INFLUENCE OF VARIOUS CARBON, NITROGEN AND MINERAL SOURCES ON GROWTH AND PRODUCTION OF FUNGAL TANNASE BY *A. FLAVUS AND A. ORYZAE.*

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The influence of carbon and nitrogen sources and of mineral salts on the growth and tannase production by *A. flavus* and *A. oryzae* was investigated. Both the aspergillus species were found to utilize sucrose as the principal carbon source for maximum growth and tannase production. As a nitrogen source, glutamic acid and urea were found responsible for optimum growth and tannase activity by *A. flavus* and *A. oryzae* respectively. Thiourea greatly affected growth and tannase activity of both the aspergillus species. Tannase production and mould growth were appreciably increased by the presence of Mg^{++} Cu^{++} and Mn^{++} in the medium. Co^{++} and Mo^{++} salts suppressed tannase formation by both the mould species.

Tannase, produced by various moulds, is responsible for the hydrolysis of tannin in vegetable tan liquors and the factors contributing to the increase in tannase production are expected to cause greater hydrolysis of tannin. In two earlier publications,[1,2] effect of some environmental factors on growth and tannase production by different species of aspergillus and penicillium groups was reported. These studies revealed that tannase production by the aspergillus group was comparatively higher than the penicillium group and, of the aspergillus species, *A. flavus* and *A. oryzae* showed more tannase activity. In the present study, efforts have been made to find out the influence of some nutritional elements, e. g. carbon, nitrogen and mineral salts, on growth and tannase production by *A. flavus* and *A. oryzae.*

Materials and methods

Czapek's medium composed of sucrose-30.0 g; $NaNO_3$ 2.0g; K_2HPO_4 1.0 g; $MgSO_4$ $7H_2O$ 0.5g; KCl 0.5g; $FeSO_4$ $7H_2O$ 0.01g, was used as the basal medium for culturing the fungi. Sucrose in the medium was replaced by 18 different carbon compounds in case of *A. oryzae* and 15 in case of *A. flavus*, at the rate of 12.6 g carbon per litre of the medium. Similarly sodium nitrate in the medium was replaced by different nitrogen compounds at the rate of 330 mg N_2 per litre.

To find out the effect of different metallic elements, magnesium sulphate and ferrous

sulphate present in the medium were replaced by 7 different mineral salts, namely : $FeSO_4$ $7H_2O$; $CoCl_2$ $6H_2O$; $MnSO_4$ H_2O; $CuSO_4$ $5H_2O$; Na_2MoO_4 $2H_2O$; $ZnSO_4$ $7H_2O$ in the proportions of 0.005 mg and 0.1 mg metal per 100 ml of the medium. The basal medium having no magnesium and ferrous sulphate was treated with chromatographic alumina to reduce trace element contamination according to the procedure (method 5) described by Donald *et al*[3]. Double distilled water was used throughout the experiment. The pH of the medium was adjusted to 3.0 in the case of *A. flavus* and 5.0 in the case of *A. oryzae*. 25 ml of the medium were taken in 100 ml Erlenmayer flasks to which tannic acid was added in the proportion of 1.5% and 0.5% for the growth of *A. flavus* and *A. oryzae* respectively. The flasks were then sterilized and inoculated with 0.5 ml of uniform spore suspension of 10 day old culture. The culture flasks were incubated at 30±1°C for a week. The thick felt, covering the surface in the flasks were separated and used for the determination of growth and tannase activity. Mould growth and tannase activity were determined according to the procedures described in an earlier publication.[1]

Results

Effect of carbon source on growth and tannase activity

Spore suspensions of *A. flavus* and *A. oryzae* were cultured in modified Czapek's medium containing various carbon sources. Medium containing no carbon source was considered as control. Growth and tannase activity of the mycelial felt were determined and the results obtained are presented in Table 1.

