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*Rhizopus*의 아밀라아제에 관한 연구(第一報)

한국산 *Rhizopus*屬의 分離 및 同定

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Studies on the Amylase of *Rhizopus*[1](1)

—Isolation and Identification of the Genus *Rhizopus* Distributed in South Korea—

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ABSTRACT

151 strains which belong to the genus *Rhizopus* were isolated from 750 specimens collected through all over South Korea, and 19 species of the genus, all of which are unrecorded in Korea, were identified according to the modified Takeda-Yamamoto's classification key.

The species identified are as follows; *R. nigricans*, *R. formosaensis*, *R. achlamydosporus*, *R. tritici*, *R. javanicus*, *R. chiuniang*, *R. liquefaciens*, *R. chungkuoensis*, *R. acidus*, *R. chinensis*, *R. delemar*, *R. hangchow*, *R. niveus*, *R. japonicus*, *R. arrhizus*, *R. bahrnensis*, *R. tonkinensis*, and *R. shanghaiensis*.

INTRODUCTION

The genus *Rhizopus* belongs to the family Mucoraceae and was established in 1820 by Ehrenberg, who named "*R. nigricans* Ehrenberg" distinguishing it from mucor. Later van Tieghem described in 1875 the characteristics of the genus *Rhizopus*. Since Calmette(1892) had pointed out that the fermenting kneaded cereals contained *Rhizopus* species and that the species were the agent causing saccharification of starchy materials in alcolic fermention, a great deal of work has been done on the subject and as a result a number of new fungal strains has been isolated.

Taxonomical study on this genus was performed extensively. Fisher(1892), Vuillemin(1902), and Lendner(1908) classified the species of the genus *Rhizopus* based upon morphological characteristics. On the other hand, Hagem(1910), Hanzawa(1914), and Weimer(1923) tried to classify the genus *Rhizopus* according to physiological characteristics. Takahashi and Sakaguchi(1925) studied the organic acids production by the *Rhizopus* species, and based upon organic acid formation, they classified them into three groups. However, Kitahara and

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Fukui(1949) insisted that their classification based upon the organic acid formation was meaningless because of no parallelism with morphological characteristics.

Yamamoto(1930) studied extensively the morphological characteristics of *Rhizopus* and favored morphological characteristics as the principal keys of *Rhizopus* classification instead of physiological characteristics, while Yamazaki (1934) suggested a method of *Rhizopus* classification based upon temperature relations. On the contrary, Takeda(1949) classified the species of the genus *Rhizopus* based upon the turf type on Pfeffer -Oryzanin agar slant.

Most of the species belonging to this genus show very active growth and are widely distributed in crops, fruits, vegetables and fermenting kneaded cereals. Some strains of *Rhizopus* species have a very important economic value in the application to the fermentation industry because of their enzymatic activities, especially amylase activity.

The microfloral distribution is different according to the locality, and even in the same species their biological activities are able to change by their environment. Therefore, it is important to classify the microflora in our natural environment in order to develope the new strains of microbe. Nevertheless, there are few researches on Korean *Rhizopus* strains. Recently, In and Lee(1968) isolated *Rhizopus* strains distributed in South Korea from the fermenting kneaded cereal named "meju", and divided it into four groups, but they did not identify the species.

In the present study, the strains of *Rhizopus* distributed in South Korea were collected, isolated and identified.

MATERIALS AND METHODS

1. Organisms

The organisms used in the present study were isolated from about 750 samples collected throughout South Korea from June to September 1972. Original substrates from which *Rhizopus* strains were isolated were bread, Korean rice cake, potato, sweet potato, fruits, vegetables, cereals, and fermenting kneaded cereals, etc.

2. Isolation and maintenance of *Rhizopus* strains.

To reduce other fungal contamination to the minimum a small piece of sample was soaked in 0.1% mercuric chloride solution for 3—4 hours, and then it was washed two times in sterilized water. After grinding the sample it was diluted tenfold with sterilized water, and then 0.5m*l* of the diluted solution was inoculated on Czapek's agar medium. After incubation at 30°C for 10 days the strains of *Rhizopus* were isolated based on their morphological characteristics.

The fungus could be readily purified by streaking sporangiospore on the solid media. Further pure culture were attained by two successive subculture of single colonies from the agar medium, and pure culture was maintained on potato starch plate at 17°C and subcultured every two months.

3. Identification of species

The isolated 151 strains of *Rhizopus* were incubated on Pfeffer-Oryzanin agar slant at 30°C for 14 days and divided into four groups by the turf type, accor-

ding to modified Takeda method (1949).

(Ⅰ) The turf on Pfeffer-Oryzanin agar slant consists of only sporangial layer.

(Ⅱ) The turf consists of both sporangial and mycelial layers, but sporangial layer is greater than mycelial layer.

(Ⅲ) Mycelial layer is greater than sporangial layer.

(Ⅳ) The turf consists of only mycelial layer.

The strains of each group were tested for their morphological characteristics, such as shape and size of sporangiophore, sporangium, sporangiospore, columellae, chlamydospore and rhizoid, by the classification key of Yamamoto(1933). The temperature relation of each fungal strains was tested according to the classification key of Yamazaki(1934), and fermentation test was done for inulin, galactose, and raffinose when necessary, by Hanzawa method(1915). Based upon the results of all these tests the species of *Rhizopus* were identified.

RESULTS AND DISCUSSION

Among the 151 strains of *Rhizopus* isolated 118 strains were identified into 19 species according to their morphological, cultural and physiological characteristics, but the rest of them couldn't be identified.

1. Morphological characteristics

Table 1 shows the morphological characteristics of sporangiophores. Sporangiophores of *R. nigricans* and *R. chinensis* are straight and those of *R. arrhizus* and *R. niveus* are clearly curved. However, sporangiophores of most species are straight or slightly curved. The sporangiophore walls of most species are smooth, but some species like *R. tonkinensis* have sometimes rough walls. Branching and swelling of sporangiophores are observed in some fungi, such as *R. oryzae*, *R. acidus* and *R. delemar*, and *R. tritici* has branching sporangiophore but no swelling part.

As shown in table 2, sporangia of most species are usually globose or subglobose in shape. The walls of sporangia are either smooth or slightly rough. In many species of this genus, small spines are observed radially along the outward membrane of sporangia. However, the walls of sporangia of some species such as *R. niveus* and *R. formosaensis* are smooth without spines. Although the range of sporangial size is rather wide even in the same culture, the mean diameter of sporangia is different in different species. Among them, *R. nigricans* has the largest sporangia and *R. niveus* has the smallest one.

