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Effect of carbon and nitrogen sources on penitrem A production by *Pencillum Puberulum*

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ABSTRACT

Influence of different carbon and nitrogen sources on growth and penitrem A production by *Penicilium puberulum* was investigated. The toxin production was analyzed by thin layer chromatography method. D-mannose followed by D-glucose and D-sorbital were good carbon sources, while Potassium nitrate and sodium nitrate were ideal nitrogen sources for the growth of *P.puberulum* and optimum production of penitrem A. No correlation could be observed between fungal biomass production and penitrem A production and pH changes in the medium.

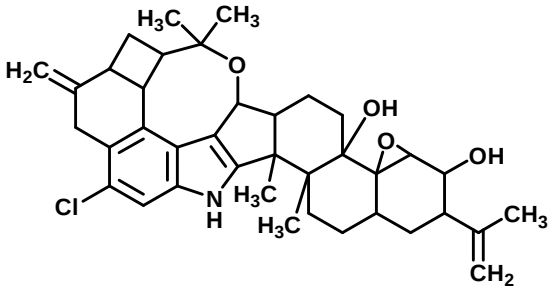
KEYWORDS

Penicillium puberulum;
Penitrem A;
Carbon source;
Nitrogen source.

INTRODUCTION

Since the first report of toxin production by *Aspergillus flavus*[1] scientific knowledge of fungal tremorgens including possible implication in the etiology of animals disease has accumulated rapidly. Fungi capable of producing tremorgenic metabolites represent species from taxonomically diverse and unrelated groups of *Claviceps paspali, Lolium endophyte, A.flavus, A.fumigatus, A.clavatus, P.simplicissimum, P.crustum and P.puberulum* are some of the fungi which elaborate these mycotoxins. These fungi are reported to be widely distributed and contaminate variety of agriculture commodities[2]. Wilson et al.[3] first reported the production of tremorgenic mycotoxin by *Penicillium cyclopium*. Subsequently, various workers have reported the production of penitrems by different species of *Penicillium*[4,5]. The penitrems have received increasing attention over the past decade on account of their termorgenic activity. The structure of penitrem A was elucidated by jesus et al.[6]. Penitrem A ($C_{37}H_{44}NOCl_6$) is the most commonly occurring family of tremorgenic compounds [Penitrem A to F]. These compounds have a complex ring structure comprising indolic and isoprenoid, derived components and differ in their functional groups.

Studies on toxic effects of penitrems are limited.



Penitrem A

These tremorgens are reported to cause tumors, limb weakness, atoxia and convulsions in mice. Penitrem A was toxic to sheep and other animals[7]. Neuron chemical studies showed that pentitrem A acts by interfering with amino acid neuro transmitter release mechanism[5].

A recent report of natural human intoxification characterized by several symptoms including tumors resulted in the consumption of bread contamined with *P. crustosum*[8].

Though penitrem B production by *P. aurantigriseum* was studied earlier[9], no information is available on the factors influencing *P.puberulum* for production of penitrem A. Several studies revealed the critical role of carbon compounds present in the medium on the production of metabolites of by the fungi studied by them[10]. Though there are several studies on the influence of nitrogen source on the production of different mycotoxins by fungi[11] no such studies have been made on the production of penitrem A by *P. puberulum*, Hence in the present study influence of different carbon and nitrogen sources on penitrem A production by *P. puberulum* was studied and the results are discussed.

MATERIAL AND METHODS

Chemicals and organism

All chemicals of highest available purity were obtained from Himedia, Mumbai, India. The culture Penicillium puberulum was procured from IMTECH, Chandigarh. Stock cultures were maintained on potato dextrose agar slants at 4°C and subcultured for every three months.

Cultural conditions

50ml of YES medium (Yeast extracts 20g, sucrose 40g, D/W 100ml) was taken in 250ml conical flasks and inoculated with *Penicillium puberulum* and incubated at 27°C for 15 days. Studies were performed to determine the suitable carbon and nitrogen source for higher production of penitrem A by Penicillium puberulum.

