

LEAF LITTER FUNGI ISOLATED IN BACH MA NATIONAL PARK, VIET NAM

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ABSTRACT

A survey of fungi from leaf litter at Bach Ma National Park in Viet Nam was initiated. Using surface disinfection and moist chamber with single spore isolation methods, 102 isolates were obtained and of those, 70 isolates were selected for further morphological study. They belong to 5 classes, 9 orders, 14 families, 33 genera, and 51 species. Among them, *Ceratosporella deviata*, *Lateriramulosa* sp., *Isthmolongispora ampuliformis*, *Polylobatispora quinquecornuta*, *Scolecobasidium tricladiatum*, *Triglyphium labamense*, *Tricladiella pulvialis*, *Tripospermum myrti*, *Triscelophorus* sp., *Varicosporium elodeaea* are newly recorded in Viet Nam.

Keywords: Bach Ma, Hyphomycetes, leaf litter, taxonomy.

1. INTRODUCTION

Litter decomposition plays an important role in nutrient recycling of forest ecosystems. Animals, bacteria and fungi living in soil are main decomposers. Fungi can decompose plant derived lignin-rich polymers because they are capable of breaking down lignin [1]. Previous studies have investigated fungal diversity in leaf litter in Japan [2], China [3], India [4, 5, 6], Thailand [7, 8], Australia [9] but not yet in Viet Nam. The tropical forests in Viet Nam are characterized by high plant diversity [10] so high fungal diversity is expected in leaf litter. The aim of this study is to shed light on recording the list of fungi on leaf litter collected at Bach Ma National Park in Viet Nam.

2. MATERIALS AND METHODS

2.1. Sampling

Bach Ma National Park is located in the Thua Thien Hue Province. The latitude is 16°05'-16°15' N and the longitude is 107°43'-107°53' E. The temperature ranges from 19-31 °C with a mean of 23-24 °C. Humidity is ranging from 27-57 % (mean: 47 %). The park covers a total area of about 22 031 ha. The plant flora of Bach Ma includes at least 1,400 species. This represents around 19 % of the entire flora of Viet Nam in only 0.07 percent of Viet Nam's total land area. The park is located within the transition zone of northern (Sino-Himalayan, Indo-Burmese) and southern (Malesiana) floras and is regarded to be an important 'Floristic Biodiversity Centre' for Indochina [10].

Freshly decaying leaf litter was collected from forest floor of Bach Ma National Park in November of 2005. They were placed into clean polythene bags for transport to the laboratory.

2.2. Fungal isolation

Fungi were isolated using surface sterilization and incubation in moist chambers. To surface sterilize, leaf pieces were submerged in 70 % ethanol (v/v) for 1 min and surface-sterilized for 1 min in a solution of 0.005 % Aerosol OT (di-iso-octyl sodium sulfosuccinate) (Wako, Japan) (v/v) and again submerged for 1 min in 70 % ethanol (v/v). The leaf pieces were rinsed with sterile distilled water 3 times, transferred to sterile filter paper in Petri dishes (9 cm diam) and dried for 24 h to suppress bacterial growth [7]. Then five leaf pieces were placed on LCA (glucose 0.1 %, KH₂PO₄ 0.1 %, MgSO₄·7H₂O 0.02 %, KCl 0.02 %, NaNO₃ 0.2 %, yeast extract 0.02 %, and agar 1.3 % (w/v) in 9 cm Petri dishes) [11].

For moist chamber and single spore isolation, leaves were washed with distilled water and then placed into a small polythene boxes (10 × 20 cm) containing 50 mL of water. The boxes were connected to an air pump to form aeration chambers. Each aeration chamber contained at least 20 leaf discs and was aerated for two- three days at room temperature (25-28 °C). One- two drops of the resulting conidial suspensions were streaked on LCA and examined under the light microscope. Staurosporoc fungi found on these samples were examined and single spores transferred to new LCA plates. Spore germination was observed weekly for 4 weeks.

2.3. Identification of fungi

Fungi were first identified on the basis of their growth characteristics, morphological characteristics and ontogeny with the consultation of monographs and taxonomic papers [12-19]. Morphological examination was carried out with the stereo and light microscopes (Olympus BX 51, Japan and Axio Scope A, Carl Zeiss).

