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Isolation and Partial Characterization of Lignin Degrading Microorganisms from Termite Gut

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Investigation on the isolation and partial characterization of lignolytic microflora from matured termite gut showed the presence of three fungi such as *Penicillium spp.*, *Fusarium spp.* and *Aspergillus niger* and one type of bacteria, *Pseudomonas aeruginosa* by their primary lignolytic ability to degrade saw dust which was incorporated as lignin source in the medium. All the fungal and bacterial isolates were further characterized for lignolysis in various pH (i.e pH 4.0, 7.0 and 9.0) conditions and the results revealed effective lignin degradation by both fungal and bacterial isolates in the acidic condition. Higher ligninolytic activity was recorded in *Penicillium spp.*

Keywords: Lignolytic microflora, *Penicillium* spp., *Fusarium* spp., *Aspergillus niger, Pseudomonas aeruginosa* and termite gut.

Lignin is the second most abundant aromatic compound on earth (Crawford 1982) and is considered to be the most recalcitrant chemical. The higher the proportion of lignin in the organic matter, lower in the bioavailability and thus reduction of soil fertility. This recalcitrant material has to be broken down in order to maintain carbon cycle in the soil. Lignin degradation is mostly an aerobic process and in the anaerobic environment lignin can persist for a longer period. In nature, various groups of micro organisms such as bacteria, fungi, and actinomycetes are found involved in lignin degradation. Several works has been carried out by scientists on microbial degradation of lignin (Kirk and Farrel, 1987; Akin and Ronald Benner 1988; Pasti, B., *et al,* 1990; Zimmer man, 1990 and Kluczet, 2003). Symbiotic termite gut microorganisms such as bacteria, fungi, and actinomyctes are efficient in degradation of lignin and hence play an important role in biodegradation of plant litter (Odier *et al.,* 1981 and Ohkuma *et al.,* 2001). With this view the present study was undertaken on the isolation and partial characterization of lignin degrading microorganisms form termite gut.

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MATERIAL AND METHODS

The gut extract from matured termites was collected aseptically and serially diluted from 10^{-1} to 10^{-8} using sterile distilled water. Lignolytic bacteria and fungi were isolated from the gut extract using Crawford medium and malt extract agar medium respectively.

Isolation of lignolytic bacteria

0.1 ml of the sample from the gut extracts of 10^{-5} and 10^{-6} dilutions were spread on Crawford medium incorporated with 0.1 percent of saw dust as lignin source. The medium contained 1.5g Na_2HPO_4, 4.0g KH_2PO_4, and 0.2 g $MgSO_4.7H_2O$, 0.2g Nacl and 20g Agar in 1000 ml distilled water. All the plates were incubated at 37º C for 24 to

48 hours and the predominant lignolytic bacterial colonies were isolated on the basis of the formation of clear zone around the colonies in the medium containing saw dust. All these bacterial isolates were pure cultured and identified using gram's staining, motility study and various biochemical tests such as methyl red reaction, indole production, Vogas Proskaur test, citrate utilization test, catalase test, oxidase test, nitrate reduction test, urease test and gelatin hydrolysis.

Isolation of ligninolytic fungi

0.1 ml of sample from the gut extracts of 10^{-3} and 10^{-4} dilutions were plated on malt extract agar medium incorporated with saw dust as lignin source. The medium contained 10g glucose, 10g malt extract, 28g yeast extract, 2g peptone, 1g L.aspergine, 2g KH_2PO_4, 1g $MgSO_4$, $7H_2O$, 1 mg thiamine HCl, 20g Agar and 1g saw dust powder in 1000 ml distilled water. All the inoculated plates were incubated at room temperature (i.e 28º ± 2º C) for 2 to 4 days and the ligninolytic fungi were isolated on the basis of abundance of growth and formation of clear zone around their colonies in the medium containing saw dust powder as lignin source. Selected fungal colonies were pure cultured and identified using colony characteristics on agar medium and their reproductive feature was observed under microscope.

Analysis of Lignolytic activity

Inoculum of selected bacteria *Pseudomonas aeruginosa*. and fungi *Penicillium spp., Fusarium spp.* and *Aspergillus niger* were inoculated on Crawford agar and malt extract agar medium respectively. These media contained different pH (4, 7 & 9) and were incorporated with 0.1 percent lignocellulose (extract from saw dust with ethanol and hot water) as carbon source. All the inoculated and uninoculated flaskes were incubated at 37º C and 28º C (room temperature) for bacteria and fungi respectively, upto 15 days. After incubation the residual (undergraded) lignocellulose in each treatment was analysed by modified Kalson procedure.

