UV light-induced conversion of *Pestalotiopsis microspora* to biotypes with multiple conidial forms

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Pestalotiopsis microspora is one of the most commonly isolated endophytes associated with tropical and semitropical rainforest plants. Taxonomic classification of this fungus is primarily based on conidial morphology. The conidia of this genus generally possess five cells, are borne in acervuli, and possess appendages. It has been possible, via UV irradiation, to convert conidia of *P. microspora* (2-3 apical and 1 basal appendage per conidium) into biotypes that bear a conidial resemblance to other fungi including *Monochaetia* spp., *Seridium* spp. and *Truncatella* spp. Single cell cultures of each of these biotypical biotype fungi retain 100% identity to 5.8s and ITS regions of DNA to the wild type source fungus *P. microspora*, indicating that no UV induced mutation occurred in this region of the genome. Furthermore, the conidia of these UV generated biotypes do remain true to biological form by also producing spore types in their acervuli that are identical to the biotypical culture types from which they were derived. The implications of this study are that many of the genera in this group of fungi are either closely related or identical.

Key words: appendages, biotypes, *Pestalotiopsis*, metabolites, systematics, UV radiation.

Introduction

Pestalotiopsis microspora is one of the most commonly isolated endophytes associated with rainforests (Li *et al.*, 2001). It has been found in a myriad of plant species located on every continent that supports rainforests (Strobel *et al.*, 1996a; Li *et al.*, 2001). This endophytic fungus seems to play several important roles in both protecting its host plant and in recycling nutrients in the ecosystem. Frequently, other fungal genera are also isolated from these plants including *Monochaetia* spp., *Seridium* spp. and *Truncatella* spp. Differentiation of these fungal genera is primarily based on conidial septation and on the number of appendages on the conidium (Sutton, 1980; Nag Raj, 1993). Typically, *P. microspora* has three apical appendages and one basal appendage, but commonly conidia with 2 or 4 apical appendages are

observed. Although all of the fungi mentioned above are similar in many of their conidial, acervular, and mycelial characteristics, they have been named as different species (Nag Raj, 1993). Fungi, in this group of appendage bearing coelomycetes, are of special interest because certain fungi in this group are known to produce the anticancer drugs taxol and torreyanic acid (Strobel *et al.*, 1996 a,b; Lee *et al.*, 1996). Still other biologically active molecules such as ambuic acid and jesterone have recently been isolated and characterized (Li *et al.*, 2001; Li and Strobel, 2001).

One taxol- producing isolate of *P. microspora* (WT#98) was selected for use in UV-light irradiation studies as we attempted to produce biotype clones enhanced in taxol production. Coincidently, this study led to the discovery of the UV-light induced conversion of *P. microspora* to organisms that resemble other appendage bearing fungal genera. Thus, this report presents methodology and results showing that UV irradiation of *P. microspora* spores, which have appendages at both apices, can result in a variety of spore types representative of such fungal genera as *Monochaetia* spp., *Seridium* spp. and *Truncatella* spp.

DNA sequence analyses has also been conducted on the biotypes a number of wild types (apparent counterpart) and authentic genera and conclusions from these analyses have been related to the classical taxonomy of this group of fungi. This report also discusses the importance of this group of fungi as a source of natural products and how an understanding of their possible genetic relatedness may facilitate the discovery of more bioactive novel natural products

Materials and Methods

Fungal strains

The fungal strain used in this experiment was an endophytic isolate of *Pestalotiopsis microspora* [WT#98]. It was isolated from *Cephalotaxus fortunei* by Jia-Yao Li and Gary Strobel, and maintained in the fungal collection in the Strobel lab at Montana State University. *Pestalotiopsis microspora*, isolate Ne 32 is also from the collection. Other fungal strains were either purchased from the American Type Culture Collection (ATCC), Manassa, Virginia or the Centraalbureau voor Schimmelcultures (CBS), Utrecht, The Netherlands. Fungal species considered in this study, including their isolation, original substratum, and geographical origin, are listed (Table 1).

UV irradiation and isolation

WT#98 was cultured on water agar plates with γ-irradiated carnation leaves provided by the *Fusarium* Laboratory, Department of Plant Pathology at Pennsylvania State University. The plates were incubated at 20 C under 12

Table 1. Genera used for sequencing ITS1, 5.8S rDNA and ITS2 DNA regions of various fungi for comparative purposes.

