BIOSCIENCES BIOTECHNOLOGY RESEARCH ASIA, June 2012. Vol. **9**(1), 451-456

Cellulose Degradation Potential of *Acacia dealbata* Link. Leaf Litter in Virgin Forest Ecosystem of Ooty by Microfungi in Relation to CO_2 Release

M.N. Abubacker[1]* and M. Prince[2]

[1]Department of Biotechnology, National College, Tiruchirappalli - 620 001, India.
[2]PG Department of Botany, National College, Tiruchirappalli - 620 001, India.

(Received: 05 January 2012; accepted: 12 February 2012)

Isolation, identification and cellulose degradation potentials of Microfungal flora of virgin forest soils of Ooty were investigated using soil dilution plate and warcup methods. A total of 40 microfungi were isolated from forest soils dominated by *Acacia dealbata* Link., *Cupreses torulosa* Don., *Eucalyptus globules* Labill. and *Pinus radiatia* Don. The results indicate *Acremonium* sp., 11 *Aspergillus* spp., *Botrytis* sp., 2 *Chaetomium* spp., *Cladosporium* sp., *Curvularia* sp., 4 *Fusarium* spp., *Helminthosporium* sp., *Humicola* sp., *Memnoniella* sp., *Mortierella* sp., 2 *Mucor* spp., *Paecilomyces* sp., 4 *Penicillium* spp., *Periconia* sp., *Pleospora* sp., *Rhizoctonia* sp., *Rhizopus* sp., *Trichoderma* sp., *Trichosporiella* sp. and *Trichothecium* sp. were the fungal genera. Among them the most widespread genera were *Aspergillus* spp. and *Fusarium* spp. The cellulose degradation potential of these fungi were examined. Carbondioxide (CO_2) release during degradation of cellulose was used as an index to determine the extend of biodegradation. *Acremonium* sp., *Humicola* sp., *Memnoniella* sp., *Paecilomyces* sp., *Periconia* sp. and *Trichothecium* sp. released more CO_2 with leaf litter used as substrate of biodegradation.

Key words: Biodegradation, Microfungi, CO_2 release, Forest ecosystem, Cellulose decomposition

A large amount of plant waste is being continuously accumulated on the surface of earth. A part of the waste gets into the soil due to the biodegradation process of soil microbes including microfungi. Intensity of decay and degradation process depends on different environmental factors such as species of plant, sort of soil, moisture, temperature, the type of soil microflora and their metabolic processes. In order to increase the efficiency of these metabolic processes, the scientific society is making strong efforts in this area of research. Moreover, the aim of the latest researchers are oriented to intensify plant remnants decay and degradation and modify metabolites enriching the soil by useful biologically active substances and finding materials able to enrich forage and food by important biologically valuable additions (Reid, 1989; Kelley, 1992; Varnaite, 2001).

Different substrata basically consisting of cellulose, hemicellulose and lignin are the main nutrient source of microorganisms. Degradation process of plant waste is stimulated by various enzymes based on the microorganisms activity (Yoshida *et al*., 1996; del Pilar Castillo *et al*., 1997; Ortega *et al*., 2001; Bridziuviene and Lugauskas, 2003; Tanaka *et al*., 2009).

Fungi play an important role in the biodegradation of various substances rich in cellulose and lignin (Babickaja, 1994; Daljit, 1995; Petre *et al*., 2005). Cellulose of plant material is degraded by fungal cellulolytic enzyme cellulase.

* To whom all correspondence should be addressed.
E-mail: abubacker_nct@yahoo.com

It is capable of degrading crystalline forms of cellulose. The enzyme composed of three enzyme species: endo-β-1,4 glucanase, exo-β-1,4-glucanase (cellobiohydrolases) and β-glucosidase (cellobiases). The three enzyme groups work synergistically to hydrolyse crystalline cellulose. The net effect is the release of carbondioxide and it is used as an index to find out decomposition of cellulose (Paul, 1992).

The aim of this study is to isolate fungi from virgin soil of Ooty forest ecosystem and to estimate the cellulose degradation potentials of *Acacia* sp. leaf litter by these fungi through CO_2 releasing method.

