INSTITUTE FOR FERMENTATION

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INSTITUTE FOR FERMENTATION

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FORWORD

The Institute for Fermentation was established at Osaka in November, 1944, as one of the research institutes for microbiology owing to the state policy to be pursued then. Following the termination of the World War its functions have been exercised under the financial protection of Takeda Chemical Industries, Ltd. Up to the year 1960, the activities of the Institute covered various fields for researches into applied and fundamental microbiology, including the production of antibiotics, ergot alkaloids and nucleotides, the microbial transformation of organic substances, and the physiological and taxonomical studies of microorganisms. The results of its researches were published in domestic and foreign scientific journals while these activities have hitherto been reported in the Annual Reports of the Takeda Research Laboratories as no periodical of its own was then available for that purpose.

Originally the Institute was established for forming valuable contributions to the development of fundamental microbiology essential for industry, for which purpose, a type culture collection was attached to the Institute. Under the development of things after the War researches within the Institute had been more in the nature of practical applications than in that of fundamental studies. According to an increase both in number of the reseach staff and the amount of equipment, an astronomical budget was required for administrative purposes.

In the summer of 1960 when a new department of applied microbiology was established in the Takeda Research Laboratories, the Institute for Fermentation was so reorganized as to carry on, as its main objective, studies in the basic field of industrial microbiology, this making it necessary for the Institute to publish its own annual report. It would be a great pleasure for the Institute to seek the advice of acknowledged authorities in all countries on this field of study.

Chōbei Takeda

Chairman of the Board of Trustees

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ADMINISTRATIVE REPORT

Ann. Rep. Inst. Fermentation, Osaka
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A REPORT ON THE TAXONOMY OF RED TO ORANGE *RHODOTORULA*

Takezi HASEGAWA

I. A Brief Historical Review on the Genus.

Rhodotorula is a widespread yeast which is found everywhere in natural world; for example, in the dust of the air, in soil and in human and animal bodies and their excretions. Formerly, it was called *Cryptococcus*, *Saccharomyces* or *Torula*, though its cells are tinged with red or yellow. In 1852, Fresenius first published only one species for this yeast, *Cryptococcus glutinis*, subsequent studies were done by Cohn (1875), Sartory (1907), Pringsheim and Bilewsky (1910) etc. No one had systematically classified this yeast until Saito in 1922. He classified the genus *Torula* into three colored groups. The first group was white to grayish white and included three species: *T. albida*, *T. aerius* and *T. candida*. The second was grayish yellow to yellow, with six species: *T. luteola*, *T. gelatinosa*, *T. aurea*, *T. flavescens*, *T. flava* and *T. rugosa*. The third, orange to red, with eight species: *T. aurantiaca*, *T. sanguinea*, *T. rufula*, *T. corallina*, *T. rubra*, *T. minuta*, *T. ramosa* and *T. rubescens*. To this group Okunuki (1931) added five species, i.e., *T. suganii*, *T. infirmominiata*, *T. miniata*, *T. decolans* and *T. koishikawensis*,. The characteristics used by Saito for identification of these species were the shape of cells, the assimilation of sucrose, maltose, lactose, trehalose and potassium nitrate, and the liquefaction of gelatine.

In 1928, Harrison created the generic name *Rhodotorula* for the third group of Saito's *Torula*. Since then, this generic name has generally been used.

Lodder in her taxonomic monograph, "Die anascosporogenen Hefen" (1934) changed the concept of *Rhodotorula* to the asporogenous yeasts producing carotenoid pigments, and listed thirteen species. She rearranged these taxons into seven species with her collaborator, Kreger-van Rij, in 1952, and gave a key for the identification of each species (Table 1).

Table 1.
A system of classification proposed by Lodder & Kreger-van Rij

1a Nitrate utilized (2)
b Nitrate not utilized (3)
2a Cells round to oval
***Rh. glutinis* (Fres.) Harrison**
Syn. *Cryptoc. glutinis*
Torulopsis bronchialis
Torula suganii
Torula infirmo-miniata
Rh. gracilis
***Rh. glutinis var. rubescens* (Saito) Lodder**
Syn. *Torula rubescens*
Torula rufula
Torulopsis saitoi
Rh. glutinis var. *lusitanica*
b Cells long-oval to elongate
***Rh. aurantiaca* (Saito) Lodder**
Syn. *Torula aurantica*
Torula miniata
Mycotorula colostri
Rh. longissima
3a Glucose and galactose assimilated
***Rh. pallida* Lodder**
b Glucose, galactose and sucrose assimilated
***Rh. minuta* (Saito) Harrison**
Syn. *Torula minuta*
c Glucose, galactose, sucrose and maltose assimilated (4)
d Glucose, galactose, sucrose, maltose and lactose assimilated
***Rh. flava* (Saito) Lodder**
Syn. *Torula flava*
4a Cells long-oval
***Rh. rubra* (Demme) Lodder**
Syn. *Sacch. ruber*
Cryptoc. mena
Cryptoc. corallinus
Rh. rubra var. *longa*
Rh. rubra var. *curvata*
b Cells round to oval
***Rh. mucilaginosa* (Jörg.) Harrison**
Syn. *Torula mucilaginosa*
Torula sanguinea
Cryptoc. ludwigi
Blastodendrion carbonei
Eutorulopsis dubia
Mycotorula pulmonalis
Torulopsis sanniei
Torulopsis biourgei
Blastodendrion simplex
Cryptoc. rubrorugosus
Cryptoc. pararoseus
Rhodotorula aclotiana
Torulopsis nitritophila
Cryptoc. radiatus
Torula decolans
Torulopsis mannitica
Torulopsis aurantia

After Lodder and van Rij's taxonomic work, the following eight species and one variety were published. Most of them were lactose assimilating species newly found.

Rhodotorula marina Phaff, Mrak et Williams (1952)
Rhodotorula texensis Phaff, Mrak et Williams (1952)
Rhodotorula peneaus Phaff, Mrak et Williams (1952)
Rhodotorula tokyoensis Kobayashi (1953)

Rhodotorula crocea Shifrine et Phaff (1956)
Rhodotorula macerans Sonne-Frederiksen (1956)
Rhodotorula glutinis (Fres.) Harrison var. *dairenensis* Hasegawa et Banno (1958)*
Rhodotorula lactosa Hasegawa (1959)
Rhodotorula infirmo-miniata (Okunuki) Hasegawa et Banno (1964)

Rh. tokyoensis was demonstrated to be the same species as *Rh. marina* (Hasegawa *et al*, 1960b) and *Rh. macerans* as *Rh. infirmo-miniata* (Hasegawa and Banno, 1964).

The strains in the second group of Saito's *Torula* were classified into the genus *Cryptococcus* by Lodder and Kreger-van Rij who distinguished this genus from *Torulopsis* by cell encapsulation and by mucous appearance, and from *Rhodotorula* by extracellular starch formation and lack of carotenoid pigments. However, several examples which led to confusion in the differentiation between *Cryptococcus* and *Rhodotorula* were reported (Nakayama *et al.*, 1954; Hasegawa et al. 1960). Moreover, morphological properties proved to be inapplicable for identification of *Rhodotorula* species because of the variability in the cell shapes. A new taxonomic system of the genus *Rhodotorula* was proposed by us in 1960. (Table 2)

Table 2.
Modified system of classification of *Rhodotorula* (Hasegawa et al. 1960b)

Subgenus Rubrotorula

colony color, red to orange

1a	Nitrate utilized	(2)
1b	Nitrate not utilized	(4)
2a	PABA essentially required, lactose assimilated	*Rh. lactosa* Hasegawa
2b	PABA not required	(3)
3a	Lactose assimilated, starch reaction positive	*Rh. infirmominiata* (Okunuki) Hasegawa et Banno
3b	Lactose not assimilated	*Rh. glutinis* Harrison
	Nitrate weakly assimilated	*Rh. glutinis* var. *dairenensis* Hasegawa et Banno
	Colony colored yellowish orange	*Rh. glutinis* var. *aurantiaca* (Saito) Hasegawa
4a	PABA essentially required	(5)
4b	PABA not required, lactose not assimilated	*Rh. rubra* (Demme) Lodder emend. Hasegawa
5a	Maltose, lactose and suclose assimilated	*Rh. marina* Phaff, Mrak et Williams
5b	Maltose not assimilated	(6)
6a	Lactose and sucrose assimilated	*Rh. texensis* Phaff, Mrak et Williams
6b	Lactose not or very weakly assimilated, sucrose assimilated	*Rh. texensis* var. *minuta* (Saito) Hasegawa, Banno et Yamauchi
6c	Lactose and sucrose not assimilated	*Rh. pallida* Lodder

* The name, dairenensis, has been often misprinted as "dairiensis". It comes from a geographical name, Dairen in China.

Subgenus Flavotorula

colony color, reddish yellow to pale yellow

1a Nitrate utilized ..... (2)
1b Nitrate not utilized ..... (3)
2a Lactose assimilated ..... *Rh. gelatinosa* (Saito) Hasegawa, Banno et Yamauchi
2b Lactose not assimilated ..... *Rh. diffluens* (Zach) Hasegawa, Banno et Yamauchi
3a Lactose assimilated ..... (4)
3b Lactose not assimilated ..... *Rh. luteola* (Saito) Hasegawa, Banno et Yamauchi
4a Starch reaction positive ..... *Rh. laurentii* (Kufferath) Hasegawa, Banno et Yamauchi
4b Starch reaction negative ..... *Rh. flava* (Saito) Lodder

The ancestral fungus of *Rhodotorula* is considered to belong to Basidiomycetes and the family Sporobolomycetaceae was given as the most appropriate name. Recently, Banno (1963), a member of this institute, found a sexual phenomenon among several strains of *Rh. glutinis*, that resulted in the detection of a new Basidiomycetes belonging to Ustilaginales, but not to Sporobolomycetaceae. He named it *Rhodosporidium* Banno (1964).

This report has been written to discuss the reasons for revising Lodder and van Rij's system.

2. Variation of Shapes of Cells in *Rhodotorula*.

In 1910, Pringsheim and Bilewsky stated that cells of *Torula glutinis* were spherical to ovoid, (4-5)×(5-6)μ, but under certain conditions giant cells of 10-25μ appeared. This strain was accepted as an authentic strain of the species *Rhodotorula glutinis* by Harrison. Henrici (1941) also pointed out that, in *Rhodotorula*, the size and shape of cells as well as the shade of pigment were highly variable. Actually, almost all the original species classified into the long cell type species, *Rh. aurantiaca and Rh. rubra* by Lodder and Kreger-van Rij, had first been described as the short cell types by their original authors, though the Dutch school modified these data in 1934 or in 1952 (Table 3).

This laboratory tested forty-six cultures of *Rhodotorula* maintained in eleven Japanese research organizations that could be assorted into several groups of lineal descendants of original species published by Saito (1922) and Okunuki (1931). The shape of cells, the assimilability of sugars and of potassium nitrate were compared with data described in the original reports and in Lodder and van Rij's monograph. All the examined properties perfectly coincided with those in the above reports except the shape and size of cells. Morphologically they were not always in accord even among lineal descendants of the same strain; for example, in one culture, the shape of cells was round or oval agreeing with the original description, but

Table 3.
Revision of cell size in original descriptions of *Rhodotorula* by the Dutch school

Names of strains	By original authors	By Lodder in 1934	By Lodder & Van Rij in 1952
Torula aurantiaca Saito (1922)	(3~4)×(5~9)μ	(3~4.5)×(9~14)μ	(3~4)×(9~18)μ
Torula miniata Okunuki (1931)	(3~3.6)×(3.6~4.5)μ	(3~4)×(3.4~6)μ	〃
Mycotorula colostri Castelli (1932)	(1.8~3)×(3.6~10)μ	(4.5~6)×(7~10)μ (2.5~4.5) ×(6.5~12)μ	〃
Torula koishikawensis Okunuki (1931) (=*Rhodotorula longissina* Lodder)	(3.0~3.6) ×(3.6~4.5)μ	(3.5~4.5) ×(8.5~15)μ	〃
Sdccharomyces ruber Demme (1889)	(4.5~5.5)μ	(2~3.5)×(6~9.5)μ	(2~4)×(6~11)μ
Cryptococcus mena Font. et B. (1923)	4.5μ		〃
Cryptococcus corallinus Sartory et al. (1930)	(2~4)μ	(2~4.5)×(4~5.5)μ	〃
Rhodotorula rubra var. *longa* Lodder (1934) (=*Cryptococcus ludwigi* Anderson 1918)	3.5×4.5μ	(2~4)×(7~11)μ	〃
Rhodotorula rubra var. *curvata* Lodder (1934) (=*Torula corallina* Saito 1922)	(3~5)×(3~6.5)μ	(3.5~5)×(5~11)μ	〃

another culture produced long oval or elongated forms that agreed with the description as modified by Lodder and Kreger-van Rij. Several such examples are shown in Table 4.

It was concluded that the discordance of cell morphology observed among descendant cultures of the same strain was caused by natural variation, that is, elongation of cells after the first observation by the original authors. Accordingly the two names of *Rh. aurantiaca* sensu Lodder and *Rh. rubra* sensu Lodder and Kreger-van Rij were rejected by Hasegawa (1958). The former was regarded as synonymous with *Rh. glutinis* and the latter with *Rh. mucilaginosa*. The specific epithet "mucilaginosa" was first given to a species of *Torula* by Jörgensen in 1909, but Demme's epithet, "rubra" was published in 1889. *Rh. mucilaginosa*, therefore, should offer the place of valid name to *Rh. rubra*.

3. *Rhodotorula* as A Capsulated Yeast.

Kutzing found a slimy yeast in 1833 and gave it the generic name *Cryptococcus*, though he described it imperfectly. Later, the genus was defined as asporogenous pathogenic yeast by Vuillemin (1901) and since then, *Cr. neoformans* has been regarded as the type species. Mager and Aschner reported that extracellular starch was restricted to capsulated nonfermenting yeasts which included some pale colored *Torulopsis* as well as *Cr. neoformans*. These strains were also characterized by mucous colony of capsulated cells on solid media. Lodder and Kreger-van Rij modified the definition of

Table 4.
Cell shape and size of *Rhodotorula* strains maintained in the IFO Collection (Hasegawa, 1956)

Names of strains	IFO. No.	Assimilation of						Shape and size of cells	History
		KNO_3	Glucose	Galactose	Sucrose	Maltose	Lactose		
Rhodotorula glutinis	0667	+	+	+	+	+	−	oval 5~8μ	IMAB←CBS
Torula suganii	0695	+	+	+	+	+	−	oval 3.5~6μ	NI←Okunuki
Rhodotorula gracilis	0559	+	+	+	+	+	−	oval 5~8μ	NRRL←Rennerfelt
Torula rubescens	0388	+	+	+	+	+	−	round, short oval 3~6.5μ	GRIF←Saito
"	0413	+	+	+	+	+	−	round, short oval 2.5~6μ	HUT←Saito
Torula rufula	0388	+	+	+	+	+	−	oval 5~8μ	GRIF←Saito
"	0389	+	+	+	+	+	−	oval 4~8μ	HUT←Saito
Torulopsis saitoi	0395	+	+	+	+	+	−	oval 4~7μ	GRIF←Saito
"	0414	+	+	+	+	+	−	oval 4~8μ	HUT←Saito
Torula miniata	0697	+	+	+	+	+	−	oval, long oval 5~10μ	NI←Okunuki
Torula koishikawensis	0386	+	+	+	+	+	−	elongate 8~18μ	GRIF←Okunuki
Torula aurantiaca	0754	+	+	+	+	+	−	elongate 8~18μ	CBS←Saito
Rhodotorula pallida	0715	−	+	+	−	−	−	oval, short oval 4.5~6.5μ	CBS
Torula minuta	0387	−	+	+	+	−	−	oval 3~6μ	GRIF←Saito
"	0412	−	+	+	+	−	−	oval 3~6μ	HUT←Saito
Torula corallina	0382	−	+	+	+	+	−	long oval 5~12μ	GRIF←Saito
"	0406	−	+	+	+	+	−	short oval, round 2.5~6μ	HUT←Saito
"	0709	−	+	+	+	+	−	short oval, oval 4~7μ	OUT←Saito
Torula sanguinea (?)	0417	−	+	+	+	+	−	oval, long oval 6~10μ	HUT←Saito
"	0712	−	+	+	+	+	−	oval, long oval 5~8μ	OUT←Saito
Blastodendrion carbonei	0001	−	+	+	+	+	−	round, short oval 2.5~6μ	HUT←Saito
Blastodendrion simplex	0002	−	+	+	+	+	−	round, short oval 2.5~6μ	HUT←CBS
Cryptococcus pararoseus	0003	−	+	+	+	+	−	elongate 8~18μ	GRIF←Ota
Cryptococcus rubrorugosus	0004	−	+	+	+	+	−	oval, short oval 3~7μ	GRIF←Ota
Torula decolans	0383	−	+	+	+	+	−	long oval, elongate 6~14μ	GRIF←Okunuki
"	0696	−	+	+	+	+	−	oval, short oval 3.5~6μ	NI←Okunuki

CBS: Centraalbureau voor Schimmelcultures, Holland. IMAB: Instituto de Microbiologia Agricola, Agrentina. GRIF: Government Research Institute of Formosa. NI: Nagao Institute. HUT: Faculty of Engineering, Hiroshima University. NRRL: Northern Utilization Research Branch, U.S.D.A. OUT: Faculty of Engineering, Osaka University.

the genus and brought all the capsulated nonfermenting pale-colored asporogenous yeasts into this genus.

