

Sexual Compatibility and Asexual Reproduction of *Tremellochaete japonica*

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Summary

A heterobasidiomycete, *Tremellochaete japonica*, was collected from the dead branch of a mangrove tree. Morphological and cultural studies revealed it has bifactorial mating system and produces conidia in annellidic conidiogenesis, a unique form of conidiogenesis among Exidiaceae fungi.

Keywords: bifactorial mating system, conidogenesis, heterobasidiomycete, *Tremellochaete japonica*.

A heterobasidiomycete, *Tremellochaete japonica* (Yasuda) Raitv. ("Tsubukikurage" in Japanese) (Exidiaceae, Auriculariales) has been often collected from dead branches of walnut in Japan, Russia and Papua New Guinea (1, 2) since its first collection from Japan in 1915 (14). However, because no cultural study of this fungus has been done, its no information is available about its pattern of sexual compatibility, the characteristics of its anamorphic state and cultural properties.

In the course of a biodiversity study of subtropical fungi at Amami Is. (28° 15' N, 129° 24' E), Kagoshima, Japan, we found basidiomata of *Tremellochaete japonica* on the dead branch of a mangrove tree. From the specimen, mass spore cultures and single basidiospore cultures were obtained by using a micro-manipulator. The pattern of sexual compatibility was examined by mating pairs of 10 monokaryotic strains. Secondary spore formation from basidiospores and conidium formation of the monokaryotic strains was examined by light and scanning electron microscopy. Both monokaryotic and dikaryotic stains were deposited in the IFO culture collection.

Materials and Methods

Specimen. Basidiomata of *Tremellochaete japonica* on an aerial dead branch of a mangrove tree, *Kandelia candel* Druce, which was overhanging a creek of the Sumiyo River, were collected on 4 March 2000 at Amami Island. Morphology of the basidiomata, basidia and basidiospores was compared with the previous description of this species. A dried specimen of the basidiomata was deposited in the herbarium of IFO.

Isolation. Spore-forming basidiomata were attached to the inside of the lid of a Petri dish containing corn meal agar (CMA) and set overnight. Basidiospores discharged from the basidiomata were isolated with the Skerman-type micro-manipulator. Ten single-basidiospore isolates were obtained and maintained on slants of YMC-1 agar (half-strength corn meal agar, 0.5 % malt extract, 0.1 % yeast extract) (1).

Sexual compatibility test. Pairs of the 10 strains were inoculated ca. 2 cm apart on plates of YMC-2 agar (half-strength corn meal agar, 0.1 % malt extract, 0.05 % yeast extract)(1) in 9-cm Petri dishes. The plates were incubated at room temperature (20–25°C) for 3 weeks. Mycelia in the contact zone of the paired colonies were examined under a light microscope for the presence of clamp connections and stained with alkaline Safranin O to check the nuclear phase (4).

Observation of conidia. Secondary spores produced directly from basidiospores were observed under light microscope. Conidia and conidiophores formed in the monokaryotic cultures on YMC-2 agar were observed under a scanning electron microscope (JSM 5400, JEOL).

Results

Morphology of the collected specimen

Tremellochaete japonica (Yasuda) Raitv., Eesti NSV TA Toimetised, biol. seer. 13: 30, 1964.

