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Cellulase make-up of certain facultative marine fungi isolated from Bhavnagar coast

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Abstract

Four facultative marine fungi (*Aspergillus nidulans, A. versicolor, Paecilomyces variotii* and *Pencillium citrinum*), isolated from Bhavnagar coast were examined for cellulase production using four different media. All the test isolates produced exoglucanase, endoglucanase and b-glucosidase and showed complete cellulase activity in all the four media. Mandels & Sterberg medium supported highest enzyme production and the least preferred media for enzyme production were Park's and Zobell.

Cellulose is the most abundant organic matter on earth and also highly recalcitrant to enzymic degradation. Marine cellulolytic microorganisms are found in relatively high numbers in the near shore waters, where dead plant residues and live plant materials are accumulated due to action of the tide (D' Souza & Frietas, 1976). Zobell (1946) found thousand cellulose digesters per gram of marine mud samples and observed that "Marine cellulolytic microorganisms may be demonstrated in most 10-100 ml samples of botoom deposites". This early lead, however, was not kept up and interest in cellulose degradation shifted to preparation of glucose syrups from agricultural wastes and bagasse. Elucidation of enzymes of the cellulase complex is rarely done for marine organisms. In the present study, we have examined cellulase make-up (exoglucanase, endoglucanase, b-glucosidase and complete cellulase activity) of four fungi isolated from Bhavnagar coast.

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Material and methods

The fungi used in this study were *Aspergillus nidulans* (isolated from sediment), *A. versicolor* (from water), *Paecilomyces variotii* (from sediment) and *Pencillium citrinum* (from water). These were grown on four media viz. Kadota medium (Kadota, 1956), Mandels and Sternberg medium (Mandels and Sternberg, 1989), Park's medium (Vardavakis, 1976) and Zobell 2216E medium (Schlieper, 1972) contaning carboxymethyl cellulose (CMC) or cellulose powder as the carbon source. The organisms were grown at 28 ± 2°C on a rotary shaker at 150 rpm, and cell-free culture filtrates at different intervals of growth (2, 4, 6, 8 and 10 days) were used as enzyme samples or assay of various cellulase enzymes viz. endoglucanase,

exoglucanase, and b - glucosidase and, complete cellulase activity.

Standard assay methods as described by Mandels (1982), were used in which reducing groups released from enzyme-specific substrates were measured spectrophotometrically by Dinitro salicylic acid (DNS) method of Miller (1959). A glucose standard curve was used to quantify the reducing groups released. Assay for each enzyme was done as given below.

Endoglucanase (EC 3.2.1.4) : Enzyme sample (0.5 ml) was mixed with 0.5 ml of 1% CMC in 0.05 M Citrate buffer (pH 6.8) and incubated at 50°C for 30 min. The reducing groups released were measured by DNS method.

Exoglucanase (EC 3.2.1.91) : 50 mg absorbent cotton, used as substrate, was mixed with 1 ml of 0.05 M Citrate buffer (pH 6.8) and 1 ml of enzyme sample. It was incubated at 50°C for 24 h and the reducing groups released were measured by DNS method.

B-glucosidase (EC 3.2.1.21) : B- glucosidase activity was assayed by using salicin as the substrate. The reaction mixture containing 0.5 ml of culture filtrates and 0.5 ml of 1% salicin in 0.05 M Citrate buffer (pH 6.8) was incubated for 30 min at 50°C. The reducing groups released were measured by DNS method.

Complete cellulase : 50 mg of Whatman No. 1 filter paper strip (rolled) was used as the substrate. 1 ml of enzyme and filter paper strip buffered at pH 6.8, were incubated for 1h at 50°C. The reducing groups released were measured by DNS method.

