Biological Sciences Section

Pakistan J. Sci. Ind. Res., Vol. 28, No. 1, Febuary 1985

OPTIMAL CONDITIONS FOR THE PRODUCTION OF FUNICULOSIC ACID FROM LOCALLY ISOLATED STRAIN OF *PENICILLIUM FUNICULOSUM* – THOM.

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(Received May 9, 1984)

The present report describes the efforts made to find a suitable medium to obtain higher yields of funiculosic acid, a metabolite of *Penicillium funiculosum* Thom. It was observed that funiculosic acid formation increased when 7% glucose was used as carbon source. In the presence of molasses and corn starch its yield decreased. The effect of concentration of $MgSO_4$, $NaNO_3$, KH_2PO_4, $FeSO_4$, and KCl on the yield of funiculosic acid has been reported. The duration for the maximum formation of funiculosic acid was also studied.

In an earlier report [1] we described the isolation of funiculosic acid from the culture filtrate of *Penicillium funiculosum* Thom grown on modified Czapek-Dox medium. Funiculosic acid was shown to be 2-formyl -3, 5-dihydroxy-p-toluic acid I, possessing moderate antibacterial activity. In literature many mold metabolites, having structural similarities with funiculosic acid, were successfully employed for the syntheses of useful drugs. For example, 2,3,4 -trihydroxy-6-formyl benzoic acid on reaction with 6-amino hexanoic acid produces an analogue of capobenic acid II, an antiarrythmic drug [2]. Likewise 2-formyl-3,4,5-trimethoxy benzoic acid has been transformed into compound III which is a coronary vasodilator [3]. The substitution pattern, particularily the presence of (-CHO) and (-COOH) groups located *ortho* to each other makes funiculosic acid a potential intermediate for the synthesis of a variety of compounds of pharmaceutical importance. It was thus thought worthwhile to develop optimal cultural conditions for the production of funiculosic acid in higher yields.

It is well known that secondary metabolism is species or even strain specific. Besides, it is also observed that change in the nutritive ingredients of the media affects the nature of the secondary metabolites as well as their yields. Literature survey reveals that *Penicillium funiculosum* Thom is a very versatile mold and has been shown to produce a variety of compounds under different sets of cultural conditions [4]. The present paper deals with the selection of a medium capable of producing funiculosic acid in a higher yield. For this purpose, the effect of change of medium, incubation period, concentration of carbon source and inorganic salts on the yield of funiculosic acid has been investigated.

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MATERIALS AND METHODS

Organism: Penicillium funiculosum Thom was isolated in our laboratories from the Karachi soil [5], confirmed by the Commonwealth Mycological Institute, England and catalogued under No. IMI-157575.

Preparation of Inoculum: Penicillium funiculosum Thom was first inoculated on ordinary Czapek-Dox medium in test tubes and incubated at 27° for 9 days. The nine-day old culture was then used to inoculate flasks containing the fermentation medium.

Preparation of carrot Extract Raw carrots were peeled off to remove roots and skin, washed and cut into small pieces. 200 g of these carrots were boiled for one hr. in 700 ml. of distilled water. After filtration, the volume of the extract was made upto one litre with distilled water.

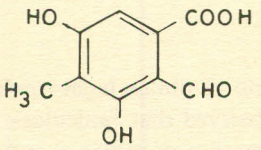
Fermentation Media. Six fermentation media were tried for the production of funiculosic acid.

(A) *Czapek-Dox Medium (Modified I) A:* The composition of this medium was as follows:
Glucose, 80.0 g; $NaNO_3$, 3.0 g; KH_2PO_4, 1.0 g; KCl, 0.5 g; $MgSO_4 7H_2O$, 0.5 g; $FeSO_4.7H_2O$, 0.01 g; and aqueous carrot extract to make upto 1 litre.

(B) *Czapek-Dox Medium (Modified II) B:* The Composition of this medium was the same as that of medium 'A' except in place of $NaNO_3$, Diammonium tartrate

(DAT) was used as nitrogen source. Diammonium tartrate (75.6 g/l) was sterilized separately and 10 ml of it was added asceptically to each one of the flasks containing culture medium.

(C) *Czapek-Dox Medium (Modified III) C;* This medium was of the same composition as medium 'A' except that 2% corn steep liquor was added as additional source of nitrogen.