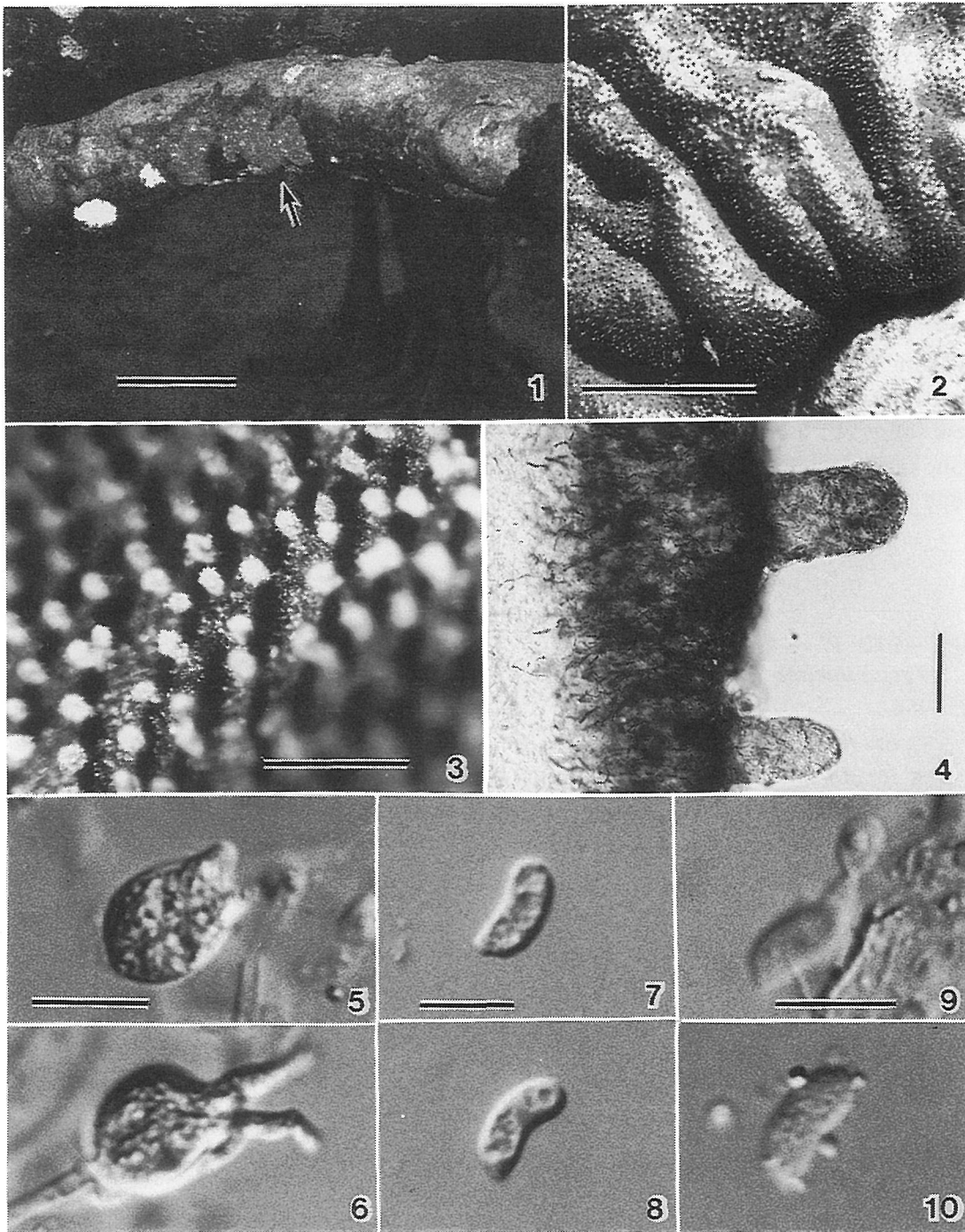
≡ *Exidia japonica* Yasuda in Lloyd, Myc. Writ. 5: 599, 1916.

≡ *Heterochaete japonica* (Yasuda) Kobayasi, Nagaoa 4: 40, 1954.

