

Full Length Research Paper

Production and partial purification of extracellular tannase by *Klebsiella pneumoniae* MTCC 7162 isolated from tannery effluent

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Accepted 20 December, 2010

Diverse bacteria belonging to various taxa were isolated from tannery effluent of leather industries located at Ranipet, India and were screened for the production of extracellular tannase. One of the strains identified as *Klebsiella pneumoniae* MTCC 7162 was found to produce tannase (3.4 U/ ml) at pH 6.0, 37°C and 100 rpm. Use of individual carbon and inorganic nitrogen sources resulted in lower tannase production. However, a combination of urea and corn steep liquor extract yielded marginal increase in tannase production (3.9 U/ ml). End-product repression was also studied with inclusion of gallic acid to the growth medium. Enrichment with various additives of metal ions and detergents resulted in inhibition of tannase production. The enzyme was partially purified using ammonium sulfate precipitation followed by the use of DEAE-cellulose. SDS-PAGE analysis indicated that the molecular weight of the protein to be 46.5 kDa. The enzyme was found to be active in a wide range of pH and temperature with an optimal activity at pH 5.5 and 40°C.

Key words: Bacterial tannase, *Klebsiella pneumoniae* MTCC 7162, tannery effluent, minimal media.

INTRODUCTION

Tannin acyl hydrolase (E.C. 3.1.1.20), commonly referred as tannase, is an inducible and hydrolytic enzyme. Tannase has been known to hydrolyze the ester and depside linkages of hydrolysable tannins into gallic acid and tannic acid. Tannase was discovered accidentally during the extraction of gallic acid from soluble tannins (Lekha et al., 1993). Tannins are polyphenolic compounds of varying molecular weights and widely occur in the plant kingdom. Also, tannins are the fourth most abundant plant constituent after cellulose, hemicellulose and lignin. Generally, tannins are accumulated as secondary metabolites in the bark, leave and stem, but do not play any direct role in plant metabolism (Lekha et al., 1993). However they have important role in plant immunity and protect them from microbial attacks (Aguilar et al., 2001).

Although tannase is present in plants, animals, and microorganisms, it is produced in substantial amounts by

the latter. Tannase is produced by bacteria, yeasts and fungi. Filamentous fungi of the *Aspergillus*, *Penicillium* genus and bacteria of the *Bacillus* and *Lactobacillus* genus have been investigated for tannase production (Banerjee et al., 2001; Mondal et al., 2001a; Pinto et al., 2001; Osawa, 1990; Murugan et al., 2007; Aguilar et al., 2007). Although tannase production by *Aspergillus* can occur in the absence of tannic acid, this fungus (mainly *Aspergillus niger*) tolerates tannic acid concentrations as high as 20% (w/v) without having a deleterious effect on both growth and enzyme production (Goel et al., 2007). Phenolic compounds such as gallic acid, pyrogallol, methyl gallate, and tannic acid induces tannase synthesis (Deschamps et al., 1983). However, the induction mechanism is not clearly understood as it is dependent on a variety of environmental factors and fermentation conditions (Deschamps et al., 1983; Aguilar et al., 2001a). For instance, gallic acid, one of the structural constituents of some hydrolysable tannins, such as tannic acid, has been reported as an inducer of tannase synthesis under submerged fermentation, while it represses tannase synthesis under solid-state fermentation (Lekha and Lonsane, 1997).

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It is observed that few of the microorganisms utilize tannins as substrates for growth and produce various secondary metabolites which are industrially important. Tannase is used in the manufacture of instant tea, wine, beer, coffee, flavored soft drinks where it is used to eliminate water insoluble precipitates (Aguilar et al., 2001). In food industry, it is used for the chemical synthesis of the preservatives such as of pyrogallol or ester gallates. Gallic acid is also used in the enzymatic synthesis of propyl gallate, which is mainly used as antioxidant in fats and oils, as well as in beverages and also being an important intermediary compound in the synthesis of the antibacterial drug, trimethoprim, used in the pharmaceutical industry (Lekha and Lonsane, 1997; Belmares et al., 2004). Microbial production of tannase, their production and industrial applications have been extensively studied (Aguilar et al., 2007; Lekha and Lonsane., 1997). In spite of these wide applications, studies on tannase production by bacteria are very obscure. The problem of pollution of water and soil from the tannery effluent is a serious environmental threat especially in the developing countries (Chowdhury et al., 2004). In the present study, tannery soil was chosen as probable source for the isolation of tannin-degrading bacteria as they had a history of either discharges of tannin-containing effluents or disposal of tannin-rich woods resulting in leaching of tannin into the soil. Investigation on the production and molecular characterization of bacterial strains from the effluents will not only unravel potential tannase producers but also help in discovering novel enzymes that can meet the industrial demand. Therefore in the present study, we have screened for bacterial strains from the effluents of leather industry and investigated for the physical and chemical conditions under which optimal amount of tannase are produced.

MATERIALS AND METHODS

Chemicals and materials used

Tannic acid, methyl gallate and rhodanine were obtained from Sigma Chemical Co., St Louis, MO, USA. The microbiological media used were dehydrated media (Hi-Media, Mumbai). Other chemicals used were of high analytical grade.

