Cultural Characteristics of Biological Species of *Armillaria from Hokkaido, Japan*

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北海道産ナラタケの生物学的種の培養特性

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Summary

Mycelial growth and rhizomorph formation of *Armillaria* species from Hokkaido were studied on different media. *Armillaria ostoyae*, *A. jezoensis*, *A. sinapina*, *A. singula* and *A. mellea* subsp. *nipponica* produced *Armillaria* rhizomorphs on PDA and PCDA. *Armillaria gallica*, however, formed no rhizomorphs on MDA, YDA, PDA and PCDA. All *Armillaria* species, except *A. gallica*, showed extra mycelial growth and rhizomorph formation on SRB (sawdust-rice bran) from broad-leaved trees. Mycelial growth and rhizomorph formation of all species were promoted on *L. kaempferi* SRB. Their rhizomorph formation on *A. sachalinensis* SRB and *P. jezoensis* SRB were inhibited. Mycelial growth and rhizomorph formation were promoted for all species on BMDA (bark extract-MDA), except *A. ostoyae* and *A. gallica* on *B. platyphylla* var. *japonica* BMDA. *Armillaria gallica*, which did not form rhizomorphs on other media, showed the promotion of rhizomorphs on BMDA of coniferous trees. *Armillaria* species which two new species, *A. jezoensis* and *A. singula*, and a new subspecies, *A. mellea* subsp. *nipponica*, from Hokkaido showed better mycelial growth and rhizomorph formation than other species on medium amended with bark extract and sawdust from local trees.

Key words: *Armillaria*, biological species, mycelial growth, rhizomorph formation, cultural characteristics

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I. Introduction

Species identification of wood-decaying fungi on the basis of their cultural characteristics has proved valuable in forest pathological research. *Armillaria* species are known to be variable in culture (Gibson, 1961; Raabe, 1966), although cultural characteristics such as colony forms and ability to fruit in culture are species characteristic in some cases (Guillaumin *et al.*, 1989; Mwangi *et al.*, 1989; Sung *et al.*, 1990). In Hokkaido, six biological species of *Armillaria* have been identified and these have also been described in conventional taxonomic terms (Cha *et al.*, 1992, 1994, 1995; Cha and Igarashi, 1994, 1995). This study was undertaken to clarify the cultural characteristics of six species of Hokkaido *Armillaria* on several media.

II. Materials and Methods

Eleven diploid isolates of six biological species were used in this study (Table 1). The media used are listed in Table 2. For bark extract, 200 g of fresh bark of six tree species was boiled in 1,000 ml distilled water for 3 hours. The boiled solutions were filtered with gauze and diluted to 1,000 ml with distilled water. Six tree species, *Fagus crenata*, *Quercus mongolica* var. *grosseserrata*, *Betula platyphylla* var. *japonica*, *Abies sachalinensis*, *Picea jezoensis* and *Larix kaempferi* used in this study for sawdust and bark extract media.

The stock cultures were maintained on MA medium in petri dishes at 5 °C in the dark. For inoculation, a mycelial plug (ϕ 5 mm) from the margin of the stock culture was removed and placed upside down on the medium. The cultures were grown in petri dishes (ϕ 90 mm) with 15 ml MDA, YDA, PDA and PCDA and incubated at 25°C for 16 days in the dark. For cultures on SRB media, 20 g of each medium were placed in 90 mm glass petri dishes and autoclaved at 121°C for one hour. After inoculation,

Table 1. Origin of diploid isolates of the six biological species of *Armillaria*

Biological species	No. of isolates	Host trees
A. ostoyae (Romagnesi) Herink	HUA9112	*Betula ermanii* Cham.
	HUA9232	*Fraxinus mandshurica* Rupr. var. *japonica* Maxim.
A. gallica Marxmüller & Romagnesi	HUA9102	*F. mandshurica* var. *japonica*
	HUA9107	*B. ermanii*
A. jezoensis Cha & Igarashi	HUA9116	*Ulmus japonica* Nakai
	HUA9120	*U. japonica*
A. sinapina Bérubé & Dessureault	HUA9115	*Salix sachalinensis* Fr. Schm
	HUA9117	*Quercus mongolica* Fisch. var. *grosseserrata* Rehd. & Wils.
A. singula Cha & Igarashi	HUA9101	*F. mandshurica* var. *japonica*
	HUA9109	*Abies sachalinensis* Masters
A. mellea (Vahl: Fr) Kummer subsp. *nipponica* Cha & Igarashi	HUA93110	*Acer mono* Maxim.