Basidiomata solitary or gregarious, effused, becoming confluent flat layer, 10×3 cm, fusing with continuous or discontinuous contact lines on the surface, sessile, firm to gelatinous, brownish gray (Figs. 1, 2). Hymenial surface plane to undulate, papillate with numerous hyphal pegs, 128–168 μm high, 56–104 μm in diam, composed of densely packed hyphae (Figs. 3, 4), covered with octahedral crystalline-like structures. Hymenia distributed over whole outer surface. Hypobasidia ovate to obpyriform, 10–14×8–11 μm, with cruciate longitudinal septa (Fig. 5). Epibasidia cylindric, tapering, 10–22 μm long, 2–4 μm in diam at the broadest point (Fig. 6). Basidiospores allantoid to reniform, 10–16×4–6 μm, hyaline, aseptate (Figs. 7, 8), germinating by germ tube or by secondary spore (ballistospore and microconidia) formation. Ballistospores were formed by repetition (Fig. 9). Microconidia (1×0.5 μm) were formed in blastic fashion from basidiospores (Fig. 10).

Colony of monokaryotic isolates reached 43–90 mm in diam on YMC-2 agar medium after 20 days of incubation at 20–25°C. Colonies hyaline on YMC-2 agar, but brown pigment was excreted on potato sucrose agar (PSA). Conidia were produced on a mononematous conidiophore, as described in detail below.

Specimen examined: IFO H-12240, on a dead branch of *Kandeliae candel*, Sumiyo River, Amami Is., Kagoshima, Japan, 4 March 2000.



Figs. 1-10. *Tremellochaete japonica*. 1. Basidiomata (arrow) on dead branch of *Kandelia candel* (in the field). 2. Surface of basidioma. 3. Close-up view of basidioma showing papillate surface with pegs. 4. Pegs. 5. Young basidium (hypobasidium). 6. Basidium with epibasidia. 7, 8. Basidiospores. 9. Basidiospore with a sterigma, from which a ballistospore was formed. 10. Basidiospore forming blastic microconidia. Scale bars: 1 = 10 cm; 2 = 5 mm; 3 = 500 μ m; 4 = 50 μ m; 5 (=6), 7 (=8), 9 (=10) = 10 μ m.

Table 1. Mating reactions among 10 monokaryotic strains of *Tremellochaete japonica*.

Mating type		A1B1				A1B2	A2B2		A3B1		A3B2
Mating type	Tj-No.	3	7	8	9	2	4	10	1	5	6
A1B1	3	-	-	-	-	-	+	+	PC	PC	+
	7	-	-	-	-	-	+	+	PC	PC	+
	8	-	-	-	-	-	+	+	PC	.*	+
	9	-	-	-	-	-	+	+	PC	.*	+
A1B2	2	-	-	-	-	-	PC	PC	+	+	PC
A2B2	4	+	+	+	+	PC	-	-	+	+	PC
	10	+	+	+	+	PC	-	-	+	+	PC
A3B1	1	PC	PC	PC	PC	+	+	+	-	-	-
	5	PC	PC	.*	.*	+	+	+	-	-	-
A3B2	6	+	+	+	+	PC	PC	PC	-	-	-

+, true-clamp formation; PC, pseudo-clamp formation; -, no reaction.

