Proc. Indian natn. Sci. Acad.
Vol. 43, Part. B, No. 3, pp. 232-235 (1977)
Plant Pathology

ENZYMATIC STUDIES ON CERTAIN FRUIT-ROT FUNGI
II. INFLUENCE OF CARBON SOURCE AND PHENOLS ON CELLULASE (Cx) PRODUCTION

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(Received 19 *December,* 1977*)*

Influence of carbon sources and phenols on *cellulase* (Cx) production by six fruit-rot fungi ; *Helminthosporium hawaiiense* Bugn., *H. spiciferum* (Bain) Nicot, *Alternaria tenuis*, Nees, *Curvularia lunata* (Wakker) Boed., *Phomopsis mangiferae* Ahmed and *Hendersonula toruloidea* Nattrass causing different fruit rots was studied. Mannitol induced more enzyme activity of *A. tenuis*, while glucose supported maximum *cellulase* activity of *P. mangiferae* and *H. toruloidea*. The rest of the organisms opted lactose as a substrate for enzyme production. Phenols varied in their activity both with the fungus and concentration of the compound.

INTRODUCTION

Cellulases have been reported to be constitutive in some fungi (Strider & Winstead 1961 and Winstead & McCombs, 1961) and adaptive in other (Hussain & Rich, 1958). The substrate has been reported to have a definite influence on enzyme secretion by the fungus. The products of hydrolysis of cellulose, glucose or cellobiose, are known to reduce the activity of some cellulases (Wood, 1967). Although recently Mandles and Reese (1963) reported naturally occurring inhibitors of cellulases, a very little work has been done to elucidate the inhibitory action of different substances *in vitro*. In this communication effect of five carbon sources and four phenols and one antibiotic on *cellulase* (Cx) production is discussed.

MATERIALS AND METHODS

Monosporic cultures of *Curvularia lunata* (Wakker) Boed., isolated from diseased fruits of banana, *Alternaria tenuis* Nees and *Helminthosporium hawaiiense* Bugn. causing fruit rot of tomato, *H. spiciferum* (Bain) Nicot inciting black rot of snake gourd, *Phomopsis mangiferae* Ahmad causing fruit rot of mango and *Hendersonula toruloidea* Nattrass, responsible for apple rot were employed. The cultures were maintained on Asthana and Hawker's medium. 25 ml. of medium (15 g glucose, 3.5 g KNO_3, 1.75 g KH_2PO_4 and 0.75 g $MgSO_4$ and distilled water 1 litre) was suspended in 100 ml Erlenmeyer flasks. Different carbon sources were substituted for glucose so as to supply 6000 mg of carbon per litre. The *p*H of the medium was adjusted to 5.5 with the help of either 6 N Hcl or 6 N NaoH.

Garett's (1936) agar disc method was followed to inoculate the flasks with respective fungi. The flasks were incubated at 29±2 °C. The *Cellulase* (Cx) was assayed on the 7th and 12th day by Fenske Ostwalds' viscometer (Hussain & Dimond, 1960) and the enzymatic activity was expressed in terms of relative enzyme activity (REA) as done by Agarwal (1971). The reaction mixture consisted of 7.5 ml 0.5% CMC, 2.5 ml of enzyme preparation and 0.5 ml McIlvaines buffer (5.5). The other details were similar to methods employed earlier (Laxminarayana & Reddy, 1977).

Results and Discussion

Effect of different carbon sources on the production of *cellulase* (Cx) was studied and the results are given in Table I. Induction of *cellulase* varied with the fungus and the source of carbon present in the medium. *H. hawaiiense*, *H. spiciferum* and *C. lunata* produced maximum amount of Cx on lactose medium, while *A. tenuis* preferred mannitol for optimum enzyme production. Glucose was found to be the best source for Cx activity for *P. mangiferae* and *H. toruloidea*. The Cx activity was maximum on the 12th day on lactose medium growing *H. hawaiiense*, and *C. lunata*. The same was the case on mannitol growing *A. tenuis*. In contrast glucose induced maximum activity in *P. mangiferae* and *H. toruloidea* by the 7th day which, however, decreased by the 12th day. Tartaric acid was useless for enzyme induction.

