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Irpex hydnoides, sp. nov. is new to science, based on morphological, cultural and molecular characters

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Abstract: *Irpex*, one of the most common polypore genera, is easily identified by macro- and microscopic characters. During field trips to Korea's Kangwon Province, some *Irpex* specimens with conspicuous morphological differences from *I. lacteus* were collected. Cultural characters and molecular evidence differentiated this new strain from *I. lacteus*, and this taxon is proposed as *I. hydnoides* sp. nov.

Key words: *Australohydnum dregeanum*, *Irpex lacteus*, *Irpex vellereus*, ITS, Polyporaceae

INTRODUCTION

Irpex Fr. is a monotypic genus belonging to the Polyporaceae (Ryvarden 1991), which is widely distributed as a saprotroph on dead wood. *Irpex lacteus* is a white-rot fungus of great commercial importance due to the production of endo- and exotype cellulases (Hamada et al 1999, Hoshino et al 1994), antibiotics and irpexans (Silberborth et al 2000). It also is important for the decolorization of chemically complex dyes (Novotny et al 2001) and bioremediation of hazardous organic compounds (Kim and Song 2000, Novotny et al 2000).

Irpex is one of the most common polypore genera in Korea (Jung 1994, Lim and Jung 2000, Yang et al 1987). Traditionally, it is recognized by its distinctly irpicoid (flattened-toothed) hymenophore, coriaceous texture, encrusted cystidia and simple-septate hyphae with a dimitic hyphal system (Gilbertson and Ryvarden 1986, Ryvarden and Gilbertson 1993). However, its morphology is variable depending on environmental conditions. In favorable conditions, it grows vigorously and fruiting bodies may reach up to 3 m in length (Jung 1987). Because of this morphological variability, numerous species have been recognized in *Irpex*. Maas Geesteranus (1974) concluded that these species names all were synonyms of *I. lacteus* or otherwise should be removed from *Irpex* to other genera, maintaining *Irpex* as a monotypic genus.

Australohydnum dregeanum morphologically is similar to *I. lacteus* and is described as possessing a hydnoid (toothed) to irpicoid hymenophore and a monomitic or dimitic hyphal system with simple-septate generative hyphae. The type species of *Australohydnum* Jül. is *A. griseofuscescens* (Reich.) Jül., but Hjortstam and Ryvarden (1990) suggested that this species is a synonym of *A. dregeanum*. *Irpex vellereus* also is treated as a synonym of *A. dregeanum* (Hjortstam and Ryvarden 1990).

Specimens of a new *Irpex* were collected from fallen branches of *Quercus* sp. in Kangwon Province. These differed from those of *I. lacteus* in basidiocarp morphology and cultural characters. The new taxon microscopically was quite similar to *A. dregeanum* but differed in hymenophoral configuration. To determine if these collections represent a new species, cultures were studied and DNA sequences of nuclear ITS1, ITS2 and 5.8S rRNA genes were analyzed.

MATERIALS AND METHODS

Isolation and characterization of strains.—The strains used in this study are listed in TABLE I. They are maintained in the Seoul National University Herbarium (SNU). Cultures were grown in a biochemical oxygen-demand incubator at 24 C on malt-extract agar (MEA). Extracellular oxidase reactions were tested as described by Stalpers (1978), using α-naphthol and *p*-cresol. Basidiomata were examined both macroscopically and microscopically. Measurements and drawings were made from slide preparations stained with 1% (w/v) aqueous phloxine and 3% (w/v) potassium hydroxide (Hawksworth et al 1995). Melzer's reagent was applied to check spore amyloidity. Color designations were adopted from Kornerup and Wanscher (1978).

DNA extraction, PCR amplification and sequencing.—DNA was extracted from mycelium grown on MEA covered with a sterile cellophane disk or directly from 300 mg of fruiting-body tissue as described by Lecellier and Silar (1994) with some modification (Lim and Jung 1998). Partial SSU rDNA and ITS regions containing the 5.8S rRNA gene were am-

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TABLE I. Fungal strains used in this study

Taxa	Source[b]	Substrate and locality	GenBank
Australohydnum dregeanum (Berk.) Hjortst. & Ryv.	SNU m-98080404	Dead *Quercus* sp., Kyonggi Province, Korea	AF479669
Irpex hydnoides	SNU m-97101112	Fallen branch of hardwood, Kangwon Province, Korea	AF479668
I. hydnoides[a]	SNU m-97121519	Dead *Quercus* sp., Kangwon Province, Korea	AF479667
I. lacteus (Fr.: Fr.) Fr.	FP-105049	*Acer* sp., New Hampshire, North America	AF163045
I. lacteus	SNU m-95100739	Dead hardwood, Kangwon Province, Korea	AF479666
I. lacteus	SNU m-97100612	Fallen branch of hardwood, Seoul, Korea	AF163046
I. vellereus Berk. & Br.	CBS 515.92	Dead log of *Shorea robusta* Gaertner, India	AF479670
Phanerochaete chrysosporium Burds.	KCTC 6293[c]	Chip of *Picea abies* (L.) Karst., Sweden	AF475146
P. sordida (Karst.) Erikss. & Ryv.	KCTC 26213	Fallen branch of *Quercus* sp., Kangwha Island, Korea	AF475150

[a] Holotype.

