Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 8, Issue, 10, pp.39558-39562, October, 2016

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

BIODEGRADATION OF WOOD BY THERMOPHILIC AND MESOPHILIC FUNGI FROM SUNDARBAN MANGROVE FOREST (WEST BENGAL)

***Archana and Jaitly, A. K.**

Mycology and Plant Pathology Laboratory, Department of Plant Science, Faculty of Applied Sciences, Rohilkhand University Bareilly –243006, India

ARTICLE INFO

Article History:
Received 10th July, 2016
Received in revised form
19th August, 2016
Accepted 08th September, 2016
Published online 30th October, 2016

Key words:

Mangrove forest, Fungal isolates,
Wood, Degradation.

ABSTRACT

Forty-three species of fungi were isolated from woods in Sundarban mangrove forest, India. The most frequently isolated species were members of *Aspergillus*, *Chaetomium*, *Curvularia*, *Fusarium*, *Mucor* and *Pestalotiopsis*. Species of *Acremonium*, *Neosartorya* and *Cladosporium* were rare, and no members of the Basidiomycotina were found. Few commonly encountered fungal isolates were tested for their wood decay ability by weight loss method in Czapek- Dox medium. The tested isolates showed soft rot of wood in laboratory tests. *Chaetomium funicolum* was found to cause 46 % weight loss being the most active in this regard. The rate of decomposition increased until about 45th day of incubation, it decrease thereafter.

Citation: Archana and Jaitly, A. K. 2016. "Biodegradation of wood by thermophilic and mesophilic fungi from sundarban mangrove forest (West Bengal)", *International Journal of Current Research*, 8, (10), 39558-39562.

INTRODUCTION

Sundarbans has a forest cover of 10,200 sq. km. shared between India and Bangladesh. India's share of this forest area is around 4,200 sq. km. Mangroves have coastal woody vegetation that fringes muddy saline shores and estuaries in tropical and subtropical regions (Gopal and Chauhan, 2006). They are characterized by high levels of productivity and fulfill essential ecological functions, harboring precious natural resources (Wang *et al.*, 2010). Wood deterioration in temperate and tropical forest ecosystems and in wood products has been widely studied, (Eaton and Hale, 1993; Eriksson *et al.*, 1990; Zabel and Morrell, 1992), however comparatively little is known from mangrove forest. Woods, like other organic goods is a nutritional product for some plants and animals. Most of the animals can be not digest cellulose and the other fiber ingredients of wood, but some fungi and insects can digest and use it as a nutritional product. Wood decay caused by fungi is mainly dependent on their ability to produce hydrolytic enzymes and cause different types of rot. Prominent members of fungi (Basidiomycetes, Ascomycetes, and Fungi Imperfecti) are capable of utilizing the cellulose and hemicellulose of the wood ray cells and lignin of the tracheids, fibers and vessels, caused their decay.

Corresponding author: Archana,
Mycology and Plant Pathology Laboratory, Department of Plant Science, Faculty of Applied sciences, Rohilkhand University Bareilly –243006, India.

Microbial degradation of lignin has considerable attention (Ander and Eriksson, 1977; Kirk *et al.*, 1980). In the forest ecosystem wood decay fungi play an important role in carbon and nitrogen cycling and help to convert organic debris into humus (Zabel and Morrell, 1992), as well as help in establishing marine and mangrove ecosystem. Fungi that selectively remove lignin without loss of appreciable amounts of cellulose, are extremely attractive for use in biological pulping processes, to improve the digestibility of highly lignified plant residues, and for bioconversion of lingo- and hemi-cellulosic waste into industrial products (Ander and Eriksson, 1977; Kirk *et al.*, 1980; Blanchette, 1984; Kang *et al.*, 2007; Fukasawa, 2011). The extreme environmental conditions of Sundarban mangrove forest have a strong impact on microbial growth and their decay ability. Mangrove environment, which has characteristically extreme ecological conditions, will certainly have some specialization in growth, development, and activity of wood-degrading mesophilic and thermophilic fungi native to this habitat. With this object in view the present investigation has been undertaken. Therefore the purpose of this study was to isolate thermophilic and mesophilic fungi and assess their abilities to degrade wood, from Sunderban Mangrove forest.

