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PRODUCTION OF CELLULOLYTIC ENZYMES BY *PLEUROTUS SAJOR-CAJU* (Fr.) *SINGER**

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Pleurotus sajor-caju was found to produce cellulases both *in vitro* and *in vivo*. The Cx activity was not inhibited by the addition of gallic acid at 100 ppm. The inhibition of Cx activity was noticed at higher concentrations of catechol and resorcinol. The C_1 activity was enhanced by the addition of 100 ppm of catechol but inhibited by 1000 ppm of catechol and 100 to 1000 ppm of resorcinol. The C_1 activity was not influenced by gallic acid. The C_1 and Cx activities were not influenced by the addition of plant hormones.

Pleurotus sajor-caju (Fr.) Singer, oyster mushroom is a well known edible fungus. During the development of mycelium biochemical changes occur in both the sporocarps as well as the substratum on which the edible fungus grows. During these processes, cellulolytic enzymes are secreted to degrade the insoluble component of the plant residues. It has been suggested that the enzymes may have a definite role in the development of sporocarps. (Wang, 1981). In the present study, an attempt has been made to study the production of cellulolytic enzymes by *P. sajor-caju* and the results are presented in this paper.

MATERIAL AND METHODS:

A pure culture of *P. sajor-caju* was isolated from fresh fruiting body by hyphal tip method and maintained on oat meal agar slants Mycelial discs of 8 mm diameter from seven day-old culture of *P. sajor-caju* were inoculated into 50 ml of Czapek's medium in which the carbon source was substituted with 3 per cent cellulose powder and incubated at room temperature (24 ± 2°C). After 5, 10 and 15 days of incubation, the cell free culture filtrates were obtained and used as enzyme extract. No dialysis was done for the assay of cellulolytic enzymes, since the dialysis is known to reduce cellulase activity (Bateman. 1964). The Cx activity was assayed by the viscosimetric method with carboxy methyl cellulose as the substrate (Hancock *et al.*, 1964). The C_1 activity was assayed by the method of Norkrans (1950).

Influence of phenolics on the *in vitro* activity of cellulases was studied. The cell free culture filtrates were obtained after 10 days of incubation at room temperature (24 ± 2°C). The phenolics viz. catechol, resorcinol

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and gallic acid at 100, 500 and 1000 ppm levels were added at 1 ml to the enzyme assay mixture before adding the enzyme source.

The culture filtrates of *P. sajor-caju* obtained as in previous case were utilised to assess the effect of certain plant hormones on the activity of cellulases. Gibberellic acid, indole acetic acid and kinetin at 0.1, 1.0, 10.0 and 100.0 ppm levels were tried. The respective concentrations of hormone were added at 1 ml to the enzyme source.

In vivo production of cellulases by the substrates was assessed by selecting waste paper and rice straw which recorded higher yields of sporophore in comparison with *coir* waste of coconut (*Cocos nucifera* L.), an unsuitable substrate. The substrates were soaked in water and sterilized at 1.4 kg/cm² for 1 hour, cooled and mushroom beds were prepared following the tray method. The crude enzyme was obtained by grinding 5g of the substrate in 20 ml distilled water following the growth of *P. sajor-caju* at 10, 20 and 30 days after inoculation. The uninoculated substrates were also taken up for the enzyme assay. The extracts were squeezed through several layers of cheese cloth and centrifuged at 18,000 G for 20 minutes at 6°C The supernatant was used as enzyme source (Maxwell and Bateman, 1967).

RESULTS AND DISCUSSION:

In vitro production of cellulases C_1 and Cx was observed in culture filtrates of the fungus. Maximum activity of C_1 and Cx enzymes was observed in 16 and 10 days old culture filtrates respectively.

The Cx activity was not inhibited by the addition of gallic acid at 100 ppm. The inhibition of Cx activity was noticed at higher concentrations of catechol and resorcinol. The C_1 activity was enhanced by the addition of 100 ppm of catechol but inhibited by 1000 ppm of catechol and all concentrations of resorcinol. The C_1 activity was not influenced by gallic acid (Table 2).