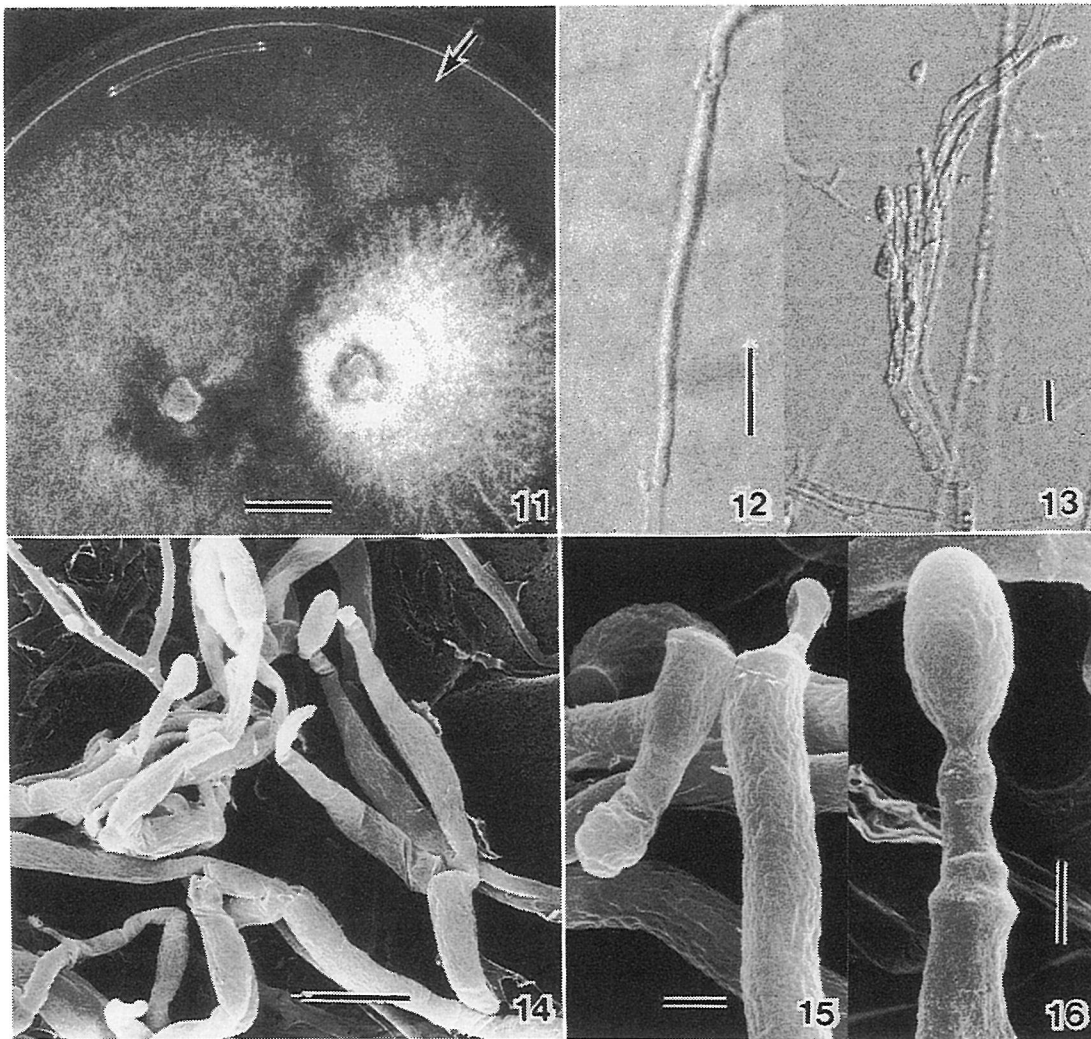
*, unexpected reaction.

Sexual compatibility

The mating tests showed this species has a bifactorial mating pattern, i.e., monokaryotic strains mated and formed true clamp connections on the secondary hyphae when both A and B mating factors were compatible, but they formed pseudoclamps when the factor A was compatible and the factor B was incompatible, and no clamps when the factor A was incompatible, even when the factor B was compatible (Table. 1). The 10 monokaryotic strains tested were found to include 4 strains of A1B1, 1 strain of A1B2, 2 strains of A2B2, 2 strains of A3B1 and 1 strain of A3B2. No strain of A2B1 type was detected, probably due to the small number of isolates tested. The presence of A3B1 and A3B2 type strains can be explained as the result of isolation of basidiospores from different basidiomata. Typical clamp connections were observed on the hyphae in the contact zone of the matching pair. The secondary hyphae grew faster than the primary hyphae (Fig. 11). Incompatible mating resulted in pseudoclamp formation (Fig. 12) or no response. Since nuclear staining reaction with Safranin O was not stable in this experiment, matching was confirmed mostly by repetitive examination of the presence of true or pseudoclamp connections for over one month. The following five representative strains of each mating type were deposited in the IFO culture collection: Tj-8=IFO 33184 (A1B1), Tj-2=IFO 33185 (A1B2), Tj-4=IFO 33186 (A2B2), Tj-1=IFO 33187 (A3B1), Tj-6=IFO 33188 (A3B2). A culture derived from mixed basidiospores was also deposited in the collection as IFO 33189 (=Tjmass-2).

