© 2016 *RELS*
http://rels.comxa.com
rel_sci@yahoo.com

**ISSN:** 0974-4908
*Res. Environ. Life Sci.*
**9(1)** 78-80 (2016)

# Evaluation of various parameters for the optimum production of tannase by *Bacillus cereus*

**Nivedita Prasad and Jane C. Bejamin**
Department of Microbiology and Fermentation Technology, SHIATS, Allahabad, India
***e-mail:** nivedita.prasad01@gmail.com

(Received: March 31, 2015; Revised received: November 04, 2015; Accepted: November 09, 2015)

**Abstract:** Tannase or tannin-acyl-hydrolase (E.C.3.1.1.2.0) catalyzes the hydrolysis of ester and depside bonds in hydrolysable tannins, as tannic acid, releasing glucose and gallic acid. The present work explored the production, optimization of various parameters like pH, temperature, incubation period, salt-concentration, carbon sources & nitrogen sources and growth profile of tannase by *Bacillus cereus*. Tannase from the bacterium showed optimal activity at 48 h and 35°C with 1M salt concentration and initial pH 4.5. The peak enzyme activity was observed while supplementing sucrose at 1% conc. and maltose at 1 % & 2 % conc. as carbon sources, ammonium chloride at 3% concentration as nitrogen source. The maximum tannase activity 0.1586 U/ml was recorded in the mid-exponential phase of *Bacillus cereus*. *Bacillus cereus* thus has high potential and may be useful for industrial production of tannase.

**Keywords**: *Bacillus cereus*, Tannase, Fermentation and incubation period

## Introduction

Tannin Acyl Hydrolase (E.C. 3.1.1.20) is commonly referred to as tannase. The important commercial condensed tannins are extracted from wattle (*Acacia millissima and M. mearnsily, Quberacho, Schinopsis loretizii and S. balansae*) and tree barks. Catechin gallate occupies an intermediate position in the tannin hierarchy between hydrolysable and condensed tannins (Bhat *et al*., 1998). Tannins have wide applications in the food, beverage, brewing, cosmetic, and chemical industries (Lekha and Lonsane, 1997). The most commercial application of tannase are in the elaboration of instantaneous tea or of acron liquor and in the production of the gallic acid (Coggon *et al.,* 1975; Chae and Yu, 1983; Pourrat *et al.,*1985; Gracia, 2002), which is an important intermediary compound in the synthesis of the antibacterial drug trimethoprim, used in the pharmaceutical industry (Sittig,1988) and also in the food industry.

Tannase is produced by a number of microorganisms like fungi (*Aspergillus, Penicillium, Rhizopus* sp.), yeast (*Candida* sp.), and bacteria (*Bacillus* sp) (Iibuchi *et al.*, 1967). Most of the bacterial strains belonging to the genera *Bacillus, Staphylococcus, and Klebsiella* use tannic acid as sole source of carbon (Deschamps *et al.,* 1983). Tannic acid is also a major constitute of tannery effluent and is also used as a sole source of carbon and energy by microorganisms. In addition to its environmental importance, the organism hydrolyses tannins may be useful as a microbial cell for effective conversion of wastes in tannery effluent to wealth. Further salt tolerant property of the enzyme provides potential utility in the treatment of hard and slightly acidic effluent containing tannin residues in pollution control mechanisms. Therefore, more work on tannase is needed to increase its application. The objective of this study was thus to produce tannase from *Bacillus cereus*, to determine the effect of different factors (pH, temperature, salt conc., incubation period, carbon and nitrogen source in production medium) on production of tannase by *Bacillus cereus* and to determine the growth profile of tannase enzyme.