Table 2. Composition of media

Media	Formulae
MA (malt agar)	1.25% malt extract + 15 g agar in 1000 m*l* distilled water
MDA (malt-dextrose agar)	30 g malt extract + 20 g dextrose + 15 g agar in 1000 m*l* distilled water
YDA (yeast-dextrose agar)	30 g yest extract + 20 g dextrose + 15 g agar in 1000 m*l* distilled water
PDA (potato-dextrose agar)	39 g potato dextrose agar (Nissui) in 1000 m*l* distilled water
PCDA (PDA-carrot)	PDA + peeled and grated carrots 20 g in 1000 m*l* distilled water
SRB (sawdust-rice bran)	35 g sawdust* + 16 g rice-bran + 80-120 m*l* distilled water (about 67-72%)
BMDA (MDA-bark extract)	30 g malt extract + 20 g destrose + 15 g agar in 1000 m*l* bark extract

*Air dried at room temperature for a month

they were incubated at 25°C for 21 days in the dark. Cultures on BMDA media contained 15 ml/plate and were incubated at 25°C for 15 days in the dark. Colony size and rhizomorph formation were determined for each biological species in three replicates of each isolates. The colony diameter, the form of colonies and rhizomorph formation were observed. Color names are indicated from Munsell (1990).

III. Results

1. Growth on MDA, YDA, PDA and PCDA

Colony diameters and rhizomorph formation of the six *Armillaria* species are shown in Table 3. *Armillaria ostoyae* produced a slight crustose mycelium covered with fine brownish yellow (10YR-6/6) or sometimes white hyphae on all media (Fig. 1). The marginal zones of the colonies produced whitish hyphae. The YDA medium was stained very dusky red (2.5YR-2.5/2) by *A. ostoyae*. *Armillaria gallica* produced no crust on any medium (Fig. 2). The colony surface was covered with fine reddish brown (2.5YR-4/3) hyphae. This species also stained the media very dusky red (2.5YR-2.5/2). *Armillaria jezoensis* produced a slight crustose mycelium on the colony surface which was covered with fine reddish brown (2.5YR-5/4) hyphae on all media (Fig. 3). This species, except on MDA, stained the media strong brown (7.5YR-5/4). *Armillaria sinapina* produced red (2.5YR-5/6) to dark red (2.5YR-3/6) crustose mycelium on all media (Fig. 4). On YDA particularly, the colony was covered with fine pinkish (7.5YR-8/3) hyphae on the surface with the medium stained dark reddish-brown (5YR-2.5/2). *Armillaria singula* had a crustose mycelium on all media. On MDA and PCDA cultures were covered with fine reddish brown (5YR-5/6) hyphae (Fig. 5). *Armillaria mellea* subsp. *nipponica* produced a crustose mycelium of dark reddish gray (5YR-4/2) to reddish brown (2.5YR-5/4) on all media, though MDA supported only a slight formation of crustose mycelium covered with fine reddish brown (2.5YR-6/3) hyphae with no staining of the medium (Fig. 6).

In *A. ostoyae*, the mycelium grew faster on PDA and PCDA than on MDA and YDA. Rhizomorphs were produced on MDA, PDA and PCDA but not on YDA. *Armillaria gallica* did not grow well compared with other species, and no rhizomorphs

Table 3 Mycelial growth and rhizomorph formation of the six biological species of *Armillaria*

Biological species	Media*							
	MDA		YDA		PDA		PCDA	
	1	2	1	2	1	2	1	2
A. ostoyae	21	+	17	−	29	+/−	28	+
A. gallica	14	−	16	−	13	−	15	−
A. jezoensis	25	+/−	25	+/−	21	−	28	+/−
A. sinapina	35	+	25	++	74	++++	85	+++++
A. singula	22	+	19	+	20	+	43	+++
A. mellea subsp. *nipponica*	21	+	57	+++	70	++++	85	+++++

*1 : Diameter of colony (mm) ; means derived from measurements of three colonies each of two isolate, except for *A. mallea* subsp. *nipponica* (one isolate), 2 : Degree of rhizomorph formation ; +, formation ; −, no formation ; +/−, no or slightly formation. Number of + shows the degree of rhizomorph formation.

were produced on any of the media tested. *Armillaria jezoensis* showed almost the same mycelial growth on all media. Rhizomorphs production were sparse. *Armillaria sinapina*, like *A. ostoyae*, showed extra mycelial growth on PDA and PCDA. *Armillaria sinapina* produced rhizomorphs on all media but most readily on PDA and PCDA. Generally, *A. singula* showed better mycelial growth and rhizomorph formation on PDA and PCDA than on MDA and YDA. *Armillaria mellea* subsp. *nipponica* showed better growth on YDA, PDA and PCDA than on MDA.

