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Optimization of Tannase Biosynthesis from Two Local *Aspergilli* using Commercial Green Tea as Solid Substrate

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Abstract: This study aimed to the production of tannase through SSF techniques by using some local fungal strain. Eleven fungal strains able to produce extracellular tannase (tannin acyl hydrolase; E.C.3.1.1.20) were isolated from tannins rich soil. On the basis of tannin degradation and tannase activity, two of them were selected and identified as *Aspergillus aculeatus* and *Aspergillus awamori*. Cultural fermentation conditions including; incubation time, culture pH, incubating temperature, initial moisture level, nitrogen source, minerals and tannin rich wastes substrate were evaluated to increase the yield of tannase production and tannin degradation. The results showed that *A. aculeatus* produced the highest tannase level (41.4 IUg^{-1}) compared with *A. awamori* (27.6 IU g^{-1}) under SSF using 1.0 g of fermentable green tea at 30°C and pH 6.0 in presence of $CaCl_2$ and peptone with 80% initial moisture level. The results also revealed 2.52 and 2.04 folds increase in tannase biosynthesis from *A. aculeatus* and *A. awamori* respectively after fermentation conditions investigation.

Key words: Tannin degradation, remaining tannins, SSF, *A. aculeatus*, *A. awamori*

INTRODUCTION

Tannins are water-soluble polyphenols naturally occurred as secondary metabolites in higher plants. It has been considered as the fourth abundant constituents after cellulose, hemicellulose and lignin (Bhat *et al*., 1998; Rana and Bhat, 2005). Tannase (Tannin acyl hydrolase, E.C.3.1.1.20) is industrially important inducible enzyme produced by large number of fungi and a few bacteria (Bhat *et al*., 1998). It hydrolyzes the ester and depside bonds of hydrolyzable tannin. The fungal tannase is used in many industrial processing including clarification of fruit juice; de-tannification of food, high grade leather tannin and coffee flavored soft drinks, but the major commercial applications of the tannase was in the manufacture of instant tea and in the production of gallic acid (Cordova *et al*., 1996). The enzyme is also used in the pre-treatment of animal feed additives, to clean-up highly polluting tannin from the effluent of leather industry, pharmaceutical and chemical industries (Lekha and Lonsane, 1997; Aguilar *et al*., 2001; Mahendran *et al*., 2006). Gallic acid, a hydrolytic product of tannin, was used in preparation of trimethoprim, pyrogallol, propyl gallate, dyes and inks (Das Mohapatra *et al*., 2006).

Solid state fermentation involves non-aseptic conditions with the use of cheap, simple and easily available raw materials as substrates, along with several economical and engineering advantages including low capital cost, low energy expenditure, less expensive downstream processing, less water usage and lower wastewater output, potential higher volumetric productivity, higher concentration of the products, high reproducibility, lesser fermentation space, easier control of contamination and generally simpler fermentation media (Krishna, 2005).

When compared to SmF techniques, SSF has advantages for tannase production in terms of an increment in purity and stability. It was observed that the tannase produced by SSF was more stable and total proteins were lower; moreover, undesirable proteolytic enzymes production was not observed during SSF (Cordoba-Salgado *et al*., 1998). Many workers reported that tannase production by SSF is more advantageous over submerged or liquid surface fermentation (Aguilar *et al*., 2001).

This study aimed to the production of tannase through SSF techniques by using some local fungal strain

MATERIALS AND METHODS

Microorganisms: The fungal cultures used in the present study were locally isolated in March 2009 by dilution plate method on tannin rich substrate namely; green tea, from soil samples collected from Mansoura University garden according to the procedures adopted by Johnson *et al.* (1960). Fungal strains were subjected to full identification

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using the most recent sophisticated facilities; an Imaging analysis system using soft-imaging GbH software (analy SIS Pro ver.3.0) at the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Egypt. The stock culture is maintained routinely on PDA slants. The freshly grown slant cultures are (28±2°C) subsequently used for further work or stored at 4°C. The slants were sub-cultured routinely at an interval of 4.0-5.0 weeks.

Solid state fermentation medium: One gram of commercial green tea used as solid substrate were added to 250 mL Conical flasks, moistened with 3.0 mL of mineral salt solution (The composition of started mineral solution was: 3.0 g $NaNO_3$; 0.1 g KH_2PO_4; 0.5 g $MgSO_4.7H_2O$ and 0.5 g $CaCl_2$ all contents were dissolved in 1000 mL 0.1 M acetate buffer pH 5.5). The contents were autoclaved at 121°C for 20 min and then the flasks were cooled and inoculated with 2.0 mL (2.0×10^7 spores mL^{-1}) of spore suspension.