The shape of sporangiospore is globose, elliptical, polygonal, oval or irregular, as shown in Table 3. The sporangiospores of *R. nigricans* are globose, oval or elliptical with distinct striation on their walls, while those of *R. niveus* are elliptical or polygonal with faint striation. The size of sporangiospores is different by the species. *R. nigricans* has the largest spore, 8μ and *R. chinensis*, the smallest, 5μ in long axis.

Table 4 shows morphological characteristics of columellae, chlamydospores and rhizoid. Columellae are generally globose, oval or oblate, and sometimes, they are cylindrical or pyriform. The walls of columellae are usually smooth,

Table 1. Morphological characteristics of sporangiophores

Species	Straight or Curved	Wall	Branching and Swelling
R. nigricans	Straight	R or S	
R. formosaensis	Straight or Curved	S	
R. achlamydosporus	〃	〃	
R. tritici	〃	R or S	Branching
R. javanicus	〃	〃	
R. chiuniang	〃	S	Branching and Swelling
R. acidus	〃	〃	〃
R. chinensis	Straight	〃	
R. oryzae	Straight or Curved	R or S	Branching and Swelling
R. delemar	〃	S	〃
R. chungkuoensis	〃		
R. hangchow	〃	〃	
R. japonicus	〃	〃	
R. bahrnensis	〃	〃	
R. tonkinensis	〃	R or S	
R. arrhizus	Curved	〃	
R. niveus	〃	S	
R. shanghaiensis	Straight or Curved	〃	
R. liquefaciens	〃	〃	

Abbreviation: S, smooth; R, rough.

Table 2. Morphological characteristics of sporangia

Species	Shape	Wall	Formation	Diameter(μ)	
				mean	range
R. nigricans	Gl Gl′	Sp	G	170	50—360
R. formosaensis	Gl	S	〃	120	60—170
R. achlamydosporus	〃	〃	〃	115	50—230
R. tritici	Gl Gl′	Sp	〃	130	50—180
R. javanicus	Gl	〃	〃	125	50—200
R. chiuniang	Gl Gl′	〃	〃	110	40—200
R. acidus	〃	〃	〃	120	40—210
R. chinensis	〃	S	〃	80	20—150
R. oryzae	〃	Sp	〃	130	60—180
R. delemar	〃	〃	〃	130	40—230
R. chungkuoensis	〃	〃	〃	110	50—180
R. hangchow	〃	〃	〃	110	40—180
R. japonicus	〃	〃	〃	120	50—270
R. bahrnensis	〃	〃	〃	120	60—250
R. tonkinensis	〃	〃	〃	130	80—230
R. arrhizus	〃	〃	P	110	50—200
R. niveus	Gl	S	〃	70	25—100
R. shanghaiensis	Gl Gl′	〃	〃	90	30—150
R. liquefaciens	〃	〃	〃	85	30—150

Abbreviation: Gl, globose; Gl′, subglobose; S, smooth; Sp, spinous; G, good; P, poor,

Table 3. Morphological characteristics of sporangiospores.

Species	Shape	Striation	Long axis(μ)	
			mean	range
R. nigricans	Gl, Ov, Ell, Pol,	+	8	4—12
R. formosaensis	Ell, Gl, Pol, Irr,	+	7	6—15
R. achlamydosporus	〃	+	7	6—15
R. tritici	Ell, Gl,	+	6	4—8
R. javanicus	Ell, Pol,	+	7	5—12
R. chiuniang	Pol, Gl, Ell, Ang	+	7	4—10
R. acidus	Gl, Pol, Ell,	+	7	4—13
R. chinensis	Gl, Gl′	−	5	4—7
R. oryzae	Ell, Gl, Irr,	+	6	5—8
R. delemar	Ell, Pol.	+	7.5	5—13
R. chungkuoensis	Ell, Gl, Pol, Irr.	+	7	5—11
R. hangchow	Ell, Gl,	−	6	5—8
R. japonicus	Ell, Pol, Gl,	+	6	5—9
R. bahrnensis	〃	+	7	5—8
R. tonkinensis	Ell, Gl, Pol,	+	6	4—8
R. arrhizus	Ell, Pol,	+	7	4—10
R. niveus	Ell, Pol, Gl,	−	6	5—9
R. shanghaiensis	〃	−	6	5—10
R. liquefaciens	Ell, Gl, Gl′	−	6	4—8

Symbols and abbreviations;
+:striated. −:faintly striated.
Ell:elliptical. Gl:globose. Gl′:subglobose. Pol:polygonal. Ov:oval. Irr:irregular. Ang:angular.

Table 4. Morphological characteristics of columellae, chlamydospore and rhizoid.

Species	Columellae		Chlamydospore	Rhizoid
	Shape	Wall	Shape	Shape
R. nigricans	Gl, Gl′, Ov,	S	—	root-shaped
R. formosaensis	Gl, Gl′	〃	〃	〃
R. achlamydosporus	Gl, Gl′ Ov,	〃	〃	〃
R. tritici	Gl, Gl′	〃	Gl, Ell, Lem,	〃
R. javanicus	Gl, Ov,	〃	Gl, Ell, Cyl.	〃
R. chiuniang	Ov, Gl, Gl′,	S or R	Gl, Ell,	root- or finger shaped
R. acidus	Ov, Gl,	〃	Gl, Gl′, Ell,	finger-shaped
R. chinensis	Gl′, Py, Cyl,	S	Gl, Ell, Cyl,	〃
R. oryzae	Gl, Gl′, Ov,	〃	Cyl, Gl, Ell,	root-shaped
R. delemar	Gl, Ov, Cyl,	S or R	Gl, Ell, Cyl,	〃
R. chungkuoensis	Gl, Gl′, Ov,	〃	Cyl, Gl, Ell,	〃
R. hangchow	Gl, Gl′	S	Gl, Lem,	〃
R. japonicus	Gl, Ov, Gl′,	〃	Gl, Ell, Cyl,	root- or finger shaped
R. bahrnensis	Gl, Gl′, Ov,	〃	Gl, Ell,	〃
R. tonkinensis	Gl, Gl′,	〃	Gl, Ell, Lem,	root-shaped
R. arrhizus	Ov, Gl,	S or R	Ell, Gl, Lem	finger-shaped
R. niveus	Gl, Gl′,	S	—	〃
R. shanghaiensis	Gl, Gl′,	〃	Ell, Gl,	〃
R. liquefaciens	Gl, Ov,	〃	〃	〃

Abbreviations; Gl:globose. Gl′:subglobose. Ov:oval. Cyl:cylindrical Ell:elliptical. Py:pyriform. Lem:lemon-shaped. S:smooth. R:rough.

but in some species such as *R. chiuniang* and *R. acidus* they are slightly rough with spines. In most species chlamydospores are formed on stolons and are globose, elliptical, cylindrical or oval shaped. However, some species, such as *R. nigricans*, *R. formosaensis*, *R. achlamydosporus* and *R. niveus*, do not form chlamydospore at all. In most species rhizoids are readily formed. They usually sprout at nodes or at any point on the stolons and are root- or finger-shaped. In some species, such as *R. nigricans* and *R. formosaensis*, rhizoids are usually root-shaped, but they are short and rarely formed in *R. niveus*.

