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Effect of Various Biotic and Abiotic Factors on the Activity of Tannase

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Abstract: Extracellular tannase production by *Trichophyton rubrum* has been evaluated using submerged fermentation (SmF) at different abiotic factors like pH, temperatures and biotic factors like carbon, nitrogen and concentration of gallic acid. *T. rubrum* produced the highest tannase level at a temperature of 30°C where the enzyme production was 23.52 U/mL and pH of 5.5 where enzyme production was 32.62 U/mL) on 3rd of inoculation. Among the different carbon and nitrogen sources tested, sucrose and casein hydrolysate respectively were found to the best in terms of the maximal production of tannase. Further, the enzyme production was coincided with the growth pattern of the fungus. The study may be more useful to optimize the media for the maximum production of tannase in which gallic acid as the substrate.

Key words: Tannase · Tannin · Gallic acid · pH · Temperature · Culture conditions

INTRODUCTION

Tannase (tannin acyl hydrolase) hydrolyzes the ester and depside linkages of tannic acid to give gallic acid and glucose. It is extensively used in food, beverages like tea and coffee, pharmaceutical and chemical industries. The enzymatic product, gallic acid, is also used in dye making, pharmaceutical and leather industries. The major commercial application of this enzyme is in the hydrolysis of gallotannin to Gallic acid, is an intermediate required for the synthesis of an antifolic antibacterial drug trimethoprim. Tannase is extensively used in the preparation of instant tea, wine, beer and coffee-flavored soft drinks and also as additive for detannification of food. Tannins are defined as water soluble phenolic compounds with molecular weights ranging from 500-3000 daltons that have the property of combining with proteins, cellulose gelatin and pectin to form an insoluble complex [1]. It has been classified into two distinct groups such as hydrolysable tannins and condensed tannins. Tannic acid is regarded as an anti-nutrient and anti-microbial agent although some fungi [2] and a few bacteria [3] can degrade tannic acid by producing an extracellular tannase. Due to the inducible nature of tannase, tannic acid itself acts as the sole carbon source as well as inducer [4].

A large number of microorganisms which are potential sources in producing tannase to degrade tannin molecules. On the other hand, in the case of tannase producing microorganisms, the enzyme is found to occur remarkably in the mycelium of fungi like *Aspergillus flavus* and *Penecillium* species which are grown in the medium containing tannic acid as a sole carbon source. The enzyme formation might be inducible and was dominant at the initial stage of microbial growth. There are some investigations which are revealed that tannase has been isolated from hydrolysable tannin-containing plant, divi-divi (*Caesalpinia coriaria*) fruits and dhawa (*Anogeissus latifolia*) leaves. In the case of condensed tannin, tannase helps to synthesize, at one stage or another's, some intermediates or precursors which in turn undergo transformation into the complex tannin molecules [5].

Optimizations of tannase production by several fungal strains like *Aspergillus niger* and *A. flavus* and bacterial strains like *Bacillus licheniformis* have been studied by different workers [6, 7]. They found that additional carbon sources like tannic acid in the culture media enhance the tannase production to the maximum extent. It has been reported that some of the bacteria and fungi are able to secrete tannase enzyme in the medium supplemented with suitable substrate like tannic acid.

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But no attempts have so far been made for optimal production of tannase using suitable biotic and abiotic factors like pH and temperature.

MATERIALS AND METHODS

Fungal Strain: *Trichophyton rubrum* have been selected for the present study which is obtained from Microbial Culture collection centre (MTCC), Chandigarph, India.

Inoculum Preparation: The fungal spore inoculum was prepared by adding 10ml of the sterile distilled water containing Tween 80 to the PDA slants. The spores were dislodged using a sterile inoculation loop under aseptic conditions. The volume of 1 ml of spore suspension was used as the inoculums.

Enzyme Production by Submerged Fermentation Method: Czapeks dox medium supplemented with 2% tannic acid as a sole carbon source was used for the production of tannase. Different additional carbon sources were added separately to the above-mentioned medium for studying their effect on enzyme production. Enzyme production was performed in 250 ml Erlenmeyer flasks containing 100 ml liquid medium with 1% (v/v) inoculum and incubated at 27°C in a rotary shaker (200 rpm) for 3 days. Cells were removed by centrifugation and the supernatant was assayed for tannase activity. The growth of the organism was estimated on the basis of biomass dry weight (mg ml).

Assay of Tannase: Tannase was assayed following Sharma *et al.* [8] method using gallic acid as standard. The pink color developed was read at 520 nm using a spectrophotometer. The enzyme activity was calculated from the change in absorbance. One unit of tannase activity was defined as the amount of enzyme required to liberate one micromole of gallic acid per minute under defined reaction conditions. Enzyme yield was expressed as units/gram dry substrate (U/g/min).

Effect of Ph and Temperature: The medium supplemented with tannic acid was adjusted with different pH ranging from 4 to 8. Similarly, the cultures were incubated in different temperatures regimes between 15 and 55°C to find out the optimum pH and temperature for the maximum production of tannase. The fungal mat was harvested and subjected to measure the dry weight to calculate the growth *T. rubrum*.