MATERIAL AND METHODS

Isolation and identification of microfungi from leaf litter degrading virgin soils

The surface of the soil profile was cleaned and vertical samples were taken from 10 cm depth with a disinfected spatula. The spatula was applied perpendicular to the vertical surface of the soil profile. The samples were stored in a sterilized cooled bottle until they reached the laboratory. The samples were processed in an isolation process using the soil dilution plate (Waksman, 1922) in Czapek's sucrose-nitrate-agar medium (Booth, 1971) in the inoculation chamber in Petri dishes and then incubated at 28 ± 1° C. Further subcultures were made and the pure cultures were maintained in the laboratory for further studies. The identification of fungi was performed following Domsch *et al.* (1980). The 40 fungal species isolated and identified are listed below:

1. *Acremonium murorum* (Corda) W. Gams (Moniliaceae)
2. *Amorphotheca resinae* Parbery (Amorphothecaceae)
3. *Aspergillus alutaceus* Berk and Curt. (Moniliaceae)
4. *Aspergillus candidus* Link ex Link (Moniliaceae)
5. *Aspergillus erythrocephalus* Berk. and Curt. (Moniliaceae)
6. *Aspergillus flavus* Link ex. Gray (Moniliaceae)
7. *Aspergillus fumigatus* Fres. (Moniliaceae)
8. *Aspergillus niger* van Tieghem (Moniliaceae)
9. *Aspergillus oryzae* (Ahlburg) Cohn (Moniliaceae)
10. *Aspergillus restrictus* G. Sm. (Moniliaceae)
11. *Aspergillus terreus* Thom. (Moniliaceae)
12. *Aspergillus ustus* (Bain) Thom and Church (Moniliaceae)
13. *Aspergillus versicolor* (Vuill.) Tiraboschi (Moniliaceae)
14. *Botrytis cinera* Pers. ex Nocca and Balb (Moniliaceae)
15. *Chaetomium crispatum* (Fuckel) Fuckel (Melanosporaceae)
16. *Chaetomium funicola* Cooke (Melanosporaceae)
17. *Cladosporium herbarum* (Pers) Link ex Gray (Dematiaceae)
18. *Curvularia lunata* (Wakker) Boediju (Dematiaceae)
19. *Fusarium chlamydosporum* Wollenw and Reink (Tuberculariaceae)
20. *Fusarium oxysporum* Schlect emend. Sny. and Hans. (Tuberculariaceae)
21. *Fusarium poae* (Peck) Wollenw (Tuberculariaceae)
22. *Fusarium solani* (Mart) Sacc (Tuberculariaceae)
23. *Helminthosporium solani* (Pleosporaceae)
24. *Humicola fuscoatra* Traaen (Deuteromycotina)
25. Memnoniella echinata (Riv) Galloway (Deuteromycotina)
26. *Mortierella alpina* Peyronel (Deuteromycotina)
27. *Mucor mucedo* Mich. ex St-Am (Mucoraceae)
28. *Mucor racemosus* Fres. (Mucoraceae)
29. *Paecilomyces carneus* (Duche and Heim) A. H. S. Brown and G. Sm. (Deuteromycotina)
30. *Penicillium chrysogennm* Thom. (Moniliaceae)
31. *Penicillium funiculosum* Thom. (Moniliaceae)
32. *Penicillium janthinellum* Biourge (Moniliaceae)
33. *Penicillium verrucosum* Dierckx (Moniliaceae)
34. *Periconia prolifica* Lefebvre and A. G. Johnson (Dematiaceae)
35. *Pleospora herbarum* (Fr ex Fr) Rabenh (Pleosporaceae)
36. *Rhizoctonia solani* Kuhn (Ceratobasidiaceae)
37. *Rhizopus oryzae* Went and Prinsen Geerligs (Mucoraceae)
38. *Trichoderma viride* Pers ex Gray (Tuberculariaceae)
39. *Trichosporiella cerebriformis* (de Vries and Kleine-Natrop W.Gams) (Deuteromycotina)
40. *Trichothecium roseum* (Pers) Link ex Gray (Moniliaceae)

Fungal cultures for biodegradation studies

The pure cultures of all the forty fungal species were tested for cellulose biodegradation studies. From the pure culture, a loopful of fungal spores along with mycelia were inoculated in 250 ml conical flask under sterile condition in 50 ml Czapek's-Sucrose-Nitrate liquid medium in an inoculation chamber. After inoculation the culture bottles were incubated at 28 ± 1° C. The growth of fungus appeared after 24 hours of inoculation.

