

Production of Cellulolytic Enzymes by the Fungi Associated with the Spoilage of Copra*

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Among the fungi isolated from spoiled copra, *Botryodiplodia theobromae*, *Aspergillus flavus* and, *A. niger* produced more cellulolytic enzyme (C1) while *Curvularia senegalensis* and *penicillium citrinum* produced more Cx enzyme. The enzyme activity increased with the period of incubation and was maximum on 16th day. *In vivo* preparations showed very little activity.

The cellulolytic enzyme, C1, acts upon native insoluble cellulose to produce linear chains susceptible to attack by the second enzyme, Cx, which acts and degrades reprecipitated or soluble celluloses (wood, 1967). Rao *et al.* (1971) studied the elaboration of cellulase in stationary as well as in shaken cultures, by *B. theobromae* isolated from coconut kernel, while Olutiola (1976 a and b) studied cellulase enzyme (C1) in the culture filtrates of *P. citrinum* and *A. flavus*.

MATERIAL AND METHODS

Seven fungi commonly associated with the spoilage of copra viz., *Aspergillus niger*, *A. flavus*, *Rhizopus oryzae*, *Mucor hiemalis*, *Pencillium citrinum*, *Botryodiplodia theobromae* and *Curvularia senegalensis* were studied *in vitro* and *in vivo*. For *in vitro* studies, the cell free extracts were prepared by growing in a liquid medium (Kannaiyyan *et al.*, 1975) and enzyme extract was prepared by filtering and centrifuging at 10,000 g

for 20 minutes at 6° C. The upernatant was decanted and dialysed against distilled water for 16 hours. Autoclaved culture filtrate served as control.

For *in vivo* studies, fungi were inoculated on copra and enzyme extract prepared by grinding 5 g of infected material in 10 ml of distilled water. The tissue homogenates were squeezed through several layers of cheese cloth, centrifuged and dialysed as in *in vitro* studies. The extract from uninfected copra treated as above served as control. The enzyme activity was studied at 4, 8 and 16 days after incubation.

Cellulase (C1) activity was assessed by adopting the procedure of Norkrans (1950) To assess cellulase (CX) enzyme activity, one ml of enzyme sample was added to 5 ml of 1.2 percent carboxy methyl Cellulose (CMC) in 0.1M citrate buffer and incubated at 30°C for 2 hours (Rai and Dhawan, 1976). The flow rate of the reaction mixture was measured using an Ostwald viscometer and per-

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centage reduction in viscosity was calculated. (Morrall *et al.* 1972).

RESULTS AND DISCUSSION

The enzyme activity increased with the period of incubation and reached maximum on 16th day (Table I). *In vivo* preparation showed the minimum activity. Among the fungi tested *B. theobromae*, *A. flavus* and *A. niger* showed maximum activity being on par for C1 activity. In the case of Cx activity, *C. senegalensis* recorded the maximum followed by *P. citrinum*. All the fungi tested showed C1 and Cx enzyme activity *in vitro* and *in vivo* with maximum activity on 16th day showing that the fungus produced enzymes only at the later stages of its growth. Ramasami and Shanmugam (1976) also obtained similar results on cellulase C1 with *Rhizoctonia bataticola* while Kannaiyan *et al.* (1975) observed maximum enzyme activity on 8th day by *Claviceps microcephala* in *in vitro* studies *in vivo* preparations showing the minimum activity. Rao (1977) obtained maximum Cellulase (Cx) activity on 12th day of incubation with an *Aspergillus* strain isolated from soil while Kannaiyan *et al.* (1975) could not detect any activity in *in vivo* preparations by *C. microcephala* but obtained considerable *in vitro* production. In the present investigation also *in vivo* preparations showed the least activity.

Comparatively *M. hiemalis* produced less C1 and Cx enzyme activity. This coincided with the negative results of white *et al.* (1948) and marsh *et al.* (1949) with mucorales.

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Table I. Production of cellulase (CI) by fungi infecting copra (Mean of three replications).
(Absorbance at 610 m μ)

Fungi	Days after inoculation			
	4	8	16	16
<i>A. niger</i>	0.2445	0.5711	0.8394	0.1631
<i>A. flavus</i>	0.3167	0.5714	0.8114	0.1547
<i>R. oryzae</i>	9.2883	0.4828	0.5347	0.0829
<i>M. hiemalis</i>	0.0368	0.6745	0.0917	0.0256
<i>p. citrinum</i>	0.1706	0.3511	0.4314	0.0322
<i>B. theobromae</i>	0.4448	0.6136	0.7165	0.0700
<i>c. senegalensis</i>	0.0918	0.1760	0.2030	0.0597
Control	0.0000	0.0000	0.0000	0.0000