Profiles from Biolog FF plates and morphological characteristics support the recognition of *Oidiodendron fimicola* sp. nov.

Adrianne V. Rice* and Randolph S. Currah

*Department of Biological Sciences, University of Alberta, Edmonton, AB T6G 2E9 Canada***

**Correspondence*: Adrianne V. Rice, arice@NRCan.gc.ca

Abstract: *Oidiodendron fimicola* sp. nov., represented by two collections from mushroom compost, is distinct from other species in the genus in having scaly conidiophores and asperulate, hyaline to melanized, barrel-shaped to irregular conidia. By light microscopy, it most closely resembles *Oidiodendron flavum*; by scanning electron microscopy the two species can be clearly distinguished based on perispore morphology and on the formation of distinctive scale-like protrusions on conidiophores of *O. fimicola*. We were unable to obtain DNA from *O. fimicola* despite repeated attempts. As a result, we investigated the use of Biolog profiles as a source of taxonomic characters for delimiting the new species among a selection of related taxa. Biolog FF profiles for 42 isolates, representing 19 species of *Oidiodendron* and *Myxotrichum*, were analysed using cluster analyses in PC-ORD. Because the reliability of Biolog FF kits with *Oidiodendron* species has not previously been assayed, multiple replicate tests were done for some isolates. Each of the resulting 54 data sets was unique; that is to say, variation occurred among isolates of the same species and in replicate trials of individual isolates, in addition to being seen in connection with differences among species. Despite this degree of test variability, it was possible to reliably distinguish *O. fimicola*, *O. rhodogenum*, *O. truncatum*, and most isolates of *O. maius* and *O. periconioides* with Biolog FF profiles. Four isolates of an as yet undescribed species of *Oidiodendron* also gave consistent profiles supporting their conspecificity.

Taxonomic novelties: *Oidiodendron fimicola* Rice & Currah sp. nov.
Key words: Biolog FF plates, cluster analyses, mushroom compost, *Myxotrichum*, *Oidiodendron*, *O. fimicola*, *O. maius*, *O. periconioides*, *O. rhodogenum*, *O. truncatum*.

INTRODUCTION

In 1976, two isolates (DC 60, 61) of an undescribed *Oidiodendron* species from mushroom compost were deposited as "*Oidiodendron* sp." in the culture collection of the Penn State Mushroom Spawn Laboratory, University Park PA, U.S.A. A putative subculture of DC 60, labelled "*Oidiodendron sindenia* Beyer" (Beyer pers. comm. 2002), was deposited as ATCC 36074 in the American Type Culture Collection (Manassas, VA, U.S.A.), but its accession data were lost, the culture later died, and the name was never published. Recently, we obtained live cultures of DC 60 and 61 from the Penn State Mushroom Spawn Laboratory and recognized them as representing a hitherto undescribed species. The lost accession data and the resulting uncertainty concerning the relationship of these isolates to "*O. sindenia*", as listed in the ATCC database, prompted us to describe it under a new name.

Several morphological features associated with the new taxon are unique and probably sufficient on their own to justify recognizing it as distinct. Nonetheless, DNA evidence, which might support or challenge assumptions concerning its identity and placement among other species in *Oidiodendron*, was considered desirable. Repeated attempts to obtain DNA sequences were unsuccessful and, as a result, we turned to using profiles obtained in the Biolog FF (filamentous fungi) physiological identification kit (Biolog Inc., Hayward, CA, U.S.A.) as a potential source of additional characters. *Oidiodendron* was not listed among the numerous hyphomycete genera in the Biolog fungal database and thus, for comparative purposes, we needed to generate substrate utilization profiles for a representative set of species.

In the present study, we provide a description of *Oidiodendron fimicola* sp. nov. based on the two isolates mentioned above, and we compare its Biolog profile with those of 40 additional isolates representing 18 distinct taxa in either *Oidiodendron* or its teleomorphic counterpart, *Myxotrichum*.

MATERIALS AND METHODS

Isolates, including the ex-type culture of *O. fimicola*, are deposited at the University of Alberta Microfungus

**Current address for Adrianne V. Rice; Northern Forestry Centre, Natural Resources Canada, 5320-122 St., Edmonton, AB T6H 3S5 Canada

Collection and Herbarium (UAMH, Edmonton, Alberta, Canada), Centraalbureau voor Schimmelcultures (CBS, Utrecht, the Netherlands), or maintained by the authors at the Department of Biological Sciences, University of Alberta (Edmonton, Alberta, Canada). Seven isolates with unknown or unconfirmed identities were included among the taxa studied.

