

A CULTURAL STUDY OF SEVERAL SPECIES OF ANTRODIA (POLYPORACEAE, APHYLLOPHORALES)

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ABSTRACT

Cultural characteristics of five species of *Antrodia* are described: *A. gossypia*, *A. juniperina*, *A. odora*, *A. sitchensis*, and *A. sordida*. Each causes a brown-rot decay of conifers. Key patterns and species codes are given for each. Additional data are also provided for six other species of *Antrodia*.

Key Words: *Antrodia gossypia*, *A. juniperina*, *A. odora*, *A. sitchensis*, *A. sordida*, cultures

The genus *Antrodia* Karst. as treated in “North American Polypores, Vol. 1” (Gilbertson and Ryvarden, 1986) includes species causing brown rots and having a dimittic hyphal system, generative hyphae with clamps, skeletal hyphae, and cylindrical to oblong-ellipsoid basidiospores. Cultures of the 20 species so-treated have two characteristics in common: clamped hyphae and negative or weakly positive oxidase reactions. Of the species that have been studied in culture to date, 14 have one hyphal element in common, *i.e.*, clamped hyphae with irregularly thickened walls. They may develop fiber hyphae and chlamydospores.

The present paper reports on a cultural study of five species, three for which cultural descriptions are published. Unpublished cultural data for additional species are included also.

MATERIALS AND METHODS

TABLE I lists the cultures used in this study. All isolates are maintained in the Reference Culture Collection of the Center for Forest Mycology Research (CFMR), Madison, Wisconsin.

The methods employed in studying the cultures, the arrangement of the descriptions, and the explanation of the “Key patterns” were the same as used in previous studies (Davidson *et al.*, 1942). Mat descriptions and growth rates were based on 2-wk-old cultures inoculated in the centers of 90-mm Petri dishes on 1.5% malt extract

agar (MEA) and incubated at 25 C. The “Species code” of Nobles (1965, as modified by Boidin, 1966) was based on 6-wk-old cultures inoculated at the sides of Petri dishes containing MEA.

Extracellular oxidase production was detected by the Bavendamm test described by Davidson *et al.* (1938), in which cultures are grown on malt agar containing either 0.5% gallic (GAA) or tannic (TAA) acids. Test-tube cultures were grown at room temperature (*ca* 25 C) in diffuse light. For the constant temperature study, cultures on MEA in Petri dishes were placed in incubators 24 h after plating and were measured at the end of 6 da. Measurements of mat diameters were averages of three replications of individual isolates. Lethal temperatures were determined by removing those cultures having no observable growth from the high test temperatures and incubating them at 25 C for 3 wk. Cultures that did not grow were presumed killed at the high temperatures. In designating incompatibility groups (*i.e.*, A, B), the A and B factors are designations of different factors but not identified as to function. Capitalized color names are from Ridgway (1912).

CULTURE DESCRIPTIONS

ANTRODIA GOSSYPPIA (Speg.) Ryv., *Norw. J. Bot.* 20: 8. 1973.

Poria gossypium Speg., *Buenos Aires Mus. Arg. Cien. Nat. Anal.* 6: 169. 1898.

Keypatterns. — A-O-S-10, A-O-S-1-10.

Species code. — 1.3r.7.32.36.38.47.(50).55.

Growth characteristics. — Growth on MEA slow, forming mats-12–14 mm diam in 7 da, 25–35

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TABLE I
CULTURES USED IN THE PRESENT STUDY

