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Production of Tannase by *Aspergillus niger* From Palm Kernel

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Abstract: Tannase production by *Aspergillus niger* was evaluated under solid-state fermentation and submerged fermentation cultures. The optimum conditions for maximum enzyme production including deferent inoculum ratios, incubation periods, initial pH values, nitrogen and carbon sources were investigated. *A. niger* was grown as stand cultures in 250 mL conical flasks containing tannic acid powder medium. The maximum production of tannase by *A. niger* was achieved at inoculum ratio of 2% (v/v), 96 h of incubation period, initial pH 5.0, yeast extract as a nitrogen sources at a concentration of 0.33 g N L^{-1} and palm kernel powder (PKP) as a carbon source at a concentration of 25% (w/v). PKP was found to be the best carbon source supporting production of 931.27 U L^{-1} min^{-1} compared with 6.25 U L^{-1} min^{-1} for wheat straw.

Key words: Tannase production, *Aspergillus niger*, yeast extract, palm kernel, wheat straw

INTRODUCTION

Tannin acyl hydrolyase (EC, 3.1.1.20) commonly called tannase is present in plants, animals and microorganisms, it is mainly produced by several microorganisms like fungi (*Aspergillus*, *Penicillium*, *Rhizopus* sp., *Trichoderma viride*, *Mucor* sp.), yeast (*Candida* sp., *Saccharomyces cerevisiae*, *Mycotorula japonica*) and bacteria (*Bacillus sp.*, *Corynebacterium* sp., *Streptococcus bovis*, *Klebsiella pneumoniae*, *Selenomonas ruminantium*) (Ayed and Hamdi, 2002; Nishitani and Osawa, 2003). Tannase can be catalyzes the hydrolysis of ester and depside bonds in hydrolysable tannins, releasing glucose and gallic acid (Aguilar *et al*., 2007; Aguilar and Gutierrez-Sanchez, 2001). Tannase is extensively used in the industrial processes such as; clarification of fruit juices, manufacture of coffee-flavored soft drinks, manufacture of instant tea and as an analytical probe for determination the structure gallic acid esters (Seth and Chand, 2000; Srivastava and Kar, 2010). Gallic acid possesses wide range of biological activities, such as antioxidant, antibacterial, antiviral, analgesic etc. As antioxidant gallic acid acts as an antiapoptotic agent and helps to protect human cells against oxidative damage. Gallic acid is also found to show cytotoxic activity against cancer cells, without harming normal cells (Bajpai and Patil, 2008).

The present study, reports the production of tannase from *Aspergillus niger* in liquid and solid culture medium by submerged fermentation and solid-state fermentation technique. Some properties of tannase have also been studied.

MATERIALS AND METHODS

Fungal strain: *Asperigillus niger* was used for screening its ability of utilizing tannic acid as main carbon source for production of fungal tannase. *Asperigillus niger* was obtained from the Laboratory of Plant Pathology of National Research Center, Cairo, Egypt.

Microorganism maintenance and inoculum preparation: *Asperigillus niger* was grown on potato dextrose agar medium (PDA). It grows rapidly at room temperature 25 to 37°C. The slant cultures were then used for further work or stored in refrigerator at 4°C. Malt medium containing malt extract (30 g L^{-1}); yeast extract (5 g L^{-1}) was used for preparing the activated fungal inocula; Tannic acid Powder Medium (TAPM) recommended by Lekha and Lonsane (1994) and Bradoo *et al.* (1997) was used for growth and tannase production.

Two media were used for tannase enzyme production the first medium (submerged fermentation), composition was as following (g L^{-1}) malt extract 30.0, tannic acid 20.0 and adjusted to pH 5.0. The second medium (solid stat fermentation), composition was as the following of ammonium sulphate 1.7 %, sodium chloride 0.1 %, sodium

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phosphate 2.0 %, PKP 25% and adjusted pH to 5.0. Spores of fungi were transferred from surface of the actively growing slants of (PDA) medium to 250 mL. conical flasks which contained 50 mL of malt medium. After incubation on a rotary shaker (120 rpm) at 32°C for 48 h, the grown cultures were employed as inocula for experimental flasks (250 mL) contained the previous media at rate of 2% (V/V) inoculum size.