Three replicates of each of the two isolates of the new species were grown as single-point-inoculated cultures on cornmeal agar [CMA; 17.0 g BBL cornmeal agar (Becton Dickinson Co., Sparks, MD, U.S.A.), 1 L dH_2O] and oatmeal agar [OA; 20.0 g Quaker oatmeal cereal, 20.0 g Bacto agar (Becton Dickinson), 1 L distilled water] at room temperature in the dark. Colonies were described and diameters measured at 28 d. Three slide cultures (Sigler & Flis 1998) of each isolate were mounted after 14 d incubation at room temperature in the dark. Conidia and conidiophores were measured and described according to Rice & Currah (2005–this volume). Dimensions were calculated from the mean of at least 10 measurements per slide per isolate. Observations were made using an Olympus BX 50 light microscope fitted with an Olympus DP 12 digital camera (Olympus Optical Co., Tokyo, Japan). Conidiophore and conidial ornamentation were observed using scanning electron microscopy (SEM). Mycelial plugs (2 mm × 2 mm) from sporulating cultures were freeze-dried in liquid nitrogen and examined using cryo-stage preparation in a JEOL #JSM6301FXV SEM (JEOL U.S.A. Inc., Peabody, MA, U.S.A.).

To obtain Biolog profiles, Biolog FF microplates were prepared for 42 isolates representing 19 species (Table 1). Each isolate was first grown on five plates of 2 % malt extract agar [MEA; 20 g malt extract (Becton Dickinson), 18 g Bacto agar, 1 L distilled water] at room temperature in the dark so that sufficient quantities of conidia could be obtained for preparing conidial suspensions (see below). Isolates that failed to sporulate on MEA were grown on CMA or OA at room temperature in the dark. Seven isolates (UAMH 1405, 1523, 1525, 1540, 5715, 8511, 10464) were grown on OA plus either MEA or CMA; one isolate (UAMH 1399) was grown on all three media; and two replicates each of three isolates (UAMH 1399, 1523, 5715) were grown on OA to test the amount of intra-isolate variability and the effect of growth media (See Table 1 for the number of Biolog FF plates prepared per isolate). After 35 d, conidia were collected with sterile cotton swabs. The swabs were dipped into screw-top culture tubes containing 16 mL Biolog FF inoculating fluid [2.5 g Phytagel (Sigma Chemical Co., St. Louis, MO, U.S.A.), 0.3 g TWEEN 40 (Sigma), 1 L distilled water] and the mixture was vortexed briefly. The conidial suspension was prepared to 75 % transmission as measured by a turbidimeter (Biolog) and 100 µL of suspension was pipetted into each of the 96 wells of a single Biolog FF plate. The resulting 54 Biolog plates were incubated at room temperature in the dark and read using a microplate reader (Biolog) at 1, 2, 3, 4, 7, and 10 d as suggested in the Biolog product information. Results were scored using 0 = no reaction, 1 = borderline positive reaction, and 2 = positive reaction (as indicated by the plate reader). The most consistent readings came from the 10-d-old Biolog plates and these were used in the analyses (below).

Data derived from study of all isolates were compared using the clustering program in PC-ORD (MjM Software, Gleneden Beach, OR, U.S.A.). Dendrograms were produced using seven linkage methods (nearest neighbour, farthest neighbour, group mean, median, centroid, McQuitty's method, and Ward's method) and seven distance measures (χ^2, Jaccard, Correlation, Euclidean, Relative Euclidean, Sorensen, and Relative Sorensen). Groups obtained in this manner were compared with distinctions among taxa based on morphological characters, simple substrate tests (Rice & Currah 2005–this volume), and ribosomal DNA (rDNA) sequences where available (Hambleton *et al*. 1998, Lacourt *et al*. 2001, Calduch *et al*. 2004, Sigler & Gibas 2005–this volume).

RESULTS

Biolog

Each of the 54 Biolog FF data sets was unique. The cluster analysis computed using the linkage methods and distance measures given above yielded 37 different dendrograms. Dendrograms varied but five main clusters were generally consistent in approximately 80 % of the topologies. A representative dendrogram is shown in Fig. 1.