Genera	Strain no.	Original substrate	Origin
Monochaetia camelliae	ATCC# 60625	*Camellia japonica*	New Zealand
Pestalosphaeria hansenii	ATCC48245	*Pinus carribaea*	New Guinea
Pestalotia rhododendri	ATCC24306	*Rhododendron* sp.	-
Pestalotia palmarum	ATCC10085	*Coconut palm*	India
Pestalotiopsis funereroides	CBS175.25	*Juniperus* sp.	-
P. microspora	NE32, MSU isolate	*Taxus wallachiana*	Nepal
P. microspora	WT#98,MSU isolate	*Cephalotaxus fortunei*	China
P. microspora	CBS171.26	-	Italy
P. microspora	CBS364.54	Bath Towel at Florida beach	USA (Florida)
P. neglecta	CBS200.65	*Taxus baccata*	UK
P. thujae	CBS303.75	*Thuja occidentalis*	The Netherlands
Seridium cardinale	ATCC52521	*Juniperus communis*	New Guinea
S. unicorne	ATCC48159	*Cryptomeria japonica*	New Zealand
Truncatella angustata	ATCC96024	*Prunus avium*	USA (Washington)

hour light/12 hour dark /light cycles employing fluorescent light for three weeks to produce acervuli and conidia. The conidia were diluted to a concentration of 10^3/ml with sterile distilled water, and 1 ml was spread on 1/10 × potato dextrose agar (PDA). The conidia were allowed to initiate the germination process for 1-2 hours at room temperature, and then exposed to a short-wave germicidal UV lamp (254 nm, PHILIPS TUV 8W/GB T5) at an 8-cm distance for 4-5 minutes. A pure culture of each germinating spore that was sampled was obtained by cutting single hyphal tips from germinating conidia 24 to 48 hours after exposure to UV light. This critical procedure was done based on previous observations that conidium germination proceeds in *P. microspora* conidium via the basal cell that contains one nucleus (Ford, unpublished). Therefore, each colony arising from that individual hyphal fragment essentially does so under the control of the same nucleus found in the germinating basal conidial cell. In the process of finding variant fungal forms after UV irradiation, some 4,000 single spore cultures were cultured and examined for morphological changes. Of these, 7 cultures having apparent atypical morphologies were picked for more intensive microscopic and molecular studies eg. UV3, UV6 UV9, UV10, UV11, UV12, and UV20.

Microscopic observations

At least three hundred individual conidia produced by WT#98, as well as three hundred conidia from each of the selected clones with altered conidium morphology were observed by laser and scanning electron microscopy. The fungal material was critical point dried, gold sputter coated and examined with

a JEOL 6100 scanning electron microscope (Strobel *et al.*, 1996b). Laser scanning micrographs were taken with a Zeiss confocal microscope equipped with an argon laser with 488 and 514 wavelengths. Results were tabulated as percentage of each group of spores showing common appendage numbers and type as well as the number of cells per conidium.

DNA isolation

For DNA isolation, all fungi were grown in 1.5 ml of potato dextrose broth for 18 to 24 hours at 23 C. The mycelium was harvested by centrifugation and washed twice with sterile double distilled H_2O. Total genomic DNA was extracted by the methods of Lee and Taylor (1990).

Amplification of Internal Transcribed Spacer (ITS) and 5.8S rDNA Sequences

The ITS regions of each fungus were amplified using PCR and the universal ITS primers ITS5 (5'GGA AGT AAA AGT CGT AAC AAG G) and ITS4 (5'TCC TCC CTT ATT GAT ATG C) (White *et al.*, 1990). PCR was performed in a 50 µl reaction containing 0.1 µg genomic DNA, 0.4 µM of each primer, 0.16 mM four dNTPs and 5µg of *Taq* polymerase (Promega) in a buffer of 10 mM tris-HCl (pH 9 at 25 C), 50 mM KCl, 3 mM $MgCl_2$, 0.1% Triton X-100. PCR cycling conditions consisted of denaturation at 94 C for 1.5 min, annealing at 55 C for 2.5 min, and extension at 72 C for 3 min for 40 cycles, with a final extension at 72 C for 10 min (Willits and Sherwood, 1999). The PCR products were purified by gel electrophoresis and desalted using a QuickStep PCR purification kit (Edge Biosystems).

Cycle Sequencing ITS Regions and 5.8S rDNA

Ten to 40ng of PCR product was sequenced using ABI prism BigDye terminator chemistry (Perkin-Elmer) and the primers used for amplification (White *et al.*, 1990). Sequencing conditions were 25 cycles of 96 C for 10 seconds, 50 C for 5 seconds and 60 C for 4 min. Isopropanol precipitation was used to purify extension products (Protocol ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit, 1998). The reactions were resolved on an ABI prism 310 Genetic Analyzer (Perkin-Elmer). Nucleotide sequences were determined on both strands with Sequencer program (Gene Codes Corporation Inc., 1995).

