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OPTIMIZATION OF TANNASE PRODUCTION BY LOCAL ISOLATE OF *ASPERGILLUS ACULEATUS*

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Abstract

Tannase have potential application in many industrial processes such as food, beverage, pharmaceutical and chemical industries. In the present study, the production of tannase by *Aspergillus aculeatus* was optimized by adjusting some process conditions included studying the effect of incubation time, temperature, pH, nitrogen and carbon sources. Results show that the maximum tannase production found at pH 6 when incubated at 30°C for 96 hrs with tannic acid as carbon source and 0.5% yeast extract as nitrogen source.

Key words: *Aspergillus aculeatus*, Tannin acyl hydrolase, enzyme production.

Introduction

Enzymes have been widely used in the food industry to obtain the enzymatic hydrolysate or improve the rate of reaction (Toushik *et al.,* 2017).

Tannin acyl hydrolase or tannase (EC 3.1.1.20) catalyzes the hydrolysis of bonds present in tannins and gallic acid esters and release of gallic acid (Yen *et al.,* 2002). The increase in the content of gallic acid could improve the antioxidant capacities of the extracts (Chemat *et al.,* 2011).

Gallic acid is a substrate for the enzymatic or chemical synthesis of propyl gallate , the antioxidant used in the food industry (García-Najera *et al.,* 2002).

Amongst a wide variety of microbes studied, fungi from genus Aspergillus (Batra and Saxena, 2005) and bacteria from genus Bacillus (Beniwal *et al.,* 2010) are used by various industries. Recently, tannases have been isolated from bacteria that populate environments rich in vegetable content (Chaitanyakumar *et al.,* 2016).

The industrial process makes use of chemical tannic acid for tannase enzyme production but this process including synthetic substrates has untoward environmental effects. gallic acid can be produced by the microbial hydrolysis of tannic acid (synthetic or natural) by using the enzyme tannase, hence any advancement in making the production of tannase more economical and environmental friendly would have far reaching benefits (Nandini *et al.,* 2014).

This study aims to determine the optimal conditions for the production of tannase enzyme using local isolation of fungus *Aspergillus aculeatus*.

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Materials and Methods

Microorganism and inoculum: *Aspergillus aculeatus* used in this study was isolated from soil by Sedrah and ahmaed (2016).

Spores of *A. aculeatus* were inoculated on potato dextrose agar using 250 mL Erlenmeyer flasks and incubated at 30°C for 7 days. The spore suspension was prepared as described by Silveira, *et al.,* (2006). The spores were accounted according to Bind *et al.,* (2014), using hemocytometer slide and the number of spores for all isolates was adjusted to 10^7 spore/ml.

Tannase production: Submerged fermentation was carried out in 250 mL Erlenmeyer flask by taking 50 mL of mineral salt medium (in g/L): potassium dihydrogen phosphate (2.2), ammonium sulfate (5.0), magnesium sulfate heptahydrate (0.44), calcium chloride heptahydrate (0.045), manganese chloride hexahydrate (0.01), Sodium molybdate dihydrate (0.004), Ferrous sulfate heptahydrate (0.06) and tannic acid (10.0) as a carbon Source. The medium was adjusted at pH 4.5 and sterilized at 121ºC for 15 min. The solution of tannic acid was sterilized by filtration and adjusted at pH 4.5, then added to the medium (Bradoo *et al.,* 1996). Each flask was inoculated with 10^7 spores and incubated for 96 h at 30ºC (Saxena and

Saxena, 2004). Static cultures (with intermittent shaking four times a day) were used for studying tannase enzyme production under variable environmental condition. Crude enzyme was obtained by filtering.

Tannase assay: Tannase activity was estimated according to Sharma *et al.,* (2000). One unit of tannase was defined as the amount o f enzyme that release 1 µmol of gallic acid per min under specific conditions (temperature and time).

Incubation time: tannase activity assay was determind after various incubation time (24 to 144h) at 30ºC.

Temperature: The effect of five temperatures (20, 25, 30, 35 and 40ºC) on tannase production were studied. The flasks were incubated for 96 hrs.

pH: the medium pH adjasted to 3, 4, 5, 6, 7 and 8.0 by using 1N HCL and 1 N NaOH. The media was inoculated and incubated at 30ºC for 96 hrs.

Nitrogen source: The effect of various organic and inorganic nitrogen sources (yeast extract, beef extract, peptone, ammonium sulfate, potassium nitrate and sodium nitrate) at 0.5% level were tested. These sources replace the original nitrogen source in the production medium.

Carbon sources: several carbon sources such as galactose, glycerol, sucrose, sorbitol and glucose at concentrations 1% were supplemented to the culture medium.

Results and Discussion

Incubation time

incubation period play an important role in microbial metabolic activity, growth and biosynthesis of defferent enzymes (Smith *et al.,* 1996).

flasks were incubated at different time duration (24 to 144 hrs) and tannase production by *A. aculeatus* determined at 24 hrs intervals.