Intraspecific variation was considered low in *O. fimicola*, *O. rhodogenum*, *Oidiodendron* sp. 1, and *O. truncatum* because all tested isolates of these species were included in well-supported clusters (congruence among at least 80 % of the dendrograms) that were only distantly connected to isolates of other species (Fig 1). Variation was moderate in *Oidiodendron maius* and *O. periconioides*, because some isolates of these species were included in well-supported clusters but others were outliers. Four of five isolates of *O. maius* formed a well-supported (81 %) cluster while the fifth one (UAMH 9749) clustered most frequently with the ex-type of the morphologically similar anamorph of *Myxotrichum arcticum*. Two of three *O. periconioides* isolates formed a well-supported (97 %) cluster while the third (UAMH 8527) clustered with them in one dendrogram variant but remained isolated in other dendrograms. In *Oidiodendron cerealis*, variation was

Table 1. Strain, species, collector, and collection information for isolates of *Oidiodendron* and *Myxotrichum* species used in the Biolog assessment.

Species	Strain	Collector	Collection Information
M. arcticum	UAMH 7565	Udagawa	Forest soil, U.S.A., ex-type
	UAMH 9243	Lumley	Decayed spruce, Canada
M. cancellatum	UAMH 1996	Udagawa	Soil, Japan
M. setosum	UAMH 3835	Bissett	Soil, Canada
	UAMH 4535	Bissett	Soil, Canada
O. cerealis	CBS 349.62	Dal Vesco	Soil, Italy
	UAMH 504	Carmichael	Human hair, Canada
	UAMH 1522	Barron	Peat soil, Canada
O. chlamydosporicum	UAMH 6520	Morrall	Boreal forest soil, Canada, ex-type
	UAMH 6521	Söderström, Bååth	Spruce forest humus, Sweden
	UAMH 9751	Thormann	*Sphagnum* bog, Canada
O. echinulatum	UAMH 8467	Barron	Peat soil, Canada, authentic
O. fimicola	UAMH 10459	Beyer	Mushroom compost, U.S.A., ex-type
	UAMH 10523	Beyer	Mushroom compost, U.S.A.
O. flavum	UAMH 1524	Barron	Peat soil, Canada
O. fuscum	UAMH 8511[a]	Robak	Wood pulp, Norway, ex-type
O. griseum	UAMH 1403[a]	Melin	Wood pulp, Norway, authentic
	UAMH 4080	Sigler	Wood chips, Canada
O. cf. *griseum*	DC 195	Davenport	Mushroom compost, U.S.A.
O. maius var. *citrinum*	UAMH 1525[a]	Barron	Cedar bog, Canada
	UAMH 7089	Malloch	Stream drift, Canada
	UAMH 9275	Hambleton	Ectomycorrhizal root tip, Canada
O. maius var. *maius*	UAMH 1540[a]	Barron	Peat soil, Canada, ex-type
	UAMH 9749	Thormann	*Sphagnum* bog, Canada
O. periconioides	UAMH 8527	Morrall	Forest soil, Canada, ex-type
	UAMH 10463	Rice	*Sphagnum* bog, Canada
	UAMH 10522	Rice	*Sphagnum* bog, Canada
O. pilicola	UAMH 7526	Nylund	Forest soil, Sweden
O. rhodogenum	UAMH 1405	Robak	Pulp sludge, Norway, authentic
	UAMH 10462	Rice	*Sphagnum* bog, Canada
	UAMH 10521	Rice	*Sphagnum* bog, Canada
O. setiferum	UAMH 5715[b]	Udagawa	House dust, Japan
O. tenuissimum	UAMH 1523[b]	Barron	Forest soil, Canada
	UAMH 8513	Castañeda	Leaf litter, Spain
O. truncatum	UAMH 1399[c]	Barron	Forest soil, Canada, ex-type
	UAMH 8443	Mosca	Soil, Italy
	UAMH 10464[a]	Lumley	Decayed spruce, Canada
Oidiodendron sp. 1	2SMC1-1B	Rice	*Sphagnum* bog, Canada
	2SMC5-1A	Rice	*Sphagnum* bog, Canada
	3MMC3-7A	Rice	*Sphagnum* bog, Canada
	5JyMC2-5A	Rice	*Sphagnum* bog, Canada
Oidiodendron sp. 2	4SM3-3	Rice	*Sphagnum* bog, Canada

[a]Two replicate Biolog plates.