Optimization of fermentation process for tannase enzyme production: Enzyme production was carried out in 250 mL conical flasks containing 50 mL each from the previous two media. Static cultures were used for studying fungal tannase production under variable environmental condition as follows.

Effect of inoculum ratio: Inoculum ratios ranged from 1 to 8% (V/V) were used with the tested fungal cultures.

Effect of incubation period: Tannase assay was performed after various incubation periods i.e., 1, 2, 3, 4, 5 and 6 days (24 to 144 h) and the tannase activity was determined according to Mondal *et al.* (2001).

Effect of initial pH: The influence of different initial pH values was studied through adjusting pH values at 3, 3.5, 4, 4.5, 5.5, 6 and 6.5 using buffer citric.

Effect of nutrients sources

Effect of nitrogen sources: Various nitrogen sources were used separately at an equivalent concentration of 0.33 g (N/L) media as recommended by Murad (1998). The nitrogen source included three inorganic salts (ammonium sulphate, ammonium chloride and sodium nitrate) and three organic sources (meat extract, yeast extract and peptone). These sources replace the original nitrogen source in the test medium.

Effect of carbon sources: Influence of various carbon sources on tannase enzyme production were studied by testing different tannins containing waste materials including banana wastes, rice straw, wheat straw, sugarcane baggase, wheat bran and palm kernel powder in range between 5% to 30% (w/v) to the fermentation media with or without addition tannic acid powder 2% (w/v). Pure tannic acid powder was also used as a sole carbon source for comparison as a control.

Assay of tannase: Tannase enzyme activity was determined by the method of Mondal *et al.* (2001). One unit of the tannase enzyme was defined as the amount of enzyme which is able to hydrolyse 1 μmole of ester linkage of tannic acid in 1 min at specific condition (pH 5.0 and 40°C).

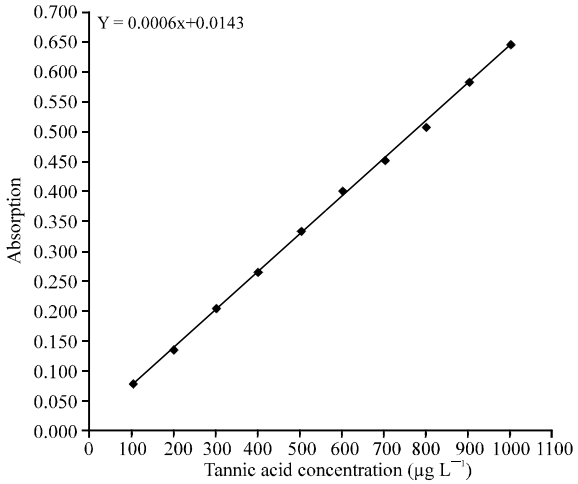

Fig. 1: Stander curve of tannic acid

Stander curve: Stander curve was design according to Mondal *et al.* (2001). Figure 1 represents the calibration curve for tannic acid, presenting linearity between 100 and 1000 μg L^{-1}.

RESULTS AND DISCUSSION

Effect of agitation on the production of tannase from *A. niger*: Data presented in Fig. 2 illustrated the difference between using shaked culture and static culture. Shaked culture exhibited the highest tannase activity than that of the static culture, being 206.25 and 178.13 (U L^{-1} min^{-1}), respectively. These results are in agreement with those reported by Purwanto *et al.* (2009) who suggested that agitation speed is a very important factor in the fermentation process since it will increase the amount of dissolved oxygen in the cultivation medium.

