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Armillaria jezoensis, a new symbiont of *Galeola septentrionalis* (Orchidaceae) in Hokkaido

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Nine *Armillaria* isolates obtained from the roots of *Galeola septentrionalis* in Hokkaido were identified as *A. jezoensis* by means of mating tests. Cultures of these isolates were similar in colony morphology, mycelial growth and rhizomorph formation on each of malt extract-dextrose agar (MDA), potato-dextrose agar (PDA), and *G. septentrionalis* root extract-dextrose agar (GDA) media, showing better mycelial growth and rhizomorph formation on GDA medium.

Key Words——*Armillaria*; *Armillaria jezoensis*; biological species; *Galeola septentrionalis*; symbiotic association.

A symbiotic relationship between *Armillaria mellea* (Vahl: Fr.) Kummer and an achlorophyllous orchid, *Galeola septentrionalis* Rechb. f. (Fig. 1) was first reported by Hamada (1940). Recent studies have shown that this fungus is really a complex of several biological species in North America (Anderson and Ullrich, 1979; Anderson et al., 1980), Europe (Korhonen, 1978) and Australia (Kile and Watling, 1983). In Japan, based on mating studies, six biological species of the *A. mellea* complex have been recorded from Honshu and Hokkaido, respectively (Cha and Igarashi, 1994, 1995 a; Cha et al., 1992, 1994, 1995; Nagasawa et al., 1991). Five biological species, *A. ostoyae* (Romagnesi) Herink, *A. gallica* Marxmüller & Romagnesi (as *A. bulbosa* (Barla) Kile & Watling), *A. jezoensis* Cha & Igarashi, *A. sinapina* Bérubé & Dessureault and *A. singula* Cha & Igarashi, associated with *Gastrodia elata* Bl. were reported by Cha et al. (1995b) in Hokkaido. Terashita and Chuman (1989) found four biological species, *A. borealis* Marxmüller & Korhonen, *A. gallica*, *A. cepistipes* Velenovsky and *A. mellea* subsp. *nipponica* Cha & Igarashi (as Japanese *A. mellea* s. s.), among *Armillaria* isolates obtained by them from *G. septentrionalis* in southern Kyushu.

In this paper we report *A. jezoensis* Cha & Igarashi (Cha et al., 1994) as a new symbiont *Armillaria* species of *G. septentrionalis* in Hokkkaido and the cultural characteristics of the Hokkaido isolates from the orchid.

Materials and Methods

Collection of *G. septentrionalis* The root samples of *G. septentrionalis* were collected from nine sites at Kaminokuni-cho, southern Hokkaido during November of 1992 and 1993 (Table 1). Host trees of symbiotic *Armillaria* were determined by tracing rhizomorphs. *Galeola septentrionalis* roots were distributed in soil at the depth of 10–15 cm, where many *Armillaria* rhizomorphs were distributed. The root surface was at-

Table 1. Origins of vegetative (diploid) isolates of *Armillaria* from *Galeola septentrionalis*.

Armillaria isolate	*G. septentrionalis* collection				
	Date	Location	Host[a)]	Habitat	
HUG9293	11/16/92	Kaminokuni	Un	Cj plantation (broad-leaved forest in origin),	12 cm in depth[b)]
HUG9294	11/16/92	Kaminokuni	Un	Cj plantation (broad-leaved forest in origin),	10 cm in depth
HUG9295	11/16/92	Kaminokuni	Un	Cj plantation (broad-leaved forest in origin),	15 cm in depth
HUG9296	11/16/92	Kaminokuni	Qm	Cj plantation (broad-leaved forest in origin),	10 cm in depth
HUG9297	11/16/92	Kaminokuni	Fc	Cj plantation (broad-leaved forest in origin),	15 cm in depth
HUG9298	11/16/92	Kaminokuni	Un	Cj plantation (broad-leaved forest in origin),	10–15 cm in depth
HUG93130	11/19/93	Kaminokuni	Fc	Broad-leaved forest (Fc), 10–15 cm in depth	
HUG93131	11/19/93	Kaminokuni	Ub	Cj plantation (broad-leaved forest in origin),	10 cm in depth
HUG93134	11/19/93	Kaminokuni	Fc	Cj plantation (broad-leaved forest in origin),	10 cm in depth

a) Cj, *Cryptomeria japonica* D. Don; Fc, *Fagus crenata* Bl.; Qm, *Quercus mongolica* Fisch. var. *grosseserrata* Rehd. & Wils.; Un, unidentified broad-leaved trees. Host tree identification was determined by tracing *Armillaria* rhizomorphal connection between roots of *G. septentrionalis* and decayed stumps and / or roots of trees.
b) Distribution of the roots in soil.

