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ORIGINAL ARTICLE

Tea stalks – a novel agro-residue for the production of tannase under solid state fermentation by *Aspergillus niger* JMU-TS528

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Abstract A novel agro-residue, tea stalks, was tested for the production of tannase under solid-state fermentation (SSF) using *Aspergillus niger* JMU-TS528. Maximum yield of tannase was obtained when SSF was carried out at 28 °C, pH 6.0, liquid-to-solid ratio (v/w) 1.8, inoculum size 2 ml (1×10^8 spores/ml), 5 % (w/v) ammonium chloride as nitrogen source and 5 % (w/v) lactose as additional carbon source. Under optimum conditions, tannase production reached 62 U/g dry substrate after 96 h of fermentation. Results from the study are promising for the economic utilization and value addition of tea stalks.

Keywords Tea stalks · Tannase · Solid-state fermentation · Agro-residue

Introduction

Tannase (Tannin acyl hydrolase, E.C.3.1.1.20) is an important hydrolytic enzyme, which specifically cracks the galloyl ester bonds of tannins to produce gallic acid and glucose. Tannase is synthesized mainly by fungi such as *Paecilomyces* spp. (Battestin and Macedo 2007), *Aspergillus* spp. and *Penicillium* spp. (Batra and Saxena 2005), and also by bacteria such as *Lactobacillus* sp. (Sabu et al. 2006), *Streptococcus gallolyticus* (Osawa et al. 1995) and *Bacillus licheniformis* (Mondal and Pati 2000). This enzyme is widely used for clarification of tea drinks, fruit juice and beer, and reduction of the antinutritional effect of tannins in animal feed; however, the major commercial applications of tannase are in the manufacture of instant tea and the production of gallic acid (Córdova-López et al. 1996). Gallic acid, a hydrolytic product of tannin, is the important substrate for chemical synthesis of propyl gallate in the food and pharmaceutical industries. Tannase is also used to remove tannin from the effluents of the leather, pharmaceutical and chemical industries (Aguilar et al. 2001; Lekha and Lonsane 1997; Mahendran et al. 2006). The methods of tannase production include solid-state fermentation (SSF), submerged fermentation and liquid surface fermentation. Compared with the other two methods, SSF is the preferred method due to its lower cost, easier operation and higher enzyme yield (Lekha and Lonsane 1994; Aguilar et al. 2001).

SSF mainly utilizes agro-industrial residues as its substrates. Application of agro-industrial residues is certainly economical and it also reduces environmental pollution. Several natural agricultural byproducts have been used for the production of tannase through SSF such as jamun leaves (Kumar et al. 2007; Yadav et al. 2008), wheat bran (Sabu et al. 2006), coffee husk (Lagemaat and Pyle 2001), and so on. Tea has been widely planted around the world and has a huge annual output. According to Commins and Sampanvejsobha

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(2008), the production of worldwide tea had reached 3.2 million tones in 2004. However, when tea is produced, tea stalks are generated as a by-product of the process. With an annual output of millions of tons, most tea stalks were generally left unprocessed and had to be discarded, which is an unnecessary waste and also leads to pollution. Meanwhile, it has been reported that processed tea contains polyphenols (20–30 %), amino acids (2–4 %), caffeine (4–5 %), sugars (2–4 %), pectic substance (4–6 %), organic acids (2.5 %), lipids (4–8 %), proteins (16 %), minerals (5 %), pigments (0.5 %), and less than 0.1 % volatile substances, besides cellulose, hemicellulose and lignin (Selvakumar et al. 1998). Similar to processed tea, the abundant phenols, sugars and amino acids in tea stalks are favorable to fungal growth. In addition, tea stalks consist largely of tannin which gives rise to tannase production. Though several natural agricultural byproducts such as jamun leaves, wheat bran and coffee husk have been used for tannase production, there are few reports on the production of tannase using tea stalks as solid substrate. In this study, tea stalks were used as a low-cost substrate for tannase production by *Aspergillus niger* JMU-TS528 through SSF. It could lay the foundation for the large-scale production and practical application of tannase.

Materials and methods

Solid substrate

Tea stalks were collected from a local tea processing factory and dried at 70 °C in an oven for 2 days until they were absolutely dry, and then powdered in a grinder.

Microorganism and inoculum preparation

Aspergillus niger JMU-TS528 was isolated from a tea plantation by the Research Center of Food Microbiology and Enzyme Engineering Technology (Jimei University) and maintained on Potato Dextrose Agar (PDA) slants at 4 °C. Before being inoculated, this strain was cultivated on PDA slants at 30 °C for 4 days and then made into spore solution (1×10^8 spores/ml) using sterile distilled water under strict aseptic conditions. Furthermore, the spore solution was cultivated synchronously at 4 °C for 1 h.