In the genus *Rhodotorula*, strains that are mucous in their colony appearance have

been isolated very often, and Hasegawa *et al* (1960a). found remarkable capsules around cells of certain strains, for example, *Rh. glutinis* (syn. *T. miniata*), *Rh. glutinis* (syn. *T. koishikawensis*), *Rh. glutinis* var. *aurantiaca*, *Rh. glutinis* var. *dairenensis*, *Rh. rubra* (syn. *T. collarina*), *Rh. rubra* (syn. *T. simplex*), etc. Moreover, extracellular starch formation was proved in two strains of the genus (*Rh. infirmominiata*), whose capsules, however, are hardly visible. (Sonne-Frederiksen 1956, Lodder *et al.* 1958)

Henrici (1930) stated: "After observing a considerable number of strains of these red yeasts from type culture collections and isolated by myself, the author is still in doubt regarding the unity or plurality of species. Some strains....have consistently yielded a moist mucoid growth on solid media....others have formed cultures which are at first mucoid but gradually become dry and markedly wrinked on the surface; this change is accompanied by the formation of short strands of pseudomycelium. But one finds so many transitions between the mucoid and the wrinkled types of growth that it is impossible to draw lines between the species on this basis." Skinner and Huxley (1956) supported this Henrici's claim.

Mager and Aschner also observed a dissociation phenomenon in a capsulated yeast. They obtained two colony types from a parent capsulated strain. One had large, flat, rapidly growing, grayish, mucoid colonies (M form) and the other small, convex, slow growing, dry-looking colonies (S form). M form cells had broad capsules like the parent cells but most of the S form cells were devoid of the capsule.

Lodder and Kreger-van Rij stated that a strain of *Cr. albidus* in their culture collection was not mucous but dry and dull in colony appearance; little capsule formation was observed, but the production of a starch-like substance could be readily demonstrated. They added that originally this strain had been mucous.

These are the reasons why *Rhodotorula* ought to be regarded as a capsulated genus, though it includes many strains seldom capsulated.

4. Taxonomic Significance of the Color Due to Carotenoid Pigments.

Some yeasts produce carotenoid pigments. Zopf (1889) first noted the presence of lipochromes in cells of a red *Torula* but nothing further was reported until Lederer (1933) found in *Rh. rubra* four pigments: an acidic pigment, β-carotene, torulene and an undefined pigment. Karrer and Rutschmann (1946) investigated the acidic pigment and named it torularhodin. Later, γ-carotene, neurosporene, phytofluene and lycopene were also found.

In 1934, Lodder reported that it was not possible to characterize the genus *Rhodotorula* according to red color shade because it contained red and yellow pigments of carotenoids. She thus modified the concept of the genus and brought a yellowish colored yeast, *Torula flava* into *Rhodotorula*.

Phaff *et al.* (1952) observed an interesting phenomenon that some of *Rhodotorula glutinis* strains were red when grown at room temperature but yellowish at lower temperatures. Nakayama *et al.* examined this further. They used a strain of *Rh. rubra* which remained red when grown at 5°C and a strain of *Rh. peneaus*, a yellow

Rhodotorula which had a paler color at 5°C. When *Rh. peneaus* was incubated at the two temperatures, 25°C and 5°C, the total pigments and β-carotene concentrations showed a marked drop at the lower temperature. The carotenoids of *Rh. rubra* were relatively little modified by the alteration of temperature. However, in *Rh. glutinis* at 25°C, the yellow carotenoids, β-and γ-carotene, comprise 43-47% and the red carotenoids, torulene and torularhodin, 53-57% of the total pigments. At 5°C, the percentages change to yellow carotenoids 92-96%, and red carotenoids only 4-8%. The absorption spectrum in petroleum ether at 5°C of the total pigments of *Rh. glutinis* coincided with that of *Rh. peneaus* at 25°C, but differed from that of *Rh. glutinis* at 25°C. According to Peterson et al., the absorption maxima of pigments extract with petroleum ether from cells of *Rhodotorula* strains showed at 480 and 450 mμ, 480 mμ or 450 mμ, but those of the yellow *Rhodotorula* and of *Cryptococcus* sensu Lodder and Kreger-van Rij were at 450 mμ with the exception of *Cr. neoformans*. Wittman reported that the composition of carotenoids in *Rh. rubra* changed depending on the culture media components.

Hasegawa (1958) reported that, when incubated at 25°C on various solid media, all the strains of *Rhodotorula* sensu Lodder showed a similar reddish color ranging from yellowish red to orange with the exception of *Rh. glutinis* var. *aurantiaca* which was yellowish orange and of *Rh. flava* which was reddish yellow and that such color differentiation could be used for the classification of the genus *.

This was reconfirmed by a further investigation which found a clear correlation between the colors of colonies on potato-yeast extract agar and the absorption maxima of pigments of cells grown in potato-yeast extract broth. Twenty-two strains of *Rhodotorula* of the IFO collection were examined. Two kinds of media were used in these studies. One was Wickerham's synthetic medium (A) which was used by Peterson *et al.* (Table 5). The other was potato-yeast extract broth (B) which was prepared by the following process. Peeled and sliced potato (200g) and press yeast (30g) were

Table 5.
Wickerham's synthetic medium

$(NH_4)_2SO_4$	0.5%	$NaMoO_2.2H_4o$	20γ%
KH_2PO_4	0.1	$ZnSO_4.7H_2O$	40
$MgSO_4$	0.05	Vitamins:	
NaCl	0.01	Biotin	0.2γ%
$CaCl_2.2H_2O$	0.01	Ca-pantothenate	40
Glucose	3.0	Folic acid	0.2
Mineral trace elements:		Inositol	200
H_3BO_3	50γ%	Niacin	40
$CuSO_4.5H_2O$	4	P-aminobenzoic acid	20
KI	10	Pyridoxine hydrochloride	40
$FeCl_3.6H_6O$	1	Riboflavin	20
$MnSO_4.H_2O$	40	Thiamine hydrochloride	40

* The colors were named according to "Guide To Color Standard" published by the Nippon Shikisai Kenkyusho in 1954, which was based on National Bureau of Standard: ISCC—N.B.S. Color Name 23 (1939).

boiled with 1000 ml of distilled water for 30 minutes and the broth filtered through cotton cloth. After cooling, it was centrifuged and the supernatant fluid, pH 5.5, used. In these two media each strain was incubated for four days or more at 28°C on a rotary shaker. The isolated cells were washed with distilled water and then extracted by grinding in a mortar three times with acetone and two times with petroleum ether. These acetone and petroleum ether fractions were combined together in a separatory funnel and distilled water was added until the pigments were completely extracted into the petroleum ether layer. Further extraction from the acetone-water layer was carried out twice with petroleum ether. Three petroleum fractions were combined and washed three times with distilled water. After drying with a small amount of anhydrous sodium sulfate, the pigmented fraction was filtered and concentrated in nitrogen gas current. Absorption spectrum of the extract was determined from 350 to 520 mμ. When necessary, the extract was saponified in 20% KOH methanolic solution. The pigment extracts from red to orange strains grown in the medium A were found to exhibit the various absorption maxima mentioned above and this was in accord with the findings of Peterson *et al.* The absorption maxima of the pigment extracts from the same strains grown in the medium B were all at 480 mμ; those of yellow-colored strains at 450 mμ; and the remainder, *Rh. glutinis* var. *aurantiaca* whose colonies were colored peculiarly yellowish orange on solid media, showed an absorption maximum at 470 mμ. (Table 6, Fig. 1)

Further investigations were also carried out on other asporogenous yeasts. Twenty-nine yellow or pale-colored strains were selected from seven hundred and forty-three cultures of the following genera and species which were listed in Lodder and Kreger-van Rij's text.

Cryptococcus (5 spp.)	*Torulopsis* (22 spp.)
Pityrosporum (1 sp.)	*Brettanomyces* (3 spp.)
Candida (30 spp.)	*Kloeckera* (5 spp.)
Trigonopsis (1 sp.)	*Trichosporon* (4 spp.)

The selected strains were incubated at 28°C for 4 days or more on a rotary shaker, and the pigment was extracted by the same method (Table 7). Among the above genera only *Cryptococcus* was found to produce carotenoid pigment which exhibited the same absorption maximum as that from yellowish *Rhodotorula*. However, none of the five authentic strains of *Cr. neoformans* tested, produced the pigment. It was also found that the pigmentation in these strains of *Cr. neoformans* was not influenced by diphenylamine in doses sufficient to inhibit the pigment production in other Cryptococci and Rhodotorulas (Table 8). From these the intracellular pigment of *Cr. neoformans* seems not to be carotenoid.

It cannot be over-emphasized that in the carotenogenic asporogenous yeasts, a clearcut relationship was observed between the colony color and the absorption maxima of the total pigments extracted when these yeasts were incubated under favorable conditions.

On the other hand, Tsuchiya et al. used the urease test recommended by Seeliger,

Table 6.
The absorption maxima of carotenoid pigments extracted from *Rhodotorula* cells gathered from two media. (Hasegawa et al. 1961c)

	Strain names	Culture nos.	Light absorption maxima	
			Medium A (mμ)	Medium B (mμ)
Rh. glutinis	*Rh. glutinis*	IFO 667	480 (450, 510)	480 (450, 510)
	Rh. gracilis	IFO 559	450, 480	480 (450, 510)
	T. suganii	IFO 695	480 (450)	480 (450, 510)
	T. rubra?	IFO 395	450 (420, 480)	480 (450, 510)
	T. rubescens	IFO 700	450 (420, 480)	480 (450, 510)
	Rh. glutinis var. *lusitanica*	IFO 388	480 (450)	480 (450, 510)
	T. miniata	IFO 697	480 (450, 510)	480 (450, 510)
	T. koishikawensis	IFO 386	450 (420, 480)	480 (450, 510)
	T. aurantiaca	IFO 754	470 (445, 500)	470 (445, 500)
	T. rubra var. *dairenensis*	IFO 415	450, 480	480 (450, 510)
Rh. macerans		IFO 943=CBS 2425	480 (450, 510)	480 (450, 510)
Rh. rubra	*Cr. ruber*	NCYC 68	480 (450, 510)	480 (450, 510)
	Rh. mucilaginosa	NCYC 63	480 (450, 510)	480 (450, 510)
	T. corallina	IFO 406	480 (450)	480 (450, 510)
	T. sanguinea	NCYC 65	480 (450)	480 (450, 510)
		IFO 471	480 (450, 510)	480 (450, 510)
		IFO 712	480 (450)	480 (450, 510)
	T. decolans	IFO 383	480, 450	480 (450, 510)
	Cr. pararoseus	IFO 003	480 (450)	480 (450, 510)
	Cr. rubrorugosus	IFO 004	480 (450)	480 (450, 510)
	Blastod. carbonei	IFO 001	480 (450, 510)	480 (450, 510)
	Blastod. simplex	IFO 002	480 (450, 510)	480 (450, 510)
Rh. minuta		IFO 387	450 (420, 480)	480 (450, 510)
Rh. tokyoensis		IFO 879	480 (450)	480 (450, 510)
Rh. flava		IFO 407	450 (420, 480)	450 (420, 480)
Rh. peneaus		CBS 2409	450 (420, 480)	450 (420, 480)
Rh. crocea		YCC 52-14	450 (420, 480)	450 (420, 480)

NCYC; National Collection of Yeast Cultures, England.
YCC ; Yeast Cultures Collection, University of California. U.S.A.

and stated that the reaction is a valuable criterion for classification purposes because their results coincided well with those of Seeliger's. According to them, the urease positive group in the yeast genera are *Schizosaccharomyces*, *Rhodotorula*, *Sporobolomyces* and *Cryptococcus* sensu Lodder and Kreger-van Rij. It is very interesting from the viewpoint of yeast taxonomy that the carotenogenic asporogenous yeasts are included.

Of the species in the genus *Cryptococcus* emended by Vuillemin, only *Cr. neoformans* (Sanfelice) Vuillemin has been maintained and so Lodder and Kreger-van Rij

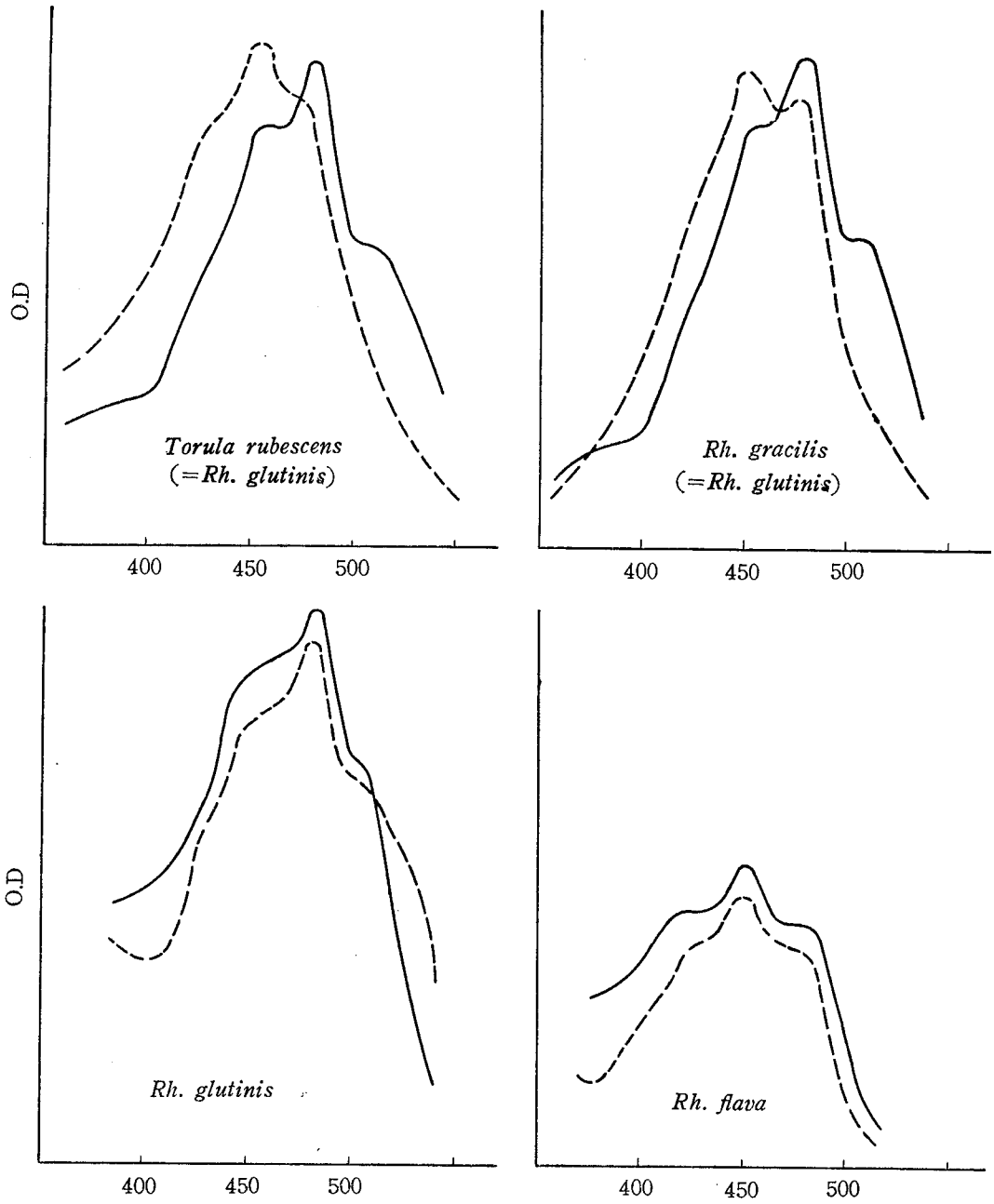

Fig. 1. The absorption curves of carotenoid pigments of *Rhodotorula* strains in two media. (Hasegawa et al. 1960a, 1961c)

accepted this as the type species of *Cryptococcus*. In addition to this species, they brought into the genus several species of *Torula* that were later proved carotenogenic; *Torula laurentii* Kufferath, *T. albida* Saito, *T. luteola* Saito, *Torulopsis diffluens* Zach, etc. and distinguished the genus from *Torulopsis* by mucous appearance caused by capsulated cells, on production of starchlike substance and on complete inability to ferment sugars.