Table 1 shows that growth and tannase activity of *A. flavus* and *A. oryzae* are influenced by carbon sources. Growth of *A. flavus* appears to be slightly affected except in the case of melibiose, where it is significantly inhibited. Sucrose encourages maximum tannase production but mannose and xylose restrict tannase activity to a considerable extent. Both fungal growth and tannase activity are appreciably supported by sucrose. Melibiose, though suppressing growth, encourages tannase production.

Carbon sources exert greater influence on the growth of and tannase production by *A. oryzae*. Growth is found to be appreciably supported by sucrose and glucose whereas raffinose, lactose, trehalose, arabinose, citric acid and malonic acid are found to discourage growth to different extents.

Tannase production by *A. oryzae* is found to be maximum with sucrose and minimum with trehalose. Raffinose also discourages tannase production. Here again, both the fungal growth and tannase production are encouraged greatly by sucrose.

Effect of nitrogen source on growth and tannase production

Spore suspensions of *A. flavus* and *A. oryzae* were cultured in modified Czapek's medium containing various nitrogen sources. Medium containing no nitrogen compound was considered as control. Growth and tannase activity of the mycelial extracts were determined. Results obtained are presented in Table 2.

Among the various N_2 compounds tested (inorganic and organic), growth of *A. flavus* is appreciably encouraged by glutamic acid, aspartic acid, ammonium nitrate, potassium nitrate and sodium nitrate. On the other hand, growth is found to be partially inhibited by ammonium sulphate, ammonium

TABLE I

Effect of carbon source on growth and tannase activity of *A. flavus and A. Oryzae*

(Incubated at 30 ± 1° C for 1 week)

Carbon source	*A. flavus*		*A. oryzae*	
	Mould growth (dry mat weight in mg)	*Tannase activity (units/ml)*	*Mould growth (dry mat weight in mg)*	*Tannase activity (units/ml)*
Control	Nil	Nil	Nil	Nil
Arabinose	273 . 6	0 . 080	182 . 8	0 . 083
Glucose	268 . 0	0 . 065	301 . 4	0 . 072
Mannose	255 . 0	0 . 057	293 . 0	0 . 084
Rhamnose	214 . 2	0 . 080	200 . 0	0 . 080
Sorbose	219 . 8	0 . 070	235 . 0	0 . 088
Sucrose	268 . 4	0 . 090	314 . 0	0 . 089
Xylose	263 . 3	0 . 059	264 . 6	0 . 084
Melibiose	23 . 4	0 . 083	262 . 2	0 . 082
Glycerol	188 . 2	0 . 080	278 . 4	0 . 083
Lactose	253 . 4	0 . 079	179 . 4	0 . 074
Maltose	236 . 4	0 . 077	232 . 0	0 . 070
Mannitol	224 . 8	0 . 075	215 . 6	0 . 067
Raffinose	233 . 2	0 . 081	135 . 4	0 . 058
Starch	233 . 6	0 . 070	209 . 0	0 . 069
Cellobiose	246 . 0	0 . 080	231 . 0	0 . 073
Trehalose			180 . 4	0 . 049
Citric acid			189 . 0	0 . 073
Melonic acid			190 . 8	0 . 082

• Carbon concentration 12.6 gm/litre

TABLE 2

Effect of N_2 source on growth and tannase activity of *A. flavus* and *A. Oryzae*
(Incubated at 30 ± 1°C for 1 week)