Sequence Analysis

Alignments of ITS regions from the different strains were performed using both the multiple alignment program CLUSTALW (Thomson *et al.*,

Table 2. GenBank accession numbers and size in base pairs of the ITS1, 5.8S and ITS2 sequences for selected taxa of *Amphisphaeriaceae*.

Species	ITS1	5.8S	ITS2	Total	GenBank
Monochaetia camelliae	192	158	166	516	AF377286
Pestalosphaeria hansenii	194	158	166	518	AF377290
Pestalotia rhododendri	194	158	167	519	AF377294
Pestalotia thujae	193	158	168	519	AF377295
Pestalotiopsis funereroides	195	158	168	521	AF377289
Pestalotiopsis microspora (CBS364.54)	142	158	162	462	AF377292
Pestalotiopsis microspora (NE32)	194	158	167	519	AF377288
Pestalotiopsis microspora (WT98)	194	158	165	517	AF377296
Pestalotiopsis neglecta	194	158	166	518	AF377293
Seridium cardinale	195	158	156	509	AF377298
Seridium unicorne	193	158	156	507	AF377299
Truncatella angustata	193	158	153	504	AF377300

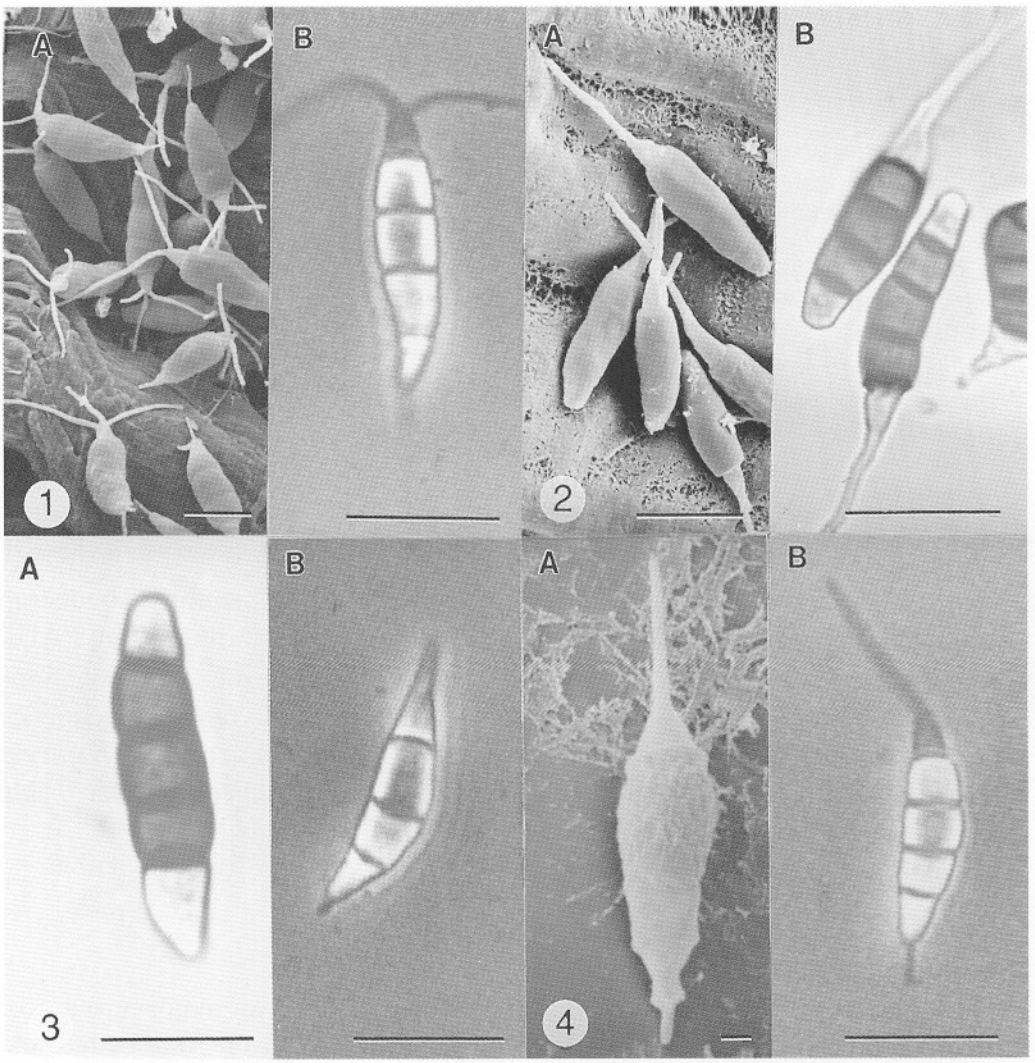
1994) and manual alignment afterward. Maximum parsimony, bootstrap method (Felsenstein, 1985) with heuristic search, were performed using PAUP (Swofford *et al.*, 1999). Bootstrap values were obtained from 100 replications of tree bisection-reconnection branch swapping with random sequence addition. All characters were weighted equally. GenBank accession numbers and size in base pairs of the ITS1, 5.8S and ITS2 sequences are given in Table 2.

