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GALLIC ACID PRODUCTION AND TANNASE ACTIVITY OF PENICILLIUM PURPUROGENUM STOLL EMPLOYING AGROBASED WASTES THROUGH SOLID STATE FERMENTATION : INFLUENCE OF CARBON AND NITROGEN SOURCES.

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ABSTRACT

The influence of various carbon sources and nitrogen sources on tannase and gallic acid production in SSF by *P. purpurogenum* BVG7 was studied. Sugars were beneficial only up to 0.2% concentrations and higher levels inhibited tannase and gallic acid production. Amongst the various N compounds (NH_4NO_3, NH_4Cl, $NaNO_3$, and KNO_3) supplemented to the substrates, NH_4NO_3 even at much lower concentration was the most beneficial even at lower concentrations than others for gallic acid production.

INTRODUCTION

Use of microorganisms for producing gallic acid through suspended solid and submerged fermentations has been of late receiving more attention since the product finds wide applications in pharmaceutical and chemical industries due to its varied biological activities (antioxidant, anti-apoptotic, antibacterial, antiviral, analgesic etc.) and also being precursor of trimethoprim, propyl gallate and some dyes (Pourrat et al., 1987; Mondal, 2001). Gallic acid production through the mediation of tannase enzyme of microbial origin through either SmF or SSF utilizes the agrobased wastes, thereby reducing environmental pollution as well as harnessing wealth from waste. Solid state fermentation is considered to be more advantageous over the submerged fermentation (Aguilar et al., 2001). Adapting SSF necessitates the process of optimization of fermentation parameters including physico-chemical parameters. Once the basic fermentation parameters like fermentation period, moisture content, inoculum size, substrate concentration, *p*H, and temperature are optimized, it becomes necessary to attempt increases in product yields. Since the agrobased wastes are generally ill-defined substrates, supplementation of various carbon and nitrogen sources, other organic substances like fatty acids, alcohols, acids, vitamins etc., have proved to be beneficial in achieving higher product yields. The present report deals with the evaluation of influence of certain C and N sources on tannase and gallic acid production by *Penicillium purpurogenum* BVG7 from agrobased wastes like Acacia pods, redgram husk, sorghum husk and spent tea powder through SSF.

MATERIALS AND METHODS

The fungal strain, *P. purpurogenum* BVG7, was isolated from the soil samples in the vicinity of leather industries, agricultural fields and cobbler's places and maintained on 2% malt extract slants at 32^0C. The slants were subcultured routinely at an interval of 4-5 weeks and the freshly grown slant cultures were used for the experimental studies. The substrates used in the present study were *Acacia* pods, redgram husk, sorghum husk and spent tea powder. Each substrate was dried, finely powdered in a mixer mechanically and then 15 g of each substrate was taken in a 50 ml Ehrlenmeyer flask. The initial culture conditions maintained were moisture level of 70%, *p*H 5.5, and inoculation with 4 ml inoculum (2×10^7 spores/ml) and incubated at 30^o C for 96 hr. In the studies involving the influence of carbon sources on the process of tannase and gallic acid production, glucose, fructose, lactose and sucrose were separately added to each substrate in the varying concentrations of 0.01, 0.05, 0.1, 0.2, .05, 1.0, 3.0, and 5.0 g/100 g substrate. In the studies pertaining to the influence of nitrogenous compounds, ammonium nitrate, ammonium chloride, sodium nitrate and potassium nitrate were separately added to each substrate in varying concentrations of 250, 500, 750, 1000, 1500 and 2000 mg/100 g substrate. Suitable controls were maintained with moisture level of 70%, *p*H 5.5, and inoculation with 4 ml inoculum (2×10^7 spores/ml) and incubated at 30^o C for 96 hr for each substrate. The results are the mean of five replicates.

