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GROWTH FACTORS OF AUXOTROPHS OF *ASPERGILLUS NIDULANS* ASSOCIATED WITH LITCHI ROT AND THEIR PATHOGENICITY[1]

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ABSTRACT

The growth factor/s requirements of four mutants of *Aspergillus nidulans* viz., E, G, M and Y which were respectively highly, mildly, weakly virulent and non-pathogenic on 'Litchi' fruits, were determined both qualitatively and quantitatively. Auxotrophs E, G, and M were found to be deficient in riboflavin and tyrosine; riboflavin and aneurine; and riboflavin respectively, whereas Y, the non-pathogenic auxotroph showed biotin deficiency. Biotin has not been reported in 'Litchi' fruits and perhaps this accounted for the non-pathogenic behaviour of Y. It was further observed that the adequacy or inadequacy of growth factor/s available in 'Litchi' for the pathogenic auxotrophs seemed to determine the nature of their virulency.

INTRODUCTION

During the recent years extensive studies on growth factor/s of auxotrophs of *Aspergillus nidulans* have been made by different workers (Pontecorvo *et al.*, 1953 ; Dorn and Rivera, 1966; Dorn, 1967; Sinha, 1967, 1969, 1970 and 1972 ; Clutterbuck, 1969 and 1974; Verma and Sinha, 1973 ; and Clutterbuck and Cove 1976).

In the present investigations both qualitative and quantitative requirements of growth factor/s of four auxotrophs of *A. nidulans* associated with 'Litchi' rot viz., E, G, M and Y which were respectively highly, mildly, weakly virulent and non-pathogenic on 'Litchi' fruits have been determined and attempts have been made to correlate these factor/s with their pathogenic capabilities.

MATERIALS AND METHOD

The growth requirements of auxotrophs on minimal medium were determined by "auxanographic" technique employed by Pontecorvo (1949). The method consists of pouring a layer of molten agar minimal medium containing a suspension of cells of the auxotrophs in a Petri dish and then introducing small crystals of various subtsances at well separated marked points on the surface of agar. After incubation, the zones of growth indicate growth inducing action of particular substances. Double requirements were also similarly detected.

Growth in liquid medium was measured by following the method of Verma and Sinha (1973). Each culture flask with 25 ml of liquid medium and the desired concentration of growth factor/s was seeded with 5×10^6 conidia. Five replicates were used for each treatment. The inoculated flasks were incubated at 30C in B.O.D. incubator for 10 days. The fungal mats were then washed on previously weighed Whatman's filter paper no. 42 and dried to constant weight in an electric oven at 60 to 65C. The differences bet-

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ween the previous and final weights of the filter papers were calculated in terms of $\mu g/ml$ of the culture fluid to determine the weight of biomass.

OBSERVATIONS

The results showing the presence or absence of growth of the auxotrophs at points of casein hydrolysate and yeast extract have been presented in Table I.

From the above table, it is quite evident that mutant E was both vitamin and amino acid requirer, whereas G, M and Y were deficient in vitamin only.

The results of specific growth factor/s in respect of vitamins for the auxotrophs G, M, and Y have been presented in table II and for auxotroph E in respect of specific vitamin and amino acid in table III.

Table II reveals that the mildly and weakly virulent auxotrophs G and M showed riboflavin and aneurine ; and only riboflavin deficiency respectively, while the non-pathogenic auxotroph Y was found to be biotin deficient.

From Table III it is evident that the highly virulent auxotroph E showed the deficiencies of riboflavin and tyrosine.

The results of average dry weight of biomass of auxtotrophs Y (biotin deficient), M (reboflavin deficient), G (riboflavin and aneurine deficient) and E (riboflavin and tyrosine deficient) at various concentrations of required growth factors have been presented in Tables IV, V, VI and VII respectively. In case of G and E 120 $\mu g/l$ of riboflavin was kept constant in the medium as 'Litchi' contains 0.012% of riboflavin (Singh *et al.*, 1967).

