

## ISOLATION OF CELLULOLYTIC FUNGI FROM THE BARIO HIGHLANDS, SARAWAK

Abdul Jalil Kader, Othman Omar & Loo Shu Feng  
Universiti Kebangsaan Malaysia

### ABSTRACT

*Soil fungi and bacteria were isolated from various locations visited throughout the expedition held at Bario Highlands. Nine fungal species were identified and among which four isolates demonstrated cellulolytic activities. Two of these isolates namely Trichoderma (isolate 17b) and Aspergillus (isolate 23a) were discovered to be highly cellulolytic compared to the rest*

### INTRODUCTION

Cellulases are a group of hydrolytic enzymes capable of hydrolysing cellulose to smaller sugar components like glucose units. Cellulolytic enzymes play an important role in nature's biodegradation processes where plant lignocellulosic material are efficiently degraded by cellulolytic fungi and bacteria. In industry, these cellulolytic enzymes have found novel applications in the production and processing of chemicals, foods and manufactured goods such as paper, rayon and cellophane. Cellulases, for instance have been extensively utilized for extraction of valuable components from plant cells, improvement of nutritional values of animal feed and the preparation of plant protoplasts in genetic research (Mandels, 1985).

Generally, fungi produces three major types of cellulolytic enzyme: endoglucanase, exoglucanase and cellobiohydrolase (Klyosov, 1990). These enzymes are extracellular and inductive in nature (Enari, 1983). The ability to produce cellulase are widespread among fungi and this has become the subject of extensive investigation. This study concentrates mainly on soil fungi isolated from the various locations visited during the scientific expedition to Bario Highlands, Sarawak organized by Universiti Malaysia Sarawak in April 1995.

### MATERIALS AND METHODS

**Samples:** Soil samples were taken from 25 locations along the trails that traversed through various forest sites visited during the expedition. Briefly, samples were obtained from five locations along the new reservoir trail, one from Airport Kem Tamar, one from Kem Tamar Slope, 3 from Kem Tamar, 2 from Jalan Tamar, 2 from Kg. Baru trail, 2 from Long Juno trail, 2 from Pa' Mor trail and 7 from Lelang trail.

**Isolation Of Fungi:** The soil samples were mixed with sterile distilled water and a series of dilutions were made. From the dilutions, 0.5ml volumes were pipetted onto potato dextrose agar (PDA) and incubated at 30°C for three days. Fungi was isolated from the mixed isolates from

each plate and subcultured on PDA. Subculturing was continued until a pure isolate was obtained.

**Determination of Cellulolytic Fungi:** From the various isolates, screening for cellulolytic fungi was made using selective media (Mandel's agar). Cellulolytic fungi creates a clearing zone around the colony on the agar.

**Enzyme Assay:** Fungi determined to be cellulolytic were then cultured in Mandels salt medium (Mandels & Reese, 1957) containing cellulose in a 1,000ml Erlenmeyer flask and incubated at 30°C for 8 days shaken at 150 rpm. Samples of 8ml were collected daily and centrifuged at 12,000 rpm (at 4°C) to obtain the extracellular enzymes. Enzymatic assays done included carboxymethylcellulase (CMCase), filter paper assay (FPase), xylanase and glucosidase. CMCase, FPase and xy-lanase were determined by the method of Mandels et al. (1976) using DNS where one IU is defined as the amount of enzyme releasing 1 µmol glucose per minute assay -glucosidase was performed using the method of Theodorou et al. (1980). The unit of activity (IU) is defined as the amount of enzyme liberating one µmol p-nitrophenyl per minute.

## RESULTS AND DISCUSSION

The number of fungi isolated from the various locations are shown in Table 1. Among the fungi isolated, only nine were identifiable to the genus level (Table 2): Microscopic photographs of several of these fungi are shown in Figure 1. Among the identified isolates, only four were found to possess cellulolytic abilities. Enzymatic activities of the 4 fungi assayed is shown in Table 3.

*Table 1: Number of fungus isolated from different locations*

Trail Location	No of Fungi	Trail Location	No of Fungi	Location	No of Fungi
New Reservoir 1	4	Kem Tamar 9	5	Pa' Mor 17	3
New Reservoir 2	3	Kem Tamar 10	2	Pa' Mor 18	3
New Reservoir 3	1	Jalan Tamar 11	1	Lelang 19	4
New Reservoir 4	3	Jalan Tamar 12	2	Lelang 20	2
New Reservoir 5	1	Kg. Baru 13	3	Lelang 21	2
Airport Kem Tamar 6	0	Kg. Baru 14	1	Lelang 22	2
Kem Tamar Hill Slope 7	5	Long Juno 15	2	Lelang 23	5
Kem Tamar 8	2	Long Juno 16	2	Lelang 24	2
				Lelang 25	2

*Table 2 : Identified fungi*

FUNGI	GENUS
1c	Aspergillus
4d	Aspergillus
11a	Penicillium

13a	Aspergillus
17b	Trichoderma
20a	Trichoderma
23a	Aspergillus
23d	Aspergillus
25b	Trichoderma

Table 3 : Maximum enzyme activity of identified fungi

FUNGI	ENZYME ACTIVITY (IU/ml)			
	CMCase	FPase	$\beta$ -glucosidase	Xylanase
1c	0.05	0.01	0.03	0.875
4d	0.05	0.01	0.04	0.05
17b	1.58	0.40	0.25	1.57
23a	0.58	0.13	0.23	1.00

#### ACKNOWLEDGEMENTS

The researchers express sincere gratitude to the expedition organizing committee and UNIMAS. The expedition was made possible with IRPA grant 4-07-03-008 from the Ministry of Science, Technology and Environment, Malaysia.

#### REFERENCES

**Enari, T.M.**

[1983] Microbial cellulases. In Fogarty, W.F. (ed.) Microbial enzymes and biotechnology. Applied Sciences Publishers, London. pp183-223

**Klyosov, A.A.**

[1990] Trends in biochemistry and enzymology Biochemistry 29:10577-10585

**Theodorou, M.K., M.J. Bazin & A.P.J. Trinci.**

[1980] Cellulose degradation in a structured ecosystem which is analogous to soil. Transaction of the British Mycological Society. 75:432-454.

**Mandels, M.**

[1985] Application of cellulases. Biochemical Society Transactions. 13:414-416.

**Mandels, M. & E.T. Reese**

[1957] Induction of cellulase in fungi by cellobiose. Journal of Bacteriology. 73:816-826.

**Mandels, M., R. Andreotti & C. Roche**

[1976] Measurements of saccharifying cellulase. Biotechnology & Bioengineering Symposium. 6: 21-33.