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PATHOGENIC CAPABILITIES OF AUXOTROPHS OF *ASPERGILLUS NIDULANS* ASSOCIATED WITH LITCHI ROT[1]

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ABSTRACT

Mutants of *Aspergillus nidulans* causing serious fruit rot disease of 'Litchi' were raised by treating with 1 mg/ml of NTG for 30 minutes at pH 6.0 and 37C temperature. Sixty auxotrophs were screened for observing their pathogenic capabilities. Twentyfive of them were found to be pathogenic, while the rest non-pathogenic. Out of the pathogenic ones, six, sixteen and three were respectively highly, mildly and wealky virulent, which suggests that the mutagen produced four different groups of auxotrophs in respect of pathogenicity. It was further observed that with the increase in incubation period in arithmetical progression, there was increase in percentage rot of fruits in geometrical progression.

INTRODUCTION

During recent years studies on the pathogenic capabilities of auxotrophs of certain bacterial and fungal pathogens have been made by different investigators (Bacon *et al.*, 1951 ; Keitt and Boone, 1954 and 1956 ; Buxton, 1956 ; Keitt *et al.*, 1959 ; Tuveson and Garber, 1959 ; Dutta *et al.*, 1960 ; and Tinline, 1963). Such studies may reveal interesting information not only on the pathological behaviour of the auxotrophs but also in respect of the intricate host parasite interactions. In the present investigation pathogenic capabilities of auxotrophs of *Aspergillus nidulans*, which has been reported to be a highly virulent pathogen, causing fruit rot of 'Litchi' (Prasad and Bilgrami, 1973), have been assessed.

MATERIAL AND METHOD

The pathogen was isolated from diseased fruits of 'Litchi' and its pure culture was obtained by the method described by Tandon and Bilgrami (1954). Culture was maintained on Czapek's medium. Pathogenicity of the organism was established by satisfying Koch's (1882) postulates. These tests were done in replicates of five. Technique described by Pontecorvo (1949), Pontecorvo *et al.*, (1953) and Chattoo and Sinha (1974) was followed for mutagenic treatment. I mg/ml of N-methyl-N-nitro-N-nitrosoguanidine (NTG) was used as mutegen and the treatment was done for 30 minutes at pH 6.0 and 37C temperature. Isolation of auxotrophs by replicaplating was made by the method described by Lederberg and Lederberg (1952) and 60 auxotrophs were selected at random. The auxotrophs were maintained on complete medium. Pathogenicity test was conducted with each auxotroph. In order to determine the degree of virulence of the pathogenic auxotrophs, percentages of fruit rot on artificial inoculation were calculated out by

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the formula given below with the mean values of replicates of three. The virulency tests were carried out at 35 ±5C.

$$\text{Percentage rot-} \quad \frac{W-w}{W} \times 100$$

Where W—weight of the fruit before inoculation.

w—weight of the fruit after inoculation.

The data were statistically analysed. Standard error (S.E.) and Critical difference (C. D.) were calculated by the formula given below :

$$\text{S.E.} = \frac{\text{Mean square of error}}{\text{Number of replicates}}$$

$$\text{C.D.} = \text{S.E.} \times P\sqrt{2}$$

Where P is probability at 5% level.

OBSERVATIONS

The results of pathogenicity tests of auxotrophs have been presented in Table I.

TABLE I

SHOWING PATHOGENICITY OF AUXOTROPHS OF *A. nidulans*

Sl. No.	Name of auxotroph	Koch's postulates satisfied/not satisfied	Remark
1	Mutant I	NS	NP
2	M-2	S	P
3	M-3	S	P
4	M-4	S	P
5	M-5	S	P
6	M-6	NS	NP
7	M-7	S	P
8	M-8	S	P
9	M-9	S	P
10	M-10	S	P
11	M-11	NS	NP
12	M-12	NS	NP
13	M-13	S	P
14	M-14	NS	NP
15	M-15	NS	NP
16	M-16	S	P
17	M-17	NS	NP
18	M-18	NS	NP
19	M-19	NS	NP
20	M-20	NS	NP
21	M-21	NS	NP
22	M-22	NS	NP
23	M-23	S	P
24	M-24	NS	NP
25	M-25	S	P
26	M-26	S	P
27	M-27	NS	NP
28	M-28	S	P
29	M-29	NS	NP
30	M-30	S	P
31	M-31	S	NP
32	M-32	NS	NP
33	M-33	NS	NP
34	M-34	NS	NP
34	M-34	NS	NP
35	M-35	NS	NP
36	M-36	S	P
37	M-37	NS	NP
38	M-38	S	P
39	M-39	NS	NP
40	M-40	S	P

TABLE I—(*Contd.*)

Sl. No.	Name of auxotroph	Koch's posulates satisfied/not satisfied	Remark
41	M-41	NS	NP
42	M-42	NS	NP
43	M-43	NS	NP
44	M-44	S	P
45	M-45	NS	NP
46	M-46	NS	NP
47	M-47	NS	NP
48	M-48	S	P
49	M-49	S	P
50	M-50	S	P
51	M-51	NS	NP
52	M-52	S	P
53	M-53	NS	NP
54	M-54	NS	NP
55	M-55	S	P
56	M-56	NS	NP
57	M-57	NS	NP
58	M-58	NS	NP
59	M-59	NS	NP
60	M-60	NS	NP

S—Koch's postulates satisfied P-Pathogenic
NS—Koch's postulates not satisfied NP-Nonpathogenic

From Table I it is quite apparent that among the auxotrophs, twentyfive were found to be pathogenic and the rest thirty five nonpathogenic i.e. only 41.6% auxotrophs proved to be pathogenic.