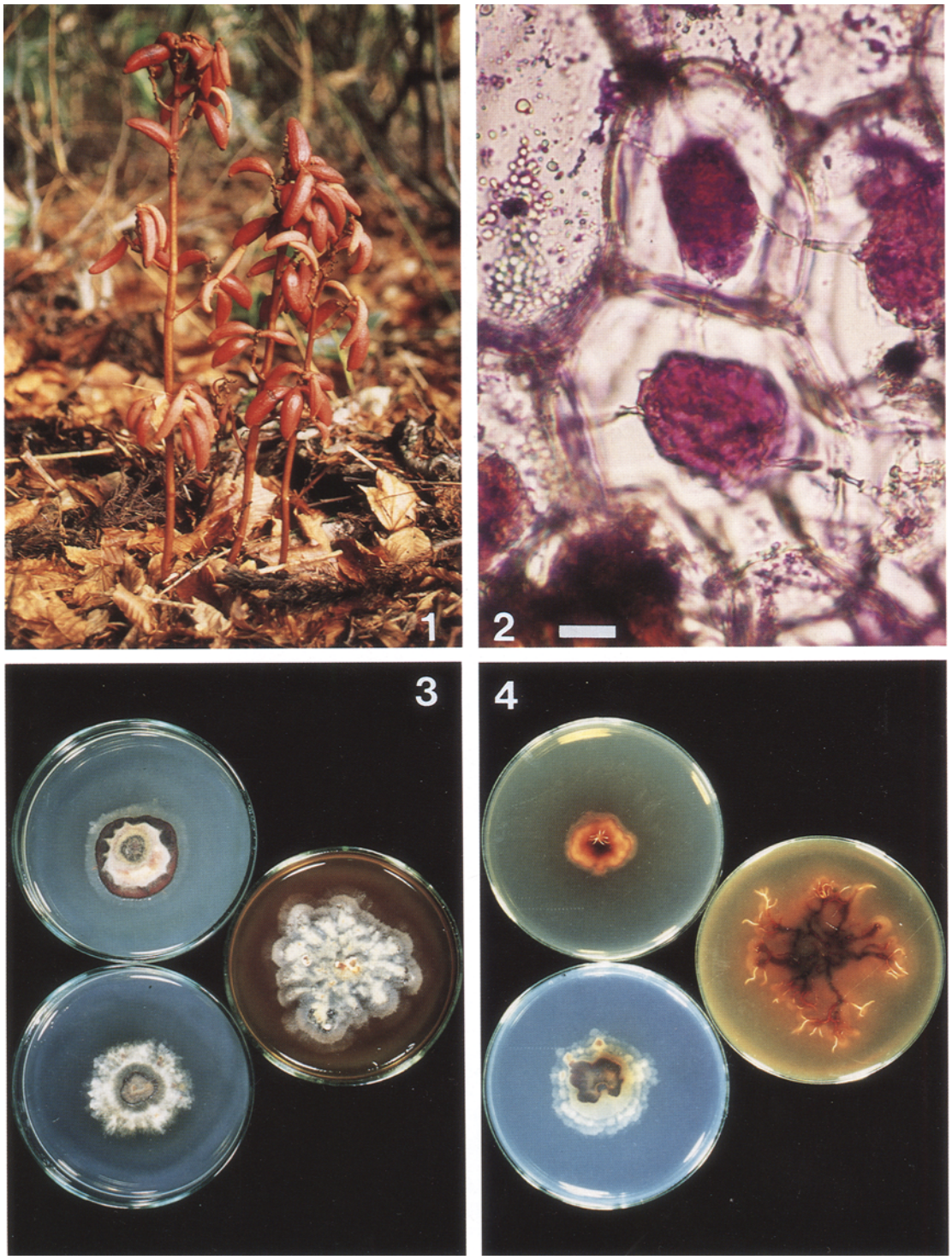

Fig. 1. *Galeola septentrionalis* in the habitat.
Fig. 2. Mycelial coils of *Armillaria jezoensis* formed in the cortical cells of a root of *G. septentrionalis* (bar = 10 μm).
Figs. 3, 4. Surface (3) and reverse views (4) of the colony morphology of cultures of *A. jezoensis* isolated from *G. septentrionalis* on MDA (upper), PDA (bottom) and GDA (right).

tached and penetrated by rhizomorphs and hyphal coils were observed within cortical cells of the roots under the microscope (Fig. 2).

Isolation An unknown *Armillaria* symbiont of each *G. septentrionalis* collection was isolated on 1.8% agar-agar medium either from hyphal coils taken from the cortical cells of the roots or from rhizomorphs attached to the surface of roots. Stock cultures of these vegetative

Table 2. Origins of haploid testers from Hokkaido *Armillaria*.

Biological species	Isolates	Host trees
A. ostoyae	HUA9112	*Betula ermanii*
	HUA9113	*Betula ermanii*
A. gallica	HUA9102	*Fraxinus mandshurica* var. *japonica*
	HUA9125	*Prunus ssiori*
A. jezoensis	HUA9116	*Ulmus japonica*
	HUA9127	*Quercus mongolica* var. *grosseserrata*
A. sinapina	HUA9115	*Salix sachalinensis*
	HUA9124	*Ulmus japonica*
A. singula	HUA9101	*Fraxinus mandshurica* var. *japonica*
	HUA9109	*Abies sachalinensis*

(diploid) isolates were maintained on a malt agar medium in Petri dishes at 25°C in the dark.

Culture studies Malt dextrose agar (MDA: 30 g of malt extract, 20 g of dextrose, 15 g of agar in 1000 ml of distilled water), potato-dextrose agar (PDA: 39 g of potato-dextrose agar (Nissui) in 1000 ml of distilled water) and orchid-dextrose agar (GDA: 20 g of dextrose, 15 g of agar in 1000 ml of *G. septentrionalis* root extract) media