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Isolation and Identification of Lignin Biodegraders from Leaf Litter

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In forest ecosystem litter play an important role, in humification and it improves the soil quality. Litter is the layer of dead plant material, on the forest floor. Because of the microbial activity litter decomposes and becomes undistinguishable. Lignin biodegradation comprises succession of microbial activity. Among these microbes lignin degrading fungi play central role in litter biodegradation because litter consist greater amount of lignin. Lignin degraders only complete the process of biodegradation. In this work 4 different strains of lignin degrading fungi were isolated from the litter of selected plants by using selective media.

Key words: Ecosystem, Humification, Succession, Biodegradation, Litter.

In ecology litter is the layer of dead plant material, which may be present on the soil surface. The litter layer may be clearly distinguishable or may not be the sharp boundary with mineral layer. The leaf litter above ground provides greater organic compounds to the soil by biodegradation process (Martinz- Yrizas, 1995 ;Montanenz, 1998).

Angiosperm leaf litter consists of 16-42% lignin was estimated based on modified Waksman method of analysis (Melin, 1930; Ohmasa and Mori, 1937; Wittich, 1943; Coldwell and Delong, 1950; Handley, 1954 ; Mikola, 1954).

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Lignin biodegradation is central to the earth's carbon cycle because lignin is second only cellulose in abundance. Decomposition of lignin is key factor controlling litter decomposition rate (Aber *et al*. 1990).

Litter decomposition is complex and involves succession of biodegradation activities by microorganisms. Especially fungi play fundamental in litter degradation in forest ecosystem (Hudson, 1986 ; Dix and Webster, 1995).

Most of lignin degraders belong to lower fungi, ascomycetes, basidiomycetes, and deteureomycetes. Lignolytic basidiomycetes are considered as major degraders of lignin (Ward, 1952; Ander and Erikson, 1978; Kirk *et al*., 1987).

Lignin biodegraders produce different extracellular enyme i.e. laccase, ligninases, Mn peroxidases, phenol oxidizing enymes and hydrogen peroxides producing enzymes. Among these laccase production is predominant and actively involved in lignin degradation process (Reinhammer, 1984)

The lignin biodegraders are widely used in different process like pulping and bleaching, in treatment of bewerges and in treatment of detoxification of polluted soil (Col *et al*., 1993; Filazzola *et al*., 1999; Jaraz-willolazka *et al*., 2001; Gianfreda *et al.*, 1999).

MATERIAL AND METHODS

Litter samples were collected from randomly selected plants of Madikeri region.

Collection of litter from selected plants

Collected 10 gm of litter along with the top soil from 5 different places around the plant samples were grinded and subjected to serial dilution method (Aneja, 2003).

Isolation and Identification of fungi by Warcup method

Serially diluted (10-2, 10-3 and 10-4) were inoculated to META (Malt Extract Tannic Acid Agar) media and then kept for 10-12 days incubation at room temperature. After incubation hallow zone developed around are fungal colony and it indicates the growth of lignin degraders. Again these isolates were confirmed with LNM (Low nitrogen) media. The isolates lignin degraders were identified by using fungal manuals .

RESULTS AND DISCUSSION

From selected plants such as *Strobilanthus barbatus*, *Dellenia pentagyra*, *Strobilanthus hyneanus*, *Dryptes oblongifolia* and *Artocarpus integrifolius*, 21 litter degrading fungi were isolated among these only 4 strains were identified up to generic level i.e. *Penicillium* spp, *Rhizopus* spp, *Aspergillus spp* and *Mucor* spp based on morphological characters by using fungal manuals. Physical characteristics of litter sample and identified fungi were listed in Table 1 and 2 respectively.

Ward (1952) made the first detailed study of litter decomposition. About 120 different fungi were recorded which are belongs to Deuteromycetes, Basidiomycetes, Ascomycetes and lower fungi. But from this work fungi are belongs to Ascomycetes only. In each litter sample *Penicillium spp* commonly occur compared to others. The 4 strains were decomposes litter at temperature about 23 to 24 ^{o}C and pH ranges 5.88 to 7.05 of natural condition. By using these data we can characterize the lignin degraders based on industrial requirements.