Effect of inoculum size on the production of tannase from *A. niger*: Fungal culture exhibited different responses to variations in inoculum size from 1 to 8% (V/V). Data presented in Fig. 3 illustrated that the production of tannase enzyme by *A. niger* was increased significantly ($p<0.05$) by increasing inoculum ratio up to 2% (V/V) being (193.75 U L^{-1} min^{-1}). Further increasing in inoculum ratio up to 8% led to decrease in tannase production by fungal cultures. These results are in line with those reported by Lokeswari and Raju (2007).

Effect of incubation period on the production of tannase from *A. niger*: The effect of different incubation periods (24 to 144 h) on tannase production is shown in Fig. 4. the tannase production was gradually increased with a rise of incubation period until 96 h and then decreased. Maximum tannase production was found after 96 h reaching

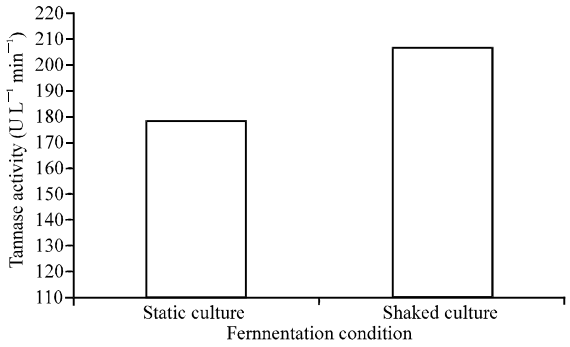

Fig. 2: Effect of agitation on the production of tannase from *A. niger*

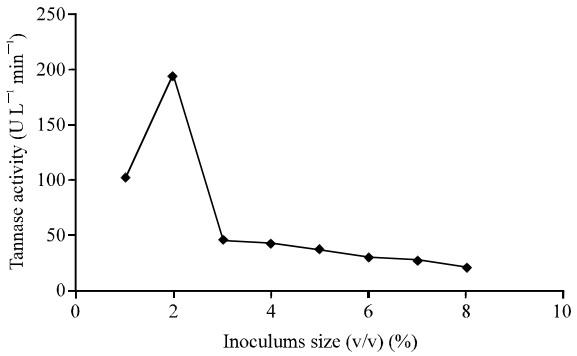

Fig. 3: Effect of inoculum size on the production of tannase from *A. niger*

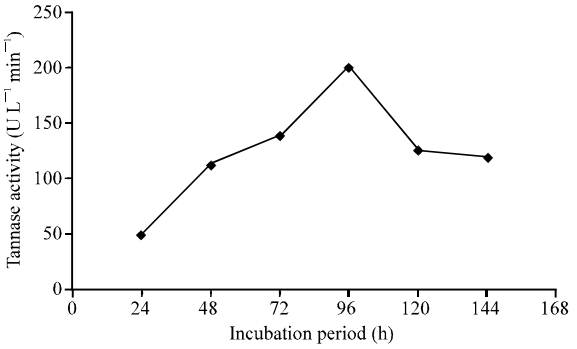

Fig. 4: Effect of incubation period on the production of tannase from *A. niger*

200.00 U L^{-1} min^{-1}. These results are in agreement with those reported by Lekha and Lonsane (1997) and Paranthaman *et al.* (2009b) who reported that maximum extra-cellular tannase production by *Aspergillus oryzae* was reached after 96 h of incubation. While, Lal *et al.* (2012) found that maximum tannase production from *A. niger* was shown at 7th day.