Screening of bacterial tannase producers

Tannery effluent soil samples were collected in sterile polypropylene bottles from the effluent outlets of different tannery industries located in Ranipet, Vellore district, India. One gram of tannery effluent soil was re suspended in 50 ml minimal medium (NaNO₃ 3.0% w/v, KCl 0.05% w/v, MgSO₄ 0.05% w/v, K₂HPO₄ 0.1% w/v) containing 1% (w/v) tannic acid, (designated as MMT broth). Microbial growth was observed under controlled growth condition of 37°C and pH 6.0 for 24 h at 150 rpm. Subsequently, the cultures were serially diluted with sterile saline and spread onto nutrient agar plates. Separate single colonies obtained on nutrient agar plates were considered to be tannic acid utilizing bacteria and designated as VIT SK1 - VIT SK22. In the next step, selected

colonies were screened for tannic acid utilization by plating them on minimal media containing 1% (w/v) tannic acid (MMT) as sole carbon source. The plates were then incubated at 37°C for 72 h. The strains exhibiting maximum zone of clearance were considered for further tannase production and were characterized using systemic classification of microorganism's and 16S rRNA gene sequence homology (Aguilar et al., 2007; 2001a).

Inoculum preparation and tannase enzyme assay

One percent of inoculum from an overnight grown culture (1.2 OD_{600nm}) in log phase was added to MMT broth. After incubation for 48 h, at 37 °C, and an agitation rate of 150 rpm, the culture was harvested. Production studies were carried out as batch cultures in 250 ml Erlenmeyer flasks, containing 100 ml of culture media. All the experiments were carried out independently and repeated twice. The cultures were centrifuged at 10,000 rpm for 10 min at 4°C. The cell free extract was used as crude preparation to measure tannase activity (Sharma et al., 2000). Accordingly, 0.25 ml of the cell free extract and 0.3 ml of methanolic rhodanine (0.667% w/v) solution was incubated for 5 min at 30°C and 0.2 ml of 0.5 M potassium hydroxide was added. The samples were diluted with 4 ml 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 520 nm. One unit of enzyme was defined as the amount of enzyme required to liberate one micromole of gallic acid per minute under defined assay conditions.

Effect of pH, temperature and agitation

In order to investigate the influence of pH on cell growth and tannase production, the isolate was grown in MMT broth at different pH (4.0 - 8.0) conditions and constant temperature of 37°C at 150 rpm. After 28 h, growth and tannase activity were quantified as mentioned previously. Similarly, influence of temperature was investigated by varying the growth temperature (30 to 60°C) at pH 6.0 and 150 rpm. Effect of agitation was also studied with varying speed of 50 to 250 rpm at pH 6.0 and 37°C.

Effect of carbon and nitrogen sources

Various simple and complex compounds including fructose, sucrose, molasses and maltose were used as a carbon source (0.5% w/v) in MMT broth. Similarly, the effect of nitrogen sources was studied using beef extract, urea, potassium nitrate, ammonium nitrate, ammonium chloride, corn steep liquor, and yeast extract. The respective nitrogen sources (0.5%, w/v) were used instead of sodium nitrate in MMT broth. The amount of extracellular tannase produced was monitored after 28 h growth at 37°C and with the agitation speed of 100 rpm.

End-product repression

Different concentrations of gallic acid (0.03, 0.05, 0.1, 0.15 and 0.2% w/v) were added to the production medium containing 1% tannic acid (w/v). The amount of extracellular tannase produced was monitored as mentioned above.

Effect of various salts and detergents

The effects of eighteen different salts (1 mM) were tested for tannase production. The salts included were calcium carbonate, sodium thiosulphate, ammonium ferrous sulphate, ammonium sulphate,

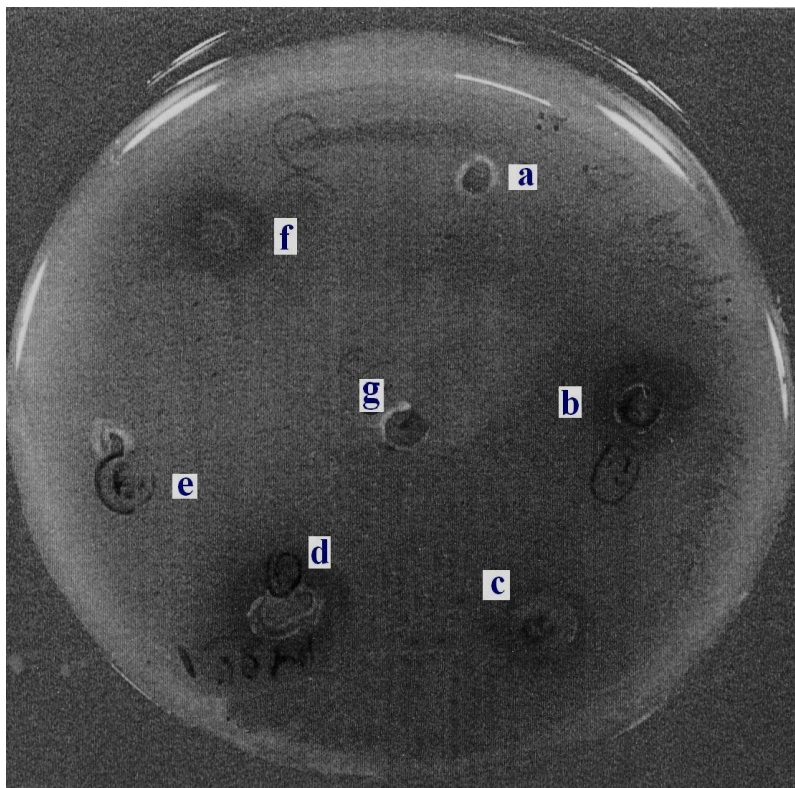


Figure 1. MMT agar plate screening for tannase producing bacteria by cup plate method. a, Control; b, VIT SK 4; c, VIT SK 5; d, VIT SK 6; e, VIT SK 7; f, VIT SK 9; g, VIT SK 20.

ammonium nitrate, ammonium chloride, ammonium azide, ammonium oxalate, ammonium molybdate, ammonium carbonate, zinc chloride, barium chloride, magnesium chloride, cuprous chloride, barium chloride, ferrous chloride and calcium chloride.