Effect of biotic factors: The carbon such as, glucose, maltose, lactose, fructose, sucrose, cellulose, pectin and starch and nitrogen sources such as ammonium nitrate, ammonium sulphate, sodium nitrate, potassium nitrate, asparagine, urea, peptone, casein hydrolysate and yeast extract were selected and replaced in the respective sources of Czapeks dox medium. Similarly, various concentrations of tannic acid were added into the production medium and incubated at 35°C for 3 days to find out the optimum level of the substrate for the maximum production of tannase.

RESULTS AND DISCUSSION

Effect of Tannic Acid Content on Tannase Production: *Trichophyton rubrum* was grown well in Czapeks dox medium under submerged fermentation. The maximum activity was found in the medium which was amended with tannic acid @ 2% concentration (Table 1). From these results tannic acid above 2% concentration had higher activity of 36.54 (U/g/min) in the crude form. Tannase activity was very least at 0.5% (11.87 U/g/min) followed by 3% (20.77 U/g/min) concentration. It was shown earlier that when tannase from *Paecilomyces variotii was* measured at 2% concentration of tannic acid [9]. A similar type of observation has been made by Bradoo *et al*. [7] using *Aspergillus japonicus.* Presently, SmF is a preferred method for production of most of the commercial enzymes like tannase, principally because sterilization and process control are easier to handle in this system. Actually, tannic acid at higher concentration produces complexes with membrane protein of the organism thereby both growth and enzyme production may be inhibited [10, 11].

It is important to note that for tannase production, the used model (substrate-support) resulted in enhanced enzyme induction. This result also indicates that *A. niger* GH1 strain can be adapted for SmF system and utilizes the nutrients in a better form than when it is grown in other culture systems. After the selection of the culture system, the effect of initial level of substrate-inducer on tannase production was evaluated. It was observed that by increasing the substrate concentration after 4% concentration, the tannase activity decreased.

Effect of Glucose Concentration on Tannase Production: To study the effect of different glucose conc. on tannase production, the glucose concentration of the medium was varied from 0.01 % to 1% (w/v). It has been found that glucose at higher concentration repressed tannase synthesis while the lower concentration is not repressive.

Table 1: Tannase activity in different concentration of tannic acid and glucose

Parameters	Tannase activity (U/g/min)
Tannic acid concentration (%)	
0.5	11.87
1.0	18.87
1.5	28.19
2.0	36.54
2.5	27.05
3.0	20.77
Glucose concentration (%)	
0.1	18.79
0.2	25.00
0.4	27.56
0.6	32.15
0.8	22.45
1.0	20.07
SE±	2.54
CD at P=0.05	4.89

Table 2: Effect of various carbon and nitrogen sources on tannase activity

S.No.	Tannic acid concentration (%)	Tannase activity (U/g/min)
Carbon sources		
1.	Glucose	34.56
2.	Maltose	26.84
3.	Lactose	28.57
4.	Fructose	32.45
5.	Sucrose	26.55
6.	Cellulose	25.50
7.	Pectin	25.00
8.	Starch	
Nitrogen sources		
1.	Ammonium nitrate	24.57
2.	Sodium nitrate	22.47
3.	Potassium nitrate	28.55
4.	Asparagine	26.45
5.	Urea	20.89
6.	Peptone	24.46
7.	Casein hydrolysate	32.68
8.	Yeast extract	27.78
SE±		3.57
CD at P=0.05	4.74	

Maximum tannase production occurred at 0.06% (w/v) glucose which was recorded as 32.15 (U/g/min). The glucose concentration above (0.04)) and below (0.08) were not supported the enzyme activity much (Table 1). Higher concentration of glucose repressed enzyme production due to the availability of ready made carbon source maximum tannase activity was 21.42 U/mL by *Aspergillus niger* after the optimum glucose concentration of 0.5 % (w/v). The obtained results were tabulated in Table 1. Earlier Banerjee *et al*. [11] also mentioned that lower concentration of glucose is not repressive for enzyme production in *A. Japonicas* but its concentration above 1.0% is inhibitory for both growth and enzyme production.

Effect of Carbon and Nitrogen Concentration on Tannase Production: In order to optimize a suitable medium for the maximal production of tannase, carbon and nitrogen sources were tested. The results indicated that among the different carbon sources, glucose was found to be better than other carbon sources (Table 2). It was due to fungal preference in which fungus prefers only monosaccharide followed by disaccharides and polysaccharide. Similarly, among the nitrogen sources tested, casein hydrolysate was found to be better than others. Because fungus prefers complex nitrogen sources followed by ammoniacal, inorganic and organic nitrogen forms (Table 1). The results were further coincided with Banerjee *et al*. [11].