2. Cultural and physiological characteristics.

Table 5 shows the turf composition of *Rhizopus* strains on Pfeffer-Oryzanin agar slant at 30°C for 14 days and fermentative activities of the fungi. The turf of *R. tritici* group, such as *R. formosaensis* and *R. acidus* consists of only sporangial layer. In *R. oryzae* group, such as *R. delemar* and *R. chungkuoensis* the turf on Pfeffer-Oryzanin agar slant consists of both sporangial and mycelial layers but sporangial layer is greater than mycelial. In some species, such as *R. japonicus* and *R. bahrnensis* mycelial layer is greater than sporangial layer. The turf on Pfeffer-Oryzanin agar slant consists of only mycelial layer in *R. arrhizus* group, such as *R. niveus* and *R. shanghaiensis*,

As to the fermentative activities of carbohydrate by *Rhizopus*, some species such as *R. achlamydosporus* and *R. javanicus* ferment inulin, while others do not. Some species such as *R. niveus* and *R. bahrnensis* ferment galactose, but most species do not ferment it at all. *R. japonicus* and *R.bahrnensis* ferment

Table 5. Turf composition on Pfeffer-Oryzanin agar medium at 30°C for 14 days and fermentative activities of *Rhizopns*.

Species	fruiting	mycelial formation	Fermentation		
			inulin	galactose	raffinose
R. nigricans	++	−	−	−	
R. formosaensis	+	−	−	−	
R. achlamydosporus	++	−	+	−	
R. tritici	+	−	−	−	
R. javanicus	++	−	+	+	
R. chiuniang	+	−	−	−	
R. acidus	+	−	−	−	
R. chinensis	+	−	−	−	
R. oryzae	++	+	−	−	
R. delemar	++	+	−	+	
R. chungkuoensis	++	+	−	−	
R. hangchow	++	+	−	+	
R. japonicus	+	++	−	−	+
R. bahrnensis	+	++	−	+	+
R. tonkinensis	+	++	−	−	−
R. arrhizus	−	++	−	+	
R. niveus	−	++	−	+	
R. shanghaiensis	−	+	−	−	
R. liquefaciens	−	++	−	−	

Symbols for fruiting and mycelial formation; ++: very good. +: good. −: none or very poor.

Table 6. Cultural characteristics of *Rhizopus*

Species	Growth			Fruiting			Air mycelium		
	30°C.	37°C.	41°C.	30°C.	37°C	41°C.	30°C.	37°C.	41°C.
R. nigricans	+	−	−	++	−	−	−	−	−
R. formosaensis	+++	++	+	+++	++	−	−	−	−
R. achlamydosporus	+++	++	+	+++	++	−	−	−	−
R. tritici	+++	++	+	+++	++	−	+++	++	+
R. javanicus	+++	++	+	+++	++	−	+++	++	+
R. chiuniang	+++	+++	++	+++	+++	+	+++	+++	+++
R. acidus	+++	+++	++	++	++	−	+++	+++	+++
R. chinensis	++	++	+	++	++	+	+	+	+
R. oryzae	+++	++	+	++	+	+	+++	++	+
R. delemar	+++	++	+	+++	+	−	+++	++	+
R. chungkuoensis	+++	++	+	+++	++	−	+++	+++	+
R. hangchow	+++	++	+	+++	+	−	+++	++	+
R. japonicus	+++	++	+	+++	++	−	+++	++	+
R. bahrnensis	+++	++	+	+++	++	+	+++	++	+
R. tonkinensis	+++	++	+	−+	++	−	+++	++	+
R. arrhizus	+++	+	−	+	−	−	+++	+	−
R. niveus	+++	++	+	−	−	−	+++	++	+
R. shanghaiensis	++	+	+	+	+	−	++	+	−
R. liquefaciens	+	+	+	−	−	−	−	−	−

Symbols: +++, very good; ++, good; +, poor; −, none;

raffinose, but *R. tonkinensis* does not ferment it.

As shown in table 6, when the fungal strains are cultured in Pfeffer's solution at 37°C, *R. nigricans* does not grow at all, but some species such as *R. liquefaciens* and *R. arrhizus* grow well without formation of sporangia, while most species including *R. chinensis* grow well and form sporangia.

3. Classification

Some morphological characteristics, including the organ dimensions of *Rhizopus* species are generally so variable that it is not resonable to classify all the species dichotomously only by using morphological characteristics, though strong emphasis should be placed on these characteristics. In the present studies, therefore, physiological and cultural characteristics, which are related closely to morphological characteristics, are included as keys of classification. The turf type on Pfeffer-Oryzanin agar slant and temperature relations are most suitable for this purpose. Based on these characteristics, a new classification may be suggested as follows:

Key to species of the genus *Rhizopus*

(I) The turf on Pfeffer-Oryzanin agar slant (at 30°C for 14 days) consists of only sporangial layer. (R. *tritici* group)

(A) Chlamydospores not formed

a) No growth at 37°C.···*R. nigricans*

b) Growth at 37°C

1) Inulin fermented

·····*R. achlamydosporus*

2) Inulin not fermented

·····*R. formosaensis*

(B) Chlamydospores formed

a) Sporangia larger than 100μ and spores distinctly striated

1) Sporangia formed on potato slice

at 41°C for 10 days
(1) Spores angular···*R. chiuniang*
(2) Spores not angular···*R. tritici*
2) Sporangia not formed on potato slice at 41°C for 10 days
(1) Inulin fermented
·····*R. javanicus*
(2) Inulin not fermented.
·····*R. acidus*
b) Sporangia smaller than 100μ and spores faintly striated···*R. chinensis*

(Ⅱ) The turf on Pfeffer-Oryzanin agar slant (at 30°C for 14 days) consists of both sporangial and mycelial layers, but sporangial layer is greater than mycelial layer. (*R. oryzae* group)
(A) Spores distinctly striated
a) Sporangia formed on potato slice at 41°C for 10 days.
(1) No branching or swelling part of sporangiophore and the walls smooth·····*R. chungkuoensis*
(2) Sporangiophores branching and swelling and the walls rough or smooth·····*R. oryzae*
b) Sporangia not formed on potato slice at 41°C for 10 days
····· *R. delemar*
(B) Spores faintly striated
····· *R. hangchow*