Extraction of tannase: After 5 days incubation, the fermented substrates were mixed properly by adding 50 mL of 0.1 M acetate buffer (pH 5.5) to the fermented medium. Then, the flasks were kept on the rotating shaker for 1.0 h at 10°C and centrifuged at 5000 rpm for 10 min to remove all fungal cells and residue of substrate (Shamala and Screekantiah, 1986), the clarified extract represented the crude enzymes was used for assaying tannase activity.

Tannase assay: Tannase activity was estimated by the modified colorimetric method of Nicholson and Rhind (1924) based on the violet colour produced when $FeSO_4$ in the presence of Rochelle salt (colour reagent) react with gallic acid. The reaction mixture contained 0.5 mL of tannic acid (2.0 % w/v in 0.1 M sodium acetate buffer, pH 5.5) was added to 0.5 mL of crude enzyme. This reaction mixture was incubated at 40°C for 30 min. The enzymatic reaction was terminated by addition of 3.0 mL of precipitant solution (100 mL Quinine HCl (1.0%) mixed with 50 mL of 10% NaCl). A control was prepared side by side using heat denatured enzyme. Then 1.0 mL of the mixture was transferred in Open Dwarf tube and centrifuged at 9000 rpm in ultra micro-centrifuge for 15 min 300 μL of the supernatant is transferred to dry clean test tube and mixed well with 0.5 mL of the color reagent. The volume was completed to 5.0 mL by adding dist water. The developed color (reddish-violet) was measured at 555 nm against boiled enzyme, using Spectro UV-VIS RS spectrophotometer (Serial No. UV-VIS 0478; Labomed Inc. USA).

Determination of remaining tannins: The residual un-hydrolyzed tannins (T) in fermentation culture are detected by estimation of total polyphenol (TP) and free polyphenol gallic acid (FP) as the follow:

Step I. determination of total polyphenol (TP): Two hundred microliter of tannin solution were added in a dry clean tube and mixed with 0.5 mL of colour reagent, and then the volume is completed to 5.0 mL by adding dist water. The developed colour was measured at 555 nm. The reading of O.D×5.0 is equivalent to the amount of "TP" in 1 mL.

Step II. determination of free polyphenol (Gallic acid) (FP): One milliliter of tannin solution is transferred to a clean test tube. The tannin compounds are precipitated by adding 2.0 mL of precipitant solution. One milliliter of the mixture is transferred to Open Dwarf tube and centrifuged at 9000 rpm for 10 min. Five hundred microliter of the supernatant is transferred to a clean and dry test tube and mixed with 0.5 mL of the colour reagent. The volume was completed to 5.0 mL by adding dist water. The developed colour was measured at 555. The reading of O.D×6.0 is equivalent to of FP in 1 mL.

The net amounts of remaining tannins in 1 mL of culture filtrate are calculated as the following:

$$T = TP - FP$$

Protein determination: Soluble protein was determined according to Bradford (1976) method by measuring optical density of developed colour at 595 nm. The μg of protein was estimated using μg standard of bovine serum albumin (BSA).

Factors affecting tannase production

Effect of different incubation periods: Each fungal culture is inoculated in the autoclaved solid medium containing green tea as a tannin source. Then, the flasks were incubated at 30°C for different time periods ranging from 1.0 to 8.0 days intervals. Then, the enzyme activity is assayed.

Effect of different incubation temperatures: The solid fermentation process was carried out at different temperatures (20 to 60°C) along for 4.0 days to *Aspergillus aculeatus,* and 5.0 days to *Aspergillus awamori*. Then, the enzyme activity is assayed.

Effect of different initial pH levels: The fungal cultures are inoculated in the autoclaved SSF flasks containing moistening agent maintained at different pH ranging from 3.5 to 8.0. The contents were mixed thoroughly and then the cultured flasks were incubated at 30°C for 4.0 days to

A. aculeatus, and 5.0 days to *A. awamori.* Then, the enzyme activity is assayed.

Effect of different moisture levels: The effect of moisture levels on *A. aculeatus* and *A. awamori* for tannase production are investigated by varying moisture ratios within a moisture range of 33, 50, 60, 67, 75, 80, 83 and 86%. The flasks are incubated at 30°C after incubation period the enzymes are extracted and then the crude enzyme activity is assayed

Effect of different nitrogen sources: On equivalent nitrogen bases; $NaNO_3$ from the basal medium was replaced by KNO_3, $(NH_4)_2SO_4$, NH_4Cl and $NH_4H_2PO_4$ as example of inorganic nitrogen sources and peptone, L-asparagine and yeast extract as example of organic nitrogen sources.

