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Cellulase (C_x) Production by Three Leaf Spot Fungi

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Ce'lulase (C_X) activity of three leaf spot fungi, viz., *Helminthosporium holmii* Luttrell., *Phaeotrichoconis crotalariae* (Salam & Rao) Subram.,and *Myrothecium roridum* Tode ex Fr. isolated from leaf spots of *Psidium guajava* L., *Abelmoschus esculentus* (L) Moensch. and *Holorrhina antidysentrica* Wall., respectively was assayed under different cultural conditions. Out of six media employed, cellulose-containing media induced cellulase formation. CMC-supplemented media gave less activity of cellulase. Among the carbon sources glucose stimulated more activity. *M. roridum* in general was a better cellulase (C_X) producer. The effect of fungicides, antifungal antibiotics, phenols and IAA on the cellulase production was studied. Aureofungin, callixin and phenols inhibited cellulase production while IAA enhanced its activity.

Key Words: *Helminthosporium holmii* **Luttrell**, *Phaeotrichoconis* **crotalarial (Salam & Rao) Subram.**, *Myrothecium roridum*, **Fungicides, Antifungal antibiotics, Phenols, IAA**

Introduction

Cellulases produced by the plant pathogens play a significant role either directly by degrading the cellulose constituents of cell wall or indirectly by releasing nutrients from disrupted cells (Mandels & Reese 1963). Inspite of voluminous work (Siu & Reese 1953, Reese 1956, Aitken et al. 1956, Whitaker 1957, Norkrans 1963 and Bateman & Basham 1976), enzymatic degradation of cellulose is imperfectly understood. The significance of cellulases in pathogenesis has been emphasized (Husain 1957, Husain & Dimond 1958, Husain & Kelman 1959 and Laxminarayan & Reddy 1978). This paper records the results of production cellulases (C_X) under different cultural conditions.

Materials and Methods

Monosporic cultures of *Helminthosporium holmii* Luttrell, *Phaeotrichoconis crotalariae* (Salam and Rao) Subram. and *Myrothecium roridum* Tode ex Fr. isolated from leaf spots of *Psidium guajava* L., *Abelmoschus esculentus* (L) Moensch and *Holorrhina antidysentrica* Wall., respectively were employed for the assay of cellulase. Unless otherwise mentioned, the fungi were grown on 25 ml modified Czapek's medium (*p*H 5.5) at 25±2°C. At the end of the incubation period the cultures were harvested and filtrate used for enzymatic assay. The filtrate was centrifuged at X 1800 g for 30 min to remove the mycelia