[b]Three replicate Biolog plates.

[c]Four replicate Biolog plates.

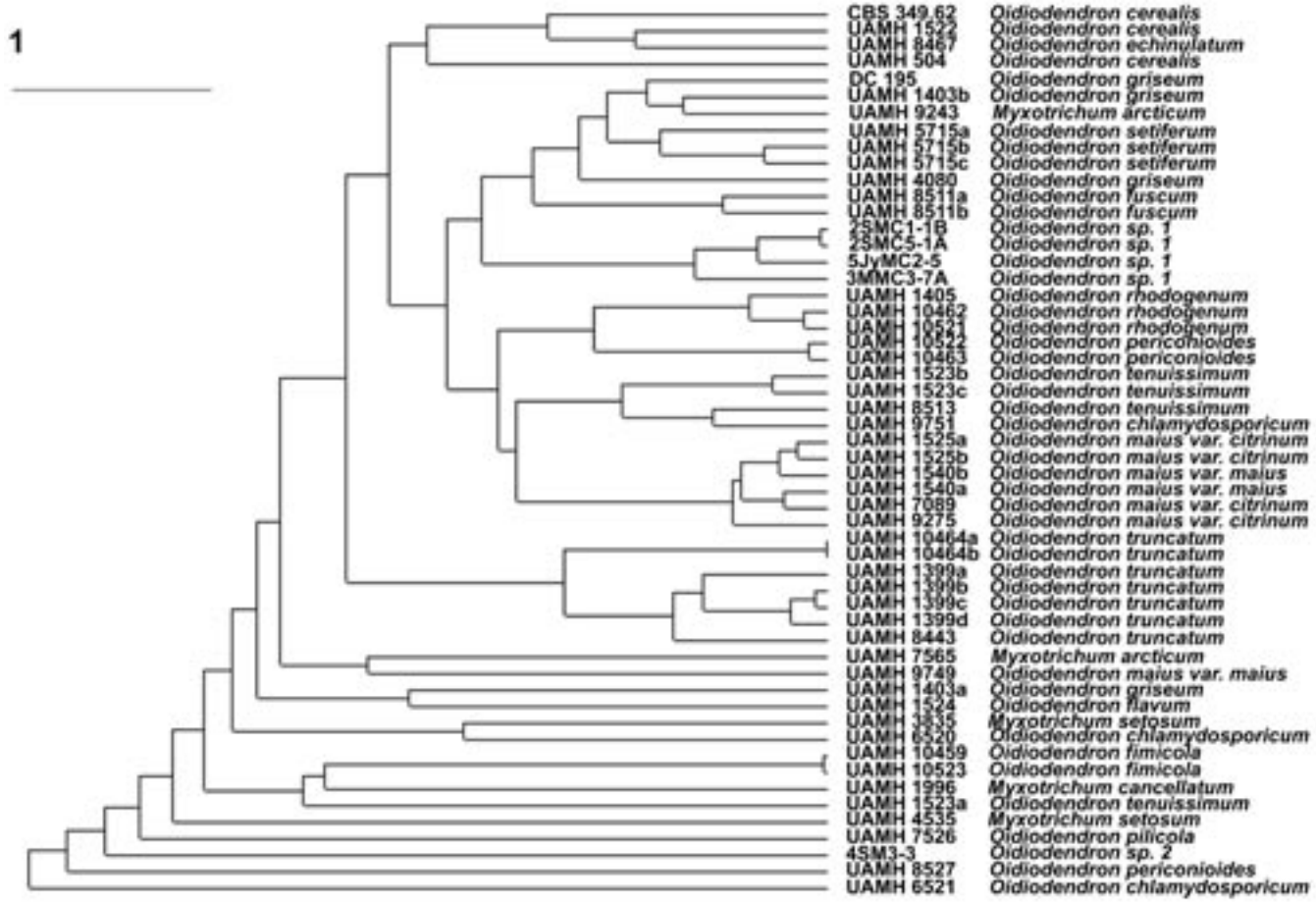


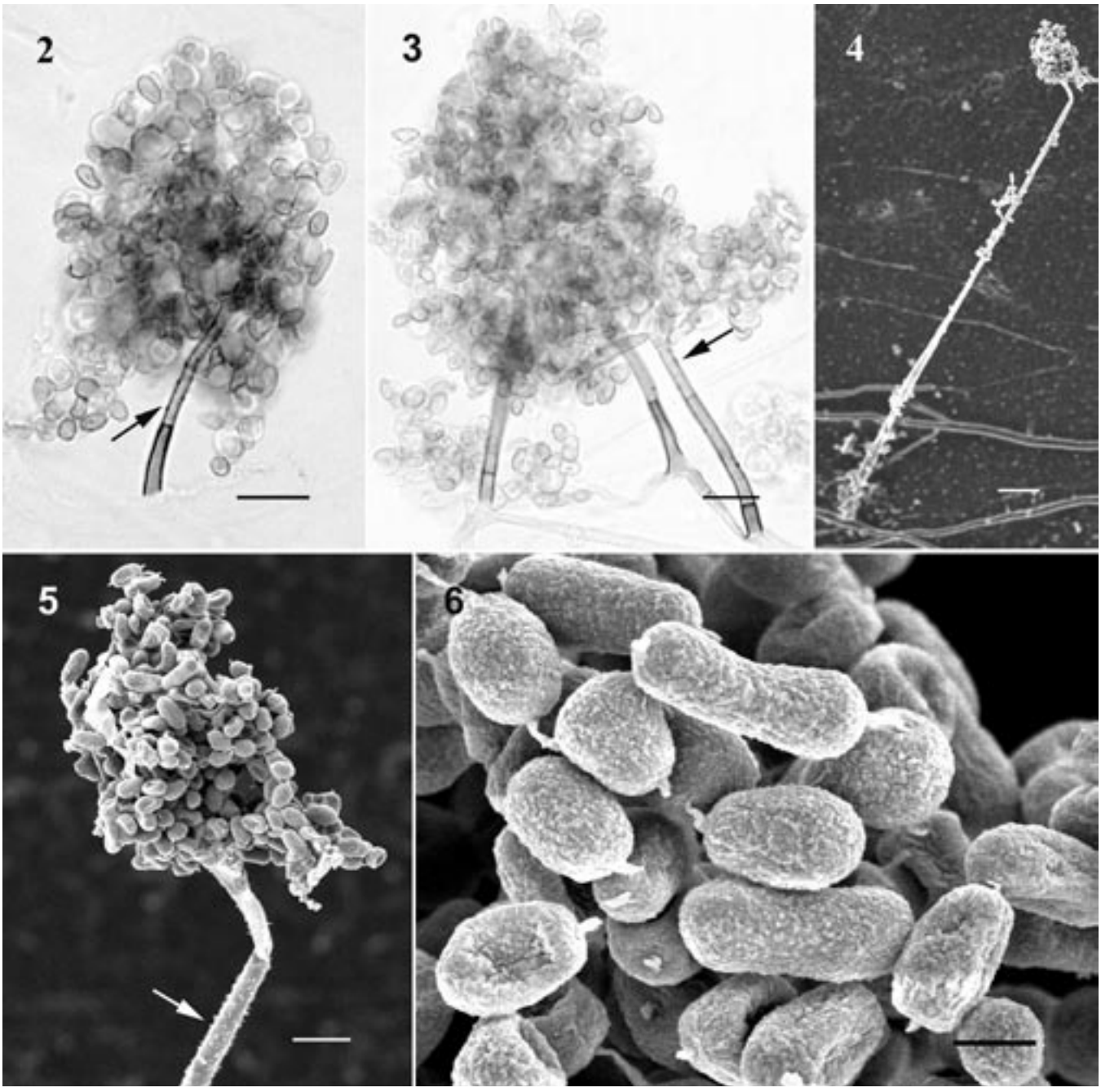
Fig. 1. Representative dendrogram of 54 Biolog FF plate data sets for 19 *Oidiodendron* and *Myxotrichum* species produced using the Correlation distance measure and the Group Average linkage method. Note the well-supported clusters for *O. fimicola*, *O. rhodogenum*, *O. truncatum*, *Oidiodendron* sp. 1, and most isolates of *O. maius* and *O. periconioides*. Bar = 25 % of information.

moderate: all three isolates clustered together in seven dendrograms (19 %) and two or more isolates were together in 19 dendrograms (51 %). Isolates of *O. cerealis* also clustered with *O. echinulatum* in about half of the dendrograms.