Tannic acid estimation was as done per the protein precipitation method of Haggerman and Butler (1978) employing bovine serum albumin (BSA) as the standard protein. Tannic acid content of the substrates and the fermentation medium were

estimated as per Ibuchi et al. (1967) and the per cent yield of gallic acid was calculated based on the estimation of available tannic acid in the fermented medium.

RESULTS

The results on the influence of sugar supplementation in different concentrations to the four substrates on tannase activity and gallic acid production of *P. purpurogenum* is presented in Fig. 1 and 2, taking the example of glucose supplemenatation. Tannase activity and gallic acid production tended to increase gradually up to 0.2 g glucose/100 g substrate, while higher supplementations of glucose sharply decreased both the enzyme activity and acid production. At 5.0 g glucose level, enzyme activity and gallic acid production were almost inhibited. Same trend was observed in all the substrates. Enzyme activity and gallic acid production were maximal in *Acacia* pods, followed by redgram husk and sorghum husk, the least being in the spent tea powder. The supplementation of lactose and sucrose also led to similar observations, maximum tannase activity and gallic acid production being recorded at 0.2 g/100 g substrate. Only in case of fructose, higher level of fructose supplementation (0.5 g fructose/100 g substrate) was necessary to yield maximum enzyme and acid production (Tables 1 and 2). Higher levels of fructose additions sharply decreased the enzyme activity and acid production, as in case of other sugars.

The results on the influence of nitrogen supplementation in different concentrations to the four substrates on tannase activity and gallic acid production of *P. purpurogenum* are presented in Fig. 3 and 4, taking example of NH_4NO_3 addition. Tannase and gallic acid production tended to increase up to 500 mg NH_4NO_3/100 g substrate, while higher supplementations of the salt sharply decreased both the enzyme activity and acid production. At 2000 mg NH_4NO_3/100 g substrate, enzyme activity and gallic acid production were almost inhibited. Similar trend was observed in all the substrates. Enzyme activity and gallic acid production were maximal in *Acacia* pods, followed by redgram husk and sorghum husk, the least being in the spent tea powder. The supplementation of $NaNO_3$ and KNO_3 also led to similar observations, maximum tannase activity and gallic acid production being recorded at 1000 mg/100 g substrate. But in sorghum husk, higher level (1500 mg/100 g substrate) of NH_4Cl_4 was required to effect maximum enzyme activity and acid production. Only in case of NH_4NO_3, a lower level of 500 mg/100 g substrate was required to cause maximum enzyme and acid productions (Tables 3 and 4).

DISCUSSION

The present study indicates that amongst the various sugars, glucose was the most beneficial sugar for the production of tannase activity and gallic acid production by *P. purpurogenum* BVG7. The next beneficial sugar was lactose, with fructose closely following and sucrose the least. All sugars were beneficial only up to concentrations of 0.2 g/100 g substrate and thereafter in their higher concentrations, enzyme activity and gallic acid production tended to decrease sharply (Table 1 and 2). Earlier workers (Hadi et al., 1994; Aguilar et al., 2001a; Van de Lagemaat and Pyle, 2001; Fumihiko and Kiyoshi, 1975) had employed 0.06 to 7.0% glucose and sucrose to promote tannase production by various fungal species and reported that excepting glucose other sugars had no beneficial effects on the activities and hence, glucose appeared to be the most favored of the sugars. Lekha and Lonsane (1997) too reported that the presence of other readily utilizable carbon sources did not enhance tannase production of *A. niger* PKL 104. The decrease in tannase and gallic acid production by higher concentrations of sugars appear to be due to the osmotic stress (Bradoo et al., 1977).