MATERIALS AND METHODS

Ecology of study area: The wood samples used in this study were collected from Sundarban mangrove forest (West Bengal)

which is located in the southern portion of the Gangetic delta bordering the Bay of Bengal, between 21°31' and 22° 15' N and between 88°10' and 87°10' E. The area is one of the tropical estuarine forests with a salinity varying between 25 and 48 ‰. Temperature is equable but due to its proximity to sea, a heavy rainfall prevails. The average annual maximum temperature is 32.7º C while the minimum is 20º C. High temperature occurs from mid-March to mid-June and low in December and January. Humidity is highest in June - October and lowest in February. The average annual rainfall is 78.81 inches (1920.38 mm) and the average humidity is over 82%. The average tidal amplitude in the estuaries of the Sundarbans ranges from 3.5 m to 4.0 m. The highest fluctuations in the water level are generally experienced in August - September when the highest tide level attained is in excess of 4.0 m. The soil is deep alluvium like that of the rest of the Bengal plains, and is alkaline (pH 7.3 to 9.0).

Sampling sites in study area and sample collection

Wood samples were collected from different localities (Canning, Gosaba, Pakhirala, Sajanakhali, Sudnnakhali, Diamond harbor, Bakhali and Gangasagar) in the Sundarban mangrove area. The samples were cut from mangrove wood lying on the swamp, were placed immediately into sterile plastic bags and brought to the laboratory for study, where they were stored at 4º C.

Isolation of fungi from wood samples

Collected wood sample were analyzed for isolation of fungal species by dilution plate method using malt extract agar medium (Malt extract 20g, Peptone 1g, Dextrose 20 g, Agar 15g, Distilled water 1L, pH 6.5). In dilution plate technique, 1 mL from each dilution was transferred to a clean sterilized Petri dish (three replicates). The medium was poured onto the samples within Petri dishes, and then plates were allowed to solidify. The plates were incubated at 28ºC and 45º C for 4–7 days. After incubation, species were isolated into pure culture and identified.

Direct examination for occurrence of fungi in wood samples

Surface sterilized wood samples were directly examined under the microscope after cutting them into small pieces for their fungal forms. Wood pieces were kept in moist chambers after soaking them with saline water having salinity level equivalent to the locality and incubated at 28± 1ºC for 12 to 16 h. The fungal mycelium thus appeared, were isolated on agar slants. Wood pieces were plated out with malt extract agar (pH 6.5), as it was proved a good medium for their isolation (Rai *et al.*, 1981). Three replicates were taken for each. Plates were incubated at 28± 1º C and at 45ºC in moist chambers and examined after every 12h. The growing hyphae were transferred on to agar slants for their identification.

Per cent frequency of isolated fungi

The per cent frequencies (Jaitly, 1982) of isolated fungal species were calculated by the formula given below-

$$\text{Frequency \%} = \frac{\text{Number of particular fungal colonies}}{\text{Total number of fungal colonies}} \times 100$$

Wood decay studies

The wood decay ability of some fungal isolates thus isolated was carried out by weight loss method in Czapek-Dox medium without carbon source. Wood decay test for each fungi was performed according to the Kang *et al.*, 2007. Wood blocks (25×12×8mm) of *Tamarindus indica* L. were dried at 80ºC to a constant weight. Blocks were sterilized by autoclaving at 15 lbs for 30 min. Sterilized wood blocks were then placed into sterilized 100 ml Erlenmeyer flasks containing 25ml sterilized Czapek- Dox medium (Sodium nitrate 3.0 gm, Dipotassium phosphate 1.0 gm, Magnesium sulphate 0.5 gm, Potassium chloride 0.5 gm, Ferrous sulphate 0.01 gm, Dist. Water 1 L) without carbon source and salinity equivalent to the collection site. Erlenmeyer flasks containing wood blocks were then inoculated with two mm disc of freshly grown mycelium taken from the edge of an actively grown colony. Flasks were incubated at 29 ± 1ºC for 15, 30, 45 and 60 days in a moist chamber. Three replicates were taken for each. After incubation, the mycelium was completely brushed off from the surface of the blocks which were then dried to a constant weight at 80ºC. Differences in weight were taken and per cent weight losses were calculated. The weight loss of each wood block was calculated by the equation as follows:

$$\text{Weight loss (\%)} = (W1 - W2)/W1 \times 100$$

Where W1 is the oven-dry weight of wood block before test, W2 is the oven-dry weight of decayed wood block after test. Based on their weight loss caused the tested species were grouped as strong (weight loss above 37%) and moderate (weight loss in between 27-37%) decomposers group (Rai *et al.*, 1981). Transverse and longitudinal sections of wood were cut and examined microscopically.