Effect of different minerals: This experiment was carried by replacement of $CaCl_2$ of starting basal medium by equivalent weight of NaCl, KCl, $CuSO_4$, $FeSO_4$, $MnSO_4$ in addition of tap water as source of mineral ions.

Effect of different tannins: This experiment was carried by replacement of green tea by commercial red tea, bark of *Acacia nilotica*, leaveas of *Acacia nilotica* and wheat bran.

RESULTS

Screening of tannase producing fungi: Eleven fungal isolates were grown with green tea containing medium through SSF technique to determine the ability of fungal isolates to degrade tannins and produce tannase. These fungi including *Aspergillus aculeatus, Aspergillus flavus, Aspergillus awamori, Fusarium oxysporum, Aspergillus wentii, Rhizopus oryzae, Aspergillus fumogatus, Penicillium chrysogenum, Aspergillus japonicus, Cladosporium herbarum* and *Emericella nidulans.* The results in Table 1 indicated that *Aspergillus aculeatus* (16.4 I Ug^{-1}) and *Aspergillus awamori* (13.5 I Ug^{-1}) are the most active tannase producing fungi compared with the activity of other isolates. The result also showed lower percentage of residual tannins at the end of incubation periods in case of *Aspergillus awamori, Aspergillus japonicus* and *Aspergillus aculeatus* (RT; 24.2, 33.2 and 36.3% respectively) compared with the other tested fungi. Due to the high levels of tannase production and the high utilization of tannins; both strains of *Aspergillus aculeatus* and *Aspergillus awamori* were used in further optimization experiments.

Table 1: Screening for the most active tannase producing fungi

Fungal strains	Tannase activity (IU g^{-1})	RT (%)
Rhizopus oryzae	10.5±0.41	55.9
Aspergillus aculeatus	16.4±0.10	36.3
Aspergillus awamori	13.5±0.03	24.2
Aspergillus japonicus	11.8±0.17	33.2
Aspergillus wentii	08.6±0.18	46.8
Aspergillus fumigatus	05.2±0.27	76.2
Emericella nidulans	03.2±0.27	79.4
Aspergillus flavus	11.6±0.17	44.5
Penicillium chrysogenum	07.9±0.29	44.0
Fusarium oxysporum	06.8±0.24	66.3
Cladosporium herbarum	03.6±0.39	87.4

RT: Residual tannins

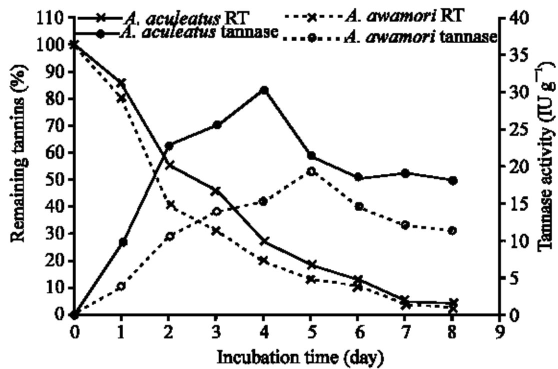

Fig. 1: Effect of different incubation periods on tannase production and tannin degradation

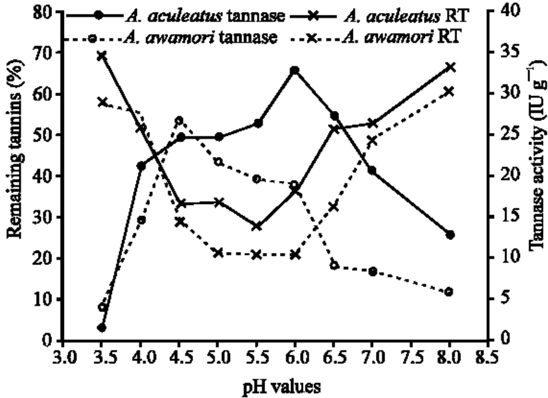

Fig. 2: Effect of different initial pH levels on tannase production and tannin degradation

Effect of different incubation periods: The time course for tannase production from *A. aculeatus* and *A. awamori* (Fig. 1) showed maximum tannase activities after 4.0 days incubation for *A. aculeatus* and after 5.0 days for *A. awamori.* The results also indicated that both fungi are able to highly consumed tannins of green tea along the studied incubation periods. However, *A. awamori* is slightly most active than *A. aculeatus.*

