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Studies on biodiversity of leaf litter fungi of Central Luzon State University and evaluation of their enzyme producing ability

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Abstract

Fungi are essential part of the ecosystem because they play an important role in the decomposition of organic materials such as plant residues. Fungi feed on dead organic matter and return the nutrients back into the soil. However, the occurrence of fungi growing on leaf litters which are responsible for decomposition are not yet fully studied and documented. Thus, in this study the different species of fungi present in leaf litters of three species of forest trees namely, rain tree (*Samanea*), orchid tree (*Clitorea*) and paper tree (*Gmelina*) were isolated and identified. These fungal species could be potentially used to hasten the decomposition of enormous leaf litters of forest trees. The isolated fungal species were screened for their ability to produce cellulase. A total of 30 species of phyllosphere fungi were isolated namely: *Absidia* sp., *Aspergillus flavus, A. fumigatus, A. japonicus, A. niger, A. niveus, A. tamarii, Colletotrichum gloeosporioides, Curvularia lunata, Eurotium repens, Fusarium acuminatum, F. semitectum,* three unidentified species of *Fusarium, Monascus ruber, Mucor piriformis, Neosartorya fischeri, Penicillium chrysogenum, P. citrinum, P. decumbens, P. hirsutum, P. implicatum, P. olsonii, P. oxalicum, P. purpurogenum*, two unidentified species of *Penicillium, Rhizopus microsporus* and *Trichoderma hamatum.* Out of 30 species of phyllosphere fungi isolated, 22 can degrade cellulose as shown by the formation of clear zone around the colony of the organism. The five species of fungi that produced the largest clear zone were *P. citrinum, P. olsonii, P. purpurogenum, A. niveus* and *P. chrysogenum.*

Key words – carboxymethylcellulose – cellulase – CLSU – phyllosphere

Introduction

Leaf litters are major component at the top layer of natural soils caused by fallen leaves. Leaves are chemically composed of lignocellulosic substances that have to be broken down by microorganisms to maintain the carbon cycle (Hammel 1997). Phylloplane microorganisms are chemoorganotrophic species requiring organic nutrients for growth (Dickinson 2012). Fungi play a central role in leaf litter decomposition through nutrient cycling and humus formation in soil because they colonize the lignocellulose matrix in litter that other organisms are unable to decompose. It has been described that cellulase is an adaptive enzyme in most fungi capable of degrading lignocellulosic materials and the most common carbohydrate on earth having a wide range of applications (Swift et al. 1979, Kjøller &

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Struwe 1982, Cooke & Rayner 1984) Microbial enzymes, involved in the degradation and transformation of plant cell-wall polysaccharides, have found many biotechnological applications (De vries & Visser 2001). Since these polysaccharides are widely used in various industries such as food and dairy, pulp and paper, textile, animal feed, pharmaceutical, detergent, cosmetic, and chemical-synthesis processes, development of enzyme systems that break down the polysaccharides has been looked for. Thus, fungi present in leaf litters of three species of forest trees namely rain tree (*Samanea*), orchid tree (*Clitorea*) and paper tree (*Gmelina*) present in Central Luzon State University (CLSU) campus were isolated and identified. These species of forest trees are abundant in the campus and the major contributors of the fallen leaf litters. Isolated phyllosphere fungi were further evaluated for their cellulose degrading ability.

Materials & Methods

Collection of leaf litters

Fallen leaves of the three species of forest trees namely rain tree, paper tree and orchid tree were randomly collected from each leaf litter at different points of sampling site at CLSU. The collected leaf litters were air dried inside an air conditioned room. The air dried leaf litters for each species were mixed thoroughly and pulverized using miller. One hundred (100) grams of each species served as the composite sample.

Serial dilution and plating

Ten (10) grams of each leaf litter from the pulverized samples was transferred to a flask containing 90 ml sterile water to obtain 10^{-1} dilution. The flask was sealed tightly and shaken vigorously for 5 minutes. One ml from the 10^{-1} dilution was added to a 9 ml sterile water to obtain 10^{-2} dilution. The same procedure was done for 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} dilutions. One ml of the aliquot from each of the dilution was taken from the homogenized suspension and transferred using a pipettor into sterile Petri dishes poured with potato dextrose agar (PDA) supplemented with streptomycin sulfate. The plates were incubated for seven days at room temperature.