[b] Cultures were obtained from the following collections: CBS, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; FP, USDA Forest Products Laboratory, Madison, WI 53705-2398; KCTC, Korean Collection for Type Cultures, Taejon 305-600, Korea; SNU, Seoul National University Herbarium, Seoul 151-742, Korea.

[c] The KCTC accession number corresponding to ATCC 32629, CBS 264.84, CCRC 36201, CECT 2777 and DSM 1547.

plified with primers NS7 and ITS4 (White et al 1990). Amplification was performed as described by Lee et al (2000). Amplified products were purified with Wizard PCR Prep (Promega), and purified products were cycle sequenced using the chain termination method with ^{35}S-labled ATP (Hillis et al 1996) of a Top™ DNA sequencing kit (Bioneer Corp., Korea). In addition to new sequences produced from nine strains in TABLE I, the ITS sequence of *Bjerkandera fumosa* (Pers. : Fr.) Karst. was obtained from GenBank (AJ006637) and sequences of *Ceriporia viridans* (Berk. & Br.) Donk and *C. excelsa* (Lund.) Parm.were provided by J. Mugnier (Rhône-Poulenc, France).

Phylogenetic analyses.—ITS sequences initially were aligned with Clustal X (Thompson et al 1997) and optimized with PHYDIT version 3.0 (Chun 1995). Ambiguously aligned regions were omitted from analyses. Maximum-parsimony (MP) analyses were performed with PAUP* 4.0b4a (Swofford 1999). Heuristic searches usingg tree-bisection reconnection (TBR) branch swapping and 100 random-taxon sequence additions were employed. Gaps were treated as missing data in all analyses. Support for individual branches was determined by bootstrap analysis (Hillis and Bull 1993) based on 1000 replicates (simple addition sequence, TBR swapping, and MAXTREES unrestricted). Based on the phylogenetic results of previous studies (Boidin et al 1998, Hibbett et al 1997, Hibbett and Thorn 2001), *Bjerkandera fumosa* was chosen as outgroup.

RESULTS

Irpex hydnoides Y. W. Lim et H. S. Jung, sp. nov.

FIG. 1

Basidiocarpus annuus, resupinatus vel effuso-reflexus; superficies albido, tomentoso-hirsuto; hymenium cremeo-lutea vel ochraceo, hydnoidea, 4 mm longis; systema hypharum dimiticum; hyphae generatoriae tenuitunicatae; hyphae skeletaleae crassitunicatae; cystidia incrustata, 25–45 × 6.5–11 μm; basidia clavata, 4-sterigmatibus, 28–32 × 6–7 μm; basidiosporae ellipsoideae, hyalinae, laeves, non-amyloideae, 5.5–6.5 × 3.5–4 μm. Holotypus in herbarium SNU; numero accessiono m-97121519.

Basidiocarps annual, resupinate or effused-reflexed, confluent and effused up to 25 cm or more; pilei often laterally fused, up to 5 mm wide, upper surface whitish to dingy white, densely tomentose to hirsute; hymenophore cream (4A3) to yellow (4A4–4A5), becoming ochraceous, hydnoid, teeth up to 4 mm long, mostly flattened, sometimes united at the base or laterally fused, often incised or denticulate at the apex; margin distinctly bounded; consistency coriaceous. Hyphal system dimitic; contextual generative hyphae thin-walled with frequent branching, simple-septate, 2.5–5 μm in diam; contextual skeletal hyphae hyaline, thick-walled with rare simple septa, 4–6 μm in diam. Cystidia conspicuous, abundant, thick-walled, apically heavily incrusted, incrusted part 25–45 × 6.5–11 μm, cylindrical to conical, originating from tramal skeletal hyphae in the subhymenium and projecting up to 55 μm beyond the hymenium. Basidia clavate, 4-sterigmate, 28–32 × 6–7 μm, simple-septate at the base. Basidiospores ellipsoid, hyaline, smooth, negative in Melzer's reagent, 5.5–6.5 × 3.5–4 μm.