2. Growth on sawdust-rice bran media (SRB)

The results of mycelial growth and rhizomorph formation on SRB are shown in Table 4. *Armillaria ostoyae* showed extra mycelial growth on *Q. mongolica* var. *grosseserrata* SRB (Fig. 7). Sawdust of broad-leaved trees particularly by *Q. mongolica* var. *grosseserrata*, were generally supported better rhizomorph development than coniferous trees, though *L. kaempferi* supported slight rhizomorph formation. For *A. gallica*, *B. platyphylla* var. *japonica* SRB produced poor rhizomorph formation (Fig. 8). *Armillaria jezoensis* showed extra mycelial growth and rhizomorph formation on all media containing sawdusts from broad-leaved trees (Fig. 9). This species also showed extra mycelial growth and rhizomorph formation on *L. kaempferi* SRB. *Armillaria jezoensis* in particular did not show rhizomorph formation on *A. sachalinensis* SRB and *P. jezoensis* SRB. *Armillaria sinapina* showed extra mycelial growth and rhizomorph formation on *Q. mongolica* var. *grosseserrata* SRB and *B. platyphylla* var. *japonica* SRB (Fig. 10). This species, however, showed poor mycelial growth and rhizomorph formation on *F. crenata* SRB. On the other hand, it showed the similar mycelial growth on all coniferous tree SRB, and produced rhizomorphs only on *L. kaempferi* SRB. *Armillaria singula* showed extra mycelial growth and rhizomorph formation on every broad-leaved tree SRB. Of coniferous tree SRB, however, *L. kaempferi* supported extra mycelial growth and rhizomorph formation in contrast to *A. sachalinensis* and *P. jezoensis* SRB which showed no rhizomorph formation (Fig. 11). *Armillaria mellea* subsp. *nipponica*, as *A. singula*, showed extra mycelial growth and rhizomorph formation on every broad-leaved tree SRB (Fig. 12). *L. kaempferi* and *A. sachalinensis* SRB gave rise to a fairly

Table 4 Mycelial growth and rhizomorph formation of the six biological species of *Armillaria* on SRB

Biological species	Sawdust medeia*											
	Fc		Qm		Bp		As		Pj		Lk	
	1	2	1	2	1	2	1	2	1	2	1	2
A. ostoyae	30.5	++	43.3	+++	28.8	++	24.6	−	23.3	−	25.7	+
A. gallica	22.0	−	22.0	−	26.0	+/−	15.7	−	23.3	−	21.7	−
A. jezoensis	59.0	+++	67.0	++++	63.8	++++	30.7	−	28.3	−	51.3	+++
A. sinapina	35.5	++	54.8	++++	51.3	+++	27.0	−	28.3	−	31.3	++
A. singula	50.7	++++	67.0	+++++	64.8	+++++	28.0	−	26.7	−	48.8	+++
A. mellea subsp. *nipponica*	55.7	++++	58.5	++++	63.0	+++++	32.8	+/−	25.7	−	33.7	+++

*Abbreviation; Fc, *Fagus cernata* Bl.; Qm, *Quercus mongolica* Fisch. var. *grosseserrata* Rehd. & Wils.; Bp, *Betula platyphylla* Sukatchev var. *japonica* Hara; As, *Abies sachalinensis* Fr. Schmidt; Pj, *Picea jezoensis* Carr.; Lk, *Larix kaempferi* (Lamb.) Carriére. 1: Diameter of colony (mm); means derived from measurements of three colonies each of two isolate, except for *A. mellea* subsp. *nipponica* (one isolate), 2: Degree of rhizomorph formation; +, formation; −, no formation; +/−, no or slightly formation. Number of + shows the degree of rhizomorph formation.

extra mycelial growth as compared with *P. jezoensis* SRB.