## Materials and Methods

To observe the production of Tannase the culture of *Bacillus cereus* (MCCB0005) was collected from microbial culture collection bank, Department of Microbiology and Fermentation Technology, SHIATS. It was maintained on nutrient agar slants. The culture supernatant obtained by centrifugation was assayed periodically for enzymatic activity. (Mondal *et al.,* 2001). Various parameters were evaluated for optimized production of tannase by *Bacillus cereus.* (Mondal *et al.,* 2001). Profile of tannase production by *Bacillus cereus* was studied at pH 4.5, 3% glucose conc., temperature 35ºC from 0-10 hrs of incubation period. The data recorded during the course of investigation was statistically analyzed by using analysis of variance (ANOVA) One-way classification and two-way classification

## Results and Discussion

In the present study the relative activity was found to be 0.211469 U/ml (Table 1). Tannase production studied by Mohapatra *et al*. (2006) using *Bacillus licheniformis* KBR6 recorded the maximum tannase activity 0.62 ± 0.04 U/ml inducted by *A.occidentale* which is much higher than reported in the presented study as this was not inducted by *A.occidentale*. The optimum pH for the maximum tannase activity was found to be 4.5 (Table 2) with 0.2908 U/ml of enzyme activity. Upon varying the pH from (3-7.5), the tannase enzyme was active at acidic pH and activity decreased as the pH approached to the alkaline pH range and finally reached 0 at pH 7.5. Mondal *et al.* (2001) reported the maximum growth and enzyme production occurred with initial medium pH of 4.5-5.0. This may be dependent on the ionic environment around the active site of enzyme. Thus the acidic environment is more suitable for this enzyme to catalyze.

**Table-1:** Production of tannase by *Bacillus cereus*

| Organism | Avg. OD | Relative activity (U/ml) |
|---|---|---|
| *Bacillus cereus* | 0.016666 | 0.1321 |

**Table-2:** Effect of pH on Tannase production

| pH | Average OD | Relative activity (U/ml) |
|---|---|---|
| 3.0 | 0.020000 | 0.1586 |
| 3.5 | 0.026667 | 0.2115 |
| 4.0 | 0.026667 | 0.2115 |
| 4.5 | 0.036667 | 0.2908 |
| 5.0 | 0.016667 | 0.1322 |
| 5.5 | 0.013333 | 0.1057 |
| 6.0 | 0.010000 | 0.0792 |
| 6.5 | 0.006667 | 0.0529 |
| 7.0 | 0.006667 | 0.0529 |
| 7.5 | 0.000000 | 0.0000 |

$F_{Cal.}$ = 4.083 > F (5%) = 2.408; Significant **(S)**

**Table-3:** Effect of temperature on Tannase production

| Temperature | Average OD | Activity (U/ml) |
|---|---|---|
| 20° | 0.003333 | 0.0264 |
| 25° | 0.003333 | 0.0264 |
| 30° | 0.013333 | 0.1055 |
| **35°** | **0.026667** | **0.2109** |
| 40° | 0.023333 | 0.1848 |
| 45° | 0.013333 | 0.1055 |
| 50° | 0.006667 | 0.0529 |
| 55° | 0.003333 | 0.0264 |
| 60° | 0.003333 | 0.0264 |
| 65° | 0.000000 | 0.0000 |

$F_{cal}$ = 5.104 > F (5%) = 2.408; Significant **(S)**

**Table-4:** Effect of incubation period on tannase production

| Incubation period | Average OD | Relative activity (U/ml) |
|---|---|---|
| 12 h | 0.010000 | 0.0793 |
| 24 h | 0.013333 | 0.1057 |
| 36 h | 0.016667 | 0.1321 |
| 48 h | 0.023333 | 0.1850 |
| 60 h | 0.006667 | 0.0529 |
| 72 h | 0.003333 | 0.0264 |
| 84 h | 0.000000 | 0.0000 |
| 96 h | 0.000000 | 0.0000 |
| 108 h | 0.000000 | 0.0000 |
| 120 h | 0.000000 | 0.0000 |

$F_{cal}$ = 5.616 > F (5%) = 2.408; Significant **(S)**

**Table-5:** Effect of salt concentration on Tannase production

| Salt conc. | Average OD | Relative activity (U/ml) |
|---|---|---|
| **1M** | **0.013333** | **0.1057** |
| 2M | 0.006667 | 0.0528 |
| 3M | 0.003333 | 0.0262 |
| 4M | 0.000000 | 0.0000 |
| 5M | 0.000000 | 0.0000 |
| 6M | 0.000000 | 0.0000 |
| 7M | 0.000000 | 0.0000 |
| 8M | 0.000000 | 0.0000 |
| 9M | 0.000000 | 0.0000 |
| 10M | 0.000000 | 0.0000 |