Moistening medium

A salt solution containing (%, w/v): NH_4NO_3 1.0, $MgSO_4{\cdot}7H_2O$ 0.1, NaCl 0.1 and KH_2PO_4 0.1 was used as the moistening medium for solid-state fermentation. Final pH of the medium was adjusted to 5.0.

Preparation of SSF medium for inoculation

Five grams of tea stalks powder were added to 250-ml Erlenmeyer flasks, moistened with 5 ml of salt solution, autoclaved at 121 °C for 20 min, then cooled to room temperature and inoculated with 1 ml of the fungal spore inoculum (1×10^8 spores). The contents were mixed thoroughly by stirring and then incubated at 28 °C in an incubator for the desired period of time.

Enzyme extraction

Crude enzyme was extracted from the fermented substrate by adding 100 ml of citrate buffer (pH 5.0) to each flask. The flasks were then kept on a rotary shaker for 1 h at 180 rpm. The extract was centrifuged at 3,500 *g* for 10 min. Supernatant was collected in vials and stored at 4 °C for further analysis.

Enzyme assay

Tannase activity was estimated by a modified method (Sharma et al. 2000) which is based on the formation of a chromogen between gallic acid (released by the action of tannase on propyl gallate) and rhodanine. In this modified method, propyl gallate was used as substrate to produce gallic acid. After finishing all the steps, the absorbance was read at 520 nm using a spectrophotometer. All the assays were conducted in triplicate. One unit of enzyme activity is defined as the amount of enzyme needed to liberate one micromole (1 μmol) of gallic acid per minute under defined conditions. Enzyme yield was expressed as units/gram dry substrate (U/g ds).

Determination of fungal biomass

Glucosamine in the cell wall of culture was estimated to determine the fungal biomass in fermented substrate and was expressed in mg/g ds. A given quantity of the fermented matter was taken in a tube and hydrolysed with concentrated sulphuric acid for 24 h. This mixture was then diluted and autoclaved at 121 °C for 1 h. After cooling, the mixture was filtered and the filtrate was neutralized with 1 mol/L NaOH to pH 7. The glucosamine was then estimated by the method of Sakurai et al. (1977).

Optimization of process parameters

Changes of tannase activity and biomass were detected under the initial condition to study the relationship of tanase yield to fungal biomass. The SSF medium containing the solid substrate and moistening medium described earlier

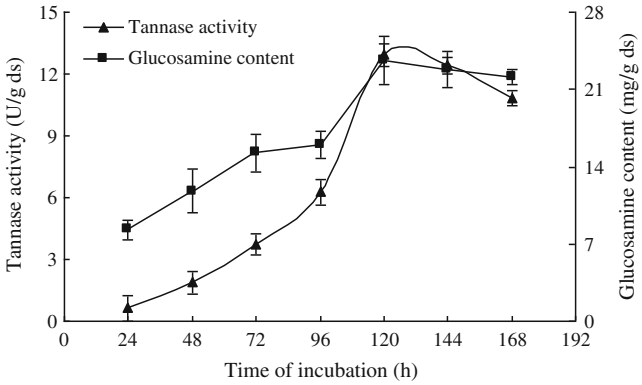

Fig. 1 Tannase production by *Aspergillus niger* JMU-TS528 under solid state fermentation (SSF) at initial fermentation condition. *Error bars* standard deviations

were taken as a basal medium and the process parameters under study were altered: pH of moisturizing agent (3–8), moisture level (moistening agent: substrate ranging 1:1–2:1), incubation temperature (20–33 °C), inoculum size (0.5–3 ml), supplementation with different nitrogen sources (ammonium chloride, sodium nitrate, ammonium sulfate, bean flour, peptone and urea at 1 %, w/v—ammonium nitrate was excluded in the SSF moistening media during this study), supplementation of additional carbon sources (lactose, starch, glucose, maltose and sucrose at 1 %, w/v) and finally a time course experiment was conducted incorporating all the optimized parameters where the biomass content (in terms of glucosamine) was also determined. The procedure adopted for optimization of various process parameters was to estimate the effect of individual parameters (keeping all other parameters as constant) and to incorporate it at the optimized level in the experiment before optimizing the next parameter. All the experiments were carried out in triplicate and the mean values were reported with standard deviation.