Some difference between *Cr. neoformans* and the carotenogenic Cryptococci has

Table 7.
Extraction test of the carotenoid pigments from colored Torulas (Hasegawa et al, 1961c)

Species	Strains	Color of cells	Color of P.E. extract	Light absorption maxima
Cryptoc. albidus	*T. gelatinosa* (3 *cul.*)	Pale yellow	Yellow	450 (420, 480)mμ
	T. albida (2 cul.)	Yellowish white	Slightly yellow	450
	Tp. liquefaciens	Pale yellowish brown	Slightly yellow	450
	CBS, L-142	Pale yellow	Yellow	450 (420, 480)
Cryptoc. diffluens	CBS, L-160	Pale yellow	Yellow	450 (420, 480)
Cryptoc. laurentii	*T. laurentii*	Dull yellow	Yellow	450 (420, 480)
	T. aurea (2 cul.)	Pale yellow	Yellow	450 (420, 480)
	T. flavescens (2 cul.)	Pale yellow	Yellow	450 (420, 480)
	CBS, L-140	Dull yellow orange	Yellow	450 (420, 480)
Cryptoc. luteolus	*T. luteola* (2 cul.)	Yellow orange	Yellow	450 (420, 480)
Cryptoc. neoformans	CBS, L-132	Yellow orange -dull yellow	Colorless	—
	NI,7496 from CONANT		Colorless	—
	T. histolytica, from TANNER		Colorless	—
	T. histolytica, original		Colorless	—
	Sacch. lithogenes		Colorless	—
	CBS, L-1830	White	Colorless	—
Torulopsis aeria	*T. aeria* (2 cul.)	Pale yellowish brown	Colorless	—
Candida krusei	NI,7492 from CONANT	Yellowish gray	Colorless	—
Candida pseudotropicalis	NI,7494 from CONANT	Dull yellow	Colorless	—
Candida pseudotropicalis var. *lactosa*	*Tp. kefir*	Pale yellow	Colorless	—
Candida scottii	DIETRICHSON's strain	Dull yellow	Colorless	—
Candida humicola	authentic strain	Yellowish gray	Colorless	—
	WICHERHAM's strain		Colorless	—
Candida mycoderma	*Mycoderma vini*	Pale yellowish brown	Colorless	—
Trichosporon cutaneum	*Parend. asteroides*	Pale yellowish brown	Colorless	—
	Parend. balzeli		Colorless	—
	Hemisp. rugosa		Colorless	—
Trichosporon pullulans	*Oidium pullulans*	Pale yellow	Colorless	—
Trichosporon beigelii	authentic strain	Pale yellowish brown	Colorless	—

also been recognized. The former is distinguished from the latter group by growth at 37°C and virulence for mice. Benham (1956) stated that the medical mycologists had found the properties necessary not only for identification but also for pathogenicity and non pathogenicity determination.

Wickerham (1952) suggested that *Cryptococcus* could be related to an ascogenous yeast, *Lipomyces*. Benham, observing plurispored asci in one strain of *Cr. neoformans* isolated from a dog brain, concluded that *Cr. neoformans* and *Lipomyces starkeyi* might be the same organism.

Before Benham, Todd and Herrmann (1936) and Redaelli et al. (1937) reported on the ascospore formation in *Cr. neoformans*. However they concluded that this ascosporogenous organism belongs to *Debaryomyces*. Emmons *et al.* stated that many old cells in culture are surrounded by a burr-like envelope which is interrupted only at one point where the cell continues to bud and that it was probably this appearance

Table 8.
Inhibition of the carotenoid production by DPA (Hasegawa et al. 1961c)

strain names	DPA concentration (p.p.m)							
	0		20		40		80	
	Growth	Color	Growth	Color	Growth	Color	Growth	Color
Rh. glutinis	+++	Pink	+++	Yellowish Pink	++	Yellow	−	−
Rh. glutinis var. *aurantiaca*	+++	Orange	+++	Orange	+++	Orange	±	White
Rh. glutinis	+++	Reddish Pink	++±	Orange	++±	Yellow	++	White
Rh. mucilaginosa	+++	Pink	+++	Cream	+++	White	+++	White
Rh. rubra	+++	Pink	+++	Yellowish cream	+++	White	++	White
Rh. flava	+++	Yellow	+++	Yellow	+++	Cream	++	White
Cr. albidus	+++	Pale yellow	+++	Pale yellow	+++	White	++	White
Cr. laurentii	+++	Yellow	+++	Yellow	+++	Cream	+	White
Cr. neoformans 1	+++	Yellow	+++	Yellow	+++	Yellow	++	Yellow
Cr. neoformans 2	+++	Yellowish cream	+++	Yellowish cream	+++	Yellowish cream	+++	Yellowish cream
Cr. neoformans 3	+++	Yellowish cream	+++	Yellowish cream	+++	Yellowish cream	+++	Yellowish cream

that led Todd and Herrmann to interpret the cell within the apparently dense and "coagulated" capsule as an ascospore and led them to place the fungus in the genus *Debaryomyces*. They also added that Benham's report on ascospore production in the genus had not been generally confirmed.

Recently, Kobayashi and Tubaki studied the asexual reproduction of three Heterobasidiomycetes; *Tremella fuciformis*, *Naematelia encephala* and *Holtermannia corniformis* and found that the monobasidiospore cultures of these organisms were uniformly *Cryptococcus*-like yeasts. These organisms *in vitro* are not able to grow at 37°C. The presence of carotenoid pigments was recorded in uredospores of several Heterobasidiomycetes. Lederer (1938) proved α-, β- and γ- carotenes in *Puccina coronifera*, *Coleosporium senecioni* and *Tremella mesenterica*, and only β- carotene in *Aleuria aurantia*.

It is quite probable that the carotenogenic Cryptococci are related to the Basidiomycetes.

But the sexual stage of *Cr. neoformans* is difficult to ascertain. This was the reason that, on the basis of the system of Lodder and van Rij's classification of the asporogenous yeast, Hasegawa *et al.* (1960b) proposed to remove the carotenogenic Cryptococci into the genus *Rhodotorula* of the carotenogenic subfamily Rhodotoruloideae in the family Cryptococcaceae leaving *Cr. neoformans* in the subfamily Cryptococcoideae. They also proposed two subgenera Flavotorula and Rubrotorula Hasegawa, Banno et Yamauchi in the genus *Rhodotorula*. Flavotorula includes three

yellowish Rhodotorulas, *Rh. flava* Saito, *Rh. paneaus* Phaff et al. and *Rh. crocea* Phaff et al. together with carotenogenic Cryptococci and Rubrotorula includes the red to orange Rhodotorulas.

5. Serological Information about *Rhodotorula* spp.

An application of the serological methods like Kauffman employed for the genus *Salmonella* has been developed to classify yeast species by Yukawa *et al.*, Benham, Tsuchiya *et al.* and Seeliger. All of them indicated that serological characteristics can serve for classification. For example, Benham (1935) reported that yeasts parasitic on man might be divided into four groups on the basis of colony appearance and that these four groups can be also sharply differentiated by agglutination tests.

According to Tsuchiya *et al.*, almost all species of yeasts can be classified into five independent antigenic groups represented by *Candida albicans*, *Schizosaccharomyces pombe*, *Rhodotorula glutinis*, *Rh. minuta* and *Cryptococcus neoformans*. They distinguished many yeast species by slide agglutination methods with monospecific or absorbed antisera homologous to each of the thermostable and thermolabile antigens prepared from the five standard organisms mentioned above. Their findings showed that some remarkable relationships exist between serological data and biological characteristics commonly used for yeast classification criteria.

Concerning the genus *Rhodotorula*, Tsuchiya *et al* and Seeliger published results of their examinations independently. (Table 9 and 10)

Both stated that *Rh. mucilaginosa* is closely related to *Rh. rubra and Rh. minuta* to *Rh. pallida* serologically. Tsuchiya *et al.* also reported that *Rh. glutinis* is closely related to *Rh. aurantiaca* (they used the strain named *T. aurantiaca* by Saito). These results are coincident with a taxonomic conclusion of Hasegawa that from the morphological point of view, *Rh. mucilaginosa* is the same species as *Rh. rubra* and *T. aurantiaca* is a peculiarly colored variety of *Rh. glutinis*.

Seeliger maintained red to orange Rhodotorulas could be divided into three groups, while Tsuchiya *et al.* asserted that they are divided into two groups. Previously Skinner and Huxley argued that these Rhodotorulas should be considered as monotypic, and that seventy-six synonyms and three nomina nuda of the *Rhodo-*

Table 9.
Antigenic structures of six species of the genus *Rhodotorula* published by Tsuchiya et al.

Species	Thermostable antigens					
Rh. glutinis	1	2		5		
Rh. aurantiaca	1	2		5		
Rh. mucilaginosa	1		3			
Rh. rubra	1	2	(3)			
Rh. minuta				4		7
Rh. pallida				(4)	6	

Table 10.
Precipitation reaction with antisera of *Cryptococcus* and *Rhodotorula* published by Seeliger

Antigen	Serum						
	Cryptococcus			*Rhodotorula*		*Candida albicans*	*Saccharomyces cerevisiae*
	neoformans	*luteolus*	*laurentii*	*mucilaginosa*	*minula*		
Cryptococcus neoformans (6 strains)	+++	−	+++	−	−	−	−
C. *diffluens* (8 strains)	+++	−	+++	−	−	−	−
C. albidus (5 strains)	+++, + or −	−	++/−	−	−	−	−
C. luteolus	−	+++	−	−	−	−	−
C. laurentii	++	−	+++	−	−	−	−
C. terreus	−	−	−	−	−	−	−
Rhodotorula mucilaginosa	−	−	−	+++	−	−	−
Rh. rubra	−	−	−	++	−	−	−
Rh. minuta	−	−	−	−	+++	−	−
Rh. pallida	−	−	−	−	+++	−	−
Rh. glutinis, Rh. aurantiaca, Rh. glut. var. rubescens	−	−	−	−	−	−	−
Rh. flava	−	−	±	−	−	−	−

torula species listed in Lodder and van Rij's text could belong to only one species, *Rh. glutinis*. This opinion, however, can not be accepted on the basis of serological data. Seeliger showed that *Rh. flava*, a yellowish *Rhodotorula*, is related to *Cryptococcus laurentii*, a carotenogenic *Cryptococcus* but not to red *Rhodotorula*. Tsuchiya *et al.* also stated that *Rh. flava* did not have any positive cross agglutination reaction against red to orange *Rhodotorula*.

6. Discussion on Some Properties Used for Classification of the Red to Orange *Rhodotorula*.

Property to Utilize Potassium Nitrate

Assimilation of nitrate has been regarded as highly useful for yeast taxonomy. This was used by Saito and the Dutch school also in the classification of *Rhodotorula*, but not by Skinner and Huxley who pointed out a correlation between this property and the vitamin requirements in the genus and concluded that it only was a subspecific criterion. According to Skinner and Huxley (1956), in red to orange *Rhodotorula*, all strains utilizing nitrate required no vitamins and belonged to only one genus, *Rh. glutinis*. However, two other species and two varieties having this property were found later to require specific vitamins. These are *Rh. macerans* Sonne-Frederiksen, *Rh. lactosa* Hasegawa, *Rh. glutinis* var. *infirmominiata* (Okunuki) Lodder and *Rh. glutinis* var. *dairenensis* Hasegawa et Banno. Among these, *Rh. macerans* and *Rh. glutinis* var. *infirmominiata* were proved to be the same species for the following reasons and a new

combination, *Rh. infirmominiata* (Okunuki) Hasegawa et Banno was made. (Hasegawa and Banno, 1964)
The reasons are:

1. According to the original descriptions, these two coincide with each other in sugar and nitrate utilization, though the living culture of *Rh. glutinis* var. *infirmominiata* lost the ability to assimilate lactose. The nature of this subsequent loss will be further discussed.

2. Both strains possess an optimum growth temperature at about 20°C, though almost all the other red to orange strains are 25-33°C.

3. Among red to orange Rhodotorulas, only these two produce a starch-like compound in culture media.

4. The two strains require biotin for their growth while other authentic cultures of *Rhodotorula* do not.

The name *Rh. glutinis* var. *dairenensis* was given to one of Saito's strains, *Torula rubra* var. α, which assimilated nitrate weakly. This weak assimilability has remained unchanged since publication by Saito in 1922, as ascertained with five living descendants maintained in separate research organizations in Japan (Table 11). No stronger testimony could be given to the reliance of this property as a taxonomic key for classification of the genus.

Table 11.
Abilities to utilize nitrate of red to orange species of *Rhodotorula*
(Hasegawa and Banno 1958)

Species	KNO_3-medium	$(NH_4)_2SO_4$-medium
Rh. glutinis	45	75
Rh. glutinis var. *aurantiaca*	18	25
Rh. glutinis var. *dairenensis*	10	70
Rh. rubra	0	80

growth in two kinds of liquid media was estimated by the optical density ($\times 100$) after seven days' incubation at 25°C with Coleman's colorimeter. The basal medium contains NaH_2PO_4, $MgSO_4 \cdot 7H_2O$, NaCl, $CaCl_2 \cdot 2H_2O$, glucose and vitamins.

Property to Assimilate Sugars

Lodder, and Lodder and Kreger-van Rij used physiological characteristics in diagnosing *Rhodotorula* species. These characters are principally abilities to assimilate glucose, sucrose, maltose, galactose and lactose as the sole source of carbon and to utilize potassium nitrate as the sole source of nitrogen. In their papers all species of *Rhodotorula* were reported not to utilize lactose. Skinner and Huxley, however, reported the ability in five strains of *Rh. minuta* including the authentic culture of the species that had been kept in Centraalbureau voor Schimmelcultures, Baarn, Netherlands. Besides the above sugars, these authors used twenty-one carbon compounds for assimilation tests and offered the following comment on Lodder and van Rij's system.

"Except for tests which were all negative or all positive (soluble starch, melibiose, i-erythritol, d-xylose, succinic acid and i-inositol utilization and starch production) there was no consistency in any of the other physiological tests."

In their conclusion, Skinner and Huxley, however, did not suggest revision of the genus by substituting new criteria and instead, they regarded it as a monotypic genus. This conclusion was put wrongly as mentioned in the preceding chapter. The important fact is that they pointed out a variation in the property to assimilate sugars. Lodder (1934) reported that *Torula infirmominiata* Okunuki were not able to utilize lactose regardless of its original description and she concluded that this strain belonged to *Rhodotorula glutinis*. Skinner and Huxley (1956) proved weak assimilation of lactose in the authentic culture of *Rh. minuta* (Saito) Lodder, though both Saito and Lodder had gained negative results.

Such a confusing results seem to be because assimilability of lactose in red to orange *Rhodotorula* sometimes changes during successive transfers.

We observed that several newly isolated strains (for example, *Rh. marina* and *Rh. texensis* sent from Phaff) were variable in lactose assimilation unless lactose was added to the medium as the sole source of carbon. Ahearn *et al.* had the same results.

Hasegawa (1959) and Hasegawa *et al.* (1960b), however, detected a taxonomical meaning in the assimilability of lactose. On vitamin requirement, the lactose assimilating strains can be divided into two groups. One group is sensitive to para-aminobenzoic acid (PABA). *Rh. lactosa*, *Rh. marina*, *Rh. texensis*, *Rh. minuta* and *Rh. pallida* belonge to it. The other group requires biotin and contains two strains of *Rh. infirmominiata*. The latter group is also characterized by positive iodine reaction of extracellular accumulated polysaccharide in contrast to the negative reaction of other red to orange Rhodotorulas. Another group is not able to assimilate lactose originally and consists of two species: *Rh. glutinis* and *Rh. rubra*. It is important that there is a certain correlation between such a classification and serological data as mentioned before. (Table 12)

The author wishes to discuss next the taxonomic position of two strains previously identified as *Rh. aurantiaca*.

Robbins and Ma (1944) reported that a strain of *Rhodotorula* requiring PABA and thiamine was identified as *Rh. aurantiaca*. A few years ago, the author obtained the strain given the accession number ATCC, 9536 from the American Type Culture Collection and examined its morphological and physiological characters. The following results were obtained;

On malt extract agar, the shape of cells is long oval, 2.0-2.5$\times$5.0-10.0μ, single or often in pairs. Colony color after one month at 24°C is yellowish red. Glucose, galactose, sucrose, maltose, lactose and ethanol are assimilated. It utilizes potassium nitrate as the sole source of nitrogen in a simplified synthetic medium. Splitting of arbutin is positive and iodine reaction of polysaccharide accumulated in the medium is negative. Of eight water-soluble vitamins tested, it required PABA essentially and thiamine stimulatively.

Table 12.
The requirement of vitamins by Rubrotorula (Hasegawa et al. 1960b)

Species	Strain names	Cultures No.	B_1HCl	PABA	Biotin	Niacin	Pantothenate	Inositol	B_6HCl	B_1
Rhodotorula rubra	*Cryptoc. ruber*	NCYC 68	S	—	—	—	—	—	—	—
	Cryptoc. rubrorugosus	IFO, 0004	S	—	—	—	—	—	—	—
	Cryptoc. rubrorugosus	NCYC 64	S	—	—	—	—	—	—	—
	Cryptoc. rubrorugosus	NRRL Y-843	(S)	—	—	—	—	—	—	—
	Cryptoc. pararoseus	IFO, 0003	S	—	—	—	—	—	—	—
	Cryptoc. pararoseus	NRRL Y-844	S	—	—	—	—	—	—	—
	Blastodendrion carbonei	IFO, 0001	S	—	—	—	—	—	—	—
	Blastodendrion simplex	IFO, 0002	S	—	—	—	—	—	—	—
	Torula mucilaginosa	NCYC 63	S	—	—	—	—	—	—	—
	Torula mucilaginosa	NRRL Y-168	S	—	—	—	—	—	—	—
	Torula sanguinea	NCYC 65	S	—	—	—	—	—	—	—
	Torula sanguinea	NCYC 66	(S)	—	—	—	—	—	—	—
	Torula sanguinea	NRRL Y-174	S	—	—	—	—	—	—	—
	Torula corallina	IFO, 0406	S	—	—	—	—	—	—	—
	Torula decolans	IFO, 0383	S	—	—	—	—	—	—	—
	Torula sanguinea?	IFO, 0712	—	—	—	E	—	—	—	—
Rhodotorula minuta		IFO, 0387	S	E	—	—	—	—	—	—
Rhodotorula pallida		CBS, L-320	(S)	E	—	—	—	—	—	—
Rhodotorula glutinis var. *dairenensis*		IFO, 0415	S	—	—	—	—	—	—	—
Rhodotorula lactosa		IFO, 1006	—	E	—	—	—	—	—	—
Rhodotorula marina		CBS, 2365	—	E	—	—	—	—	—	—
Rhodotorula marina (*Kobayashi strain*)		IFO, 0879	S	E	—	—	—	—	—	—
Rhodotorula texensis		CBS, 2177	—	E	—	—	—	—	—	—
Rhodotorula macerans		CBS, 2425	—	—	E	—	—	—	—	—
Torala infirmominiata		CBS, 0323	—	—	E	—	—	—	—	—

S: stimulative requirement E: essential requirement

According to Ahearn *et al.* (1952), *Rh. aurantiaca* NRRL Y-1581 required PABA and was incapable of assimilating lactose (Table 12). The author reconfermed this on the strain sent from the ARS Culture Collection (NRRL), but after a few successive cultivations on a synthetic medium containing lactose, this strain gained the ability to assimilate lactose. However, when subcultured on a medium without lactose this ability weakened easily.

