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## Gallic Acid Production by Submerged Fermentation of *Aspergillus aculeatus* DBF9

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**Abstract:** Tannin from *Cassia siamea* was fermented in a bioreactor by *Aspergillus aculeatus* DBF9 for gallic acid production. Production of gallic acid was observed to be highest at 30°C with an initial pH of 5.5 after 36 h. Aeration at the rate of 4 L min<sup>-1</sup> and agitation speed of 100 rpm was found to be suitable for maximum gallic acid production. Medium containing 3% tannin was found suitable for highest gallic acid production.

**Key words:** *Aspergillus aculeatus*, fermentation, gallic acid, tannase, plant tannin

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### INTRODUCTION

Gallic acid (3,4,5-tri hydroxy benzoic acid), one of the byproducts of tannin hydrolysis has several applications in chemical and pharmaceutical industries. It has huge demand in India though it is an imported item. It is mainly used for manufacture of propyl gallate (Weetal, 1985; Gathon *et al.*, 1989) and preparation of trimethoprim (Hadi *et al.*, 1994). Gallic acid is also used in the manufacture of pyrogallol, inks, photographic developer, in testing free mineral acids, dihydroxy acetone and alkaloids (Budavari, 1989). The worldwide annual demand of gallic acid is about 8000 tons. Conventionally gallic acid is produced by acid hydrolysis of tannins, but this process releases a large amount of toxic effluent that causes environmental hazards. Production of gallic acid through the fermentation of tannic acid using suitable tannase producing microorganism is preferred today.

A different fungal (Banerjee *et al.*, 2001; Mukherjee and Banerjee, 2004; Misro *et al.*, 1997; Pourrat *et al.*, 1982, 1985; Vermeire and Vandamme, 1990) and bacterial (Deschamps *et al.*, 1980; Deschamps and Lebeault, 1984; Mondal *et al.*, 2001a) strain has been utilized for tannase and gallic acid production. Pourrat *et al.* (1985) recovered 30% gallic acid through fermentation of tara pod powder by *A. niger*. Deschamps *et al.* (1980) studied chestnut tannin degradation by *Corynebacterium* sp. in a 2 L jar-fermenter. Production of gallic acid by *Aspergillus* sp. from gallo-tannin was studied by Vermeire and Vandamme (1990). Misro *et al.* (1997) studied the production of gallic acid using the immobilized cells of *Rhizopus oryzae*. They found that 78.5% tannin conversion was possible after 4 day of incubation. Mukherjee and Banerjee (2004) reported gallic acid production from tannins of myrabolan and teri pod powder through modified solid state fermentation by two fungal strains *Rhizopus oryzae* and *Aspergillus foetidus*. Therefore it has been found that production of gallic acid through fermentation is possible provided that plenty of raw material (tannin) and desired organism for bioconversion are in hand.

In the present investigation for the first time gallic acid production by *Aspergillus aculeatus* DBF9 has been carried out in Eylea 5 L bioreactor through liquid submerged fermentation of locally available raw plant tannin.

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## MATERIALS AND METHODS

### Microorganism

One of a potent gallic acid producing fungal strain *Aspergillus aculeatus* DBF9, has been used in the present study.

### Culture Condition

Production of gallic acid was made from raw tannin through fermentation. Fermentation was carried out in a 5 L jar-fermenter (Eyela, MBF-500PE, Japan) connected with automatic control devices consisting of a standard cylindrical glass culture vessel with a working volume of 3 L. The tank diameter was 15 cm and height to vessel diameter ratio was 1.6. The culture medium consisted of raw tannin (*Cassia siamea* plant extract), 20.0 g L<sup>-1</sup>; K<sub>2</sub>HPO<sub>4</sub>, 0.5 g L<sup>-1</sup>; KH<sub>2</sub>PO<sub>4</sub>, 0.5 g L<sup>-1</sup>; MgSO<sub>4</sub>·7H<sub>2</sub>O, 1.0 g L<sup>-1</sup> and (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub>, 3.0 g L<sup>-1</sup>. For fermentation, the culture medium was inoculated with active preculture of *A. aculeatus* spores and was cultivated for a period of 72 h. Fermented broth was removed from the vessels at every 6 h interval by a hand pump for analysis.

