agriculture field area, Meerut (farming soil) | – | – | – |
| **S3** | Aminagar, Partapur, Meerut (garden soil). | – | – | – |
| **S4** | R.D foundation group of institution, Ghaziabad (garden soil) | 4 | S4RD1 (black)<br>S4RD2 (white)<br>S4RD3 (green)<br>**S4RD4 (light green)** | 12<br>7<br>8<br>**30** |
| **S5** | Girls hostel (MIET Campus) Meerut, garden soil | 3 | S5M1 (gray)<br>S5M2 (brown)<br>S5M3 (black) | 6<br>19<br>9 |
| **S6** | Puranakila, New-Delhi (garbage site soil) | 3 | S6PK1 (brown)<br>S6PK2 (green with white ring) | 8<br>9<br>7 |
| **S7** | Patiala house court, New Delhi (garden soil) | 4 | S7PH1 (black)<br>S7PH2 (green with white ring)<br>S7PH3 (dark green)<br>S7PH4 (white) | 16<br>8<br>10<br>8 |
| **S8** | Subharti Hospital Meerut (garbage site) | 2 | S8SH1 (white)<br>S8SH2 (green with light gray ring) | 6<br>7 |
| **S9** | Kitchen waste soil sample, Meerut area, Meerut | 4 | S9KW1 (yellow)<br>S9KW2 (gray)<br>S9KW3 (white)<br>S9KW4 (brown with white ring) | 6<br>7<br>15<br>10 |
| S10 | Meerut, (soil enriched with guava) | 4 | S10GT1 (brown with white ring)<br>S10GT2 (white)<br>S10GT3 (yellow)<br>S10GT4 (green) | 23<br>10<br>6<br>7 |
| S11 | Sugar mill, Mohiddinpur, Meerut (soil from garbage site) | 3 | S11SM1 (gray with white ring)<br>S11SM2 (yellow with white ring)<br>S11SM3 (green) | 18<br>6<br>9 |
| S12 | Soil from Animal waste, rural area of Meerut | 3 | S12AW1 (green with light green ring)<br>S12AW2 (yellow)<br>S12AW3 (white) | 8<br>7<br>19 |
| S13 | MIET, Meerut (humus waste soil sample) | 4 | S13HA1 (green with white ring)<br>S13HA2 (black)<br>S13HA3 (white)<br>S13HA4 (light yellow) | 7<br>11<br>10<br>06 |
| S14 | T.C Health care, Modinagar (garden soil). | 3 | S14TC1 (green)<br>S14TC2 (dark brown with light brown ring)<br>S14TC3 (light green) | 07<br>14<br>10 |
| S15 | Padamshri dairy, Modinagar (dairy waste soil) | 2 | S15DW1 (light brown with white ring<br>S15DW2 (light yellow) | 18<br>7 |
| S16 | Mahrolli village, Meerut (soil enriched with imli) | 5 | S16IT1 (black)<br>S16IT2 (brown)<br>S15IT3 (green)<br>S15IT4 (white)<br>S15IT5 (gray) | 19<br>10<br>7<br>7<br>8 |
| S17 | Soil from legume tree, Meerut (root soil) | 4 | S17LT1 (white)<br>S17LT2 (yellow with white ring)<br>S17LT3 (black) | 9<br>7<br>10 |

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|---|---|---|---|---|
| | | | S17LT4 (gray) | 8 |
| S18 | Partapur hostels area, Meerut (kitchen soil) | 4 | S18PHA1 (dark green)<br>S18PHA2 (white)<br>S18PHA3 (light green with white ring)<br>S18PHA4 (blue with white ring)<br>S18PHA5 (brown)<br>S18PHA6 (gray) | 7<br>10<br>8<br>10<br>17<br>8 |
| S19 | Heranpur village, Gardh Ganga (agriculture soil) | 3 | S19GJ1 (black with white ring)<br>S19GJ2 (light green)<br>S19GJ3 (White) | 11<br>7<br>9 |

**Comparison of initial and optimized culture conditions for tannase production from *Penicillumduclauxii***

To improve the production of tannase from *Penicilliumduclauxii*, various culture conditions were optimized including incubation time, incubation temperature, and initial pH of the medium, carbon source and nitrogen source.The optimization of various nutritional and cultural conditions resulted in an increase in the level of tannase production to 1.36-fold as compared to initial conditions (Fig. 12). The results of thetime-course study before and after optimization of culture conditions are shownin Figure 2. Under optimized culture conditions such as malt extract medium with 1% carbon source, 4 days incubation at $30^0$C and pH 5.5, the production level of enzyme increased compared to initial unoptimized conditions (malt extract medium with 1% tannic acid as carbon source, 5 days incubation at $37^0$C and pH 5.5).