Results

Microscopic observations

Microscopic examination of WT #98 revealed two major groups of conidial types. In the first group, 89% of the conidia were five-celled and possessed two apical appendages and one basal appendage, while those in the second group possessed three apical appendages and were otherwise identical to the first group (Table 3, Fig. 1). It was also noted that these two conidial types appeared within any individual acervulus.

In each biotype studied, with one exception (UV10), between 1% and 9% of the conidia had only one appendage (Table 3). Many of these conidia, with 4 septa and one terminal appendage bear similarities in overall structure, but not in exact size measurements to the fungus *Monochaetia kansensis* (Sutton, 1980; UV20 Fig. 2). Similarly, in all other cases with the irradiated fungi, conidia appeared with no appendages as with UV9 and UV12. These conidia usually had 4 septa making the spores having resemblance to *M. saccardiana* (Sutton, 1980, Tables 3 and 4, Fig. 3). Still another group of irradiated fungi,



Figs. 1-4. Scanning and laser light scanning micrographs of WT #98 and UV generated biotype conidia of *P. microspora.* **1A, B.** WT # 98 conidia. **2A, B.** Representative conidia of UV20 having a single terminal and apical appendages. **3A, B.** Conidia of UV6 with no appendages. **4A, B.** Conidia of UV6 having single appendages at each end of the conidia. Please note that comparable conidia were found in UV9 and UV12. Bars: 1-3, 4B = 10 μm; 4A = 1 μm.

specifically UV3, 6, 9, 10, 11, and 12, had representative conidia (between 5% and 35%) bearing a single appendage at each end of the conidium (Table 3). This is quite unlike the wild type in which only 1% of the conidia possessed one apical and one basal appendage (Table 3). Fungal types such as *M. ceratoniae*, *M. monochaeta* and *M. carissae* each possess a single apical and a basal conidial appendage more or less as represented by 35% of the conidia in

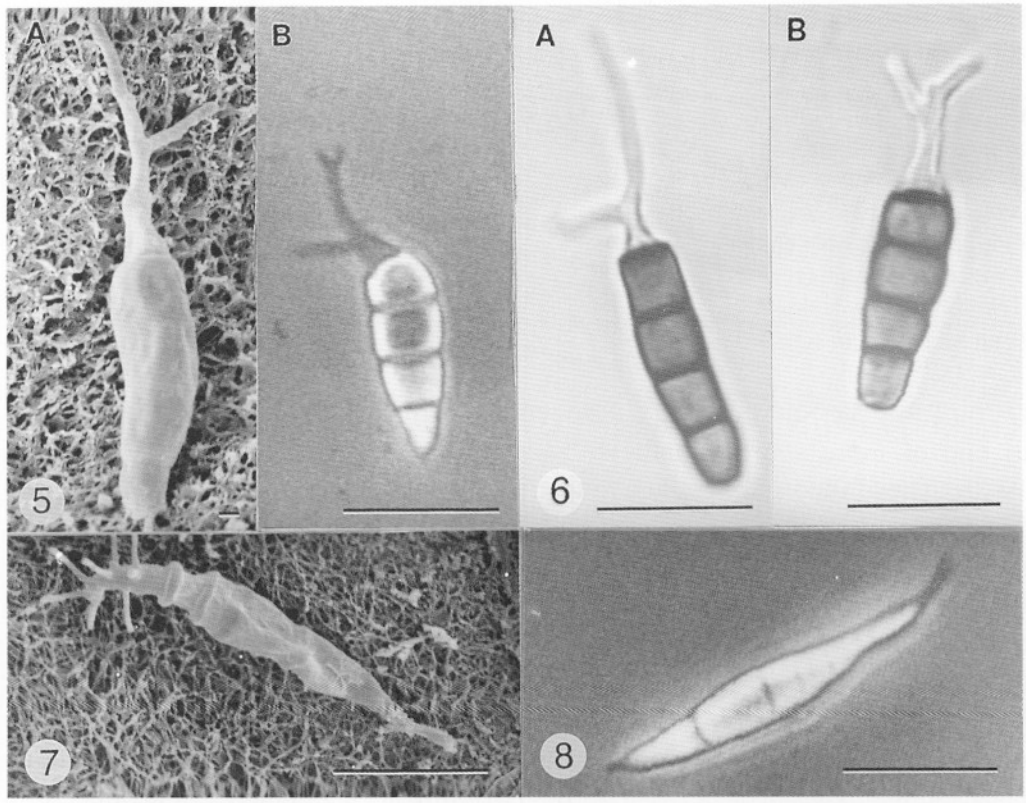
Table 3. Number of different types of appendage bearing conidia derived from *P. microspora* WT#98 and biotypes originating from it by UV-irradiation.