3. RESULTS AND DISCUSSION

A total of 102 fungal isolates were obtained from 50 leaf litter samples collected at Bach Ma National Park in Viet Nam. Strains that were morphologically similar were discarded leaving 70 isolates, which were selected for further study and identification. These were identified based on morphological observation. The isolates were found to belong to 5 classes (Eurotiomycetes, Dothideomycetes, Sordariomycetes, Orbiliomycetes and Leotiomyces); 9 orders (Capnodiales, Diaporthales, Pleosporales, Eurotiales, Helotiales, Orbiliales, Chaetosphaeriales, Hypocreales, Xylariales); 14 families (Amphisphaeriaceae, Apiosporaceae, Bionectriaceae, Chaetosphaeriaceae, Gnomoniaceae, Glomerellaceae, Helotiaceae, Hypocreaceae, Hyponectriaceae, Nectriaceae, Mycospharalaceae, Orbiliaceae, Pleosporaceae,

Trichocomaceae); 33 genera, and 51 species (Table 1). Morphology and taxonomy of some Vietnamese staurosporid fungi are illustrated in this study.

Table 1. Diversity of leaf litter fungi isolated in Bach Ma National Park, Viet Nam.

Class (cetes)	Order (ales)	Family (aceae)	Species
Incertaesedis (Pucciniomycotina)	Incertaesedis	Incertaesedis	<i>Tritirachium</i> sp.
Dothideomycetes	Pleosporales	Pleosporaceae	<i>Curvularia senegalensis</i> <i>Curvularia eragrostidis</i> <i>Drechslera</i> sp.
Eurotiomycetes	Eurotiales	Trichocomaceae	<i>Talaromyces amestolkae</i> <i>Penicillium herquei</i>
Leotiomycetes	Helotiales	Helotiaceae	<i>Idriella</i> spp. <i>Varicosporium edodes</i> *
Orbiliomycetes	Orbiliales	Incertaesedis Orbiliaceae	<i>Dactylaria</i> sp. <i>Arthrobotrys</i> spp.
Sordariomycetes	Capnodiales	Mycosphaeraceae	<i>Ramichloridium</i> sp.
	Chaetosphaeriales	Chaetosphaeriaceae	<i>Chloridium</i> spp. <i>Chloridium virescens</i>
	Diaporthales	Gnomoniaceae	<i>Cochliobolus kusanoi</i>
	Hypocreales	Bionectriaceae	<i>Clonostachys</i> spp. <i>Fusarium graminearum</i>
		Nectriaceae	<i>Fusarium poae</i> <i>Trichoderma</i> sp. <i>Trichoderma reesei</i> <i>Trichoderma atroviride</i>
		Hypocreaceae	<i>Trichoderma koningiopsis</i> <i>Cylindrocladiella</i> sp. <i>Tricladiella pulvialis</i> *
		Incertaesedis	<i>Myrothecium cinctum</i>
	Xylariales	Hyponectriaceae	<i>Beltraniella portoricensis</i>
		Amphisphaeriaceae	<i>Pestalotiopsis photiniae</i>
	Incertaesedis	Apiosporaceae	<i>Arthrinium sacchari</i>

			<i>Colletotrichum acutatum</i>
		Glomerellaceae	<i>Colletotrichum fructicola</i>
			<i>Ceratosporella deviate</i> *
			<i>Chalara</i> sp.
Incertaesedis	Incertaesedis	Incertaesedis	<i>Condylospora vietnamenensis</i> *
			<i>Isthmolongispora ampulliformis</i> *
			<i>Isthmolongispora intermedia</i> *
			<i>Isthmolongispora</i> spp.*
			<i>Lateriramulosa</i> sp.*
			<i>Bahusakala longispora</i>
			<i>Polylobatispora</i> sp.*
			<i>Polylobatispora quinquecornata</i> *
			<i>Scolecobasidium tricladiatum</i> *
			<i>Triglyphium alabamense</i> *
			<i>Triscelophorus</i> sp.*
			<i>Tripospermum myrti</i> *

*Strain isolated by moist chamber and single spore method.

Taxonomy of some staurosporidic fungi isolated in Viet Nam

***Ceratosporella deviate* Subram. 1957**

(Figure 1A)

Colony grows slowly on LCA medium, immerses in agar, brown to dark brown. Mycelium submerged in medium, composed of smooth, branched, septate, subhyaline to pale yellow colour, 1.5–3.0 µm wide hyphae. Conidiophore absent. Conidia produced direct from hypha, composed one main axis 4–7 septate, 30–52 × 4–6 µm wide and 3–5–(8) arms, constricted at the apex, 3–6 septate, 22–38 µm long, 3.5–5.5 µm wide.