RESULTS

Occurrence of fungi in wood samples

Microbial analysis of wood using malt extract medium at 28°C and 45°C, resulted in the isolation of 43 fungi. Out of these 25 fungal forms were thermophilic, while the rest were mesophilic. The majority of species were of Deuteromycotina (29 species). Ascomycotina (including ascosporic Aspergilli and Penicillia), Zygomycotina and Mycelia sterillia were represented by six species, three species and five strains respectively. The most frequently isolated species were of *Aspergillus* followed by *Pencillium*, *Mucor*, *Fusarium*, *Pestalotiosis*, *Chaetomium*, *Trichoderma* and *Curvularia* (Table 1). Maximum number of mesophilic fungi were isolated from Pakhairala and the least from Gangasagar, while thermophilic fungi were encountered in maximum number from Sajanakhali, while the least from Gangasagar.

Per cent frequency of tested fungi

Per cent frequency of occurrence of various fungi is given in Table 1, which indicate that among the Zygomycotina, *Mucor* spp were isolated in higher frequency. Of the Ascomycotina, *Chaetomium* spp were more frequent than the species of other genera (Rai and Chowdhery, 1978; Rai *et al.*, 1981). Aspergilli were more prevalent amongst Deuteromycotina, of which *A. carneus*, *A. fumigatus*, *A. niger*, *A. terreus* have a high frequency of occurrence. *Acremonium* and *Cladosporium* species were rather rare as compared to *Alternaria*, *Curvularia*,

Table 1. Frequency of occurrence of fungi from woods in mangrove swamps (Sundarban) on malt extract agar

Fungi	Per cent frequency
Zygomycotina	
Mucor racemosus Fres.	28
Mucor milvaticus Hagern	26
Mucor sp.	18
Ascomycotina	
Chaetomium arcuatum Rai *et* Tewari	21
Chaetomium funicolum Cooke	24
Chaetomium globosum Kunza	33
Emericella nidulans Vuill. Lata (Raper *et* Thom) Subram	23
Neosartorya fischeri (Wehmer) Malloch *et* Cain	09
Talaromyces wortmanni (Klocker) C.R. Benjamin	15
Deuteromycotina	
Acremonium strictum W. Gams	08
Alternaria alternata (Fr.) Keissler	21
Alternaria humicola Dudem	32
Aspergillus carbonarius (Bain.) Thom	20
Aspergillus carneus (V. Teigh.) Blochwitz	30
Aspergillus flavus Link	45
Aspergillus fumigatus Fres.	59
Aspergillus japonicas Saito	19
Aspergillus niger Van Tiegh.	55
Aspergillus Penicilliformis Kamyschko	16
Aspergillus tamarii Kita	15
Aspergillus terreus Thom	53
Cladosporium cladosporioides (Fres.) de Vries	12
Curvularia lunata var.*aeria* (Batista, Lima *et* Vas) M.B. Ellis	34
Curvularia sp.	30
Fusarium oxysporum Schlecht. *et* Fr.	33
Fusarium roseum Link	25
Fusarium solani (Mart.) Sacc.	35
Humicola grisea Traaen	22
Penicillium chrysogenum Thom	49
Penicillium citrinum Thom	45
Penicillium funiculosum Thom	28
Penicillum sp. I (Unidentified)	50
Pestalotia sp.	52
Pestalotiopsis veriscolor (speg.) Steyaert	47
Pestalotiopsis sp.	45
Sporotrichum sp.	28
Trichoderma album Preuss	36
Trichoderma lignorum (Tode) Harz	34
Mycelia Sterilia (5 strains)	26

Table 2. Average per cent weight losses in wood as caused by some fungi

Test Fungi	Average weight loss (in Per cent)			
	Incubation period in days			
	15	30	45	60
Control	3	4	5.5	6
Alternaria humicola	10	19	33	40
Aspergillus carneus	9	17	29	39
Aspergillus flavus	9	20	33	42
Aspergillus japonicus	6	14	23	29
Aspergillus niger	6	14	24	30
Aspergillus penicilliformis	7	15	24	30
Chaetomium arcuatum	9	21	37	43
Chaetomium funicolum	9	21	36	46
Fusarium roseum	9	18	34	40
Fusarium solani	11	23	36	44
Humicola grisea	8	19	31	39
Neosartorya fischeri	9	17	27	33
Penicillium chrysogenum	8	16	29	38
Trichoderma album	9	20	35	42

Fusarium, *Penicillium*, *Pestalotia*, *Pestalotiopsis* and *Trichoderma*. The members of Basidiomycotina were not isolated.