Effect of pH: To study the effect of initial pH on tannase production, the pH of the medium was varied from 4.0-8.0 using 1N HCl and 1N NaOH and fermentation was done as usual. The enzyme was active at acidic pH and activity decreased as the pH approached the alkaline range. The optimum tannase production was recorded at pH 5.5 (Fig. 1). Maximum tannase activity was 32.62 U/mL by the *T. rubrum* after the optimum pH of 5.5. The tannase activity was getting increased from pH 4 onwards upto 5.5 and thereafter it was declined sharply. The result was due to the rhythmic growth of the fungus. It could be concluded from the results that tannase from the tested fungus needed an acidic environment to be active. Fungal tannase is an acidic enzyme in general.

Effect of Temperature: To study the effect of different temperatures on tannase production, the flasks containing medium kept at temperature range was varied from 15-55°C (Fig. 2). With a rise in temperature, the tannase production increased and optimum activity 23.52 U/mL was recorded at 30°C (Fig. 2). With a further increase in temperature, there was a decrease in activity. The optimum temperature for tannase production was 30°C. An optimum temperature around 30°C has been reported for tannase activity in case of *A. oryrae* and *P. chysogenum* around 35°C in case of *A. niger* and 50°C in case of *Candida*. In our study the maximum activity of (52 U/g/min) was found at 30°C in tannic acid. With a rise in temperature the tannase production was decreased. This was in good agreement with the results obtained earlier for tannase

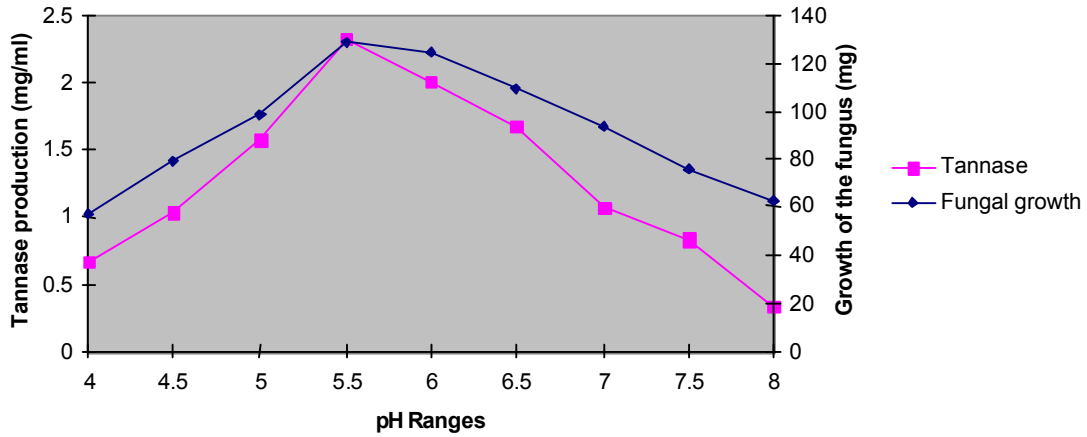

Fig. 1: Effect of various pH ranges on the production of tannase

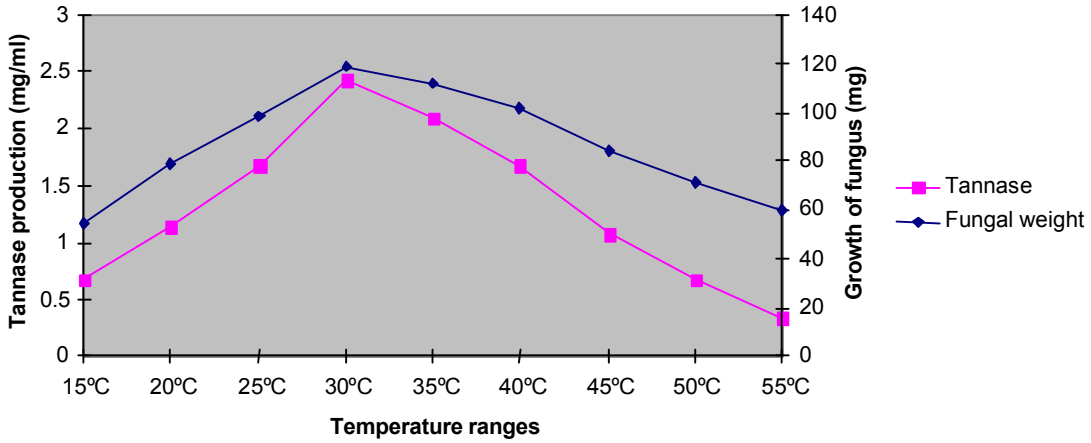

Fig. 2: Effect of various temperature regimes on the production of tannase

from *Bacillus cereus* [12]. Tannase produced by most of the potent strains like *Aspergillus oryzae, Penicillium chrysogenum* and *Aspergillus niger* also showed temperature optima at 30°C [13]. An optimum temperature of 35°C was reported for tannase from *Aspergillus awamori nakazawa* and in case of *Penicillium variable* [11], the optimum temperature was at 50°C.

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