Etymology. ''hydnoides'': denticulate.

Habitat. Fallen twigs of *Quercus* sp.

Distribution. Known only from the temperate oak-

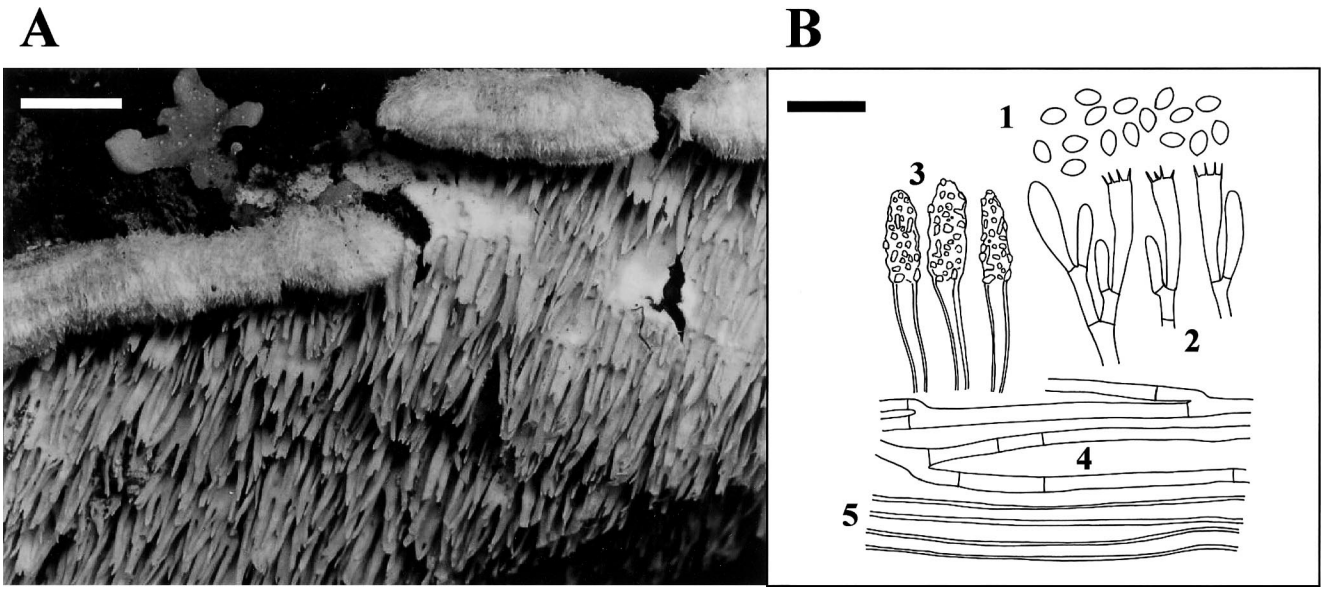

FIG. 1. Basidiocarps (A) and microscopic features (B) of *Irpex hydnoides* (holotype). 1. basidiospores. 2. basidia. 3. cystidia. 4. generative hyphae. 5. skeletal hyphae. Scale bars: A = 5 mm, B = 20 μm.

pine forests of the Taebaek Mountains, Kangwon Province, Korea.

Specimens examined. KOREA. Mount Odae, Kangwon Province, on *Quercus* sp., 11 Oct 1997, *Y. W. Lim* (SNU m-97101112); Mount Chiak, Kangwon Province, on *Quercus* sp., 15 Dec 1997, *Y. W. Lim* (HOLOTYPE: SNU m-97121519).

Cultural characteristics.—Growth on MEA slow, forming mats of 11.2 mm diam in 7 d at 25 C. Advancing zone even, hyaline and slightly raised; hyphae distant, branched, 2–5.5 μm wide. Aerial mycelium at first downy, becoming thinly cottony, white, hyphae 2–3 μm wide. Reverse bleached (FIG. 2).

Species code (Stalpers, 1978). 1, 2, 9, 12, 15, 17, 30, 31, 37, 46, 50, 52, 53, 89.

Extracellular oxidase reactions. α-naphthol +; *p*-cresol ++.

Culture examined. SNU m-97121519 isolated from the tissue of the holotype basidiocarp.

Remarks. Irpex hydnoides is recognized by its effused-reflexed fruit bodies and conspicuous hydnoid hymenophore. It is similar to *I. lacteus*, and these two species often are confused in the field. *Irpex hydnoides* can be distinguished easily by its long pale yellow to ochraceous teeth, large basidia and basidiospores, slow growth on MEA and ITS sequences. *Schizopora paradoxa* (Fr.) Donk resembles *I. hydnoides* in basidiospore morphology and the shape and color of its basidiomata but forms a poroid hymenophore with daedaleoid-hydnoid dissepiments and clamped generative hyphae. Species of *Steccherinum* Gray share some characters with *I. hydnoides*, but the members of this genus generally possess odontioid hymenophores up to 1 mm in length and clamped generative hyphae when the teeth are longer than 1 mm.