The nitrogen sources supplemented to the various substrates in the present study were all equally slightly beneficial to *P. purpurogenum* BVG7 as far as tannase production is concerned (Table 3). But their influence on gallic acid production was more pronounced. NH_4NO_3 was most beneficial even at 500 mg/100 g substrate concentration. Other forms of N sources (NH_4Cl, $NaNO_3$ and KNO_3) to were beneficial to the organism under study but considerably at higher levels of 2000 mg/100 g substrate for gallic acid production (Table 4). Earlier workers reported NH_4NO_3 to be a better nitrogen source for tannase production since the fungal species were able to utilize N from both NH_4 and NO_3 (Nagib and Saddik, 1960; Bradoo et al., 1997; Lekha and Lonsane, 1997; Shamina Begum, 2006). Battestin and Macedo (2007) too reported that NH_4NO_3 had significant influence on tannase production than KNO_3. In the present study too, NH_4NO_3 not only stimulated tannase and gallic acid production at lower concentrations but also proved to be more beneficial than other inorganic N sources.

On the whole it can be concluded that glucose was the most favored C source and NH_4NO_3 was the most favored N source for the tannase and gallic acid production respectively, by *P. purpurogenum* BVG7 from the various agrobased substrates through suspended solid fermentation.

Table 1. Influence of C sources on Tannase activity in different substrates in SSF

Sugars, mg/100 g substrate	Acacia pods	Redgram husk	Sorghum husk	Spent tea powder
Glucose, 0.2 mg	46.5±1.2	44.0±1.8	43.5±2.1	38.0±1.2
Fructose,0.2 mg	43.5±1.0	43.0±1.9	42.0±2.0	35.5±1.0*
Lactose, 0.2 mg	45.5±2.3	44.0±1.1	43.7±2.1	37.5±1.7
Sucrose, 0.2 mg	44.1±1.0	43.7±1.7	43.0±2.3	36.7±2.0
Control	40.5±1.4	39.0±1.4	38.5±2.1	30.0±1.2

* at 0.5 mg/100 g substrate

Table 2. Influence of C sources on Gallic acid production in different substrates in SSF

Sugars, mg/100 g substrate	Acacia pods	Redgram husk	Sorghum husk	Spent tea powder
Glucose, 0.2 mg	85.7±2.3	83.0±1.2	82.0±1.5	78.5±2.2
Fructose,0.2 mg	79.7±1.9	77.0±1.2	75.0±1.5	65.8±2.5*
Lactose, 0.2 mg	82.7±1.2	82.0±1.9	81.0±2.5	73.5±2.6
Sucrose, 0.2 mg	80.7±2.5	79.0±1.2	77.0±1.9	69.5±2.0
Control	70.7±1.8	69.0±1.7	68.4±1.5	58.5±2.2

* at 0.5 mg/100 g substrate

Table 3. Influence of N sources on Tannase activity in different substrates in SSF

mg/100 g substrate	Acacia pods	Redgram husk	Sorghum husk	Spent tea powder
Amm. chloride, 1000 mg	42.5±1.4	41.0±1.4	40.5±1.2*	36.0±1.2
Amm. Nitrate, 500 mg	41.2±2.2	40.5±2.0	40.9±1.0	36.0±1.2
Sod. nitrate, 1000 mg	42.5±1.4	41.0±1.4	40.5±1.2	36.0±1.2
Pot. nitrate, 1000 mg	43.0±1.4	41.0±1.4	39.5±1.2	36.0±1.2
Control	40.5±1.4	39.0±1.4	38.5±2.1	30.0±1.2

* at 1500 mg/100 g substrate

Table 2. Influence of N sources on Gallic acid production in different substrates in SSF

mg/100 g substrate	Acacia pods	Redgram husk	Sorghum husk	Spent tea powder
Amm. chloride, 1000 mg	77.7±1.8	75.0±1.7	74.3±1.9*	62.5±2.2
Amm. Nitrate, 500 mg	75.0±1.9	77.0±1.2	75.0±1.5	65.8±2.5
Sod. nitrate, 1000 mg	73.7±1.8	71.0±1.7	70.3±1.9	62.5±2.2
Pot. nitrate, 1000 mg	74.7±1.8	69.0±1.7	67.3±1.9	60.5±2.2
Control	70.7±1.8	69.0±1.7	68.4±1.5	58.5±2.2

* at 1500 mg/100 g substrate

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