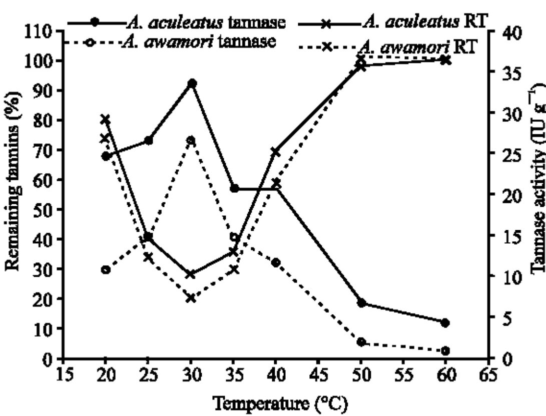

Fig. 3: Effect of different incubation temperatures on tannase production and tannin degradation

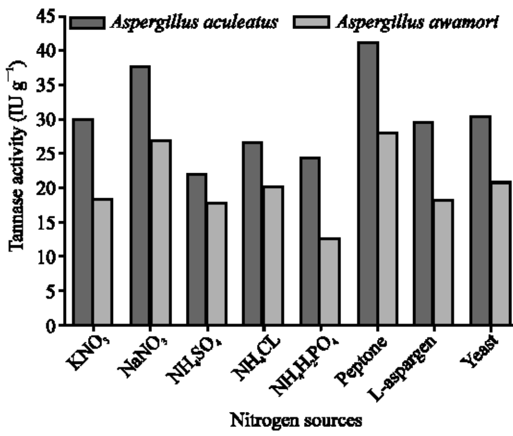

Fig. 5a: Effect of different nitrogen sources on tannase production

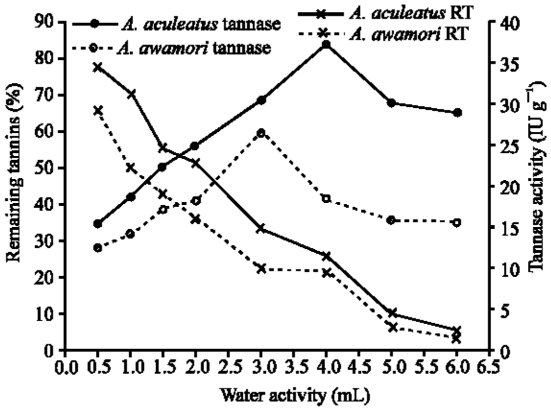

Fig. 4: Effect of different initial moisture levels on tannase production and tannin degradation

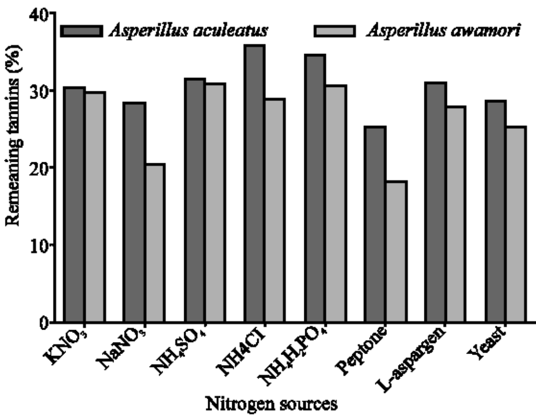

Fig. 5b: Effect of different nitrogen sources on tannin degradation

Effect of different initial pH levels: The maximum production of tannase was observed at pH 6.0 for *A. aculeatus* and at pH 4.5 for *A. awamori* as shown in Fig . 2. The result also showed that the rate of tannins degradation was highly observed at pH 5.5 in both *A. aculeatus* and *A. awamori* and the low degradation was occurred in both highly acidic and alkaline pH levels. With increasing pH levels, the rate of enzyme productivity as well as tannins consumption were decreased.

Effect of different incubation temperatures: The results in Fig. 3 showed maximum enzyme production at 30°C and also obtained that the rate of tannin degradation was highly observed at 30°C incubation in both *A. aculeatus* and *A. awamori.* However, the degradation was completely stopped at high temperature above 50°C in both *A. aculeatus* and *A. awamori.*

Effect of different initial moisture levels: The results obtained in Fig. 4 indicated that the maximum production of tannase was obtained at 80% moisture level in case of *A. aculeatus* and 75% in case of *A. awamori.* The results also showed that with increasing the moisture levels the degree of tannin degradation of green tea increased in both tested fungi.

