

- 1' PD-AAx-380  
54786

X

4.429

The International Centre  
of Insect Physiology and Ecology  
P.O. Box 30772, Nairobi, Kenya

Your Ref: Grant No. 936-5543-G-00-4023-00

Our Ref: S4/USAID/102

Third Scientific Report (6 monthly) On: Mission-Oriented  
Research on the Microorganisms from African termites for Improved  
Biomass Degradation, covering the period July - December, 1985.

Submitted to: Programme in Science and Technology Cooperation  
(PSTC), United States for International Development  
(USAID) Washington D.C. Through

East African Regional Economic Development Services  
Office (REDSO/ESA)

January 1986

Rec'd in SOI FEB 11 1986

1. Introduction

Since our last report, June 1985, efforts have been made to optimise the conditions for culturing termite associated microorganisms in the laboratory and to study factors which are responsible for the production of laccase, one of the enzymes implicated in the depolymerisation of lignin. One set of organisms that shows promising results is Termitomyces ICIPE isolate 1 and 2 isolated from Macrotermes michaelsoni and Odototermes species respectively.

In this report, we have included (a) details of the isolation of Termitomyces (b) observation relating to laccase production in Termitomyces and finally (c) the growth of Termitomyces on different lignocellulosic media.

2. Isolation of Termitomyces

Termitomyces species is one of the microorganisms associated with higher termites. This organisms is present in the termite mound all year round in the form called synnemata (Syn. mycotete, conidia or white modules). It also exists in the mushroom form seasonally during the major rainy periods for a limited period of time. Both forms have been isolated as discussed below.

2:1 Isolation of Termitomyces synnemata from the fungus-comb of Macrotermes michaelsoni:

Synnemata were collected from fresh fungus-comb and surface sterilized as follows: (a) they were first washed in 0.5% sodium hypochlorite for 2 minutes and then (b) washed in sterile distilled water for 5 minutes and finally (c) rinsed in 0.85% sterile saline. The surface sterilized synnemata were then inoculated onto Potato Dextrose Agar plates and incubated at 29°C. Observations were made everyday for signs of growth.

In each trial, five synnemata were placed on each of 20 plates. Pure cultures were isolated and propagated for further studies. This isolate was named Termitomyces ICIPE isolate number 1.

2:2: Isolation of Termitomyces mushroom from the Odontotermes species.

In this culture, mushroom tissue was used following the method illustrated in Chang (1982). Fresh mushroom, collected from Odontotermes species mound, were prepared on the same day for cultures as follows: (a) mushrooms were washed to remove any soil (b) disinfected with 75% alcohol, (c) the volva was removed and (d) the mushrooms were opened by hand longitudinally in halves. Small pieces were aseptically removed from the centre and inoculated onto Potato Dextrose Agar. Observations were carried out everyday as normal for signs of growth.

Successful pure cultures were isolated and propagated for further studies. This isolate was named Termitomyces ICIPE isolate number 2.

3. Optimal pH and temperature requirements for the growth of Termitomyces.

3:1 pH

Termitomyces were grown on Potato Dextrose Agar prepared at varying pH of 3.5, 4.5, 5.6, 7.2, 8.1, 9.1, 10.5, 11.5 and 12.5. These cultures were incubated at 29°C.

Termitomyces was found to growth at pH 6.5. No growth was observed below pH 4.5 and above pH 10.5.

3:2 Temperature

Termitomyces was inoculated on Potato Dextrose Agar plates and incubated at the following temperatures: 24°C, 28°C, 35°C and 42°C. Our observations showed that Termitomyces grows best at 28°C. No growth was observed at temperatures 35°C and above.

4. Laccase production in Termitomyces

Many higher basidiomycete fungi and particularly those associated with wood decay produce the enzyme laccase (Wood 1979). Laccases are normally blue, copper-containing proteins capable of oxidizing both ortho- and para-diphenols and aromatic amines. It is believed to be one of the key enzymes in the initial reactions of the side-chain carbon atoms of lignin and the subsequent cleavage of the quantitatively important C<sub>α</sub> - C<sub>β</sub> linkages (Shimada 1980, Iwahara et al. 1980).

Termitomyces is a basidiomycete which grows on the termite fungus-comb made up of lignocellulosic material. What enables Termitomyces to survive on lignocellulosic material? Is it capable of producing lignin degrading enzymes which would allow it access to cellulose and other nutrients? If so, under what conditions ligninolytic activity occurs? To answer the above questions, the following experiments were carried out.