Effect of carbon source

Carbon sources used are D-fructose, D-glucose, D-mannose, L-sorbose, D- xylose, Sorbitol, Mannitol, Sucrose, Lactose, maltose, melibiose, starch, dextrin, glycerol, citric acid, succinic acid and tartaric acid.

Effect of nitrogen source

Nitrogen sources used were potassium nitrate, sodium nitrate, alluminium nitrate, zirconyle nitrate, ammonium nitrate, ammonium chloride, ammonium molybdate, ammonium sulphate, aceranilyde, urea, thiourea, D-L aspartic acid, L- aspergine, L-glutamic acid, glutamine, glycine, L-arginine, L-tyrisine, D-L alanine, L-methionine, L-histidine, L-lysine, L-tryptophan, P-amino benzoic acid, p-nitrobenzoic acid and p- niroaline.

Penitrem A extraction and estimation

At the end of incubation period the mycelial mat was dried and extracted with diethyl ether in soxhlet apparatus for 24 hrs. Ether extract was evaporated to dryness and redissolved in acetone. A portion of acetone solution was spotted on TLC plates and developed in hexane: ethyl acetate (6:4) mixture. The plates thus developed were sprayed with cerium sulphate (1% solution in 6N H_2SO_4) and heat at 110°C. Penitrems appeared as green colour spot immediately which changed to purple after heating. Penitrems gave grey spots when sprayed with the $FeCl_3$ and heated. Penitrems A-F was identified by Rf values. Penitrem A was estimated quantitatively by colorimetric method as suggested by Hou et al.[12].

RESULTS AND DISCUSSION

Effect of carbon source

The influence of carbon source on penitrem A production by *P.puberulum* was studied by substitution sucrose of basal medium by different carbon compounds so as to supply equal amount of carbon source and the results are précised in TABLE 1.

TABLE 1 reveal a great specificity of *P. puberulum* for the carbon source present in the medium. The critical role of carbon compounds present in the medium on the production of metabolites by the fungi was revealed in many studies[11]. In general organic acids were poor substrates for the growth of *P. puberulum*. D-mannose followed by D-glucose and D-sorbital were very good carbon source and responsible for maximum growth of *P. puberulum*. In contrast to present observation *P. griseofulvum* preffered glycerol for maximum

TABLE 1 : Effect of different carbon source on the growth and penitrem A production by *P.puberulum*

Carbon source	Final pH	Dry weight (mg/ml)	Penitrem A (mg/g)
D-glucose	6.7	4.62	1.68
D-fructose	6.9	3.50	1.54
D-mannose	6.4	4.82	1.73
L-sorbose	6.9	3.12	1.42
D-xylose	6.5	4.24	1.62
Sorbitol	6.3	4.21	1.58
Mannitol	6.2	4.21	1.58
Lactose	7.2	2.48	1.32
Maltose	7.1	3.55	1.54
Melibiose	6.4	2.06	0.95
Starch	7.2	2.42	0.84
Dextrin	6.8	2.18	0.48
Glycerol	6.4	0.98	0.15
Citric acid	5.9	0.78	0.09
Succinic acid	3.8	1.12	0.62
Tartaric acid	3.6	NG	Nil
Sucrose	6.5	3.84	1.42

growth and production of CAP[13]. *P.citrinum* is reported to prefer source for production of citrinin[14]. On the other hand, glycerol was unfavourable carbon source for the growth of *P.puberulum*.

Rest of the carbon sources was intermediate in their efficiency to induce the growth of *P. puberulum*. D-mannose followed by D-glucose and sorabital were ideal carbon source for production of penitrem A by *P.puberulum*. Carbon which is the major structural and functional component plays a vital role in the nutrition of the fungus. The unfavorable nature of organic acids for the growth of *P. puberulum* may be attributed to their strong condition. Similarly Krishna Reddy and Reddy[13] have reported poor growth of *P.griseofulvum* and CAP production in the medium containing organic acids. In general simple sugars are preferred. Krishna Reddy et al.[11] reported effect of carbon and nitrogen source on the interaction of mycotoxigenic fungi and mycotoxin production. The response of *P.puberulum* was different to different carbon source. These Fungi may be capable of using wide variety of carbon compounds with varying degree of efficiency depending on the inherent potentials of these organisms and molecular configuration of carbon compounds used in the present study.