Extracellular tannase production was also investigated with the addition of varying detergents like sodium lauryl sulfate, Tween 20, Tween 80, and the non-ionic surfactant Triton X-100 (0.1 - 1)% (w/v) along with production medium.

Properties of the extracellular tannase

The optimum pH was determined using the following buffer systems: Acetate buffer (pH 2.0 to 5.5), potassium phosphate buffer (pH 6.0 - 8.0) and glycine-NaOH buffer (pH 9.0 to 10.0). The optimum temperature was determined for the tannase at different temperatures (10 to 70°C) at pH 5.5.

Purification of tannase

Cell free culture was subjected to ammonium sulphate precipitation (80% w/v). After centrifugation (9,000 \times g, 25 min) the precipitate was dissolved in 5 ml of citrate buffer (0.05 M, pH 5.5) and dialyzed for 24 h. The dialysates were further purified by column chromatography.

The dialysate was applied to a DEAE-cellulose column (2 \times 10 cm) that was previously equilibrated with 0.05 M citrate buffer (pH 5.5). The elution was performed with a linear gradient of 0 to 2 M NaCl at a flow rate of 30 ml/h. The eluted fractions were collected in an automated fraction collector (Pharmacia Biotech) and the

absorbance of the fractions was measured at 280 nm. The major peak fractions were then assayed for tannase activity, and only the fractions possessing tannase activity were pooled.

Molecular mass determination by SDS-PAGE

The properties of the purified tannase were analysed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). The protein bands were detected by Coomassie blue staining and then de-stained using a mixture of methanol, glacial acetic acid and distilled water. The molecular weights of the proteins were determined using the standard protein mixture of 220, 97, 66, 45, 30, 20 and 14 kDa.

RESULTS AND DISCUSSION

Selection of bacterial tannase producing strains

Twenty two strains of bacterial origin isolated from tannery effluent soil samples containing rich tannin sources (Ranipet, Vellore, India), were screened for tannase production. A total of 9 strains producing halo zone on MMT agar plate (Figure 1) were considered to be positive tannase producers (Osawa et al., 2006; Murugan et al., 2007; Sharma et al., 2000) and their tannase yield was quantified using tannase assay spectrophotometrically. The following enzyme activities were recorded (per ml):

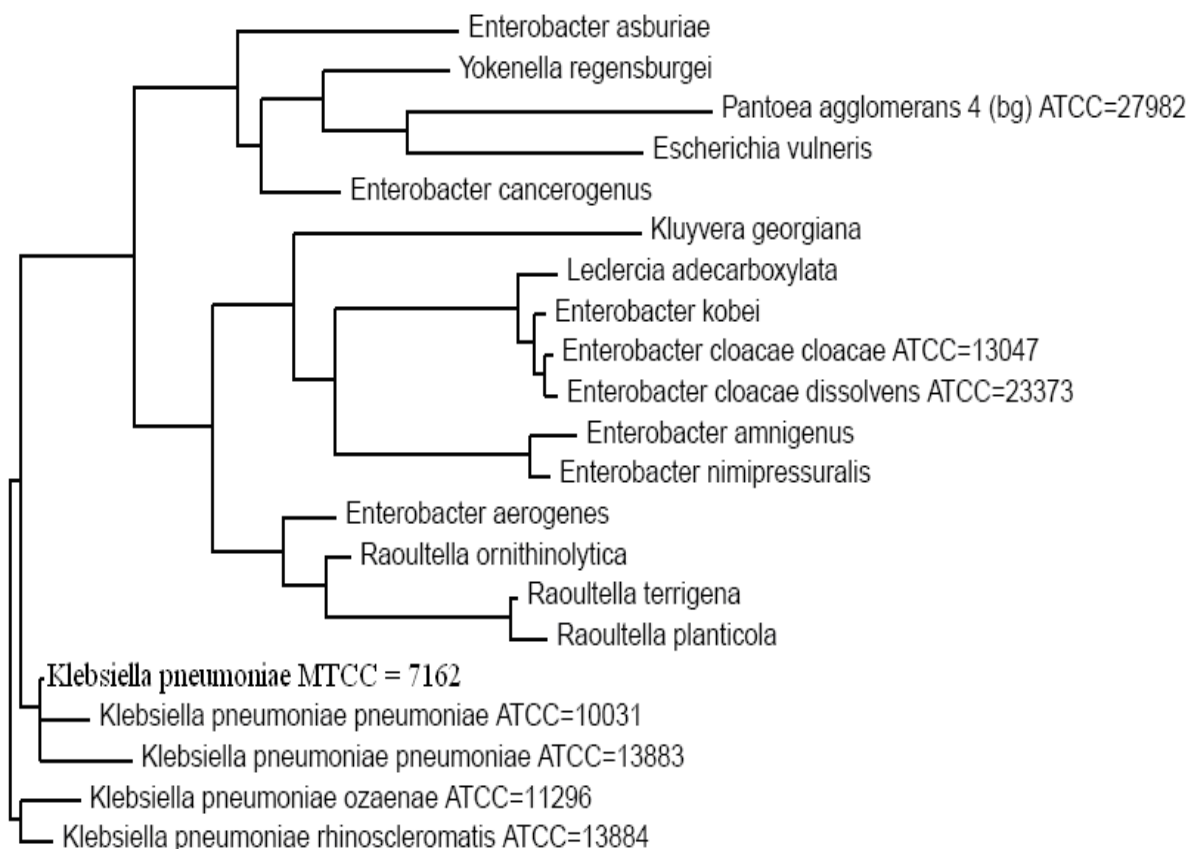


Figure 2. Neighbor-joining tree showing the position of isolate *Klebsiella pneumoniae* VIT SK 6 to a selected number of members of the moderately enterobacteriaceae. It was observed that the bacterial strain VIT SK 6 belonging to the cluster of *Klebsiella* family. *Klebsiella pneumoniae* MTCC 7126 was found to have maximum similarity of 99.91% towards *Klebsiella pneumoniae* ATCC 13884.