Effect of different nitrogen sources: On an equivalent nitrogen basis, the nitrogen source in the basal medium (0.3% $NaNO_3$) was substituted by different nitrogen sources. These included organic (Yeast extract, peptone, and L-asparagine) and inorganic nitrogen sources (KNO_3, NH_4Cl, $(NH_4)_2SO_4$ and $NH_4H_2PO_4$). The results in Fig. 5a showed that peptone (organic nitrogen source) is the most suitable nitrogen source for tannase production by the both fungi followed by $NaNO_3$ (inorganic source). The

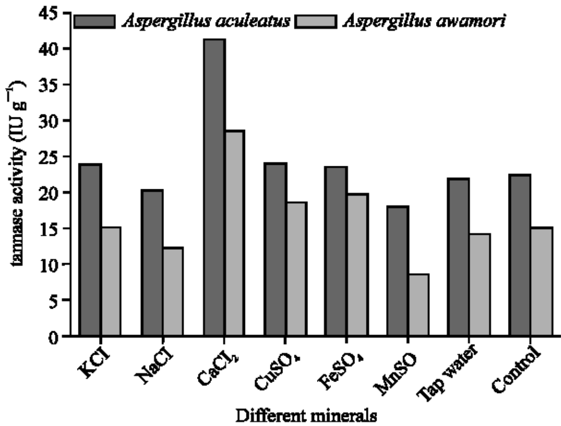

Fig. 6a: Effect of different minerals on tannase production

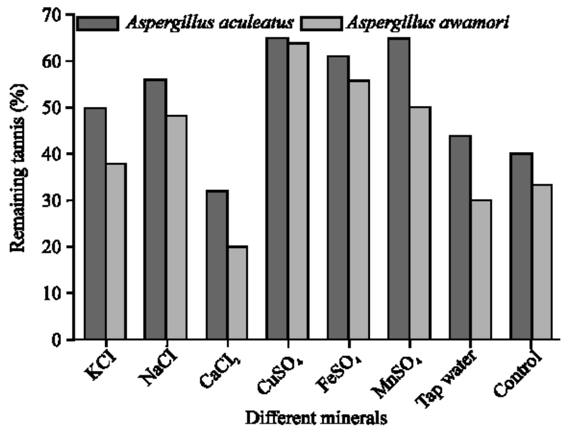

Fig. 6b: Effect of different minerals on tannin degradation

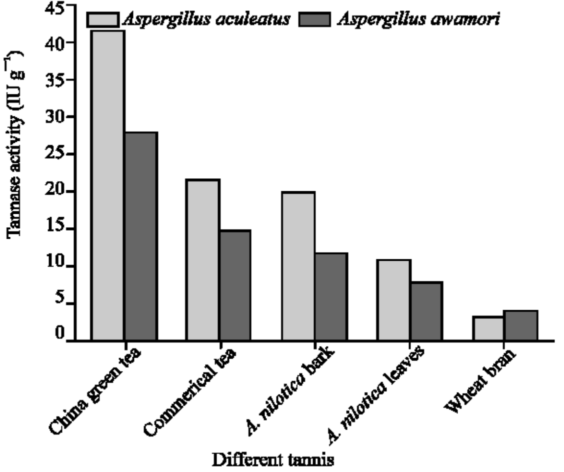

Fig. 7: Effect different tannin rich materials on tannase production

Table 2: Fold increase of tannase production after optimization culture conditions

Condition	*Aspergillus aculeatus*	*Aspergillus awamori*
Original activity (IU g^{-1})	16.4±0.10	13.5±0.03
After optimization (IU g^{-1})	41.4±0.15	27.6±0.27
Fold increases of tannase activity	2.52	2.04

results in Fig. 5b also showed high degradation of tannins by both fungi in presence of peptone and $NaNO_3$ as nitrogen source.

Effect of different minerals: Effect of different minerals on tannase production was carried out by replacement of $CaCl_2$ of the origin medium by NaCl, KCl, $FeSO_4$, $MnSO_4$, $CuSO_4$ and tap water as source of minerals. The results in Fig. 6a and 6b showed that $CaCl_2$ enhanced both tannase production and tannin degradation by both fungal strain compared with the other used minerals, while, $MnSO_4$ gave the lowest tannase yield. The lower tannin degradation was also recorded in presences of $MnSO_4$, $FeSO_4$ and $CuSO_4$ in green tea medium.

Effect of different tannin sources: The results obtained in Fig. 7 showed that media containing green tea as carbon source was the optimum for higher tannase production by both *A. aculeatus* and *A. awamori* and the lowest level of tannase was obtained in case of wheat bran.

Finally, the optimized culture medium consisted of 1.0 g of green moistened with 4.0 mL of mineral salt solution in case of *A. aculeatus* and 3.0 mL in case of *A. awamori* using 0.1 M acetate buffer pH 6.0 in case of *A. aculeatus* and pH 4.5 in case of *A. awamori* incubated at 30°C yielded 2.52, 2.04 folds increase of tannase activity from *A. aculeatus* and *A. awamori*, respectively compared with the original medium as shown in Table 2. Therefore we can conclude that both *A. aculeatus* and *A. awamori* could be used as good source for production of industrial tannase under the previous mentioned conditions.