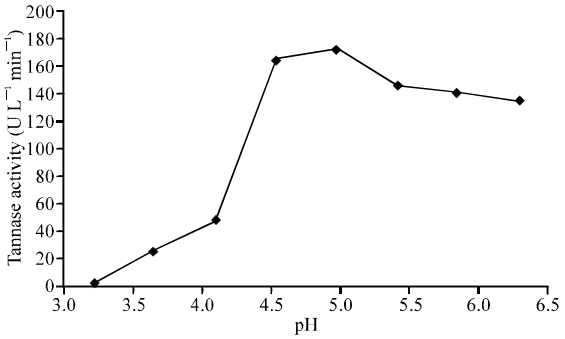

Fig. 5: Effect of the initial pH values on the production of tannase from *A. niger*

Paranthaman *et al.* (2009a) reported that the decreased enzyme yield on prolonged incubation could also be due to inhibition and denaturation of the enzyme. It has been reported before that tannase was produced during the primary phase of growth and thereafter the activity decreases either due to the decrease in production or due to enzyme degradation.

Effect of the initial pH values on the production of tannase from *A. niger*: The influence of different initial pH values (from 3.0-6.0) on tannase production was studied. The highest value of tannase activity was recorded at pH 5.0 (173.44 U L^{-1} min^{-1}) (Fig. 5). These results are in line with those reported by Barthomeuf *et al.* (1994) who confirmed that the tannase from *A. niger* contained both esterase and depsidase activity with the esterase and tannase activities peaking at a pH of 5.0. Also, Iibuchi *et al.* (1968) found that optimum pH for tannase isolated from *A. niger* was shown to be between 5.0 and 6.0, with instability occurring at a pH above pH 6.0. In addition, Lal *et al.* (2012) found that optimum pH for tannase production from *A.niger* was shown at 5.0.

In the present study, a decrease in enzyme yield with increased pH values until pH 5 was noted. This might be due to that tannase enzyme was active at acidic pH and the activity decreased as the pH approached the alkaline range. And any change of pH affects the protein structure and decline enzyme activation or its instability (Lokeswari and Raju, 2007). It could be concluded from the results that tannase from the *A. niger* needed an acidic environment to be active. Lekha and Lonsane (1997) reported that fungal tannase is an acidic protein in general.

Effect of nitrogen sources on the production of tannase from *A. niger*: The effect of supplementation of different organic (meat extract, yeast extract and peptone) and

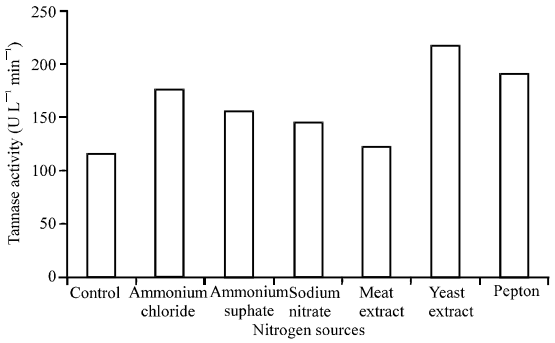

Fig. 6: Effect of nitrogen sources on the production of tannase from *A. niger*

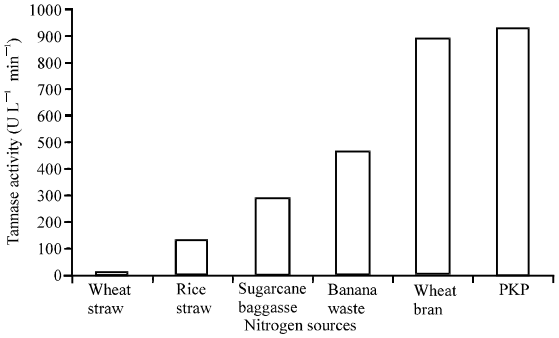

Fig. 7: Effect of substrate on the production of tannase from *A. niger*

inorganic (ammonium chloride, ammonium sulfate and sodium nitrate) nitrogen sources on tannase production was evaluated and results are shown in Fig. 6. The yeast extract was found to be the best organic nitrogen source producing the highest level of tannase activity by *A. niger* being 215.63 U L^{-1} min^{-1}. Similar results were reported by Reddy and Kumar (2012) who found that the maximum enzyme production was observed with yeast extract, but Kulkarni *et al.* (2012) found that the addition of beef extract yielded the highest enzyme activity. Ammonium chloride gave the highest level of tannase activity by *A. niger* being 175.00 U L^{-1} min^{-1} as an inorganic nitrogen source, compared with control (malt).