VIT SK 1 (1.01 U), VIT SK 4 (2.30 U), VIT SK 5 (2.34 U), VIT SK 6 (3.27 U), VIT SK 7 (2.14 U), VIT SK 8 (1.22 U), VIT 14 (0.78 U), VIT SK 18 (0.69 U), VIT SK 20 (1.68 U). It is obvious that the bacterial strain VIT SK 6 exhibited maximal activity under the conditions used in the study and therefore this strain was selected for further investigation.

Characteristics of the potential strain

Morphological characterization indicated that the strain VIT SK 6 is a gram negative, non motile, capsulated, rod shaped bacteria. It fermented most of the carbohydrates such as arabinose, lactose, sorbitol and sucrose. The strain was negative for methyl red and hydrogen sulphide but positive for Voges Proskauer and citrate biochemical tests. Growth was observed in 0 to 3% (w/v) NaCl (optimally with 1 to 2%, w/v), at pH 5.5 to 8.0 (optimally at pH 7.0) with an optimal growth temperature of 37°C. According to Bergey's manual of Determinative Bacteriology, and considering the physiological and biochemical tests performed, the strain was tentatively named as

Klebsiella pneumoniae sp. VIT SK 6. Later it was characterized and identified to be *K. pneumoniae* MTCC 7126 by MTCC, Chandigarh (India). Furthermore on the basis of 16S rRNA gene sequence studies, the isolate was found to be having 99.91% sequence similarity with *K. pneumoniae rhinoscleromatis* ATCC 13884. The phylogenetic tree (Figure 2) constructed by the neighbor-joining method indicated that the isolate VIT SK 6 was a novel strain in the *K. pneumoniae* cluster. The ribosomal RNA gene sequence has been submitted to GenBank (ID: FJ868492.1).

Tannase production by *K. pneumoniae* MTCC 7126

The growth of *K. pneumoniae* MTCC 7126 was studied with reference to tannase production in MMT broth. Conventional method of monitoring the growth of the organism by measuring the optical density at 600 nm was not possible, because of the back ground absorbance of the media components in this region. The results in the initial stages of growth indicated that there was no substantial production of tannase and this could correspond

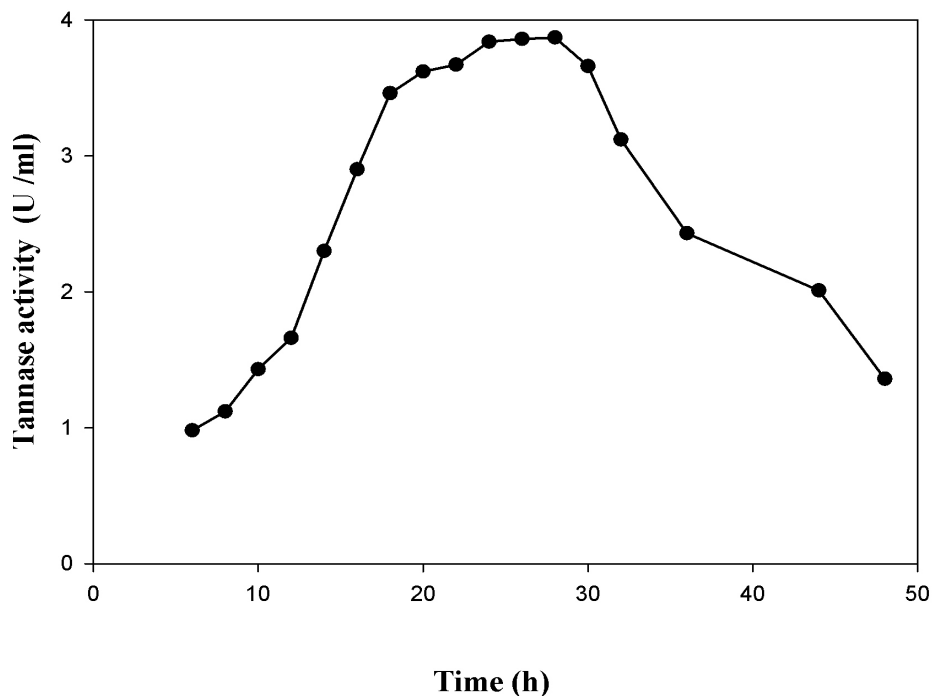


Figure 3. Growth kinetics of extracellular tannase production by bacterial strain VIT SK 6 studied for 48 h. Tannase assay was performed for every 2 h of growth.

Table 1. Effect of additives on extracellular tannase produced by *K.pneumoniae* MTCC 7162. The enzyme activity of 3.45 U/ml in MMT is taken as 100.

Additives	Relative activity (%)
Calcium carbonate	42.6
Sodium thiosulphate	43.1
Sodium hydroxide	21.4
Ammonium ferrous sulphate	0
Ammonium sulphate	45.5
Ammonium nitrate	48.9
Ammonium chloride	60.2
Ammonium azide	22.5
Ammonium oxalate	33.2
Ammonium molybdate	27.7
Ammonium carbonate	4.4
Zinc chloride	26.3
EDTA	0
Magnesium chloride	37.2
Cuprous chloride	0
Barium chloride	0
Ferrous chloride	4
Calcium chloride	3.4

to the lag phase of the organisms' growth. Following this 6 h period, the production of tannase was found to increase exponentially till 20 h and remained stationary till 30 h (Figure 3) After this period, decrease in tannase

production was observed. The maximal activity observed was 3.46 U/ml which was comparable to the amount of tannase secreted (Table 1) by group of bacterial strains of taxa *K. pneumoniae* (0.75 U/ml), *Lactobacillus* (0.3 U/ml), *Bacillus* sp. (1.03 U/ml) and *Pseudomonas aeruginosa* IIB 8914 (13.65 and 12.90 U/ml using amla and keekar leaves (2% w/v) respectively under SmF). (Deschamps et al., 1983; Hadi et al., 1994; Kar et al., 2003; Selwal et al., 2010).