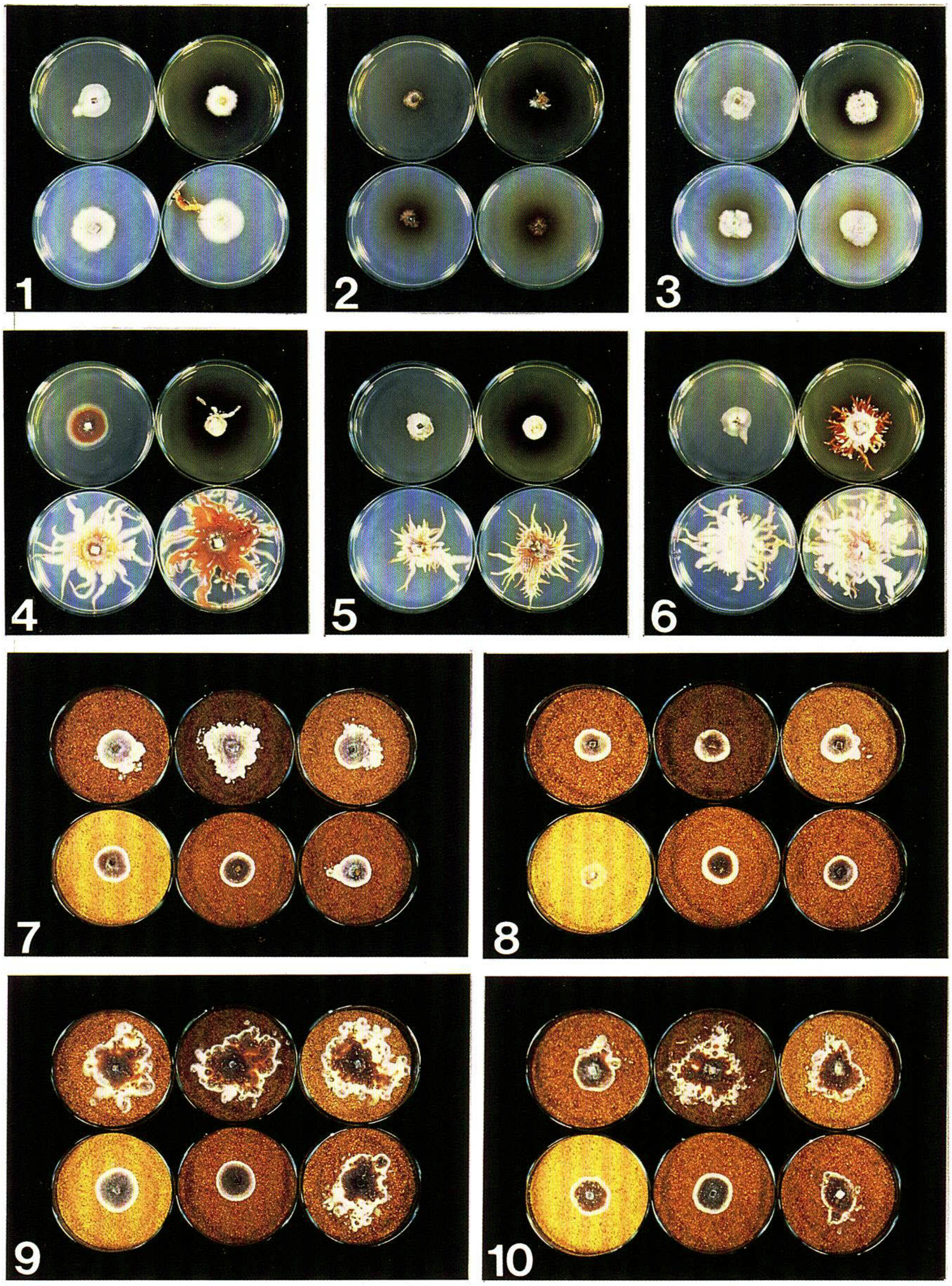
3. Growth on MDA-bark extract media (BMDA)

