MICROBIAL DEGRADATION OF COCONUT WASTE MATERIALS

V. P. POTTY, K. V. JOSEPH, MATHEW GEORGE AND N. P. JAYASANKAR

CPCRI Regional Station, Krishnapuram-690 533 Kayangulam, Kerala, India.

ABSTRACT

Retting of coconut husk yields coir fibre and coconut pith both of which are resistant to microbial attack. The former finds a place in commerce, while the disposal of the latter is a problem. Enrichment culture methodology using lignocellulose as source of carbon yielded a fungus identified as *Poria ravanulae*. The fungus could utilise coconut pith which is essentially a ligno cellulose complex, as source of carbon for its growth. The coconut *P. ravanulae* isolates were lignoclastic, its polyphenol oxidase was noninducible and could use a range of phenolic substrates for growth. Replacement culture techniques indicated the conversion of trihydric phloroglucinol to dihydric resorcinol.

INTRODUCTION

The hard fibre obtained from the mesocarp of the fruit of *Cocos nucifera* Linn. or the coconut palm is known the world over as coir. It is possibly a transliteration of the malayalam '*Kayar*' by which the yarn of the fibre is known in Kerala State where the cultivation of the palm, the extraction of fibre and the manufacture of its products are known to exist since several centuries. The oldest available document describing the extraction of coir from its natural setting is perhaps that of the 14th century. Persian traveller Ibn Batuta (Panikkasseri and Moulani, 1967). This traditional method of natural retting consists in soaking the husks in saline back waters and remains the same even at present. The process which is essentially microbiological results in the liberation of fibre without disintegration along with the coconut pith which constitutes about 50 – 70 % of

the total weight of the husk. While the fibre finds a place in commerce the disposal of the pith is a problem at the moment. These materials are usually resistant to microbiological degradation in view of the nature of its lignin complexed with cellulose (Menon, 1936). Against this background certain experiments were designed to isolate microorganisms capable of attacking the ligno-cellulosic complexes and utilising these waste materials. Certain characteristics of a resultant fungus that have a relevance to the kind of investigation are presented in this paper.

Materials and Methods

A basal solution containing $(NH_4)_2 SO_4$ – 0.5 g, $Na_2 HPO_4$ – 0.8 g, KH_2PO_4 – 0.2 g, Nacl 0.05 g, $MgSO_4$ 7 H_2O –0.2 g, $FeSO_4$ $7H_2$ 0–0.001 g, in 1000 ml water containing macerated lignocellulosic pulp (0.1 %) as source of carbon was used for the isolation of organisms. Enrichments were set up by inoculating decaying coconut petioles in 25 ml media dispensed in 250 ml Erlenmeyer flasks and incubation at room temperature (25–28°C) for 10 days. After three successive transfers final enrichments were streaked on the same medium solidified with 2% agar. For subsequent studies the organism was cultivated in potato extract (2.5 g in 100 ml water) containing 100 mg of tannic acid or other phenolic substrates. The ability of the isolates to utilise the range of aromatic compounds was determined in the same medium inoculated with 10 mm diameter agar discs. The rate of growth was ascertained after 10 days. The extent of polyphenol oxidase production was assayed by measuring the optical density of culture filtrates/reaction mixtures, at 400 nm in a Beckman model DU-2 spectrophotometer after appropriate dilutions. Replacement cultures were set up with washed mycelial mats in 0.01 M catechol (30 ml) and 0.05 M Phosphate buffer, pH 7.0 (3 ml). For chromatographic detection during different periods of incubation culture filtrates (10 ml) were acidified to pH 2.5 with 1 N HCl and extracted with Ethyl Acetate (10 ml). The extracts were

evaporated to dryness after treatment with anhydrous sodium sulphate and the residue dissolved in methanol (2 ml.) Chromatographic separation was achieved in TLC (cellulose) using formic acid (2%) as solvent.

Results and Discussion

Enrichments with lignocellulosic pulp using decaying coconut petiole as the source of inoculum yielded predominently a fungus whose identity as *Poria ravenalae* was confirmed by Dr. D. E. Etheridge. The fungus grew in a medium containing powdered coconut pith as source of energy. The coconut *P. ravenalae* isolates presumably could cleave ligno - cellulosic bondage but were not cellulolytic. In a medium containing tannic acid – a phenolic substrate, the isolates elaborated polyphenol oxidase (PPO). Results are indicative of the potentialities of the isolates for the disintegration of these waste materials possibly in combination with other microorganisms.