were used in this study. The orchid root extract for GDA was prepared by boiling 200 g of the dried roots in 1000 ml of distilled water in a flask for 3 h, then filtering the resultant liquid through gauze and bringing volume up to 1000 ml with distilled water. Inoculation plugs (5 mm in diam) for each *Armillaria* isolate were cut from stock culture plates and inoculated in the center of each Petri dish containing 15 ml of agar medium. For each isolate, three plates of each medium were inoculated and incubated at 25°C for 1 mo in the dark.

Identification of species The unknown *Armillaria* vegetative (diploid) isolates from *G. septentrionalis* were mated with haploid tester strains of *Armillaria* biological species known in Hokkaido. Testers are listed in Table 2. Matings were performed by placing inoculum blocks 1 mm apart on a 1.25% malt agar Petri plates (one pair per plate), which were incubated at 22°C for about 4 wk. The results of mating tests were judged by the methods of Guillaumin et al. (1991).

Table 3. Comparison of mycelial growth and rhizomorph formation among *Armillaria* isolates from *G. septentrionalis* in three culture media (30 d, incubation in the dark, 25°C).

Isolate (diploid)	Media[a]					
	MDA		PDA		GDA	
	1	2	1	2	1	2
HUG9293	38.2	−	22.5	+	60.7	+++++
HUG9294	44.8	−	24.3	+	66.0	+++++
HUG9295	40.7	−	30.8	++	57.5	+++++
HUG9296	45.0	−	33.5	+	46.0	++++
HUG9297	39.0	−	33.8	+	55.0	+++++
HUG9298	40.0	−	34.2	−	41.5	++++
HUG93130	44.5	−	39.7	+	55.7	+++++
HUG93131	38.3	−	24.7	+	59.2	+++++
HUG93134	38.7	−	24.3	+	62.5	+++++

a) 1: Diameter of colony (mm; mean value of three plates), 2: Rhizomorph formation: +, recognized; −, not recognized; number of + shows the degree of rhizomorph production.