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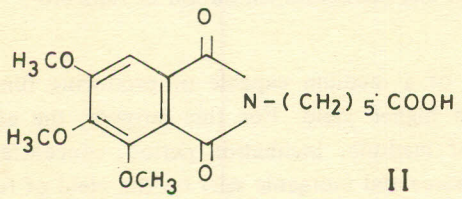

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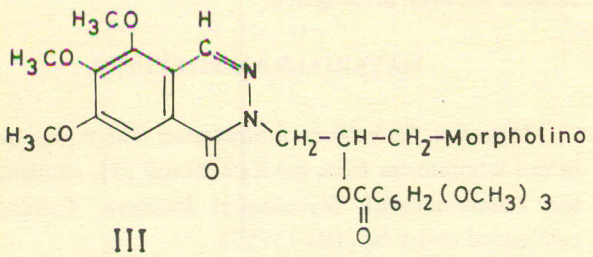

III

(D) *Modified Moyer and Coghill Medium D:* The composition of this medium was as follows: Corn starch, 20.0 g; molasses, 120.0 g; glucose, 2.75 g; $NaNO_3$, 3.0 g; KH_2PO4, 0.5 g, $MgSO_4 \cdot 7H_20$, 0.25 g; $ZnSO_4$, 0.44 g and aqueous carrot extract to make upto 1 litre.

(E) *Findlays Medium E:* This medium was of the following composition: Glucose, 37.7 g; NH_4C1, 2.5 g, $MgSO_4 \cdot 7H_2O$, 1. Og, $FeSO_4 \cdot 7H_2O$, O. Olg; $ZnSO_4$ O. Olg, $MnSO_4 \cdot 7H_2O$, 0.005 g; $CuSO_4 \cdot 5H_2O$, 0.005 g; $Na_2B_4O_7 \cdot 10H_2O$, 0.005 g; Vitamin B_1 0.005g, CH_3COONa, 1.0 g; and aqueous carrot extract to make upto 1 litre.

(F) *Raulin-Thom Medium F:* The Composition of this medium was as follows: Sucrose, 70.0 g; tartaric acid, 4.0 g diammonium tartrate 4.0 g; K_2CO_3, 0.6 g; $NH_4H_2PO_4$, 0.6 g, $MgCO_3$, 0.4 g; $(NH_4)_2SO_4$, 0.25 g; $ZnSO_4$, 0.07 g; $FeSO_4 \cdot 7H_2O$, 0.07 g, potassium silicate, 0.07 g; and aqueous carrot extract to make upto 1 litre.

In a batch of fifteen 1-litre conical flasks each containing 350 ml. one of the above media was inoculated with nine-days old tube culture of *Penicillium funiculosum* and incubated at 27° for 22 days. The mycelium was separated from the broth by filtration.

Production and Extraction of Funiculosic acid: The broth from each batch was extracted thrice with ethyl acetate. The combined extracts were dried over sodium sulphate (anhydrous) and the solvent was removed *in vacuo.* In the resulting residue, the amount of funiculosic acid produced was estimated by measuring the intensity of UV absorption at λmax 297nm (6) on the SPEKTROMOM-204 instrument in methanol. It was noted that the best yield of funiculosic acid was obtained from Czapek-Dox (modified I) medium A, and this medium was therefore selected for further studies.

In the next step *Penicillium funiculosum* was grown on medium A in sets of 15 flasks (350 ml in 1-litre conical flask) and incubated for different durations i.e. 8, 12, 14, 16, 18, 22 and 24 days. It was observed that the maximum yield of funiculosic acid was obtained after 22 days incubation.

In order to further improve the yield, the medium was modified and the concentration of each ingredient which gave high yields, of funiculosic acid was selected for further studies.

RESULTS AND DISCUSSIONS

During preliminary investigations, it was observed that when *Penicillium funiculosum* was grown on different media without carrot extract, the growth of the mold was very slow, producing only a thin felt of mycelium. Also extraction of the broth gave only negligible amount of an oily material. On the other hand the media enriched with carrot extract showed rapid and healthy growth of the mold producing crystalline metabolites [1]. In the present studies, therefore *Penicillium funiculosum* was grown on six different media enriched with carrot extract for the production of funiculosic acid. The results are shown in Table 1. It was concluded that the best yield of funiculosic acid was obtained on medium A.

Sugar consumption, change in pH and duration required for maximum production of funiculosic acid was studied and the results are shown in Table 2.

The effect on the yield of funiculosic acid was also studied by varying the concentration of each ingredient. The best concentration, of each ingredient was selected and the medium thus prepared was studied for the production of funiculosic acid. The results are shown in Table 3-8.