Determination of CO_2 release during cellulose biodegradation

To the conical flask with 24 hr of fungal inoculum, 500 mg of dried leaf material as substrate was added in small pieces. Vials containing 5 ml of 5 N sodium hydroxide solution was suspended with the help of a thread. The conical flask was closed with stopper and sealed with parafilm membrane to ensure air-tight condition and incubated at 28° ± 1° C. The fungus colonizes the dried *Acacia dealbata* leaf material degrading cellulose material with the release of CO_2. It was absorbed by sodium hydroxide in the vials. During each estimation the content of the vials was quantitatively transferred to a flask followed by the addition of 5 ml of saturated solution of barium chloride to precipitate the CO_2 as barium carbonate. Two drops of phenolphthalein was added. The residual amount of sodium hydroxide in the flask was measured by titrating against 0.1 N hydrochloric acid. The end point is the disappearance of pink colour (Gaur *et al*., 1971).

Calculation

1 ml of 0.1 N hydrochloric acid = 1 ml of 0.1 N sodium hydroxide = 2.2 mg of carbondioxide evolved

RESULTS AND DISCUSSION

Forty fungal species which colonize the forest ecosystem of Ooty have been isolated and their cellulose biodegradation potentials were estimated through CO_2 release method on 10th, 20th and 30th days of degradation process and the results are presented in Table-1.

On the 10th day of cellulose biodegradation, CO_2 release by *Humicola fuscoatra* showed a maximum of 72 mg followed by 68 mg by *Paecilomyces carneus*, 66 mg by *Memnoniella echinata* and *Rhizotonia solani*, 62 mg by *Acremonium murorum*, 60 mg by *Fusarium oxysporum* and *F. solani*, the minimum of 28 mg by *Aspergillus fumigatus* and *Penicillium verrucosum*. On the 20th day of cellulose biodegradation CO_2 release by *H. fuscoatra* showed a maximum of 96 mg followed by 92 mg by *A. murorum* and *M. echinata*, 86 mg by *P. carneus*, 82 mg by *R. solani*, 80 mg by *F. solani* and 72 mg by *A. versicolor, Cladosporium herbarum* and *F. oxysporum*, the minimum of 38 mg by *Helminthosporium solani* and *P. chrysogenum*. On the 30th day of cellulose biodegradation CO_2 release by *H. fuscoatra* showed a maximum of 64 mg followed by 58 mg by *P. carneus*, 56 mg by *A. murorum, C. herbarum* and *P. carneus*, 54 mg by *R. solani* and *Trichoderma viride*, 52 mg by *Trichothecium roseum*, the minimum of 24 mg by *Aspergillus candidus, A. flavus* and *Mucor racemosus*.

At the end of 30th day of cellulose biodegradation the maximum total amount of CO_2 release of 232 mg was found in *H. fuscoatra*, followed by 220 mg by *P. carneus*, 216 mg by *M. echinata*, 210 mg by *A. murorum*, 202 mg by *R. solani*, 186 mg by *F. solani*, the minimum of 92 mg by *A. restrictus*.

From this result it can be grouped that *A. murorum, H. fuscoatra, M. echinata, P. carneus* and *R. solani* are the fast cellulose degrading fungi. *Amorphotheca resinae, Aspergillus alutaceus, A. erythrocephalus, A. niger, A. oryzae, A. terreus, A. versicolor, Botrytis cinera, Chaetomium funicola, C. herbarum, Curvularia lunata, F. chlamydosponum, F. oxysporum, F. solani, Rhizopus oryzae, Trichoderma viride* and *Trichothecium roseum* are moderate cellulose degrading fungi, whereas *Aspergillus candidus, A. flavus, A. fumigatus, A. restrictus, A. ustus, Chaetomium crispatum, Fusarium poae, Helminthosporium solani, Mortierella alpina, Mucor mucedo, M. racemosus, Penicillium chrysogenum, P. funiculosum, P. janthinellum, P. verrucosum, Periconia prolifica, Pleospora herbarum* and *Trichosporiella cerebriformis* are the slow cellulose degrading fungi.

The various levels of biodegradation are due to the activities of fungal enzymes. These fungi are capable of producing cellulose enzymes such as endoglucanase, exoglucanase and β-glucosidase in their system (Fahnrich *et al*., 1981; Schuabel, 1981; Kannan *et al*., 1990). Cellulose enzyme is capable of degrading crystalline forms of cellulose of endo β-1, 4-glucanases, exo-β-1,4-glucanases (Cellobiohydrolases) and β-glucosidases (Cellobiases). The net effect of these three enzymes is to rapidly decrease the polymer length with a slow increase in reducing group (Moore and Landecker, 1972; Coughlan, 1989). The fast cellulose degrading fungi in this study namely *A. murorum, H. fuscoatra, M. echinata, P. carneus*

and *R. solani* have such high potential of producing cellulose enzymes in their system so as to degrade the cellulose in leaf litter substrate more effectively and the result is in accordance with the study conducted by Abubacker *et al.* (2001) with respect to cellulose degradation of commercial paper as substrate. Cellulolytic enzymes play an important role in natural biodegradation process in which plant lignocellulose materials are efficiently degraded by fungi (Varnaite *et al*., 2011). Many fungi probably produce β-glucosidase, a large enzyme with a molecular mass of 32 KDa, active with glucose and cello oligosaccharides (Goodell, 2003). β-glucosidase enzyme responsible