Effect of pH on fermentation was studied by adjusting the initial pH of the medium at various values (5.0, 5.5 and 6.0). Fermentation was conducted at different temperature (30-40°C), aeration (4.0-6.0 L min<sup>-1</sup>) and agitation (50-150 rpm). Effect of different concentration of tannin (1.5-6.0%) was examined with raw tannin from *Cassia siamea* plant.

### Tannase Activity

Tannase activity was determined following the method of Mondal *et al.* (2001b) Enzyme solution (0.05 mL) was incubated with 0.3 mL of 1.0% (w/v) tannic acid, in 0.2 M acetate buffer (pH 5.0) at 40°C for 10 min and then the reaction was stopped by addition of 2.0 mL bovine serum albumin (BSA) (1 mg mL<sup>-1</sup>), which precipitated the remaining tannic acid. A control reaction was done side by side with heat denatured enzyme. The tubes were then centrifuged (5,000 × g, 10 min) and the precipitate was dissolved in 2.0 mL of SDS-triethanolamine (1% w/v SDS in 5% v/v triethanolamine) solution and the absorbency was measured at 550 nm after addition of 1.0 mL of FeCl<sub>3</sub> (0.13 M) (Systronics spectrophotometer 105).

One unit of tannase activity is defined as the amount of enzyme required to hydrolyze 1.0 μmol of ester linkage of tannic acid in 1 min under specified condition.

### Estimation of Gallic Acid

Gallic acid in the culture broth was estimated by the method of Bajpai and Patil (1996). Culture supernatant was diluted to 100 fold in 0.2 M acetate buffer, pH 5.0. The absorbance was recorded at two selective wavelengths of 254.6 and 293.8 nm. The concentration of gallic acid was measured using specific extinction coefficient, by the following equation; Concentration of gallic acid (μg mL<sup>-1</sup>) = 21.77 (A<sub>254.6</sub>)-17.17 (A<sub>293.8</sub>).

### Biomass Estimation

Growth of the organism was estimated after drying the biomass at 60°C for 24 h.

All the experiments were performed in triplicate and the results represented here are the mean of the three.

## RESULTS AND DISCUSSION

Selection of a substrate for enzyme and subsequent product formation by fermentation depends on several factors like cost, availability and suitability of the substrate for obtaining the desired product

of fermentation and thus requires screening of several agro-industrial residues (Pandey *et al.*, 1999). In the present experiment, cost-effective substrates for biotransformation were selected on the basis of their tannin content and it was found that *Cassia siamea* plant tannin gives better result (results are not shown). Tannin obtained from *Cassia siamea* was selected as a substrate for optimization of gallic acid production through fermentation of *A. aculeatus* DBF9. Mukherjee and Banerjee (2004) used tannin rich plant material myrabolan and teri pod powder as a source of raw material for tannase and gallic acid production by *Rhizopus oryzae* and *Aspergillus foetidus*.

### Effect of Temperature

The effect of cultivation temperature (25-40°C) on the growth, tannase and gallic acid production by *A. aculeatus* in batch fermenter was studied and is presented in Fig. 1. It was observed that during fermentation the growth of the organism was highest at temperature 30°C (Fig. 1A). The tannase activity in culture media increased rapidly during the initial period of cultivation (Fig. 1B). The highest tannase activity of 3.93 U mL<sup>-1</sup> was noted in the medium after 36 h of cultivation at 30°C. Negligible amount of tannase was produced at 25 and 40°C. It was also found that production of gallic acid varied