Termitomyces ICIPE 1 and 2 were cultured on Potato Dextrose Agar. Each organism was cultured in ten different plates. These cultures were incubated at 29°C and on every second day, the presence of laccase was tested using the method by Harkin and Obst (1973). The point at which laccase secretion commenced was determined and it was found to be between the 5<sup>th</sup> and the 6<sup>th</sup> day of culture.

Laccase production was detected from solid cultures of Termitomyces isolate 1 and 2 and from Termitomyces naturally growing in the fungus-comb. Laccase in the fungus-comb was also tested using the method mentioned above. The fungus-comb laccase has been extracted and freeze-dried for further studies.

#### 4.1

In contrast to solid media, Termitomyces grown on liquid media has failed to show evidence of laccase production. The reason for this is not clear, but the availability of some key chemical stimulus present in the solid media may be important in triggering the production of laccase.

Different carbohydrates were included in solid media to determine their effect on the growth of Termitomyces and on laccase production. The carbohydrates used included the following individually: mannitol, maltose, sucrose, mannose, glycogen, cellulose, D-glucose, galactose, starch, arabinose, fructose and xylose. In two media, wheat straw and fungus-comb material were used respectively instead of the pure carbohydrate. The complete media consisted of:

$\text{KH}_2\text{PO}_4$	1.0g
$(\text{NH}_4)_2\text{SO}_4$	0.5g
KCL	0.5g
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.2g
$\text{CaCl}_2$	0.1g
Yeast Extract	0.5g
Carbohydrate/Straw/fungus-comb material	20.0g
Distilled water	1 litres
pH adjusted to 6.5	

After two weeks it was found that although all carbohydrates supported the growth of Termitomyces to different degrees, not all stimulated the production of laccase. The media containing the fungus-comb materials, straw, mannitol, starch, glycoen and arabinose in that order, were the only ones that gave positive tests for laccase.

5. Growth of Termitomyces on different lignocellulosic media

One of our current activities and also a recommendation of IAEA sponsored review expert, is to develop assay procedures that measures the efficiency with which lignocellulose degrading microorganisms can demask cellulose by selective degradation of lignin.

As an initial aspect of this work, we are processing the major agricultural waste material that can be used as feedstock for biogas fermenters such as cornstover, cereal straws, sugar-cane baggasse and cassava stems for use in culture media. The materials have been ground to specific mesh sizes for incorporation into different media. The ability of these materials to support the growth of microorganisms isolated from the fungus-combs is then assessed.

So far, the growth of Termitomyces, Fuserium semitectum, Aspergillus niger, A. flavus and Trichoderma harzianum on media containing maize straw, sorghum, wheat straw and cassava stem has been screened. It was noted that for all the above organisms, growth was best in media containing (a) cassava stem and (b) sorghum straw. Scanty growth was observed in maize and wheat straw. It is hoped that chemical analysis of the different lignocellulosic materials currently underway will help identify the factors responsible for the above differences.

6. Current studies

Current work on the project is focussed on (a) developing an assay for estimating the rate and extent of demasking of cellulose from lignocellulosic materials during the growth of the termite associated fungi; (b) defining the conditions for optimal growth of the isolated fungi and for the production of laccase.

Reference

- S.T. Chang (1982) Mushroom Spawn, In "Tropical Mushrooms: Biological Nature and Cultivation Methods". Ed. S.T. Chang and T.H. Quimio.
- Paul Ander and Karl-Erik Eriksson (1978) Lignin Degradation and Utilization by Micro-organisms Prog. Ind. Microbiology Vol. 14 (1) pg 1-58.
- J.M. Harkin and Obst, J.R. (1973) Syringaldazine, and effective reagent for detecting laccase and peroxidase in fungi. Experientia 29: 381-387.
- S. Iwahara, Nishahara, T., Jomori, T., Kowahara, M, Higuchi, T. (1980) Enzymic oxidation of unsaturated alcohol in the side-chains of lignin-related aromatic compounds J. of Fermentation Technology 58, 183-188.
- M. Shimada (1980) Stereobiochemical approach to lignin biodegradation. In "Lignin Biodegradation: Microbiology, Biochemistry and Potential Applications". eds Kirk, T.K., Higuchi, T. and Chang H.M. Vol. 1 pp 195-213. Boca Raton CRC Press.
- D.A. Wood (1979) Production, Purification and Properties of Extracellular laccase of Agaricus bisporus. J. of Gen. Micro. 117 pg 327-338.