Table 1. CO_2 release (mg) during *Acacia dealbata* leaf litter cellulose biodegradation of fungi isolated from forest ecosystem of Ooty

S. No.	Fungi	10th day	20th day	30th day	CO_2 released in 30 days
1.	*Acremonium murorum*	62	92	56	210
2.	*Amorphotheca resinae*	50	62	30	142
3.	*Aspergillus alutaceus*	46	68	32	146
4.	*Aspergillus candidus*	30	40	24	94
5.	*Aspergillus erythrocephalus*	54	66	42	162
6.	*Aspergillus flavus*	32	40	24	96
7.	*Aspergillus fumigatus*	28	48	36	112
8.	*Aspergillus niger*	42	62	46	150
9.	*Aspergillus oryzae*	40	56	44	140
10.	*Aspergillus restrictus*	32	42	28	92
11.	*Aspergillus terreus*	32	71	36	139
12.	*Aspergillus ustus*	30	42	32	104
13.	*Aspergillus versicolor*	46	72	32	150
14.	*Botrytis cinera*	52	62	46	160
15.	*Chaetomium crispatum*	42	46	32	120
16.	*Chaetomium funicola*	46	48	40	134
17.	*Cladosporium herbarum*	46	72	56	174
18.	*Curvularia lunata*	40	58	52	150
19.	*Fusarium chlamydosporum*	32	60	42	134
20.	*Fusarium oxysporum*	60	72	40	172
21.	*Fusarium poae*	34	46	32	112
22.	*Fusarium solani*	60	80	46	186
23.	*Helminthosporium solani*	32	38	32	102
24.	*Humicola fuscoatra*	72	96	64	232
25.	Memnoniella echinata	66	92	58	216
26.	*Mortierella alpina*	30	46	40	116
27.	*Mucor mucedo*	36	42	32	112
28.	*Mucor racemosus*	48	52	24	124
29.	*Paecilomyces carneus*	68	86	56	220
30.	*Penicillium chrysogennm*	32	38	28	98
31.	*Penicillium funiculosum*	30	42	32	104
32.	*Penicillium janthinellum*	36	46	30	112
33.	*Penicillium verrucosum*	28	42	36	106
34.	*Periconia prolifica*	42	50	36	128
35.	*Pleospora herbarum*	34	52	32	118
36.	*Rhizoctonia solani*	66	82	54	202
37.	*Rhizopus oryzae*	36	60	42	138
38.	*Trichoderma viride*	42	66	54	162
39.	*Trichosporiella cerebriformis*	30	52	30	112
40.	*Trichothecium roseum*	46	64	52	162

for most of the glucosidase activity is optimum in certain fungi which are capable of degrading cellulose. The cellulolytic and hemicellulolytic enzymes systems cannot be separated, since several enzymes show activity against more than one substrate (Cohen *et al*., 2005). However the measurement of carbondioxide release during the biodegradation process is used as an index of cellulose decomposition (Gaur *et al*., 1971). From this study it is to be concluded that the fungi like *A. murorum, H. fuscoatra, M. echinata, P. carneus* and *R. solani* are capable of producing cellulose degrading enzymes at a faster rate to decompose the leaf litter substrate cellulose and released more CO_2 and hence these potential fungi can effectively add more nutrients by way of degradation in forest ecosystem.

ACKNOWLEDGEMENTS

The authors thank DST-FIST, Government of India, New Delhi for providing the infrastructure facilities for the Department of Botany, National College, Tiruchirappalli. The authors also thank Shri. K. Ragunathan, Secretary and Dr. K. Anbarasu, Principal, National College, Tiruchirappalli for their encouragement.