As to taxonomic position of these two strains, the author believes neither of them belongs to *Rh. aurantiaca* but to *Rh. lactosa.*

Among the three groups, only the PABA-requiring one has several species that

were described originally to differ from each other by kinds of sugars assimilated (Table 13). However, some conflicting data were reported on these differentiation regarding to not only lactose but also other sugars as well. Several authors published different results of sugar assimilation test on a strain belonging to Rh. marina that was named Rh. tokyoensis by Kobayashi (Table 14). Moreover, Yonezawa reported that this strain was quite in accord with *Rh. minuta* serologically. Ahearn et al. (1962) also reported that all the species of this group failed to assimilate maltose and the assimilation of sucrose as well as galactose in *Rh.* pallida is variable.

From the above facts, it is concluded that it is difficult to differentiate species of the group with assimilability of sugars.

Table 13.
Difference among patterns of sugar assimilation with PABA requiring species of *Rhodotorula* described by original authors.

Rh. lactosa	*Rh. marina*	*Rh. texensis*	*Rh. minuta*	*Rh. pallida*
Assimilates				
KNO_3	galactose	galactose	galactose	galactose
galactose	maltose	sucrose	sucrose	
maltose	sucrose	lactose		
sucrose	lactose			
lactose				

Table 14.
Discordance among data of sugar assimilation with *Rh. marina* strain Kobayashi by several authors.

authors	galactose	maltose	sucrose	lactose
Kobayashi 1953	±	±	+	w+
Hasegawa 1959	+	w+	+	+
Yonezawa 1959	+	−	+	−
Ahearn et al. 1962	+	−	+	+

w+ means weakly positive

Requirement of Vitamins

As mentioned above, the first group in the red to orange *Rhodotorula*, with few exceptions, required no vitamins other than thiamine. The second one was characterized by dependence on para-aminobenzoic acid and the third one by a biotin requirement (Table 11). The requirement of thiamine was very common among all kinds of *Rhodotorula*. Previously, Hasegawa *et al.* (1960b, 1961) concluded that the two subgenera of *Rhodotorula* can be distinguished by their response to each of the two moieties of the vitamin. Later, stability of the vitamin requirement was reexamined by Hasegawa and Banno (1963) with several strains of *Rhodotorula* on two different media. These tests also compared two methods of incubation, shaking and stationary. It was found that requirement for thiamine and its pyrimidine and thiazole moieties

varied considerably with culture conditions. But requirement for PABA did not vary under different conditions of preservation and incubation, indicating its essentiality in accordance with the previous results. (Table 15)

Table 15.
Stability or variability of the degree of vitamin requirement under different culture conditions. (Hasegawa and Banno 1963)

Names of strains	Preservation media	Methods of incubation	Growth rates in each medium					
			I	II	III	IV	V	VI
Rh. rubra	P.Y.G	Stationary	29	24	93	140	124	100
	"	Shaking	13	15	100	100	100	100
	M	Stationary	9.1	8.8	98	98	88	100
	"	Shaking	0	0	100	95	100	100
Rh. lactosa	P.Y.G	Stationary	5	33	3.3	95	41.5	100
	"	Shaking	2	10	2	50	15	100
	M	Stationary	0	30	0	76	37.5	100
	"	Shaking	2	2	2	60	10	100
Rh. marina strain *tokyoensis*	P.Y.G	Stationary	11.94	8.9	9.6	38.8	15.7	100
	"	Shaking	5.1	6.9	6.9	6.9	6.5	100
Rh. texensis	P.Y.G	Stationary	10.1	7.8	9.8	12.7	7.8	100
	"	Shaking	3.9	6.25	4.5	4.4	3.9	100
Rh. flava	P.Y.G	Stationary	37	37	89	124	59.5	100
	"	Shaking	8	10	90	100	30	100
Rh. laurentii strain *peneaus*	P.Y.G	Stationary	15.9	14	123	116	8.9	100
	"	Shaking	8.9	4.6	73	93.4	9.7	100

Medium I is the basal medium; Medium II contains para-aminobenzoic acid; Medium III thiamine; Medium IV para-aminobenzoic acid and 4-methyl-5-(β-hydroxyethyl)-thiazole; Medium V para-aminobenzoic acid and 2-methyl-4-amino-5-(aminomethyl)-pyrimidine hydrochloride; Medium VI para-aminobenzoic acid and thiamine (complete medium). P.Y.G.—potato-yeast extract agar added glucose; M—malt extract agar.

Table 16.
Characteristic properties of the three groups of Rubrotorula.

Characteristics	Groups		
	I	II	III
Specific vitamin required	—	PABA	biotin
Iodine reaction of culture broth	—	—	+
Assimilability of lactose	—	+(losable)	+(losable)
Optimum temperature for growth	25-33°	25-33°	18-20°
Antigen nos. detected by TSUCHIYA et al.	1, 2, 3, 5	4, 6, 7	

Table 17.
Three groups of Rubrotorula distinguished by their vitamin requirement

	Group I	Group II	Group III
Assim. of KNO_3 +	*Rh. glutinis* *Rh. glutinis* var. *dairenensis* *Rh. glutinis* var. *aurantiaca*	*Rh. lactosa*	*Rh. infirmominiata*
Assim. of KNO_3 −	*Rh. rubra*	*Rh. marina* *Rh. texensis* *Rh. minuta* *Rh. pallida*	

The characteristic distinctions among three groups of the red to orange *Rhodotorula* are summarized in Table 16 and 17. These tables showed that vitamin requirement differences are related to differences in other features, such as iodine reaction, assimilability of lactose, optimum temperature for growth and kind of antigens, and that it would be better to regard *Rh. texensis*, *Rh. minuta* and *Rh. pallida* as probable synonyms of *Rh. marina*.

7. Sexual Stage of Red to Orange *Rhodotorula*.

Nadson and Philippov (1928) observed the origin of yeasts resembling *Sporobolomyces* species from asporogenous mucoid rose yeasts indistinguishable from *Rhodotorula* species, and Derx (1930) observed the same sort of transformation to occur. Lodder (1934) recorded that cultures of *Sporobolomyces* maintained in the type culture collection at Delft have irreversibly lost the power to form basidiospores and become indistinguishable from the ordinary rose asporogenous yeasts. Henrici (1941) stated that these facts indicate stronly *Rhodotorula* to be considered as imperfect forms of the basidiospore-forming yeast, *Sporobolomyces*. But the formation of basidiospore (ballistospore) by *Rhodotorula* yeast has never been reported.

Recently, Banno (1963) observed sexual interaction between a couple of well-known strains of *Rh. glutinis* named *Torula koishikawensis* and *Rh. gracilis*. After ascertaining that they were haploid (Banno, 1963a), he made a conjugation test between a methionine-, pantothenic acid-less mutant of the former and a para-aminobenzoic acid-less, yellow-colored mutant of the latter.

Equal numbers of the two mutant cells were mixed in a synthetic medium. The mixture was incubated on a slow rotator at 25°C for 24 hr., and then spread on a minimal medium. a few pale brownish pink colonies appeared after incubation of about four days. They were prototrophs and contained cells of mycelial type, whereas all cells of the mutants as well as of their original strains had oval shapes. In addition, a clamp-like structure was observed at the septum of hyphae developed from each conjugated paired cells. Banno successfully followed its life cycle. (Fig. 2)

He, however, could not observe any ballistospores during the life cycle. He did

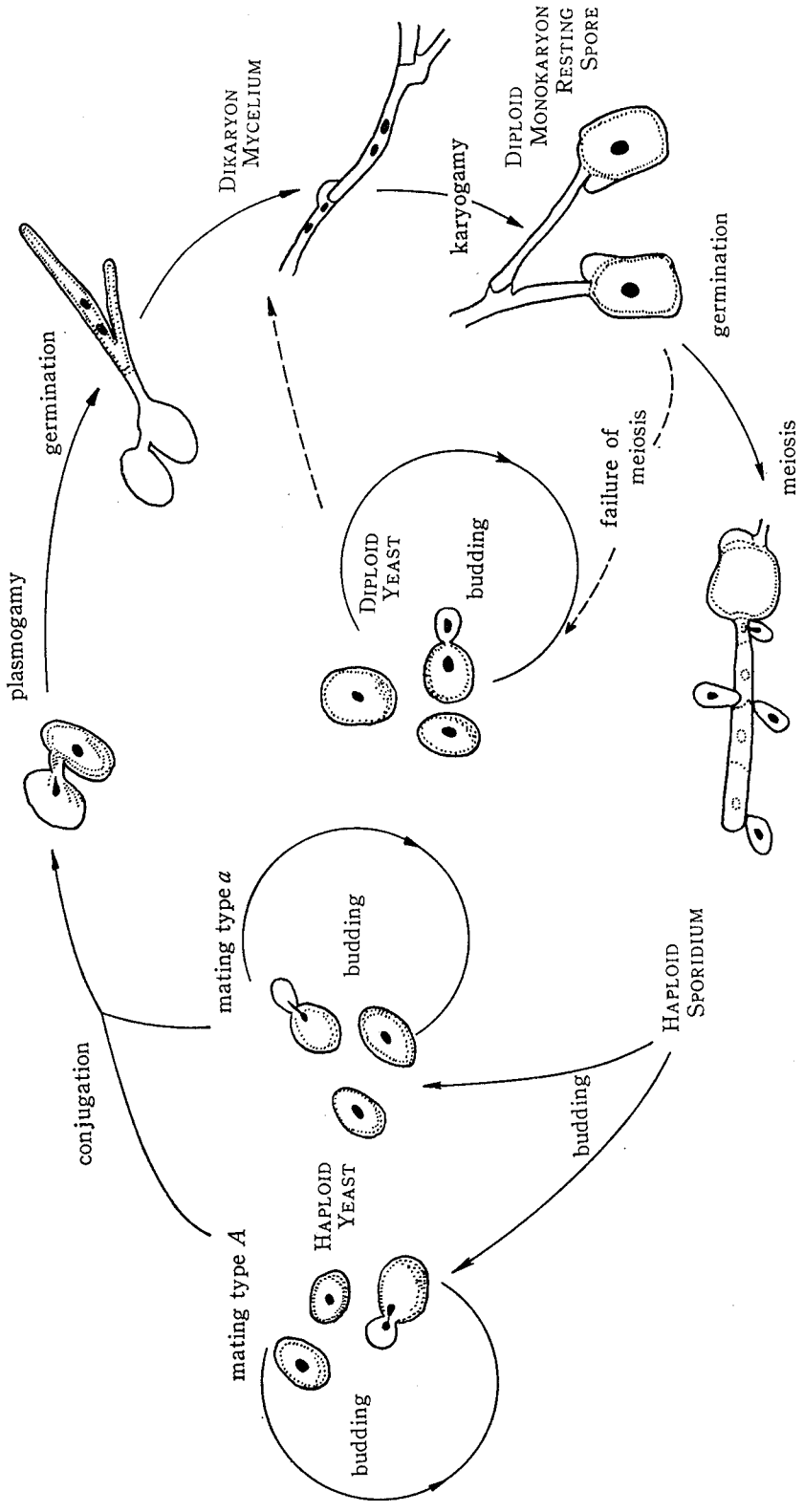

Fig. 2. Life cycle of *Rhodotorula glutinis*.

see pink sporidia budded laterally from promycelia and large dark colored resting spores. The sexual stage closely resembled that in the family Ustilaginaceae of Heterobasidiomycetes. Similar sexual phenomenon was observed among other couples of the mutants and even between the original strains and also among several others of the species. On the other hand, Tsuchiya *et al.* (1965) reported that they could confirm a certain relationship between *Rhodotorula* and *Sporobolomyces* serologically. It may be also possible that some other sporogenous stage will be detected in the red to orange *Rhodotorula* in the future.

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MICROBIOLOGICAL ACTIVITIES OF THIAMINE AND ITS RELATED COMPOUNDS FOR NINETEEN STRAINS OF LACTIC ACID BACTERIA

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Introduction

Since the discovery of allithiamine, a number of thiamine analogues have been synthesized. Some of them were found to have several advantages over thiamine itself such as better absorbability and stability. Their microbiological activities have also been studied (Hosoda et al., 1958a, 1958b; Fujiwara, 1960a, 1960b, 1960c; Kawasaki et al., 1961, 1963a, 1963b; Hiraoka and Shimamoto, 1961; Fukui et al., 1961; Hayashi and Kamikubo, 1962; Chibata et al., 1963; Kawasaki and Yamada, 1963; Kawasaki and Shinoda, 1963, 1964; Kawasaki and Nagayama, 1963; Kawasaki and Kishi, 1964; etc.), employing mainly *L. fermenti* 36.

Investigation was made whether or not the patterns of the microbiological activities vary with species or even with strains.

In this paper are presented the relative activities of fourteen thiamine related compounds for nineteen strains of heterofermentative lactic acid bacteria.

Experimental

Strains. The nineteen lactic acid bacteria strains tested are shown in Table 1.

Basal Medium. The composition of the basal medium used is shown in Table 2. This medium was used for all the organisms tested except *L. brevis* IFO 3345, *L. brevis* IFO 3960, and *L. fructosus* IFO 3516.

When *L. brevis* IFO 3345 or *L. brevis* IFO 3960 was used as the test organism, glucose was replaced by the same amount of arabinose as it gave better growth. When *L. fructosus* IFO 3516 was used, both 100 mg of glucose and 200 mg of fructose were used as the carbon-sources instead of glucose alone. Nicotinic acid was replaced by the same amount of nicotinamide in accordance with the nutritional requirements of this organism.

Measurement of growth. Growth was measured turbidimetrically by a Coleman spectrophotometer (650 mμ); and expressed by the optical density.

Relative activities were calculated in terms of thiamine hydrochloride percentage.

Samples. Samples tested are listed in Table 3. These samples were added aseptically to the medium after boiling for three minutes.

Table 1. Strains tested

	Incubation
1) *Lactobacillus batatas* 4005*	36°
Lactobacillus batatas 4115*	36°
Lactobacillus batatas T-8**	30°
2) *Lactobacillus brevis* IFO 3345	30°
Lactobacillus brevis IFO 3960	36°
Lactobacillus brevis IFO 3966	36°
3) *Lactobacillus buchneri* IFO 3961	30°
Lactobacillus buchneri 14-2***	36°
4) *Lactobacillus fermenti* IFO 3071	36°
Lactobacillus fermenti IFO 3954	36°
Lactobacillus fermenti IFO 3955	36°
Lactobacillus fermenti IFO 3957	36°
Lactobacillus fermenti IFO 3959	36°
5) *Lactobacillus fructosus* IFO 3516	36°
6) *Lactobacillus pastorianus* IFO 3325	30°
7) *Lactobacillus viridescens* IFO 3949	30°
8) *Leuconostoc dextranicum* IFO 3349	30°
9) *Leuconostoc mesenteroides* IFO 3832	24°
Leuconostoc mesenteroides IFO 3426	30°

*** Stock culture in our laboratory
** Kodama and Nishio, 1961
* Kodama, 1957

Table 2. Composition of basal medium (Quantities per final volume of 10 ml)

Adenine sulfate	200 γ	L-Ascorbic acid	10 mg
Guanine hydrochloride	200	L-Cysteine hydrochloride	1
Xanthine	200	L-Tryprophan	1
Uracil	200	DL-Alanine	2
Riboflavin	10	L-Asparagine	1
Calcium pantothenate	10	Fumaric acid	5
Nicotinic acid	10	Sodium acetate	60
Biotin	0.05	Tween 80	10
Folic acid	5	Glucose	200
Pyridoxine hydrochloride	20	Vitamin Free Casamino Acids	50
Pyridoxal hydrochloride	20	Salts A*	0.1 ml
Pyridoxamine dihydrochloride	20	Salts B*	0.1
P-Aminobenzoic acid	5	Adjusted to pPH 7.0	
B_{12}	0.002		

* Prepared according to Snell and Wright (Snell and Wright, 1941)

Table 3. Thiamine-related compounds tested

	Abbreviation
Thiamine monophosphate hydrochloride	TMP
Thiamine diphosphate hydrochloride	TDP
Thiamine disulfide	TDS
S-Carboethoxy thiamine hydrochloride	CET
Thiamine propyl disulfide hydrochloride	TPD
Thiamine tetrahydrofurfuryl disulfide hydrochloride	TTFD
S-Benzoyl thiamine monophosphate hydrochloride	BTMP
2-Methyl-4-amino-5-hydroxymethylpyrimidine	OMP
4-Methyl-5-(2-hydroxyethyl) thiazole hydrochloride	B_1-Thiazole
1-[(2-Methyl-4-amino-5-pyrimidinyl)-methyl] pyridinium chloride hydrochloride	Pyridinium-B_1
O, S-Dibenzoylthiamine hydrochloride	DBT
3-[(2-Methyl-4-hydroxy-5-pyrimidinyl)-methyl]-4-methyl-5-(2-hydroxyethyl) thiazolium chloride hydrochloride	Oxy-B_1
3-[(2-Methyl-4-hydroxy-5-pyrimidinyl)-methyl]-4-methyl-5-(2-hydroxyethyl) imidazolium chloride hydrochloride	Imidazole-B_1
1-[(2-Methyl-4-amino-5-pyrimidinyl)-methyl]-2-methyl-5-ethylpyridinium chloride hydrochloride	2-Me-5-Et-pyridinium-B_1

Results and Discussion

Results are summarized in Table 4. Of the fourteen compounds tested, TMP, TDP, TDS, CET, TPD, TTFD and BTMP were more or less active for all strains, while OMP, B_1-thiazole, Pyridinium-B_1, Oxy-B_1, Imidazole-B_1 and 2-Me-5-Et-pyridinium-B_1 were inactive or only slightly active.