Species and number	Source ^a	Host	Locality
<i>Antrodia gossypia</i>			
L-6187	Sp	Conifer log	Arapaho Natl. Forest, Grand Co., Colorado
L-6202	Sp	<i>Picea sp.</i> log	Arapaho Natl. Forest, Grand Co., Colorado
<i>Antrodia juniperina</i>			
WM-284	S	<i>Juniperus virginiana</i> L.	Blain, Perry Co., Pennsylvania
SRM-403	S	<i>Juniperus virginiana</i> L.	Lincoln, Lancaster Co., Nebraska
HHB-8540	S	Living <i>Juniperus deppeana</i> Steud.	Coronado Natl. Forest, Santa Cruz Co., Arizona
FP 71540	R	Dead <i>Juniperus virginiana</i> L.	Port Republic, Calvert Co., Maryland
FP 71583	R	<i>Juniperus virginiana</i> L. post	Arlington, Arlington Co., Virginia
FP 71585	R	<i>Juniperus virginiana</i> L. post	Arlington, Arlington Co., Virginia
FP 71586	R	<i>Juniperus virginiana</i> L. post	Arlington, Arlington Co., Virginia
FP 90014	Sp	Dead <i>Juniperus virginiana</i> L.	Beltsville, Prince Georges Co., Maryland
FP 97452	S	<i>Juniperus procera</i> Hochst. ex Endl. castle beam	Port of Gorgora, Ethiopia, East Africa
FP 103280	R	<i>Juniperus virginiana</i> L. post	Oconee Natl. Forest, Clarke Co., Georgia
FP 103360	S	Living <i>Juniperus virginiana</i> L.	Athens, Clarke Co., Georgia
<i>Antrodia odora</i>			
RLG-6951	R ^b	<i>Pinus ponderosa</i> Doug. ex Laws. log	Paradise Park, Graham Co., Arizona
<i>Antrodia sitchensis</i>			
RLG-15170	Sp	<i>Pinus ponderosa</i> Doug. ex Laws. log	Coconino Natl. Forest, Coconino Co., Arizona
<i>Antrodia sordida</i>			
RLG-9497	S	<i>Picea sp.</i> log	Lake Itasca State Park, Clearwater Co., Minnesota

^a S from basidiocarp tissue. Sp from basidiospore print, and R from rot in host wood.

^b From rot in host wood with associated basidiocarp.

mm diam in 14 da; mats white, appressed, very thin subfelty, near sodden, adherent; margins distinct, even or finely fimbriate; odorless or faintly fragrant; reverse unchanged oxidase reactions negative, mats on GAA at 7 da 11–19 mm diam. no growth on TAA.

Hyphal characteristics.—All hyphae staining in phloxine, thin-walled, mostly simple-septate, with very rare simple clamps, 1.5–4.5(–5.5) μm diam.

Test-tube cultures.—In 28 da mycelium white but so thin that color of substratum shows through, appressed, very thin subfelty covering the slant and extending onto the cylinder; no reverse discoloration.

Temperature relations.—See FIG. 1; optimum 22 C; killed at 36 C.

Type of incompatibility system.—Heterothallic, type unknown.

Cultures studied.—See TABLE I.

Remarks.—The two cultures were isolated in 1955 from basidiospore prints. At that time, as now, clamp connections were very rare in both isolates. The “3r” in the Species code refers to rare simple clamps (Boidin, 1966).

For descriptions of the basidiocarps, see Gilbertson and Ryvarden (1986) and Lowe (1966, as *Poria*).

ANTRODIA JUNIPERINA (Murr.) Niem. *et* Ryv.,
Trans. Brit. Mycol. Soc. **65**: 427. 1975.

Agaricus juniperinus Murr., *Bull. Torrey Bot. Club*
32: 85. 1905.

Daedalea juniperina (Mum.) Murr., *North Amer.*
Flora **9**: 125. 1908.

Key patterns.—A-O-M-1-2-10-16, A-O-M-1-10-16, A-O-M-1-2-11-16.

Species code.—1.3.(8).9.(34).36.38.46-47.
(48).50.55.58.

Growth characteristics.—Growth on MEA moderate, forming mats 24–36 mm diam in 7 da, 72–89 mm diam in 14 da; mats white, appressed, ranging from thin cottony-woolly to thin sub-felty, usually homogeneous, later developing small scattered floccose patches; by 6 wk one isolate developed a raised white mound, with irregular pores and thick dissepiments, from which a white spore print was obtained margins distinct, finely fimbriate; odor fragrant, near coconut; reverse unchanged; oxidase reactions negative, mats on GAA at 7 da 15–22 mm diam, no growth to trace² on TAA.

