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RESEARCH ARTICLE

PRODUCTION OF PENITREM B A TREMOGENIC TOXIN PRODUCED BY *Penicillium aurantiogriseum*

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ABSTRACT

Production of penitrem B by *Penicillium aurantiogriseum* under different cultural conditions was investigated. The toxin production was analysed by employing different media. Among the different media employed Richards medium was good substratum for both vegetative growth and toxin production. Except beef extract, all the other microbial nutrients tried enhanced the production of penitrem B.

INTRODUCTION

Penitrems have received increasing attention over the past decade on account of their tremogenic activity. Wilson *et al.,* (1968) for the first time reported the production of tremogenic mycotoxin by a species of *Penicillium.* Subsequently, several investigators have reported its production by different species of *Penicillium* (Ciegler and Pitt 1970; Wagenar *et al.,* 1980; Jesus *et a.,* 1983; Bridge *et al.,* 1989; Frisvad *et al.,* 2004). Penitrems were responsible for out breaks of neurological disorders and death in sheep, horses and dogs (Hocking *et al.,* 1988; Walter *et al.,* 2001). Though there are several studies on penitrem A and its toxicity little is known about penitrem B. Hence in the present investigations production of penitrem B (Fig. 1) by *Penicillium aurantiogriseum* under different cultural conditions was investigated.

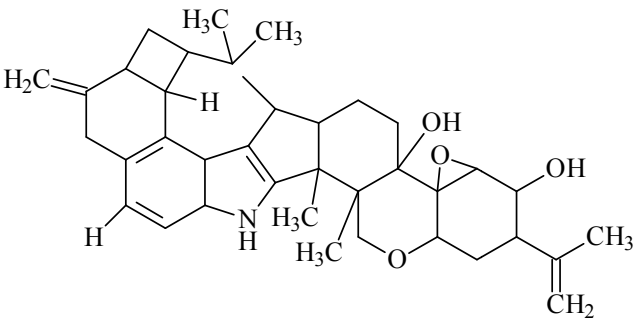

Fig. 1. Structure of Penitrem B

MATERIALS AND METHODS

Monosporic cultures of *P. aurantiogriseum* were grown in 50ml of different synthetic media contained in 250ml Erlenmeyer conical flasks. The different media employed for the present study are:

1. Adye and Matele's (A): Glucose 50g; (NH_4) So_4 3g; KH_2PO_4 10g; $MgSO_4.7H_2O$ 2g; yeast extract 3g and distilled water 1000ml.
2. Czapek's (B): $NaNO_3$ 2g; KH_2PO_4 1.0g; $MgSo_4.7H_2O$ 0.5g; KCl 0.5g; sucrose 30g and distilled water 1000ml.
3. Glucose ammonium nitrate (C): Glucose 50g; NH_4No_3 2.4g; KH_2PO_4 10g; $MgSO_4.7H_2O$ 2g and distilled water 1000ml.
4. Minimal Liquid (D): Glucose 40g; $NaNO_3$ 2g; KCl 0.52g; $MgSO_4.7H_2O$ 0.52g; $FeSO_4$ 0.01g; KH_2PO_4 1.52 and distilled water 1000ml.
5. Maize flour (E): Maize flour 40g; sucrose 30g; yeast extract 1g and distilled water 1000ml.
6. Rice flour (F): Rice flour 40g; sucrose 30g; yeast extract 1g and distilled water 1000ml.
7. Richard's (G): KNO_3 10g; KH_2PO_4 5g; $MgSo_4.7H_2O$ 2.5g; sucrose 30g; $FeCl_2$ traces and distilled water 1000ml.
8. Semi-synthetic (H): Glucose 20g; NH_4No_2 0.4g; KH_2PO_4 0.1g; KCl 0.3g; $MgSO_4.7H_2O$ 0.049g; $CaCl_2$ 0.04g; $CuSO_4$ 0.1g; sodium molybdate 0.1g; $ZnSO_4$ 0.01g and distilled water 1000ml.
9. SMKY (I). Sucrose 20g; $MgSO_4$. $7H_2O$ 0.5g; KNo_3 3g; yeast extract 7g and distilled water 1000ml.
10. YES (J): Yeast extract 20g; sucrose 40g and distilled water 1000ml.
11. Cyclopiazonic acid (K): Glucose 50g; tartaric acid 5g; ammonium tartarate 5g; $(NH_4)_2HPO_4$ 0.5g; K_2CO_3 0.5g; $MgCO_3$ 0.5g; $(NH_4)_2SO_4$ 0.25g; $ZnSO_4$ 0.07g; $FeSO_4$ $.7H_2O$ 0.07g and distilled water 1000ml.
12. Asthana and Hawker's (L): Glucose 10g; KNO_3 3.5g; KH_2PO_4 1.75g; $MgSO_4.7H_2O$ 0.75g and distilled water 1000ml.
13. Singh and Wood (M): Glucose 5g; asparagine 4g; KH_2PO_4 1g; $MgSO_4.7H_2O$ 0.5g; Pectin 10g and distilled water 1000ml.