Conidium formation

Conidia were produced in the monokaryotic cultures, among which the strain Tj-8 (IFO 33184) formed conidia abundantly. Conidiophores are mononematous, branched, 28-110 μm long, 2-6 μm in diam, and often the composing branches are detached from the septa (Figs. 13-15). Conidiogenous cells taper toward the apex and proliferate percurrently by successive formation of conidia, which leaves annellation at the apex of the cell (Fig. 16). Conidia are blastic, obovate, elliptical or reniform, aseptate, 6-13 \times 3-7



Figs. 11-16. *Tremellochaete japonica* in culture. 11. A matching pair forming fast growing secondary hyphae (arrow). 12. Pseudoclamp connections. 13. Conidia and conidiophore. 14-16. Scanning electron micrographs of conidiophores and conidia. 14. Branching conidiophores. 15. Conidiogenous cell detaching at septum. Note a new conidiogenous cell is growing from the septum. 16. Conidiogenous cell showing annellation with a newly forming conidium at the apex. Scale bars: 11=1 cm; 12-14=10 μ m; 15, 16=2 μ m.

μ m, hyaline.

Discussion

The morphology of the mangrove material of this fungus fits well with the previous descriptions (e.x., 1), though the pegs on the hymenial surface are larger than in the material observed by Yasuda (1917) and Kobayashi (1954) (100-150 \times 80-200 μ m and 90-

110×45–70 μm , respectively). This fungus has been reported from wide range of geography, i.e., Japan (Miyagi, Iwate, Nagano, Okinawa), Russia (Vladivostok) and Papua New Guinea (1). In Japan, it has often been found on broad-leaf trees, especially walnut, *Juglans mandshurica* Maxim. var. *sieboldiana* Makino (3, 14). This is the second report of this fungus from a mangrove tree since its collection by Nakagiri (T. Aoki bB1) from Iriomote Is., Okinawa in Feb. 1984 (1).

The present study revealed that *T. japonica* has a bifactorial mating system. Genera in the family Exidiaceae show unifactorial or bifactorial systems. *Exidia glandulosa* (Bull.) Fr., *E. recisa* Ditm., *E. saccharina* Fr. and *E. repanda* Fr. were found to have unifactorial systems (2, 5), whereas other species of *Exidia*, such as *E. candida* Klett, *E. populina* Klett and *E. pithya* Fr., were reportedly bifactorial (2). Bifactorial systems were also demonstrated in *Exidiopsis plumbescens* (Burt) Wells (12) and *Myxarium nucleatum* Wallr. (7). Among the order Auriculariales, species of the genus *Auricularia* Bull. ex Juss. were reported to have unifactorial or bifactorial mating systems (6, 13). Thus, both mating systems coexist in a genus and family within the Auriculariales.

Conidium formation on monokaryotic hyphae of *T. japonica* was observed for the first time in this study. This species showed annellidic conidiogenesis. Phialidic conidial formation has been observed among *Exidia* species and related heterobasidiomycetes. *Exidia glandulosa* and *E. thuretiana* (Lév.) Fr. were reported to produce C-shaped conidia in succession from a phialide (8, 9). This type of conidial formation was also observed in *E. uvapassa* Lloyd, *A. auricula* (Hook.) Underw. and *Heterochaete delicata* (Kl. ex Berk.) Bres. (1). On the other hand, *E. recisa* produces rod-shaped conidia on a phialide (10). Thus, *T. japonica* is unique in its conidiogenesis among Exidiaceous fungi and this may support delimitation of *Tremellochaete* from the allied genus *Exidia*, but further research and reexamination of conidiogenesis under a scanning electron microscope are necessary to clarify the taxonomic implication of conidiogenesis in this group of fungi.

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