The activities of TMP, TDP, TPD, TTFD, TDS, Imidazole-B_1, Oxy-B_1, Pyridinium-B_1 and 2-Me-5-Et-pyridinium-B_1 conformed to the same pattern in all strains, but those of CET, BTMP, DBT and B_1-thiazole plus OMP varied as follows:

1) CET was more active for *L. buchneri* 14-2 than the others, and was 35 to 50% as active as thiamine.

2) B_1-Thiazole has a slight activity for all strains except *Leuc. mesenteroides* T-5, which had an activity of 60 to 85% of thiamine.

3) DBT was only 0.01 to 0.1% as active as thiamine for most strains tested, but for *L. buchneri* 14-2 and *Leuc. mesenteroides* T-5 activity was approximately 1 to 5% of that of thiamine.

4) BTMP was utilized by *L. fructosus* IFO 3516 more than the other strains with approximately equal activities of BTMP and thiamine calculatcd on their molar bases. For the other strains BTMP was only one tenth to a quarter as active as thiamine.

5) With both OMP and B_1-thiazole in the medium, there was a high thiamine activity in the cultures of most strains, although each compound individually has no or only slight activity for all the strains except *Leuc. mesenteroides* T-5. However, even in the presence of both thiamine activity was never observed in the cultures of

Table 4. Relative activivities of thiamine-related compounds for lactic acid bacteria

species / strain	*Lactobacillus batatas*	*Lactobacillus brevis*	*Lactobacillus buchneri*		*Lactobacillus fermenti*	*Lactobacillus fructosus*	*Lactobacillus pastorianus*	*Lactobacillus viridescens*	*Leuconostoc mesenteroides*		*Leuconostoc dextranicum*
	4005 4115 T—8	IFO 3345 IFO 3960 IFO 3966	IFO 3961	14-2	IFO 3071 IFO 3954 IFO 3955 IFO 3957 IFO 3959	IFO 3516	IFO 3325	IFO 3949	IFO 3832	IFO 3426	IFO 3349
TMP	65- 80%	60- 80%	70- 90%		60- 90%	60- 80%	60- 80	70- 90%	60- 80%		70- 90%
TDP	60- 90	60- 80	70- 90		50- 90	70- 80	70- 90	70- 80	60- 80		70- 85
TDS	100-220	130-180	100-160		100-240	200	100-110	110-130	120-170		120-160
CET	10- 20	10- 20	10-20	35-50	10- 25	15- 20	15- 20	15- 20	15- 25		15- 25
TPD	90-150	90-130	80-150		100-140	140-150	90-100	100-120	90-130		100-110
TTFD	100-150	100-150	100-120		100-150	100-120	120-150	100-130	100-140		100-130
BTMP	5- 15	5- 15	5- 15		5- 15	60- 80	5- 15	10- 15	15- 20		10- 15
OMP	NS*	NS	NS		NS	NS	NS	NS	NS		NS
B_1-Thiazole	NS	NS	NS		NS	NS	NS	NS	60-85	NS	NS
OMP+B_1-Thiazole	150-250	150-230	180-250		110-270**	200-240	NS	NS	120-150		240-270
Pyridinium-B_1	NS	NS	NS		NS	NS	NS	NS	NS		NS
DBT	NS	NS	NS	2-4	NS	NS	NS	NS	1-2	NS	NS
Oxy-B_1	NS	NS	NS		NS	NS	NS	NS	NS		NS
Imidazole-B_1	NS	NS	NS		NS	NS	NS	NS	NS		NS
2-Me-5-Et-pyridinium-B_1	NS	NS	NS		NS	NS	NS	NS	NS		NS

* NS indicates no or only slight activity (less than 0.1% as active as thiamine)
** 50-80% as active as thiamine only for *L. fermenti* IFO3071.

either *L. pastorianus* IFO 3325 and *L. viridescens* IFO 3949. Because of this, these two organisms could be used in microbiological assays of thiamine.

Summary

The relative activities of fourteen thiamine-related compounds for nineteen strains of heterofermentative lactic acid bacteria, leuconostocs and lactobacilli, were investigated.

TMP, TDP, TDS, CET, TPD, TTFD and BTMP were more or less active for all the strains tested, while OMP, B_1-thiazole, Pyridinium-B_1 and 2-Me-5-Et-pyridinium-B_1 were inactive or only slightly active.

Althouth the pattern of the relative activities of the compounds were similar in all strains, the activities of CET, BTMP, B_1-thiazole, and DBT varied depending on the strain. CET was more active for *L. buchneri* 14-2 than for the other strains, and BTMP for *L. fructosus*. B_1-thiazole showed definite activity only for *Leuc. mesenteroides* T-5 and DBT for *L. buchneri* 14-2 and *Leuc. mesenteroides* T-5.

Even when both B_1-thiazole and OMP were in the culture medium, they did not show any thiamine activity for *L. pastorianus* and *L. viridescens*.

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LOCALIZATION OF ϕ 170 PROPHAGE ON THE CHROMOSOME OF *ESCHERICHIA COLI* K 12

Teiji IIJIMA

Introduction

Temperate phages which are active on *Escherichia coli* K 12 occupy their own specific site on the chromosome of the host when they lysogenize as prophage. The locus of each prophage can be mapped by genetic conjugation experiments between a lysogenic and a nonlysogenic strain (Lederberg and Lederberg 1953, Wollman 1953). Several temperate phages were isolated in this laboratory and the prophage locus on the chromosome of K 12 was determined by genetic conjugation.

This paper reports the chatacteristics of an Hfr strain which was isolated to facilitate the determination of prophage locus and an analysis of recombinants to determine the specific site of ϕ 170 prophage which is now known as a vector of transduction of the galactose markers among K 12 mutants (Iijima 1963, in press).

Materials and Methods

Hfr strain: An Hfr strain which transfers a large portion of the chromosome to the conjugal F^- strain would be necessary to facilitate the determining of the prophage locus. An Hfr strain F 84 (methionine requiring and streptomycin resistant) which has been preserved in this laboratory, was found to lose high fertility, so a new Hfr strain was isolated from this Hfr. The logarithmically growing culture of F 84 was irradiated by UV light and plated on nutrient agar plates. Colonies which appeared on the plates were tested for fertility by replica plating on a selective plate such as EMS-lactose, EMS-galactose or EMS-xylose (Lederberg 1951), on which a recipient, W4573, had been seeded previously. The fertility was checked by numbers of recombinant colonies appearing on the replica plates where the W 4573 recipient cells were mixed with the Hfr cells which were transferred by replica plating from the master plates.

An Hfr strain HX 201 which can transfer its chromosome in an order *Mal-Xyl-Lac-Gal* to the conjugal F^- was isolated. This Hfr was lambda lysogenic, so ϕ 170 lysogenic cell HX 201 (ϕ 170) was isolated by superinfecting this strain with ϕ 170. Recipient W 4573 is a prototroph, sugar-negative and lysogenic for lambda (X^+ *Lac*$^-$ *Gal*$^-$ *Xyl*$^-$ *Mal*$^-$ S^R).

Recombination procedures: HX 201 (ϕ 170) and W 4573 were cultured separately in nutrient broth overnight. Cells were centrifuged (3000 rpm) and resuspended in a synthetic medium (Davis and Mingioli 1950). The cell suspensions were combined and incubated with gentle agitation. After 2 hours, aliquots of the mixture were withdrawn and plated on selective media, for example, EMS-lactose, EMS-galactose, and

EMS-xylose. The recombinants appeared after 2 days incubation. The recombinants were picked from the selective plates and inoculated on the same selective medium. After serial purification on the same medium they were tested for their unselected markers, such as sugar utilization and lysogeny. Lysogeny was tested by plating on an appropriate lambda lysogenic indicator strain. All incubations in these experiments were at 37C.

Results and discussions

An Hfr, HX201 (ϕ 170), was crossed with an F^-, W 4573, and the mating mixture was plated on EMS-lactose, EMS-galactose and EMS-xylose medium, respectively. The numbers of recombinants are shown in Table 1.

Table 1. Numbers of recombinants appearing on plates by various selection.

dilution	selective madia		
	EMS-xylose	EMS-lactose	EMS-galactose
10^{-3}	246	73, 75	11, 16
10^{-4}	26	26	0

HX 201 (ϕ 170) was crossed with W 4573. After 2 hours incubation, aliquots were withdrawn from the mixture and plated on selective plates. The figures in the table represents numbers of recombinants appearing on the selective plates.

From this table the order of the chromosome transfer of the Hfr was assumed to be *Xyl-Lac-Gal*. This order of chromosome transfer was confirmed by blendor experiments (Wollman and Jacob 1955) with the same combination of mating pairs (Fig. 1).

The recombinants from the cross were purified and tested for their unselected markers. Results of the distribution of unselected markers in the recombinants from various selections are shown in Table 2.

Table 2. Unselected markers found in recombinants.

selective medium			
EMS-lactose		EMS-xylose	
Lac$^+$ *Gal*$^+$	155/193*	*Xyl*$^+$ *Lac*$^+$	4/151
Lac$^+$ *Xyl*$^+$	27/193	*Xyl*$^+$ *Gal*$^+$	3/151
Lac$^+$ *Mal*$^+$	63/193	*Xyl*$^+$ *Mal*$^+$	116/151

HX 201 (ϕ 170) was crossed with a recipient W 4573. Aliquots were withdrawn from the mixture and plated on selective media. Recombinants appearing on the selective plates were picked and purified by inoculating on a medium of the same composition. After purification the recombinants were tested for their unselected markers by replica pating on EMB-lactose, EMB-galactose, EMB-xylose and EMB-maltose.

* Numbers of recombinants having the unselected marker indicated per total recombinants tested.

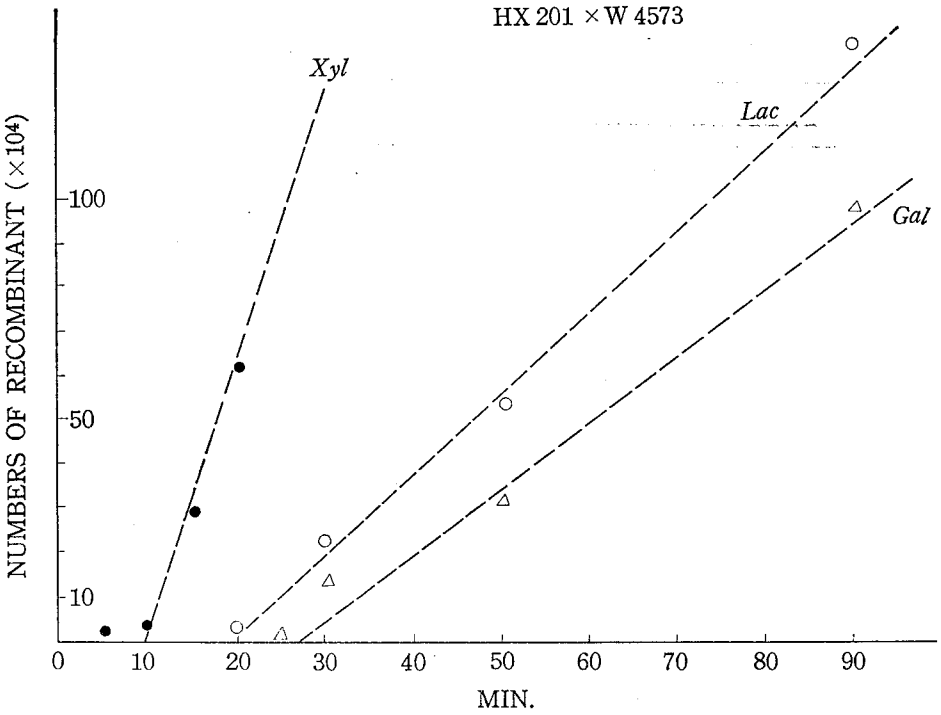

Fig. 1. Cell suspensions of HX 201 and W 4573 from logarithmically growing cultures were mixed in a tube. Aliquots were withdrawn at intervals and diluted to 100 fold with a synthetic medium. The cell suspension was treated with mechanical agitation for 30 sec. to break mating pairs. The treated sample was plated on selecting media such as EMS-xylose, EMS-lactose and EMS-galactose. Numbers of recombinants which appeared after 2 days incubation were plotted.

These results show that *Lac* and *Gal* or *Xyl* and *Mal* are closely linked to one another, but in EMS-xylose selection, the ratio of Lac^+ alleles found in Xyl^+ recombinants (4/151) or Gal^+ alleles in Xyl^+ recombinants (3/151) were very low, in comparison with Mal^+ alleles in Xyl^+ recombinants (116/151), although the blendor experiments (Fig. 1) give an evidence of linear transfer of (*Mal-*)*Xyl-Lac-Gal* markers. This discrepancy is due to the genetic marker of the Hfr used in these experiments. As shown in materials and methods, the Hfr used in these experiments is methionine requiring. When the Hfr are mated with F^- and plated on EMS-galactose medium, the recombinant types which can grow on the selective medium are prototroph and galactose positive. This type of recombination should be type (A) in Fig. 2, because this type of copying minimizes the numbers of crossing over. The same situation occurred in the EMS-lactose selection (Fig. 2).

The recombinants which grow on the EMS-xylose medium should be type (B) as in Fig. 2, where the numbers of crossing over are also minimal. This type of recombinant, X^+ Xyl^+, resulted from the cell which copied the Xyl^+ on the Hfr chromosome and then copied the met^+ marker on the F^- chromosome and afterwards copied the genetic marker along the F^- chromosome. In this type of recombinant we would not find linkage between *Xyl* and *Lac* or between *Xyl* and *Gal*.

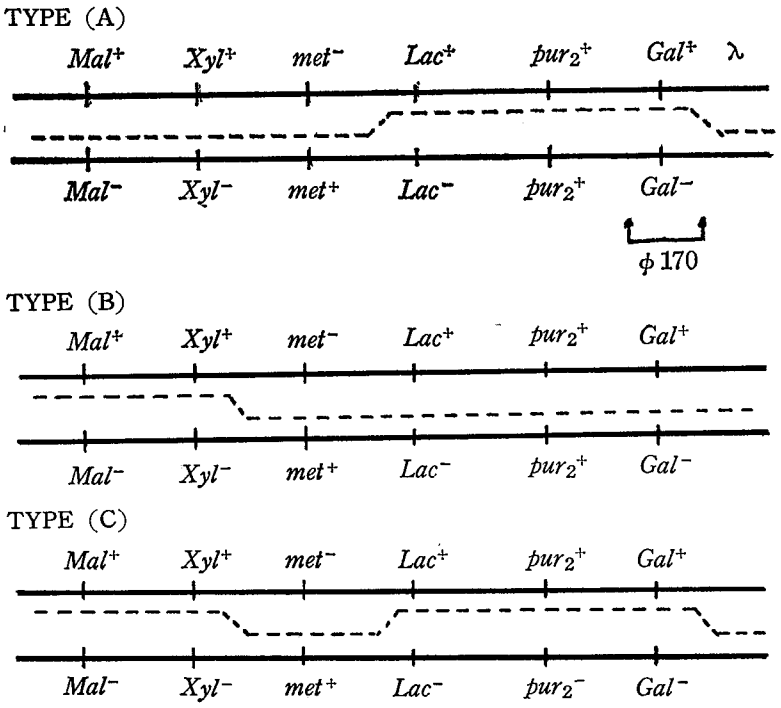

Fig. 2. Types of chromosomal copying
(A) EMS-xylose or EMS-maltose selection
(B) EMS-lactose or EMS-galactose selection.
(C) EMS-xylose or EMS-maltose selection.
-----: a recombinant chromosome which was derived from the selective medium.

This estimation was confirmed by crossing the Hfr with another F^- recipient, F333, whose genetic markers were *pur*$^-$ *Lac*$^-$ *Gal*$^-$ *Xyl*$^-$ *Mal*$^-$ *try*$^-$ and streptomycin resistant. This *pur*$^-$ was a mutation of *pur*$_2$ located between *Lac* and *Gal* as confirmed by F′ infection (Hirota and Sneeth 1961). HX 201 (ϕ 170) was crossed with F333 and the resulting recombinants were examined, for their unselected markers. The distribution of unselected markers are shown in Table 3.