$F_{cal}$ = 1.988 < F (5%) = 2.408; Non-significant **(NS)**

**Table-6:** Effect of different carbon sources on tannase production.

| Carbon Source | Glucose Relative activity (U/ml) | Fructose Relative activity (U/ml) | Sucrose Relative activity (U/ml) | Maltose Relative activity (U/ml) |
|---|---|---|---|---|
| 1 % | 0.0793 | 0.0264 | 0.1586 | 0.1586 |
| 2 % | 0.0793 | 0.0264 | 0.1322 | 0.1586 |
| 3 % | 0.1057 | 0.0793 | 0.1057 | 0.1057 |
| 4 % | 0.0793 | 0.0529 | 0.1322 | 0.0793 |
| 5 % | 0.0264 | 0.0529 | 0.1057 | 0.1057 |
| 6 % | 0.0264 | 0.0264 | 0.0793 | 0.0529 |
| 7 % | 0.0000 | 0.0264 | 0.0529 | 0.0264 |
| 8 % | 0.0000 | 0.0000 | 0.0264 | 0.0000 |
| 9 % | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| 10% | 0.0000 | 0.0000 | 0.0000 | 0.0000 |

S.S. due to carbon sources, $F_{cal}$ = 8.22 > F (5%) = 2.96; Significant (S) S.S. due to different conc., $F_{cal}$ = 11.07 > F (5%) = 2.25; Significant (S) (C.D. = 0.0204)

**Table-7:** Effect of nitrogen source on tannase production

| Nitrogen Source | $NH_4NO_3$ Relative activity (U/ml) | $NH_4Cl$ Relative activity (U/ml) | $KNO_3$ Relative activity (U/ml) | Urea Relative activity (U/ml) |
|---|---|---|---|---|
| 0.5% | 0.1321 | 0.1321 | 0.0793 | 0.1321 |
| 1 % | 0.0793 | 0.1057 | 0.0528 | 0.1057 |
| 1.5% | 0.1057 | 0.1057 | 0.0264 | 0.0793 |
| 2 % | 0.0528 | 0.0528 | 0.0264 | 0.0264 |
| 2.5% | 0.0528 | 0.0793 | 0.0000 | 0.0000 |
| 3 % | 0.0264 | 0.1586 | 0.0000 | 0.0000 |
| 3.5% | 0.0000 | 0.0528 | 0.0000 | 0.0000 |
| 4 % | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| 4.5% | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| 5 % | 0.0000 | 0.0000 | 0.0000 | 0.0000 |

S.S. due to nitrogen sources, $F_{cal}$ = 1.414 > F (5%) = 2.96; Non Significant (NS); S.S. due to different conc., $F_{cal}$ = 0.572 > F (5%) = 2.25; Non Significant (NS) (C.D. = 0.0425)

**Table-8:** Growth profile of *Bacillus cereus* for optimized tannase production

| Time (hrs.) | pH | Tannase (U/ml) | Cellmass (U/ml) | Glucose (U/ml) |
|---|---|---|---|---|
| 0 | 4.5 | 0 | 0 | 0 |
| 1 | 4.4 | 0 | 0.1 | 0.0793 |
| 2 | 4.1 | 0 | 0.12 | 0.0793 |
| 3 | 4.0 | 0.0528 | 0.15 | 0.1057 |
| 4 | 3.8 | 0.1057 | 0.25 | 0.0793 |
| 5 | 3.9 | 0.1321 | 0.31 | 0.0264 |
| 6 | 3.9 | 0.1586 | 0.32 | 0.0264 |
| 7 | 3.7 | 0.0793 | 0.32 | 0 |
| 8 | 3.6 | 0.0528 | 0.31 | 0 |
| 9 | 3.5 | 0.0264 | 0.3 | 0 |
| 10 | 3.5 | 0.0264 | 0.3 | 0 |