Purification

Three–point inoculation of the isolated fungal organisms was done in previously plated PDA. The plates were incubated at room temperature until profuse growth of fungi was seen on the petri dishes. It was considered pure if only one colony was seen growing in the medium.

Identification of fungi

Identification of the fungal isolates was done at the Laboratory Services Division (LSD), Philippine Center for Postharvest Development and Mechanization (PhilMech), CLSU Compound, Science City of Munoz, Nueva Ecija. The isolates were identified based on their cultural and morphological characteristics.

Evaluation for cellulose degrading ability

Each isolated leaf litter fungi were inoculated using three–point inoculation method in Carboxymethylcellulose (CMC) agar medium (Hankin & Anagnostakis 1975) and incubated for five days at room temperature. The CMC agar media is composed of 0.1 g of yeast extract, 0.5 g of peptone and 16 g of agar and dissolved in 1000 mL distilled water supplemented with 0.5% Na–Carboxymethylcellulose then boiled until homogenous mixture was attained. After five days of incubation, the plates were flooded with 0.2% congo red solution for 15 minutes and rinsed with 1 M NaCl solution for 15 minutes (Hankin & Anagnostakis 1975). The ability of the fungi to degrade cellulose was evaluated by measuring the clear zone around colonies on CMC agar (Patel et al. 2013, Waing et al. 2015).

Results

The fungal species isolated from the leaf litters of the three species of forest trees are presented in Table 1. Thirty species of phyllosphere fungi were isolated from the three species of forest trees. Out of this number, 18 species of phyllosphere fungi were isolated from the leaf litters of rain tree, and 15 species each from orchid tree and paper tree.

Table 1 Isolated species of fungi from the different species of forest trees.

Species	Rain Tree	Orchid Tree	Paper Tree
Absidia sp.	–	+	–
Aspergillus flavus	+	+	+
A. fumigatus	+	+	–
A. japonicus	+	+	–
A. niger	+	+	+
A. niveus	+	–	–
A. tamarii	+	–	–
Colletotrichum gloeosporioides	+	+	+
Curvularia lunata	+	–	–
Eurotium repens	–	+	–
Fusarium acuminatum	+	–	–
F. semitectum	+	–	–
Fusarium sp. 1	–	–	+
Fusarium sp. 2	–	–	+
Fusarium sp. 3	–	+	–
Monascus ruber	+	+	+
Mucor piriformis	–	–	+
Neosartorya fischeri	–	–	+
Penicillium chrysogenum	–	–	+
P. citrinum	+	+	+
P. decumbens	+	–	+
P. hirsutum	–	+	–
P. implicatum	–	–	+
P. olsonii	+	–	+
P. oxalicum	–	+	–
P. purpurogenum	+	–	+
Penicillium sp.1	+	+	–
Penicillium sp. 2	+	–	–
Rhizopus microsporus	+	+	+
Trichoderma hamatum	–	+	–

Legend: (+) present (-) absent

A large number of microorganisms are capable of degrading cellulose. Clear zone around the colony of fungal species indicated its ability to degrade cellulose. The diameter of clear zone produced by different fungal isolates is presented in Table 2. Out of the 30 species evaluated, 22 species can degrade cellulose as indicated by clear zone around the colony of the organism (Figure 1). The five species of fungi that produced the largest clear zone were *Penicillium citrinum, P. olsonii, P. purpurogenum, Aspergillus niveus* and *P. chrysogenum.* Nine species of *Penicillium*, five species of *Aspergillus*, five species of *Fusarium* and one species of *Absidia*, *Colletotrichum* and *Neosartorya* can degrade cellulose. Statistical analysis revealed that the diameter of clear zone differed significantly among species. This result indicates that the different species have different ability to degrade the cellulose.