Hyphal characteristics.—Generative hyphae staining in phloxine, with thin hyaline walls and abundant clamps, 1–4 μ m diam; hyphae with irregularly thickened walls frequently broken at the clamps, lumina staining, well developed in most isolates, poorly developed in others, 3.5–5.5 μ m diam; fiber hyphae few to lacking at 14 da. not present in all isolates by 6 wk, non-branching, with thick non-staining hyaline walls, 1–3 μ m diam; chlamydospores present in most isolates at 7 da, terminal or intercalary, ovoid to elongate, occasionally triangular, with medium thick walls, 8.8–22 \times 6.6–11.5 μ m; crystals small to medium octahedrons and small irregular thin plates.

Test-tube cultures.—In 28 da mats white, appressed, fine thin downy to downy-cottony, covering the slant and extending nearly to the base of the cylinder, becoming thinner toward the base; no reverse discoloration.

Temperature relations.—See FIG. 1; optimum 30 C; not killed at 44 C.

² Less than 11 mm diam including 4 mm inoculum plug.

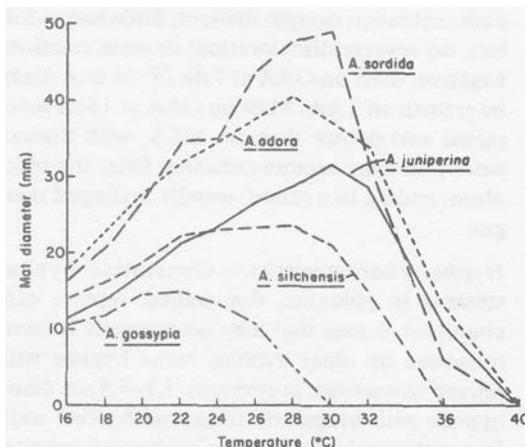


FIG. 1. Average mat diameters of *Antrodia* spp. grown on MEA after 6 da incubation at 11 constant temperatures.

Type of incompatibility system.—Heterothallic, type unknown.

Cultures studied.—See TABLE I.

Remarks.—Niemelä and Ryvardeen (1975) published a description for this species, based on one isolate from East Africa collected in 1973. Our newest isolate is 10 years old, and our oldest isolates have been in culture for more than 50 years. This difference may account for the fresher African isolate filling a Petri dish in 3 wk, while ours filled dishes in 6 wk or more. The Species code reflects these differing growth rates.

Descriptions of the basidiocarps of *A. juniperina* are found in Gilbertson and Ryvardeen (1986), Niemelä and Ryvardeen (1975), and Overholts (1953, as *Daedalea*).

ANTRODIA ODORA (Pk. *ex* Sacc.) Gilbn. *et* Ryv.,
Mycotaxon **22**: 363. 1985.

Poria odora Pk. *ex* Sacc., *Syll. Fung.* **6**: 294. 1888.

Key patterns.—A-O-M-1-2-11-16, A-O-I-1-2-11-16.

Species code.—1.3.8.9.34.36.38.44.55.60.

Growth characteristics.—Growth on MEA medium to moderately rapid, forming mats 48–52 mm diam in 7 da, 80–90+ mm diam in 14 da; mats white, appressed, with scant fine radiating downy aerial mycelium, some isolates becoming raised in woolly or silky patches against side of

dish. adherent; margin distinct, fimbriate; odorless; no reverse discoloration; oxidase reactions negative, mats on GAA at 7 da 28–36mm diam, no growth on TAA. Mats on GAA at 14 da more raised and denser than on MEA, with distinct woolly to silky sectors radiating from the inoculum, ending in a raised, usually scalloped margin.