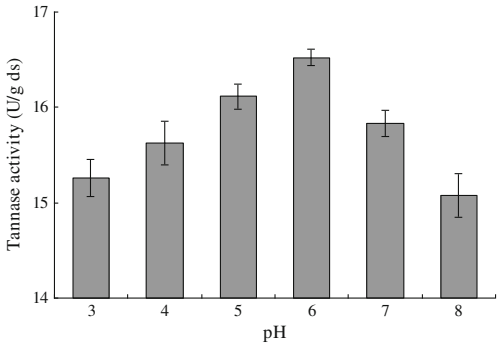

Fig. 2 Effect of pH of moisturizing agent (salt solution) on tannase production by *Aspergillus niger* JMU-TS528 in SSF. *Error bars* standard deviations

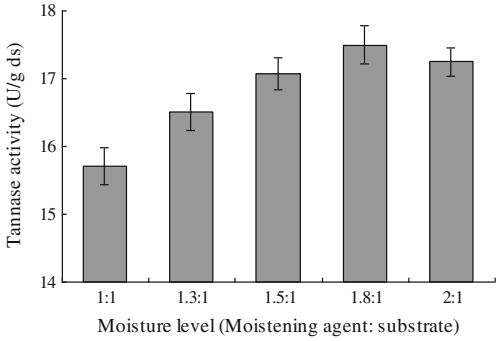

Fig. 3 Tannase production by *Aspergillus niger* JMU-TS528 in SSF with different moisture level (moistening agent: substrate v/w). *Error bars* standard deviations

Results and discussion

Changes of tannase activity and biomass under initial condition

Considering the relationship of tanase yield to fungal biomass, the changes of tannase activity and biomass were detected under the initial condition. The biomass of the fungi was estimated through the analysis of glucosamine. Glucosamine is an integral part of the cell wall and the content is positively correlated with the cell quantity, thus the fungi biomass in fermented substrate can be estimated with the content of glucosamine. From the result of Fig. 1, it can be found that tannase yield was positively correlated with biomass. This result was in agreement with earlier reports on enzyme production through SSF by fungal strains, which indicated that tannase yield was growth associated (Sabu et al. 2000). Considering our aim was to obtain the maximum tannase yield, not the ratio of enzyme yield to biomass, tannase activity was used as the index in the optimization process to reduce the amount of work.

The result (Fig. 1) demonstrated that a maximum tannase production (12 U/g ds) was obtained after 120 h of incubation. Thereafter, the enzyme production started decreasing. Significant variation ($P<0.05$) in tannase production was observed during different incubation times. The decrease in production after 120 h may be due to the excessive

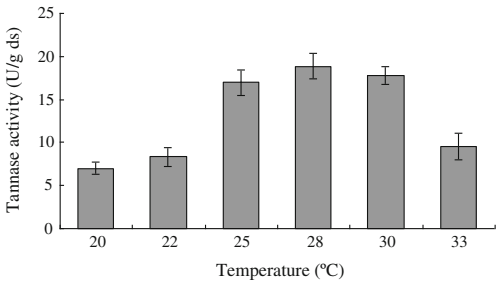

Fig. 4 Tannase production by *Aspergillus niger* JMU-TS528 in SSF at different incubation temperature. *Error bars* standard deviations

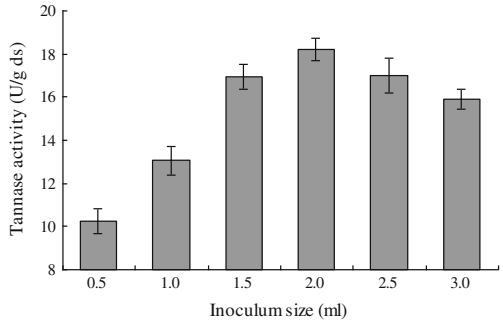

Fig. 5 Effect of inoculum size on tannase production by *Aspergillus niger* JMU-TS528 in SSF. *Error bars* standard deviations

consumption of medium, causing less biomass and affecting the enzyme synthesis (Sabu et al. 2005). Decreased enzyme yield on prolonged incubation time could also result from the accumulation of end product and toxic metabolites excreted during fermentation which hinders tannase production (Kar et al. 1999).

The effect of pH

SSF was carried out using salt solution with different pH ranging from 3.0 to 8.0. Maximum production of enzyme (16 U/g ds) was observed at pH 6.0 (Fig. 2). A significant variation ($P<0.05$) was observed in tannase production at varying pH. Similarly, Li et al. (2009) also reported an optimum pH of 6.0 for tannase production by *A. niger* B0201. The optimum pH for tannase production by different fungi has been found to vary from 4.5 to 6.5 (Rajakumar and Nandy 1983; Barthomeuf et al. 1994; Hadi et al. 1994).

Acidic environment was favorable to the growth of fungi (Yadav et al. 2008) and led to more biomass, and maybe more biomass caused higher enzyme production at pH 6.0. Any change in pH can affect the protein structure, and a decrease in enzyme activity beyond the optimum pH is likely due to enzyme inactivation or its instability.