The C_1 and Cx activities were not influenced by the addition of plant hormones at different concentrations (Table 3).

The data on *in vivo* test indicated the maximum activity of both C_1 and cx enzymes at 50 days after inoculation. maximnm activity of both the enzymes was observed in waste paper followed by rice straw (Table 4).

In the present study, *P. sajor-caju* was found to produce cellulases the *in vitro* and *in vivo*. *in vivo* activity of cellulases was high during the initiation of sporophores at 20 days after inoculation but decreased later. According to Turner *et al* (1975) the level of cellulase activity rose in the bedding material reaching at fruiting a high level which was maintained through out the subsequent cropping during the life cycle of the cultivated mushroom, *Agaricus bisborus*. The cellulase activity was most in the susbstrates which recorded higher yields of sporophore. This indicated a positive relationship between enzme activity and yield

It is well known that phenolics interfere in the activity of various enzymes produced dy fungi (singh and chand, 1969) as well as cellulases(Mandels and Reese 1965., Koti Reddy and Mahadevan, 1967). In the present study plant hormones did not significantly influence the activity of cellulases *in vitro*. The finding that the spraying of plant hormones on the initials of sporophore did not significantly influence the development of fruiting bodies (Sivaprakasam 1980). corroborates this conclusion.

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Table 1. *In vitro* production of cellulases

Age of culture (days)	Cx enzyme (per cent loss in viscosity of CMC)			C^1 enzyme 11 unit — change in absorbance of 0.01)
	15	30	60	
5	5.00	8.00	11.00	1.00
10	38.00	46.33	55.00	6.33
15	30.00	37.67	39.33	8.67
20	24.00	30 33	35.00	4.00

Table. 2. Influence of phenolics on the *in vitro* activity of cellulases

Phenolics		Cx enzyme (per cent loss in viscosity of CMC)	C_1 enzyme (1 unit= change in absorbance of 0.01)
Catechol	100 ppm	35.3 (36.42)	12.33
	500 ppm	32.2 (34.57)	7.67
	1000 ppm	26.0 (30.65)	4.33
Gallic acid	100 ppm	37.4 (37.76)	8.33
	500 ppm	33.0 (35.06)	7.67
	1000 ppm	29.5 (32.89)	7.67
Resorcinol	100 ppm	35.7 (36.69)	5.67
	500 ppm	33.0 (35.05)	6.33
	1000 ppm	30.3 (33.41)	4.67
Control		40.2 (38.96)	7.67
C.D. ($P=0.05$)		2.05	1.34

Table 3. Effect of plant hormones on the *in vitro* activity of cellulases

Plant hormone		Cx enzyme (per cent loss in viscosity of CMC)	C_1 enzyme (1 unit = change in absorbance of 0.01)
GA	0.1 ppm	36.20	6.5
	1.0 ppm	34.40	5.0
	10.0 ppm	35.70	7.0
	100.0 ppm	37·60	6.5
IAA	0.1 ppm	38.25	3.0
	1.0 ppm	41.00	5.0
	10.0 ppm	43.70	4.0
	100 0 ppm	47.00	7.0
Kinetin	0.1 ppm	41.40	4.0
	1.0 ppm	38.00	4.5
	10.0 ppm	38 80	3.0
	100.0 ppm	38.10	3.5
Control		41.60	6.0
C.D. (P=0.05,		6.39	NS

Table 4. *In vivo* production of cellulases

No. of days after inoculation	Cx enzyme (per cent loss in viscosity in CMC)			C_1 enzyme [1 unit = Change in absorbanced of 0.01]		
	Paper waste	Rice straw	*Coir* waste	Paper waste	Rice straw	*Coir* waste
0	0	0	0	0	0	0
10	35.00	33.33	25 00	5.00	4 33	4.00
20	43.00	40.67	31.00	7 33	6.00	5 00
30	39.33	40 00	15.00	7.00	4 67	1.00