Effect of pH, temperature and agitation

K. pneumoniae MTCC 7126 was found to grow and produce extracellular tannase over acidic pH of 4.0 - 7.0 (Figure 4a) whereas the production was found to decrease substantially at pH 8.0. It is observed that the tannase producing organisms investigated till date has the optimal pH for production in the acidic pH. For instance, the optimum pH was 6.0 in case of tannase obtained from *A. niger* and *Paecilomyces variotii* (Pinto et al., 2001; Battestin and Alves-Macedo, 2007), while tannase produced from *P. chrysogenum* (Rajkumar and Nandy, 1983) and *Aspergillus oryzae* (Yamada et al., 1968) was higher in the ranges of (4.5 to 6.0) pH and (5 to 5.5) pH, respectively. As in the present study, tannase production (3.46 U/ml and 1.01 U/ml at pH 7.0 and 8.0 respectively) was found to decrease with further increase in the pH of the production medium (Lekha and Lonsane, 1997; Mukherjee and Banerjee, 2005).

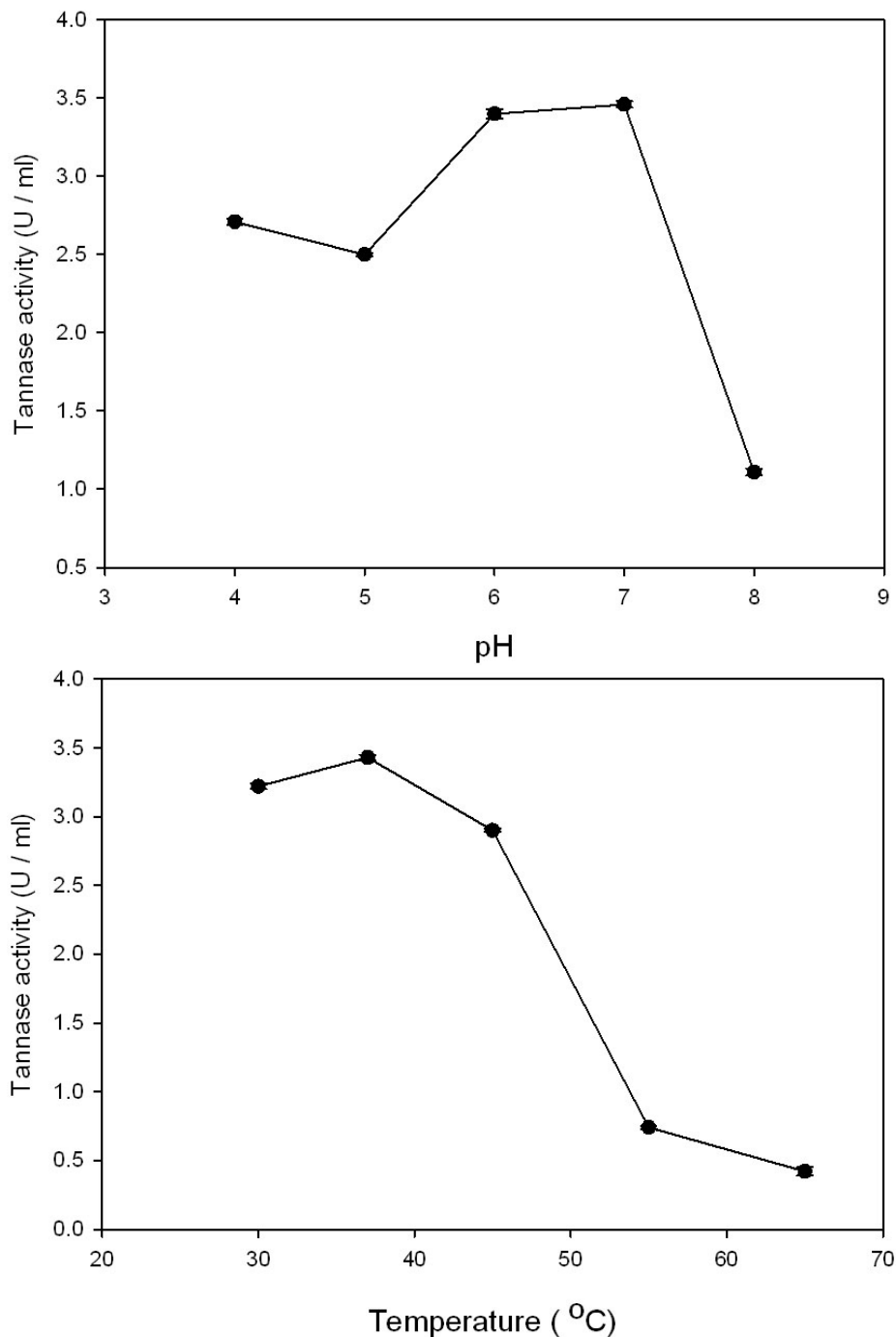


Figure 4. Effect of various parameters on extracellular tannase production by *K.pneumoniae* MTCC 7162. (a) pH, (b) temperature, (c) agitation rate, (d) tannic acid concentration.