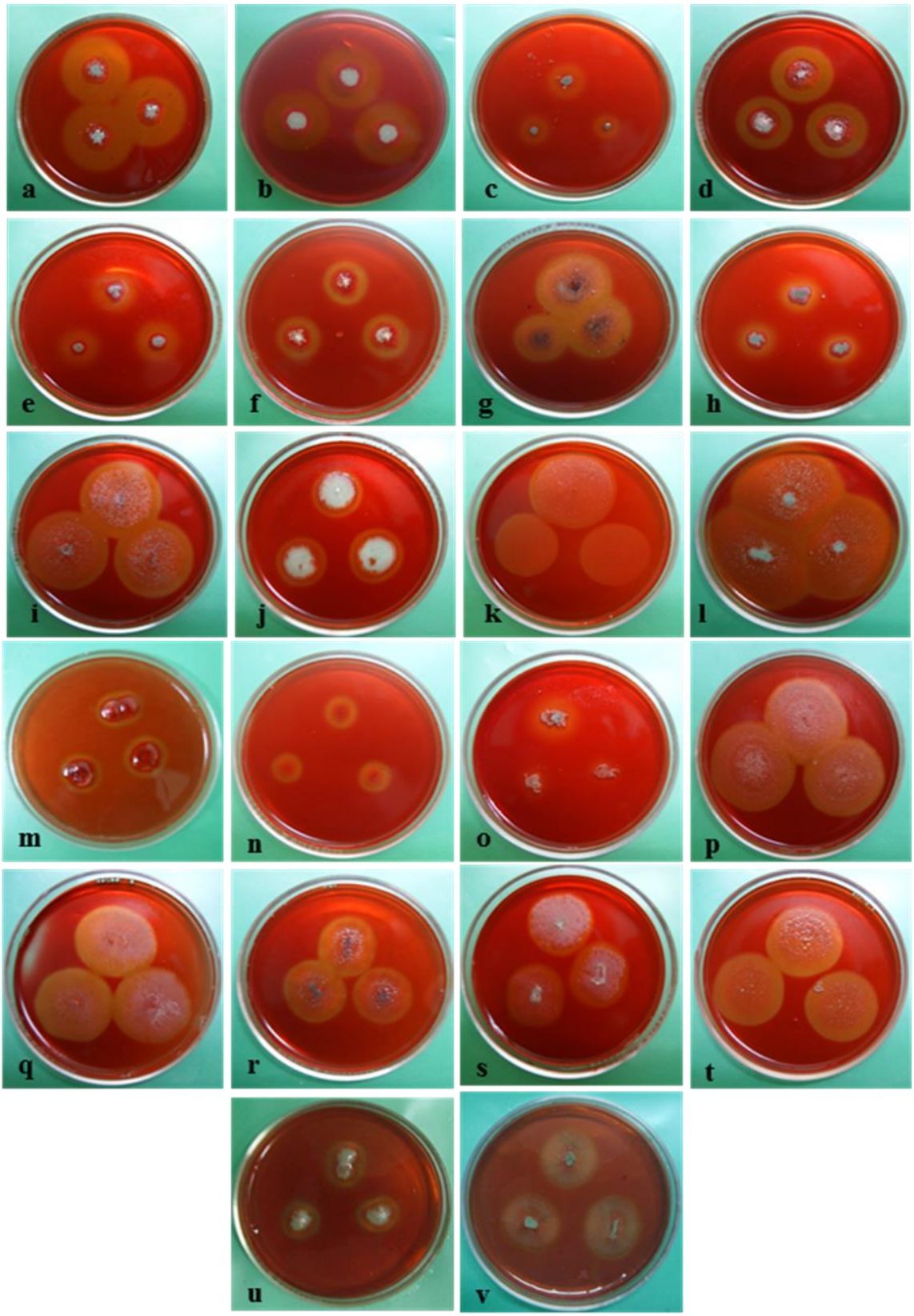

Fig. 1 – Clear zones produced by different isolated leaf litter fungi: *Penicillium citrinum* (a), *P. olsonii* (b), *P. purpurogenum* (c), *Aspergillus niveus* (d), *P. chrysogenum* (e), *Neosartorya fischeri* (f), *P. oxalicum* (g), *A. niger* (h), *P. implicatum* (i), *Penicillium* sp. 1 (j), *Fusarium semitectum* (k), *A. fumigatus* (l), *F. acuminatum* (m), *Fusarium* sp. 2 (n), *P. hirsutum* (o), *Colletotrichum gloeosporioides* (p), *Fusarium* sp. 3 (q), *A. japonicus* (r), *Absidia* sp. (s), *Fusarium* sp. 1 (t), *P. decumbens* (u) and *A. flavus* (v).