Generally, BMDA media supported better mycelial growth and rhizomorph formation than MDA which contained no bark extract, with the exception of *B. platyphylla* var. *japonica* BMDA for *A. ostoyae* (Table 5). *Armillaria ostoyae* showed extra mycelial growth and abundant rhizomorphs on *L. kaempferi* BMDA. *Armillaria ostoyae* in particular showed only limited mycelial growth and rhizomorph formation on *B. platyphylla* var. *japonica* BMDA (Fig. 13). Mycelial growth and rhizomorph formation of *A. gallica* was better on BMDA of coniferous tree species (*A. sachalinensis*, *P. jezoensis* and *L. kaempferi*) than on BMDA of broad-leaved tree species (*F. crenata*, *Q. mongolica* var. *grosseserrata* and *B. platyphylla* var. *japonica*) (Fig. 14). *Armillaria jezoensis* showed extra mycelial growth and profuse rhizomorph formation on BMDA of all tree species (Fig. 15). Although *A. sinapina* and *A. singula* showed a slightly inhibited mycelial growth and rhizomorph formation on the *B. platyphylla* var. *japonica* BMDA, their mycelial growth and rhizomorph formation were better on other BMDA media.

Figs. 1-6 Colonies of *A. ostoyae* (1), *A. gallica* (2), *A. jezoensis* (3), *A. sinapina* (4), *A. singula* (5) and *A. mellea* subsp. *nipponica* (6) on malt-dextrose agar medium (top left), yeast-dextrose agar medium (top right), potato-dextrose agar medium (bottom left) and potato-carrot-dextrose agar medium (bottom right).

Figs. 7-12 Colonies of *A. ostoyae* (7), *A. gallica* (8), *A. jezoensis* (9), *A. sinapina* (10), *A. singula* (11) and *A. mellea* subsp.*nipponica* (12) on sawdust-rice bran medium of *F. crenata* (top left), *Q. mongolica* var. *grosseserrata* (top center), *B. platyphylla* var. *japonica* (top right), *A. sachalinensis* (bottom left), *P. jezoensis* (bottom center) and *L. kaempferi* (bottom right).

Figs. 13-18 Colonies of *A. ostyae* (13), *A. gallica* (14), *A. jezoensis* (15), *A. sinapina* (16), *A. singula* (17) and *A. mellea* subsp. *nipponica* (18) on BMDA of *F. crenata* (top left), *Q. mongolica* var. *grosseserrata* (top center), *B. platyphylla* var. *japonica* (top right), *A. sachalinensis* (bottom left), *P. jezoensis* (bottom center), *L. kaempferi* (bottom right). The far right is control (MDA).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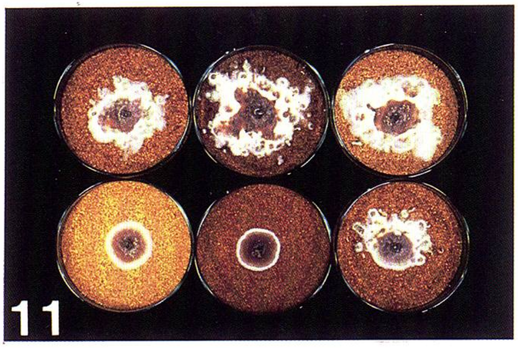
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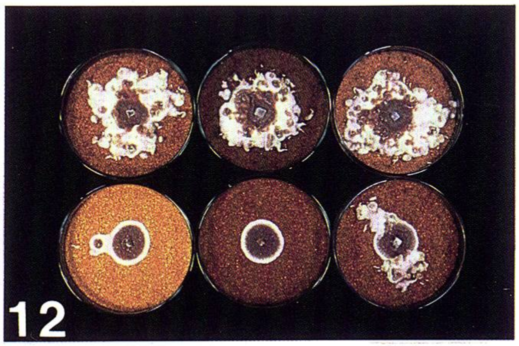
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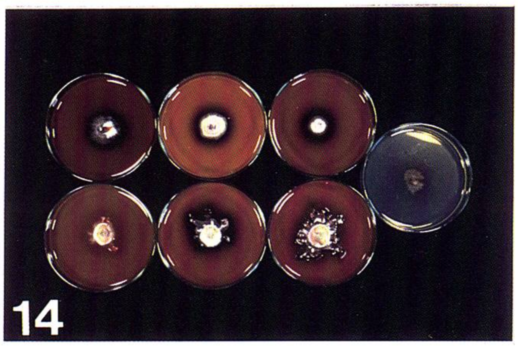
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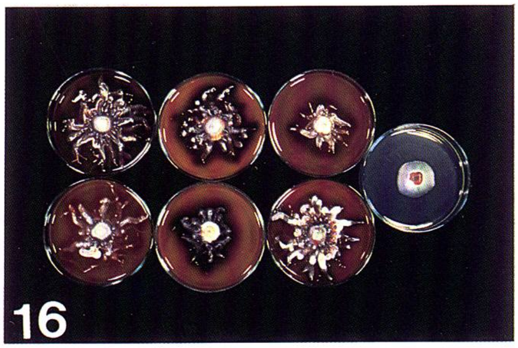
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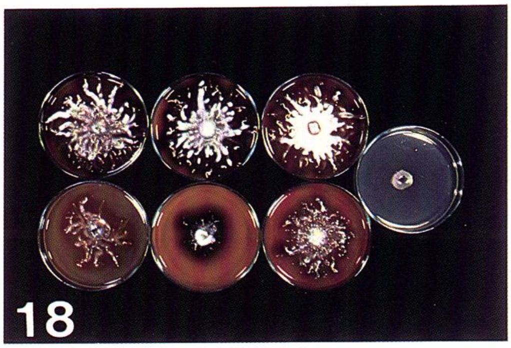
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Table 5 Mycelial growth and rhizomorph formation of the six biological species of *Armillaria* on BMDA