Tannase production by *K. pneumoniae* MTCC 7126 was found optimal in the range of 30 and 45°C and significantly reduced at 50°C (Figure 4b). An optimum temperature for growth around 30°C has been reported for tannase production in *Aspergillus oryzae* (Lekha and Lonsane., 1997) and *A. niger* (Pinto et al., 2001). Agitation rate in the range of 50 to 200 rpm was chosen to determine the optimal rate. It was found that an agitation

rate of 100 rpm (at pH 6.0 and 37°C) yielded maximum tannase production of 3.47 U/ml (Figure 4c). It was noted that increase in agitation rate beyond 200 rpm resulted in a drastic fall in enzyme production. The agitation speed below 100 rpm resulted in inadequate mixing of the broth towards the later stages of growth.

As tannase is an inducible enzyme, effect of tannic acid on tannase production was studied by including various

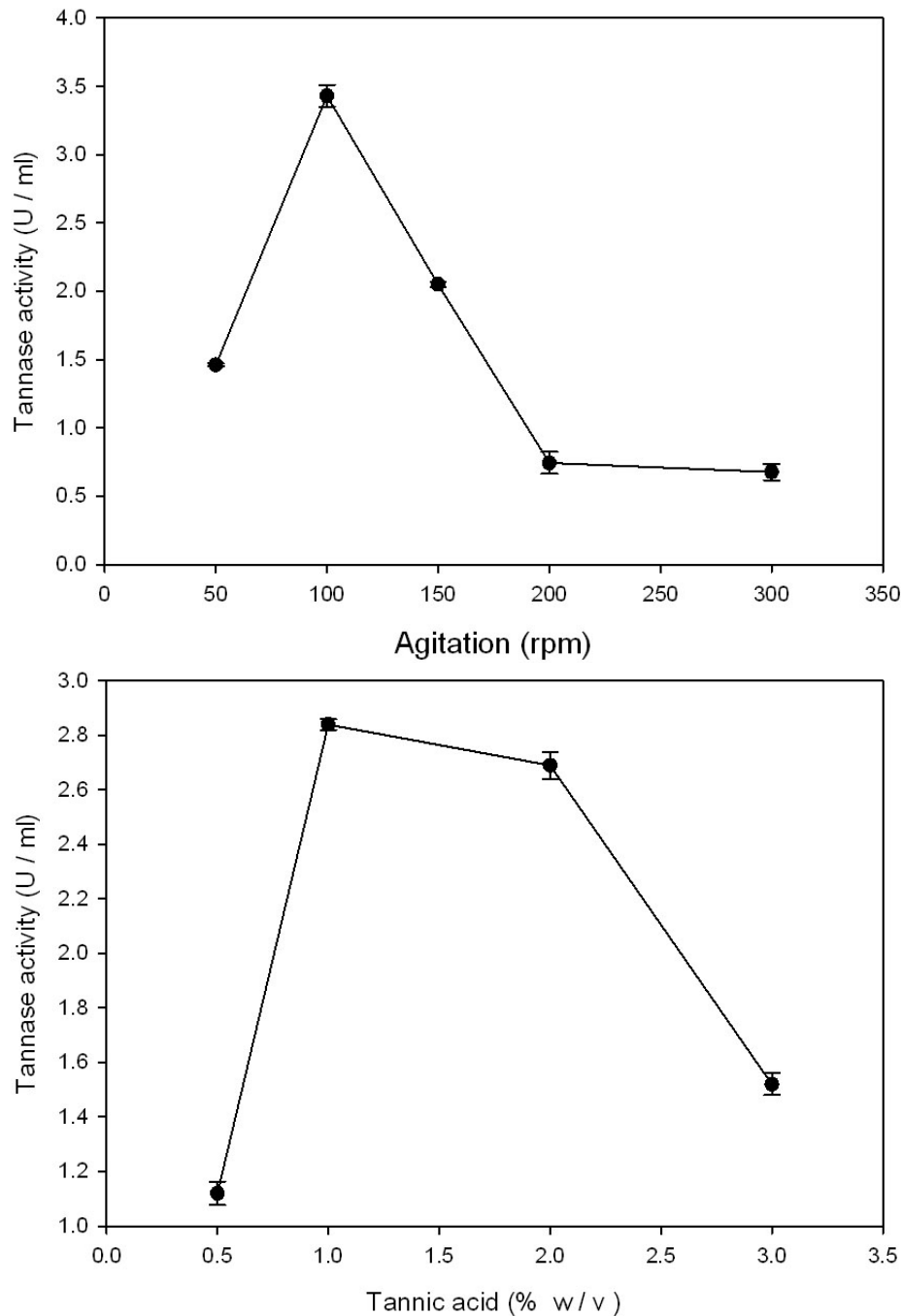


Figure 4. Continued.

concentrations of tannic acid in the media. It was observed that presence of 1- 2% w/v of tannic acid resulted in maximal tannase production (Figure 4d).

Effect of carbon and nitrogen sources

Tannase production depends on the availability of both carbon and nitrogen sources in the medium. Both have

been shown to have regulatory effects on enzyme synthesis (Bradoo et al., 1996). In the present study, addition of sugars as potential carbon source did not have any positive effect on extracellular tannase production (Figure 5a). It should be noted that degradation of tannic acid releases glucose which can be efficiently utilized by the organism for its growth as carbon source. Therefore, any disaccharide or polysaccharide that releases glucose on degradation will act as a better carbon source for tannase