Table 1. Physical characteristics of litter sample

S. No.	Sample	Temperature		Moisture Percentage	pH of soil sample
		Env. temp	Sample temp.		
1.	*Strobilanthus barbatus*	23^{o}C	22^{o}C	39.2 %	7.05
2.	*Dellenia pentagyra*	24^{o}C	23^{o}C	48.76 %	6.26
3.	*Strobilanthus hyncanus*	24^{o}C	24^{o}C	26.75%	6.1
4.	*Dryptes oblongifolia*	23^{o}C	26^{o}C	35.92 %	6.34
5.	*Artocarpus integrifolius*	23^{o}C	25^{o}C	24.27%	5.88

Table 2. List of Identified isolates from each sample

Plants Fungi	*Strobilanthus barbatus*	*Dellenia pentagyra*	*Strobilanthus hyncanus*	*Dryptes oblongifolia*	*Artocarpus integrifolius*
Penicillium sp	+	+	+	+	+
Rhizopus sp	+	+	_	_	_
Mucor Sp	_	_	+	+	+
Aspergillus sp	_	_	+	+	+

REFERENCES

1. Aber, J. D., Melillo, J. M. and McClaugherty, C. A. Predicting long-term patterns of mass loss, nitrogen dynamics and soil organic matter formation from initial litter chemistry in temperate forest ecosystem. *Can. J. Bot*. 1990; **1**(8): 2201-2208.
2. Ander, P .and Eriksson, K.E., Lignin degradation and utilization by microorganisms. *Progress in Industrial Microbiology*. 1978; **14**: 1-58.
3. Aneja, K.R., Preparation of stains. In: Experiments in Microbiology, plant pathology and biotechnology (4th ed.), pp.571. New Age International publishers, New Delhi 2003.
4. Coldwell, B.B. and Delong, W.A., Studies on the compositon of decidious forest tree leaves before and after partial decomposition. *Sci. Agric Ecology*. 1950; **63** :621-626.
5. Coll, P.M., Fernandez–Albalos, J.M., Villanueva, R.S.and Peres, P., Purification and characterization of phenoloxidase (laccase) from lignin-degrading Basidiomycetes PM1 (CECT 2971). *Appl. Environ. Microbial*. 1993; **59**(8): 2607-2613.
6. Dix, N. J. and Webster, J., Fungal Ecology. Chapma 1995.
7. Filazzola, M.T., Sannino, F., Rao, M.A. and Gianfreda, L., Effect of various pollutants and soil-like constituents on laccase from Cerrena uncoiled. *J. Environ. Qual*. 1999; **28**: 1929-1938.
8. Gianfreda, L., Xu, F. and Bollag, J.M., Laccases: a useful group of oxidoreductive enzymes. *Bioremediation J*. 1999; **3**(1): 1-26.
9. Handley, W.R.C., Mull and mor formation in relation to forest soils. *For. Comm.. Bull*. 1954; **23**: 1-115.
10. Hudson, H. J., In: Fungal Biology. Edward Arnold Publishers Ltd. Cambridge University press 1986.
11. Jarosz–Willolazka, A., Marlarcyte, E and Leonowicz, A., Bioremediation of lignin-containing industrial waste materials and dyes by white rot fungi. Proceedings of the 8th International Conference on Biotechnology in the pulp and paper Industry,4-8 June.Helsinki, Finland 2001.
12. Kirk, J.K., S. Croan. M. Jien., K.E., Murtagh. and R.L. Farrell., Production of multiple ligninares by phanerochacte chrysosporum : Effect of selected growth conditions and use of a mutant strain. *Enzyme microb. Technol*. 1987; 27-32.
13. Martinez –Yrizar, A., Bismass distribution and primary productivity of tropical dry forests. In: Bullock S.H., Mooney H.A., Medina, E. Seasonally Dry tropical forests, pp.326-345. Cambridge University Press, Cambridge 1995.
14. Melin, E., Biological composition of some types of litter from North American forests. *Ecology* 1930; **11**: 72-101 .
15. Mikola, P., Experiment on the rate of decomposition of forest litter. *Communicationes Instituti Forestalis Fenniae*. 1954; **43**: 1-50.
16. Montanenz, E.P.A., Production de hojarasea, y aporte de nutrimenlos in los hurtor familiareas de Hocaba y Saheaba, Yucatan, Mexico. Master's Thesis. Faculated de medicina veterinaria y zooteenia. Univessidad autonoma de yucatan, Mexico, 1998; 110.
17. Ohmasa, M and Mori, K., The amount of fall and decomposition of the leaf litter of the forest trees of japan. *Bull. For. Exp. Stn. Imp. Household*. Tokyo- Fu, 1937; **3**: 39-101.
18. Wittich, W., Untersuchungen über den Verlauf der Streuzersetzung auf einem Boden mit Mullzustand II. *Forstarchiv*. 1943; **19**: 1-18.
19. Reinhammer, B. R. M., Laccase. In: Copper Proteins andCopper Enzymes, vol 3, pp. 1±35. Edited by R. Lontie. Boca Raton, FL: CRC Press 1984.
20. Ward, G.M., Studies on the Succession of fungi in decomposing litter of Foerst soil. *Ph.D thesis.* University of Nottingham 1952.