(Ⅲ) The turf on Pfeffer-Oryzanin agar slant (at 30°C for 14 days) consists of both sporangial and mycelial layers but mycelial layer is greater than sporangial layer.(*R. japonicus* group)
(A) Sporangia formed on potato slice at 41°C for 10 days··· *R. bahrnensis*
(B) Sporangia not formed on potato slice at 41°C for 10 days
a) Raffinose fermented
····· *R. japonicus*
b) Raffinose not fermented
····· *R. tonkinensis*

(Ⅳ) The turf on Pfeffer-Oryzanin agar slant (at 30°C for 14 days) consists of only mycelial layer. (*R. arrhizus* group)
(A) Sporangiophores straight or curved and galactose not fermented.
a) Sporangia formed in Pfeffer solution at 37°C for 10 days
····· *R. shanghaiensis*
b) Sporangia not formed in Pfeffer solution at 37°C for 10 days
····· *R. liquefaciens*
(B) Sporangiophores mostly curved and galactose fermented. (at 30°C for 10 days)
a) Spores distinctly striated
·····*R. arrhizus*
b) Spores faintly striated···*R. niveus*

The strains of *Rhizopus* isolated are tested to identify the species according to this classification key, and the results are shown in Table 7.

DESCRIPTION OF SPECIES

1. *R. nigricans* Ehrenberg

Grayish dark in color on Pfeffer-Oryzanin agar medium. Sporangiophores are usually straight and their walls are slightly rough or smooth. Sporangia are globose or subglobose in shape and their walls are rough with spines. Sporangiospores are globose, oval, polygonal, or elliptical, in shape, and there are distinct striation on their walls. Columellae are globose, subglobose or oval in shape and their walls are usually smooth. Chlamydospores are not observed on stolon. Root-shaped rhizoids are abundant. The turf on Pfeffer-Oryzanin agar medium (at 30°C for 14 days)

Table 7. Relationship between species and strain number

Species	Strain No.
R. nigricans	1, 2, 4, 6, 13, 19, 31, 37, 40, 41, 44, 45, 47, 55, 56, 87, 90, 92, 98, 99, 100, 105, 110, 115, 126, 127, 134, 140, 142, 143, 146, 151
R. formosaensis	16, 88, 93
R. achlamydosporus	80, 86, 131
R. tritici	7, 8, 18, 29, 34, 46, 59, 67, 76, 77, 79, 81, 91, 133, 135, 144, 148
R. javanicus	32, 97, 128
R. chiuniang	139
R. acidus	49, 50, 65, 89, 106, 111, 113, 120, 141
R. chinensis	96
R. oryzae	5, 9, 14, 17, 63, 69, 84, 94, 101, 116, 121, 123, 130, 137, 149
R. delemar	73
R. chungkuoensis	20, 42, 57, 60, 74, 78, 85, 102, 103, 107, 108, 119, 125
R. hangchow	104
R. japonicus	27, 28, 43
R. bahrnensis	12, 15, 48, 64, 70, 150
R. tonkinensis	62, 72, 118
R. arrhizus	109
R. niveus	10, 35
R. shanghaiensis	23
R. liquefaciens	21, 25, 83
Unidentified strains	3, 11, 22, 24, 26, 30, 33, 36, 38, 39, 51, 52, 53, 54, 58, 61, 66, 68, 71, 75, 82, 95, 112, 114, 117, 122, 124, 129, 132, 136, 138, 145, 147

consists of only sporangial layer. This species does not grow above 37°C in Pfeffer solution and has not the fermentative activity of carbohydrates at all.

2. *R. formosaensis* Nakazawa

Dark brownish gray in color. Sporangiophores are straight or curved, rarely branched, and their walls are smooth. Sporangia are globose in shape and their walls are smooth without spines. Sporangiospore are elliptical, subglobose, polygonal or irregular in shape and their walls are distinctly striated, Columellae are globose or subglobose in shape and their walls are smooth. Chlamydospores are not observed on stolons and analogous organs are rarely formed. Root-shaped rhizoids are abundant. The turf on Pfeffer-Oryzanin agar medium (at 30°C for 14 days) consists of only sporangial layer. This species grows well at 37°C in Pfeffer solution and forms sporangia but not at 41°C on potato slice and ferments neither inulin nor galactose.

3. *R. achlamydosporus* Takeda

R. achlamydosporus T. is very similar to *R. formosaensis* N. in morphological and cultural characteristics. Both grow well at 37°C but do not form sporangia on potato slice at 41°C. Chlamydospore is rarely formed on stolons while sporangia and rhizoids are readily formed. Sporangiophores are mostly straight and pale yellowish brown or dark brownish gray in color. However, they are quite different in fermentative activity. *R. achlam-*

ydosporus ferments inulin but *R. formosaensis* does not.

4. R. *tritici* Saito

Pale yellowish orange in color. Sporangiophores are straight, curved or sometimes branched, and their walls are smooth or slightly rough. Sporangia are globose or subglobose in shape and the walls are rough with spines. Sporangispores are elliptical or globose in shape, and the walls are distinctly striated. Globose, elliptical or lemon shaped chlamydospores are observed on stolons. Root-shaped rhizoids are abundant. Columellae are globose or subglobose in shape and their walls are smooth. The turf on Pfeffer-Oryzanin agar medium (at 30°C for 14 days) consists of only sporangial layer. This species forms sporangia on potato slice at 41°C. It does not ferment either inulin or galactose.

5. *R. javanicus* Takeda

Yellowish brown in color on Pfeffer-Oryzanin agar. Sporangiophores, sprouting from the points to which root-shaped rhizoids are attached, are straight or curved and the walls are smooth. Sporangia are globose in shape, and the walls are rough with spines. Sporangiospores are elliptical or polygonal in shape, and the walls are distinctly striated. Columellae are globose or oval in shape, and the walls are smooth. Globose, elliptical, or cylindrical chlamydospores are observed on stolons. The turf on Pfeffer-Oryzanin agar medium (at 30°C for 14 days) consists of only sporangial layer. This species forms sporangia in Pfeffer solution at 37°C, but not on potato slice at 41°C and ferments both inulin and galactose.