As showed in Fig. 1, the tannase production was increased with the increase in the incubation period at initial and found to be maximal after 96 hrs of inocubation (88 U/ml).

Further increase in incubation time will decrease the enzyme production. It might be due to lack of nutrients or aggregation of toxic materials.

These results are compatible with paranthaman *et al.,* (2009) who found that the maximum tannase production by *A. oryzae* was reached after 96 hrs. While, the maximum tannase production from *A. niger* was

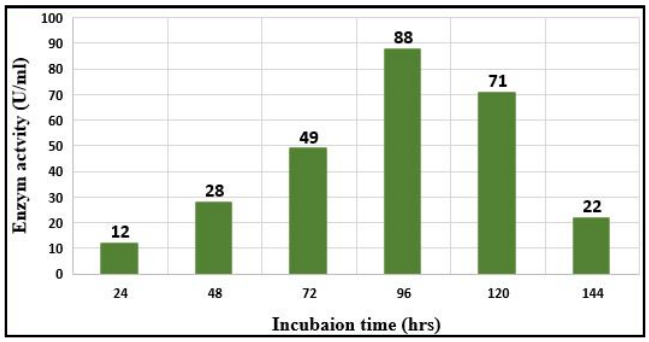

Fig. 1: Effect of Incubation time on Tannase activity from *A. aculeatus*.

shown in 168 hrs (Lal *et al.,* 2012).

Temperature

The effect of different incubation temperature, 20ºC, 25ºC, 30ºC, 35ºC and 40ºC checked on the tannase production by *A. aculeatus*.

Maximum tannase activity (89 U/ml) was observed at an incubation temperature of 30°C Fig. 2. Similar optimum temperatures were reported for tannase from fungal isolates by Lokeshwari and Reddy (2010).

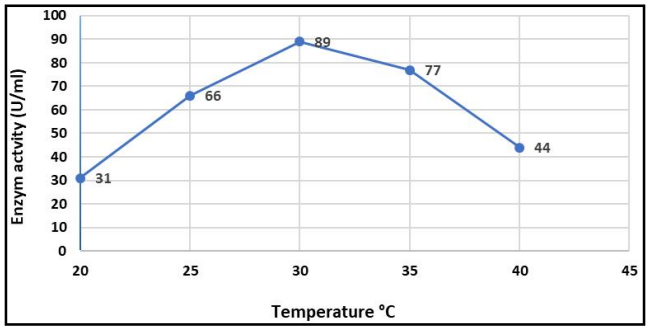

Fig. 2: Effect of temperature on Tannase activity from *A. aculeatus*.

The temperature is the most important factor among all the Physical conditions affecting the performance because both cell growth and the enzyme production are dependant on temperature (Krishna, 2005).

The decrease in tannase activity at high temperature because of change in membrane composition and cause protein degradation and fungal growth inhibition. Also the detrimental effect of high and low temperature on spore germination, cell growth, product formation, sporulation and the overall productivity of the fermentation process is reported by Govindarajan *et al.,* (2016).

Effect of pH:

The hydrogen ion concentration has a important effect on enzyme production. Different pH values (3, 4, 5, 6, 7 and 8) were chosen to studied the effect on tannase activity Fig. 3.

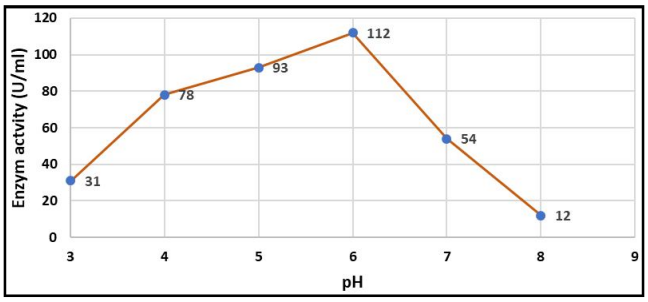

Fig. 3: Effect of pH on Tannase activity from *A. aculeatus*.

Tannase production was maximum 112 U/ml at pH 6. Similar optimum pH was reported for tannase production by Shrivastava and Kar (2009). At lower or higher pH, may be the affect stability of extracellular enzyme values and causes the rapid denaturation (Kalra and Sandhu, 1986).

Tannase production by fungal strains depends on the extracellular pH as it influences many enzymatic reactions as well as the transport of various components across the cell membrane (Ellaiah *et al.,* 2002).

Nitrogen sources

The effect of various organic (yeast extract, beef extract and peptone) and inorganic nitrogen sources (ammonium sulfate, potassium nitrate and sodium nitrate) on tannase production were tested.

