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ACTIVITY OF SOME GROWTH FACTORS ON *CEPHALOSPORIUM SACCHARI* BUTL. CAUSING SUGARCANE WILT.

By

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The test-fungus, *C. sacchari*, which causes sugarcane wilt, showed maximum mycelial growth when externally supplied with inositol at 100 ppm concentration revealing thereby its auxoheterotrophic nature for the vitamin while it is auxoautotrophic for thiamine as it did not show any significant increase when externally supplied with thiamine. On the other hand, maximum spore production in thiamine at higher concentration (100 ppm) of the fungus revealed that an excess thiamine is required which the fungus is unable to synthesize. The higher mycelial growth in biotin and pyridoxine than the control revealed the partial auxoheterotrophic nature of the fungus under consideration.

Introduction

Fungi require minute amounts of specific growth factors for their normal development. A complete documentation on growth factor requirements in fungi, have been made by previous workers (Schopfer, 1943 ; Robbins and Kavanagh, 1942). A number of workers (Sadasivan and Subramanian 1954 ; Tandon *et al*, 1957 ; Cochrane, 1958 ; Fries, 1961 1965 ; Singh, 1963 and Sankhla and Mathur, 1967) have shown that vitamins are essential for normal development of some fungi. Amongst fungi, auxoautotrophic forms are independent of the externally supplied vitamins and indicate their ability to synthesise the substance, while auxoheterotrophic forms suffer from complete deficiencies for one or more vitamins and do not grow at all in a vitamin-free medium because of their inability to synthesise any of the required vitamins. Most of the known vitamins have a catalytic function in the cell and are known to be precursors of coenzymes or

constituent parts of coenzymes. As such, the deficiency of a vitamin interferes with the activity of an enzyme-system and prevents normal metabolic changes performed through the agency of that system (Tanber, 1939). Of the large number of chemically pure vitamins, relatively a few have been tested for their role in fungal growth. The present investigation has been undertaken to study the effect of some vitamins on mycelial growth and sporulation of *Cephalosporium sacchari* Butl., on which any information is still lacking. Four vitamins, *viz.*, thiamine, biotin, inositol and pyridoxine, for which fungi are most frequently deficient, have been selected for investigation.

Material and Methods

Cephalosporium sacchari Butl., isolated from infected stem of sugarcane, was collected from Hooghly District, 1972. For determining the vitamin requirements, the pathogen was grown in *Richard's liquid* medium. The medium was prepared in double distilled water and was boiled with activated charcoal (Norit 5g/L) for 5 minutes (Mathur *et al*., 1950) and then filtered to remove any trace of vitamins. The *p*H of the medium was adjusted, before autoclaving, to *p*H 6.6, found to be optimum for the test-fungus in an earlier experiment.

Stock solutions of thiamine, biotin, inositol and pyridoxine were prepared in double distilled water and were mixed with the medium in desired quantities (100 ppm, 10 ppm, 1 ppm, 0.1 ppm and 0.01 ppm) separately in flasks. Twentyfive ml of the medium was then poured in each of 250 ml. Erlenmyer flasks which was then plugged. One set of flasks with the vitamin-free medium was kept as control. The flasks were then subjected to fractional sterilization for three successive days to avoid thermo degeneration of growth factors at higher temperature. The flasks were inoculated aseptically with very thin and uniform disc of mycelium (5 mm. diam.) of the fungus from actively growing cultures on vitamine-free *Richard's medium* For each treatment three replicates were taken. The inoculated flasks were incubated at 30°C (±1°C) for 8 days, 12 days and 16 days respectively. After regular intervals, the mycelial mats were harvested by filtering and washing thoroughly over previously dried and weighed Whatman's filter paper No. 42 with distilled water. These were then dried to a constant weight at 60°C in an oven and weighed. The average of three replicates was recorded. The data were analysed statistically and results have been discussed at 5% level of probability.

Counting of spores was made in Hæmocytometer of 0.1 mm. depth and the number was calculated in 1 ml. of original solution (Paul, 1929).

RESULTS

The average dry weight of mycelia and quantum of sporulation of *C. sacchari* in different concentrations of vitamins are presented in Tables 1 and 2 respectively.