Moisture level

The tea stalks powder was moistened using salt solution with pH 6.0 at different moisture levels. The ratios of salt solution to tea stalks powder (v/w) were 1:1, 1.3:1, 1.5:1, 1.8:1 and 2:1. A ratio of 1.8:1 was found to be the best (17 U/g ds) for tannase production (Fig. 3). Significant variation ($P<0.05$) was observed in tannase production at varying moisture levels. Initial moisture content of the solid substrate is an important factor which dictates the growth of the organism and enzyme production (Pandey et al. 1992).

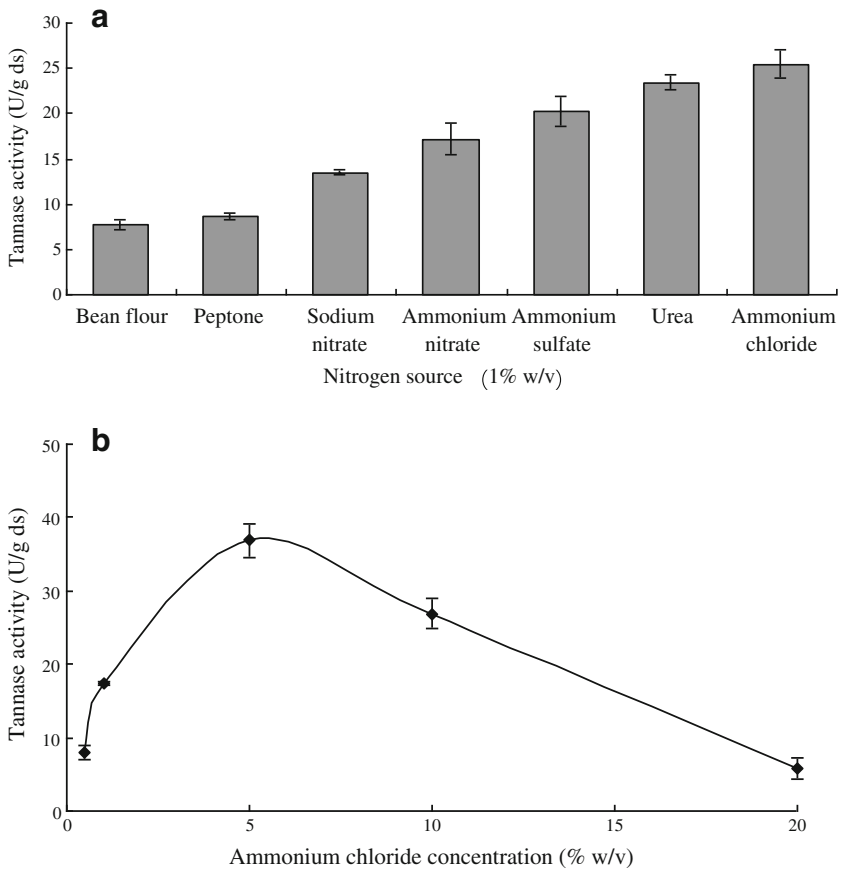

Fig. 6 Effects of nitrogen source on tannase production by *Aspergillus niger* JMU-TS528 in SSF. **a** Effect of different nitrogen sources on tannase production; **b** effect of selected nitrogen source content. *Error bars* standard deviations