Biological species	Tree species of bark*													
	Fc		Qm		Bp		As		Pj		Lk		Control	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2
A. ostoyae	63.5	++++	60.0	++++	10.0	−	62.5	++++	48.0	+++	80.0	+++++	21.3	+/−
A. gallica	17.0	−	20.0	−	15.0	−	31.0	+	40.0	++	47.0	+++	17.0	−
A. jezoensis	85.0	+++++	85.0	+++++	85.0	+++++	85.0	+++++	80.0	+++++	85.0	+++++	25.0	+
A. sinapina	80.0	+++++	65.0	++++	40.0	+++	65.0	++++	50.0	+++	72.5	++++	33.0	+
A. singula	86.0	+++++	70.0	++++	63.0	++++	85.0	+++++	88.0	+++++	86.0	+++++	21.0	+
A. mellea subsp. *nipponica*	86.0	+++++	85.0	+++++	84.0	+++++	55.0	+++	36.0	++	60.0	++++	16.0	+

*Abbreviation; Fc, *F. crenata*; Qm, *Q. monogolica* var. *grosseserrata*; Bp, *B. platyphylla* var. *japonica*; As, *A. sachalinensis*; Pj, *P. jezoensis*; Lk, *L. kaempferi*. 1: Diameter of colony (mm); means derived from measurements of three colonies each of two isolate, except for A. *mellea* subsp. *nipponica* (one isolate), 2: Degree of rhizomorph formation; +, formation; −, no formation; +/−, no or slightly formation. Number of + shows the degree of rhizomorph formation.

(Figs. 16, 17). Mycelial growth and rhizomorph formation of *A. mellea* subsp. *nipponica* was greater on BMDA of broad-leaved tree species than on BMDA of coniferous tree species (Fig. 18) .

IV. Discussion

Many studies on *Armillaria* have reported that undefined media such as yeast extract or potato-dextrose agar stimulate rhizomorph formation. Hokkaido *Armillaria* were distinctive in cultural form and growth according to biological species on MDA, YDA, PDA and PCDA. All *Armillaria* species showed poor crustose mycelium and stained very dusky red (2.5YR-2.5/2) on their media. Rhizomorph formation of *A. ostoyae*, *A. jezoensis*, *A. sinapina*, *A. singula* and *A. mellea* subsp. *nipponica* species was promoted on PDA and PCDA. Rhizomorph formation of *A. gallica*, however, was not promoted on any media of MDA, YDA, PDA, PCDA. On the other hand, Rishbeth (1986) reported that *A. gallica* from England had two types of colonies one with few or no rhizomorphs and the second type often formed rhizomorphs. Although it is considered that *A. gallica* in this study is similar to the former, the latter also may be distributed in Hokkaido if it is broadly investigated.

Raabe (1962) reported on the suitability of wood-based culture media for their stimulatory effect on rhizomorph formation. Moreover, Lin *et al.* (1985) studied the rhizomorph formation by substances present in bark. Their promoting effects that various plant constituents are able to induce rhizomorphs and have been confirmed by recent studies with auxins and phenolic compounds. *Armillaria* has been reported to respond in various ways to phenolic compounds (Garraway and Weinhold, 1970). Shaw (1985) found differences in the growth habits of twenty-one isolates of several *Armillaria* species depending on the phenol amendments used, i. e. gallic acid (the hydrolyzed form of tannic acid) or tannic acid. All species, except *A. gallica*, were extra mycelial growth and rhizomorph formation on SRB of broad-leaved tree species.

