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Short Communication

Cerrena unicolor isolated from the mycangia of a horntail, *Tremex longicollis*, in Kochi Prefecture, Japan

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A fungus was found to be stored in the mycangia of a horntail, *Tremex longicollis*, as hyphal fragments. All fungal isolates from the mycangia of 31 adult females of the horntail produced the same colonies on PDA. Basidiocarps of *Cerrena unicolor* occurred near the emergence hole of the horntail on a dead hackberry tree (*Celtis sinensis*). The cultures of this fungus were similar to those from the mycangia of the horntail in cultural characteristics. Mating between single-basidiospore mycelia of *C. unicolor* and single-arthrospore mycelia from the mycangia of the horntail showed that they were compatible. These results revealed that the fungus isolated from the mycangia of *T. longicollis* was *C. unicolor*.

Key Words——*Celtis sinensis*; *Cerrena unicolor*; horntail; mating tests; *Tremex longicollis*.

We found horntails (*Tremex longicollis* Konow) occurring on *Celtis sinensis* Per. in October in 1994. All horntails, which belong to the Tremecinae, attack broad-leaved trees (Okutani, 1967). Stillwell (1964) found that one of the horntails in the Tremecinae, *Tremex columba* (L.), infested *Fagus grandifolia* Ehrh. in New Brunswick, Canada and was associated with the fungus *Cerrena unicolor* (Fr.) Murr. There have been no reports about the fungus associated with the horntails of the Tremecinae in Japan. We isolated and studied fungi from the mycangia of horntails and those from a fungal fruit body on a dead hackberry tree. The purpose of this paper is to identify the fungus associated with *T. longicollis*.

Many fungal fruit bodies occurred near the emergence hole of *T. longicollis* on the trunk of a dead hackberry tree (*C. sinensis*) in October in 1994 (Fig. 1). These were collected, and the fungus was identified as *C. unicolor*.

Eighteen adult males and 31 adult females of *T. longicollis* (Fig. 2), which emerged from the dead hackberry tree, were caught by means of a net covered on the trunk from the 5th to 19th Oct. 1994, at Ino Town, Kochi Prefecture. Collected horntails were put in the −20°C freezer for 15-30 min to paralyze them and dissected under a stereoscopic microscope. Fungi taken from the mycangia were mounted in lactophenol and observed on microscopic features under a light microscope. The 31 adult females of *T. longicollis* were found to have mycangia filled with hyphal fragments (Fig. 3), whereas the 18 adult males apparently lacked mycangia. Stillwell (1964) reported that the mycangia of the horntail were filled with arthrospores. However, we found the fungus in the mycangia to be present as hyphal fragments, in which cells were not separate, but joined by clamp connections.

Mycangia were partly removed from dissected horntails with two sterilized forceps under a stereoscopic microscope, put on potato-dextrose agar (PDA, Eiken) in Petri plates, and incubated at 20°C in darkness for 4–7 days. Mycelia arising from the mycangia in Petri plates were transferred onto PDA in Petri plates and incubated at 25°C in darkness to examine the cultural characteristics. Basidiospores from collected fruit body were isolated on PDA in Petri plates by the method of Aoshima (1983), and basidiospores which fell on PDA in Petri plates from the fruit body, were cultured on PDA slants. The isolates were transferred onto PDA in Petri plates and incubated at 25°C in darkness to examine the cultural characteristics. All the isolates from the mycangia of 31 adult females of *T. longicollis* produced the same colonies on PDA, suggesting that a single species of fungus was stored in the mycangia of the horntail. The cultures originating from the basidiospores of *C. unicolor* were similar to those from the mycangia of the horntail in cultural characteristics. Both cultures of *C. unicolor* and cultures from the mycangia of *T. longicollis* produced cottony, finally subfelty, white mycelia on PDA (Fig.4), with an offensive odor. Generative hyphae and fiber hyphae were produced in these cultures and mycelia filled the Petri plates after 7–10 days at 25°C in darkness. The reverse of these cultures was bleached. The widths of hyphae in advancing zone, submerged hyphae, and fiber hyphae were 2–5 μm, 1.5–4.5 μm, and 1–2 μm respectively. The cultural characteristics of cultures of *C. unicolor* and cultures from the mycangia of *T. longicollis* were in Nobles's (1965) and Stalpers's (1978) species codes as follows. Nobles's species code: 2, 3, 8, 32, 36, 38, 40, 41, 42, 53, 54, 59. Stalpers's species