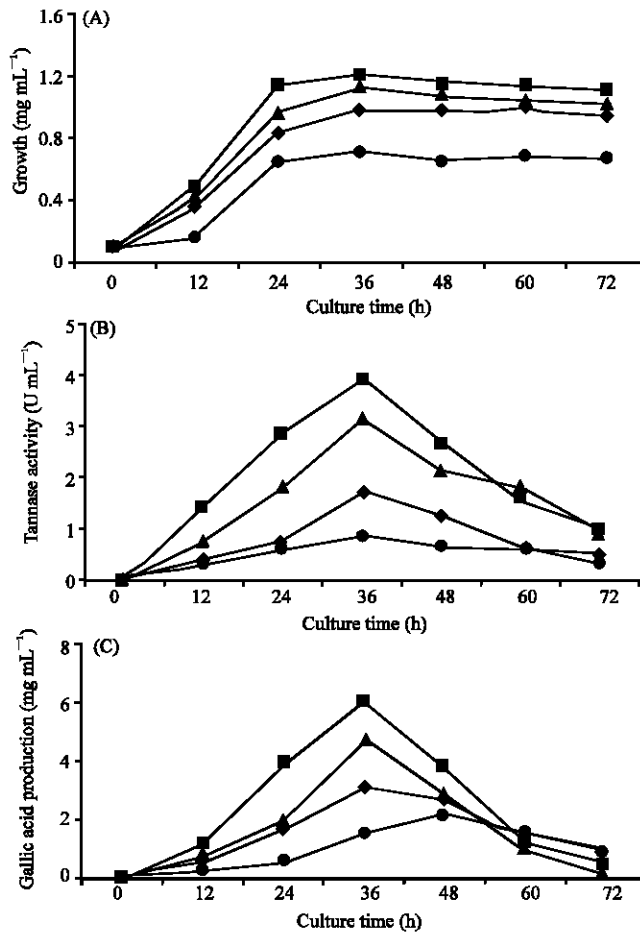


Fig. 1: Effect of temperature on growth (A), tannase (B) and gallic acid production (C) (●, 25°C; ■, 30°C; ▲, 35°C; ◆, 40°C)

with incubation temperature. Maximum gallic acid (Fig. 1C) of  $6.0 \text{ mg mL}^{-1}$  was produced at  $30^\circ\text{C}$  after 36 h of incubation. Temperature of the fermentation medium is one of the crucial factors that have direct effect on the production of tannase and gallic acid. Earlier  $35^\circ\text{C}$  was found suitable for tannase production by *A. niger* (Pourrat *et al.*, 1985).

### Effect of pH

Effect of pH on gallic acid production was studied in relation to tannase production for 72 h and is presented in Fig. 2. Maximum amount of gallic acid ( $6.21 \text{ mg mL}^{-1}$ ) was obtained after 36 h when the initial pH of fermentation medium was kept at 5.5 (Fig. 2B). This may be due to maximum production of tannase in the same pH. Further increase of medium pH decreases gallic acid production. The pH is very important factor for tannase and gallic acid production (Barthomeuf *et al.*, 1994). The maintenance of a favourable pH is very essential for the successful fermentation of gallic acid (Vermeire and Vandamme, 1988).

### Effect of Aeration

Tannase and gallic acid production by *A. aculeatus* was tested at different aeration rate ( $3.0$ ,  $4.0$  and  $5.0 \text{ L min}^{-1}$ ); the results are shown in Fig. 3. The highest tannase production was obtained at an aeration level of  $4 \text{ L min}^{-1}$  (Fig. 3B). But above or below this aeration level productions of tannase become reduced. Gallic acid ( $6.4 \text{ mg mL}^{-1}$ ) production was also found maximum after 36 h with the same aeration rate of  $4 \text{ L min}^{-1}$ . Sufficient aeration of the medium as well as supply of oxygen is essential for adequate growth of the microorganism and production of tannase. Pourrat *et al.* (1987) mentioned that a critical level must be maintained for aeration as in high oxygenation causes oxidation