These results agree with those reported by Paranthaman *et al.* (2009a) who stated that the maximum activity in all nitrogen sources was observed when ammonium chloride and ammonium nitrate were used. This data indicate that the source of nitrogen should be organic for better results. Nitrogen can be an important limiting factor in the microbial production of enzymes. The presence of an additional nitrogen source in the substrate may have promoted cell growth and enzyme production (Sabu *et al.*, 2005).

Effect of substrate on the production of tannase from *A. niger*: Data in Fig. 7 illustrated the use of available agro-industrial residues as a carbon source in the growth medium with addition of Tannic Acid Powder (TAP). It was know that using agro-industrial residues are generally considered the best substrates for the process of enzyme production based on SSF and reduce the costs of enzyme production (Ellaiah *et al.*, 2002). Among of the available substrate material tested palm kernel powder (PKP) gave the maximum tannase production (931.27 U L^{-1} min^{-1}) when was fermented with *A. niger* flowed by wheat bran with activity reached 893.76 U L^{-1} min^{-1}, while wheat straw produced the lowest activity (6.25) followed by rice

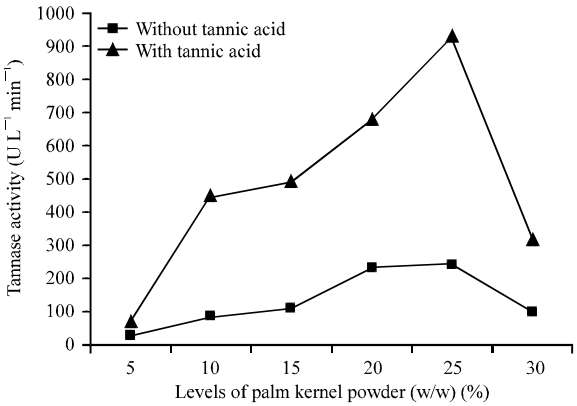

Fig. 8: Effect of PKP concentration in the growth medium on the production of tannase from *A. niger*

straw 131.25 U L^{-1} min^{-1}. These results are in coincide with those reported by Sabu *et al.* (2005) observed that the maximum tannase production was reached with palm kernel cake as a substrate after 96 h of fermentation period by *A. niger* ATCC 16620, using medium containing TAP, methylgallate and gallic cid. Also, Reddy and Kumar (2012) who found that the maximum enzyme production of tannase was achieved with using wheat bran using medium containing 2% TAP.

In addition production of tannase enzyme was observed when these materials were used with addition of 2% (w/v) TAP. While there was no production of tannase enzyme was observed when these materials were used without addition of TAP, except the case when PKP was used as substrate. The results in the present study indicated that tannase production was varied with the type of by-product used as substrate. This could be attributed to that solid materials have dual roles supply of nutrients to the microbial culture (Reddy and Kumar, 2012).

Effect of PKP concentration in the growth medium on the production of tannase from *A. niger*: Among several factors affecting tannase production, moisture level content is one of the most critical factors (Pandey *et al*., 2000). Data in Fig. 8 showed the impact of PKP concentration in the growth medium on the production of tannase from *A. niger* with or without TAP supplementation. The general results showed that maximum of tannase production were attained in medium containing TAP. The maximum tannase production was observed at 25% (w/v) PKP in both media (with or without TAP) being 931.26 and 243.75 U L^{-1} min^{-1}, respectively.

CONCLUSION

The production of tannase from *Aspergillus niger* under solid-state fermentation and submerged fermentation cultures were evaluated and standardized. These results found that tannase production from solid-state fermentation cultures superior over tannase production from submerged fermentation cultures under the optimum conditions cultures.

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