From Tables IV, V, VI and VII, it is quite clear that for optimum growth of auxotrophs Y, M, G and E, minimum quantities of 4 $\mu g/l$ of biotin, 180 $\mu g/l$ of riboflavin, 140 $\mu g/l$ of aneurine and

TABLE I

SHOWING THE PRESENCE OF ABSENCE OF GROWTH OF THE AUXOTROPHS E, G, M AND Y AT POINTS OF CASEIN HYDROLYSATE AND YEAST EXTRACT ON MINIMAL MEDIUM

Auxotroph	Minimal medium	Substance made available to the auxotrophs		
		Casein hydrolystate on minimal medium	Yeast extract on minimal medium	Casein hydrolysate and Yeast extract on minimal medium
E	(—)	(—)	(—)	(+)
G	(—)	(—)	(+)	(+)
M	(—)	(—)	(+)	(+)
Y	(—)	(—)	(+)	(+)

(+) and (—) denote respectively presence and absence of growth.

TABLE II

SHOWING THE PRESENCE OR ABSENCE OF GROWTH OF THE AUXOTROPHS G, M AND Y AT POINTS OF SPECIFIC VITAMINS OR IN-BETWEEN THEM ON MINIMAL MEDIUM

Treatment No.	Vitamins added at opposite points on the minimal medium	Auxotroph		
		G	M	Y
1	2	3	4	5
1	(a) Riboflavin	(—)	(+)	(—)
	(b) Biotin	(—)	(—)	(+)
2	(a) Riboflavin	(—)	(+)	(—)
	(b) Nicotinamide	(—)	(—)	(—)
3	(a) Riboflavin	(—)	(+)	(—)
	(b) para amino-benzoic acid	(—)	(—)	(—)
4	(a) Riboflavin	(—)	(+)	(—)
	(b) Pyridoxine	(—)	(—)	(—)
5	(a) Riboflavin	(++)	(+)	(—)
	(b) Aneurine		(—)	(—)
6	(a) Biotin	(—)	(—)	(+)
	(b) Nicotinamide	(—)	(—)	(—)
7	(a) Biotin	(—)	(—)	(+)
	(b) Para aminobenzoic acid	(—)	(—)	(—)
8	(a) Biotin	(—)	(—)	(+)
	(b) Pyridoxine	(—)	(—)	(—)
9	(a) Biotin	(—)	(—)	(+)
	(b) Aneurine	(—)	(—)	(—)
10	(a) Nicotinamide	(—)	(—)	(—)
	(b) Para aminobenzoic acid	(—)	(—)	(—)
11	(a) Nicotinamide	(—)	(—)	(—)
	(b) Aneurine	(—)	(—)	(—)
12	(a) Nicotinamide	(—)	(—)	(—)
	(b) Pyridoxine	(—)	(—)	(—)
13	(a) Para aminobenzoic acid	(—)	(—)	(—)
	(b) Pyridoxine	(—)	(—)	(—)
14	(a) Para aminobenzoic acid	(—)	(—)	(—)
	(b) Aneurine	(—)	(—)	(—)
15	(a) Pyridoxine	(—)	(—)	(—)
	(b) Aneurine	(—)	(—)	(—)

(+) and (—) denote respectively presence and absence of growth of auxotrophs at points of specific vitamins and (++) denotes presence of growth in-between the two vitamin points.

TABLE III

SHOWING THE PRESENCE OR ABSENCE OF GROWTH OF AUXOTROPH E IN-BETWEEN THE TWO POINTS OF SPECIFIC VITAMIN AND AMINO ACID ON MINIMAL MEDIUM

Amino acid	Riboflavin	Biotin	Nicotina-mide	Para amino-benzoic acid	Pyridoxine	Aneurine
1	2	3	4	5	6	7
L-proline	(—)	(—)	(—)	(—)	(—)	(—)
L-Serine	(—)	(—)	(—)	(—)	(—)	(—)
L-threonine	(—)	(—)	(—)	(—)	(—)	(—)
Aspartic acid	(—)	(—)	(—)	(—)	(—)	(—)
L-Valine	(—)	(—)	(—)	(—)	(—)	(—)
L-arginine	(—)	(—)	(—)	(—)	(—)	(—)
Tyrosine	(+)	(—)	(—)	(—)	(—)	(—)
L-iso-leucine	(—)	(—)	(—)	(—)	(—)	(—)
L-leucine	(—)	(—)	(—)	(—)	(—)	(—)
Glutamic acid	(—)	(—)	(—)	(—)	(—)	(—)
Lysine	(—)	(—)	(—)	(—)	(—)	(—)
L-phenylalanine	(—)	(—)	(—)	(—)	(—)	(—)
Tryptophane	(—)	(—)	(—)	(—)	(—)	(—)
L-methionine	(—)	(—)	(—)	(—)	(—)	(—)

(+) and (—) denote respectively presence and absence of growth of the auxotroph in between the specific amino acid and vitamin points.

