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Factors influencing penitrem A production by *Pencillum puberulum*

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ABSTRACT

Influence of different media, PH and temperature on growth and penitrem A production by *Penicilium puberulum* was investigated. The toxin production was analyzed by thin layer chromatography method. Among different media studied, SMKY and Yeast extract sucrose media, PH of 6.5 and temperature 30°C was found to be optimum for maximum production of penitrem A.

KEYWORDS

Penicillium puberulum;
Penitrem A;
Media;
pH;
Temperature.

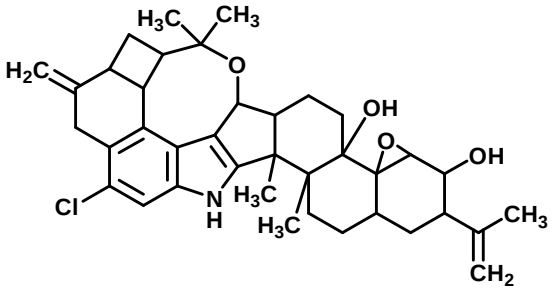
INTRODUCTION

Scientific knowledge of fungal tremorgens including possible implication in the etiology of animals disease has accumulated rapidly ever since in the first report of toxin production by *Aspergillus flavus*[1]. 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].

Trermorgenic mycotoxins can be divided into 4 different groups. The largest group consist of aflatrem, paxilline, paspalitrems, lolitrems, penitrems and janthitrems. The other three chemical goups include the verrculogen and fumitrogens tryptoquivalines and territrems. The latter represents the only group that is not derived from tryptophan and therefore, does not contain nitrogen. The tryptoquivalines and territrems have not yet been implicated in animal disease.

The penitrems have recieved increasing attention over the past decade on account of their termorgenic activity. Wilson et al.[3] first reported the production of tremorgenic mycotoxin by *Penicillium cyclopium*. Subsequntly, various workers have reported the production of penitrems by different species of *Penicillium*[4,5]. The structure of penitrem A was elucidated by Jesus et al.[6].

Penitrem A was originally reported from *P. cyclopium*, the major contaminant of feed responsible for out breaks of neurological disorders and deaths in sheep and horses[3]. Penitrem A has been reported to



Penitrem A

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contaminate cheese, walnuts and mouldy bread[7]. 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. These tremorgens are reported to cause tumors, limbweakness, atoxia and convulsions in mice[5]. Penitrem A was toxic to sheep and other animals[8]. 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*[9]. Though penitrem B production by *P. aurantigriseum* was studied earlier [10], no information is available on the facts on influencing *P. puberulum* for production of penitrem A. Hence, it was considered with while to investigate various factors influencing penitrem A production by *P. puberulum*.

EXPERIMENTAL

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.

Effect of media

To find a sutable media for maximum production of penitrem A, Different media were screened. The compositions (per Lit.) of media used were given below,