In particular, *L. kaempferi* SRB promoted mycelial growth and rhizomorph formation to the same extent as SRB of broad-leaved tree species. But all *Armillaria* species showed reduced rhizomorph formation on *A. sachalinensis* and *P. jezoensis* SRB. As the results, it is considered that sawdust from broad-leaved trees contain the promoting constituents and from coniferous trees contain the inhibiting ones on *Armillaria* growth.

All *Armillaria* species, except *A. ostoyae* and *A. gallica* on *B. platyphylla* var. *japonica* BMDA, showed extra mycelial growth and rhizomorph formation. Moreover, *A. gallica* showed rhizomorph formation on BMDA from coniferous tree species, though those are more slight than others. As the results, it is considered that the bark of both broad-leaved and coniferous tree species contained the substance(s) for promotion of *Armillaria* growth. As a differentiation to SRB of coniferous trees, the result on BMDA that no bark substance(s) of coniferous trees contained inhibiting constituents on *Armillaria*. On the other hand, SRB and BMDA of *B. platyphylla* var. *japonica* may contain a substance inhibitory to mycelial growth and rhizomorph formation on *A. ostoyae* and *A. gallica*. All *Armillaria* species showed similar mycelial growth and rhizomorphs on BMDA from both coniferous and broad-leaved trees. On the other hand, *A. ostoyae* and *A. gallica* showed similar mycelial growth and rhizomorphs on SRB from both trees, but other species showed better the former than the latter. It is supported that the fruit-bodies of *A. ostoyae* and *A. gallica* often form on deacy trunks and wood of both coniferous and broad-leaved trees, but other species form chiefly on broad-leaved trees in forests. Moreover, *Armillaria* species which new species, *A. jezoensis* and *A. singula*, and new subspecies, *A. mellea* subsp. *nipponica,* from Hokkaido showed better mycelial growth and rhizomorph formation than other species on medium amended with bark extract and sawdust from local trees. It is considered that the native species of *Armillaria* show the adaptability on the Hokkaido tree species.

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要　　約

通常よく用いられる4種の栄養培地，6樹種のおかくず・米ぬか培地及び樹皮抽出物・麦芽・ブドウ糖寒天培地での北海道産ナラタケの生物学的種6種の菌糸束の形成および菌糸の成長について調べた。4種の栄養培地で培養した結果，ツバナラタケ（*A. ostoyae*），コバリナラタケ（*A. jezoensis*），ホテイナラタケ（*A. sinapina*）とナラタケ（*A. mellea* subsp. *nipponica*）はジャガイモ寒天培地（PDA）とジャガイモ・ニンジン・ブドウ糖寒天培地（PCDA）で菌糸束の形成が認められたが，ヤワナラタケは麦芽・ブドウ糖寒天培地（MDA），酵母・ブドウ糖寒天培地（YDA），ジャガイモ・ブドウ糖寒天培地，ジャガイモ・ニンジン・ブドウ糖寒天培地のいずれでも菌糸束は形成されなかった。6樹種のおかくず・米ぬか培地（SRB）ではヤワナラタケを除いた全てのナラタケで菌糸束の形成および菌糸の成長は針葉樹より広葉樹の培地で優れていた。また，カラマツのおかくず・米ぬか培地では全てのナラタケの菌糸束の形成および菌糸の成長が見られたが，トドマツとエゾマツのおかくず・米ぬか培

地では菌糸束の形成が抑制された。樹皮抽出物・麦芽・ブドウ糖寒天培地（BMDA）でのツバナラタケとヤワナラタケはシラカンバ培地で成長の抑制が認められたが，他のナラタケ菌は広葉樹と針葉樹に関係なく，全ての樹種で成長の促進が認められた。また 4 種の栄養培地，6 樹種のおかくず・米ぬか培地で菌糸束の形成が見られなかったヤワナラタケは全ての針葉樹の樹皮抽出物・麦芽・ブドウ糖寒天培地で菌糸束形成の促進が認められた。また北海道の自生樹種である 6 種のおかくず・米ぬか培地及び樹皮抽出物・麦芽・ブドウ糖寒天培地で北海道産新種であるコバリナラタケ，ヒトリナラタケと新亜種であるナラタケは，他 3 種より菌糸束の形成および菌糸の成長が優れていた。