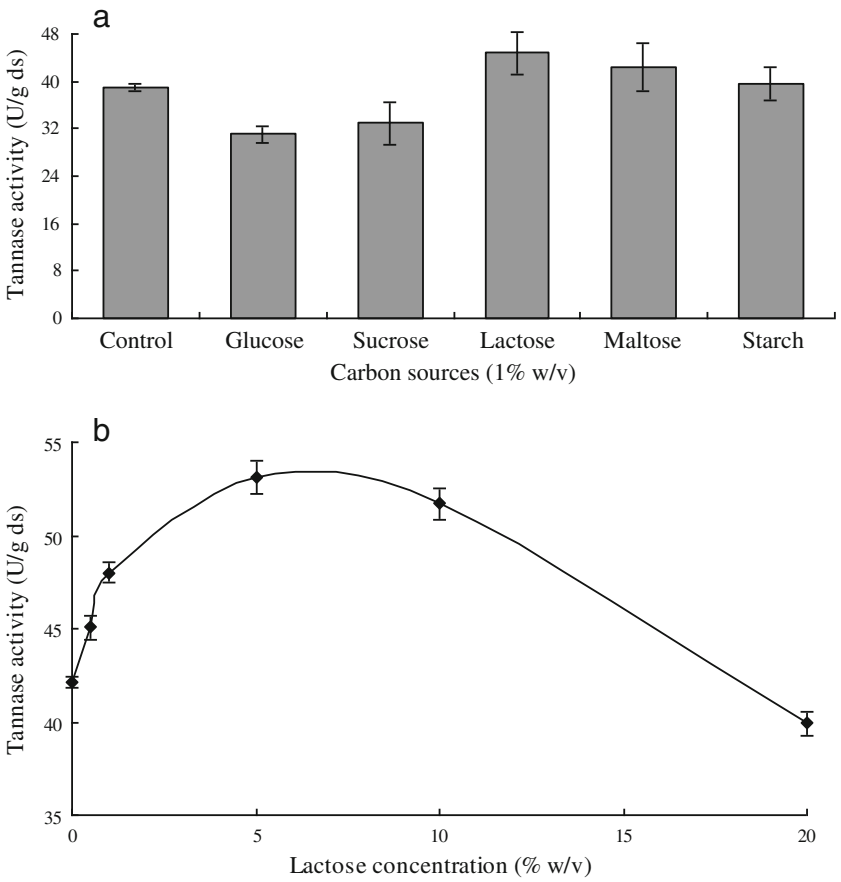

Fig. 7 Effects of additional carbon source on tannas production by *Aspergillus niger* JMU-TS528 in SSF. **a** Effect of different additional carbon sources; **b** effect of selected carbon source content. *Error bars* standard deviations

An increase in moisture level beyond a certain level was found to inhibit the production of enzyme. With increasing water content, the air content of the substrate (air occupied within the interparticle space) decreased, lower enzyme yield at higher moisture level may be due to the poor oxygen supply with increasing moisture level. However, the production of enzyme was also inhibited when the water content was too low, because insufficient water content reduced the growth and metabolism of the organism and affected enzyme production.

Incubation temperature

The study on the optimization of the incubation temperature demanded for the tannase production demonstrated that maximum enzyme yield (18 U/g ds) was observed at 28 °C (Fig. 4). Significant variation ($P<0.01$) was observed in tannase production at varying incubation temperatures. Above 28 °C, there was a decrease in enzyme production. This may be due to the fact that with increasing temperature sporulation was induced, therefore hindering mycelial growth (Kumar et al. 2007) and affecting the production of enzyme. However, when the temperature was too low, it was unfavorable to the growth of organism; the organism grew slowly at lower temperatures, leading to less biomass. An optimum temperature of around 30 °C has been reported by many workers for various fungi (Bradoo et al. 1997; Kar et al. 1999; Kar and Banerjee 2000; Sabu et al. 2005; Banerjee et al. 2007; Banerjee and Pati 2007), except Kasieczka et al. (2007) and Manjit et al. (2008) who reported optimum temperatures of 16 °C and 25 °C for the maximum tannase production, respectively. Also, a new fungal strain was found in our investigation to produce tannase, which may

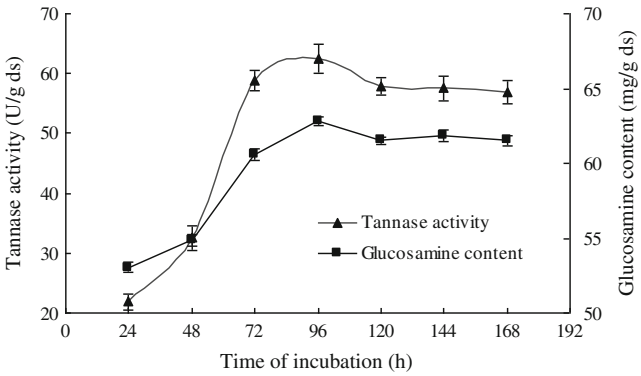

Fig. 8 Tannase producton by *Aspergillus niger* JMU-TS528 in SSF under optimum fermentation condition. *Error bars* standard deviations

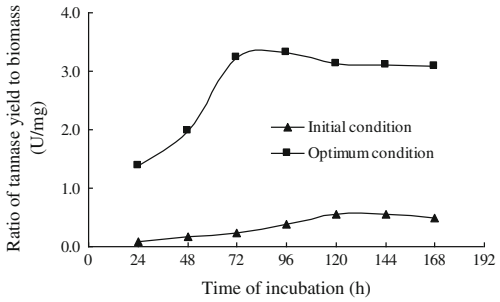

Fig. 9 Ratios of tannase yield to biomass in SSF by *Aspergillus niger* JMU-TS528 under initial and optimum fermentation condition

account for the difference in optimum temperature from other workers.

Inoculum size

Different inoculum sizes (0.5, 1.0, 1.5, 2.0, 2.5, 3.0) were tested in order to enhance tannase yield. Figure 5 showed the effect of inoculum size on tannase production; maximum tannase production (18 U/g ds) was observed at 2 ml (Fig. 5). Significant variation ($P<0.05$) was observed in tannase production at varying inoculum sizes. It is important to provide an optimum inoculum size in the fermentation process (Buzzini et al. 1993; Ray and Banik 1994). Lower levels of inoculum may not be sufficient for initiating growth of microorganisms, resulting in less biomass and lower enzyme production. An increase in inoculum size, however, ensures a rapid proliferation of biomass and enzyme synthesis (Kashyap et al. 2002). After a certain point, the enzyme production may decrease due to the depletion of nutrients, which causes a decrease in metabolic activity. A balance between proliferating biomass and available material would yield maximum enzyme production (Pandey et al. 2000; Kashyap et al. 2002).