REFERENCES

1. Abubacker, M. N., Anbu, P., Hilda, A. and Senthilkumar, S., Cellulose Biodegrdative Potential of some Fungi. *Asian J. Microbiol. Biotech. Env. Sci*. **3**: 49-51 (2001).
2. Babickaja, V. G., Enzymatic Lignin Degradation in Plant Substrates by Mycelial Fungi. *Engineering Biochemistry and Microbiology.* **30**: 827-835 (1994).
3. Booth, C., Fungal culture media. In: *Methods in Microbiology*. Vol. 4. Eds. Norris, J. R. and Ribbons, D. W. Academic Press, London. p. 66 (1971).
4. Bridziuviene, D. and Lugauskas, A., Evaluation of the efficiency of some on offers. *Wood Preservatives, Materials Science.* **9**: 363-367 (2003).
5. Cohen, R., Suzuki, M. R. and Hammel, K. E., Processive endoglucanse active in crystalline cellulose hydrolysis by the brown rot basidiomycete *Gloeophyllum trabeum*. *Appl. Environ. Microbiol.* **71**: 2412-2417 (2005).
6. Coughlan, M. P., *Enzyme System for Lignocellulose Degradation*. Coughlan, M.P. (Ed.), Elsevier Appl. Sci., London, (1989).
7. Daljit S. Arora, Biodelignification of Wheat Straw by Different Fungal Associations. *Biodegradation*. **6**: 57-60 (1995).
8. del Pilar Castillo, M., Ander, P. and Stenstrom, J., Lignin and Manganese Peroxidase Activity in Extracts from Straw Solid Substrate Fermentations. *Biotechnology Techniques*. **11**: 701-706 (1997).
9. Domsch, K. H., Gams, W. and Anderson, T. H., *Compendium of Soil Fungi*. Academic Press, London (1980).
10. Fahnrich, P., Irrgang, K., Rulten, B. and Schimz, K. L., *Abban Von Cellulose in Cellulosehaltigen material dutch mikroorganismen sowie durch mikrobielle Enzymsystem. Das Papier*. 35. **9** (1981).
11. Gaur, A. C., Sadasivam, K. V., Vimal, O. P. and Matur, R. S., A study of decomposition of organic matter in an alluvial soil: CO_2 Evolution, Microbiological and Chemical Transformation. *Plant Soil*. **34**: 17 (1971).
12. Goodell, B., Brown-rot fungal degradation of wood: Our Evolving View. *Wood Deterior Preserv.* **845**: 97-118 (2003).
13. Kannan, K., Oblisami, G. and Loganathan, B. G., Enzymology of Ligno-Cellulose Degradation by *Pleurotus sajor-Caju* growth on paper mill sludge. *Biol. Waste*. **33**: 1-8, (1990).
14. Kelley, J., Upgrading of Waste Cereal Straws. *Outlook of Agriculture*. **21**: 105-108 (1992).
15. Moore, E. and Landecker, *Fundamentals of the Fungi*. Prentice-Hall India Pvt. Ltd., New Delhi. pp. 295-296 (1972).
16. Ortega, N., Busto, M. D. and Perez-Mateos, M., Kinetics of Cellulose Saccharification by *Trichoderma reesei*. Cellulases, *International Biodeterioration and Biodegradation*. **47**: 7-14 (2001).
17. Paul, J., Different aspects of microbial degradation of cellulose. *Indian J. Microbiol.* **32**: 127-147 (1992).
18. Petre, M., Teodorescu, A. and Dieu, G., The Growing Effect of Vineyard and Winery Wastes on the Production of Mycelia and Fruit Bodies of Edible and Medicinal Fungi. *International Journal for Medicinal Mushrooms.* **7**: 444-445 (2005).
19. Reid, I. D., Solid State Fermentations for Biological Delignification. *Enzyme Microbial Technology*. **11**: 786-803 (1989).
20. Schuabel, W., *Polymer Degradation*. Hanser International, Munchen and Wien, (1981).
21. Tanaka, H., Koike, K., Itakura, S. and Enoki, A., Degradation of wood and enzyme production

by *Ceriporiopsis subvermispora*. *Enzyme and Microbial. Technology*. **45**: 384-390 (2009).

22. Varnaite, R., Bioconversion of Rye Strand by Micromycetes. *Biologija.* **4**: 34-36 (2001).
23. Varnaite, R., Raudoniene, V. and Bridziuviene, D., Enzymatic Biodegradation of Lignin-Cellulose Complex in Plant Origin Material. *Materials Science.* **17**: 99-103 (2011).
24. Waksman, S. A., A Method of Counting the Number of Fungi in the Soil. *J. Bacteriol.* **7**: 339-341 (1922).
25. Yoshida, S., Yonehara, S., Minami, S., Ha, H., Iwahara, K., Watanabe, T., Honda, Y. and Kuwahara, M., Production and characterisation of Ligninolytic Enzymes by *Bjerkandera adusta* grown on wood meal / wheat bran culture and production of these enzymes using a rotary solid fermenter. *Mycoscience.* **37**: 417-425 (1996).