DISCUSSION

This study indicated the dominance of *Aspergillus* species for tannase production when grown on commercial green tea as solid substrate compared with the other isolated fungi. These results are closed related with that obtained by Batra and Saxena (2005) for both *Aspergillus* and *Penicillium* genera. Furthermore, the most results reported that *Aspergillus* species are the most tannase producers and tannins degrading fungi during SSF process by different fungal strains including; different *Aspergillus niger* strains (Lekha and Lonsane,

1994; Pinto *et al.*, 2001), *Aspergillus foetidus* and *Aspergillus aculeatus* (Mukherjee and Banerjee, 2006; Banerjee *et al.*, 2007) and *Aspergillus ruber* (Kumar *et al.*, 2007).

Four and five days incubation was the optimum for higher tannase production and tannins degradation by *A. aculeatus* and *A. awamori* respectively. These results are in agreement with the results obtained from *Rhizopus oryzae* which showed maximum tannase production after 5.0 days incubation (Chatterjee *et al.*, 1996) and after 4.0 days incubation from *Aspergillus niger* (Lekha and Lonsane, 1997; Sabu *et al.*, 2005). In addition, 60 and 72 h incubation period for *Rhizopus oryzae* and *Aspergillus foetidus* respectively were optimum for maximum tannase production and tannin degradation when grown on powder of myrobalan and teri pod (Mukherjee and Banerjee, 2004). The decrease of tannase productivity after the optimum incubation periods for *A. aculeatus* and *A. awamori* could be due to the accumulation of gallic acid which increase tannase production or due to appearance of toxic metabolites during fermentation as reported by Kar *et al.* (1999).

Initial pH 6.0 and pH 4.5 was the optimum for maximum tannase production in case of *A. aculeatus* and *A. awamori* respectively. However, pH 5.5 was optimum for tannin degradation in both tested fungi. Low tannin degradation and tannase productivity was occurred in both tested highly acidic and alkaline pH levels. The optimum pH for tannase production has been reported to vary from 4.5 to 6.5 by different fungal strains such as; *Penicillium chrysogenum* (Rajkumar and Nandy, 1983), *Aspergillus niger LCF8* (Barthomeuf *et al.*, 1994), *Rhizopus oryzae* (Hadi *et al.*, 1994), *Rhizopus oryzae* and *Aspergillus foetidus* (Mukherjee and Banerjee, 2004).

This optimum temperature for tannin degradation and tannase production by *A. aculeatus* and *A. awamori* was reported at 30°C. These results are in agreements with that obtained by many investigators using different fungal strains such as; *Aspergillus japonicus* (Bradoo *et al.*, 1997), *Rhizopus oryzae* and *Aspergillus foetidus* (Mukherjee and Banerjee, 2004) and *Aspergillus aculeatus* (Banerjee *et al.*, 2007). On the other hand, Kasieczka *et al.* (2007) reported that 16°C was the optimum temperature for the maximum tannase production by *Verticillium* sp. *P9*. 80% and 75% was the optimum initial moisture level for higher tannase productivity as well as tannin degradation in case of *A. aculeatus* and *A. awamori*. Similarly, 80% initial moisture was optimum for tannase production from *Aspergillus aculeatus* DBF9, *Rhizopus oryzae* and *Aspergillus foetidus* under SSF (Mukherjee and Banerjee, 2004; Banerjee *et al.*, 2007).

The results showed that peptone is the most suitable nitrogen source for tannase production and tannin degradation by the both fungi followed by $NaNO_3$. The effective of inorganic nitrogen source; $NaNO_3$ was reported during the production of tannase and tannin degradation by *Asergillus japonicus* (Bradoo *et al.*, 1997). Similarly, Paranthaman *et al.* (2009a) reported maximum tannase production by *Aspergillus flavus* in the medium containing $NaNO_3$.

The results indicated that $CaCl_2$ enhanced tannase production and tannin degradation by both fungal strain compared with the other used minerals, while, $MnSO_4$ gave the lowest tannase yield. The knowledge about the effect of minerals on tannin degradation and tannase production under SSF is rare. However, Yamada *et al.* (1967) and El-Tanash (1997) showed that $CaCl_2$ was the most essential element for the tannase production by *Aspergillus oryzae* and *Aspergillus japonicus* under submerged fermentation medium containing tannic acid as main carbon source.