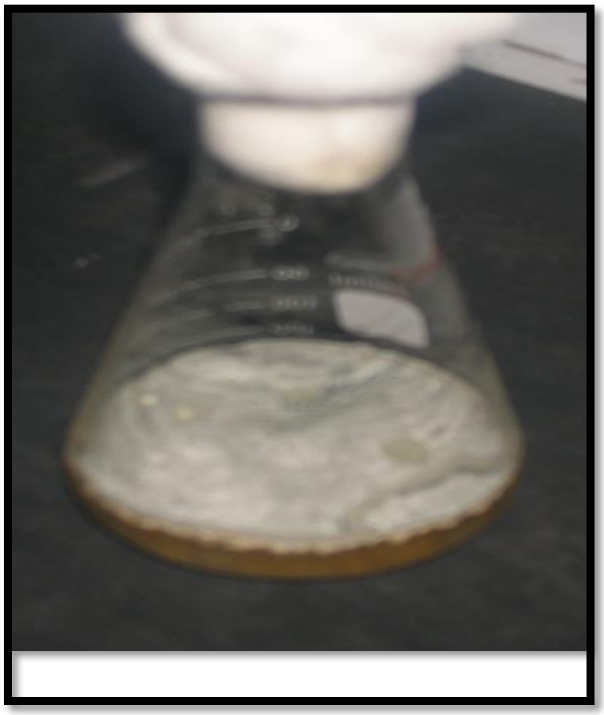

**Figure 1:Tannase production (Quantitative estimation)**

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**Figure 2: Fungal culture (S4RD4)showing tannase activity on tannic acid agar plate, (identified as *Penicilliumduclauxii*)**