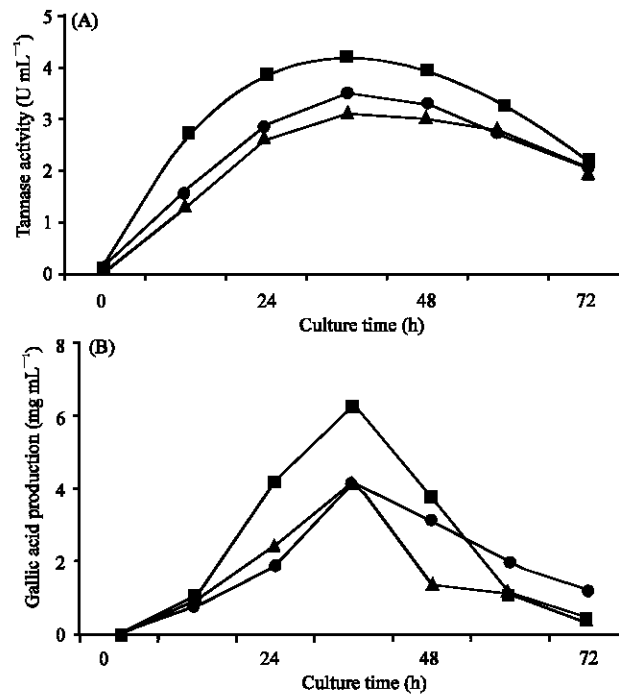


Fig. 2: Effect of initial medium pH on tannase (A) and gallic acid (B) production (●, 5.0; ■, 5.5; ▲, 6.0)

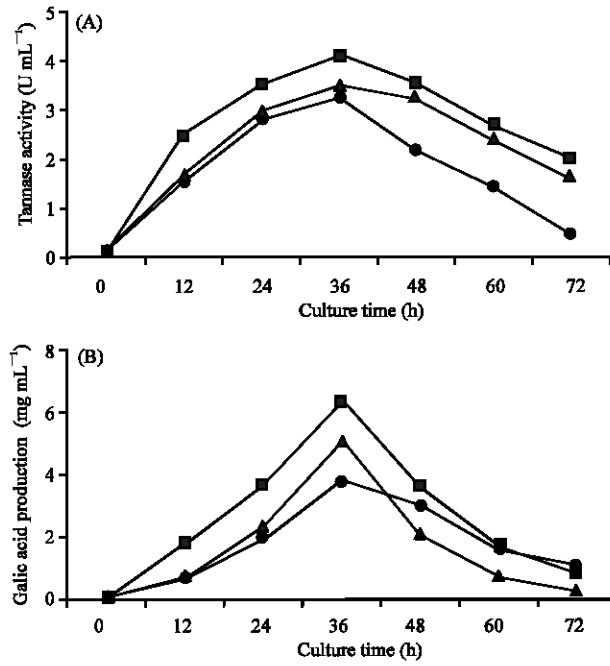


Fig. 3: Effect of aeration on tannase (A) and gallic acid (B) production (●, 3 L min<sup>-1</sup>; ■, 4 L min<sup>-1</sup>; ▲, 5 L min<sup>-1</sup>)

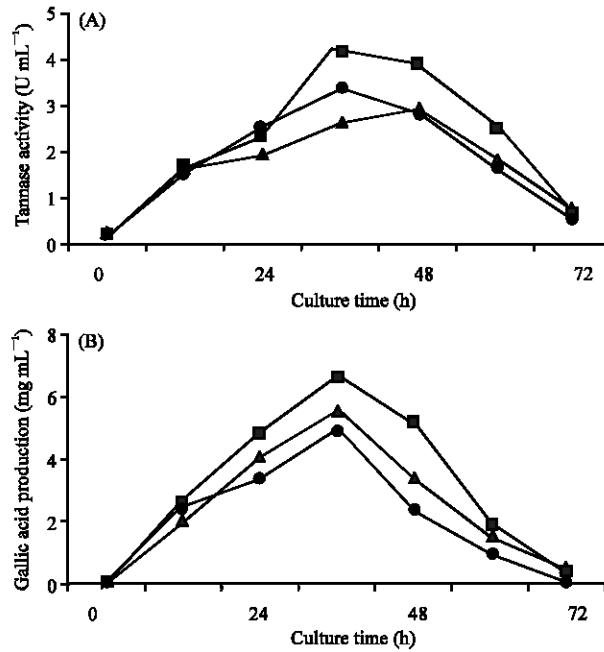


Fig. 4: Effect of agitation speed on tannase (A) and gallic acid (B) production (●, 50 rpm; ■, 100 rpm; ▲, 150 rpm)