***Lateriramulosa* sp. Matsush. 1971**

(Figure 1B)

Colony grows slowly, immerses in agar, hyaline. Mycelium submerged in medium, composed of smooth, branched, septate, hyaline, 0.5–2.0 µm wide. Conidiophore absent. Conidia consist of one main axis (6–8 µm long × 1.2–1.5 µm wide); two arms oblong, 9–11 µm long, base constrict, 2–2.5 µm wide; and the remaining arm 7–11 µm long × 2–2.5 µm wide, base constrict 1 µm wide.

***Isthmolongispora ampuliformis* (Tubaki) de Hoog & Hennebert 1983**

(Figure 1C)

Colony grows slowly on LCA medium, immerses in agar, hyaline. Mycelium submerged in medium, composed of smooth, branched, septate, hyaline, 0.5–2.0 µm wide. Conidiogenous cells integrated in hyphae, short, simple, cylindrical, thin-walled; denticles. Conidia elongate, hyaline, 2–cell with an isthmus connection at the medium, total 24–42.5 µm long (one basal cell 15–20 × 2–2.5 µm, one ampuliform at the apex 6–10 × 0.7–1 µm).

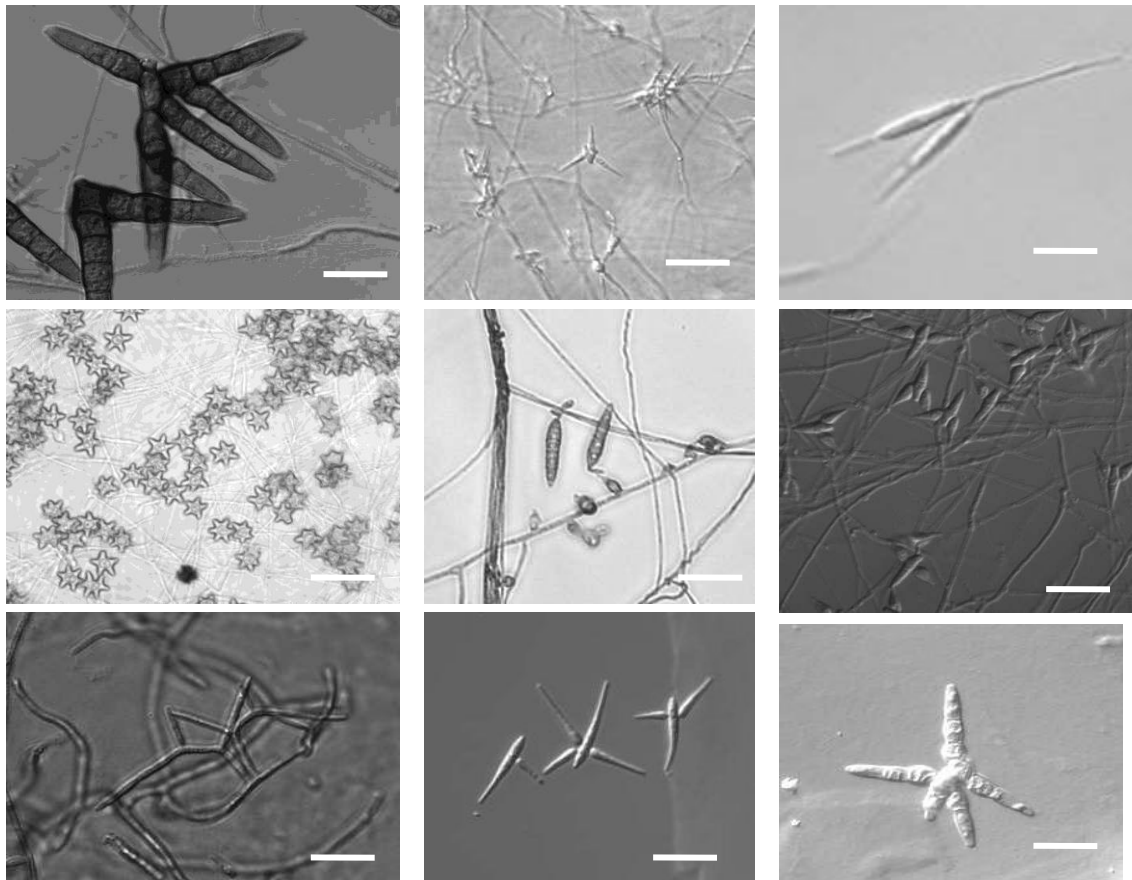


Figure 1. Morphology of some aquatic fungi isolated in Bach Ma National Park, bar = 10 μm .