Effect of nitrogen source

The SSF production medium was added with 1 % different nitrogen sources to replace ammonium nitrate. With the addition of bean flour, peptone and sodium nitrate, there was a decrease in enzyme production. Synchronously with the supplementation of ammonium chloride, ammonium sulfate and urea, there was an increase in enzyme production. Maximum yield (25 U/g ds) was recorded when medium was added with ammonium chloride (Fig. 6a). In view of the effectiveness of ammonium chloride, it was thought desirable to estimate the response to different concentrations in tannase production. Accordingly, ammonium chloride was added in the SSF medium at 0.5–20 % concentrations. There was a further increase in enzyme production, and maximum activity (37 U/g ds) was obtained with 5 % addition (Fig. 6b). Significant variation ($P<0.01$) was observed in tannase production at varying ammonium chloride concentrations. Nitrogen can be a significant limiting factor in the microbial production of enzymes. Possibly, the presence of ammonium chloride along with nitrogenous compounds present in the substrate promoted enhanced growth of the microorganism and consequent enzyme production (Chandrasekaran et al. 1991). Similarly, Sabu et al. (2005) also reported an increase in tannase production by fungal culture in the presence of different nitrogen sources in the case of tamarind seed powder (TSP). That tannase production was reduced when ammonium

Table 1 Comparison of tannase production using different substrates and microorganisms

Reference	Microorganism	Substrate	Fermentation style[a]	Tannase yield[b] (U/ml or U/g ds)
Sabu et al. 2005	*Aspergillus niger*	Tamarind seed powder	SSF	6.44
Sabu et al. 2005	*Aspergillus niger*	Palm kernel cake	SSF	13.03
Sabu et al. 2006	*Lactobacillus* sp.	Wheat bran	SSF	0.5
Sabu et al. 2006	*Lactobacillus* sp.	Palm kernel cake	SSF	0.5
Sabu et al. 2006	*Lactobacillus* sp.	Tamarind seed powder	SSF	0.63
Sabu et al. 2006	*Lactobacillus* sp.	Coffee husk	SSF	0.85
Kumar et al. 2007	*Aspergillus ruber*	Jamun leaves	SSF	69
Yadav et al. 2008	*Aspergillus fumigatus*	Jamun leaves	SSF	174.32
Das Mohapatra et al. 2009	*Bacillus licheniformis*	Tannic acid	SmF	0.356
Selwal et al. 2010	*Pseudomonas aeruginosa*	Amla leaves	SmF	13.65
Selwal et al. 2010	*Pseudomonas aeruginosa*	Keekar leaves	SmF	12.9
Selwal and Selwal 2012	*Penicillium atramentosum*	Amla leaves	SmF	32.8
Selwal and Selwal 2012	*Penicillium atramentosum*	Keekar leaves	SmF	34.7

[a]*SSF* solid-state fermentation; *SmF* submerged fermentation

[b]When the fermentation style is SSF, tannase yield is expressed in U/g ds; when the fermentation style is SmF, tannase yield is expressed in U/ml

chloride concentration was above 5 % could be due to the too high extracellular permeation pressure, impeding the microorganism to absorb water and nutriment from the culture medium, thus affecting the growth of the microorganism and the enzyme production.

Supplementation of carbon sources

On the basis of the addition of 5 % ammonium chloride, the SSF production medium was supplemented with 1 % different carbon sources. With glucose and sucrose being added, there was a decrease in enzyme production. Simultaneously, with supplementation of lactose, maltose, and starch, there was an increase in enzyme production. Maximum production (45 U/g ds) was gained when medium was supplemented with lactose (Fig. 7a). In this case, it was determined to evaluate different concentrations of lactose to get the optimal level for tannase production. Consequently, the medium was supplemented with 0.5–20 % lactose. Maximum enzyme yield (53 U/g ds) was obtained with 5 % concentration (Fig. 7b). A significant variation ($P<0.01$) was observed in tannase production at various lactose concentrations. Available reports on the effect of additional carbon sources on the extracellular tannase differ. Yadav et al. (2008) reported the positive effect of glucose on tannase production by *Aspergillus fumigatus* MA, while Sabu et al. (2006) found that tannase production was inhibited due to the presence of additional carbon sources. In this study, lactose was found to be an optimal additional carbon source due to the nature of the substrate and microorganism. In the matter of the decrease of tannase yield when the lactose concentration was above 5 %, this could be due to there being too much lactose in the medium causing the microorganism to use lactose as the sole carbon source, so that the tannin present in the substrate was not used, thus the induction of tannase was hampered.