Table 2 Cellulase activity of the different isolated leaf litter fungi after five days of incubation.

Fungi	Diameter of Clear Zone (mm)
Penicillium citrinum	21.20[a]
P. olsonii	18.03[b]
P. purpurogenum	11.44[c]
Aspergillus niveus	11.07[c]
P. chrysogenum	10.20[c]
Neosartorya fischeri	7.38[d]
P. oxalicum	7.28[d]
A. niger	7.16[d]
P. implicatum	7.02[d]
Penicillium sp. 1	6.04[de]
Fusarium semitectum	5.96[de]
A. fumigatus	4.59[ef]
F. acuminatum	3.96[efg]
Fusarium sp. 2	3.84[efg]
P. hirsutum	3.60[fg]
Colletotrichum gloeosporioides	3.29[fg]
Fusarium sp. 3	2.88[fg]
A. japonicus	2.77[fg]
Absidia sp.	2.55[fg]
Fusarium sp. 1	2.37[fg]
P. decumbens	2.10[gh]
A. flavus	1.91[gh]
A. tamarii	0.00[h]
Curvularia lunata	0.00[h]
Eurotium repens	0.00[h]
Monascus ruber	0.00[h]
Mucor piriformis	0.00[h]
Penicillium sp. 2	0.00[h]
Rhizopus microsporus	0.00[h]
Trichoderma hamatum	0.00[h]

Means with the same letter superscript are not significantly different at P>0.05.
Mean values represent the clear zone produced by isolated leaf litter fungus with three replications.

Discussion

Leaf litters are major contributor to waste generated because many forest trees like rain tree, paper tree and orchid tree are present in the campus. Fallen leaves from these forest trees are being decomposed by a variety of microorganisms. In view of this, the study was conducted to isolate species of fungi from leaf litter that can be used to hasten the decomposition process. Thirty species were identified namely: *Absidia* sp., *Aspergillus flavus, A. fumigatus, A. japonicus, A. niger, A. niveus, A. tamarii, Colletotrichum gloeosporioides, Curvularia lunata, Eurotium repens, Fusarium acuminatum, F. semitectum,* another three species of *Fusarium, Monascus ruber, Mucor piriformis, Neosartorya fischeri, Penicillium chrysogenum, P. citrinum, P. decumbens, P. hirsutum, P. implicatum, P. olsonii, P. oxalicum, P. purpurogenum,* another two species of *Penicillium, Rhizopus microsporus* and *Trichoderma hamatum.*

Many fungal species have been reported to be associated with decomposition of organic matter such as plant residues. For instance, Kannangara and Deshappriya (2005) reported that *Acremonium,*

Alternaria, Aspergillus, Botrytis, Broomella, Cladosporium, Curvularia, Cylindrocarpon, Fusarium, Penicillium, Rhizopus and *Trichoderma* species were associated with decomposition of *Michelia* and *Semercarpus* leaves. Moreover, Manoch et al. (2008) identified 29 species from various fallen leaves of bamboo, jack fruit, lan thom, banana, Kaffir lime (ma-krut), star gooseberry, Pra du, rose apple and Java plum (wha). These were *Arthrinium phaeospermum, Bipolaris maydis, Beltrania rhombica, Chaetospermum camelliae, Cladosporium cladosporioides, Colletotrichum capsici, Corynespora* sp., *Curvularia eragrostidis, Cylindrocladium* sp., *Ellisiopsis gallesiae, Fusarium semitectum, Gilmaniella humicola, Gyrothrix* sp., *Helicomyces* sp., *Lophodermium* sp., *Memnoniella echinata, Myrothecium verrucaria, Nigrospora sphaerica, Periconia digitata, Pestalotiopsis guepinii, Pithomyces* sp., *Pseudorobillarda* sp., *Stachybotrys nephrospora, S. kampalensis, Tetraploa aristata, Torula herbarum, Volutella concentrica* and *Wiesneriomyces javanicus*.

