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Production of enzyme tannase by solid state fermentation using Tamarind seed powder

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ABSTRACT

The objective of this study is to produce tannase using pure culture *Aspergillus foetidus* MTCC 3557, *Rhizopus oryzae* MTCC 1987 and co-culture (*Aspergillus foetidus* + *Rhizopus oryzae*) through solid-state fermentation. Using tamarind seed powder as substrate. To compare the yield of tannase by pure and co-culture, to enhance the yield of tannase by using carbon (glucose and tannic acid) and nitrogen sources (ammonium nitrate and sodium nitrate). Results revealed that co-culture system yielded maximum tannase production under desired incubation period of three days. Tannic acid increased the production of tannase when compared to glucose, ammonium nitrate showed minor increase in the production of tannase and the enzyme tannase capable to degrade the tannin by catalyzing the hydrolysis of ester bonds in hydrolysable tannins.

Key words: Tannase, carbon source, tamarind seed poweder, fungi, debittering.

INTRODUCTION

Tannin acyl hydrolase commonly called as tannase, catalyses the hydrolysis of ester bonds in hydrolysable tannins such as tannic acid, there by releasing glucose and gallic acid. Tannase finds its applications in debittering fruit juice (Rout S and Banerjee R, 2006), production of gallic acid (Ignacio Vaquero *et al*., 2004), detannification of food and in industrial effluent treatment. Tannase can be obtained from plant, animal and microbial sources (Sabu *et al*., 2006). Tannase can be obtained from microbes by solid-state fermentation and submerged fermentation. Solid-state fermentation is the fermentation process occurring in the absence of free water, employing a natural substrate or an inert support. Solid-state fermentation is a batch process using natural heterogeneous materials containing complex polymers like lignin, pectin and lingo cellulose. Bacteria, fungi and yeast can grow on solid substrate and find application in solid-state fermentation.

The objective of this study is to produce tannase using pure culture *Aspergillus foetidus* MTCC 3557, *Rhizopus oryzae* MTCC 1987 and co-culture (*Aspergillus foetidus* + *Rhizopus oryzae*) through solid-state fermentation. Using tamarind seed powder as substrate, to compare the yield of tannase by pure and co-culture and to enhance the yield of tannase by using carbon (glucose and tannic acid) and nitrogen sources (ammonium nitrate and sodium nitrate).

MATERIAL AND METHODS

Fungi

Aspergillus foetidus MTCC 3557, *Rhizopus oryzae* MTCC 1987 and co-culture (*Aspergillus foetidus* + *Rhizopus oryzae* (1:1)) were grown and maintained in potato dextrose agar.

Substrate

Tamarind seed powder (TSP) was obtained after removing the fruit pulp from tamarind fruit pod.

Production medium

Tamarind seed powder (solid substrate), moistening media (Magnesium sulphate 0.1 g, Sodium chloride 0.1 g, Distilled water 100 ml) and various carbon (glucose and tannic acid) and nitrogen sources (ammonium nitrate ad sodium nitrate) were used.

Experimental set up

1. 10 g TSP + 20 ml moistening media + 1 % glucose + 0.1 % ammonium nitrate
2. 10 g TSP + 20 ml moistening media + 1 % tannic acid + 0.1 % ammonium nitrate
3. 10 g TSP + 20 ml moistening media + 1 % glucose + 0.1 % sodium nitrate
4. 10 g TSP + 20 ml moistening media + 1 % tannic acid + 0.1 % sodium nitrate

Inoculation (Sabu et al., 2005)

To each experimental setup, 1 ml of spore suspension (11 x 10^9 spores) of *Aspergillus foetidus* and *Rhizopus oryzae* were added. Then spore suspensions of *Aspergillus foetidus* and *Rhizopus oryzae* in the ratio 1: 1 were added (pure culture). The inoculated flasks were incubated at 30^{0}C for 3, 5, 7 days.

Enzyme extraction

The fermented substrate was mixed thoroughly by keeping the flasks on rotary shaker at 150 rpm for 10 minutes after adding 50 ml of distilled water with 0.01 % tween 80 (Sabu *et al*., 2005). Crude enzyme from the fermented substrate was extracted by direct filtration using watmann no.1 filter paper. The filtrate was collected in vials and preserved at 4^{0}C.

Tannase assay (Mondal et al., 2007)

The activity tannase was analyzed by the colorimetric method, based on the changes in the optical density of the substrate tannic acid after enzymatic reaction at 530 nm and tannase activity had been calculated by using this formula

$$\text{Volume activity (V/ml)} = \frac{(A_0\text{-}A_s)\times 20.3\times 10\text{ml}\times 1.04}{0.71\times 0.05\text{ ml}\times 10\text{ min}}$$

A_0 – Absorbance of blank

A_S – Absorbance of test

20.3 – Micromoles of tannic acid in 1.0 ml of substrate solution

0.71 - Change in absorbance after template hydrolysis of 20.3 µm if tannic acid under assay conditions

1.04 – a factor for correction between the methods of Iibuchi *et al*., 1968

Application of tannase enzyme in fruit juice debittering

Juice preparation

Pomegranates (*Punica granatum*) were washed with water to remove any adhering substances. The rind, rag and membrane of the fruit sacs were removed mechanically. Juice was extracted using mixer grinder, followed by filtration through cheese cloth. The extracted juice was stored at 4°C until use.