TABLE IV

SHOWING AVERAGE DRY WEIGHT OF BIOMASS OF AUXOTROPH Y AT VARIOUS CONCENTRATIONS OF BIOTIN

Treatment	Concentration (μg/l)	Dry weight (μg/ml)
1	1.0	350.2
2	2.0	700.2
3	3.0	1750.5
4	4.0	2000.2
5	5.0	2000.0
6	6.0	2000.0

TABLE V

SHOWING AVERAGE DRY WEIGHT OF BIOMASS OF AUXOTROPH M AT VARIOUS CONCENTRATIONS OF RIBOFLAVIN

Treatment	Concentration (μg/l)	Dry weight (μg/ml)
1	100.0	825.2
2	120.0	900.0
3	140.0	1500.1
4	160.0	2400.0
5	180.0	3000.0
6	200.0	3000.0

TABLE VI

SHOWING AVERAGE DRY WEIGHT OF BIOMASS OF AUXOTROPH G AT VARIOUS CONCENTRATIONS OF ANEURINE IN THE MEDIUM CONTAINING 120 μg/l OF RIBOFLAVIN

Treatment	Concentration (μg/l)	Dry weight (μg/ml)
1	60.0	750.2
2	80.0	1500.0
3	100.0	2000.25
4	120.0	3010.10
5	140.0	3500.5
6	160.0	3500.6

TABLE VII

SHOWING AVERAGE DRY WEIGHT OF BIOMASS OF AUXOTROPH E AT VARIOUS CONCENTRATIONS OF TYROSINE IN THE MEDIUM CONTAINING 120 μg/l OF RIBOFLAVIN

Treatment	Concentration (μg/l)	Dry weight (μg/ml)
1	4.0	500.0
2	6.0	800.5
3	8.0	1600.0
4	10.0	2375.0
5	12.0	2550.2
6	14.0	2550.2

12 μg/l of tyrosine respectively were required.

DISCUSSION

Auxotrophs E (highly virulent), G, (mildly virulent), M (weakly virulent) and Y (non-pathogenic) were requirers of riboflavin and tyrosine ; riboflavin and aneurine ; riboflavin ; and of biotin as growth factor/s respectively.

Biotin is lacking in 'Litchi' fruits, but riboflavin and aneurine (Singh *et al.*, 1967) and also tyrosine (Bilgrami, 1970) are present in it and hence the auxotroph Y which is biotin deficient proved to be non-pathogenic, while the rest three pathogenic. In similar studies Kline *et al.*, (1957) observed that some mutants of *Venturia inaequalis* which were deficient in chloine and riboflavin were unable to cause scab.

Auxotroph M and G required 0.018% riboflavin and 0.014% aneurine respectively in the medium for optimum growth, whereas 'Litchi' fruits contain only 0.012% of riboflavin and 0.009% of aneurine (Singh *et al.*, 1967). Thus the requirements for optimum growth fall short of 0.006% riboflavin and 0.005% aneurine and, therefore, quite likely M proved to be weakly and G mildly virulent. The highly virulent auxotroph Y of *A. nidulans* required 0.0012% tyrosin for optimum growth. It is quite likely that at least this quantity of tyrosine might be present in the 'Litchi' fruits for supporting optimum growth of this auxotroph.

The above observations regarding the pathogenic capabilities of the auxotrophs in respect of adequacy, inadequacy or total absence of the required nutrients in the host are in confirmity with those of several investigators (Garber and Schaeffer, 1957 ; Lippincott *et al.*, 1965 ; and Bajaj *et al.*, 1965).

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