Table 1. Yield of funiculosic acid in various media

Medium	No. of flasks	Volume of broth (1)	Total extract (g)	Funiculosic acid (mg/1)
A	15	3.924	3.537	382
B	15	4.125	1.218	218
C	15	4.149	4.920	137
D	15	3.450	6.180	45
E	15	4.269	0.402	70
F	15	4.176	0.855	83

Table 2. Duration for the maximum production of funiculosic acid along with changes in pH and sugar concentration

Days	Volume of broth (1)	pH	Sugar Concentration (%)	Funiculosic acid (mg/l)
8	5.10	3.5	1.90	No.
10	4.95	3.5	1.51	32.
12	4.80	3.5	1.37	39
14	4.80	3.5	1.18	41
16	4.80	3.5	0.97	46
18	4.50	4.0	0.69	49
20	4.50	4.0	0.59	62
22	4.50	4.0	0.32	119
24	4.10	4.0	0.32	75

Table 3. Effect of varying concentration of $FeSO_4$ in medium A on the yield of funiculosic acid.

$FeSO_4$ (g/1)	Volume of broth (1)	Total extract (g)	Funiculosic acid (mg/1)
0.0025	4.312	1.151	102.5
0.005	4.562	0.939	113.5
0.01	4.562	1.551	154.0
0.02	4.500	1.33	96.0
0.03	4.312	1.078	57.0

Table 4.Effect of varying concentration of KCl in medium A on the yield of funiculosic acid.

KCl (g/l)	Volume of broth (1)	Total extract (g)	Funiculosic acid (mg/1)
0.125	4.55	1.788	31
0.25	4.46	2.07	136
0.5	4.62	1.52	171
0.75	4.52	1.44	143
1.0	4.56	1.78	86

Table 5. Effect of varying concentration of KH_2PO_4 in medium A on the yield of funiculosic Acid.

KH_2PO_4 (g/1)	Volume of broth (1)	Total extract (g)	Funiculosic acid (mg/l)
0.50	4.312	1.154	156
0.75	4.575	1.102	163
1.00	4.250	0.782	178
1.25	4.162	0.886	150
1.50	4.374	0.975	58

Table 6. Effect of varying concentration of $MgSO_4$ in medium A on the yield of funiculosic acid.

$MgSO_4$ (g/l)	Volume of broth (1)	Total extract (g)	Funiculosic acid (mg/l)
0.125	4.68	1.68	194
0.25	4.52	2.27	355
0.5	4.41	1.83	198
0.75	4.7	2.01	64
1.0	4.38	2.01	25

From the above data it may be concluded that higher yield of funiculosic acid was obtained in Czepek Dox modified-I medium A. It was also noted that 7% glucose concentration gave a better yield of funiculosic acid as

compared to its normal concentration of 10%. In the case of $FeSO_4$, the best concentration was found to be 0.01 g/l as higher concentration showed adverse effect on yield. Similarly, it was observed that the normal concentration of KCl (0.5 g/l) gave better results. In the case of KH_2PO_4 higher production of funiculosic acid was obtained by using 1 g/l which is the normal concentration of the Czapek Dox medium. It was also noted that lower concentration of $MgSO_4$, i.e. 0.25 g/l favoured higher production of funiculosic acid. The normal concentration of $NaNO_3$ in Czapek Dox medium is 3 g/l, which was found to be the best concentration for the production of funiculosic acid.

Table 7. Effect of varying concentration of $NaNO_3$ in medium A on the yield of funiculosic acid.

$NaNO_3$ (g/l)	Volume of broth (1)	Total extract (g)	Funiculosic acid (mg/1)
1.0	4.61	1.42	60
2.0	4.39	1.49	101
3.0	4.48	1.57	122
4.0	4.62	2.11	97
5.0	4.44	2.29	89

Table 8. Effect of varying concentration of glucose in medium A on the yield of funiculosic acid.

Glucose (g/l)	Volume of broth (1)	Total extract (g)	Funiculosic acid (mg/l)
60.0	4.625	0.943	83
70.0	4.562	0.846	110
80.0	4.625	0.870	93
90.0	4.575	0.814	67
100.0	4.466	1.000	55

REFERENCES

1. I.H. Qureshi, Tahira Begum, and N. Murtaza, Pakistan J. Sci. Ind. Res., **23**, 16 (1980).
2. M.T. Shipchandler, Riv. Farmacol ter., 8, 272 (1977).
3. S. Adolf, S. Tosef and B. Rudi, Can. Pat. 956308 (1974), C.A. 83, 43363 G (1975).
4. Tahira Begum Ph.D. Thesis University of Karachi (1982).
5. S.I. Ahmed and N. Murtaza., Pakistan J. Sci. Ind. Res., **12**, 389 (1970).
6. Gridgemen, Analyst., **76**, 449 (1951).