Green tea as carbon source was the optimum for higher tannase production and tannin degradation by *A. aculeatus* and *A. awamori*. These results are attributed to the richness of green tea compared with other used substrates with polyphenolic compounds of tannins especially hydrolysable forms as shown by He and Kies (1994). Production of fungal tannase was previously recorded by many investigators using the extracts of tannins rich substrates such as China green tea, commercial black tea and different parts of *Acacia* species under submerged culture using *A. oryzae* and *A. japonicus* (El-Tanash, 1997). However, many literatures were used inert substrate such as wheat bran, sugarcane pith bagasse, Paddy straw, rice straw or Cashew Apple Bagasse in presence of tannic acid for fungal tannase induction during solid state fermentation by different *Aspergillus* sp. (Lekha and Lonsane, 1994; Chatterjee *et al.*, 1996; Battestin and Macedo, 2007; Rodrigues *et al.*, 2008; Paranthaman *et al.*, 2008, 2009b). Other natural tannin rich substrates were reported to use alone for tannase production such as *Terminalia chebula* (myrobalan) and *Caesalpinia digyna* (teri pod) (Mukherjee and Banerjee, 2004), Tamarind seed powder and palm kernel cake (Sabu *et al.*, 2005), tannin-rich desert plant: *Larrea tridentata* Cov. (Trevio-Cueto *et al.*, 2007) and jamun leaves (Kumar *et al.*, 2007).

REFERENCES

Aguilar, C.N., C. Augur, E. Favela-Torre and G. Viniega-Gonzalez, 2001. Production of tannase by *Aspergillus niger* Aa-20 in submerged and solid state fermentation. J. Ind. Microbiol. Biotechnol., 56: 296-302.

Banerjee, D., K.C. Mondal and B.R. Pati, 2007. Tannase production by *Aspergillus aculeatus* DBF9 through solid state fermentation. Acta Microbiologica Immunologica Hungarica, 54: 159-166.

Barthomeuf, C., F. Regerat and H. Pourrat, 1994. Production, purification and characterization of a tannase from *Aspergillus niger* LCF 8. J. Ferment. Bioeng., 77: 320-323.

Batra, A. and R.K. Saxena, 2005. Potential tannase producers from the genera *Aspergillus* and *Penicillium*. Process. Biochem., 40: 1553-1557.

Battestin, V. and G.A. Macedo, 2007. Effects of temperature, pH and additives on the activity of tannase produced by *Paecilomyces variotii*. Electronic J. Biotechnol., 10: 191-199.

Bhat, T.K., B. Singh and O.P. Sharma, 1998. Microbial degradation of tannins: A current perspective. Biodegradation, 25: 343-357.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., 72: 248-254.

Bradoo, S., R. Gupta and R.K. Saxenaa, 1997. Parametric optimization and biochemical regulation of extracellular tannase from *Aspergillus japonicus*. Process Biochem., 32: 135-139.

Chatterjee, R., A. Dutta, R. Banerjee and B.C. Bhattacharyya, 1996. Production of tannase by solid-state fermentation. Bioprocess. Eng., 14: 159-162.

Cordoba-Salgado, M.A., J. Soriano-Santos, C. Augur and G. Viniegra-Gonzalez, 1998. Comparison of Tannase Production (Tannin-Acyl Hydrolase) by *Aspergillus niger* on Liquid and Solid State Fermentation on Polyurethane Foam. In: Biotech India, Mota, M. and E.C. Ferreira (Eds.). Prentice Hall, India, pp: 163-167.

Cordova, J., M. Gutierrez-Rojas, S. Huerta, G. Saucedo-Castaneda and E. Favela-Torres, 1996. Biomass estimation of *Aspergillus niger* growing on real and model supports in solid state fermentation. Biotechnol. Tech., 10: 1-6.

Das Mohapatra, P.K., K.C. Mondal and B.R. Pati, 2006. Production of tannase through submerged fermentation of tannin-containing plant extracts by *Bacillus licheniformis* KBR6. Pol. J. Microbiol., 55: 297-301.

El-Tanash, A.B., 1997. Studies on some fungi degrading tannins. M.Sc. Thesis, Botany Department, Faculty of Science, Mansoura University, Egypt.

Hadi, T.A., R. Banerjee and B.C. Bhattacharyya, 1994. Optimization of tannase biosynthesis by newly isolated *Rhizopus oryzae*. Bioproc. Eng., 11: 239-243.

He, Y.H. and C. Kies, 1994. Green and black tea consumption by humans: Impact on polyphenol concentrations in feces, blood and urine. Plant Foods Human Nutr. (Formerly Qualitas Plantarum), 46: 220-229.

Johnson, I.F., E.A. Curl, J.H. Bond and H.A. Fibourg, 1960. Method for Studying Soil Microflora. Burgess Publishing Co., Minnwapolis, USA.