N_2 source*	*A. flavus*		*A. oryzae*	
	Mould growth (dry mat weight in mg.)	*Tannase activity (units/ml.)*	*Mould growth (dry mat weight in mg.)*	*Tannase activity (units/ml.)*
Control	14 . 3	0 . 004	17 . 0	0 . 002
Ammonium sulphate	149 . 2	0 . 015	149 . 2	0 . 067
Ammonium phosphate	160 . 4	0 . 022	196 . 6	0 . 072
Ammonium nitrate	289 . 0	0 . 062	291 . 2	0 . 070
Sodium nitrite	141 . 6	0 . 042	147 . 8	0 . 020
Sodium nitrate	256 . 6	0 . 078	263 . 4	0 . 087
Potassium nitrate	255 . 8	0 . 073	265 . 6	0 . 086
Thiourea	7 . 8	0 . 002	25 . 2	0 . 002
Urea	233 . 8	0 . 074	293 . 2	0 . 087
Aspartic acid	251 . 5	0 . 072	251 . 5	0 . 085
Asparagine	227 . 6	0 . 074	293 . 9	0 . 074
Cysteine	217 . 0	0 . 049	142 . 5	0 . 065
Cystine	163 . 7	0 . 046	136 . 0	0 . 060
Methionine	208 . 6	0 . 089	143 . 6	0 . 080
Proline	247 . 7	0 . 071	299 . 9	0 . 074
Tryptophan	220 . 2	0 . 093	251 . 0	0 . 080
Glutamic acid	267 . 2	0 . 085	–	–

* N_2 concentration 330 mg/litre.

phosphate, sodium nitrite and cystine and almost completely by thiourea.

Tannase production, however, seems to be optimum when tryptophan is used as the N_2 source, which is successively followed by methionine and glutamic acid. Tannase production is found to be greatly reduced by ammonium sulphate, ammonium phosphate, sodium nitrite, thiourea and cystine, which are also responsible for reduced mould growth. On the other hand, glutamic acid encourages both the growth and tannase activity to a considerable extent.

In the case of *A. oryzae*, results obtained with regard to its growth and tannase production differ from that of *A. flavus*. In this case, optimum growth is recorded with proline, which is closely followed by asparagine, and ammonium nitrate whereas growth is found to be moderately inhibited by different compounds like ammonium sulphate, sodium nitrite, cystine, cysteine, and methionine. Again, thiourea is found to inhibit the growth of *A. oryzae* quite appreciably.

Sodium nitrate, urea and potassium nitrate are found to encourage tannase production by *A. oryzae* to a considerable extent. In general, both the growth and tannase activity are encouraged by urea. Tannase production by *A. oryzae* is reduced by N_2 compounds like ammonium sulphate, sodium nitrite, thiourea, cystine and cysteine.

Effect of metallic elements on growth and tannase activity

Spore suspensions of *A. flavus* and *A. oryzae* were cultured in modified Czapek's medium containing different mineral salts. Medium containing no metallic elements was used as control. Growth and tannase activity of the mycelial felts were estimated and the results obtained are tabulated in Table 3.

Among the seven mineral salts used, magnesium, manganese and copper, at the concentration of 0.1 mg/100 ml. stimulate both the growth and tannase production by *A. flavus*. On the other hand, iron and zinc slightly inhibit tannase activity at higher concentration and cobalt and molybdenum are found to suppress both the growth of and tannase production by *A. flavus*.

In the case of *A. oryzae*, magnesium, zinc, copper and iron salts support tannase production only at the higher concentration. Mould growth is also encouraged at 0.1mg/100ml concentration. Cobalt and molybdenum salts are found to have no influence on growth and tannase production.

Discussion

Tannase production by *A. flavus* and *A. oryzae* are considerably influenced by different carbon and nitrogen compounds used in the medium. 2% tannic acid was found to serve as the sole source of carbon for the growth of *A. niger*[4]. In the present study, no mould growth is noted in the medium containing tannic acid (1.5% and 0.5%) upto the period of observation. From the results obtained (Table 1) maximum growth and tannase formation are found to be obtained both for *A. flavus* and *A. oryzae* when sucrose is used as the carbon source. Olutiola[5] also reported that sucrose was the best carbon source for growth and sporulation of *A. flavus*. In the case of *A. flavus*, disaccharides are better sources of carbon for tannase production compared to monosaccharides. Reverse is the case with *A. oryzae*. Glycerol favours