Wood decay ability (Weight loss of wood blocks)

All the tested fungi showed the highest increasing rate of weight loss in the first 45 days of test period. Weight loss of wood was found to increase throughout the decay period, but the increasing rate of weight loss decreased after 45 days of incubation. *Chaetomium funicolum* caused the greatest per cent weight loss (45.5 %) of *Tamarindus* wood followed by *Fusarium solani* (43.7%), *Chaetomium arcuatum* (43%), *Trichoderma album* (42%), while *Aspergillus japonicus* caused the least (28.9). Per cent weight losses for fungi tested on different days are given in Table 2.

DISCUSSION

During the present investigation members of Deuteromycotina were isolated in the highest number indicating that it is the major group inhabiting mangrove habitat (Rai and Chowdhery, 1978; Rai *et al*., 1981). The species of *Aspergillus*, *Penicillium*, *Chaetomium*, *Curvularia*, *Fusarium*, *Pestalotiopsis* and *Trichoderma* were more frequently isolated. It has conformity with the results obtained by Rai *et al.* (1969), Rai and Chowdhery (1978) and Rai *et al.* (1981). Among Deuteromycotina Aspergilli were most prevalent. It is in agreement with the result obtained by Rai and Chowdhery (1978) from mangrove mud and Rai *et al.* (1981) from woods. The dominance of Aspergilli has been reported to be the characteristic of the soil of warmer regions (Saxena, 1955; Rai *et al.*, 1968). Swart (1958) also observed the dominance of Aspergilli and Penicillia in the mangrove mycoflora of Inhaca islands. Isolation of large number of terrestrial forms in mangrove swamp indicate after being transmitted to this habitat either by wind or by river and see tides, they have developed certain degree of adaptability to extreme conditions of the mangroves and slowly and slowly become natural inhabitant of this habitat (Swart, 1958). Manglicolous filamentous fungi have been isolated from different parts along the Indian West Coast (Borse, 1988; Maria and Sridhar, 2002; Marbaniang and Nazareth, 2006), East Coast (Rai and Chowdhery, 1978; Vittal and Sharma, 2006; Nambiar and Raveendran, 2008) and from around the globe (Sahoo and Dhal, 2009) have also shown the same trend. During present investigation a total of 43 fungi were isolated. It does not however reflect that these are the only species that are native inhabitants of wood but it is merely a chance that fungi other than these have not been observed and isolated. Frequent isolation and high decomposition rate of *Chaetomium*, *Penicillium*, *Fusarium*, *Pestalotiopsis* and *Trichoderma* showed that they are the forms more capable to draw the nutrients from woods (natural organic matter). The high rate of decomposition by the species of these fungi has also been observed by Rai *et al.* (1981). Ananda and Sridhar (2004) have also reported the occurrence of more terrestrial fungi than marine fungi on mangrove leaves. It has also been reported that even after 12 months of immersion of wood in the mangroves, anamorphic fungi (Deuteromycotina) dominated during the subsequent monsoon (Maria and Sridhar, 2003). Fungi living in different saline environment are generally adapted to extreme conditions of low Aw, temperature, pH and salinity. During the present investigation we have also isolated terrestrial fungi. All the tested fungi caused maximum weight loss during first 45 days of incubation. Subsequent decrease in the rate of weight loss may be due to the production of toxic by-products during rapid fungal growth which in turn may be inhibitory to further growth. Microscopic examinations of the sections show the presence of fungal hyphae in parenchyma cells, tracheids and vessels. The absence of Basidiomycotina might be due to their inability to grow with extreme ecological condition of the mangroves (Rai *et al.*, 1981). Duncan and Eslyn (1966) have also stated that soft-rot fungi are prevalent in situation of extreme wetness (as found in mangrove environment) or frequent dryness conditions that restart or inhibit development of the most aggressive wood destroying Basidiomycotina.

Acknowledgement

The authors are grateful to the Forestry authorities of West Bengal for the help during the collection of samples from remote areas in Sundarban.

REFERENCES

Ananda K. and Sridhar K.P. 2004. Diversity of filamentous fungi on decomposing leaf & woody litter of mangrove forest in the south-west coast of India, *Curr. Sci*., 87, 1431-1437.

Ander P. and Eriksson K.E. 1977. Selective degradation of wood components by white rot fungi, *Physiol. Plant*., 41, 239-248.