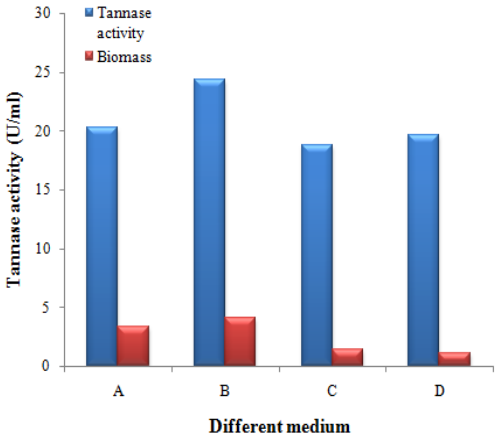


**Figure 3:Effect of different medium production**

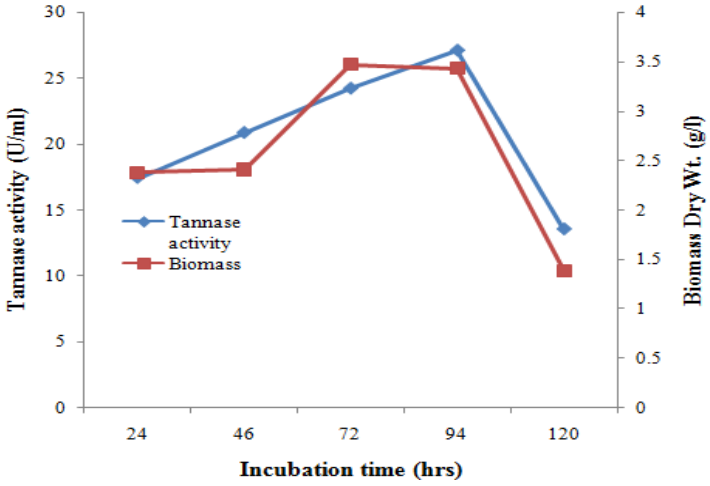


**Figure 4:Different time course of tannase on tannase for maximum tannase production**

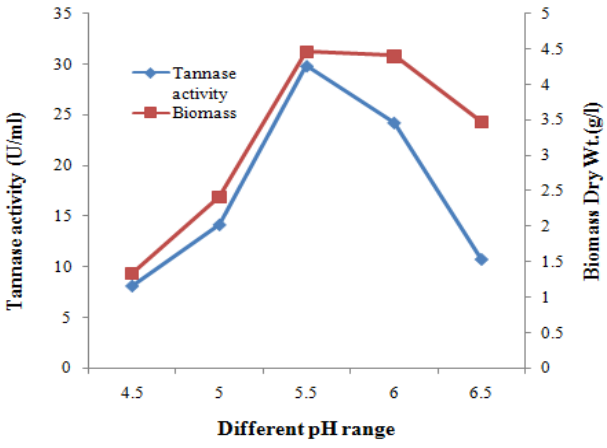


**Figure 5:Effect of different pH**

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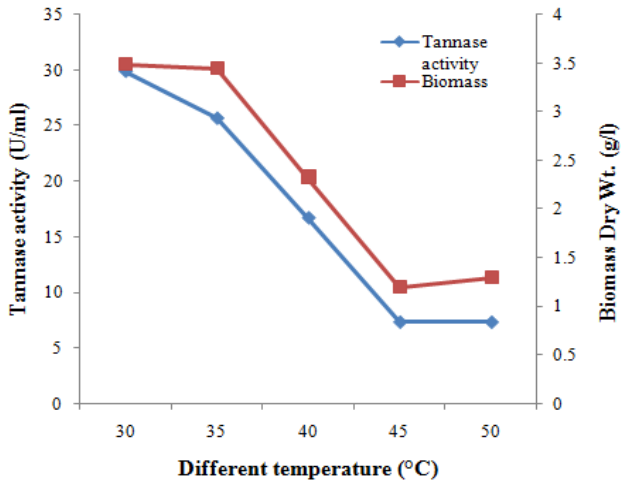


**Figure6:Effect of different incubation temperature**

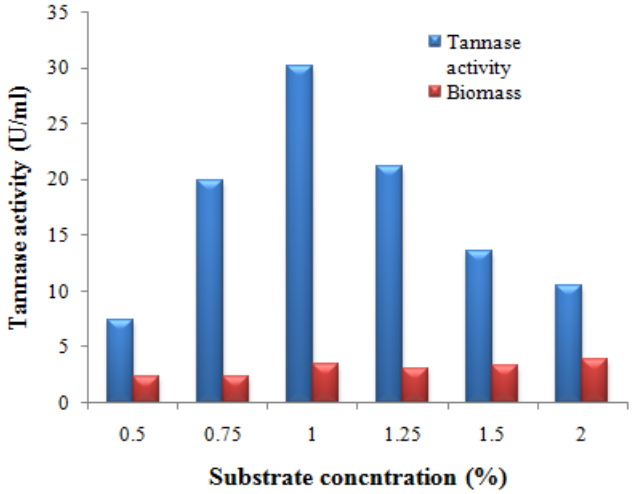


**Figure 7:Effect of Substrate concentrationon tannase production**

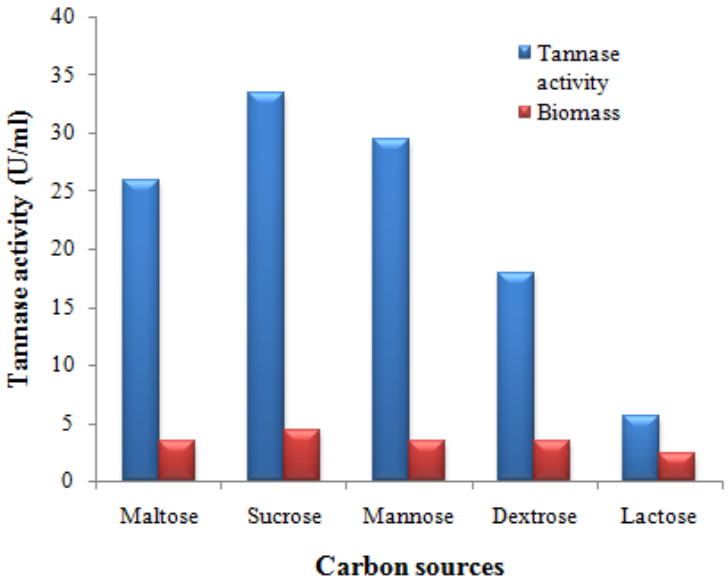


**Figure 8: Effect of different carbon sources**

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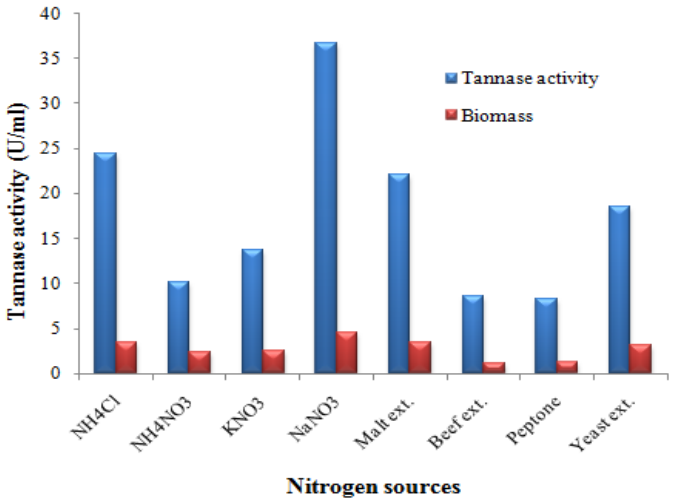