Hyphal characteristics. —Generative hyphae staining in phloxine, thin-walled, septate with abundant clamps that may occasionally become refractive on older hyphae, some hyphae with refractive wart-like projections, 1.1–5.5 μ m diam; hyphae with irregularly thickened hyaline walls frequently broken at the clamps, lumina staining, 3.3–7.2 μ m diam; fiber hyphae with thick hyaline non-staining walls, few to abundant by 14 da, 1.5–2.7 μ m diam; chlamydospores few, intercalary, ovoid to limoniform, staining, with thin hyaline walls, 6–15.5 \times 3.5–9 μ m; crystals thin irregular plates.

Test-tube cultures.—In 28 da mats white, densely compacted at top of slant, appressed, thin downy on slant and upper part of agar cylinder allowing substratum to show through, becoming long radiating woolly or near silky to bottom of cylinder; reverse at tip of slant Russet.

Temperature relations. —See FIG. 1; optimum 28 C; killed at 44 C.

Type of incompatibility system. - Tetrapolar with the following distribution of incompatibility types among a sample of 16 single basidiospore isolates from dikaryotic isolate RLG-6951-R:

A₁B₁: 1, 6, 7.

A₁B₂: 2, 4, 8, 9.

A₂B₂: 10, 12, 15.

A₂B₁: 3, 5, 11, 13, 14, 16.

Culture studied. —See TABLE I.

Remarks. —Noble(1958) included *A. odora* (as *Poria*) in a group of white-rot fungi with positive oxidase reactions but omitted it from her 1965 paper.

For descriptions of the basidiocarps, see Gilbertson and Ryvarden (1986) and Lowe (1966, as *Poria*).

ANTRODIA SITCHENSIS (Baxt.) Gilbn. et Ryv., *Mycotaxon* 22: 364. 1985.

Poria sitchensis Baxt., *Pap. Michigan Acad. Sci.* 23: 293. 1938.

Key pattern. —A-O-M-1-2-11-16.

Species code. - 1.3.8.9.34.36.38.43.50.55.58.

Growth characteristics. —Growth on MEA medium, forming mats 22–31 mm diam in 7 da, 58–77 mm diam in 14 da; mats white, cleared around inoculum, appressed short downy at 7 da, by 14 da becoming widely zonate, thin subfelty around inoculum alternating with bands of denser subfelty, raised downy-woolly at margin, by 6 wk thin subfelty around inoculum, rest of mat appressed felty; margin distinct, even; odor slight, fruity; no reverse discoloration; oxidase reactions negative, mats on GAA at 7 da 20–37 mm diam, no growth on TAA.

Hyphal characteristics. —Generative hyphae staining in phloxine, septate with abundant simple clamps, 1.5–3.5(4) μ m diam; hyphae with irregularly thickened hyaline walls frequently broken at the clamps, lumina staining, 3.5–6.5 μ m diam; fiber hyphae thick-walled, non-staining, hyaline, 1.5–3 μ m diam; chlamydospores intercalary, staining, with medium-thick hyaline walls, ovoid to elongate-ovoid, 12–30 \times 6–9 μ m.

Test-tube cultures. —In 28 da mat Drab on raised woolly mound at top of slant, rest of mat white, dense cottony covering slant and extending to bottom of cylinder; no reverse discoloration.

Temperature relations. —See FIG. 1; optimum 26 C; killed at 44 C.

Culture studied. —See TABLE I.

Remarks. —Baxter(1938) and Baxter and Varner (1942) gave brief characteristics of this species in culture.

Descriptions of the basidiocarps of *A. sitchensis* are found in Baxter (1938, as *Poria*), Gilbertson and Ryvarden (1986), and Lowe (1966, as *Poria*).

ANTRODIA SORDIDA Ryv. et Gilbn., *Mycotaxon* 19: 143. 1984.

Poria oleagina Overh., *Pa. Expt. Sta. Tech. Bull.* 418: 34. 1943 (invalid name).