From this table the effect of addition of adenine was more striking in the *Xyl*$^+$ or *Mal*$^+$ selection than in the *Lac*$^+$ or *Gal*$^+$ selection. This means that almost all of the recombinants isolated from *Xyl*$^+$ or *Mal*$^+$ selection were adenine requiring, that is, the type (B) in Fig. 2. This table also shows that addition of tryptophan alone also increase the numbers of recombinants, but this effect was not because of the increase of true recombinants but because of miscount of the background recipient colonies which formed somewhat larger colonies with the addition of tryptophan. Distribution of unselected markers in these recombinants was different from that of HX 201 (ϕ 170) $\times$W 4573 as shown in Table 2. In the mating of HX 201 (ϕ 170) $\times$W 4573, copying type in *Xyl*$^+$ selection was type (B) in Fig. 2; hence the ratio of *Lac*$^+$ alleles in *Xyl*$^+$ recombinants was low (4/151). However, in the mating of HX 201 (ϕ 170) $\times$F 333, copying type should be type (C) in Fig. 2; consequently the ratio of *Lac*$^+$ alleles in *Xyl*$^+$ recombinants was raised to 6/10.

Table 3. Numbers of recombinants appearing on various selecting media and the unselected markers found in the recombinants.

media	No. of recombinants	unselected markers
EMS-Lac	2.5×10^4	*Mal*$^+$ 7/34 *Xyl*$^+$ 4/34 *Gal*$^+$ 29/34
EMS-Lac + try	9.7×10^4	
EMS-Lac + ade	3.5×10^4	
EMS-Lac + try + ade	1.1×10^5	
EMS-Gal	6.2×10^4	*Mal*$^+$ 24/96 *Xyl*$^+$ 20/96 *Lac*$^+$ 67/96
EMS-Gal + try	1.7×10^5	
EMS-Gal + ade	8.3×10^4	
EMS-Gal + try + ade	4.4×10^5	
EMS-Xyl	4.0×10^5	*Lac*$^+$ 6/10 *Gal*$^+$ 8/10
EMS-Xyl + try	3.6×10^3	*Lac*$^+$ 12/62 *Mal*$^+$ 52/62
EMS-Xyl + ade	1.5×10^4	
EMS-Xyl + try + ade	7.0×10^5	
EMS-Mal	4.0×10^3	
EMS-Mal + try	9×10^5	
EMS-Mal + ade	8.3×10^4	
EMS-Mal + try + ade	5.5×10^4	

Determination of the prophage locus: As described above, the mode of chromosomal copying was modified according to the selective medium used. In *Xyl*$^+$ or *Mal*$^+$ selection, the most frequent type of copying would be type (A), while in *Lac*$^+$ selection, it would be type (B). The results of distribution of unselcted markers in HX 201 (ϕ 170) $\times$ W 4573 mating is shown in Table 4.

Table 4. Numbers of recombinants which have various combinations of unselected markers.

EMS-lactose selection						EMS-galactose selection						EMS-xylose selection					
Gal	*Xyl*	*Lys*	No. of recombinants	A	B	*Lac*	*Xyl*	*Lys*	No. of recombinants	A	B	*Lac*	*Gal*	*Lys*	No. of recombinants	A	B
+	+	+	3	3	3	+	+	+	2	3	3	+	+	+	0	3	3
+	+	−	0	5	3	+	+	−	0	5	3	+	+	−	0	5	3
+	−	+	12	2	2	+	−	+	35	2	2	+	−	+	2	3	5
+	−	−	0	4	2	+	−	−	1	4	2	+	−	−	12	3	3
−	+	+	8	3	5	−	+	+	1	3	3	−	+	+	2	3	3
−	+	−	13	3	3	−	+	−	1	3	3	−	+	−	0	3	3
−	−	+	29	2	4	−	−	+	52	2	2	−	−	+	68	3	3
−	−	−	47	2	2	−	−	−	4	2	2	−	−	−	150	1	1

In this table, column A in each selection indicates the numbers of crossing over if the prophage locus is located between *Lac* and *Gal*. Column B indicates the numbers of crossing over if the prophage locus is located on the right hand of the *Gal* locus as in Fig. 2. The numbers of recombinant types which were isolated from these experiments are consistent with the former case (column A). From the analysis of the recombinant types, the locus of prophage ϕ 170 may be determined to be located between *Lac* and *Gal*. The close linkage of prophage ϕ 170 to *Gal* marker is shown in the ratio Lys^+/Lys^- (90/6) in Gal^+ selection.

These results are obtained from the experiments of Hfr (ϕ 170) × W 4573, but as shown elsewhere (Iijima in press), double lysogenic strain ϕ 170 and lambda was unstable and segregates the single lysogenic strains of ϕ 170 and lambda as well as a small fraction of nonlysogenic strains, but this phenomenon does not affect the linkage relationship.

Summary

A temperate phage ϕ 170 which is active to but temperate for *Escherichia coli* K 12 was isolated. Locus of the prophage of ϕ 170 was determined by conjugation experiments with an Hfr, HX 201 (ϕ 170), and F^-, W 4573. By genetic analysis of the recombinants resulting from the cross, the locus of the prophage was determined to be in the neighborhood of the *Gal* locus. The mode of chromosomal copying in the conjugation experiment was discussed.

Acknowledgement

The author wish to thank to Mr. T. Sakane in the Institute for Fermentation for his technical assistance during this work.

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CONTRIBUTION TOWARDS THE FUNGUS FLORA OF AUSTRALIA AND NEW ZEALAND

Keisuke TUBAKI

From November to December of 1965, Tubaki attended a mycological expedition to Australia and New Zealand with Mr. D. Shimizu, Yonezawa Municipal Museum under the leadership of Dr. Y. Kobayasi, National Science Museum and during the expedition the present author studied a fungus flora of both countries with special reference to those members of lower fungi. Because of the technical difficulties involved, cultural studies of fungi were very insufficient at that time. Therefore, the soil fungi were isolated in our laboratory in January, 1964, nearly one month after their collection. For isolation, the dilution plate method was carried out using malt agar, soil extract agar and plane agar. Collections from New Zealand were arrived to the laboratory by sea on February and June respectively, then the taxonomical work started.

Studies in the present expedition were largely of preliminary nature, and for Japanese mycologists it was the first expedition in the southern hemisphere. Time allowed for the expedition was rather limited. In addition, covering a large area within a short period of time, we travelled during the early summer season of these countries, which was apparently suited for trips but not always for fungus studies.

In spite of these handicaps, the result obtained by the present author in the field of lower fungi was enough to serve as the starting point for future investigations. Fungus collections were made by us including 240 dried materials and nearly 700 cultures in two months following their arrival. The author felt that a long-termed stay in favourable field from summer through autumn seems indispensable for the advance of fungal flora.

I wish to express my heartfelt gratitude to Dr. Y. Kobayasi, leader of our party, and to Mr. D. Shimizu for their constant encouragement and various suggestions. For field and laboratory investigations we received a great support from the Division of Science Services (Department of Agriculture, N.S.W.), Tasmanian Government Tourist Bureau (Hobart, Tasmania), Lincoln College (Christchurch, New Zealand), D.S.I.R. (Christchurch and Auckland, New Zealand) and other organizations. Without the staffs of those, studies in both countries could not been successful. I also owe much to many rangers in the national parks for their constant aids in the field work.

Financial aids to the expedition were given by the Takeda Chemical Ind. Ltd. To this support, my sincerest thanks are also due. Much of this work was suggested by Dr. T. Hasegawa, a director of the Institute for Fermentation, whom I owe many

thanks. Mr. I. Asano identified most of *Aspergillus* & *Penicillium* and Mr. T. Ito was of major assistance in carrying out the experiments, whom I owe many thanks.

Results and Observations

Fungi isolated from the soils are listed in Table 1, and those found on the natural habitats are listed in Table 2. Of course, a number of unidentified species were encountered, but they are not discussed in the present paper and those fungi which seemed to be new species will be described in other papers.

Among them listed in the Table 1, some of the dominant groups are described and discussed specially in the following items.

I. Aquatic Hyphomycetes
II. Keratinophilic fungi
III. *Penicillium*, *Aspergillus* and related genera
IV. *Mortierella isabellina* group

Table 1. Fungi Isolated from the Soil

Species	Number of Strains Isolated		
	Aust.	Tasmania	N.Z.[1)]
Absidia butleri Lendner		2	
Ab. cylindrospora Hagem		2	
Ab. glauca Hagem		1	2
Aspergillus nidulans (Eidam) Winter	3		
Asp. cervinus Massee		1	
Asp. niger v. Tieghem	1	1	
Asp. unilateralis McLennann et al		1	
Asp. ustus (Bain.) Thom et Church	1		
Aureobasidium pullulans (de Bary) Arnaud	2	2	1
Beauveria sp.	1	1	5
Bispora antennata (Pers.) Mason		1	
Bisporomyces chlamydosporis v. Beyma	1		
Calcarisporium arbuscula Preuss	2	1	
Cephalosporium acremonium Cda.	1	2	
Cephalosporium mycophilum Tubaki		1	
Cephalosporium spp.	4	7	3
Chrysosporium asperatum Carmichael			1
Ch. pannorum (Link) Hughes	1		
Chrysosporium spp.		1	1
Cladosporium herbarum (Pers.) Link ex Fr.	2	1	2
Cladosporium spp.	1	1	2
Cylindrium griseum (Link) Lindau	1		
Cylindrocladium spp.		1	2
Fusarium spp.	4	2	3
Gliocladium deliquescens Olsen-Sopp	1		1

Table 1 continued

Species	Number of Strains Isolated		
	Aust.	Tasmania	N.Z.[1)]
Gliocladium roseum (Link) Bainier	2	4	3
Heterosporium sp.	1		1
Humicola grisea Traaen		1	1
Hyaloflorae namosa Batista et Maia	1	1	2
Keratinomyces ajelloi Vanb.	8	3	
Moeszia cylindroides Bubak	1		9
Mortierella isabellina Oudemans	1	1	3
M. humicola Oudemans	1		2
M. longicollis Dixon-Stewart			4
M. nana Linnemann			
M. ramanniana (M.) Linnemann	1	1	2
M. ramanniana var. *augulispora* (N.) Linn.	2	1	
Nannizzia cajetata Ajello (=*M. cookei*)	1(3)		
N. incurvata Stack. (=*M. gypseum*)	1(3)		
Oidiodendron flavum Szilvinyi			1
Paecilomyces carneus (D. et H.) Brown et G. Smith	1		5
P. farinosus (Dicks ex F.) B. et G. S.	2	2	
P. javanicus (F. er B.) B. et G. S.	1		1
Pachybasium hamatum (Bon.) Sacc.		1	2
Penicillium aculeatum Raper et Fennel			
P. adametzi Zaleski	1	1	
P. canescens Sopp		5	
P. citreo-viride Biourge	2	1	1
P. cyclopium Westling	2		2
P. frequentans Westling	1	1	2
P. herquei Bainier et Sartory			1
P. janthinellum Biourge		8	7
P. miczynskii Zaleski	4	1	
P. pinetorum Christ. et Backus	1	1	
P. radulatum G. Smith		2	2
P. restrictum Gilman et Abbott		1	
P. roseo-purpureum Dierckx		1	
P. spinulosum Thom			1
P. stoloniferum Thom		1	
P. thomii Maire	2	3	1
P. vinaceum Gilman et Abbott	1		
P. waksmanii Zaleski			1
Scolecobasidium variabile Barron et Busch	1		
Scopulariopsis brevicaulis (Sacc.) Bainier		1	1
Trichoderma viride Pers. ex Fr.	6	8	17
Zygorhynchus moelleri Vuillemin			1

Table 2. Fungi Found on the Natural Habitats

Species	Habitat	Loaclity
Cordana pauciseptata Preuss	dead leaves	Tasmania
Corynelia uberata Fries	*Podocarps* sp.	Te Anau, N. Z.
Cyttaria gunnii Berk.*	*Nothofagus cunninghamii*	Cladle Mt. Nat. Park, Tasmania, Australia
	N. menziesii	Te Anau, N. Z.
C. nigra Rawlings*	*N. menziesii*	Te Anau, N. Z.
Doratomyces stemonites (Pers. ex Fr.) Morton et G. Smith	antelope-dung; deer-dung	Te Anau, N. Z.
Monocillium humicola Barron	dead bark of *Cyathea*	N. Z.
Sordaria fimicola (Rob.) Ces. et De Not	deer-dung	N. Z.
Sporendonema sp.		Piha Coast, N. Z.
Verticicladiella serpens (Goid.) Kendrick	*Not. menziesii*	Hermitage, N. Z.

* Kobayasi, Y., 1964. On the genus Cyttaria I. Jour. Jap. Bot. 39: 216-224.

I. AQUATIC HYPHOMYCETES

During the mycological survey in Australia and New Zealand, samples of foam and scum from streams and other waters were collected and kept in small plastic bags containing formalin during the trip, and was examined at this laboratory. This is a short account of the species found during a visit to both countries and much more research is needed because of the abundance of these aquatic fungi inspite of the fact that our trip was not quite timely for the collection of fungi. Unfortunately, it was unable to bring back any living materials to Japan; therefore, no cultural studies could be made.

Collections were made mainly in the following six regions: Tasmania (Australia)–Mt. Field National Park and Cradle Mountain National Park; New Zealand–Hermitage, Te Anau, Fiordland National Park, Auckland and Waipoua. In only one foam sample collected by Dr. Y. Kobayasi from streams in Bulolo, East New Guinea, a very rich flora of aquatic fungi was found and these fungi were already described in a previous paper (Tubaki, 1965).

As already known, the spores of aquatic Hyphomycetes are readily trapped in foam and scum which may accumulate behind a barrier of twigs or stones or at the foot of a small waterfall in a stream. Many fungi were already found by Ingold, Nilsson, Petersen and others. Since the first report principally on the aquatic Hypho mycetes by Ingold (1942), considerable attention has been paid to these members by many mycologists, and recently, Nilsson (1964) summarized morphology, ecology, physiology and taxonomy of these fungi in his comprehensive work with the discussion of the general considerations on Hyphomycetes.

1) Aust. and N.Z. stand for Australia and New Zealand respectively.

There is the only report made on aquatic Hyphomycetes from Australia by Cowling and Waid (1963) and none from New Zealand. The species found in the present trip are arranged below with brief notes. Species identified on basis of detached spores are indicated by plus sign.

Table 3. Species Identified from Foam and Scum

Species	Locality	
	Australia	New Zealand
*Actinospora megalospora**		+
*Alatospora acuminata**,**	+	+
*Anguillospora crassa**	+	
*A. longissima**	+	+
*Articulospora tetracladia**	+	
Clavariopsis aquatica		+
Clavatospora longibrachiata	+	
Culcidispora aquatica		+
Dactyllela aquatica	+	
*Heliscus lugdunensis***	+	+
Lemonniera terrestris	+	
*Tetrachaetum elegans***	+	
*Tetracladium marchalianum**,**		+
*T. setigerum**	+	
Tricladium eccentrichum	+	
*Triscelophorus monosporus**,**	+	
Total number of samples	10	9

* Found from East New Guinea (Tubaki, 1965).
** Already reported from Australia by Cowling & Waid (1963).

Species Identified

In Australia most of foam and scum samples were collected in Tasmania which is conceded as the most mountainous island in the world. The stream are rather small and shallow with running water ensuring good aeration through natural wood with a rich flora of *Eucalyptus*, *Nothofagus*, *Arthrotaxia*, *Richea*, tree-ferns, etc. and the soil was generally water-logged. It was early summer in Tasmania around November 25th through 30th and was fairly cold in Cradle Mountain National Park with heavy rainfalls. Therefore, much more species is expected to be found in January and February.

Habit of the collections are abbreviated as follows:

MF.....Mt. Field National Park

CM.....Cradle Mountain-Lake St. Clair National Park

In New Zealand, the collection was made on both islands as already noted. It was early summer and too early a season for the fungus survey. On both islands,

foam and scum were collected mainly around Lake Te Anau, Fiordland National Park and a Waipoua forest.

Actionospora megalospora Ingold

Trans. Brit. Mycol. Soc. 35: 66 (1952)

This fungus was rather rare and found only from one place in Piha coast, near Auckland.

Alatospora acuminata Ingold

Trans. Brit. Mycol. Soc. 25: 381 (1942); Cowling & Waid, in Aust. J. Sci. 26: 122 (1963).

This species has been found in abundance, especially richest in the stream near Lake Drake (C M) and was fairly common in Te Anau and Piha coast of New Zealand.

Anguillospora crassa Ingold

Trans. Brit. Mycol. Soc. 41: 367 (1958)

In scum collections, several spores of another type having thick *Anguillospora*-like conidia were observed. There are some differences in size, but they are unable to be classified because no culture was obtained.
Collected in C M.

Anguillospora longissima (Sacc. et Syd.) Ingold

Ingold, in Trans. Brit. Mycol. Soc. 25: 389 (1942)

Collected in M F, C M, and also in Te Anau of New Zealand.

Articulospora tetracladia Ingold

Trans. Brit. Mycol. Soc. 25: 339 (1942)

This is one of the common species in foam and scum in the present survery. Collected in M F, Nov. 27, 1963 and C M.

Clavariopsis aquatica de Wild

Ann. Soc. Belge. Microsc. 19: 197 (1895); Dowing & Waid, in Austr. J. Sci. 26: (1963).

This fungus was fairly abundance in Te Anau and Piha coast.

Clavatospora longibrachiata (Ingold) Nilsson

Symb. Bot. Upsal. 18: 88 (1964)

Syn. *Heliscus longibrachiatus* Ingold, in Trans. Brit. Mycol. Soc. 25: 360 (1942)

Only found in scum collected in C M. Though monilioid aerial arthrospores were reported by Ingold & Cox (1957), no such spore was found in the present samples. Collected in C M.