6. *R. chiuniang* Yamazaki

Yellowish brown in color. Sporangiophores are straight or curved and their walls are smooth, sometimes swollen and branched. Sporangia are globose or subglobose in shape, and the walls are rough with spines. Sporangiospores are polygonal, globose, angular or elliptical in shape, and the walls are distinctly striated. Columellae are generally globose, subglobose, or oval, and the walls of columellae are smooth or slightly rough. Elliptical or globose chlamydospores are observed on stolons. Rhizoids of this species are root or finger-shaped. The turf on Pfeffer-Oryzanin agar medium (at 30°C for 14 days) consists of only sporangial layer. This species forms sporangia in Pfeffer solution at 37°C and on potato slice at 41°C. It ferments neither inulin nor galactose.

7. *R. acidus* Yamamoto

This species turns from white to yellowish brown in color. Sporangiophores are straight or curved, and the walls are smooth, sometimes swollen and branched. Sporangia are globose or subglobose, and the walls are rough with spines. Sporangiospores are polygonal, globose or elliptical in shape, and the walls are distinctly striated. Various chlamydospores, globose, subglobose or elliptical in shape are observed on stolons. Rhizoids are finger-shaped. Columellae are oval or globose and their walls are generally smooth, sometimes rough. The turf on Pfeffer-Oryzanin agar medium (at 30°C for 14 days) consists of only sporangial layer. This species forms sporangia in Pfeffer solution at 37°C but not on potato slice at 41°C. It does not ferment either inulin or galactose.

8. *R. chinensis* Saito

Grayish white or brownish gray in color. Sporangiophores are straight and short. The walls of sporangiophores are smooth. Sporangia are globose or subglobose, and the walls are smooth. Sporangiospores are mostly globose or subglobose in shape, and the walls are faintly striated. Columellae are subglobose, pyriform or cylindrical in shape, and the walls are usually smooth. Chlamydospores on stolons are globose, elliptical or cylindrical in shape. Rhizoids are poor, finger-shaped. The turf on Pfeffer-Oryzanin agar slant (at 30°C for 14 days) consists of only sporangial layer. The species forms sporangia both in Pfeffer solution at 37°C and on potato slice at 41°C and does not ferment either inulin or galactose.

9. *R. oryzae* Went et Prinsen Geerligs

Brownish gray or dark gray in color. Sporangiophores are straight or curved, and the walls are sometimes rough with spines, sometimes swollen and branched. Sporangia are globose, or subglobose and small spines are observed radially along the outward membrane of sporangia. Sporangiospores are elliptical, globose or irregular in shape, and the walls are distinctly striated. Chlamydospores of cylindrical, globose or elliptical shape are observed on stolons. Columellae are globose, subglobose or oval in shape, and the walls are smooth. Root-shaped rhizoids are abundant.

The turf on Pfeffer-Oryzanin agar medium (at 30°C for 14 days) consists of both mycelial and sporangial layer, but sporangial layer is greater than mycelial layer. In Pfeffer solution at 37°C or on potato slice at 41°C, this species forms sporangia but ferments neither inulin nor galactose. Inui(1965) regarded that *R. oryzae*, *R. tritici* and *R. tonkinensis* are the same species. In this study, however, these species are identified separately according to modified Takeda's classification key. They are quite different in the shape of the turf on Pfeffer-Oryzanin agar medium (at 30°C for 14 days), i.e. the turf of *R. tritici* consists of only sporangial layer while the turf of *R. tonkinensis* consists of both sporangial and mycelial layers, but mycelial layer is greater than sporangial layer. The turf of *R. oryzae* consists of both sporangial and mycelial layers but sporangial layer is greater than mycelial layer.

10. *R. delemar* Wehmer et Hanzawa

Pale yellowish brown or brown colored. Sporangiophores are straight or curved, often swollen or branched, and their walls are usually smooth. Sporangia are globose or subglobose, and their walls are rough with spines. It exhibits brown or black color at maturity. Sporangiospores are elliptical or polygonal, and their walls are distinctly striated. Chlamydospores are globose, elliptical or cylindrical and abundant. Root-shaped rhizoids are abundant. Columellae are globose, oval or cylindrical, and the walls are smooth or slightly rough. It exhibits pale brown in color.

On Pfeffer-Oryzanin agar slant, the turf consists of both sporangial and mycelial layers, but sporangial layer is greater than mycelial layer and is yellowish white to light brownish gray in color from bottom to the top of slant. This species forms sporangia in Pfeffer solution at 37°C but not on potato slice at 41°C and ferments galactose but not

inulin. Inui(1965) regarded that *R. chiuniang*, *R. acidus* and *R. chungkuoensis* are synonyms of this species but in this study, they are indentified separately according to temperature effect and the morphological characteristics of the turf on Pfeffer-Oryzanin agar slant (at 30°C for 14 days). For example, in *R. acidus* and *R. chiuniang*, both turfs on Pfeffer-Oryzanin agar slants (at 30°C for 14 days) consist of only sporangial layer but on potato slice at 41°C. *R. chiuniang* forms sporangia, while *R. acidus* does not. On the other hand, the turfs of *R. delemar* and *R. chungkuoensis* on Pfeffer-Oryzanin agar slant (at 30°C for 14 days) consist of both mycelial and sporangial layers, but *R. chungkuoensis* forms sporangia on potato slice at 41°C, while *R. delemar* does not.

11. *R. chungkuoensis* Yamazaki

Brownish gray in color. Sporangiophores are straight or curved, and the walls are smooth. Sporangia are globose or subglobose, and the walls are rough with spines. Sporangiospores are elliptical, globose, polygonal or irregular in shape, and the walls are distinctly striated. Rhizoids are root-shaped. Chlamydospores on stolons are globose, cylindrical or elliptical. Columellae are globose, subglobose or oval, and the walls are generally smooth but sometimes rough. The turf on Pfeffer-Oryzanin agar slant(at 30°C for 14 days) consists of both mycelial and sporangial layer, but the sporangial layer is greater than mycelial layer. This species forms sporangia on potato slice at 41°C and ferment neither inulin nor galactose.

12. *R. hangchow* Yamazaki

Brownish gray in color. Sporangiophores are straight or curved, and the walls are smooth. Sporangia are globose or subglobose, and the walls are rough with spines. Sporangiospores are elliptical or globose, and the walls are faintly striated. Columellae are globose or subglobose, and the walls are smooth. Chlamydospores on stolons are globose or lemon shaped. Rhizoids are root-shaped. The turf on Pfeffer-Oryzanin agar slant (at 30°C for 14 days) consists of both mycelial and sporangial layers, but sporangial layer is greater than mycelial layer. This species does not form sporangia on potato slice at 41°C but forms sporangia in Pfeffer solution at 37°C, and ferments galactose, but not inulin.