Changes of tannase activity and biomass under the optimum condition

After optimizing the various process parameters, a time course study was carried out to see the cumulative effect of various physical parameters and nutrient sources on tannase production and biomass. Similarly to the initial condition, tannase yield was also positively correlated with biomass under the optimum condition (Fig. 8). After 96 h of incubation, the highest biomass and enzyme activity reached 63 mg/g ds and 62 U/g ds, respectively. The yield of tannase under optimum condition was about 5.17 times as high as the yield under initial condition.

To determine whether tannase yield is or is not related to biomass, the ratio of tannase yield to biomass (Yp/x) was calculated. As shown in Fig. 9, Yp/x at optimum condition was much higher than that under initial condition. These results could clearly illustrate the increase of tannase yield is not only related to the increase of biomass but also the other factors mentioned above (such as increasing enzyme stability).

A number of reports on the production of tannase using different substrates and microorganisms are shown in Table 1. From the results, it was found that tannase yields varied greatly when the production of tannase was carried out using different substrates and microorganisms. Among these reports, when Jamun leaves were used as substrate for tannase production by *Aspergillus ruber* and *A. fumigatus*, tannase yields could reach 69 U/g ds and 174.32 U/g ds, respectively. In this study, tea stalks were used as substrate for tannase production by *A. niger* JMU-TS528, and the maximum yield reached 62 U/g ds, which was higher than most of the yields listed in Table 1. This indicated that tea stalks had a good application prospect for tannase production.

Conclusions

In this study, tea stalks were used as a low-cost substrate for tannase production by *Aspergillus niger* JMU-TS528, developing a new way to use tea stalks. Various parameters were evaluated for maximum tannase production and, under optimum conditions, the enzyme production reached 62 U/g ds. The application of tea stalks is certainly economical and also minimizes environmental pollution.

This study has laid a foundation for the large-scale production of tannase and its practical application.

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References

Aguilar CN, Augur C, Favela-Torres E, Viniegra-Gonzalez G (2001) Production of tannase by *Aspergillus niger* Aa-20 in submerged and solid-state fermentation: influence of glucose and tannic acid. J Ind Microbiol Biotechnol 26(5):296–302

Banerjee D, Pati BR (2007) Optimization of tannase production by *Aureobasidium pullulans* DBS66. J Microbiol Biotechnol 17 (6):1049–1053

Banerjee D, Mondal KC, Pati BR (2007) Tannase production by *Aspergillus aculeatus* DBF9 through solid state fermentation. Acta Microbiol Immunol Hung 54(2):159–166

Barthomeuf C, Regerat F, Pourrat H (1994) Production, purification and characterization of a Tannase from *Aspergillus niger* LCF 8. J Ferment Bioeng 77(3):320–323

Batra A, Saxena RK (2005) Potential tannase producers from the genera *Aspergillus* and *Penicillium*. Process Biochem 40:1553–1557

Battestin V, Macedo GA (2007) Tannase production by Paecilomyces variotii. Bioresour Technol 98:1832–1837

Bradoo S, Gupta R, Saxena RK (1997) Parametric optimization and biochemical regulation of extracellular tannase from *Aspergillus japonicus*. Process Biochem 32:135–139

Buzzini P, Gobbetti M, Rossi J, Ribaldi M (1993) Utilization of grape must and concentrated rectified grape must to produce gluconic acid by *Aspergillus niger*, in batch fermentations. Biotechnol Lett 15(2):151–156

Chandrasekaran M, Lakshmanaperumalsamy P, Chandramohan D (1991) Combined effect of environmental factors on spoilage bacteria. Fish Tech India 28:146–153

Commins T, Sampanvejsobha S (2008) Development of the tea industry in Thailand. Asian J Food Agric Ind 1(01):1–16

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

Das Mohapatra PK, Maity C, Rao R, Pati BR, Mondal KC (2009) Tannase production by *Bacillus licheniformis* KBR6: Optimization of submerged culture conditions by Taguchi DOE methodology. Food Res Int 42(4):430–435

Hadi TA, Banerjee R, Bhattacharyya BC (1994) Optimization of tannase biosynthesis by a newly isolated *Rhizopus oryzae*. Bioprocess Biosyst Eng 11(6):239–242

Kar B, Banerjee R (2000) Biosynthesis of tannin acyl hydrolase from tannin-rich forest residue under fermentation conditions. J Ind Microbiol Biotechnol 25:29–38

Kar B, Banerjee R, Bhattacharyya BC (1999) Microbial production of gallic acid by modified solid state fermentation. J Ind Microbiol Biotechnol 23:173–177