DNA amplification and sequence analyses.—The amplified products obtained with primers NS7 and ITS4 were approximately 1 kb in size and ITS and 5.8S rDNA products were estimated to be between 630 and 650 bp in length. The aligned sequences of species of *Irpex* were 651 bp in length. Sequences of strains of *I. hydnoides* were invariant but sequences of *I. lacteus* from Korea and North America differed by 0.3%. Sequence divergence between *I. lacteus* and *I. hydnoides* ranged from 2.15 to 2.31%. The final alignment for 12 species consisted of 612 characters of which 150 were parsimony informative.

Maximum-parsimony analyses yielded two equally most-parsimonious trees (tree length = 359 steps, consistency index = 0.7911, retention index = 0.7741, rescaled consistency index = 0.6124), one of which is presented in FIG. 3. These trees were identical except for altered positions of strains in each of *I. hydnoides* clade and *I. lacteus* clade. The two strains of *I. hydnoides* formed a well-supported clade (100% bootstrap support) that is closely related to strains of the type specie of *Irpex*, *I. lacteus* (FIG. 3). *Irpex vellereus*, a species treated as a synonym of *A. dregeanum* (Hjortstam and Ryvarden 1990), was not inferred as a member of the *I. hydnoides*–*I. lacteus* clade but grouped with a high level of support (100%) with the single isolate of *A. dregeanum* sequenced in this study. This clade was sister of the group that includes members of the genus *Phanerochaete* Karst.

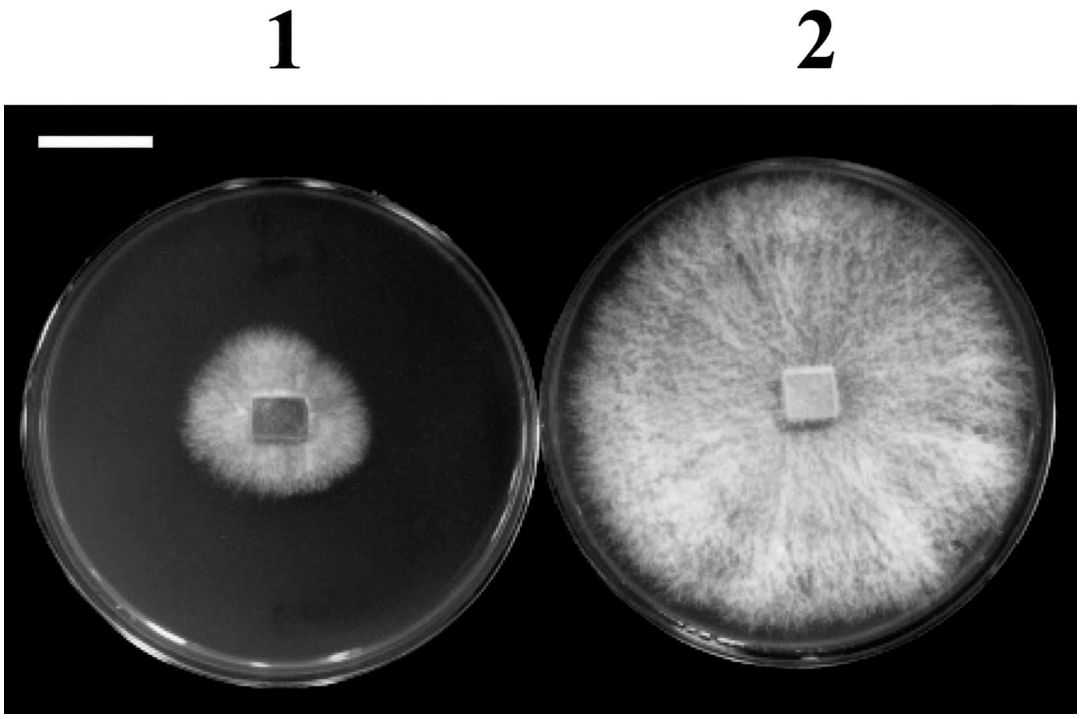

FIG. 2. Mycelial cultures after five days of growth on MEA at 25 C. 1. *Irpex hydnoides* (ex-holotype). 2. *Irpex lacteus* FP-105049. Scale bar: 2 cm.