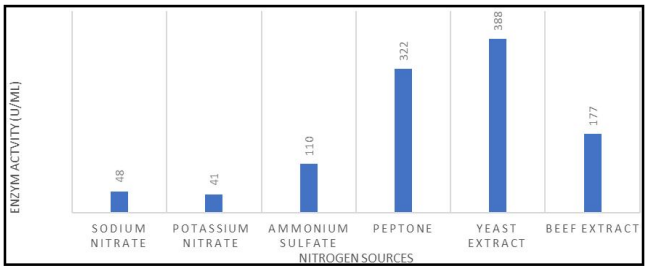

Fig. 4: Effect of Nitrogen sources on tannase activity from *A. aculeatus*.

The yeast extract was found to be the best nitrogen source producing the highest level of tannase activity by *A. aculeatus* being 388 U/ml Fig. 4.

Reddy and Kumar (2012) and Murad *et al.,* (2014) reported similar results. They found that the maximum tannase production was noticed with yeast extract, while Kulkarni *et al.,* (2012) found that the beef extract yielded the highest tannase activity.

Ammonium sulfate (as an inorganic source) gave the highest tannase activity being 110 U/ml, compard with other inorganic sources.

Nitrogen is an important factor in the enzyme production by microbs becaouse of the presence of an sdditional nitrogen source in the substrate may have assisted cell growth and production of enzymes (Sabu *et al.,* 2005).

Effect of different carbon sources

Different carbohydrates (galactose, glycerol, sucrose, sorbitol and glucose at concentrations 1%) were added in the culture medium Fig. 5.

All the carbon sources showed a adverse effect on tannase production by the Aspergillus aculeatus. Tannic acid (without added sugers) was the most suitable carbon

Table 1: Effect of carbon sources on tannase activity from *A. aculeatus*.

Carbon sources	Enzyme activity (U/ml)	Reduction (%)
control (tannic acid only)	385	-
galactose	222	42.5
lactose	147	61.9
sucrose	301	21.8
sorbitol	188	51.2
glucose	249	35.4

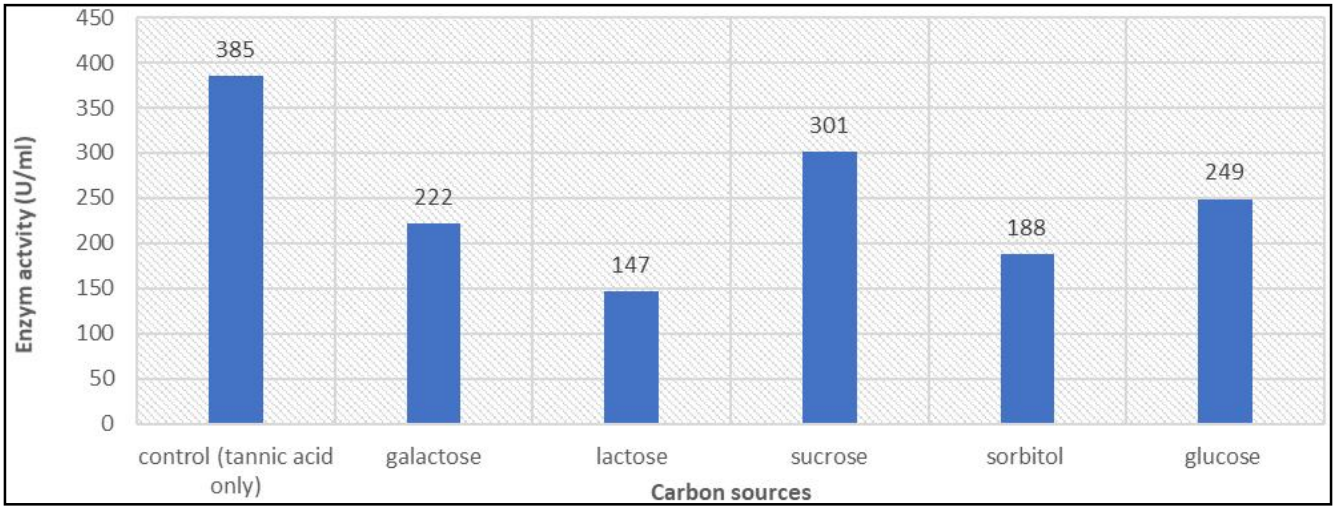

Fig. 5: Effect of carbon sources on tannase activity from *A. aculeatus*.

source for tannase induction (385 U/ml).

The reduction in tannase production varied with the t type of sugar. The highest reduction was for lactose (61.9%) followed by sorbitol (51.2%), galactose (42.5%), glucose (35.4%) and sucrose (21.8%).

Similar results were reported by Paranthaman *et al.,* (2009) and Lal and Gardner (2012).

Conclusion

The research indicated that the local isolate of Aspergillus aculeatus is very important for optimizing tannase production using tannic acid as a substrate.

The obtained results show that A. aculeatus and produced a considerable amount of the tannase. Under optimum conditions, fermentation period of 96 hrs, temperature of 30°C, pH 6.0, tannic acid (1%) and yeast extract as nitrogen source (0.5%).

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