1 Adye and mateles: Glucose 50g; $(NH_4)SO_4$ 3g; KH_2PO_4 10g; $MgSO_4$ $7H_2O$ 2g; Yeast extract 3g; distilled water 1000ml.
2 Czapek's: $NaNO_3$ 2g; KH_2PO_4 0.5g; KNO_3 3g; yeast extract 7g; distilled water 1000ml.
3 Glucose Ammonia nitrate: Glucose 50g; NH_4NO_3 2.4g; KH_2PO_4 10g; $MgSO_4$ 2g; distilled water 1000ml.
4 Richard's medium: KNO_3 10g; KH_2PO_4 1g; $MgSO_4$ $7H_2O$ 2.5g; Sucrose 35g; $FeCl_2$ traces; distilled water 1000ml.
5 Semisynthetic medium: Glucose 20g; NH_4NO_2 0.4g; KH_2PO_4 0.1g; KCl 0.3g; Ng SO_4 $7H_2O$ 0.4g; $CaCl_2$ 0.04g; $CuSO_4$ 0.1g; sodium molys date 0.1g; $ZnSO_4$ 0.1g; distilled water 1000ml.
6 SMKY: Sucrose 20g; $MgSO_4$ $7H_2O$ 0.5g; KNO_3 3g; Yeast extract 7g; distilled water 1000ml.
7 YES: Yeast extract 20g; Sucrose 40g; Distilled water 1000ml.
8 Minimal Liquid: Glucose 40g; $NaNO_3$ 2g; KCL 0.52g; $NgSO_4$ $7H_2O$ 0.52g; $FeSO_4$ 0.01g; KH_2PO_4 1.52g and distilled water 1000ml.
9 Maize flour: Maize flour 40g; Sucrose 30g; yast extract 1g; distilled water 1000ml.
10 Rice flour: Rice flour 40g; Sucrose 30g; yeast extract 1g; and distilled water 1000ml.
11 Asthana & Hawker's: Glucose 5g; KNO_3 3.5g; KH_2PO_4 1.75g; $MgSO_4$ 0.75g and distilled water 1000ml.
12 Singh & wood: Glucose 5g; aspargine 4g; KH_2PO_4 1g; $MgSO_4$ $7H_2O$ 0.5g; Pectin 10g; and distilled water 1000ml.
13 Malt extract: Glucose 20g; Malt extract 20g; peptone 1g; and distilled water 1000ml.
14 Nutrient agar: Bacto beef extract 3g; peptone 5g; and distilled water 1000ml.
15 Czapek's + 2% yeast extract: $NaNO_3$ 2g; KH_2PO_4 1g; $MgSO_4$ $7H_2O$ 0.5g; KCl 0.5g; Sucrose 30g; yeast extract 20g and distilled water 1000ml.
16 Glucose aspargine: Glucose 20g; aspargine 5g; KH_2PO_4 3.4g; $MgSO_4$ $7H_2O$ 1.9g; NaCl 0.01g; and distilled water 1000ml.

Isolates of different species of *Penicillium puberulum* were screened for their ability to produce penitrem A. They were grown in YES medium for 15 days at 27-29°C. 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 color spot immediately

TABLE 1 : Production of penitrem A by *P. puberulum* in different synthetic media

Medium		Final PH	Dry weight (mg/ml)	Penitrem A (mg/g)
Adye and Mateles	(A)	3.6	0.86	0.10
Asthana Hawkers	(B)	5.0	2.86	1.18
Beef extract + 2% yeast extract	(C)	7.6	1.65	1.10
Czapecks	(D)	5.8	1.12	0.45
Czapecks + 2% yeast extract	(E)	7.8	4.35	1.72
Glucose Ammonium nitrate	(F)	3.5	3.45	1.62
Glucose Aspargine	(G)	7.5	2.56	1.12
Maize flour	(H)	5.0	2.89	1.05
Malt extract	(I)	3.5	2.95	1.10
Minimal liquid	(J)	4.5	2.42	0.95
Nutrient broth	(K)	6.6	1.85	0.86
Peptone + 2% yeast extract	(L)	4.0	2.42	1.20
Rice flour	(M)	5.5	1.64	0.82
Richards	(N)	5.0	3.19	1.12
Semisynthetic	(O)	6.8	0.98	1.75
Singhwood	(P)	5.2	3.78	1.20
Smky	(Q)	7.5	4.92	1.98
Yeast extract sucrose	(R)	6.5	4.86	1.92

which changed to purple after heating. Penitrems gave grey spots when sprayed with the $FeCl_3$ and heated. Penitrems A-F were identified by Rf values. Penitrem A was estimated quantitatively by colorimetric method as suggested by Hou et al.[11].

Effect of pH

Studies were performed to determine the suitable pH for higher production of penitrem A by *pencillium puberulum.* The experiment was studied at pH viz 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0.

Effect of temperature

Studies were performed to determine the sutable temperature for higher production of penitrem A by *pencillium puberulum.* The experiment was studied at temperature viz 10, 15, 20, 25, 30, 35, 40, 45.