The optimum temperature was found to be 35°C (Table-3) with activity of 0.2109 U/ml when the effect of temperature (20-65°C) on enzyme activity was studied. There was an increase in enzyme activity with an increase in temperature upto 35°C, and a subsequent decrease. At 65°C zero activity was recorded. The tannase activity in the present study was markedly reduced above 35°C. In more previous researches optimum temperature around

35°C with enzyme activity 23.52 U/ml was reported in case of *Aspergillus niger* by Lokeswari & Raju (2007). This indicated that the rate of protein enzyme denaturation increased at higher temperature leading to the loss of enzyme activity. With a rise in incubation period, the tannase production increased and optimum activity was recorded at 48 h (Table-4) with 0.1850 U/ml of activity which gradually decreases upto 72 h and 0 activity was recorded from 84-120 h. The results were in agreement with the findings of Hota *et al.* (2007) and Bradoos *et al.* (1997). Decrease in the enzyme production on further incubation may be due to depletion of nutrients and feedback inhibition due to the production of gallic acid from tannic acid present in the medium.

The maximum enzyme activity 0.1057 U/ml was observed at 1M conc. (Table-5) which gradually decreases upto 3M conc. and 0 enzyme activity was recorded from 4-10M salt conc. The statistical analysis revealed the result to be non-significant (P>0.05). Similar result was reported by Mondal *et al.* (2001) according to which enzyme was stable upto 2M and retains 82% activity at 3M of NaCl. Mohapatra *et al*. (2006) reported that both growth of the organism and enzyme production increased two-fold when it was grown in salt containing crude tannin extract rather than enriched pure tannin acid medium. Maximum tannase production was observed at 1% conc. of sucrose and 1 & 2% conc. of maltose (Table-6) with enzyme activity of 0.1586 U/ml. After that a decline was observed in activity and 0 activity was recorded from 9-10% conc. Srivastava and Kar, (2009) reported tannase activity 30.8 U/ml with 0.5% of glucose whereas the activity decreased at 0.75% of glucose. The addition of carbon sources such as glucose, sucrose, fructose, maltose and arabinose to the culture medium at initial conc. from 10 to 30 g/l improved the production of tannase by *A.niger* (Belmares *et al.,* 2004). This shows that carbon source at higher conc. repressed tannase synthesis while the lower conc. was not repressive as it is one of the products of tannic degradation hence in higher conc. it perhaps show feed- inhibition.

The maximum tannase production was observed at 3% conc. of $NH_4Cl$ with activity of 0.1586 U/ml and 0 activity was reported from 4 to 5% conc. in all nitrogen sources (Table-7). Paranthaman *et al.* (2009b) observed the maximum activity in all substrates when used ammonium nitrate and ammonium chloride at 1.5% conc. as a nitrogen source. The presence of additional nitrogen sources along with nitrogenous compounds present in the substrate promotes enhanced growth and consequent enzyme production (Chandrasekaran *et al.,* 1991). The tannase production was found to increase gradually from 4 hrs of the fermentation period when the growth of the microorganism reached the early exponential phase which shows that the tannase production was growth associated product (Table 8). The maximum tannase activity was found in the mid-exponential phase and early stationary phase of *B. cereus*. Similar result was reported in case of *Lactobacillus plantarum* by Ayed and Hamdi (2002).

*Bacillus cereus* is very suitable for tannase production because it can grow easily and produce a huge amount of tannase within a short period. The novelty of this enzyme is that it is salt-tolerant as well as stable over a wide pH range. These features make the strain promising for industrial exploitation in various fields. Various reports on microbial production of tannase revealed that the fermentation parameters namely culture condition, culture composition, and substrate concentration had significant effect on production of tannase and biomass. In addition to its environmental importance, the tannin degrading organism may also be useful as a microbial cell and may be utilized for effective conversion of wastes in tannery effluent to wealth. Further works can be conducted on widespread prospective application of tannase producers in bioremediation and it could also be very valuable in industry for the production of tannase and also of the detoxification of the bark content of animal feed. Moreover, tannase producers may find its application in food and pharmaceutical industries.

## Acknowledgement

The authors are grateful to the Department of Microbiology and Fermentation Technology, SHIATS, Allahabad for financial support to the presented research work.