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14. Malt extract (N): Glucose 20g; malt extract 20g; peptone 1g and distilled water 1000ml.
15. Glucose-aspargine (O): Glucose 20g; asparagine 5g; KH_2PO_4 3.4g; $MgSO_4$. $7H_2O$ 1.9g; NaCl 0.01g and distilled water 1000ml.
16. Nutrient agar (P): Bacto beef extract 3g; peptone 5g and distilled water 1000ml.
17. Czapek's + 2% yeast extract (Q): $NaNO_3$ 2g; KH_2PO_4 1.0g; $MgSO_4$. $7H_2O$ 0.5g; KCl 0.5g; sucrose 30g; yeast extract 20g and distilled water 1000ml.

Effect of different microbial nutrients (Peptone, yeast extract, beef extract and malt extract) was studied by adding them aseptically to the basal medium (Buffered Richards medium) before inoculation of the fungus. Flasks were sterilized at 15 lbs pressure for 30min. after sterilization flasks were inoculated with 7 days old cultures of *P. aurantiogriseum* and incubated at 27±29°C for 15 days. At the end of incubation period a set of flasks were harvested on previously dried and weighed Whatman filter paper No.42 to determine the biomass of the fungus. The pH of the medium was also determined. Mycelium was employed to extract penitrem B in soxhlet for 24 hours using. The extracts were pooled and concentrated to 1ml, 0.5 ml of extract was taken and penitrem B was estimated by method suggested by Hou *et al.,* (1970). The amount of penitrem B per gram of mycelium was calculated as described earlier. The rest of the details were similar to those described by Surekha and Reddy (1991).

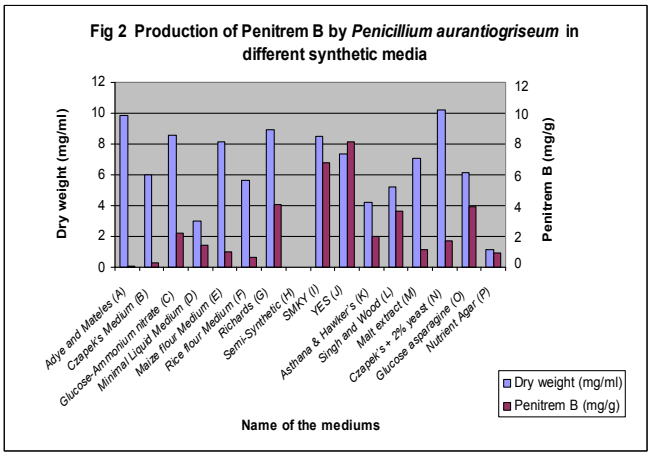
RESULTS AND DISCUSSION

From table 1 it is clear that *P. aurantiogriseum* responded differently towards different media for production of penitrem B. YES (J) was the best for synthesis of penitrem B, while SMKY (I) and Richards medium (G) were next preferred substrates. Semi synthetic medium (H) failed to induce synthesis of penitrem B. Similarly Adye and Matele's (A), Czapek's (B), Rice flour (F) and Nutrient agar (P) were able to induce the production of penitrem B only in meager amounts. Minimal liquid (D), Maize flour (E), Singh and Wood (M) and Malt extract medium were very poor substrates for production of penitrem B, while glucose ammonium nitrate (C), Asthana and Hawkers medium (L) and Glucose asparagine medium (O) were moderate in their efficiency in induction of penitrem B.

