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Quantitative Estimation of Percent Conversion of Tannins to Gallic Acid by High Gallotannin Tolerant Mutant *Penicillium Granulatum* PM-33 Strain Isolated from Kanpur Tannery Leachate

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Abstract

Kanpur is one of the largest industrial metropolis of U.P. having more than two million population. About 480 leather industries are situated in the Jajmau area along the river Ganga at the downstream of the city. The tannery effluents are flown in the river Ganga. These effluents are highly toxic and contain tannin in addition to chloride, sulphide, chromium, etc. In the present work tannin tolerant fungal strain, *Penicillium granulatum* PM-33 has been isolated from tannery soil and evaluated for its tannase activity.

The strain has the potentiality to produce the enzyme Tannin Acyl Hydrolase (E.C.3.1.1.20) in presence of the substrate tannin as carbon source in the medium. Minimal medium containing 0.2% tannic acid as the sole carbon source was used for the isolation of the fungal strain. Tannase is an inducible and regulatory enzyme. With the increase in the concentration of tannic acid in the culture broth the production of the enzyme tannase by the *P. granulatum* PM-33 strain is accordingly enhanced. The enzyme tannase(TAH) catalyses the hydrolysis of ester and depside bonds of hydrolysable tannins to liberate gallic acid and glucose. Gallic acid has undisputed commercial importance. It is a phenolic compound (3,4,5, trihydroxy benzoic acid) and is used mainly in pharmaceutical industries for manufacturing trimethoprim(TMP). The percent conversion of Tannic acid to Gallic acid increases with the increase in TA concentration in the medium upto 100 $mgml^{-1}$. At this concentration, the percent conversion is 84. Any further increase in TA concentration results in the decline of percent conversion. This may be either due to toxic nature of the tannic acid or due to end product repression. High tannin tolerant *Penicillium granulatum* PM-33 strains may be developed either through isolation and selection or through genetic manipulation and they may be used in the biotechnological processes like industrial production of gallic acid from the hydrolysable tannins. They can also be used for bioremediation of tannins from tannery effluents and tannin contaminated soils. Immobilization of TAH has added benefit.

Keywords: *Tanneries; Tannase; Gallic Acid; Percent Conversion; Bioremediation; Penicillium granulatum PM-33 Strain*

Introduction

Kanpur is one of the largest industrial metropolis of U.P. having more than two million population. More than 400 leather tanning industries are situated in Jajmau area along the river Ganga at the downstream of the city. These tanneries are discharging more than 12 million liters per day (mL/d) of highly polluted and noxious wastes to the river Ganga. Burhiya Ghat received the maximum volume of direct tannery effluents. The effluents from tanneries is a dangerous pollutant (Figure 1). It causes many health hazards, mainly it is affecting the residing communities, as they uses the polluted water for their domestic purpose. The main polluting constituents of the composite effluent are chlorides, sulphates, sulphides, chromium, suspended organic matters and tannins. Various undesirable effects are brought about by the constituents of the untreated effluents when discharged in milieu.

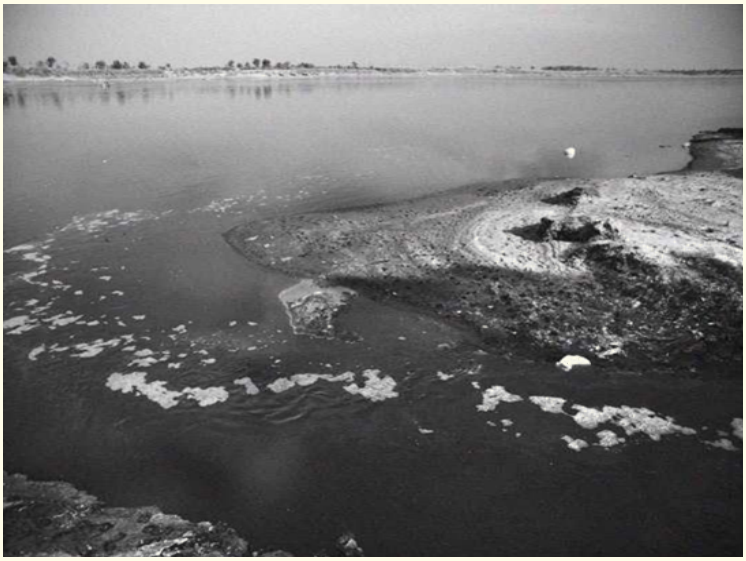

Figure 1: *Kanpur Tannery Effluents.*

Tannins are naturally occurring water soluble phenolic compounds having molecular weight between 500 and 3000. They are considered to be the plant's secondary metabolic products because they play no direct role in the plant metabolism. After lignin, tannins are the second most abundant group of plant phenolics. The large amount of phenolic hydroxyl groups allow the tannins to form complexes with proteins and to a lesser extent with other macromolecules like cellulose and pectin [1]. Because of these protein binding properties, they are of considerable importance in food processing, fruit ripening, manufacture of cocoa, wine, beer, etc. [2]. The tannins are used for tanning animal hides and skins for producing leather. The large number of phenolic hydroxylic groups present in tannins form effective cross links with proteins of the skin and increase its stability to water, bacteria and abrasion [3]. They are also used in wood dyeing, black hair colorant and in treatment of burns and fevers in tonsillitis, laryngitis, hemorrhoids and skin problems.

Materials and Methods

Collection of Tannery Soil

Soil samples were collected in sterile polypropylene bottles from Jajmau area of Kanpur, India, where deposition of tannin rich waste has been occurring for past many years. The pH of the soil and the effluent was determined.