Table 1. *Results of assays of cellulase enzymes produced by four fungi in different media. The data show maximum enzyme activity in International Units (IU) and the day it was recorded. (For a 30 min assay, 1 mg of glucose = 1/30 x 0.18 = 0.185 units)*

Medium	*Aspergillus nidulans*			*A. versicolor*			*Paecilomyces variotii*			*Penicillium citrinum*		
	Exo-Glucanase	Endo-Glucanase	b-gluco-Sidase	Exo-Glucanase	Endo-Glucanase	b-gluco-sidase	Exo-glucanase	Endo-glucanase	b-gluco-sidase	Exo-glucanase	Endo-glucanase	b-gluco-sidase
Kaduta	0.036, 6th	0.044, 8th	0.06, 8th	0.052, 6th	0.05, 6th	0.05, 6th,8th	0.06, 6th,8th	0.09, 8th	0.09, 6th	0.047 10th	0.045, 8th	0.060 10th
Mandels& Sternberg's	0.045, 6th,8th	0.09, 6th	0.15, 10th	0.068, 6th	0.11, 6th	0.10, 10th	0.07, 8th	0.07, 6th,8th	0.10, 8th	0.068, 10th	0.150, 8th	0.081, 8th
Park's	0.024, 10th	0.038, 8th	0.029, 8th	0.038, 8th	0.05, 6th,8th	0.04, 6th,8th	0.05, 6th	0.06, 6th,8th	0.07, 6th,8th	0.027, 8th	0.045, 8th	0.053, 10th
Zobell	0.032, 6th	0.029, 6th	0.042, 8th	0.031, 8th	0.05, 8th	0.04, 8th	0.04, 6th,8th	0.04, 4th,6th	0.05, 6th	0.044, 8th	0.053, 10th	0.045, 8th

All enzyme activities were expressed in International Units (IU) in which one enzyme unit equals one micromole of substrate hydrolyzed per minute (for cellulose, the micromole glucose released per minute).

Results and discussion

Table 1 shows that all the test fungi produced the three component enzymes of the cellulase complex and also showed complete cellulase activity. However, the activities varied with the organism, period of growth and composition of the medium. The organisms showed a distinct preference for Mandels and Sternberg medium, in which all the four enzyme activities were maximum, but for the lone exception of higher endoglucanase activity in *Paecilomyces variotii.* Kadota medium was similarly, the next best medium for enzyme production, while Zobell and Park's media, especially the former, were the media that supported least enzyme activity.

It is also to be noted that while the organisms showed a uniformly distinct preference for one medium (Mandels and Sternberg medium), no single organism showed highest activity for all the component enzyme on any of the four media. Thus, while *Aspergillus versicolor* showed highest exoglucanase and complete cellulase activities, the endoglucanase was maximally produced by *Pencillium citrinum,* and b-glucosidase by *Aspergillus nidulans.*

In period of growth for highest enzyme production also, the organisms differred for the different enzymes. But, mostly the highest activity was recorded by 6th or 8th day, and occasionally on the 10th day. It is worth emphasizing that no single fungus was the best producer of all enzymes. Rather, the fungus, which showed highest activity for one enzyme was poor for other activity. It is depicted below (enzyme activity in IU):

Exoglucanase :	*A. versicolor* >	*P. variotii* >	*P. citrinum* >	*A. nidulans*
	0.11	0.07	0.067	0.045
Endoglucanase :	*P. citrinum* >	*A. versiclor* >	*P. variotii*	= *A. nidulans*
	0.15	0.10	0.09	0.09
b-*glucosidase* :	*A. nidulans* >	*P. variotii* =	*A. versicolor*	> *P. citrinum*
	0.15	0.10	0.10	0.089
Complete cellulase :	*A. versicolor* >	*P. citrinum* >	*A. nidulans* >	*P. variotii*
	0.09	0.08	0.068	0.06

Thus, a mixed mileu of organisms is necessary for maximum utilization of cellulosic materials in the marine ecosystem. Our observation of Mandels and Sternberg medium as the best medium is supported

by similar observations of Mogal (1993) for marine fungi, and Abhay Kumar (1990) for marine bacteria.

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