Kashyap P, Sabu A, Pandey A, Szakacs G (2002) Extra-cellular L-glutaminase production by *Zygosaccharomyces rouxii* under solid-state fermentation. Process Biochem 38:307–312

Kasieczka BM, Kuk K, Kalinowska K, Turkiewicz M (2007) Purification and characterization of two cold-adapted extracellular tannin acyl hydrolases from an Antarctic strain *Verticillium* sp. P9. Appl Microbiol Biotechnol 77(1):77–89

Kumar R, Sharma J, Singh R (2007) Production of tannase from *Aspergillus ruber* under solid-state fermentation using jamun (*Syzygium cumini*) leaves. Microbiol Res 162(4):384–390

Lagemaat J, Pyle DL (2001) Solid-state fermentation and bioremediation: development of a continuous process for the production of fungal tannase. Chem Eng J 84(2):115–123

Lekha PK, Lonsane BK (1994) Comparative titres, location and properties of tannin acyl hydrolase produced by *Aspergillus niger* PKL 104 in solid-state, liquid surface an submerged fermentations. Process Biochem 29(6):497–503

Lekha PK, Lonsane BK (1997) Production and application of tannin acyl hydrolase: state of the art. Adv Appl Microbiol 44:215–260

Li YZ, Qiu SY, Bao YX, Wu XY (2009) Research on Conditions of Tannase Production from Aspergillus Niger B0201 Under Solid-state Fermentation Using Gallnut. Microbiology 36(8):1123–1129

Mahendran B, Raman N, Kim DJ (2006) Purification and characterization of tannase from Paecilomyces variotii: hydrolysis of tannic acid using immobilized tannase. Appl Microbiol Biotechnol 70 (4):444–450

Manjit AY, Aggarwal NK, Kumar K, Kumar A (2008) Tannase production by *Aspergillus fumigatus* MA under solid-state fermentation. World J Microbiol Biotechnol 24:3023–3030

Mondal KC, Pati BR (2000) Studies on the extracellular tannase from newly isolated *Bacillus licheniformis* KBR 6. J Basic Microbiol 40:223–232

Osawa R, Fujisawa T, Sly LI (1995) *Streptococcus gallolyticus* sp. nov.; gallate degrading organisms formerly assigned to *Streptococcus bovis*. Syst Appl Microbiol 18:74–78

Pandey A, Soccol CR, Larroche C (1992) Current developments in solid-state fermentation. Process Biochem 7:109–116

Pandey A, Soccol CR, Mitchell DA (2000) New developments in solid-state fermentation: I-bioprocesses and products. Process Biochem 35:1158–1169

Rajakumar GS, Nandy SC (1983) Isolation, purification, and some properties of *Penicillium chrysogenum* tannase. Appl Environ Microbiol 46(2):525–527

Ray S, Banik AK (1994) Development of a mutant strain of *Aspergillus niger* and optimization of some physical factors for improved calcium gluconate production. Indian J Exp Biol 32(12):865–868

Sabu A, Keerthi TR, Rajeev Kumar S, Chandrasekaran M (2000) L-glutaminase production by marine *Beauveria* sp. under solid state fermentation. Process Biochem 35(7):705–710

Sabu A, Pandey A, Jaafar Daud M, Szakacs G (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(11):1223–1228

Sabu A, Augur C, Swati C, Pandey A (2006) Tannase production by *Lactobacillus* sp. ASR-S1 under solid-state fermentation. Process Biochem 41(3):575–580

Sakurai Y, Lee TH, Shiota H (1977) On the convenient method for the glucosamine estimation in koji. Agric Biol Chem 41:619–624

Selvakumar P, Ashakumary L, Pandey A (1998) Biosynthesis of glucoamylase from *Aspergillus niger* by solid-state fermentation using tea waste as the basis of a solid substrate. Bioresour Technol 65(1–2):83–85

Selwal MK, Selwal KK (2012) High-level tannase production by *Penicillium atramentosum* KM using agro residues under submerged fermentation. Ann Microbiol 62(1):139–148

Selwal MK, Yadav A, Selwal KK, Aggarwal NK, Gupta R, Gautam SK (2010) Optimization of cultural conditions for tannase production by *Pseudomonas aeruginosa* IIIB 8914 under submerged fermentation. World J Microbiol Biotechnol 26(4):599–605

Sharma S, Bhat TK, Dawra RK (2000) A spectrophotometric method for assay of tannase using rhodanine. Anal Biochem 279(1):85–89

Yadav A, Aggarwal NK, Kumar K, Kumar A (2008) Tannase production by *Aspergillus fumigatus* MA under solid-state fermentation. World J Microbiol Biotechnol 24(12):3023–3030