Preparation of gelatin

Gelatin was pre-swelled by dissolving 1 g of gelatin in 1 liter of dist. Water and heated at 60^{0}C for 2 hours. 1:1 volume of gelatin and tannase was mixed just before treatment.

Treatment of juice with tannase

To 10 ml of fruit juice, 1 ml of tannase was added. The control test tube was added with 1 ml of water instead of tannase, tubes were incubated at 37°C with gentle shaking up to 120 minutes and tubes were placed in a water bath at 50°C for 10 minutes to deactivate the enzyme.

1 ml of the aliquot of treated and untreated juice were subjected to the estimation of the following: Tannin content (Folin Ciocalteau reagent), total sugar (Mann and Saunder, 1960), ascorbic acid, protein (Lowry's method), total phenol (Folin Ciocalteau reagent) and pH determination.

RESULTS AND DISCUSSION

A number of microorganisms including bacteria, fungi and yeast have been reported to produce tannase that catalyzes that hydrolysis of ester bonds present in the hydrolysable tannins and gallic acid esters. (Keshab C Mondal, 2001).

In the present, study the fungi *Aspergillus foetidus, Rhizopus oryzae* obtained from MTCC and co culture from *Aspergillus foetidus, Rhizopus oryzae* was tested for the production of tannase. Rout and Banerjee (2006) have carried out a similar study.

The substrate used for the production of tannase was tamarind seed powder (a similar work was done by Sabu *et al*., (2005). Extra cellular production of tannase by *Aspergillus foetidus, Rhizopus oryzae* (pure culture) and co culture under solid-state fermentation using tamarind seed powder was evaluated. Out of these, co- cultures system yielded maximum tannase under desired incubation period of three days. Sabu et al., (2003) and Rout et al., (2006), obtained a similar result. Maximum production was at 7 days in the case of *Aspergillus foetidus* and *Rhizopus oryzae*. However, the production declined on further incubation in the case of co culture system. Rout and Banerjee (2006) obtained a similar result. Between the organism *Aspergillus foetidus and Rhizopus oryzae,* the former showed maximum production on the 7 th day.

The effect of supplementation of different carbon sources on tannase production was evaluated. Glucose and tannic acid was used as carbon source.

A similar study was carried out by Sabu *et al*., (2005). Tannic acid as a carbon source increased the production of tannase when compared to glucose.

Table 1: Glucose as carbon source and ammonium nitrate as nitrogen source

Type of culture	Name of Organism	Incubation period (days)	Enzyme activity (U/ml)
Pure culture	*Rhizopus oryzae*	3	19.1
		5	21.7
		7	24.3
	Aspergillus foetidus	3	20.6
		5	23.2
		7	27.7
Co-culture	*Rhizopus oryzae* + *Aspergillus foetidus*	3	30.6
		5	23.5
		7	16.2

Table 2: Tannic acid as carbon source and ammonium nitrate as nitrogen source

Type of culture	Name of Organism	Incubation period (days)	Enzyme activity (U/ml)
Pure culture	*Rhizopus oryzae*	3	22.9
		5	25.4
		7	28.3
	Aspergillus foetidus	3	24.5
		5	28.0
		7	30.7
Co-culture	*Rhizopus oryzae* + *Aspergillus foetidus*	3	32.4
		5	26.3
		7	19.1

The effect of supplementation of different inorganic nitrogen sources on tannase productions was evaluated. Ammonium nitrate and sodium nitrate were used as nitrogen source. Effect of ammonium nitrate showed a minor increase in the production of tannase when compared to sodium nitrate.

In the present study, an attempt had been made for degradation of tannins in pomegranate

Table 3: Glucose as carbon source and sodium nitrate as nitrogen source

Type of culture	Name of Organism	Incubation period (days)	Enzyme activity (U/ml)
Pure culture	*Rhizopus oryzae*	3	18.3
		5	20.0
		7	23.6
	Aspergillus foetidus	3	19.1
		5	22.3
		7	26.0
Co-culture	*Rhizopus oryzae* + *Aspergillus foetidus*	3	28.8
		5	21.9
		7	15.8

Table 4: Tannic acid as carbon source and sodium nitrate as nitrogen source

Type of culture	Name of Organism	Incubation period (days)	Enzyme activity (U/ml)
Pure culture	*Rhizopus oryzae*	3	21.7
		5	24.2
		7	26.9
	Aspergillus foetidus	3	23.1
		5	27.5
		7	28.7
Co-culture	*Rhizopus oryzae* + *Aspergillus foetidus*	3	31.2
		5	25.2
		7	18.2

Table 5: Effect ot tannase on the debittering of *Pomegranete juice*

	Effect of *Rhizopus oryzae* tannase on physical and chemical parameters of fruit juice		
S.No	Parameter	Non-treated juice	Treated juice
1	*Tannin content*	290 mg/100 ml	163 mg/100 ml
2	Total sugars	11.2 mg/ 100 ml	11.9 mg/100 ml
3	Abscorbic acid content	22.8 mg/ 100 ml	19.3 mg/100 ml
4	*Protein*	1.0 mg/ 100 ml	0.92 mg/100 ml
5	Total phenolics	2.1 mg/ 100 ml	1.8 mg/100 ml
6	pH	4.2	4.0

juice, which is responsible for the bitterness in the juice. A similar study was carried out by Rout and Banerjee (2006). The enzyme tannase degraded the tannins by catalyzing the hydrolysis of ester bonds in hydrolysable tannins.