Intraspecific variation was moderate to high in *Oidiodendron griseum* and *O. tenuissimum*. Isolates of these species did not form distinct, well-supported clusters. Instead, they appeared in large clusters interspersed with members of other species. Isolates of *O. griseum* clustered with isolates of the related species *O. flavum* and *M. arcticum*, as well as with *O. setiferum*. *Oidiodendron tenuissimum* isolates clustered with those of *O. maius*, *O. chlamydosporicum*, and *M. arcticum*. Isolates of each of *M. arcticum*, *M. setosum*, and *O. chlamydosporicum* never clustered together, indicating a high level of intraspecific variation in these taxa. Instead, the ex-type of *M. arcticum*, UAMH 7565, clustered with an isolate of *O. maius* var. *maius* in 9 dendrograms, and with isolates of *O. tenuissimum* and *O. chlamydosporicum* in 7 dendrograms, while the second representative, UAMH 9243, clustered 92 % of the time with isolates of *O. griseum*. One isolate of *M. setosum*, UAMH 3835, mainly clustered with the ex-type of *O. chlamydosporicum* (UAMH 6520; 57 %) while the second test isolate, UAMH 4535, was distinct but was distantly connected with *O. fimicola*, *Myxotrichum cancellatum*, and one isolate of *O. tenuissimum* in 7 dendrograms. In two dendrograms, it clustered with a second isolate of *O. chlamydosporicum*, UAMH 6521, and with *Oidiodendron pilicola*. The third isolate of *O. chlamydosporicum*, UAMH 9751, formed a well-supported (73 %) cluster with an isolate of *O. tenuissimum* UAMH 8513.

In eight isolates tested in multiple replicate trials, each trial produced a distinct profile. The variation seen was low in *O. truncatum*, both in four replicate studies of isolate UAMH 1399 and two replicates of UAMH 10464. The two replicates of UAMH 10464 differed from one another at seven wells but still formed a distinct subgroup in all analyses, while the four replications of UAMH 1399 differed at 23 wells and clustered together about 90 % of the time. Replicate trials 'c' and 'd' of UAMH 1399, based on colonies grown on the same medium favouring conidial production, differed at 11 wells and were no more similar to each other than to replicates grown on different media. In *O. maius*, the level of within-isolate variability was similar to levels of variability among isolates: replicates of the ex-type isolates of

O. maius var. *maius* and *O. maius* var. *citrinum* were included in the same cluster, but were no closer to each other than to various other isolates. *Oidiodendron fuscum* showed moderate-level variability, with two replicates for UAMH 8511 clustering together in over half the analyses. In *O. setiferum*, also rated moderately variable, UAMH 5715 replicates 'b' and 'c', grown on the same medium, clustered with each other in most analyses but grouped with replicate 'a' from a different medium only in somewhat more than one quarter of the analyses. Moderate variability was also seen in *O. tenuissimum*. Variation was highest in *O. griseum*, where UAMH 1403 replicates 'a' and 'b' were part of the same cluster in less than half the dendrograms. In many analyses, 'a' clustered with the ex-type isolate of *O. flavum* while 'b' clustered with isolates of *O. griseum* and *M. arcticum*. *Oidiodendron echinulatum*, *O. flavum*, *O. pilicola*, *Oidiodendron* sp. 2, *M. cancellatum*, each represented by a single test of a single isolate, were relatively distinct, showing distant association with isolates of other species in less than half of the analyses.



Figs 2–6. Conidiophores and conidia of *O. fimicola*. 2. Mass of irregularly shaped, thick-walled, hyaline to melanized conidia produced at the apex of an asperulate conidiophore (arrow shows asperulate region). 3. Asperulate (arrow) conidiophores bearing masses of irregularly shaped and pigmented conidia. 4. Small dense head of conidia at the apex of a solitary conidiophore. 5. Dense head of irregularly shaped, asperulate conidia in chains at the conidiophore apex. Note the scales on the conidiophore (arrow) that give the conidiophores an asperulate appearance under light microscopy. 6. Asperulate conidia. Note the varying shapes and sizes of the conidia and the connectives visible between spores. Scale bars: 2–3 = 15µm, 4 = 10 µm, 5 = 5 µm, 6 = 1 µm.