Morphology of conidia	Number of different morphologies of conidia (%)							
	WT	UV3	UV6	UV9	UV10	UV11	UV12	UV20
Terminal appendages none	0	0.5	1	5	0.6	0.5	4	1
At least one appendage	0	1	3	8	0	1	2	9
One appendage each end	1	39	35	33	14	5.0	15.5	21
Two apical appendages	89	53.5	43	41	69.4	37	28.5	58
Triple apical appendages	10	6	18	13	16	56.5	50	11

Total each column 100%

UV6 (Table 3) (Sutton, 1980). Also, certain of conidial types bore resemblance to one or more species in the fungal genus *Seridium* spp. (Fig. 4) (Sutton, 1980). These fungi possessed a single appendage at the terminus of each spore and five septa (Fig. 4). Furthermore, some spore types resembling *Truncatella* spp. appeared. For instance, 20% of the conidia of UV12 were *Truncatella*-like, that is, four-celled conidia with distorted or branched, apical appendages (Tables 3 and 4) (Sutton, 1980). In still another example, conidia appeared with two apical appendages either normal, relative to the wild type (UV10 as in Fig. 5) or abnormal in length as in UV21 in Fig. 6. These examples are comparable to the number of apical appendages in *P. microspora*, but these biotypes had no basal appendage. The presence of two apical appendages and no basal appendage is characteristic of conidia in both *M. karstenii* and *M. nattrassii* (Sutton, 1980). Furthermore, especially with biotype line UV12, enormous variation in cell numbers in the conidia was noted, ranging from five cells to as little as one (Table 4). Finally, other variants were noted that seem to have no resemblance to any previously described fungal species. These include a form with multiple apical appendages that are extremely shortened (Fig. 7) and one that has shortened appendages on a spore that is extremely narrow in width compared to its length (Fig. 8).

A number of other observations were made on the morphology of the spores in the UV radiation experiments. For instance, in some fungal biotypes, the conidia possessed no cytoplasm as in UV9 and UV12 (Table 4). Also, quite unexpectedly, one biotype line (UV11) consistently produced more apical appendages than the wild type. In this case, UV11 notably had over 56% as triple apical appendages vs the wild type that had only 10% (Table 3). The second observed change of biotype conidia was in the number of cells per conidium. *Pestalotiopsis* sp. normally produces conidia with five cells, but some of UV irradiated biotypes possessed more or less than five cells, such as UV3, UV9 and UV12 (Table 4). Single celled conidia were found in UV9 and UV12 at 0.5% and 3%, respectively. In UV9, 15% of the conidia were two-



Figs. 5-8. Scanning and laser light scanning micrographs of WT #98 and UV generated biotype conidia of *P. microspora.* **5A, B.** Conidia of UV10 with two normal appearing apical appendages, but no basal appendage. **6A, B.** Conidia of UV21 with two abnormal appearing apical appendages, but having no basal appendage. **7.** An abnormal conidium with multiple apical appendages that was found only once in UV20. **8.** A peculiarly narrow conidium with one terminal appendage, found only once in UV12. Bars: 5B-8 = 10 μm; 5A = 1 μm.

celled, whereas 20% of the UV12 conidia were four-celled. Finally, 2.5% of the conidia produced by UV3 were six-celled.

It is important to note that each of these UV biotypes, after subculturing, retains its phenotypic identity relative to spore morphology and conidial cell number within individual acervuli in fungal cultures indicating its relative genetic stability. Thus, there is no sorting out of individual biotypes at the point of acervular formation. Furthermore, as expected, cultures of representative single spore isolates of the biotypes also retained their respective distribution of conidial morphologies (Tables 3 and 4). This experiment was routinely conducted on each biotypical fungus from which cultures derived from single spores were placed on carnation leaves on the surface of water agar and incubated for 3-4 weeks. Then, acervuli were dissected from the carnation

Table 4. Number of cells per conidium (%) in the WT #98 and the biotype cultures derived therefrom.