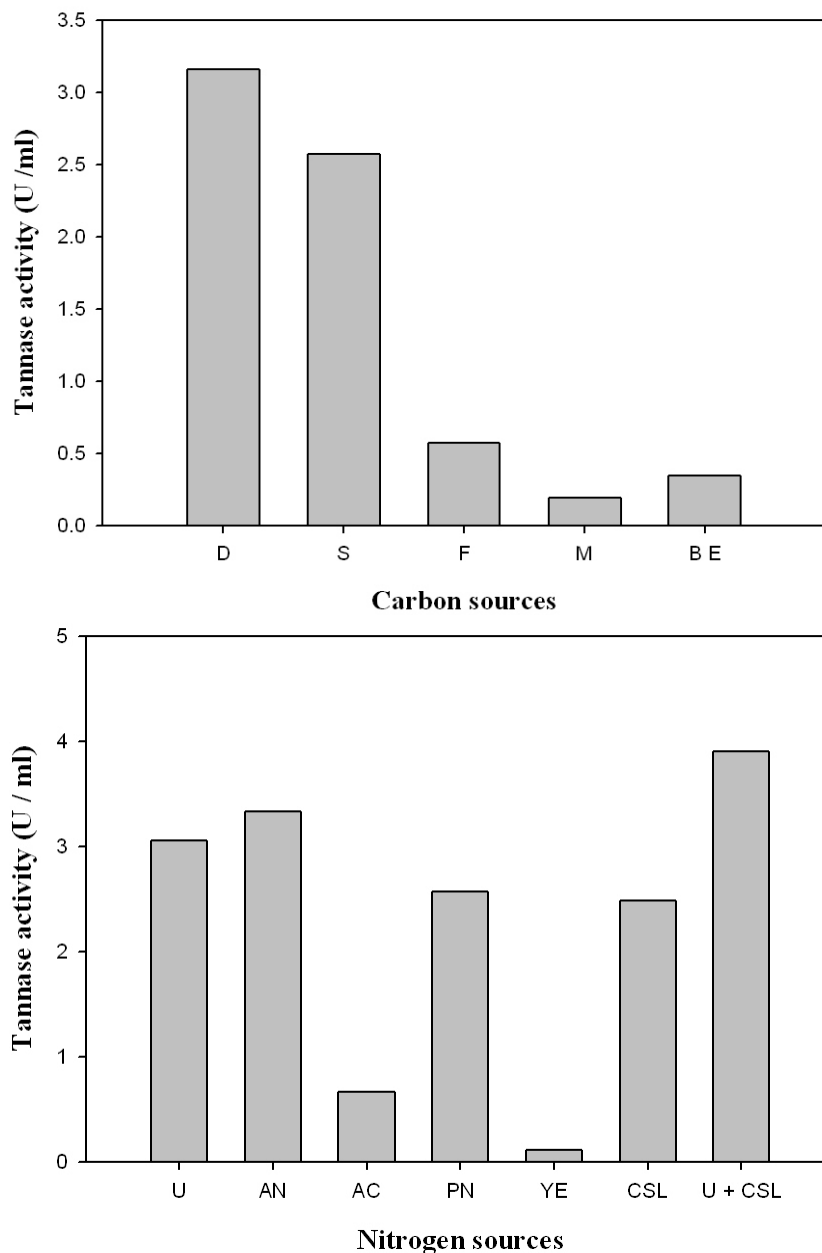


Figure 5. Effect of varying carbon sources (a) and nitrogen sources (b) on extracellular tannase production by *K.pneumoniae* MTCC 7162. D, glucose; S, sucrose; F, fructose; M, maltose; BE, beef extract, U, Urea; AN, ammonium Nitrate; AC, ammonium chloride; PN, potassium nitrate; YE, yeast extract; CSL, corn steep liquor.

production (Bradoo et al., 1997). It has been reported that pure carbon sources, in general, inhibit tannase production possibly indicating the presence of catabolite repression of protein biosynthesis (Aguilar et al., 2001a). This was substantiated by the decrease in growth of the organism when glucose was used as the sole carbon source (Figure 5a).

Among all nitrogen sources tested in our study, inclusion of ammonium nitrate resulted in the highest enzyme

production (3.33 U/ml). Ammonium nitrate stimulates the synthesis of proteins and is a source of readily utilizable nitrogen. Yeast extract resulted in the least production of enzyme in *K. pneumoniae* MTCC 7126. This decrease may be due to the complexity of nitrogen source in the media fermentation. On basis of the results obtained, it may be concluded that microorganisms necessitate a low level of nitrogen in order to produce enzymes because nitrogen may be a limiting factor (Patel et al., 2005).

Table 2. Properties of the extracellular tannase produced by *K.pneumoniae* MTCC 7162. The activity of the enzyme at its optimal pH and temperature is taken as 100.

Optimum pH	5.5
Optimum temperature	40 °C
pH	Relative activity (%)
4.0	67.0
4.5	71.3
5.0	81.3
6.0	81.7
6.5	76.3
7.0	70.9
Temperature (°C)	Relative activity (%)
30	86.0
35	91.7
45	88.2
50	82.7
55	71.5
60	55.6

However a combination of corn steep liquor and urea resulted in enhanced production of the enzyme (Figure 5b). This might indicate a positive synergetic effect of growth media as against the individual contribution of the nitrogen sources used.

Effect of other additives on tannase production

The effect of different salts supplemented along with MMT broth for the production of tannase by *K. pneumoniae* MTCC 7126 (Table 1). Various divalent cations specifically affected tannase production to different extent when added to MMT broth independently. Though all the salts used decreased the production of extracellular tannase, complete inhibition was found when CuCl_2 , BaCl_2 , CaCl_2 and FeCl_2 were included in the growth medium. The other salts with the exception of NH_4Cl resulted in moderate inhibition. Tannase production from *Aspergillus japonicus* and other fungal strains (Bradoo et al., 1997; Bajpai and Patil, 1997; Ganga et al., 1977) were also reported to have similar behavior.

End-product repression

It has been reported that gallic acid represses the production of tannase (Kar et al., 2003). Therefore to study the effect of gallic acid on tannase production was monitored using varying concentration of gallic acid in MMT broth. It was observed that the tannase enzyme production was completely inhibited even at very low concentrations (0.03 to 0.2% (w/v)) of gallic acid. This indicates that the production of tannase at the longer growth period is reduced substantially. Also inclusion of

detergents in the growth media resulted in complete inhibition of tannase production by *K. pneumoniae* MTCC 7126.