**Figure 9:Effect of different nitrogen sources**

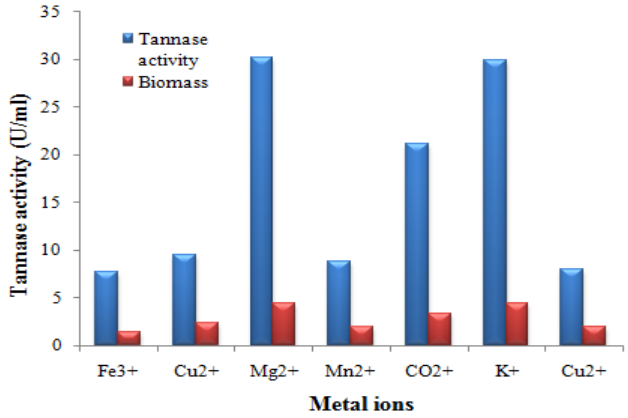


**Figure 10: Effect of different metal ions**

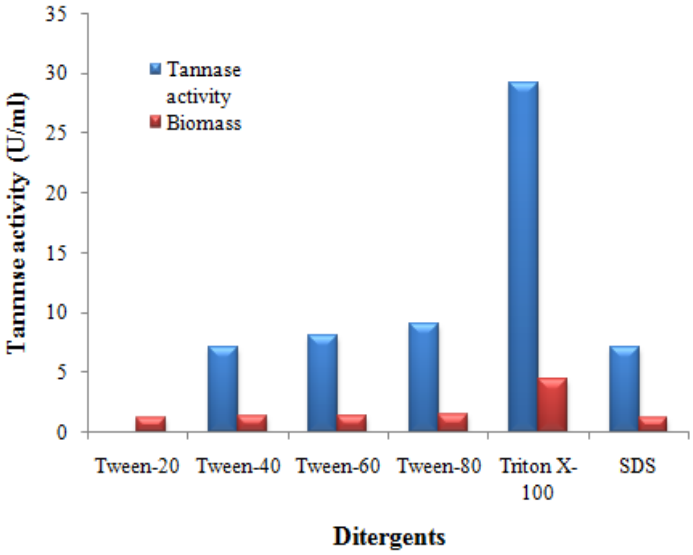


**Figure 11: Effect of different salts**

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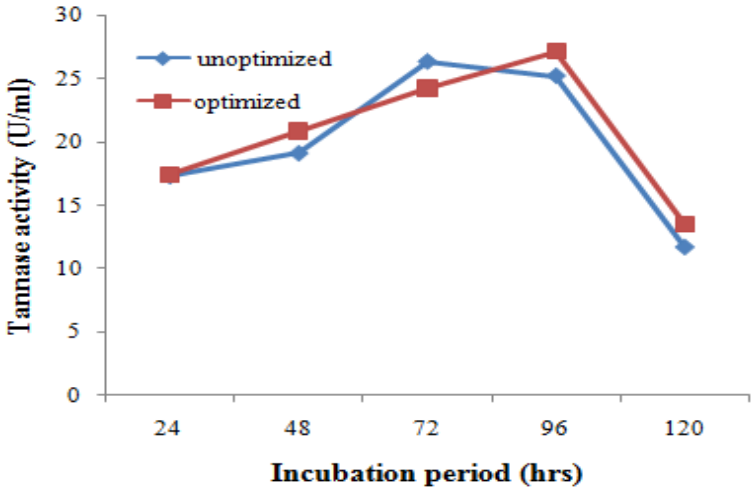


**Figure 12: Tannase production from *Penicilliumduclauxii*under optimized and unoptimized culture conditions**.

## CONCULISION

Tannase has the potential for a wide range of application, but due to higher production cost & lower yield, they currently have limited uses. This review article suggest the type of fermentation method & optimum conditions for the tannase production .Generalization of the conditions cannot be done for the production of tannase as it vary from organism to organism. In view of growing demand for the tannase for industrial application, it is very important to develop high yielding & cost effective process.Some natural tannin sources proved to be better substrates than commercial tannic acid for production of tannase. These agro-residues substrates can be substituted for costly tannic acid in the production medium for economic production of the enzyme at commercial level. Thus, there is a growing interest on basic and applied aspects of tannase.

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