of tannins and catabolism of the gallic acid. Highest gallic acid production was recorded after 36 h at aeration of  $4 \text{ L min}^{-1}$ . Higher aeration ( $5 \text{ L min}^{-1}$ ) rate resulting lower gallic acid production. This may be due to the oxidation of tannins. Under favourable conditions the fungal mycelia simultaneously synthesized an antioxidant, which prevented tannin oxidation (Barthomeuf *et al.*, 1994).

### Effect of Agitation

Tannase production was enhanced in culture media with the increase in the agitation speed upto 100 rpm (Fig. 4A). At the same agitation speed tannase production after 36 h of fermentation was found  $4.18 \text{ U mL}^{-1}$  but after 48 h its production was reduced to  $3.84 \text{ U mL}^{-1}$ . Gallic acid ( $6.64 \text{ mg mL}^{-1}$ ) production reached maximum after 36 h with 100 rpm (Fig. 4B). Lower agitation speed may limit the nutrient availability to the mycelium and at higher agitation speed, mycelial growth rate decreased due to the breakage of mycelial wall. Though Barthomeuf *et al.* (1994) found that 300-450 rpm agitation speed was suitable for tannase production in *A. niger*.

### Effect of Tannin Concentration

The effect of tannin concentration (1.5-6.0%, w/v) on tannase and gallic acid production by *Aspergillus aculeatus* DBF9 was carried out in 5 L jar fermenter with different concentrations of *Cassia siamea* tannin (Fig. 5). The maximum amount of gallic acid ( $6.8 \text{ mg mL}^{-1}$ ) was obtained after 36 h in the medium containing 3% tannin. Production of tannase was found maximum ( $4.12 \text{ U mL}^{-1}$ ) with the same concentration of tannin. Reduction of tannase production was noticed at higher concentration of tannin. This indicates that low tannin concentration is a good inducer for tannase biosynthesis. It has a significant impact on large-scale production of gallic acid in industrial basis.

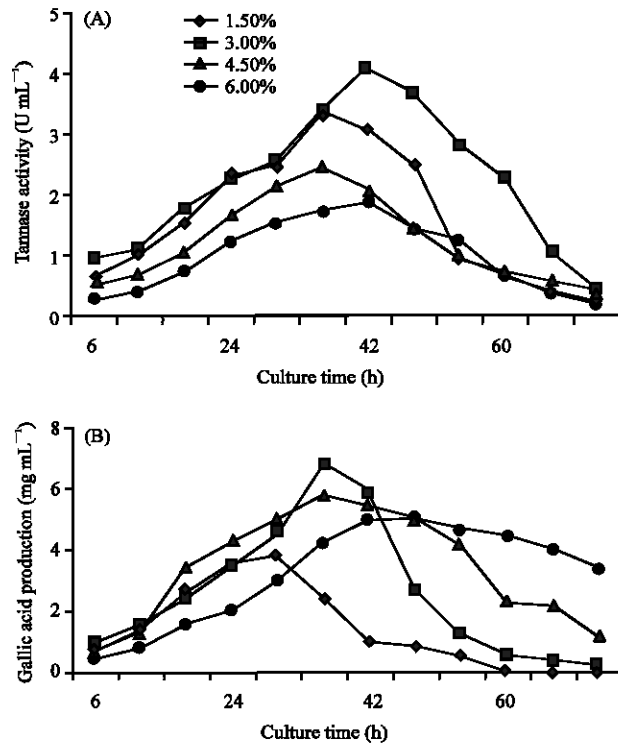


Fig. 5: Effect of tannin concentration (w/v) on tannase (A) and gallic acid (B) production

## CONCLUSIONS

This is for the first time; gallic acid production has been made from the raw tannin of *Cassia siamea* through fermentation by *A. aculeatus* DBF9. Plenty of such cost effective raw material ensured the possibility for exploitation of this organism in large scale production of gallic acid.