Properties of extracellular tannase

The conditions for optimal activity with respect to pH and temperature were investigated for the tannase produced by *K. pneumoniae* MTCC 7126. The enzyme was found to be active in the pH range of 4.0 to 7.0 with an optimal activity of 4.65 U/ml at pH 5.5. Even at pH 4.0 and to 7.0 the enzyme exhibited 67.01 and 70.96% of activity with respect to the activity of optimal pH. It has been previously reported that some extracellular tannase from bacterial sources have maximal activity at near neutral pH (Enemour and Odibo, 2009; Sabu et al., 2006; Mondal et al., 2001b; Vinod et al., 2009; Mahapatra and Banerjee, 2009; Batra and Saxena, 2005).

With respect to temperature, the enzyme was active in the temperature range of 30 to 60°C with an optimal activity at 40°C. At extremes of temperatures used in the study (30 and 60°C) the extracellular tannase had retained more than 50% of its activity. The results thus indicate that the extracellular tannase reported in the present study is active in a wide range of pH and temperature (Table 2).

To our knowledge, most of the organisms capable of degrading tannins isolated till date are either anaerobic or facultative anaerobic bacteria from the alimentary canal of ruminating animals or fungal strains associated with the degradation of wood and forest litter. In view of the above, the aim of the present study was to isolate bacteria from tannery soils that are able to degrade tannins aerobically. The occurrence of *Klebsiella* sp. in sludge has been reported (Sharma et al., 2000; Munmun et al., 2008) and they manifest up to 70% of the culturable flora along with other genera.

Partial purification of extracellular tannase

Tannase was produced extracellularly by the isolated strain of *K. pneumoniae*; MTCC 7126 were subjected to fractional precipitation with 80% ammonium sulphate (Table 3). DEAE-cellulose column chromatography led to an overall purification of 9 fold with a yield of 4.84% (Table 3), results in agreement with those of Sharma et al. (1999), who purified a tannase from *A. niger* van Tieghem. The yield of 3% was lower than the value of 7 to 19% recovery reported by other authors (Rajakumar and Nandy, 1983; Farias et al. 1994). However, the purification factor was similar to that of the purified tannase obtained from various different fungi, as reported by other workers (Rajakumar and Nandy, 1983; Sharma et al. 1999). The molecular mass of the purified enzyme determined by SDS-PAGE (Figure 6) revealed that the tannase from *K. pneumoniae* MTCC 7162 is of the

Table 3. Partial purification of tannase isolated from *K.pneumoniae* MTCC 7162.

Purification step	Total activity (U)	Total protein (mg)	Specific activity (U/mg)	Purification fold	Yield (%)
Crude enzyme	692	132.86	0.192	1	100
Ammonium sulphate	164.02	89.72	0.547	2.85	23.7
DEAE –cellulose	33.53	19.84	1.69	8.80	4.84

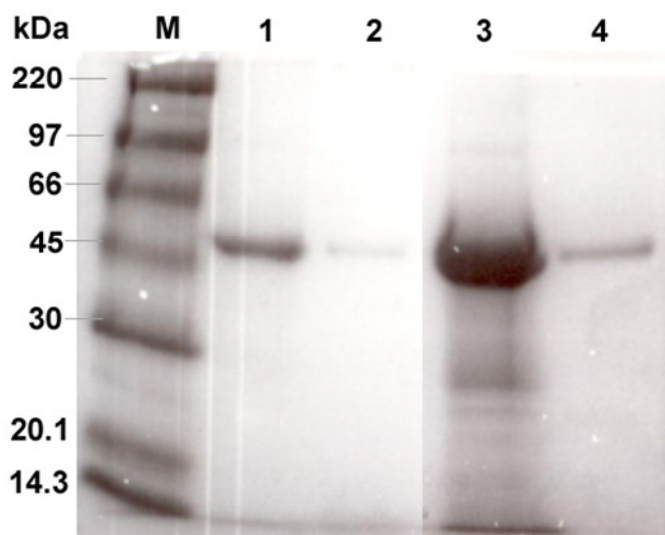


Figure 6. SDS-PAGE showing the (M) Molecular weight marker, (1) after purification by DEAE-cellulose column, (2) ammonium precipitated sample, (3) crude cell free extract and (4) dialyzed sample after purification with DEAE-cellulose column.

molecular weight 46.5 kDa. Tannase obtained from fungal sources had relatively higher molecular weight (in the range 70.0 to 180 kDa) in their associated forms as these enzymes, in general, have been reported to exist in both homomultimeric and/ or heteromultimeric forms. However, tannase from bacteria has been reported to have a lower molecular weight and are found to be homomultimeric.

In conclusion, this is the first study reporting on higher extracellular tannase production by *K. pneumoniae* isolated from tannery effluent samples. The present paper reports the effect of various physiochemical parameters for tannase production by *K. pneumoniae* MTCC 7162. Maximal tannase produced was found to be seven times greater than the tannase produced by *K. pneumoniae* (Deschamps et al., 1983). The tannase isolated and characterized in the present study gave reasonable yields compared to that reported in literature with an optimal activity at pH 5.5 and 40°C. As in the case of tannase from other sources, tannase from *K. pneumoniae* was also found to correspond with the average molecular weight of the monomeric enzyme molecule.

ACKNOWLEDGEMENTS

Financial Assistance from VIT University (Vellore, India) is gratefully acknowledged.

This article is dedicated to late Prof. Kunthala Jayaraman, FNAE, SATW, Founder Director of CBT, Anna University and SBST, VIT University.

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