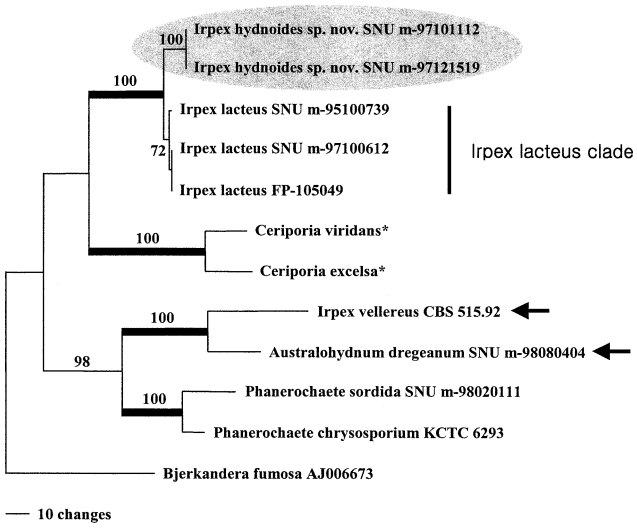

FIG. 3. One of two most-parsimonious trees of *Irpex* and related taxa based on sequence data of the ITS region. Bootstrap percentages above 60% based on 1000 replicates were indicated above internodes. The sequence from *Bjerkandera fumosa* AJ006673 was used as outgroup. Sequences noted with an asterisk were obtained from J. Mugnier (Rhône-Poulenc, France). The shaded ellipse indicates the *I. hydnoides* clade and the vertical labeled bar the *I. lacteus* clade. The arrows indicate the *I. vellereus–A. dregeanum* clade and the length difference of terminal branches suggests the degree of ITS sequence divergence between two taxa (10.1%).

DISCUSSION

Irpex hydnoides is distinguished from *I. lacteus* by its conspicuous teeth up to 4 mm long, cream or yellow to ochraceous hymenophore, densely tomentose pileal surface, and ellipsoid spores (FIG. 1). Although the teeth of *I. lacteus* sometimes measure up to 3 mm long (Jung 1987), its hymenophore is always white to cream in color. Basidia of *I. lacteus* and *I. hydnoides* are similar, but those of *I. hydnoides* are larger (28–32 × 6–7 μm) than the basidia of *I. lacteus* (20–25 × 4–5 μm). The spores of *I. lacteus* are oblong to cylindric and straight to slightly curved and 5–6 × 2–3 μm, while those of *I. hydnoides* are ellipsoid to broadly ellipsoid and 5.5–6.5 × 3.5–4 μm (FIG. 1B).

Cultural characters, growth rate and extracellular oxidase reactions also distinguish *I. hydnoides* from *I. lacteus*. The growth rate of our isolates of *I. lacteus* was 4.9–5.3 mm per day (reported as 3.9–5 mm per day by Stalpers 1978), while that of *I. hydnoides* is 1.6 mm per day (FIG. 2). A strong color change occurred in a *p*-cresol test for *I. hydnoides* but there was no such reaction for *I. lacteus*.

The phylogeny inferred from the analysis of ITS sequence data indicates that the genus *Irpex* includes two species, *I. hydnoides* and *I. lacteus*. This result, coupled with morphological and cultural characters, supports adding *I. hydnoides* as a new species in the previously monotypic genus *Irpex*. *Irpex vellereus* is

more closely related to *A. dregeanum*, a species that also possess a dimitic hyphal system, metuloids and skeletocystidia. Although *A. dregeanum* and *I. vellereus* have been treated as synonyms (Hjortstam and Ryvarden 1990), ITS sequence divergence between these taxa (10.1%) is greater than has been reported within other basidiomycete species (Anderson and Stasovski 1992, Gardes et al 1991, Vasiliauskas et al 1999). Testing the hypothesis that these morphologically similar taxa are conspecific awaits the study of a greater number of isolates, including *A. griseofuscescens*, the type species of *Australohydnum*.

The *A. dregeanum–I. vellereus* clade was sister of the group that included two species of *Phanerochaete*. These four taxa formed a well-supported clade (98%), but the relationship of this group to *Ceriporia* and *Irpex* clades was not resolved in our ITS phylogeny. A number of morphological characters also support the supposition that *Australohydnum* is more closely allied to *Irpex* than to *Phanerochaete*, but there are several tuberculate, raduloid to aculeate *Phanerochaete* and such a suggestion is not as strongly supported by some microscopic features and molecular data (Hjortstam and Ryvarden 1990, Lim 2001). Elucidating phylogenetic relationships within this clade will require sequencing of a greater number of taxa.

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