***Polylobatispora quinquecornuta* Matsush. 1996**

(Figure 1D)

Colonies grow slowly, immerse in agar, dull white to light yellow. Mycelium composed of smooth, branched, septate, hyaline to suhyaline, 0.5–2.0 μm wide hyphae. Conidiophore absent. Conidiogenous cells monoblastic to enteroblastic, 3–20 μm long \times 3.0–4.5 μm wide, hyaline, 1–(3–4) conidia forming from one locus. Conidia continue, solitary, star-shaped, consisting of (4)–5 hyaline, 6–7 μm height, and truncate-conical lobes. The circumscription circle is 11–15 μm .

***Scolecobasidium tricladiatum* Matsush. 1971**

(Figure 1E)

Colonies olive to black, mostly immersed, aerial hyphae sparse. Mycelia develop in and on the medium, compose of branch, septa, hyaline, 2– 3.5 μm wide. Conidiophore absent. Conidiogenous cell growth direct from hypha, oblong and broaden at the middle, constrict at the tip. The broaden part is 5–7 \times 4–5 μm , the constricted part 3–5 \times 0.2–0.3 μm ; conidia develop from this constricted part. Conidia solitary, dry, acropleurogenous, composed of 2 parts, a main axis part and two arms at the tip to make a T-or Y shaped conidia. The main axis is oblong and round at the end, fusiform, pale to mid brown, smooth, echinulate, 0–3 septate, 25–35 \times 5–7 μm .

***Triglyphium alabamense* Matsush. 1981**

(Figure 1F)

Colonies grow slowly, smooth and whitish. Mycelium consisting of smooth, branched, septate, hyaline 1–2 μm wide hyphae. Conidiophore absent. Conidia hyaline, grow directly from hyphae, composting one oblong, 1 septa, 8–10 μm long \times 2–2.5 μm wide main axis and two obovate, strongly curve, continue, base obsolete, none septate, 5–7.5 μm long \times 1.5–2.0 μm wide arms.

***Tricladiaella pulvialis* K. Ando & Tubaki 1984**

(Figure 1G)

Colonies olive to black, mostly immersed, aerial hyphae sparse. Mycelia develop in and on the medium, composed of branch, septa, hyaline, 2.0–3.5 μm wide. Conidiophore absent. Conidia blastospore hyaline or pale olivaceous, smooth, composed of a main axis and 2 arms. Main axis curve, (2)–5–7(–9) septate, 52–99 μm \times 1.8–3.2, 1.0–1.5 μm at base, two short, 0–4 septate, 6.5–32 μm long \times 1.5–3 μm wide arms which develop from different levels of the main axis. The immature conidia easily detach from the vegetative hyphae.

***Triscelophorus* sp. Ingold 1994**

(Figure 1H)

Colonies grow slowly in LCA media, light brown. Mycelium consists of smooth, branched, septate, pale brown 1–2 μm wide hyphae. Conidiophores germinate from hyphae, 3–20 long \times 1.5–2 wide, no septa were found. Conidia composed one main axis, 20–30 μm long \times 3.5–4 μm wide, (1)–2 septate; (0–1)–2–3 oblong, 0–1 septa arms, 12–25 μm long \times 2–2.5 μm wide, base constrict 1.2–2 μm wide.

***Triperspermum myrti* (Lind) S. Hughes 1975**

(Figure 1I)

Colonies olive to black, mostly immersed, aerial hyphae sparse. Mycelium develops in and on the medium, composed of branch, septa, hyaline, 2.0–3.5 μm wide. Conidiophore absent. Conidia arising from hyphae, pale brown to pale olive, consisting of 4 awl-shaped, up to 35 μm long \times 4.4–8 μm wide, 1–3 septate, constricted at septate.

4. CONCLUSION

Two isolation methods we used were applied for the first time to isolate leaf litter fungi in Viet Nam. However, some fungi isolated with the surface disinfection isolation method, such as: *Trichoderma* spp., *Chloridium* spp., *Penicillium* spp., and *Clonostachys* spp. are common in soil in Viet Nam as they were all recorded before in Viet Nam. All fungi isolated by the moist chamber combined with single spore isolation method are new to Viet Nam, they are *Ceratosporella deviata*, *Condylospora vietnamensis*, *Lateriramulosa* sp., *Isthmologispora ampuliformis*, *Polylobatispora quinquecornata*, *Scolecobasidium tricladiatum*, *Tricladiaellapulvialis*, *Triglyphium alabamense*, *Triperspermum myrti*, *Triscelophorus* sp. and *Varicosporium elodeae*. These fungi are usually found in aquatic habitats and most of them were identified as Ingoldia fungi. The results in this study showed that staurosporidic fungi which usually regarded as aquatic habitat fungi are also can be isolated from leaf litter collected in terrestrial habitat.

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