RESULTS AND DISCUSSION

P. puberulum was grown in different synthetic media and pentitrem A production was investigated and results are precised in TABLE 1. Medium Q and R most probable substrates for growth of pentitrem A production. On the other hand Adye and mateles, Rice flour, nutrient broth, and czapecks medium were most unfavorable for the growth of *P.puberulum.* Rest of the medium supported intermediate amount of biomass and penitrem A production. Rest of the media were intermediate in their ability to support the growth of *P.puberulum.* The final pH of the medium was changed due to the growth of *P. puberulum* which varied with the medium. In medium C, B, G and Q the final pH was above 7.0. In medium A, F, I the pH was below 3.6. Medium A, D, K and M were very poor substrates they were able to induce the production of penitrem A only in meager amounts. The pH changes in different media was found to be different. In medium A, F and I were strongly acidic. While in the medium C, E, G, O and Q it was neutral or near neutral. In rest of the media pH changes were in range of 4 to 5.8. Critical perusal of table reveals no correlation between pH of the medium and growth of the fungus. For instant final pH of the medium G and Q was the same but mycelium growth was entirely different. On the other hand medium G and Q was the same but mucelium growth was entirely different. On the other hand medium Q and R supported almost equal amount of mycelial growth but the final pH was entirely different such instances were not common with media. Similarly no correlation could be observed between penitrem A production and mycelium growth. On the other hand medium Q was good for both mycelial growth and pentrium A production. From present investigation it is clear that substrates which induces vegetative growth but not promote toxin production and vice versa. It is also clear that pH maybe playing little role on penitrem A production. In general it

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

PH	Final PH	Dry weight (mg/ml)	Penitrem A (mg/g)
2.0	2.2	0.42	0.09
3.0	3.4	1.89	0.45
4.0	4.5	2.24	1.18
5.0	5.5	3.58	1.34
6.0	6.5	4.66	1.56
7.0	7.4	3.62	1.39
8.0	8.6	2.94	1.06
9.0	8.4	0.98	0.42
10.0	9.8	--	--
Control (6.5)	6.5	4.86	1.92

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TABLE 3 : Effect of temperature on the growth and penitrem A production by *P. puberulum*

Incubation temperature (°C)	Final PH	Dry weight (mg/ml)	Penitrem A (mg/g)
10	5.0	1.12	0.68
15	5.5	2.42	1.10
20	5.5	2.92	1.28
25	5.8	3.86	1.47
30	6.0	4.52	1.62
35	6.5	3.98	1.34
40	5.4	1.05	0.56
45	5.2	--	--

can be concluded that medium R and Q was good for both vegetative growth and toxin production.

Effect of pH

TABLE 2 reveals that a distinct of pH on growth and penitrem A production by *P. puberulum* accomplished good pH of increase in growth and penitrem A production at pH 5.5. Acidity or alkalinity resulted in gradual decrease in the biomass production. Penitrem A production was maximum at pH 5.5 decreased with further increased with further increase in medium acidity or alkalinity. *P. puberulum* could elaborate penitrem A only in trace amount at pH 2.0. A positive correlation could be observed biomass and penitrem A production relation to pH of the medium.

Effect of temperature

The effect of temperature on growth and penitrem A production by *P.puberulum* could grow over a temperature on growth and penitrem A production by *P.puberulum* was investigated and the results are presented in TABLE 3. The fungus which could grow over a temperature range of 10 to 40°C. Maximum growth at 30°C, marginal growth was recorded both at 10°C and 40°C. The fungus was totally inhibited at 45°C. The final pH was acidic in nature. The *P.puberulum* could elaborate penitrem A in the temperatures ranged from 10 to 40°C with a maximum at 30°C. Similarly Chary and Reddy[12] have reported that the temperature of 30°C was optimum for production of citrinin by *P.citrinum*. Rajkumari[13] have reported that *P.griseofulvum* and *P.citrinum* could elaborate maximum amount of citrinin and CAP respectively at 30°C and preffered incubation temperature at 25°C for production of roridin by *M.roridum*. Penitrem A production was totally inhibited at 45°C. Decrease or increase incubation temperature resulted an adverse effect on penitrem A production.

CONCLUSION

Based on the above results it can be concluded that factors [different media pH and temperature] should be optimized for maximum production penitrem A.

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