In the present study, out of the 30 species of fungi isolated, 18 species were isolated from the leaf litters of rain tree, 15 species from orchid tree and 15 species from paper tree. The difference in the number of species isolated may be due to the substratum, nutrients and succession of other organism that support their growth. Fungal communities on different niche comprising integral fungal communities will gradually change with the succession of other organism such as plant communities (Suzuki 2002). Likewise, the diversity of saprobic fungi is higher in areas with higher host diversity and resource abundance (Lodge et al. 1995). Fungi depend entirely on other organisms such as animals and plants for substrata for their growth. Host preferences among microfungi and ascomycetes that decompose leaf litter are common but usually involve differences so in this case their diversity may be loosely correlated with species richness of host trees (Lodge 1997).

The identified fungal isolates were evaluated for their ability to produce cellulase. Cellulase is a group of hydrolytic enzymes acting together in degrading cellulose. This enzyme actually plays a very important role in natural biodegradation process. Fungi are well-known agents of decomposition of organic matter (Lynd et al. 2002) and are very good source of enzymes capable of degrading natural polymeric compounds such as cellulose. Based on the results of our study, 22 fungal species were capable of degrading cellulose namely: *Penicillium* namely *P. citrinum, P. olsonii, P. purpurogenum, P. chrysogenum, P. oxalicum, P. implicatum, Penicillium* sp. 1, *P. hirsutum* and *P. decumbens.* Among the different species evaluated, *Penicillium citrinum* (21.20 mm), *P. olsonii* (18.03 mm), *P. purpurogenum* (11.44 mm) produced the largest diameter of clear zones. *Penicillium* strains have the ability to produce extracellular enzymes and metabolize hydrocarbons and have the capacity to produce cellulase, mannanase and pectinase (Leitão 2009). Likewise, different *Aspergillus* species such as *A. niveus, A. niger, A. fumigatus, A. japonicus* and *A. flavus* are also cellulose degraders. In the study of Shahriarinour et al. (2011), active cellulase producers were selected from isolates of genus *Aspergillus. Neosartorya fischeri* was also reported to possess cellulase activity. Ugwuanyi and Obeta (1999) disclosed that *N. fischeri* had the ability to produce cellulase which supported the result of this study. Same is true with the species of *Fusarium semitectum, F. acuminatum,* and *Fusarium* sp. 1, 2 and 3. Bowen and Harper (1988) reported that *Fusarium* species were one of the most frequently isolated cellulose-decomposers during the autumn and early spring in all treatments on the decomposition of wheat straw. *Fusarium* species are also potential cellulose degrading fungal pathogens *(*Makeshkumar 2011). *Colletotrichum* sp. was able to grow and produce extracellular cellulolytic activity in a defined medium containing cellulose as the main carbon substrate (Falcon et al. 1995). This finding is congruent with the results of the present study. *Absidia* sp. can degrade cellulose which Lennox et al. (2010) reported that *Absidia* sp. can degrade sawdust which is primarily made up of cellulose, hemicelluloses and lignin. This species was also evaluated by Tan et al. (1986) for extracellular amylase, protease, cellulase and lipase activity and showed limited range of enzyme activities.

The results obtained on the evaluation of cellulose degradation are in conformity with previous reports. For example, Hao et al. (2005) found out that *Alternaria* sp., *Penicillium* sp., *Acremonium* sp., and *Trichoderma* sp., are efficient decomposers of cellulose while *Pestalotiopsis* sp. and *Aspergillus fumigatus* are efficient decomposers of lignocelluloses. Meanwhile, Gautam et al. (2010) reported that 16 out of 20 fungal culture isolates possess cellulose degrading ability. These were species of *Aspergillus, Penicillium, Fusarium, Humicola, Alternaria* and *Torula.* Moreover, Jahangeer et al.

(2005) identified species of cellulolytic fungi which were *Aspergillus*, *Fusarium*, *Alternaria* and *Penicillium.*

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