Key patterns. —A-O-M-1-2-10-16, A-O-I-1-2-10-16.

Species code. - 1.3.8.9.34.36.38.44.(48).55.59.

Growth characteristics. —Growth on MEA medium to moderately rapid, forming mats 42–58 mm diam in 7 da, (80–)89–90+ mm diam in 14

da; mats white, appressed, with scant fine thin downy aerial mycelium, with faintly distinct tiny streaks radiating toward the margin, homogeneous, adherent at 14 da, by 6 wk mats subfelty around inoculum, with small patches that may coalesce giving the mat a speckled appearance, flat minutely-pored fertile cream-colored fruiting areas may develop around the periphery of the mat in centrally inoculated dishes, peeling readily from the substratum; margin distinct, finely fimbriate; odorless; no reverse discoloration; oxidase reactions negative, mats on GAA at 7 da 28–39 mm diam, no growth on TAA.

Hyphal characteristics. —Generative hyphae staining in phloxine, septate with abundant simple clamps, some hyphae with refractive wart-like projections, 1.5–4.5 μm diam; hyphae with irregularly thickened hyaline walls frequently broken at the clamps, lumina staining, 3.5–6 μm diam; fiber hyphae present by 6 wk, with thick non-staining hyaline walls, 2.5–3 μm diam; chlamydospores terminal or intercalary, ovoid to elongate, staining, with moderately thick hyaline walls, 10–19 \times 7.5–11.5 μm ; crystals small plates, singles or in layers.

Test-tube cultures.—In 28 da mats white, appressed, thin short downy on slant, with a large woolly mound at tip of slant, cottony extending to bottom of cylinder; reverse at tip of slant Tawny.

Temperature relations. —See FIG. 1; optimum 30 C; not killed at 44 C.

Type of incompatibility system. —Bipolar with the following distribution of incompatibility types among a sample of 22 single basidiospore isolates from dikaryotic isolate RLG-9497-S:

A₁: 1, 3, 5, 7, 8, 9, 10, 12, 13, 15, 16, 17, 18, 19, 20, 22, 23.

A₂: 2, 4, 6, 11, 14, 21.

Culture studied. —See TABLE I.

Remarks. —David and Tortic (1984) have described basidiocarps and a culture of *A. sordida* (as *Amyloporiella*). There is a significant difference in the basidiospore measurements between their Polish and Yugoslavian collections and those of North America. The basidiospores from their collections were described as “ellipsoid, 4–5.5(–6) \times 2–2.8 μm ” (David and Tortic, 1984). They reported the spores from the type of *A. sordida* as 4.2–4.9 \times 1.5 μm . Lowe (1966) gave

the basidiospore range for this species as 3.5–5 \times 1.5–2 μm and Gilbertson and Ryvarden (1986) gave the basidiospore size as 4–5 \times 1.5–1.8 μm .

Furthermore, the nodose-septate hyphae of their culture were reported to be “often very irregular with numerous swellings (vesicles) up to 25 μm diam” (David and Tortic, 1984). Vesicles are lacking in our culture, although hyphal tips may be slightly swollen. David and Tortic reported their species to be tetrapolar; our species is bipolar. Their culture has a pleasant odor; our culture is odorless. Based on these differences, I strongly suspect that the species treated by them is not the same as the species treated here.

Descriptions of the basidiocarps of *A. sordida* are found in Gilbertson and Ryvarden (1986) and Lowe (1966, as *Poria oleagina*).

NOTES ON OTHER SPECIES

Sarkar (1959) reported *Antrodia albida* (Fr.) Donk [as *Coriellus sepium* (Berk.) Murr.] to be heterothallic. The species has a bipolar type of incompatibility system, with the following distribution of incompatibility types among a sample of 14 single basidiospore isolates from the dikaryotic culture ME-642:

A₁: 2, 3, 4, 5, 10, 12.

A₂: 1, 6, 7, 8, 9, 11, 13, 14.