Reagents

The various chemicals used in the present study were obtained from SRL, Merck, Ranbaxy, Qualigens, Hi-media, etc. and were of analytical grade reagent.

Enrichment and isolation of *Penicillium* strains from the soil

A screening programme was undertaken for the isolation of *Penicillium* spp. exhibiting tannase activity from the collected soil sample. Minimal medium containing 0.2% tannic acid as sole carbon source in place of sucrose was used for isolation of the fungi. A sterile petriplate was inoculated with 0.1ml of the sample prepared by adding 1 gm. of the soil to 9 ml of sterile distilled water. Morphologically distinct colonies developed after 48 - 72 hrs. of incubation at 30 ± 2^0C [4]. From the different isolates *Penicillium granulatum* PM-33 was selected for knowing its bioconversion potentiality. Subsequently Czapek Dox medium containing 1% gallotannin was inoculated with preinduced *Penicillium granulatum* PM-33 strain. Similarly, the medium containing higher concentrations of gallotannin (2%, 3%, 4%

……..25%) were inoculated with the isolated strain growing in the preceding concentration. Inoculation in each concentration was carried out in triplicate. In this way, *P. granulatum* PM-33 was transferred from 1% to 25% concentration of gallotannin gradually. The flasks were incubated at 30^0C in static condition. The incubation period for the growth of the strain varied with the gallotannin concentration. At each concentration, the percent hydrolysis of gallotannin (tannic acid) was calculated on the basis of the residual gallotannin and gallic acid formed following the method described by Bajpai and Patil [5] and the bioconversion percentage has been calculated.

Results and Discussion

The tannery effluents contain different amount of hydrolysable tannins like tannic acid. The tannery effluent and soil is expected to contain conidia of *Penicillium* strains and by their characteristic conidial colour they can be easily identified. Enrichment medium containing tannic acid as sole carbon source when inoculated with tannery soil suspension showed decrease in the brown black colouration of the medium. Repeated enrichment in the medium containing tannic acid as sole carbon source ensured the multiplication of the Penicillium strain that could utilize tannin for their growth. Appropriately diluted aliquots from these enrichment cultures produced pure isolated colonies. The pure culture of *Penicillium granulatum* PM-33 was seen to show apparent tannase activity.

From the experimental result (Table 1) it is apparent that the strain *P. granulatum* PM-33 can tolerate higher concentration of tannic acid provided the increase of tannic acid concentration in the medium is gradual. The highest percent conversion was noted at 10% concentration of tannic acid. Initially at 10 $mgml^{-1}$ concentration of tannic acid the bioconversion percentage was 62. The other parameters viz. temperature, pH, incubation period, etc. were kept at optimum. The gradual increase in tannic acid concentration was made upto 250 $mgml^{-1}$ and at this concentration the bioconversion percentage was calculated to be 44, which is comparatively lower. The maximum bioconversion was observed at 100 $mgml^{-1}$ concentration and it was 84%. The experimental result agrees with the conclusion of Van Tiegham [6], that *A. niger* can withstand a saturated solution of tannic acid and that it is not toxic for the strain. Kundson [7] in his experiment found that *Aspergillus oryzae* could withstand 10% tannic acid concentration and *Fusarium oxysporium* was found to grow in 11.6% tannic acid concentration. Pourrat., *et al.* [8] reported the growth of *A. niger* in 13% TA concentration. Kar and Banerjee [9] found that *Rhizopus oryzae* grow well in 2.5 to 20% tannic acid containing medium. But in this connection, it is to be noted that the enzyme tannase is inducible and adaptive in nature [10]. When the strain was inoculated directly in the medium containing 10% or more tannic acid, the strain exhibited very slow or apparently, no growth. Knudson [7] reported that tannase (TAH) is induced only when the microorganism is grown in presence of tannic acid, giving gallic acid and glucose as final products [11]. This observation is contradictory to the reports published by Bradoo., *et al.* [12]. The decline in percent conversion after reaching a peak may be due to toxic nature of tannic acid or due to end product repression [12,13] or both. High tannin tolerant *Penicillium* strains may be developed either through isolation and selection or through genetic manipulation and they may be used in the biotechnological processes like industrial production of gallic acid from the hydrolysable tannins. They can also be used for bioremediation of tannins from tannery effluents and tannin contaminated soils. Immobilization of TAH has added benefit [14-19].

Concentration of tannic acid ($mg\ ml^{-1}$)	pH of the broth	Concentration of gallic acid ($mg\ ml^{-1}$)	Bioconversion Percentage
10	2.4	6.2	62%
20	2.5	12.4	62%
30	2.5	19.5	65%
40	2.3	28.0	70%
50	2.2	36.0	72%
60	2.1	43.8	73%
70	2.1	52.2	75%
80	2.2	63.2	79%

90	2.4	73.8	82%
100	2.3	84	84%
110	2.3	88.0	80%
120	2.4	90.0	75%
130	2.2	94.9	73%
140	2.2	100.8	72%
150	2.3	105	70%
160	2.4	108.8	68%
170	2.3	110.5	65%
180	2.2	111.6	62%
190	2.2	114.0	60%
200	2.2	116	58%
210	2.1	121.8	58%
220	2.1	110.0	50%
230	2.1	115.0	50%
240	2.1	115.2	48%
250	2.0	110.0	44%

Table 1: *Effect of Tannic Acid Concentration on the Bioconversion Percentage of Tannic Acid to Gallic Acid By Penicillium granulatum PM-33.*

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