Table No. 1. *Data (mean) showing the effect of different concentrations of vitamins on the growth of* C. sacchari *at different incubation periods.*

Dry weight of mycelium (mg.)

Vitamins	Con. in ppm	Incubation periods		
		8	12	16
Thiamine	100	48.0	54.6	72.0
	10	34.5	56 2	64.0
	1	31.5	60.0	63.5
	0.1	30.6	66.5	75.6
	0.01	30.0	50.0	70.0
Biotin	100	49.5	56.0	74.5
	10	52.5	57.5	76.0
	1	52.0	66.3	78.6
	0.1	74.5	82.5	95.6
	0.01	59.0	64.5	73.3
Inositol	100	45.5	100.5	126.0
	10	38.0	80.5	86.2
	1	30.6	55.0	106.5
	0.1	37.3	76.0	92.5
	0.01	39.5	72.0	90.0
Pyridoxine	100	32 3	72.0	74.0
	10	39.0	73.5	77.5
	1	63.0	75.0	80.5
	0.1	64.0	85.5	90.0
	0.01	37.5	66.5	89.5
Control	0	33.9	53.3	68.9

'F'—value for days significant at 1% level
'F'—value for vitamin significant at 1% level
'F'—value for vitamin × days significant at 1% level

C.D. at 5% level for days = 16.79
C.D. at 5% level for vitamin = 27.60
C.D. at 5% level for vitamin × days = 14.43.

Table No. 2 *Data (mean) showing the effect of different concentrations of vitamins on the sporulation of* C. sacchari *at different incubation periods.*

Number of spores in millions/ml.

Vitamins	Conc. in ppm	Incubation periods		
		8	12	16
Thiamine	100	1.08	2.72	3.04
	10	.40	1.08	2.04
	1	.24	1.48	1.72
	0.1	.16	1.08	1.80
	0.01	.16	1.04	1.32
Biotin	100	.24	.04	.36
	10	.24	.032	.24
	1	.12	.18	.22
	0.1	.08	.012	.20
	0.01	.24	.04	.16
Inositol	100	.04	.04	.04
	10	.24	.03	.08
	1	.024	.04	.031
	0.1	.04	.12	.44
	0.01	.04	.20	.32
Pyridoxine	100	0	.032	.08
	10	.032	.08	.24
	1	.16	0	.08
	0.1	.08	0	.04
	0.01	.04	.08	.08
Control	0	.03	.03	.12

It is evident from Table 1 that externally supplied thiamine does not show any appreciable increase in the mycelial growth when compared with the control even after 16 days of incubation. In all other cases the test-fungus responds well to the external supply of vitamins. Maximum dry weight of mycelium was obtained at 100 ppm inositol. In this case, however, lower concentration showed lesser growth, while at 0.1 ppm. biotin and pyridoxine exhibited much better result than at higher concentrations. It has also been observed that with increase in incubation period the growth also gradually increases in biotin, pyridoxine and inositol.

From Table 2 it appears that thiamine, which did not show any stimulatory effect on mycelial growth, exhibited maximum spore production at 100 ppm. With decrease in concentration. the spore production decreased considerably. Pyridoxine, on the other hand, induced less spore production in all concentrations except at 10 ppm than even the control. Biotin and inositol appeared to induce

much higher spore production at 100 ppm and 0.01 ppm respectively. This, however, decreased gradually at lower concentrations in biotin and higher in inositol. In almost all cases the sporulation increased with increase in period of incubation.

Discussions

From the results it can be concluded that not all the vitamins are equally effective in inducing better mycelial growth and sporulation of the test-fungus. Moreover, the same vitamin may not be the most favourable one for both the purposes as is evident from maximum mycelial growth in inositol and maximum sporulation in thiamine. The present experiment reveals that the test-fungus may be an auxoautotroph for thiamine and partial auxoheterotroph for all other vitamins under consideration. The increased production of spores in externally supplied thiamine probably suggest that for sporulation a higher concentration of thiamine is more favourable and the fungus is incapable of producing this excess thiamine.

The 'F' values were found to be significant for vitamins, days and their interactions together at 1% level of probability.

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