Blanchette R.A. 1984. Screening wood decayed by white rot fungi for preferential lignin degradation, *Appl. Environ. Microbiol*., 48, 647-653.

Borse B.D. 1988. Frequency of occurrence of marine fungi from Maharastra coast, India. *Indian Journal of Marine Sciences*, 17, 165-167.

Duncan C.G. and Eslyn W.E. 1966. Wood-decaying Ascomycetes and Fungi Imperfecti, *Mycologia*, 58, 642-645.

Eaton R. A. and Hale M. D. C. 1993. Wood: decay, pests and protection, (Chapman and Hall, London, England).

Eriksson K. E., Blanchette R. A. and Ander P. 1990. Microbial and enzymatic degradation of wood and wood components, in: wood science, (Springer-Verlag, Heidelberg, Germany) pp. 407.

Fukasawa Y. 2011. Wood decomposing abilities of diverse lignicolous fungi on nondecayed and decayed beech wood, *Mycologia*, 103**,** 474-482.

Gopal B. and Chauhan M. 2006. Biodiversity and its conservation in the Sundarban mangrove ecosystem, *Aquat. Sci.*, 68, 338–354.

Jaitly A.K. 1982. Ecological studied of thermophilic fungi native to mangrove swamps, *Trans. Mycol. Soc. Japan*., 23, 65-71.

Kang K.Y., Sung J.S. and Kim D.Y. 2007. Evaluation of White-rot Fungi for Biopulping of Wood, *Mycobiology*, 35, 205-209.

Kirk T. K., Higuchi T. and Chang H. M. 1980. Lignin biodegradation: summary and perspectives, in: Lignin biodegradation: microbiology, chemistry and potential applications, edited by T. K. Kirk, T. Higuchi and H. M. Chang (Fla: CRC Press. Inc., Boca Raton), pp. 235-244.

Marbaniang B.T. and Nazareth S. 2006. Isolation of halo-tolerant *Penicillium* species from mangroves and solterns and their resistance to heavy metals, *Curr. Sci*., 92, 895-895.

Maria G.L. and Sridhar K.R. 2002. Richness and diversity of filamentous fungi on woody litter of mangrove along the west coast of India, *Curr. Sci*., 83, 1573-1580.

Maria G.L. and Sridhar K.R. 2003. Diversity of filamentous fungi on woody litter of five mangrove plant species from the southwest coast of India, Fungal Diversity, 14, 109-126.

Nambiar G.R., and Raveendran K. 2008. Diversity of mangrove fungi of North Malabar, Karala, India, Indian Forester, 134, 1658-1662.

Rai J.N. and Chowdhery H.J. 1978. Micro fungi from mangrove swamps of West Bengal, *India Geophytol*., 8, 103-110.

Rai J.N., Agarwal S.C., Tewari J.P. and Wadhwani K. 1968. Natural Occurrence of buff and tan mutants of *Aspergillus fumigatus* in Indian alkaline soils, *Can. J. Bot*., 46, 1330-1331.

Rai J.N., Garg K.L. and Jaitly A.K. 1981. Saprophytic fungi isolated from woods Mangrove swamps and their wood-decaying capability, *Trans. Mycol. Soc. Japan*, 22, 65-74.

Rai J.N., Tewari J.P. and Agarwal S.C. 1969. Mycoflora of mangrove mud. Mycopath, *Mycol. Appl*., 38, 17-31.

Sahoo K. and Dhal N.K. 2009. Potential microbial diversity in mangrove ecosystem: A review, *Indian J. Mar. Sci*., 38, 249-256.

Saxena S.B. 1955. Ecological factors governing the distribution of micro- fungi in some forest soil of Sagar, *J. Indian Bot. Soc*., 34, 262-298.

Swart H. J. 1958. An investigation of the mycoflora in the soil of some mangrove swamps, (North-Hill and publishing Company, Amsterdam), pp. 741-768.

Vittal P.R. and Sharma V. 2006. Diversity and ecology of fungi on mangroves of Bay of Bengal region: An overview, *Indian J. Mar. Sci*., 35, 308-317.

Wang Y., Qiu Q., Yang Z., Hu Z., Tam N.F.Y. and Xin G. 2010. Arbuscular mycorrhizal fungi in two mangroves in South China, Plant Soil, 33, 181–191.

Zabel R. A. and Morrell J. J. 1992. Wood microbiology: Decay and its prevention, (Academic Press Inc, New York).

****