TABLE 2 : Effect of nitrogen source on the growth and penitrem A production by *P.puberulum*

Nitrogen source	Final pH	Dry weight (mg/ml)	Penitrem A (mg/g)
Potassium nitrate	6.3	4.12	1.65
Sodium nitrate	6.5	4.04	1.58
Barium nitrate	4.5	NG	Nil
Aluminium nitrate	3.6	NG	Nil
Zirconyle nitrate	3.5	NG	Nil
Ammonium nitrate	4.8	2.85	1.42
Ammonium chloride	4.5	2.65	1.37
Ammonium molybdate	5.3	1.12	0.62
Ammonium sulphate	4.6	2.42	1.14
Aceranilyde	4.9	1.98	1.25
Urea	6.0	2.75	1.38
Thiourea	4.5	3.80	1.49
DL Aspertaic acid	6.2	2.61	1.34
L-Asperagine	6.2	0.98	0.38
L-Glutamic acid	5.9	2.98	1.10
Glutamine	6.0	3.12	1.42
Glycine	6.4	1.24	0.64
L-Arginine	5.5	1.98	0.96
L-tryrosene	4.3	1.62	0.87
D-L alanine	5.4	1.45	0.74
L-methionine	4.2	2.69	1.12
L-histidine	5.0	1.98	0.96
L-lysin	4.5	0.92	0.35
L-tryptophan	4.0	2.75	1.29
P-aminobenzoic acid	3.4	NG	Nil
P-nitrobenzoic acid	3.5	NG	Nil
P-niroailne	4.6	NG	Nil

Effect of nitrogen source

The influence of different nitrogen sources on penitrem A production by *P.puberulum* was studied by substituting yease extract of basal medium by different nitrogen compounds so as to supply equal amount of nitrogen source and the results are precised in TABLE 2.

The structural and functional importance of nitrogen is well documented, an enormous account reflecting their varied effect on fungal metabolism is enumerated in different reports. *P. puberulum* exhibited varied response towards different nitrogen source present in the medium (TABLE 2) *P.puberulum* failed to grow in medium containing barium nitrate, aluminium nitrate, zirconyl nitrate, p-amino benzoic acid, p-nitrobenzonic acid and p-nitroaniline. L-aspargine and l-lysine were

responsible for decreased biomass yield of *P.puberulum*. Potassium nitrate and sodium nitrate were best nitrogen source for the growth of *P.puberulum*. Ammonium molybdate, L-asparagine, glycine, L-arginine and L-tyrosine were also responsible for poor growth of *P.puberulum* pH of the medium was drift towards acidic side and final pH was below 6.0. No correlation could be observed between fungal biomass production and penitrem A production and pH changes in the medium.

The efficiency of organic and inorganic form of nitrogen in including the growth of fungus depends upon the capacity of the organism to convert the complex, nitrogen source into assimilable form. Fungi are reported to exhibit great specificity for nitrogen source present in the medium[15,16]. Potassium nitrate and sodium nitrate were best nitrogen source for production of penitrem A by *P. puberulum*. Ammonium nitrate, Ammonium chloride, thiurea, urea were next preferred nitrogen substrates for production of penitrem A by *P. puberulum* Rest of the substance were intermediate nutritive value for penitrem A production. *P. griseofulvum* elaborate maximum amount of CPA in medium containing L-aspagine[13] various environmental.

CONCLUSION

This work demonstrated the feasibility of using experimental design tools to screen significant influential factors and nutritional conditions like carbon and nitrogen sources for the growth of *P.puberulum* and optimum production of penitrem A.

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