Culcidispora aquatica Petersen

Bull. Torrey Bot. Club 87: 342 (1960)

This interesting fungus was hitherto described only by Petersen. Therefore, the present investigation is the second and this extend the flora of this species. Matured conidia were collected only in Te Anau in a scum.

Dactylella aquatica (Ingold) Ranzoni

Farlowia 4: 359 (1953)

Several conidia were found in the scum, collected in C M, December 5, 1963.

Heliscus lugdunensis Sacc. et Therry

Michelia 2: 1 (1880)

Syn. *Heliscus aquatics* Ingold, in Trans. Brit. Mycol. Soc. 25: 367 (1942)

Perfect stage of this fungus was found by Webster (1959) and was named as *Nectria Lugdunensis* Webster. As described by Nilsson (1964), this fungus is not so common and only few conidia are found in a scum collected in C M, in Te Anau and Hermitage of New Zealand.

Lemonniera terrestris Tubaki

J. Hattori Bot. Lab. 20: (1958)

Syn. Lemonniera brachycladia Ingold, in Trans. Brit. Mycol. Soc. 41: 365 (1958); Nilsson, in Symb. Bot. Upsal. 18: 100 (1964)

Tubaki (1958) found the fungus on the capsule of *Juglans*, not in aquatic condition; and sporulation occurred in cultures on agar. Ingold's and Tubaki's descriptions and figures agree with each other very well. Therefore, Nilsson (1964) referred *L. brachycladia* as synonymous with *L. terrestris*.

In the present study, the fungus was found only in M F.

Tetrachaetum elegans Ingold

Trans. Brit. Mycol. Soc. 26: 380 (1942)

This fungus was found in fairly abundance in C M and M F.

Tetracladium marchalianum de Wild

Ann. Soc. Belge Microsc. 17: 39 (1893); Cowling & Waid, in Austr. J. Sci. 26: (1963)

This fungus was found in abundance in all the scum-samples from the Piha coast and Te Anau.

Tetracladium setigerum (Grove) Ingold

Trans. Brit. Mycol. Soc. 25: 369 (1942)

Only found from C M.

Tricladium eccentrichum Petersen

Mycologia 54: 117 (1962)

This species was established in 1962 from the fungus on the rotten submerged *Quercus*-leaves in the New York Botanical Garden and was also found in Sweden (Nilsson, 1964). Therefore, the present investigation extends the flora of this species. This fungus was collected in C M and M F.

Triscelophorus monosporus Ingold

Trans. Brit. Mycol. Soc. 26: 148 (1943)

This fungus was collected from M F only.

Discussion: As a result, 16 species were identified. Among them, *Alatospora acuminata*, *Articulospora tetracladia* and *Triscelophorus monosporus* are fairly common.

As already described by Nilsson (1964) or by myself (1960), some of terrestrial spora are usually found in scum and foam samples. In the present samples, those of terrestrial fungi were also found in addition to the aquatic spora. Spore found in

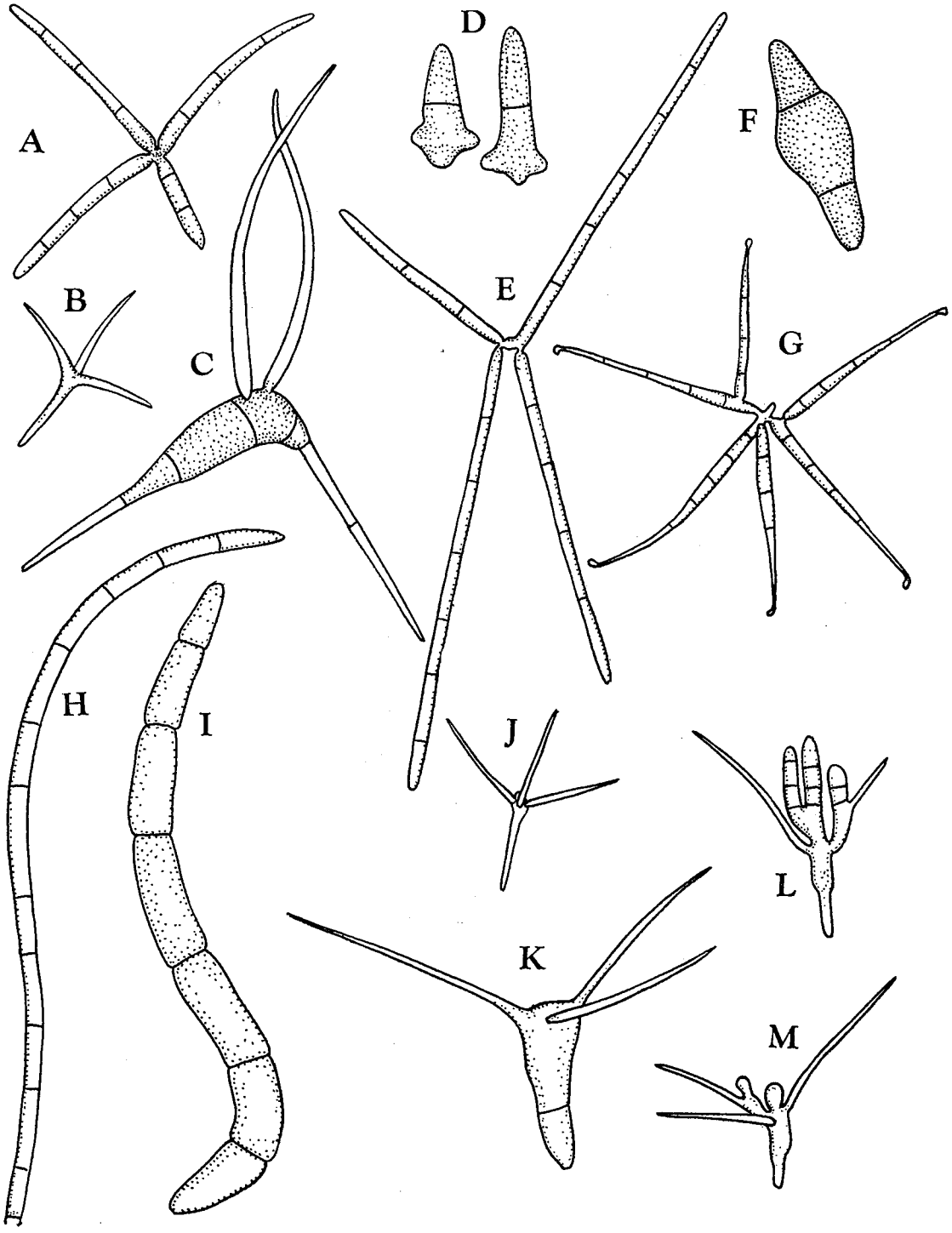

Fig. 1. Aquatic Spora. A. *Articulospora tetracladia* B. *Alatospora acuminata* C. *Culcidispora aquatica* D. *Heliscus lugdunensis* E. *Geniculospora* sp. F. *Dactylella aquatica* G. unidentified fungus H. *Anguillospora longissima* I. *A. crassa* J. unidentified fungus (*Clavatospora* sp. ?) K. *Clavariopsis aquatica* L. *Tetracladium setigerum* M. *T. marchalianum*.

Tasmania (Fig. 1-G) is remarkable because of its fan-like arms which developed near the base of main axis and branched again in the same manner. It resembles spores pictured by Ingold (1952, fig. 1-H), by Nilsson (1958, fig. 12-C; 1964, fig. 18-E) and also by Arnaud (1952) as *Magdalenaea monogramma* Arnaud, but quite differs from them in the septate arms which have small knobs at each spex. Spores shown in pl. V-E, collected from Tasmania, was already pictured by Ingold (1959, etc.) as one of the common in Britain and no name has been given. It consists of a small central globose part with four to five septate arms grow out. Curious spores are found from Te Anau, New Zealand. It consists of a short septate, clavate main axis giving off at the top a pair of opposite lateral arms; the result of the branching is a two-prolonged Y shape (pl. V-F). This spore is similar to that of *Ceratosporium* or *Ceratosporella*, terrestrial fungi. Many spores are found from Te Anau and also from New Guinea as already described (Tubaki, 1965). They are consisted of one-septate and crescent cells. Spores shown in pl. V-I were also already described from New Guinea (Tubaki, 1965) and from Japan (Tubaki, 1960). It will probably be a undescribed species of *Angulospora* Nilsson (1964).

II. KERATINOPHILIC FUNGI

Keratinophilic fungi from Australia and New Zealand were reported by Durie and Frey (1955, 1957, 1962), Frey and Durie (1957), Ridley (1961), Donald (1962), Dunne and Morahan (1964), Frey and Griffin (1961), and Frey (1961, 1965); those from New Zealand were reported by Di Menna and Marples (1954).

Soil samples were taken from a total of 24 areas in the vicinity of Sydney, Melbourne, Tasmania, in Australia and Christchurch, Hermitage, Arthur's Pass, Te Anau, Auckland, Waipoua, in New Zealand. A small portion of the soil of these places was brought back in sterilized sacks and was proceeded for the isolation of the keratinophilic fungi after returning to Japan. These soil samples were taken from various types of habitats including zoological gardens, botanical gardens, wooded areas, sheep yards, kangaroo woods, pathways in the field, parks, caves, beech areas, etc.

The methods for the isolation were similar to those used by Vanbreuseghem (1952). Portions of each soil samples were placed in petri dishes, moistened with sterile water, and baited with short pieces of human hair. Human hair, baited, was washed successively with alcohol and ether, then sterilized with propylene-oxide. Periodically, during a period of 2 to 4 weeks these hair pieces were examined by the naked eye for the development of mycelial growth. A pure culture was obtained by hanging the conidia from the infected hair by sterile needles onto malt agar plates (PH 4.5). The fungi isolated by the above method were inoculated on the sterilized human hair according to Carmichael's method. On a DSA plate-(Carmichael, 1962), fungi were streaked, and then cut-up, propylene-oxide sterilized human hair was sprinckled. Pieces of hair were examined microscopically at intervals. This method was suitable for the examination of erosion or penetrating bodies of the keratinophilic fungi as described by Carmichael (1962).

As a result, nearly 27 strains of keratinophilic fungi were isolated using the hair baiting technique. It is noteworthy that the distribution of these fungi is apparently restricted to those soil samples of botanic gardens and of areas where animals (sheep or kangaroo) live or have lived. The details are described in the following table.

Table 4. Species and Location of The Keratinophilic Fungi

Location	Habit	Number of strains	Species	Number of positive samples
Royal Botanic Garden, Melbourne, Australia	Flower bed	9	*K. ajelloi*	4
			M. cookei	1
Tasmania (Hobart to Cr. Mt. National Park), Australia	Sheep yards	6	*K. ajelloi*	2
			M. gypseum	1
Botanic Garden, Christchurch, N.Z.	Flower bed	2	*M. gypseum*	1
Botanic Garden, Sydney	Flower bed	7	*K. ajelloi*	4
			M. cookei	3
			M. gypseum	1
			Ch. asperatum	1
Tasmania (Cr. Mt. National Park-Launceston)	Field	1	*K. ajelloi*	1
	Cow dung	2		2

From the table above, it appears that many keratinophilic fungi were collected in Australia, and, on the contrary, only one species was found from the soil sample of a botanic garden in Christchurch, New Zealand. The reason is uncertain, but more detailed study will make it clear. Abundance of these organisms in soil samples of botanic gardens will due to manuring to the flower beds of those obtained from zoological gardens.

Species Identified

Chrysosporium asperatum Carmichael

Canad. J. Bot. 40: 1153, 1962

Growth on malt agar, rather restrict, dry, powdery, yellowish brown to olive color, reverse yellowish brown. Aleuriospores are borne directly along the side of hyphae or on short, ampulliform side branches, pyriform or clavate, markedly verrucose, 9.0-11.2×6-9 μ, pale yellow or brown.

Growth on sterilized human hair, good, with scattered colonies. Aleuriospores in abundance, pale yellow or brown with greenish tint.

Isolated from the soil, Botanic Garden, Christchurch, N.Z. (95S-108K-1).

The gross morphology and microscopic characters of this fungus fit to the description of Carmichael who already reported the keratinolytic activity of this species. Because the present species was hitherto found from Edmonton and California (Carmichael, 1962), the present isolation extends the flora of it.

Keratinomyces ajelloi Vanbreuseghem

Bull. Acad. royal de Belgique 5 ser, 1952 38: 1068,

Growth on Sabouraud's glucose agar, rapid, velvety or powdery, with sterile marginal areas, at first white, becoming gradually to cinnamon buff as the aleuriospores develop; reverse and agar dark olive brown to Victoria Lake. Macroconidia (aleuriospores) abundant, varies in diameter, usually 35-65×9-11 μ, ranging from 17-75 μ in length and 7-15 μ in width, distoseptate (Luttrell, 1963) with 8-12 cross-walls, smooth walled, abruptly flattened at one end with little frill, pale brown colored.

Growth on malt agar, same as on Sabourand's agar except for the radiating marginal areas.

On sterilized human hair, growth luxuriant with aleuriospores in abundance, yellowish brown to cinnamon buff.

Isolated from the soil, Sydney, Melbourne, Tasmania.

Nannizzia cajetana Ajello

Sabouraudia 1: 173 (1961)

Cleistothecia on human hair globose, pale yellow, μ in diam. Peridial hyphae branched verticillately or dichotomously, slightly constricted at the septa, echinulate, hyaline. Asci globose or ovate, 6-10 μ in diam. 8-spored. Ascospores ovate, smooth walled, 3.0-3.5×1.5-1.8 μ, golden yellow in color.

Conidial stage: *Microsporum cookei* Ajello.

Growth on Sabourand's agar, good, velvety to powdery, with rediate marginal areas, pale grayish yellow to yellowish tan; reverse, deep purplish red through the surface downy area. Microconidia (aleuriospore-type) borne singly, clavate, smooth walled, 3-5×1-6-2μ. Macroconidia (aleuriospore-type) abundant, elliptical, distoseptate, many celled, echinulate, 45-65×12-13 μ, hyaline or pale colored.

On malt agar, the growth is more sparce than that on Sabourau's agar, rather restricted; the reverse color is darker.

On sterilized human hair, aleuriospores are abundant, pale yellowish brown, then cleistothecia developed after two or three weeks at 25°C.

Four strains were isolated from the soil of botanic gardens of Sydney and Melbourne. Among them, only one strain (95S-103K-1) from Sydney developed the cleistothecia.

In addition to the above strains, one isolate was made in the Tasmanian soil (sheep yard on the way from Hobart to Cradle Mountain National Park) which is similar to *M. cookei* in purplish-red pigment on the reverse of the malt agar colony. However, the colony on Sabouraud-dextrose agar differs from that in the dark reddish brown colony of the reverse. The macroconidia are distoseptate, markedly spinulose and the cell wall is 2.5-3.3 μ in diam.; on the contrary, on Sabouraud's agar, the cell wall is thinner, 1.5-2.5 u in diam., smooth or slightly verrucose, longer and often distorted. These characters somewhat differ from those of the typical strain of *M. cookei*. However, the development of the distoseptate, echinulate macroconidia and the reddish pigment are criteria that serve to include this strain in the varieties

of *M. cookei.*

Nannizzia incurvata Stockdale

Sabouraudia 1: 41 (1961)

Cleistothecia on human hair globose, pale yellow to yellow-buff, 300-700 u in diam. Peridial hyphae septate, branched verticillately or dichotomously which curve in towards their main axis, constricted at septa, asperulate, with slender, smooth walled and coiled appendages, 3-5 μ in diam. Asci globose to ovate, 4.5-7 μ in diam., 8-spored. Ascospores lenticular, smooth-walled, 2.5-3.5$\times$1.5-2.0 μ.

Conidial stage: *Microsporum gypseum* (Bodin) Guiart et Grigorakis.

Growth on Sabouraud agar, rapid, flat, powdery, at first white, then becoming buff, yellow-tan to light brown; reverse, yellowish tan. Microconidia borne singly, clavate, 3-5$\times$2 μ, hyaline. Macroconidia numerous, euseptate, 4 to 6 septate, elliptical, surface echinulate, 35-55$\times$9-12 μ, with or without racquet hyphae.

Isolated from the soil, Royal Botanic Garden, Sydney, Australia; Royal Botanic Garden of Christchurch, New Zealand.

Among 4 cultures, only one strain which was isolated from Sydney developed cleistothecia on the sterilized human hair.

Discussion: From the previous papers hitherto published, two dermatophytes, *Microsporum gypseum* and *Keratinomyces ajelloi*, have proven to be the commonest keratinophylic fungi. In the present survey, however, *Microsporum gypseum* was very few in contrast with many isolates of *M. cookei* and *Keratinomyces ajelloi.* In *Chrysosporium*, only one species, *C. asperatum*, was isolated. Therefore, further studies on these fungi from the "fresh soil" is necessary to find more keratinophilic fungi of *Microsporum*, *Trichophyton*, *Epidermophyton* from Australia and New Zealand.

III. ***Aspergillus, Penicillium*** and their Allied Genus

On *Aspergillus* and *Penicillium* from Australia, only one report was hitherto published by McLennann and others (1954). They isolated three new species of *Aspergillus*, *A. nutans*, *A viridi-nutans* and *A. unilateralis;* one new species, *Penicillium resedanum*, was also described.

In the present study, nearly 10 strains of *Aspergillus* and 68 strains of *Penicillium* were isolated. Among them, 6 species of *Aspergillus* and 17 species of *Penicillium* were identified as below.