I find the Species code to be 1.3.8.9.32.36.38.44–45.(48).54.55.59, which agrees with that of Roy and Mitra (1984, as *Daedalea*).

A cultural description of *Antrodia albobrunnea* (Rom.) Ryv. was published by Lombard and Gilbertson (1965, as *Poria*) based on 2-wk-old cultures. By 6 wk some hyphae developed terminal or intercalary swellings or knoblike side swellings, 6–12 μm diam, and filled with coarse, disintegrating contents. Its Species code is 1.3.9.(26).32.36.(39).45–47.(53).55.

David and Tortic (1984) published a description of the basidiocarps and cultures of *A. alpina* (Litsch.) Gilbn. et Ryv. (as *Amyloporiella*). Their cultures were said to agree with the cultural description by Lombard and Gilbertson (1965, as *Poria*), and matings between European and North American haploids were compatible. I found the Species code, based on our isolates, to be 1.3.8.9.34.36.38.46–47.55.

Antrodia oleracea (Davids. et Lomb.) Ryv. typically occurs on hardwoods in North America, but on several occasions it has been isolated

from coniferous wood products, *e.g.*, redwood [*Sequoia sempervirens* (D. Don.) Endl.] in cooling towers and *Pinus* sp. stakes in decay tests in ground. The odor of old cultures is typically that of rotten cabbage. Freshly isolated cultures frequently develop basidia and basidiospores within 14 da. Even isolates that have been maintained for up to 40 yr may develop fruiting areas by 6 wk. The species has a bipolar type of incompatibility system, with the following distribution of incompatibility types among a sample of 18 single basidiospore isolates from the dikaryotic type culture FP 48282-R:

A₁: 1, 2, 3, 4, 6, 7, 8, 9, 10, 11, 12, 14, 18.
A₂: 5, 13, 15, 16, 17.

A study of 10 isolates incubated at 13 constant temperatures showed the optimum temperature for growth to be 30 C with fair growth at 36 C. The fungus remained viable at 40 C and 44 C. David and Tortic (1984, as *Amyloporiella*), Davidson *et al.* (1942, 1947, as *Poria* sp.), and Stalpers (1978, as *Poria*) studied and described this species in culture. The Species code based on 20 isolates is 1.3.9.34.36.38.44-46.(48).53.54.(55).59.

Cultural characteristics of *Antrodia radiculosa* (Pk.) Gilbn. *et* Ryv. have been published by Lombard and Gilbertson (1965, as *Poria*) and Stalpers (1978, as *Fibroporia*). By 6 wk rare fiber hyphae, with slight- to medium-thick hyaline walls, 1.5–2.2 μm, may develop. The species is tetrapolar with the following distribution of incompatibility types among a sample of 18 single basidiospore isolates from the dikaryotic culture HHB-11414-Sp:

A₁B₁: 6, 13, 15, 17.
A₁B₂: 3, 9, 11, 16.
A₂B₂: 1, 4, 7, 8, 10, 12, 14, 18.
A₂B₁: 2, 5.

The Species code is 1.3.(8).34.36.38.44-46.54.55.60.

Antrodia vaillantii (DC. *et* Lamarck Fr.) Ryv. is tetrapolar with the following distribution of incompatibility types among a sample of 17 single basidiospore isolates from the dikaryotic culture L00-13780:

A₁B₁: 1, 3, 5, 15.
A₁B₂: 11, 13.
A₂B₂: 9, 10, 14.
A₂B₁: 2, 4, 6, 7, 8, 12, 16, 17.

The cultures may be odorless or develop a mushroom odor. Cultural descriptions were published by Cartwright and Findlay (1958), Lombard and Gilbertson (1965), Nobles (1948, 1965), Stalpers (1978), van der Westhuizen (1958) —all as *Poria* —and by Rajchenberg (1983, as *Fibroporia*). The Species code is 1.3.8.(34).36.38.43-44.(48).(53).55.60.

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