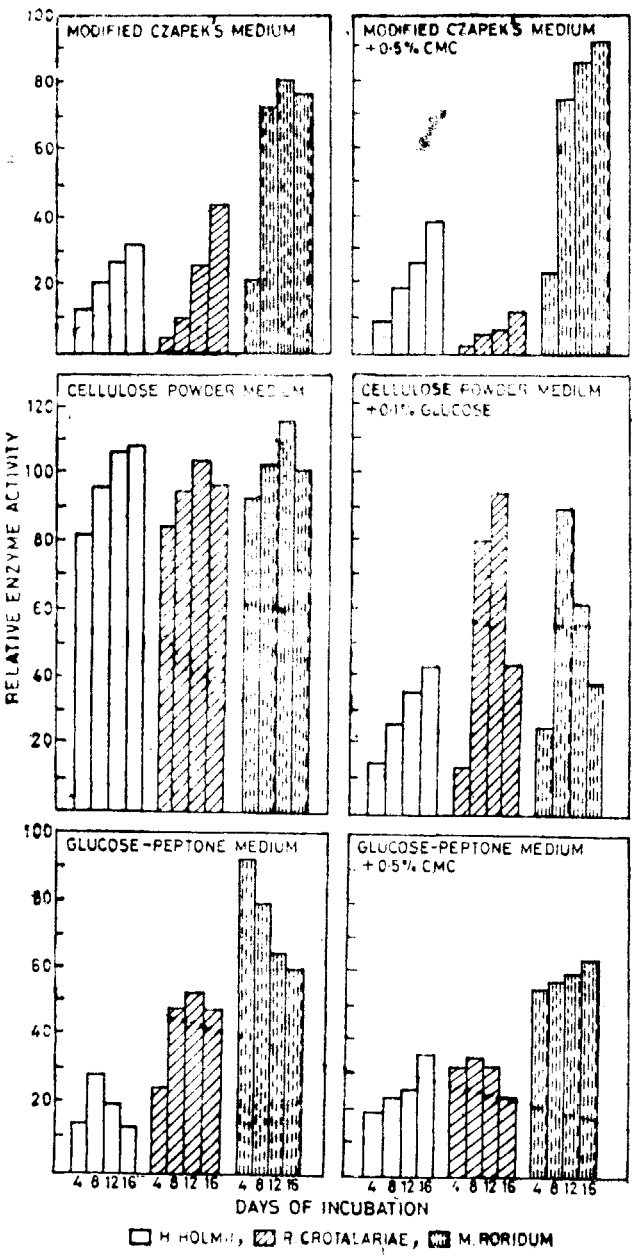

Figure 1

debris. The reaction mixture consisted 15 ml of 1% CMC solution, 5 ml of enzyme and 1.0 ml of buffer at *p*H 5.5. The loss of viscosity was measured every 10 min for a period of 1 hr at 30±2°C. The enzyme activity was expressed as relative enzyme activity (REA). $REA = 1000/t_{50}$ where t_{50} is the time taken for 50% loss of viscosity. To see the effect of various carbon and nitrogen sources, sucrose and potassium nitrate of the basal medium were replaced with equal amounts of carbon and nitrogen sources. The effect of different chemicals on cellulose production was observed by adding them aseptically to the basal medium after autoclaving.

Results and Discussion

Cellulose powder medium: It induced maximum cellulase production followed by glucose supplemented cellulose powder medium (figure 1). However, CMC-supplemented media did not enhance Cx secretion. Maximum activity on cellulose powder indicates the adoptive nature of the enzyme. These results are similar to those of earlier investigators (Wood 1960, Hortan & Keen 1966, Moore & Couch 1968 and Laxminarayana & Reddy 1978). There is a significant reduction in cellulase production by the fungi under study when cellulose powder was supplemented with 0.1% glucose, as earlier reported by Verma et al. (1975).

Carbon sources: Among the six carbon sources employed, glucose supported some activity (table 1). Starch stood in next position and it exhibited peak activity on 12th day. Only *P. crotalariae* could show some traces of cellulase in presence of sorbose. *M. roridum* seems to be more versatile as it secreted significant amount of Cx on all carbon sources tested. Ghewande and Deshpande (1975) observed that cellulase activity of *Helminthosporium apattarnae* varied with the carbon source.

Table 1 *Relative cellulase (C_x) activity of three leaf spot fungi on different carbon sources*

Carbon source	Days of incubation	*H. holmii*	*P. crotalariae*	*M. roridum*
Glucose	6	19.2	28.8	44.6
	12	41.0	16.4	30.8
Sorbose	6	—	4.2	—
	12	—	7.6	—
Lactose	6	14.8	3.0	32.2
	12	9.6	—	30.4
Starch	6	9.6	16.2	21.2
	12	8.4	24.2	24.0
Sorbitol	6	16.8	11.2	27.6
	12	7.8	15.6	30.8
Tartaric acid	6	9.0	18.8	7.2
	12	15.0	15.2	20.5

Table 2 *Relative cellulase (Cx) activity of three leaf spot fungi on different nitrogen sources*

Nitrogen source	Days of incubation	*H. holmii*	*P. crotalariae*	*M. roridum*
Potassium nitrate	6	19.2	28.8	44 6
	12	41.4	16.4	30.8
Sodium nitrite	6	28.6	2 9	34.2
	12	64.2	11.2	104 8
Ammonium chloride	6	91.0	15.2	24 6
	12	34.4	34.0	28.8
Asparagine	6	31.8	8.3	83 6
	12	40.2	21.0	116.4
Methionine	6	32.4	6.8	104 6
	12	51.6	39.6	136.8
Urea	6	69.9	18.2	70.4
	12	58.0	51.2	112.2

Nitrogen sources: Ammonium chloride, urea and methionine were excellent nitrogen sources for cellulase production of *H. holmii*, *P. crotalariae* and *M. roridum* respectively (table 2). Potassium nitrate, sodium nitrite and ammonium chloride were poor sources. On the other hand, *H. apattarnae* (Ghewande & Deshpande 1977) showed more enzymatic activity on inorganic nitrogen sources being maximum on calcium nitrate. L-asparagine which only supported moderate amounts of cellulase production by the fungi, has been reported to be the best source for cellulase production (Forbes & Dickinson 1977 and Narania & Reddy 1978).

Fungicides: A number of fungicides, antibiotics and chemicals have been reported to inhibit production of cellulases (Grover 1963, Crossman 1962 and Ghewande & Deshpande 1976). Effect of these substances on cellulase production was studied.

Aureofungin suppressed cellulase production in *H. holmii*, but enhanced in *P. crotalariae* and *M. roridum* at 10 mcg/ml concentration. However, at 20 mcg/ml concentration, the trend in Cx production of *M. roridum* continued whereas *P. crotalariae* was inhibited. Reddy and Laxminarayana (1978) reported hat two species of *Helminthosporium* studied by them reacted differently to aureofungin. Cellulase production of all the three fungi under study was inhibited at 40 mcg/ml of callixin. On the other hand, Reese and Mandels (1957) reported poor performance of fungicides against fungal cellulases. The enzyme secretion of the three fungi was stimulated significantly at 50 mcg/ml concentration of IAA and inhibited at 100 mcg/ml concentration. Both catechol and pyrogallic acid inhibited cellulase production. Similarly Ghewande and Deshpande (1976), Reddy and Mahadevan (1967) and Reddy and Laxminarayana (1978) reported the inhibition of different phenolic compounds on fungal cellulases.

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