Results

Culture studies Cultures of all isolates on each of the three media used were similar in colony morphology, mycelial growth and rhizomorph formation (Table 3). Mycelial growth and rhizomorph formation were promoted on GDA containing *Galeola* root extract (Figs. 3, 4). Although MDA yield better mycelial growth than PDA,

Table 4. Results of mating tests between *Armillaria* vegetative (diploid) isolates from *G. septentrionalis* and haploid testers originating from Hokkaido *Armillaria*.

Isolate (diploid)	Testers[a]									
	A. ostoyae		*A. gallica*		*A. jezoensis*		*A. sinapina*		*A. singula*	
	1	2	3	4	5	6	7	8	9	10
HUG9293	−	−	−	−	+	+	−	−	−	−
HUG9294	−	−	−	−	+	+	−	−	−	−
HUG9295	−	−	?	−	+	+	−	?	−	−
HUG9296	−	−	−	−	+	+	−	?	−	−
HUG9297	−	−	?	−	+	+	−	?	−	−
HUG9298	−	−	?	−	+	?	−	−	−	−
HUG93130	−	−	−	−	+	?	−	−	−	−
HUG93131	−	−	−	−	+	+	−	−	−	−
HUG93134	−	−	−	−	+	+	−	−	−	−

a) Number of strains: 1, HUA9112; 2, HUA9113; 3, HUA9107; 4, HUA9125; 5, HUA9116; 6, HUA9127; 7, HUA9115; 8, HUA9124; 9, HUA9101; 10, HUA9109: +, compatible pairing; ?, uncertain pairing, no interpretation possible; −, incompatible pairing.

rhizomorph formation was not recognized on MDA.

Identification of species The results of mating tests of the nine *Armillaria* isolates with haploid testers of Hokkaido *Armillaria* are detailed in Table 4. All the isolates were compatible with testers of *A. jezoensis*, although a few uncertain pairing reactions with testers of *A. gallica* (in HUG9295 and HUG9297) and *A. sinapina* (in HUG99295, HUG9296 and HUG9297) were observed.

Discussion

The results of mating tests (Table 4) suggest that *A. jezoensis* has a symbiotic association with *G. septentrionalis* in Hokkaido. So far we know, *A. jezoensis* is known only from Hokkaido. Although only *A. jezoensis* was detected among *Armillaria* isolates from *G. septentrionalis* in this study, further studies using more isolates from wider areas of Hokkaido may reveal that other members of the *A. mellea* complex in Hokkaido also have symbiotic relationships with the orchid, because the present study is based on material from a limited area and, further, *A. gallica*, *A. cepistipes* and *A. mellea* subsp. *nipponica*, which have been reported by Terashita and Chuman (1989) as symbionts of *G. septentrionalis* in southern Kyushu, are distributed in Hokkaido (Cha et al., 1992, 1994; Cha and Igarashi, 1995a; Mohammed et al., 1994).

In this study all collections of *G. septentrionalis* except one from broad-leaved forest were made in *Cryptomeria japonica* D. Don plantations, but *A. jezoensis* rhizomorphs attached to the orchid roots were observed to connect with decayed roots and/or stumps of broad-leaved trees (see Table 1) in the plantations, not with those of *C. japonica*.

Sagara and Takayama (1978) reported the root system of *G. septentrionalis* in situ based on their observation in Kyoto Prefecture. According to them, although the symbiont *Armillaria* species was not identified, the orchid roots were distributed at depths of 5–15 cm in soil and all the roots except the vigourously growing tips were infected by rhizomorphs of *A. mellea* s. l. In the association of *A. jezoensis* and *G. septentrionalis* observed by us, the orchid roots were distributed in soil at the depth of 10–15 cm, and many rhizomorphs were found around and on the roots, agreeing well with the case observed in Kyoto by Sagara and Takayama (1978). Why *Armillaria* rhizomorphs develop well around and on roots of *G. septentrionalis* remains unknown. In relation to this phenomenon, it might be noteworthy that the development of rhizomorphs of *A. jezoensis* was highly promoted on GDA medium containing the *Galeola* root extract in comparison with on MDA and PDA (Table 3).

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