13. *R. japonicus* Vuillemin

The colony of this species on Pfeffer-Oryzanin agar medium is white at first but turns brownish gray in color. Sporangiophores are straight or curved, and the walls are usually smooth, and pale yellowish brown or grayish red brown in color. Sporangia are globose or subglobose, and the walls are rough with spines, and black in color at maturity. Sporangiospores are elliptical, globose, or polygonal, and the walls are distinctly striated. Columellae are globose, oval or subglobose, and the walls are usually smooth. Chlamydospores on stolons are globose, elliptical or cylindrical in shape. Finger- or root-rhizoids are abundant.

The turf on Pfeffer-Oryzanin agar slant (at 30°C for 14 days) consists of both mycelial and sporangial layers, but mycelial layer is greater than sporangial layer. This species forms sporangia in Pfeffer solution at 37°C but does not on potato slice at 41°C, It does not ferment inulin or galactose.

14. *R. bahrnensis* Takeda

Gray or brownish gray in color. Sporangiophores are straight or curved, and the walls are smooth. Sporangia are globose or subglobose, and the walls are rough with spines. Sporangiospores are elliptical, globose, or polygonal, and the walls are distinctly striated. Columellae are globose, subglobose or oval, and the walls are usually smooth. Chlamydospores on stolons are globose or elliptical in shape. Rhizoids are finger- or root shaped. The turf on Pfeffer-Oryzanin agar slant (at 30°C for 14 days) consists of both mycelial and sporangial layers, but mycelial layer is greater than sporangial layer. This species forms sporangia in Pfeffer solution at 37°C and on potato slice at 41°C as well. It ferments galactose but not inulin.

15. *R. tonkinensis* Vuillemin

Colony is dark gray in color. Sporangiophores are straight or curved, and the walls are generally smooth but sometimes rough. Sporangia are globose or subglobose, and the walls are rough with spines. Sporangiospores are elliptical, globose or polygonal, and the walls are distinctly striated. Columellae are globose or subglobose, and the walls are smooth. Chlamydospores on stolons are globose, elliptical or lemon shape. Root-shaped rhizoids are abundant. The turf on Pfeffer-Oryzanin agar slant (at 30°C for 14 days) consists of both mycelial and sporangial layers, but mycelial layer is greater than sporangial layer. This species forms sporangia in Pfeffer solution at 37°C but not on potato slice at 41°C. It ferments neither inulin nor galactose, *R. tonkinensis* is similar to *R. japonicus* except that the former does not ferment raffinose but latter does.

16. *R. arrhizus* Fischer

This species is grayish white in color. Sporangiophores are mostly curved, and the walls are smooth or slightly rough exhibiting brownish white or pale yellowish brown in color. Globose or subglobose sporangia are yellowish brown or dark brownish gray in color, and the walls are rough with spines. Sporangiospores are elliptical or polygonal, and the walls are distinctly striated. Columellae are oval or globose, and the walls are generally smooth but sometimes rough. Rhizoids are poor, very short and finger-shaped. Chlamydospores on stolons are elliptical, globose, or lemon-shaped. The turf on Pfeffer-Oryzanin agar slant (at 30°C for 14 days) consists of only mycelial layer. This species does not form sporangia both in Pfeffer-solution at 37°C and on potato slice at 41°C, and ferments galactose but not inulin.

17. *R. niveus* Yamazaki

White or yellowish white in color. Sporangiophores are mostly curved, and their walls are smooth. Sporangia are globose and the walls are usually smooth and not easily broken. Sporangiospores are elliptical, globose or polygonal in shape and the walls are faintly striated. Columellae are globose or subglobose, and the walls are smooth. Chlamydospores are not observed on stolons. Rhizoids are poor, finger-shaped.

The turf on Pfeffer-Oryzanin agar slant (at 30°C for 14 days) consists of only mycelial layer. This species does not form sporangia either in Pfeffer solution at 37°C or on potato slice at 41°C. It ferments galactose but not inulin.

18. *R. shanghaiensis* Yamazaki

Gray in color. Sporangiophores are straight or curved, and the walls are smooth. Sporangia are globose or subglobose, and the walls are smooth. The sporangia formation is poor. Sporangiospores are globose, polygonal or elliptical in shape, and the walls are faintly striated. Columellae are globose or subglobose, and the walls are smooth. Chlamydospores on stolons are elliptical or globose. Rhizoids are poor, finger-shaped.

The turf on Pfeffer-Oryzanin agar slant(at 30°C for 14 days) consists of only mycelial layer. This species form sporangia poorly in Pfeffer solution at 37°C but does not form on potato slice at 41°C. It ferments neither inulin nor galactose.

19. *R. liquefaciens* Yamazaki

White in color. Sporangiophores are straight or curved, and the walls are smooth. Sporangia are globose or subglobose in shape and the walls are smooth. Sporangiospores are elliptical or globose, and the walls are faintly striated. Globose or elliptical chlamydospores are observed on stolons. Rhizoids are poor, finger-shaped. Columellae are globose or oval in shape, and the walls are smooth. The turf on Pfeffer-Oryzanin agar medium (at 30°C for 14 days) consists of only mycelial layer. It grows well in Pfeffer solution at 37°C and on potato slice at 41°C but does not form sporangia. It ferments neither inulin nor galactose.

摘 要

우리 나라 中部以南과 濟州 일원에 걸쳐 수집한 750여점의 標品중 *Rhizopns*屬에 속하는 151菌株를 분리하여 19種을 同定하였다.

同定된 種은 다음과 같다.

R. nigricans
R. tritici
R. acidus
R. delemar
R. japonicus
R. arrhizus
R. liquefaciens.
R. formosaensis
R. javanicus
R. chinensis
R. chungkuoensis
R. bahrnensis
R. niveus
R. achlamydosporus
R. chiuniang
R. oryzae
R. hangchow
R. tonkinensis
R. shanghaiensis

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Explanation of plates

Fig. 1: Rhizoids of *R. nigricans.*

Fig. 2: Curved sporagiophores of *R. niveus.*

Fig. 3: Swollen sporangiophore with spines of *R. oryzae.*

Fig. 4: Sporangial wall with spines of *R. oryzae.*

Fig. 5: Sporangium with smooth wall of *R. formosaensis.*

Fig. 6: Chlamydospore of *R. chungkuoensis.*

Fig. 7: Sporangiophores emerging from the point without rhizoids of *R. delemar.*