Taxonomy

Oidiodendron fimicola Rice and Currah, **sp. nov.**, MycoBank MB500255, Figs 2–6.

Etymology: *fimus* refers to composted dung, a reference to the mushroom compost from which the ex-type was isolated.

Conidiophora 20–(50)–100 × 2–4 µm, non ramose vel dichotomose ramosa, asperulata vel squamosa. Hyphae fertiles oriunt vel ab apicibus conidiophorum vel directe ab hyphis vegetativitis; erigunt catenas breves vel ramosas vel non ramosas arthroconidiorum. Conidia 3–(5)–6 × 2–(2.5)–3 µm, crassiter tunicata, asperulata, hyaline ad pallide-brunnea, dolioformia ad elongata vel irregularia et in extremis ambis truncata vel magis vel minus, inter se conexionibus distinctis separata. Isolatum a fimo ad cultum fungorum.

Holotypus: Colonia exsiccata ex UAMH 10459 isolato ex fimo ad cultum fungorum, St. Louis, MO, U.S.A.

Colonies on CMA 19–26 mm diam at 28 d, off-white to beige or pale grey, appressed, with concentric rings of abundant conidiophores bearing masses of off-white to beige conidia; reverse olivaceous. Colonies on OA 20–22 mm diam at 28 d, pale grey with olivaceous margins, appressed, with concentric rings of abundant conidiophores bearing masses of off-white to grey conidia; reverse dark olivaceous to black. Conidiophores melanized, unbranched or dichotomously branched, 20–(50)–100 × 2–4 µm, appearing asperulate under light microscopy, covered in small scales visible in SEM. Fertile hyphae hyaline, 2–2.5 µm diam, arising from conidiophore apices or laterally from vegetative hyphae, fragmenting basipetally to form short, dichotomously- or sparsely-branched chains of arthroconidia. Conidia thick-walled, asperulate, hyaline to beige or pale brown, barrel-shaped to elongate or irregular, more or less truncate at one or both ends, 3–(5)–6 × 2–(2.5)–3 µm; connectives visible between conidia.

Holotype: **U.S.A.** St. Louis, MO, dried culture of isolate UAMH 10459 from mushroom compost.

Additional Specimen: **U.S.A.**, California, mushroom compost, 1976, Beyer [UAHM 10523 (= DC 61, as *Oidiodendron* sp.)].

DISCUSSION

Oidiodendron fimicola is most similar to *O. flavum* in producing irregularly shaped, lightly pigmented to melanized conidia, but it differs by having roughened rather than smooth conidiophores. The smooth to asperulate conidia of *O. fimicola* and *O. flavum* are similar by light microscopy, but by SEM, the perispore can be seen to be slightly to markedly asperulate in *O. fimicola*, while it is smooth to dimpled in *O. flavum*. In both species, conidial shape varies but the conidia of *O. fimicola* are more or less elliptical to elongate while those of *O. flavum* (Rice & Currah 2005–this volume) are subglobose to pyriform.

The two *O. fimicola* isolates in Biolog FF plate profiles differed from one another only at eight wells. Five of these discrepancies involved wells that were either borderline positive or negative (scored as "0" or "1"), while the other three involved positive reactions differing in intensity (scored as "1" or "2"). These differences were of minimal import because the isolates clustered together with each other and remained relatively isolated from isolates of the other species in all of the 37 dendrograms calculated. In contrast to their superficial morphological similarities, *O. fimicola* and *O. flavum* had markedly different Biolog profiles. They differed at 55 wells and did not cluster together in any dendrogram.

Relatively consistent topologies in Biolog-based dendrograms were also observed with the three isolates of *O. rhodogenum*, three isolates of *O. truncatum*, four isolates of *Oidiodendron* sp. 1, four of the five test isolates of *O. maius*, and two of the three isolates of *O. periconioides*: all formed distinct clusters in at least 80 % of the dendrograms. With *O. truncatum*, all isolates clustered together consistently, including isolates from peat soils in Canada, mountain soil in Italy, and decaying wood in Canada. In *O. rhodogenum*, two isolates from sphagnum peat in Canada clustered well with an isolate from Norwegian wood pulp. The two well-clustered isolates of *O. periconioides* both came from sphagnum peat, while the single distinct isolate, the ex-type isolate, was from soil. It is possible that these physiological differences reflect different ecological adaptations. The varietal differences between *O. maius* var. *maius* and *O. maius* var. *citrinum* were not significantly reflected in Biolog FF profiles. The ex-type isolates of the two varieties clustered with each other while a second isolate of *O. maius* var. *maius* was an outlier.