In the present study, the combined effect of hydrolysis and precipitation of tannin by tannase (1 ml) and gelatin (1 ml) resulted in tannin degradation. A decrease in tannin content from 2.9 mg/ml to 1.6 mg/ml with above treatment could be attributed to the ability of gelatin to precipitate tannin. A similar result was obtained by Rout and Banerjee (2006).

In the present study the effect of tannase on parameters like ascorbic acid, carbohydrate, protein, total phenolics of pomegranate juice were

Table 6: Effect of *Aspergillus foetidus* tannase on physical and chemical parameters of fruit juice

S.No	Parameter	Non-treated juice	Treated juice
1	*Tannin content*	290 mg/100 ml	145 mg/100 ml
2	Total sugars	11.2 mg/ 100 ml	12.2 mg/100 ml
3	Abscorbic acid content	22.8 mg/ 100 ml	18.1 mg/100 ml
4	*Protein*	1.0 mg/ 100 ml	0.8 mg/100 ml
5	Total phenolics	2.1 mg/ 100 ml	1.8 mg/100 ml
6	pH	4.2	4.0

Table 7: Effect of Co-culture tannase on physical and chemical parameters of fruit juice

S.No	Parameter	Non-treated juice	Treated juice
1	*Tannin content*	290 mg/100 ml	120 mg/100 ml
2	Total sugars	11.2 mg/ 100 ml	13.4 mg/100 ml
3	Abscorbic acid content	22.8 mg/ 100 ml	17.4 mg/100 ml
4	*Protein*	1.0 mg/ 100 ml	0.6 mg/100 ml
5	Total phenolics	2.1 mg/ 100 ml	1.6 mg/100 ml
6	pH	4.2	4.0

studied. The ascorbic acid and phenolics act as anti-oxidant and improved the colour and palatability of juice. The oxidizing action reduces the available oxygen in the immediate environment making ascorbic as an effective oxidant. Ascorbic acid inhibits browning reaction by reducing the quinines back to original phenol components.

The action of tannase in the fruit juice was studied for the changes in ascorbic acid content, total phenolics, carbohydrates, pH. After this study, it was found that action of tannase showed a considerable increase in sugar content in fruit juice from 0.1 mg/ml to 0.2 mg/ml because the enzyme acts on the complex sugar moieties present in the fruit juice and release free sugars in the juice. Action of tannase did not show any considerable changes in parameters like ascorbic acid, total phenol, pH. A similar result was obtained by Rout S and Banerjee (2006).

With the remarkable decrease in tannin content of the juice and a slight increase in the sugar content showed the effective action of tannase and gelatin with no considerable changes in total phenol, protein content and pH.

REFERENCES

1. Ignacio V, Angela M and Rosario M. Tannase activity by lactic acid bacteria isolated from grape must and wine. *International Journal of Food Microbiology*, **96**(2): 199-204 (2004).
2. Iibuchi S, Minoda Y & Yamada K. Studies on tannin acyl hydrolase of microorganisms Part III. Purification of the enzyme and some properties of it. *Agricultural and Biological Chemistry*, **32**(7): 803-809 1968.
3. Mann F G and Saunders B C. *Practical Organic Chemistry*. Longoman 4th Ed (1960).
4. Mondal KC, Banerjee D, Banerjee R & Pati BR. Production and characterization of tannase from *Bacillus cereus* KBR9. *Journal of General and Applied Microbiology*., **47**: 263-267 (2001).
5. Mondal KC, Banerjee D, Jana M and Pati BR. Colorimetric assay method for determination of the tannin acyl hydrolase (EC 3.1.1.20) activity. *Analytical Biochem*., **295**(2): 168-171 (2001).
6. Rout S and Banerjee R. Production of tannase under mSSF and its application in fruit juice debittering. *Indian Journal of Biotechn*., **5**: 346-350 (2006).
7. Sabu A, Augur C, Swati C, Pandey A. Tannase production by *Lactobacillus* sp. ASR-S1 under solid-state fermentation. *Process Biochem*., **41**: 575-580 (2006).
8. Sabu, Abdulhameed, Kiran, G. Shegal and Pandey, Ashok. Purification and characterization of tannin acyl hydrolase from *Aspergillus niger* ATCC 16620. *Food Technology and Biotechnology*, **43**(2): 133-138 (2005).