Fig. 8: Young root-shaped rhizoid of *R. japonicus.*

Fig. 9: Branches of sporangiophores of *R. tritici.*

Fig. 10: Chlamydospores of *R. javanicus.*

Fig. 11: Granular stolon without chlamydosphores of *R. achlamydosporus.*

Fig. 12—30: Spores of various species of *Rhizopus.*

Fig. 12: *R. nigricans.*
Fig. 13: *R. chiuniang.*
Fig. 14: *R. tritici.*
Fig. 15: *R. niveus.*
Fig. 16: *R. javanicus.*
Fig. 17: *R. oryzae.*
Fig. 18: *R. japonicus.*
Fig. 19: *R. acidus.*
Fig. 20: *R. bahrnensis.*
Fig. 21: *R. delemar.*
Fig. 22: *R. achlamydosporus.*
Fig. 23: *R. tonkinensis.*
Fig. 24: *R. shanghaiensis.*
Fig. 25: *R. liquefaciens.*
Fig. 26: *R. chinensis.*
Fig. 27: *R. chungkuoensis.*
Fig. 28: *R. hangchow.*
Fig. 29: *R. arrhizus.*
Fig. 30: *R. formosaensis.*

Fig. 31: Turf compositions on Pfeffer-Oryzanin agar medium.

a. Mycelial layer only.
b. Mycelial layer greater than sporangial layer.
c. Sporangial layer greater than mycelial layer.
d. Sporangial layer only.

Fig. 32: Culture in Pfeffer solution at 37°C.

a. *R. niveus.*
b. *R. liquefaciens.*
c. *R. japonicus.*
d. *R. tonkinensis.*
e. *R. acidus.*
f. *R. oryzae.*
g. *R. tritici.*
h. *R. chiuniang.*
i. *R. formosaensis.*
j. *R. nigricans.*