The isolates could utilise a range of aromatic compounds (Table 1) for growth. The phenolic substrates which were utilised by the isolates for growth included the hydrocarbons Benzene and Toulene and 1:3 dihydroxy naphthalene. The monohydric phenols the dihydric hydroquinone and the trihydric pyrogallol were not utilised by the isolates. However growth was recorded in the dihydric catechol and resorcenol and the trihydric phloroglucinol in which cases the hydroxy groups are either in *ortho* or *meta* position. The ability of these coconut *P. ravenalae* isolates to utilise the range of aromatic compounds suggests their possible implication in aromatic degradation.

A comparison of the rate of growth of the coconut *P. ravenalae* isolates and their ability to elaborate PPO indicated an initial increase in PPO activity along with growth (Table 2). There was a subsequent cessation in the elaboration of PPO by the isolates with a further breakdown of the products of this enzyme in culture

Table 1. Utilisation of Aromatic compounds by coconut *Poria ravenalae* isolates*

Serial No.	Aromatic compound	Rate of growth
1.	Benzene	+++
2.	Toluene	+
3.	Phenol	—
4.	O. Cresol	—
5.	Catechol	++
6.	Resorcinol	+++
7.	Hydroquinone	—
8.	O. Amino Phenol	—
9.	Phloroglucinol	+++
10.	Pyrogallol	—
11.	Caffeic acid	+++
12.	L. Tyrosine	++
13.	1 : 3 dihydroxy naphthalene	+++
14.	B. Naphthol	—
15.	D. L. Aniline	++++

*Growth was recorded after 10 days of inoculation

Table 2. Rate of growth and Polyphenoloxidase (PPO) activity of coconut *Poria ravenalae* isolates

No. of days after inoculation	Growth (Dry Mycelial weights in g)	PPO* (OD of culture filtrates)
1	—	—
2	0.034	0.85
3	0.106	1.20
4	0.098	1.75
5	0.100	1.80
6	0.130	5.00
7	0.232	4.00
8	0.254	3.50
9	0.269	2.75
10	—	0.25
11	0.276	0.00

*Absorbance was measured after a fivefold dilution of the culture filtrates

filtrates. Additional properties of this isolates to degrade quinonoid compounds are indicated.

The PPO activity of the coconut *P. ravenalae* isolates is noninducible. Replacement culture with 6 days old mycelial mats in catechol shows an enhanced PPO activity in darkness compared to those cultivated and incubated in the presence of light (Towards *et al,* 1974). Consistent photo regulation has not been observed on the phenolic enzymes in fungi. In *Polyporus hispidus* Phenyl alanine ammonia lyase, Cinnamate hydroxylase and p. Coumarate hydroxylase show enhanced activity in the presence of light while the Tyrosine ammonialyase activity is more in the dark (Towers *et al.* 1974).

Table 3. Effect of light on the Polyphenoloxidase (PPO) activity of coconut *P. ravenalae* isolates

Period of Incubation (hr)	Absorbance	
	Incubation in light	Incubation in dark
0	0.05	0.09
3	0.07	0.14
6	0.26	0.44
14	0.62	1.20
18	0.68	1.50

Series of replacement cultures were set up with mycelial mats using the trihydric phloroglucinol, as no definite indications are available on the mechanism of its ring cleavage. Chromatographic analyses do not give any definite clue except the presence of Resorcinol suggesting a possible conversion of Phloroglucinol to Resorcinol before further breakdown. This observation merits consideration while screening the various microorganisms with a view to elucidating the sequential events leading to the break down of this trihydric phenol.

REFERENCES

MENON, S. R.K. 1936. *J. Textile Inst.* 27 : 241.

PANIKKASSERI, V. AND MOULANI, A. R. 1967 In *Kerala Aruno-orukollam mumpu.* National Book Stall, Kottayam, India.

TOWERS, G. H. N., VANCE, C. P. AND NAMBUDIRI, A. M. D. 1974. Photoregulation of phenyl propanoid styrylpyrone Biosynthesis in *Polyporus hispidus.* In *Metabolism and regulation of secondary plant products* (Eds. V. C. Runeckles and E. E. Conn). Academic Press, New York.