## References

Ayed, L. and Hamdi, M.: Culture conditions of tannase by *L. plantarum*. *Biotechnology Letters*. **24**: 1763-1765 (2002). .

Belmares, R., Esquivel, J.C.C., Rodriguez, R., Coronel, A.R. and Aguilar, C.N.: Microbial production of tannase: an enzyme with potential use in food industry. *Lebensm-Wiss U-Technology*, **37**:857-864 (2004).

Bhat, T.K., Singh, B. and Sharma, O.P.: Microbial degradation of tannins – A current perspective. *Biodegradation,* **9**: 343-357 (1998).

Bradoos, S., Gupta, R. and Saxena, R.K.: Parametric optimization and biochemical regulation of extra-cellular tannase from *Aspergillus japonicas*. *Process Biochemistry,* **32**: 135-139 (1997).

Chae, S.K. and Yu, T.J.: Experimental manufacture of acron wine by fungal tannase. *Hanguk Sipkum Kwahakhoechi,* **15**: 326-332 (1983).

Chandrasekharan, M., Lakshmana, P.P. and Chandramohan D.: Combined effect of environmental factors on spoilage bacteria. *Fish Tech India.* **28**: 146-153 (1991).

Coggon, P. and Sander, G.W.:Manufacture of instant tea. *Pat.Ger.Offen*, **2**: 304-307 (1975).

Deschamps, A.M., Otuk, G. and Lebeault, J.M.: Production of tannase and degradation of chestnut tannin by bacteria. *Journal of Fermentation Technology,* **61**: 55–59 (1983).

Gracia-Najera, J.A., Medina, A., Castro, Y., Reyes-Vega, M.L., Prado-Barragan, L.A., Rodriguez-Herrera, R. and Aguilar, C.N.: Accumulation and Recovery of Gallic Acid in a Submerged Culture of *Aspergillus niger* Aa-20. IFT Annual Meeting, Anaheim, CA, USA, p. 25 (2002).

Hota, S.K., Dutta, J.R. and Banerjee R.: Immobilization of tannase from *Rhizopus oryzae* and its efficiency to produce gallic acid from tannin rich agro-residues. *Indian Journal of Biotechnology*, **6**: 200-204 (2007).

Iibuchi, S., Minoda, Y. and Yamada, K.: Studies on tannin acyl hydrolase of the microorganism: Part III. *J. Agric Biol Chem*, **31**: 513-518 (1967).

Lekha, P.K. and Lonsane, B.K.: Production and application of tannin acyl hydrolase: State of the art. *Advances in Applied Microbiology,* 44, 215-260 (1997).

Lokeswari, N. and Raju, K.J.: Tannase production by *Aspergillus niger. Electronic Journal of Chemistry,* **4:** 192-198 (2007).

Mohapatra, P.K.D., Mondal, K.C. and Pati, B.R.: Production of tannase through submerged fermentation of tannin containing plant extracts by *Bacillus licheniformis* KBR6. *Polish Journal of Microbiology*, **55**: 297-301 (2006).

Mondal, K.C., Banerjee, D., Banerjee, R. and Pati, B.R.: Production and characterization of tannase from *Bacillus cereus* KBR9. *Journal of Applied Microbiology,* **47**: 263-267 (2001).

Paranthaman, R., Vidyalakshmi, R., Murugesh, S. and Singaravadivel, K.: Comparitive study on the suitability of different substrates for tannin acyl hydrolase producing using *Aspergillus oryzae. Journal of Pharmaceutical Sciences & Research,* **1**: 36-42 (2009).

Pourrat, H., Regerat, F., Pourrat, A. and Jean, D.: Production of tannase (tannin-acyl-hydrolase E.C.3.1.1.20) by a strain of *Aspergillus niger*. *Biotechnology Letters,* **4**: 583-599 (1985).

Sitting, M.: In Pharmaceutical Manufacturing Encyclopedia, 2nd (eds.), 282 (1988).

Srivastava, A. and Kar, R.: Characterization and application of tannase produced by *Aspergillus niger* ITCC 6514.07 on pomegranate rind. *Brazilian Journal of Microbiology*, **40**: 782-789 (2009).