Plate 3

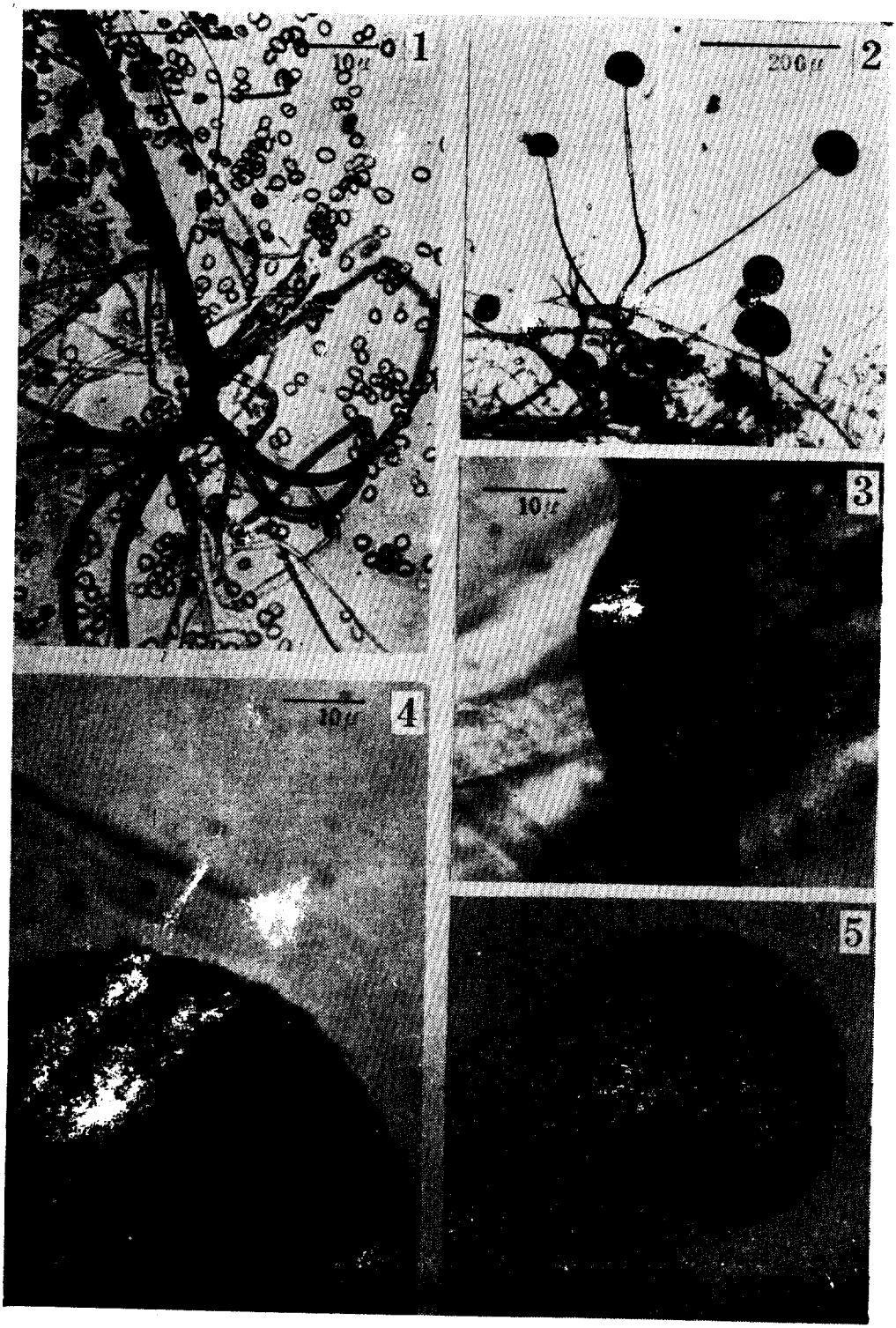

Plate 4

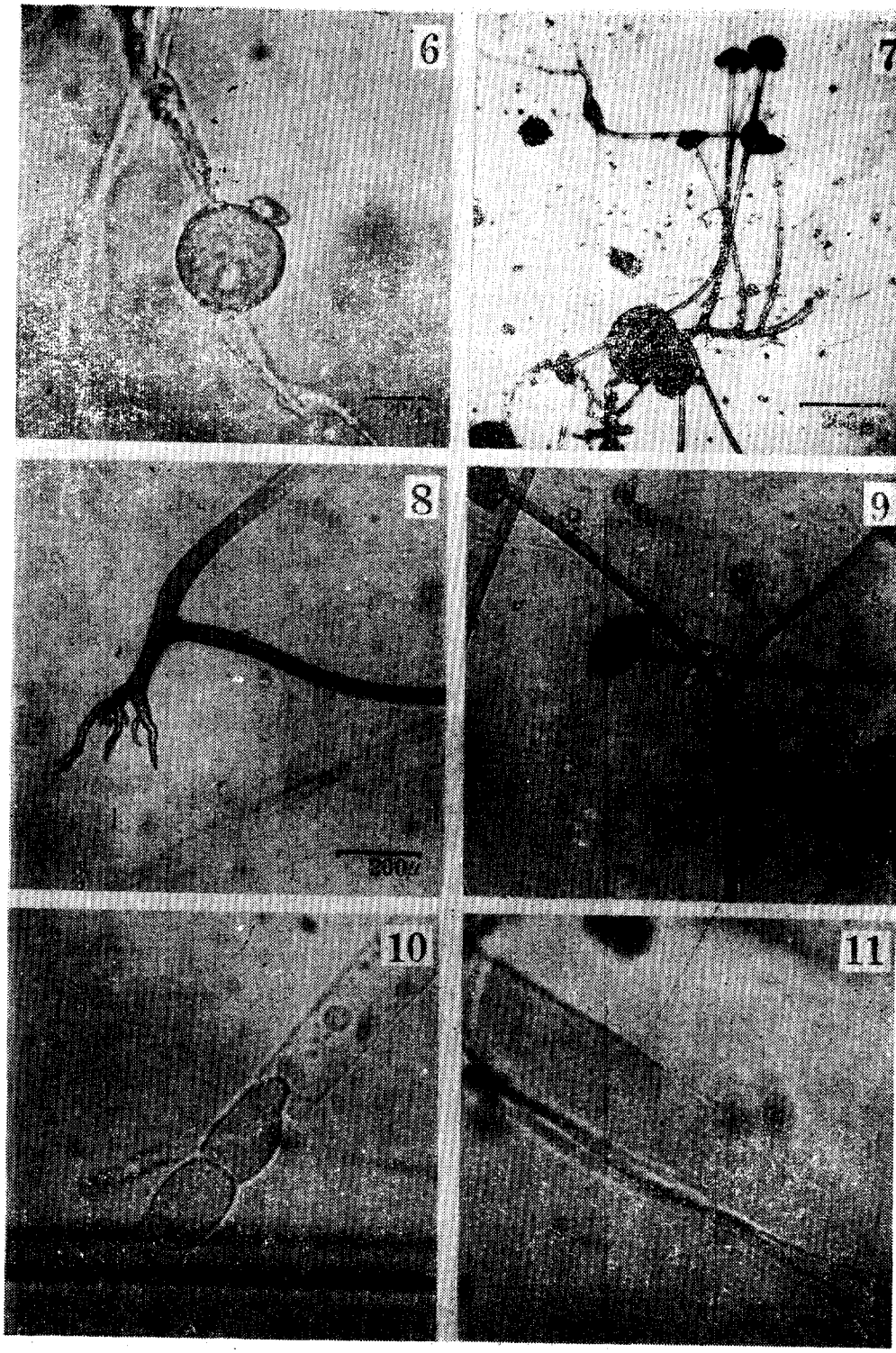

Plate 5

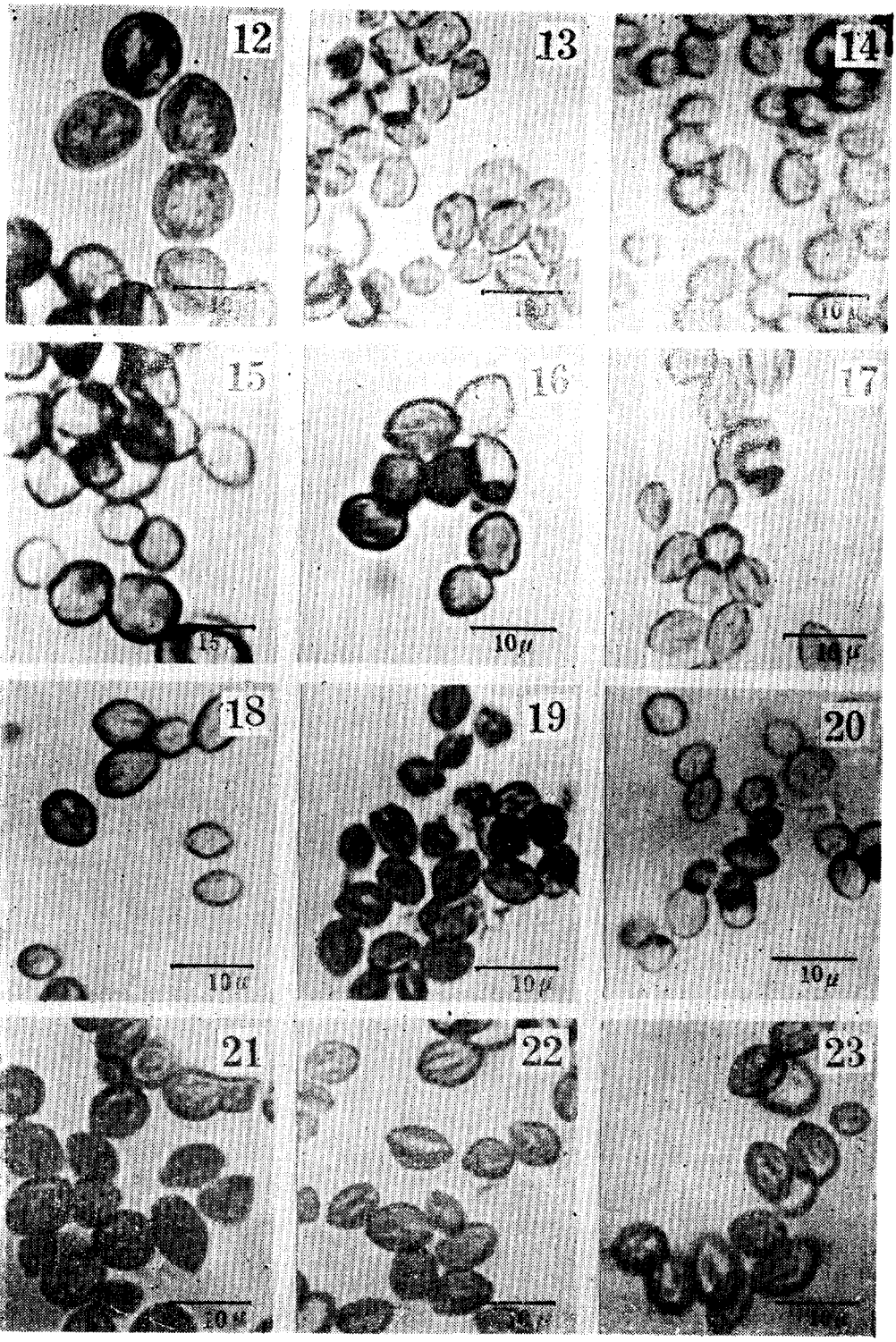

Plate 6