Five cells (normal)	100	91	99	78	96	100	56	98
Number of cells in each conidium	WT	UV3	UV6	UV9	UV10	UV11	UV12	UV20
Single cell	-	-	-	5	-	-	3	-
Two cells	-	1	1	15	-	-	8	1
Three cells	-	1	-	-	-	-	-	-
Four cells	-	1.5	-	-	-	-	20	-
Five celled distorted	-	3	-	-	3	-	3	-
Six and more than six cells	-	2.5	-	-	1	-	-	-
Spores with no cytoplasm	-	-	-	2	-	-	10	1

(-) denotes that there were no observed spores with the number of cells in this group.

Table 5. Base differences in the nuclear ITS regions and 5.8S rDNA reported between species pairs and genera pairs.

Taxon pairs	Total	No. of different basepairs	Percentage differences
P. microspora WT#98 vs. biotypes	549	0	0
P. microspora WT#98 vs. *Monochaetia camelliae*	504	13	2.5
P. microspora WT#98 vs. *Pestalosphaeria hansenii*	505	13	2.5
P. microspora WT#98 vs. *Pestalotia rhododendri*	506	14	2.7
P. microspora WT#98 vs. *Pestalotiopsis funereroides*	497	16	3.2
P. microspora WT#98 vs. *P. microspora* (NE32)	505	13	2.5
P. microspora WT#98 vs. *P. microspora* (CBS 171.26)	507	25	4.9
P. microspora WT#98 vs. *P. microspora* (CBS 364.54)	509	103	20.2
P. microspora WT#98 vs. *P. neglecta*	504	11	2.1
P. microspora WT#98 vs. *P. thujae*	506	6	1.2
P. microspora WT#98 vs. *Seridium cardinale*	525	90	17.1
P. microspora WT#98 vs. *S. unicorne*	523	85	16.2
P. microspora WT#98 vs. *Truncatella angustata*	514	77	13.6

leaves and observations made by light microscopy on the morphology of individual spores.

DNA sequence analysis

Molecular data were derived from the sequence analysis of ITS 1 and 2 regions and 5.8S rRNA in *P. microspora* # 98, each biotype, and other taxonomically related fungi (Table 5). The results showed that there was 100% sequence identity in each of these regions among WT#98 and biotypes derived

from it: UV3, 6, 9, 10, 11, 12 and 20. In contrast, different isolates of *P. microspora* revealed 1-5% sequence differences to isolate WT # 98 (Table 5). *Pestalotiopsis microspora* strain (CBS 364.54) showed a 20.2% sequence difference to isolate WT #98. The sequences of strains of *M. camelliae* and *T. angustata* differed from WT#98 by 2.5% and 13.6%, respectively.

Sequence analysis of the DNA of various isolates of *P. microspora* by themselves revealed 1-5% differences in the ITS region and 5.8 r DNA (Table 5). Wild type isolates of *M. camelliae* revealed 2.5% base sequence differences in the ITS region and rDNA. On the other hand, there was 100% sequence identity in both of these regions in the *Monochaetia* -like and *Truncatella* -like UV generated isolates obtained after irradiation of *P. microspora* cultures.

Phylogenetic tree

The inferred bootstrap phylogenetic tree was derived from sequences of the ITS1 and 2 or 5.8S rDNA regions of 16 taxa and preceded by a maximum parsimony heuristic search with 100 replications. All 530 characters were weighed equally. Characters (339) were constant and 159 characters are parsimony-informative. The wild type fungi *Monochaetia camelliae*, *Seridium* sp. and *Truncatella angustata* revealed base sequence differences from *P. microspora* # 98 at 2.5%, 13.6% and 16-17%, respectively in the complete sequences of the ITS1 and 2 and 5.8S rDNA (Table 5).

Discussion

Traditionally, according to conventional taxonomy, the appendage bearing coelomycetous fungi have been classified on the basis of the size, cell number and most importantly, the number and location of appendages on the conidia (Sutton, 1980). However, in all authentic *Pestalotiopsis* spp. some variation in conidial morphology, especially the appendage number and length, is usually noted (Sutton, 1980). This suggests that multiple genes may be controlling these morphological aspects of the phenotypic expression in the spore. For instance, in the case of WT# 98, commonly spores appear with either two or three appendages and in rare cases, only one (Table 3). After exposure of the wild type fungus to UV radiation, multiple phenotypic conidial forms were produced (Tables 3 and 4). Each of these, upon subculturing, exactly replicated the original conidial biotype. The multiple conidial forms of any biotype may possibly be explained on the basis of the interruption of signalling genes that may be controlling appendage development in the conidia. Deletion of any one or more of these genes may affect the entire processes in appendage development. There is no question that this wide ranging conidial variation in each biotype was due to the genome of only one nucleus since each biotype originated from a hypha known to carry only one

nucleus. Quite unexpectedly, however, some of the conidia arising in the various UV irradiated fungi perfectly resembled species of *Monochaetia*, *Seridium* and *Truncatella* that are appendage bearing fungal genera belonging to the coelomycetes.