Table 1: Production of Penitrem B by *P. aurantiogriseum* in different synthetic media

Medium	Final pH	Dry Weight (mg/ml)	Penitrem B (mg/g)
Adye and Mateles (A)	3.50	9.83	0.08
Czapek's Medium (B)	5.50	6.03	0.28
Glucose-Ammonium nitrate (C)	4.00	8.60	2.18
Minimal Liquid Medium (D)	5.00	3.00	1.40
Maize flour Medium (E)	5.00	8.17	1.00
Rice flour Medium (F)	5.00	5.62	0.62
Richards (G)	5.50	8.91	4.08
Semi-Synthetic (H)	7.00	--	--
SMKY (I)	7.50	8.49	6.80
YES (J)	5.80	7.37	8.12
Asthana & Hawker's (K)	5.00	4.22	2.00
Singh and Wood (L)	7.50	5.18	3.66
Malt extract (M)	4.00	7.10	1.14
Czapek's + 2% yeast (N)	7.50	10.2	1.71
Glucose asparagine (O)	7.30	6.12	3.91
Nutrient Agar (P)	7.00	1.13	0.94

P. aurantiogriseum attained maximum growth on cyclopiazonic medium (N), while medium A and G were next preferred substrates. Medium H failed to support the vegetative growth of *P. aurantiogriseum*. Medium P was again a poor substratum, while rest of the media supported intermediate amount of biomass. No correlation could be observed between penitrem B production and mycelium growth. For instance medium A induced good mycelium growth but toxin production was in meager amounts. On the other hand, medium G was good for both mycelia growth and penitrem B production (Fig 2). Medium N supported maximum growth but toxin production was poor.

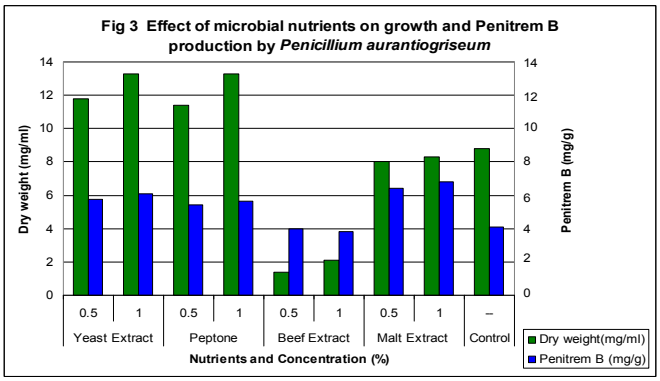


The final pH of filtrate varied with the medium. In medium A it was strongly acidic, while in medium H, I, L, N, O and P it was neutral or near neutral. In rest of the media pH changes were in the range of 4.0 to 5.8. No correlation could be observed between pH, mycelial growth and penitrem B production by the fungus. Almost all the microbial nutrients tried except beef extract which inhibited penitrem B production, enhanced the production of penitrem B (Table 2).

Table 2: Effect of microbial nutrients on growth and Penitrem B production by *P. aurantiogriseum*

Nutrients	Concentration (%)	Final pH	Dry wt. (mg/ml)	Penitrem B (mg/g)
Yeast extract	0.5	6.5	11.8	5.78
	1	6.8	13.3	6.1
Peptone	0.5	7	11.4	5.41
	1	7.2	13.3	5.63
Beef extract	0.5	5.5	1.39	4.01
	1	6	2.09	3.82
Malt extract	0.5	5.5	8	6.44
	1	5.8	8.28	6.78
Control	--	6	8.82	4.09

Similarly Krishna Reddy and Reddy (1988) reported the stimulatory effect of beef extract on CPA production by *P. griseofulvum*. Yeast extract followed by peptone also stimulated the mycelium growth of *P. aurantiogriseum* (Fig 3). The mycelial growth of the fungus increased with increase in their concentration. The final pH of medium varied with the nutrients present in the medium. From the present investigations it is clear that the substratum which induces vegetative growth need not promote penitrem B production and vice versa. In general it can be concluded that the Richard's medium (G) was good substratum for both vegetative growth and penitrem B production.



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