## ACKNOWLEDGMENT

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## REFERENCES

- Bajpai, B. and S. Patil, 1996. Tannin acyl hydrolase (EC-3.1.1.20) activity of *Aspergillus*, *Penicillium*, *Fusarium* and *Trichoderma*. World J. Microbiol. Biotechnol., 12: 217-220.
- Banerjee, D., K.C. Mondal and B.R. Pati, 2001. Production and characterization of extracellular and intracellular tannase from newly isolated *Aspergillus aculeatus* DBF9. J. Basic Microbiol., 41: 313-318.
- Barthomeuf, C., F. Regeat and H. Pourrat, 1994. Production, purification and characterization of a tannase from *Aspergillus niger* LCF 8. J. Ferment. Bioeng., 77: 320-323.
- Budavari, S., 1989. The Merck Index, MERCK and CO., INC, USA., pp: 4250, 9624.
- Deschamps, A.M., G. Mahoudeau, M. Conti and J.M. Lebeault, 1980. Bacterial degradation of tannic acid and related compounds. J. Ferment. Technol., 58: 93-97.
- Deschamps, A.M. and J.M. Lebeault, 1984. Production of gallic acid from tara tannins by bacterial strains. Biotechnol. Lett., 6: 237-242.
- Gathon, A., Z. Gross and M. Rozhanski, 1989. Propyl gallate: Enzymatic synthesis in a reverse micelle system. Enzyme Microb. Technol., 11: 604-609.
- Hadi, T.A., R. Banerjee and B.C. Bhattacharya, 1994. Optimization of tannase biosynthesis by a newly isolated *Rhizopus oryzae*. Bioprocess Eng., 11: 239-243.
- Misro, S.K., R. Kumar, R. Banerjee and B.C. Bhattacharya, 1997. Production of gallic acid by immobilization of *Rhizopus oryzae*. Bioprocess Eng., 16: 257-260.
- Mondal, K.C., D. Banerjee, R. Banerjee and B.R. Pati, 2001a. Production and characterization of tannase from *Bacillus cereus* KBR9. J. Gen. Applied Microbiol., 47: 263-267.
- Mondal, K.C., D. Banerjee, M. Jana and B.R. Pati, 2001b. Colorimetric assay method for determination of tannin acyl hydrolase (EC: 3.1.1.20) activity. Anal. Biochem., 295: 168-171.
- Mukherjee, G. and R. Banerjee, 2004. Biosynthesis of tannase and gallic acid from tannin rich substrates by *Rhizopus oryzae* and *Aspergillus foetidus*. J. Basic Microbiol., 44: 42-48.
- Pandey, A., P. Selvakumar, C.R. Soccol and P. Nigam, 1999. Solid-state fermentation for the production of industrial enzymes. Curr. Sci., 77: 149-162.
- Pourrat, H., F. Regeat, A. Pourrat and D. Jean, 1982. Production of tannase (tannin acyl hydrolase E.C.3.1.1.20) by a strain of *Aspergillus niger*. Biotechnol. Lett., 4: 583-588.
- Pourrat, H., F. Regeat, A. Pourrat and D. Jean, 1985. Production of gallic acid from tara by a strain of *Aspergillus niger*. J. Ferment. Technol., 63: 401-403.
- Pourrat, H., F. Regeat, P. Morvan and A. Pourrat, 1987. Microbiological production of gallic acid from *Rhus coriaria* L. Biotechnol. Lett., 9: 731-734.
- Vermeire, A. and E. Vandamme, 1988. Fungal production of tannin acyl hydrolase. Med. Fac. Landbouw. Rijksuniv. Gent., 53: 2047-2056.
- Vermeire, A. and E. Vandamme, 1990. Fungal conversion of gallo-tannin into gallic acid. Ferment. Technol. Ind. Applied, pp: 198-203.
- Weetal, H.H., 1985. Enzymatic gallic acid esterification. Biotechnol. Bioeng., 27: 124-127.