Table I

Relative Cellulase *activity (Cx) of six fruit-rot fungi on different carbon sources*

Name of the organism	Days of incubation	Glucose	Lactose	Sorbitol	Mannitol	Tartaric acid
H. hawaiiense	7	42·3	76·2	21·6	32·6	—
	12	26·2	98·4	34·2	46·9	—
H. spiciferum	7	82·6	102·6	96·2	53·6	—
	12	27·6	142·6	110·4	86·7	—
A. tenuis	7	90·3	18·6	38·7	110·3	—
	12	68·6	26·2	56·3	126·8	16·2
C. lunata	7	102·6	96·2	25·2	78·6	—
	12	84·7	126·4	36·4	96·3	26·2
P. mangiferae	7	261·7	67·8	18·4	46·2	27·6
	12	145·8	46·2	26·3	25·6	40·2
H. toruloidea	7	268·2	96·2	38·2	16·2	25·7
	12	201·7	76·3	64·1	29·3	41·6

Effect of some phenols and aureofungin on the Cx activity of some fruit-rot fungi was also studied and the results are precised in Table I. Aureofungin, although toxic to the fungus, induced more enzymatic activity in *H. hawaiiense*. However, the activity decreased considerably when concentration of aureofungin

increased up to 100 μg/ml. It was found to be inhibitory to enzyme production of the rest of the fungi under study. All the phenols tested, varied in their action with the fungi. Catechol inhibited completely the enzyme activity of *P. mangiferae*, while rest of the fungi showed declining trend in the *Cellulase* (Cx) activity with increase in concentration of catechol. *H. toruloidea* was an exception in secreting more enzyme at 100 μg/ml concentration of catechol which subsequently reacted adversely with increase in the concentration of phenol.

TABLE II

The relative enzyme activity (REA) of Cellulase *(Cx) on Asthana and Hawker's medium 'A' containing aureofungin and four phenolic compounds on seventh day of incubation*

	H. hawaiiense	*H. spiciferum*	*A. tenuis*	*C. lunata*	*P. mangiferae*	*H. toruloidea*
Control	42·3	82·6	90·3	102·6	261·7	168·2
Catechol						
*100	31·2	25·3	35·6	23·3	—	176·2
500	16·2	16·4	16·1	19·4	—	127·3
1000	11·2	12·5	13·1	13·8	—	85·2
Pyrogallic Acid						
200	38·1	15·7	—	36·8	83·3	86·3
500	19·2	—	—	18·2	31·9	62·8
1000	15·6	—	—	—	19·8	39·7
Gallic acid						
100	12·2	100·7	18·3	55·6	56·2	50·2
500	—	43·5	66·6	34·4	25·1	33·6
1000	—	17·2	—	23·1	16·1	—
Salicylic acid						
100	250·1	62·5	40·4	248·3	36·4	252·6
500	39·3	—	—	66·2	—	60·7
1000	14·7	—	—	23·2	—	29·2
Aureofungin						
5	166·5	28·2	62·4	19·8	146·3	84·1
10	83·3	20·1	16·1	14·6	82·1	47·6

* Concentration in μg/ml.

Pyrogallic acid, completely suppressed the enzyme activity of *A. tenuis*, while *H. spiciferum* and *C. lunata* stopped secreting Cx enzyme from 500 μg/ml and 100 μg/ml concentration respectively. Other organisms also expressed similar response at higher concentrations.

Gallic acid, was toxic to *H. hawaiiense*, while *A. tenuis* and *H. toruloidea* could secrete Cx enzyme up to 500 μg/ml after which there was no enzyme activity. *H. spiciferum* produced more enzyme at 100 μg/ml concentration of gallic acid, while *C. lunata* and *P. mangiferae* responded adversely as the concentration increased. Salicylic acid was also toxic for Cx activity of *H. spiciferum*, *A. tenuis* and *P. mangiferae* beyond 100 μg/ml concentration. However, it induced more enzymic

activity of *H. hawaiiense* and *C. lunata* in the medium containing 100 μg/ml. Higher concentrations had adverse effect.

Koti Reddy and Mahadevan (1967) reported that catechol and anthraquinone were converted into effective inhibitors which in turn inhibited the cellulase production. These substances get converted to toxic quinones and intermediate toxic chemicals in the presence of polyphenol oxidases (Lyr, 1965). Mandels and Reese (1963) reported that cellulolytic activity of fungi studied by them is reduced by tannic acid and leucoanthocyanins.

Acknowledgements

Thanks are due to Professor U. B. S. Swami, Professor and Head of the Botany Department, Kakatiya University, Warangal, for kind encouragement and providing facilities. One of the authors (PL) wish to thank Sri K. Jayashankar, Principal, C.K.M. College, Warangal, for encouragement.

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