The residual lignocellulose from each flask was harvested by adding 50ml of distilled water, homogenized and filtered through pre-weighed filter paper. The residual lignocellulose on filter paper was treated with 5ml of conc. H_2SO_4 and 25ml of HCl and digested for 16 hours. The acidity of the sample was adjusted to neutrality with Na_2CO_3 and the volume was made to 50 ml with distlled water. Then the sample was filtered through preweighted Whattman No.1 filter paper and the residue was dried in hot air oven at 100°C over night and weighed. The dried residue was ashed using muffle furnace at 500°C for 5 hours, cooled in a desiccator and the weight of the ash was determined. Finally the actual lignin content in the sample was measured by substracting the weight of the ash content from the dried residual content.

RESULTS AND DISCUSSION

Three predominant lignolytic fungal colonies were isolated from the gut extract of termite by plating on malt extract agar medium incorporated with saw dust as lignin source that in turn served as carbon source. All the three

Table 1. Identification characteristics of fungal isolates

Isolate No.	Colony Morphology	Lacto phenol cotton blue staining features	Identification result
Fis 1	Green colony	Septate hyphae with an erect conidiophore bearing conidiospores which appears like inverted street broom.	*Penicillium spp.*
Fis 2	Cottony colony with black spores	Septate hyphae with an erect conidiophore bearing chains of spherical conidispore	*Aspergillus niger*
Fis 3	Cottony, white to pink colony	Septate hyphae with crescent shaped macroconidia and spherical shaped microconidia	*Fusarium spp.*

fungal isolates showed zone of clearance around their colonies and that indicates the lignolytic efficiency of these fungal isolates. These isolates were identified as *Penicillium spp., Aspergillus niger* and *Fusarium spp.* using colony characteristics and reproductive features (Table 1).

One predominant lignolytic bacterial strain isolated from the gut extract was identified as *Pseudomonas aeruginosa* using colony morphology, staining feature, motility study and various biochemical tests (Table 2). Several investigations had already been made on isolation of lignolytic microorganisms from animal gut. Pasti, *et al* (1977) isloated spore forming bacteria (Actinomycetes) from termite gut and he described that the isolate was effective in the degradation of ligno cellulose. Certain other bacterial flora of rumina associated with the degradation of lignocellulose was observed by Akin and Ronal Banner (1998). Mannkoskiu *et al.*, (1981) found that a mixture culture of bacteria degraded lignin upto 25 percent in 36 days. Ohkuma *et al.*, (2001) found that termite and fungi accomplished efficient decomposition of lignin and complete biorecycling of plant litter.

In the present study, the primary lignolytic efficiency of the isolated fungi (*Penicillium spp., Fusarium spp. and Asperillus niger*) and bacterial (*Pseudomonas aeruginosa*) cultures were evaluated by observing the zone of clearance in the respective medium. Among the fungal isolates, *Penicillium spp.* showed higher liginolytic activity by forming a larger clearing zone on the medium with the size of 3 cm diameter followed by *Fusarium spp*. with a zone of clearance of 2.5 cm dia and *Asperillus niger* did not produce any clearance zone but it manged to grow in the medium. *Pseudomonas aeruginosa* showed comparatively less lignolytic activity with a zone size of 1.5 cm (Table 3).

Table 2. Identification characteristics of bacterial isolate

Characteristics	Results
Gram stain	-
Shape	rod
Motility	+
Indole Production	-
Methyl red reaction	-
Vogas- praskaur reaction	-
Citrate utilization	*
Catalase	-
Oxidase	+
Urease	-
Gelatin liquefaction	+
Nitrate reduction	-
Identification result	*P. aeruginosa*

Table 3. Lignolytic activity by the isolates in the medium containing saw dust

Name of the culture	Formation of clearing zone (dia in cm)
Penicllium spp	3.0
Fusarium spp	2.5
Aspergillus niger	Growth only
Pseudomonas aeruginosa	1.5

The results of the effect of various pH conditions on lignolysis by bacterial and fungal isolates (15 days) showed that effective lignin degradation took place in the acidic condition i.e pH 4.0 than in the other pH conditions. The highest lignolytic activity was recorded in *Penicillium spp*. (Fig. 1).

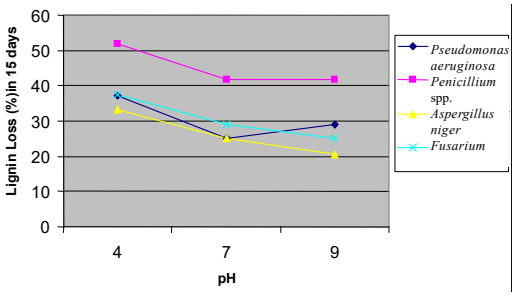

Fig. 1. Lignolytic activity by the isolates at different pH

CONCLUSION

All the three fungi i.e., *Penicillium spp., Fusarium spp.* and *Aspregillus niger* and the bacterial strain *Pseudomonas aeruginosa* isolated from the termite gut were found to degrade lignin. All the isolates degraded lignin effectively in the acidic (i.e pH 4.0) condition. *Penicillium spp.* can be used as an efficient strain to recycle lignocellulosic wastes.

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