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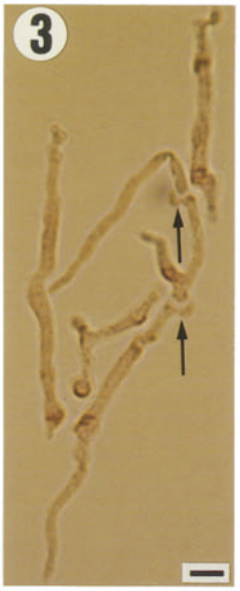
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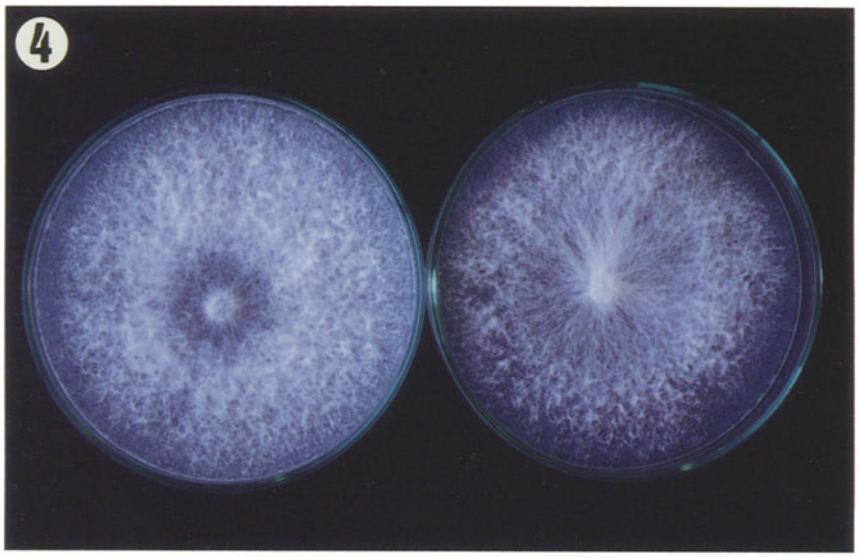
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Table 1. Intracollection matings between single-spore isolates of *Cerrena unicolor*.

Isolates	2	3	6	7	11	12	13	15	17	19	1	4	5	8	9	10	16	18	21
1	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−
4	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−
5	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−
8	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−
9	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−
10	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−
16	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−
18	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−
21	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−
2	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+
3	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+
6	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+
7	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+
11	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+
12	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+
13	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+
15	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+
17	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+
19											+	+	+	+	+	+	+	+	+

+, compatible mating, with clamp connections;
−, incompatible mating, in which clamp connections were not formed.

code: 1, 2, 3, (6), (12), (13), 19, 21, 22, (24), 27, 30, 37, 39, 45, (46), 48, 52, 53, (83), 89, 93. The cultural characteristics of the isolates from the fruit body and from the horntail were almost in accord with those for *C. unicolor* reported by Nobles (1948, 1965) and Stalpers (1978).

Basidiospores which fell on PDA in Petri plates from the fruit body of *C. unicolor* following the method of Aoshima (1983) were suspended in sterilized-distilled water, and single basidiospores were isolated with a micromanipulator (Leitz) and cultured on PDA slants. Nineteen isolates were transferred onto PDA in Petri plates and incubated at 25°C in darkness for intracollection pairings. Mycelia from single basidiospores were paired by inoculating agar disks of mycelia at a distance of 30-40 mm apart on PDA in Petri plates and incubated for 1 week at 25°C in darkness. Mycelia were taken from the contact zone and observed under a light microscope for the presence or absence of clamp connections. The results of analysis of intracollection parings are shown in Table 1. *Cerrena unicolor* was heterothallic bipolar, as reported by Nobles (1965).

After the analysis of intracollection parings, one isolate of each mating type was chosen to serve as a tester strain to be mated with single-arthrospore isolates from the mycangia of horntails. Arthrospores were induced by culturing mycelia, that had been isolated from the mycangia of horntails, on PDA plates containing 1% gall powder (Nacalai) following the method of Takemaru (1964). Single-arthrospores were isolated by using a micromanipulator and incubated on PDA plates for 1 week at 25°C in darkness. Mating tests between tester strains (CU-12, 18) of *C. unicolor* and single-arthrospore isolates (1, 2, 3, 4, 5) from the mycangia of *T. longicollis* were performed in the same manner as mentioned above. The results are shown in Table 2. Matings between CU-12 and single-arthrospore isolates were either compatible or incompatible, while those with CU-18 were compatible (Fig. 5). This result proves that the fungus isolated from the mycangia of *T. longicollis* is conspecific with *C. unicolor*. The species of horntails in Kochi Prefecture, Japan was different from that of horntails in Canada, but the fungus stored in the mycangia of the two horntails was the same species. Many fungal species are known

Fig. 1. Basidiocarps of *Cerrena unicolor* occurring near the emergence hole of *Tremex longicollis* on a dead hackberry tree. Scale bar = 1 cm.
Fig. 2. Adult female of *T. longicollis*. Scale bar = 1 cm.
Fig. 3. Hyphae with clamp connections (arrows) from the mycangia of *T. longicollis*. Scale bar = 5 μm.
Fig. 4. Cultures from the mycangia of *T. longicollis* (left) and those of *C. unicolor* (right), grown on PDA at 25°C in darkness for 1 week.
Fig. 5. Compatible mating, which gave rise to mycelia with clamp connections in the contact zone (arrow). Isolates CU-18 (left) × 1 (right).

Table 2. Intercollection matings between two tester strains and five arthrospore isolates.

Isolates	Tester strains	
	CU-12	CU-18
1	+	+
2	+	+
3	−	+
4	−	+
5	−	+

+, compatible mating, with clamp connections;
−, incompatible mating, in which clamp connections were not formed.

to be associated with insects (Gilbertson, 1984), but there have been no reports about the fungus associated with horntails of the Tremecinae since Stillwell (1964). This is the first report about the fungus associated with horntails of the Tremecinae, exclusive of the report in Canada.

All matings between CU-18 and single-arthrospore isolates showed compatibility. The horntails, which emerged from one dead hackberry tree, appeared to have two or more different compatible factors of *C. unicolor*. Further studies are required to examine how many compatible factors there are in one dead hackberry tree infested by horntails.

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