Kar, B., R. Banerjee and B.C. Bhattachaaryya, 1999. Microbial production of gallic acid by modified solid-state fermentation. J. Ind. Microbiol. Biotechnol., 23: 173-177.

Kasieczka, B. M., K. Kuk, K. Kalinowska and M. Turkiewicz, 2007. Purification and characterization of two cold-adapted extracellular tannin acyl hydrolases from an Antarctic strain *Verticillium* sp. P9. Applied Microbiol. Biotechnol., 77: 77-89.

Krishna, C., 2005. Solid-state fermentation systems-an overview. Crit. Rev. Biotechnol., 25: 1-30.

Kumar, R., J. Sharma and R. Singh, 2007. Production of tannase from *Aspergillus* ruber under solid-state fermentation using Jamun (Syzygium cumini) leaves. Microbiol. Res., 162: 384-390.

Lekha, P.K. and B.K. Lonsane, 1994. Comparative titres, location and properties of tannin acyl hydrolase produced by *Aspergillus niger* PKL 104 in solid state, liquid surface and submerged fermentations. Process Biochem., 29: 497-503.

Lekha, P.K. and B.K. Lonsane, 1997. Production and application of tannin acyl hydrolase: State of art. Adv. Applied Microbiol., 44: 215-260.

Mahendran, B., N. Raman and D.J. Kim, 2006. Purification and characterization of tannase from *Paecilomyces variotii*: Hydrolysis of tannic acid using immobilized tannase. Applied Microbiol. Biotechnol., 70: 444-450.

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.

Mukherjee, G. and R. Banerjee, 2006. Effects of temperature, pH and additives on the activity of tannase produced by a co-culture of *Rhizopus oryzae* and *Aspergillus foetidus*. World J. Microbiol. Biotechnol., 22: 207-212.

Nicholson, W.N. and D. Rhind, 1924. The quantitative estimation of the degree of hydrolysis of gallotannin by tannase. Analyst, 49: 505-509.

Paranthaman, R., R.R. Vidyalakshmi, S. Murugesh and K. Singaravadivel, 2008. Optimisation of fermentation conditions for production of tannase enzyme by *Aspergillus oryzae* using sugarcane baggasse and rice straw. Global J. Biotechnol. Biochemist., 3: 105-110.

Paranthaman, R., R. Vidyalakshmi, S. Murugesh and K. Singaravadivel, 2009a. Optimization of various culture media for tannase production in submerged fermentation by *Aspergillus flavus*. Adv. Biol. Res., 3: 34-39.

Paranthaman, R., R. Vidyalakshmi, J. Indhumathi and K. Singaravadivel, 2009b. Biosynthesis of tannase and simultaneou determination of phenolic compounds in *Aspergillus niger* fermented paddy straw by HPLC. Global J. Biotechnol. Biochemist., 4: 93-97.

Pinto, G.A.S., S.G.F. Leite, S.C. Terzil and S. Couri, 2001. Selection of tannase producing *Aspergillus niger* strains. Brazilian J. Microbiol., 32: 24-26.

Rajkumar, G.S. and S.C. Nandy, 1983. Isolation, purification and some properties of *Penicillium chrysogenum* tannase. Applied Environ. Microbiol., 46: 525-527.

Rana, N.K. and T.K. Bhat, 2005. Effect of fermentation system on the production and properties of tannase of *Aspergillus niger* van Tieghem MTCC 2425. J. Gen. Appl. Microbiol., 51: 203-212.

Rodrigues, T.H.S., G.A.S. Pinto and L.R.B. Gonçalves, 2008. Effects of inoculum concentration, temperature and carbon sources on tannase production during solid state fermentation of cashew apple bagasse. Biotechnol. Bioprocess Engin., 13: 571-576.

Sabu, A., A. Pandey, M.J. Daud and G. Szakacs, 2005. Tamarind seed powder and palm kernel cake: Two novel agro residues for the production of tannase under solid state fermentation by *Aspergillus niger* ATCC 16620. Bioresour. Technol., 96: 1223-1228.

Shamala, T.R. and K.R. Sreekantiah, 1986. Production of cellulases and D–xylanase by some selected fungal isolates. Enzyme Microbial. Technol., 8: 178-182.

Trevio-Cueto, B., M. Luis, J.C. Contreras-Esquivel, R. Rodriguez, A. Aguilera and C.N. Aguilar, 2007. Gallic acid and tannase accumulation during fungal solid state culture of a tannin-rich desert plant (Larrea tridentata Cov.). Bioresource Technol., 98: 720-724.

Yamada, K., S. Libuchi and Y. Minoda, 1967. Studies on tannin acyl-hydrolase of microorganisms. Hakko Kogaku Zsshi, 45: 233-240.