The four isolates of *Oidiodendron* sp. 1 were all isolated from lignin bait blocks that had been set in the sphagnum moss of a local bog. They may represent a new species. Morphologically, they are similar to *O. rhodogenum* but lack the characteristic diffusible red pigment and have conidia that are less heavily melanized. Both of these characters, however, can vary among *O. rhodogenum* isolates, and the new isolates could conceivably represent an ecological variant of this species. Consistent Biolog FF profiles for each set of representatives indicate that *Oidiodendron* sp. 1 might represent a distinct species but other criteria (e.g., DNA data) will be examined before a decision is made.

The groupings of *Oidiodendron* isolates based on Biolog FF profiles differ from groupings obtained in molecular studies (Hambleton *et al*. 1998, Lacourt *et al*. 2001, Calduch *et al*. 2004, Sigler & Gibas 2005–this volume). While the species that are distinct in Biolog analyses are also distinct in their relevant DNA sequences, where these have been done, the species that did not cluster well in Biolog tests have generally nonetheless been supported as distinct species in sequencing studies. Similarity in Biolog data is not expected necessarily to reflect phylogenetic relatedness, although some closely related species, including *O. flavum*, *O. griseum*, and *M. arcticum* did cluster together in most of the analyses. Conversely, some species that clustered together in the Biolog data, such as *O. cerealis* and *O. echinulatum*, and *O. rhodogenum* and *O. periconioides*, are not closely related phylogenetically. Physiological profiles would be expected to show ecological or functional similarities or to reveal differences that would not be disclosed by sequence analyses that adumbrated only a minute fraction of the genome. An example of functional similarity may be seen in the clustering of two *O. periconioides* isolates from peat with the isolates of *O. rhodogenum*, two of which are from the same peatland. Differences among isolates of individual species, for example in *O. maius*, *O. periconioides* or *O. chlamydosporicum*, may indicate some ecological differentiation or adaptations for different habitats. Differences between or among replicates of the same isolates may be the result of heterokaryosis. The high level of physiological similarity noted between the two isolates of *O. fimicola* may reflect their common adaptation to an unusual habitat, mushroom compost, as much as it reflects their intrinsic genetic similarity as conspecific isolates.

The use of Biolog profiles as ancillary taxonomic characters in the routine identification of *Oidiodendron* species can be recommended, but only with the caveat that the user must anticipate that in some cases, a very wide range of variation will occur among replicates and among conspecific isolates. Relatively few studies involving Biolog profiles of multiple isolates and sets of closely related species have been published (Wildman 1995, Talbot *et al*. 1996, Kubiček *et al*. 2003). Among conidial fungi, it may be typical to see high levels of variation in some species and low levels in others, as was observed here. Observations similar to ours have been made in examining Biolog profiles of collections of *Fusarium compactum* (Wollenw.) Gordon (Wildman 1995, Talbot *et al*. 1996) and *Trichoderma* Pers. (Kubiček *et al*. 2003) isolates. Before taxa are added to the Biolog database, screening multiple isolates (and replicates of these isolates) is necessary to allow quantification of the amount of variation that may occur. Also, such studies, interpreted in light of phylogenetic studies, will reveal whether there are intraspecific patterns that might relate to ecological differences or to the differentiation of distinct subtaxa. Expansion of such studies would be particularly valuable for species like *O. maius* and *O. periconioides* where some isolates form distinct clusters but others appear to be outliers. Certainly, the species where only one isolate has been tested should be studied in more detail. Nevertheless, our preliminary evidence, albeit based on a limited data set, suggests that this method is promising as a source of additional characters for the definition of new species (e.g. *O. fimicola*) and for the identification of species that seem to have relatively low levels of inherent variation (e.g. *O. rhodogenum*, and *O. truncatum*).

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