Theoretically, it is possible to conceive that one of these fungi gave rise to all of the other fungal genera mentioned above. Mutations may have occurred in the fungus that eventually gave rise to one or more fungal biotypes that eventually stabilized into a biotypical form that could be recognized at the genus level. Some support for this conclusion comes from the molecular biology study of the fungi in this report. For instance, the wild types of *Monochaetia camelliae*, *Seiridium* sp. and *Truncatella angustata* revealed base sequenced differences from WT#98 at 2.58%, 13.62% and 16-17%, respectively in the complete sequences of the ITS1and2 and 5.8S rDNA (Table 5). An examination of the phylogenetic tree (Fig. 9), using ITS and 5.8S rDNA supports the conclusion that *Pestalosphaeria* sp., *Pestalotia* sp., *Pestalotiopsis* sp., *Monochaetia* sp., *Truncatella* sp. and *Seiridium* sp. evolved from a common ancestor.

The high percentage of amenity among sequences of these fungi is supportive of the very early claims of Christensen (1932). Namely, he obtained monoconidial cultures isolated from the acervuli of *Pestalotia* sp. on *Pinus pavulstris* Mill. Eventually he observed that these cultures produced spore types that conformed to the characters of both *Pestalotia* and *Monochaetia*. He also concluded that the salient characters used to distinguish the two genera are not entirely dependable and that the genus *Monochaetia* could not be maintained distinctively from *Pestalotia*. In addition, in the classification of Guba (1961), *Pestalotiopsis* and *Truncatella* were included in the same genus as *Pestalotia*. Guba (1961) also noted that the Fungi Imperfecti are extremely variable in morphological characters under different conditions in nature. Nevertheless, to make stronger conclusions about the taxonomic identity or non-identity of these fungal genera, more molecular data is needed from more species of these fungi.

As a side note, its is interesting that the similarity of the ITS regions and 5.8S rDNA genes among several fungal species also provides additional support for the integration of the anamorphs *Pestalotiopsis microspora* (NE32), *Pestalotia rhododendri* and the teleomorph *Pestalosphaeria hansenii* (Metz *et al.*, 2000) (Table 5). The identity of the ITS regions and 5.8S rDNA between anamorph and teleomorph in this study is another example of research that has demonstrated that nucleotide data are a useful tool for synanamorph systematics (Kuhls *et al.*, 1996; Arenal *et al.*, 2000). The sequence strongly confirms that these three species are likely the same fungus. This coincidence