The results of virulency tests with the pathogenic auxotrophs have been presented in Table II. In order to observe the progress of disease development in terms of incubation period of the auxotrophs, percentage rot of fruits was calculated at intervals of 2 days. The final observation was taken on the 10th day.

Statistical analysis of the data of Table II revealed that the auxotrophs, incubation periods and interaction of auxotrophs and incubation periods were highly significant.

The twentyfive auxotrophs could be classified in three sharp and significant groups i.e. auxotrophs M3, M5, M7, M9, M16 and M50 which caused maximum rot have been called highly virulent. while M2, M4, M8 M13, M23, M25, M26, M28, M30, M31, M36, M38, M44, M48, M52 and M55 have been designated as mildly virulent because they caused mild infection, whereas M10, M40 and M49 which brought about the minimum rotting of fruits have been named as weakly virulent. Thus there were six, sixteen and three highly mildly and weakly virulent auxotrophs respectively among the twenty-five pathogenic mutants. None of the auxotrophs could produce perceptible rotting till the 2nd day and the weakly virulent ones even upto the 4th day.

Percentage rot in terms of incubation period indicated that with the increase in incubation period in arithmetical progression, there was proportionate increse in percentage rot of fruits in geometrical progression.

Interaction studies between auxotrophs and incubation periods revealed that the highly virulent auxotrophs caused maximum rotting on the 8th day, while the mildly and weakly virulent auxotrophs on the 10th day.

DISCUSSION

Flor (1959) and Fincham and Day (1965) have explained variation in viru-

TABLE II

SHOWING PERCENTAGE ROT IN 'LITCHI' FRUITS DUE TO THE 25 PATHOGENIC AUXOTROPHS OF *A. nidulans*

Sl. No.	Auxotroph	Incubation period in days					
		2	4	6	8	10	Mean
1	M3	Negligible	39.3	78.5	100.0	100.0	79.45
2	M5	,,	39.2	78.6	100.0	100.0	79.45
3	M7	,,	39.2	78.5	100.0	100.0	79.42
4	M9	,,	39.3	78.6	100.0	100.0	79.47
5	M16	,,	39.3	78.5	100.0	100.0	79.44
6	M50	,,	39.2	78.7	100.0	100.0	79.47
7	M2	,,	8.6	17.6	35.1	70.4	32.91
8	M4	,,	8.6	17.7	52.2	70.4	32.97
9	M8	,,	8.5	17.6	35.3	70.4	32.95
10	M13	,,	8.5	17.6	35.2	70.4	32.97
11	M23	,,	8.6	17.6	35.2	70.5	32.97
12	M25	,,	8.6	17.6	35.3	70.4	32.97
13	M26	,,	8.6	17.7	35.2	70.5	32.97
14	M28	,,	8.5	17.7	35.3	70.4	32.97
15	M30	,,	8.5	17.7	35.3	70.5	33.00
16	M31	,,	8.5	17.8	35.4	70.5	33.05
17	M36	,,	8.5	17.6	35.4	70.5	33.00
18	M38	,,	8.5	17.6	35.3	70.4	32.95
19	M44	,,	8.6	17.7	35.2	70.4	32.97
20	M48	,,	8.6	17.8	35.2	70.4	33.08
21	M52	,,	8.5	17.6	35.3	70.5	32.97
22	M55	,,	8.6	17.7	35.4	70.5	33.05
23	M10	,,	Negligible	8.3	16.2	32.5	14.25
24	M40	,,	,,	8.3	16.3	32.5	14.28
25	M49	,,	,,	8.2	16.3	32.4	14.23
Mean		,,	1.56	32.59	48.54	72.86	G.M-41.89

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EFFECT OF 2, 2-DIPYRIDYL AND ETHYLENEDIAMINETERA-ACETIC ACID (EDTA) ON AMYLASE ACTIVITY AND CARBOHYDRATE CONSTITUENTS IN GREEN GRAM (*PHASEOLUS RADIATUS* L.) SEEDLINGS[1]

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ABSTRACT

Cellulose synthesis was enhanced in green gram seedlings by chelating agents viz., EDTA and 2,2-dipyridyl at low concentration (10 ppm) with a corresponding increase in amylase activity and a decrease in sugar content. Even at higher concentration (100 ppm) cellulose content was increased with EDTA but not with dipyridyl, the former showing decreased and the latter an increased amylase activity.

INTRODUCTION

The effects of chelating agents on metabolic aspects is not well understood and the work is meagre. Only few attempts have been made to follow up this line in plants. The present study has been designed to gather more information as to their effects on growth and metabolism. The effect of 2, 2-dipyridyl and EDTA on amylase activity and changes in starch, cellulose and sugar contents was studied in order to get an insight as to their influence on cell wall synthesis. Burstrom (1958) stated that strong growth inhibitions by chelating agents do not hamper the cell wall synthesis. The present study is intended to confirm the validity of his statement.

MATERIAL AND METHODS

Seeds of green gram Var-PS. 16 were subjected to presowing soaking in 10 and 100 ppm EDTA and 2, 2,-dipyridyl for 24 h after which they were transferred to distilled water and allowed to grow for a period of 8 days in petridishes under continuous illumination of 2000 lux at 28°C ± 1. Roots and shoots of the seedlings were separately analysed at 2 day intervals for amylase and carbohydrate constituents. Amylase activity was determined by the method of Bernfiled (1955). Cellulose was extracted following the method of Asamizu *et al.*, (1977) and estimated using the anthrone method of Villes & Silverman (1949). Starch and reducing sugars were estimated according to the methods of McCready *et al.*, (1950). and Highkin & Frankel (1962) respectively.

RESULTS AND DISCUSSION

EDTA enhanced elongation growth of the seedlings both at 10 and 100 ppm. 2, 2-bipridyl caused significant inhibition on growth at 100 ppm (Table I). According to Burstrom (1963) growth pro-

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