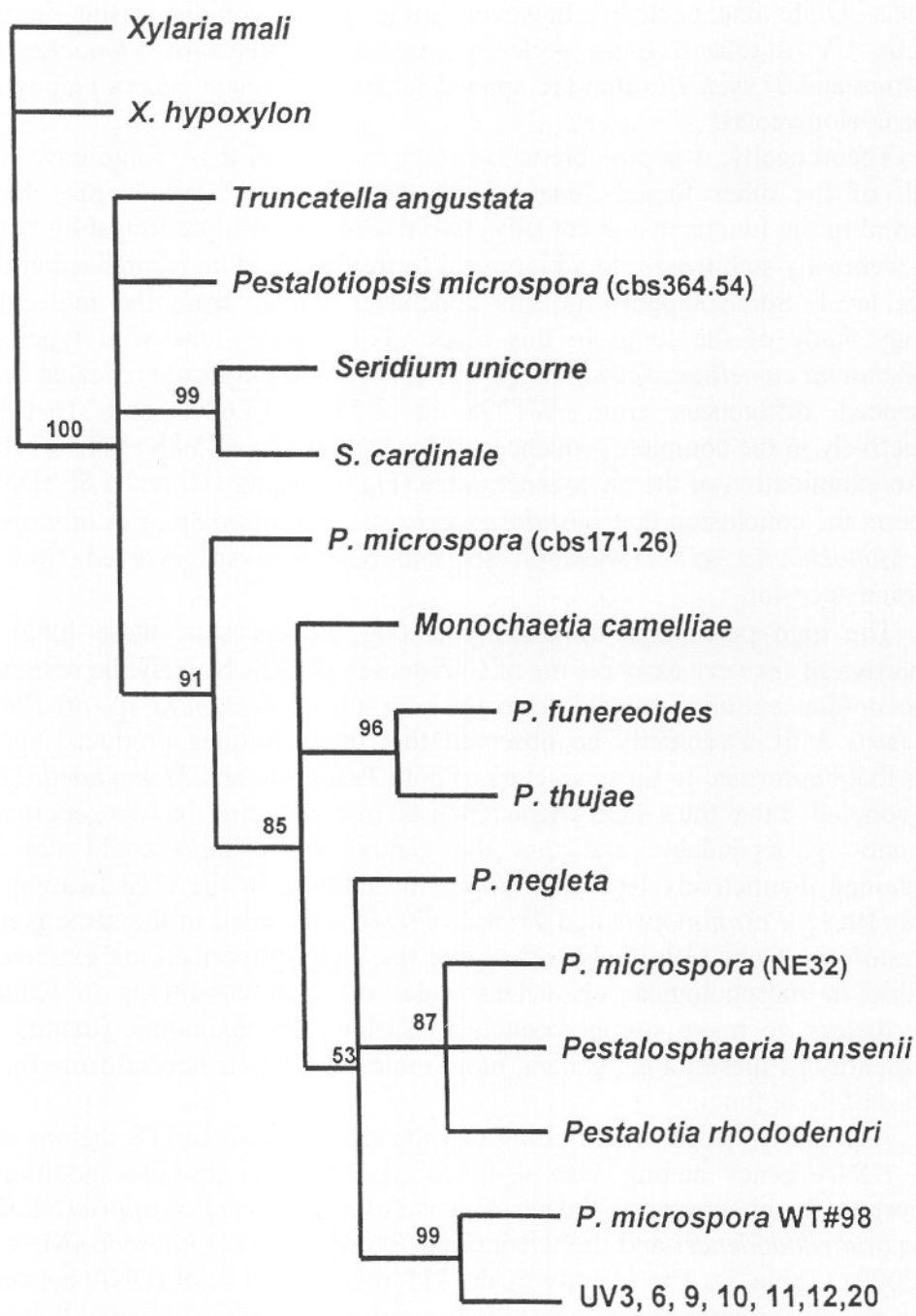


Fig. 9. Maximum parsimony phenogram of ITS and 5.8S rDNA regions of *Pestalotiopsis* sp. and related genera. *Xylaria mali* and *X. hypoxylon* were reference taxa. Bootstrap confidence measures greater than 50% from 100 replications are indicated at internodes.

shows that the molecular phylogeny study of *Pestalotiopsis* sp. and related genera can be applied and compared with the older classification schemes that are primarily based on morphological characteristics. Thus, affirming that molecular phylogeny is a useful method to help clarify the outcomes of earlier fungal taxonomic schemes.

It is possible to generate morphological variants of *Pestalotiopsis microspora* via UV radiation. This taxonomic study is of special interest since several wild types strains of *P. microspora* and related species produce the anticancer drug taxol. Thus, UV irradiation may have the potential to yield fungal strains with the capacity for increased yields of this drug. It has been noted that some of biotypes produce and accumulate not only greater quantities of taxol but also some new secondary metabolites differing from those of the WT # 98 (Ford, unpublished).

If *Monochaetia* and other fungal species such as *Seridium* and *Truncatella* are genetically related to *Pestalotiopsis*, which this work does conclude, then these organisms should be as carefully examined for bioactive secondary metabolites. Various isolates of *P. microspora* produce the anticancer drugs taxol and torreyanic acid (Lee *et al.*, 1996; Strobel *et al.*, 1996a,b). Some isolates of the fungus produce great quantities of only a few metabolites such as jesterone, or terrein, or others (Li and Strobel, 2001; Harper *et al.*, 2000). Other compounds including pestalotiopsin A and B, 2-α-hydroxydimeniol, pestaloside and pestalopyrone are also produced by isolates of this fungus (Lee *et al.*, 1995; Pulici *et al.*, 1996a,b). Most recently, a novel bioactive organic acid named ambuic acid has been described from *Pestalotiopsis* spp. obtained from representative rainforests on 4 continents (Li *et al.*, 2001). Interestingly, this novel acid was also found in *Monochaetia* sp. that was originally isolated as an endophyte from *Taxus wallichiana* in the Himalayan foothills of Nepal. This biochemical observation seems to lend some biochemical support to the genetic relatedness of these two fungal species outlined in this report.

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