Species Identified

Aspergillus cervinus Massee

This is isolated from the soil collected in Cradle Mt. National Park, Tasmania, Australia (S-95-46). Historically, this species was originally described from the soil collected near Khartoum in Africa (1914). In 1939, Neill also isolated it from the soil of North Auckland, New Zealand. Recently (1964) Christensen & Fennell isolated this species from the soil of Kuala Lumpur, Malaya, and of Wisconsin, and they proposed a new major group, the *Aspergillus cervinus* Group.

The characters of this species are a pinkish-cinnamon color on the colony surface, thick-walled conidiophores, a single series of sterigmata and globose smooth conidia. Our culture matches Massee's diagnosis and other descriptions. Our findings seem to confirm that this is widely distributed in Australia and New Zealand.

Aspergillus nidulans (Eidam) Winter

This is abundant and cosmopolitan in its distribution; isolated from Melbourne soil (S-1-3; S-4-4), and Botanic Garden soil, Sydney, Australia (S-104-5).

Aspergillus niger van Tieghem

The soils of Melbourne (S-9-3) and Tasmania (S-13-5).

Aspergillus unilateralis McLennann, Ducker et Thrower

This species was originally found in rhizosphaera of *Epacridia impressea* and of *Hibbertiae fasciculatae*, Australia (McLennann, 1954). Our isolates from the soil of Mt. Wellington, Hobart, Tasmania (S-11-3), fairly fits in the culture received from Commonwealth Mycological Institute (C M I. 62876, McLennann) though the growth is more restricted on the agar.

Aspergillus versicolor (Vuill.) Tiraboschi

The soil of Melbourne, Australia (S-4-7).

Penicillium aculeatum Raper et Fennell

The soil of Blue Mountain, N.S.W., Australia (S-113-2).

Penicillium adametzi Zaleski

The soil of Mt. Fielad National Park, Tasmania, Australia (S-12-3).

Penicillium canescens Sopp

The soil of Melbourne, Australia (S-8-5; S-2-6); Hobart and Mt. Field National Park, Tasmania, Australia (S-14-2, S-18-1, S-21-3, S-23-2).

Penicillium citreo-viride Biourge

The soil of Melbourne (S-3-3, S-6-4) and Cradle Mountain National Park, Tasmania, Australia (S-31-2); from a fruit-body of unidentified Myxomycetes, Te Anau, N.Z. (95-185).

Penicillium cyclopium Westling

The soil of Melbourne, Australia (S-3-5) and of Hermitage, N.Z. (S-62-1); from a fruit-body of *Cyttaria gunnii*, Te Anau, N.Z. (95-180).

Penicillium frequentans Westling

The soil of Cradle Mountain National Park, Tasmania, Australia (S-28-3); Arthur's Pass (S-51-3) and Te Anau, N.Z. (S-71-1).

Penicillium herquei Bainier et Sartory

The soil of Auckland, N.Z. (S-92-1).

Penicillium janthinellum Biourge

This is the most dominant species in the genus. Nineteen strains were isolated from the soil of Melbourne; Blue Mountain, N.S.W., Cradle Mountain National Park, Hobart, Mt. Field National Park, Australia; Hermitage, Waipoua, Te Anau, N.Z.

Penicillium miczynskii Zaleski

Cradle Mt. National Park (S-31-3). Additional several similar strains were

isolated.

Penicillium pinetorum Christensen et Backus

Melbourne (S-1-2) and Cradle Mountain National Park, Tasmania (S-33-2). Our strains fit in a subculture of *P. pinetorum* received from Dr. M. Christensen (WSF. 15-C).

Penicillium radulatum Smith

Mt. Field National Park, Tasmania (S-17-2; S-19-2) and Auckland, N.Z. (S-94-3; S-95-189). This species, originally found in England, was abundant in the soil of certain Wisconsin habitats (Christensen & Backus, 1961). The Australia and New Zealand isolates were found to be very similar morphologically to a subculture of the type for the present species received from the Univ. of Wisconsin (Christensen, W.S.F. 3454).

Penicillium restrictum Gilman et Abbott

The soil of Hobart, Tasmania, Australia (S-24-3).

Penicillium roseo-purpureum Dierckx

The soil of Cradle Mountain National Park, Tasmania, Australia (S-119-2).

Penicillium spinulosum Thom

The soil of Piha coast, Auckland, N.Z. (S-95-1).

Penicillium stoloniferum Thom

The soil of Hobart, Tasmania, Australia (S-24-1).

Penicillium thomii Maire

The soils of Melbourne (S-2-2, S-9-9), Cradle Mountain National Park, Tasmania (S-38-4) and Mt. Field National Park, Tasmania (S-14-5, S-16-3). New Zealand.... Hermitage (S-64-2).

Penicillium vinaceum Gilman et Abbott

The soil of Melbourne, Australia (S-2-3).

Penicillium waksmanii Zaleski

The soil of Te Anau, N.Z. (S-75-2).

Paecilomyces carneus (Ducké et Heim) Brown et G. Smith

Australia....Blue Mountain, N.S.W. (S-112-2). New Zealand....Te Anau (S-78-4, S-80-2); Auckland (S-93-2); Waipoua (S-98-2, S-100-2); Botanic Garden, Christchurch (S-90-3).

In fresh isolates, *Isaria*-type synnemata develope on malt agarwith more or less powdery and pale brownish pink overgrowth, but easily reduced after successive transfer. The greenish reverse is distinct in fresh cultures. This is a dominant species in the genus isolated in the present study.

Paecilomyces farinosus (Dicke et Fr.) Brown et G. Smith

The soil of Cradle Mountain National Park, Tasmania (S-37-3, S-37-6) and Blue Mountain, Sydney, Australia (S-112-1).

General appearance of the colony on Czapek agar resembles to *P. carneus* in many respects especially in the *Isaria*-type synnemata formation in some fresh isolates. The growth is white to pale yellowish; reverse pale yellow to orange brown.

Paecilomyces javanisus (Fried. et Bally) Brown et G. Smith

The soil of Waipoua, N.Z, (S-97-1)

Conidia are characteristically long cylindrical or fusiform, smooth walled. Our measurements of the conidia do not agree with that of Brown & Smith (4-6×1-1.5 μ) and of Friedericks & Bally (2×1-1.5 μ), situating between in the two (3-4 (5)×1-1.5 μ).

IV. *Mortierella isabellina* group

From the 127 soil samples collected in Australia and New Zealand, nearly 50 strains of *Mortierella* were isolated. Two thirds of them are to be included in the *Mort. isabellina* group and were identified as below. Zycha made studies on the *Mortierella isabellina* group (1935), and the research was continued by Turner (1963). However, the differentiation of the species is not yet clear. They are commonly known to be isolated from acid soil. From Australia, the presence of *Mort. ramanniana* and its allied species was reported by McLennan and Ducker (1951, 1954, 1957) referring to the ecology of these fungi. In the present study, nearly 40 strains were isolated from the soil of Australia and New Zealand. The key provided by Turner (1963) was used for their classification.

Species Identified

Mortierella isabellina Oudeman

Colony on the malt agar, felt-like with grey shade to "isabelline". Sporangia 10-25 μ or more in diam., and collar broad. Spores somewhat angular to globose, smooth-walled, 2-4 μ in diam.

Five strains were isolated from the soil; collected in Cradle Mountain National Park, Tasmania (S-37-2); Botanic Garden, Sydney (S-104-2); Arthur's Pass, New Zealand (S-50-3); Auckland, New Zealand (S-94-1); Waipoua, New Zealand (S-98-1).

Of the eight cultures of this species examined in the present study including typical cultures of *Mort. isabellina* (CBS strains, from Zycha and v. Beyma) and *Mort. pusilla* var. *ramifica* (CBS), the colony color varies from pale to dark gray. Moreover, the shape of spores also vary from angular to globose as described by Linnemann (1941). Spores of CBS-cultures derived from Zycha are mostly angular, but, on the contrary, a strain deposited by v. Beyma is mostly globose.

Of the five cultures isolated by the writer, two of them have angular spores and others have globose spores. But, in other respects, they are indistinguishable. The cultures of neither *Mort. atrogrisea*-type nor *Mort. fusca*-type were isolated in this study.

Mortierella ramanniana (Moller) Linnemann

Colony composed of turf, felt-like mycelium, pink with gray or lilac tint. Sporangiophores fairly long, 100-200 (300) μ or more in length, simple or branched. Sporangia 16-20 μ in diam., rose colored. Columella and collar small, but definite. Spores globose or oval, 2-3 μ in diam. Large, thick walled chlamydospores abundant, with dense oil content, globose or oval, 30-100 u in diam., usually 40-60 μ.

Four strains were isolated from the soil, Blue Mountain, N.S.W., Australia (S-111-2); Cradle Mountain National Park, Tasmania (121-3); Te Anau, New Zealand (S-88-1, S-88-3).

This is one of the dominant species in this group. Characteristically this species forms large chlamydospores in abundance, especially on weak media, 1/10 conc, malt agar extract agar, for example. They are globose or oval, terminal or intercalary on submerged hyphae, and can not be seen in other species as far as tested. This species is often treated as *Mucor* (Zycha, Indog, etc.), but Linnemann transferred it to *Mortierella* because of the similarities of the character to other species of *Mort. isabellina* group except for the distinct collumella. Turner (1963) agrees his concept.

As already known, this species is thiazol-dependent, and, in this study, this character was also recognized according to the Leaderberg's method (2 mg per ml).

Mortierella ramanniana var. ***angulispora*** (Naumov) Linnemann

Colony composed of velvety mycelium, gray with dark lilac, dark grayish russet-vinaceous. Sporangiophores 100 μ or more in length, branched sympodically or in whorls. Sporangia 10-20 μ in diam. Columella definite, spherical with a collar. Spores angular, 2-3 μ in diam. Chlamydospores fairly abundant, round to oval, usually 9 μ or more in diam.

Two strains were isolated from the soil, Blue Mountain, N.S.W. (S-110-1); Royal Botanic Garden, Melbourne (S-3-1).

This species is characteristic in its velvety, grayish lilac colored colony and angular spores. Though our strains near so much *Mort. vinacea*, the present strains were identified as the above because of the smaller spores, the grayish russet-vinaceous colony and the abundant chlamydospores. In addition, the present strains are quite different from the typical culture of *Mort. vinacea* (CBS) in colony color and spores. The present fungus is thiazol-dependent like in *Mort. ramanniana*.

Mortierella humicola Oudemans

Colony composed of cottony felty mycelium, pure white. General characters of the colony and sporangiophores are similar to those of *M. isabellina*. Spores globose, 2.6-3.0 (3.5) μ in diam.

Pure white colony and globose spores are typical characters of this species.

Three strains were isolated from the soil, Royal Botanic Garden, Melbourne (S-6-1); Te Anau, New Zealand (S-77-2, S-78-1). These strains are not thiamine-dependent.

Mortierella longicollis Dixon-Stewart

Colony composed of dense felty or wooly mycelium, pure white at first, then becoming pink with lilac tint, with fairly abundant aerial mycelium. Sporangiophores long, 100 to 500 μ in length, usually simple. Sporangia 10-25 μ in diam. Some sporangia having a long "neck" filled with spores. Spores sharply angular, 2.5-3 (4) μ in diam.

Four strains were isolated from the soil, Hermitage, New Zealand (S-57-2); Te Anau, New Zealand (S-78-5, S-88-2, S-88-4).

The definition of this species is not so clear. Characters of abundant aerial my-

celium and sharply angular spores could also be found in some strains of *Mort. vinacea*. The most distinct feature of this species is that of a "nect" of sporangium filled with spores as described by Dixon-Stewart. These strains are not thiamine-dependent.

This species was already found in Australia by McLennan (List of Cultures of CBS).

Mortierella nana Linnemann

Colony composed of fairly abundant, felty aerial mycelium, pure white. Sporangiophores developed from creeping or aerial hyphae, 20-50 μ or more in length. Sporangia monosporous; sporangia wall indistinguishable. Spores globose, 5.0-6.5 (7) μ in diam.

One strain was chosen as a typical of this species which was isolated from the soil, Cradle Mountain National Park (S-32-1).

In addition to the above, other four strains having monosporous sporangia were isolated in this study. However, their colony color is not pure white but becoming pale gray with pinkish tint. Accordingly *Mort. nana* could be regarded as the stylosporic or sporngiole-forming variant of pinkish species of *Mort. isabellina* group. These strains, including the typical one, are not thiamine-dependent.

SUMMARY

From November to December of 1963, a mycological surey was done in the Australian continent, Tasmania and New Zealand. As a result, 240 dried materials and nearly 700 cultures were made. Among them, about 85 species isolated from the soils and 9 species found on the natural habitats were listed in the present study. As soil fungi, about 58 species belonging to the dominant fungus-groups were described in the following items: Aquatic Hyphomycetes (16 species), Keratinophilic Group (10 species), *Mortierella isabellina* Group (6 species) and *Penicillium*, *Aspergillus* and related genera (25 species). Due to the trip made during the early summer of these countries, not enough collections were obtained. Therefore, a long-termed stay from January through February is indispensable for the further study of fungal flora of these regions.

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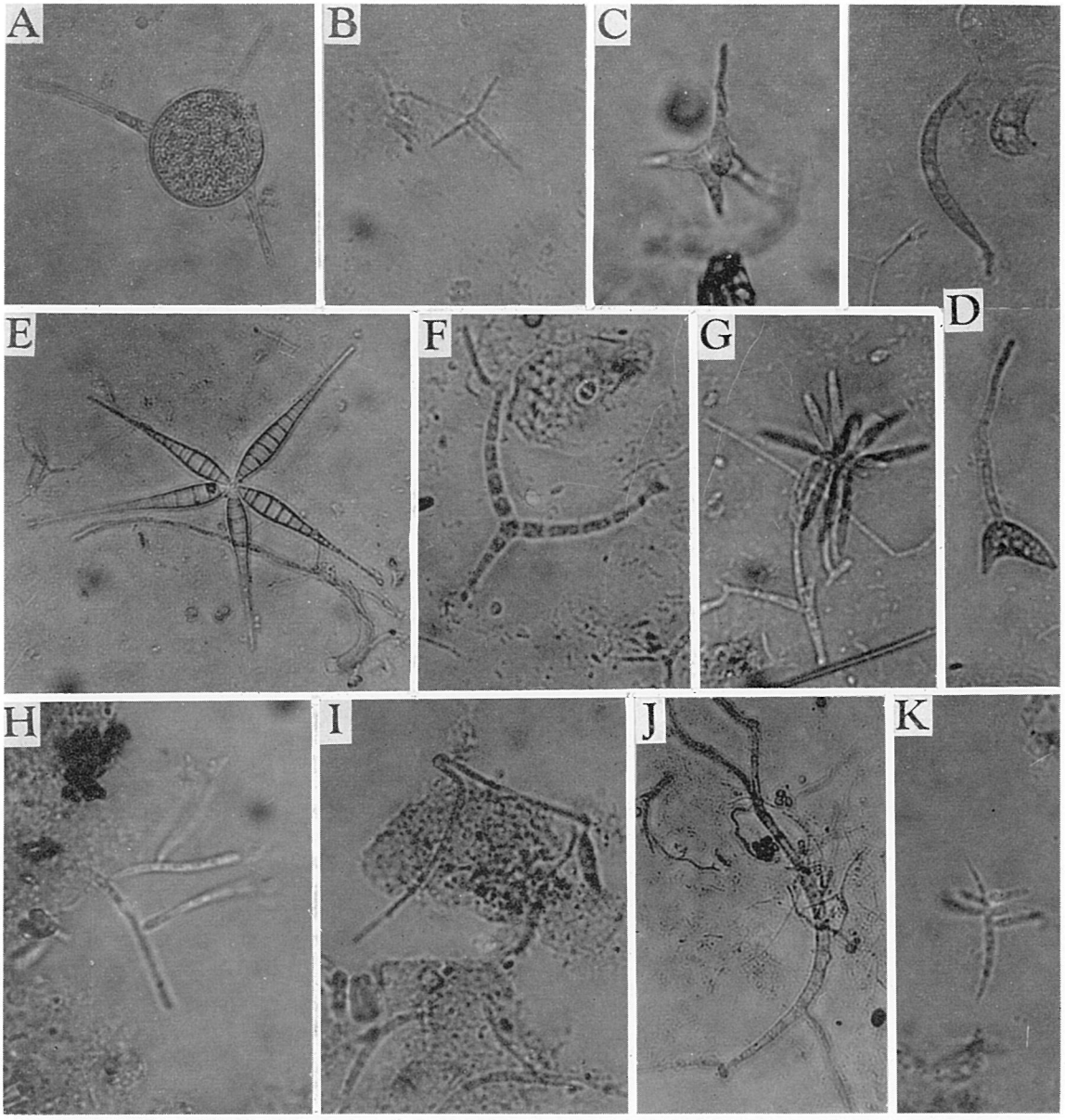

Plate I. **Aquatic Spora** A. *Actinospora megalospora* (×40) B. *Clavatospora longibrachiata* (×40). C. unidentified fungus (*Campylospora* sp. ?). D. unidentified fungus (*Centrospora* sp. ?). E. unidentified fungus (Ingold, 1958) F. unidentified fungus G. unidentified fungus (*Dendrospora* sp. ?). H. unidentified fungus (*Varicosporium* sp. ?). I. unidentified fungus (Tubaki 1960). J. unidentified fungus (*Centrospora* sp. ?).

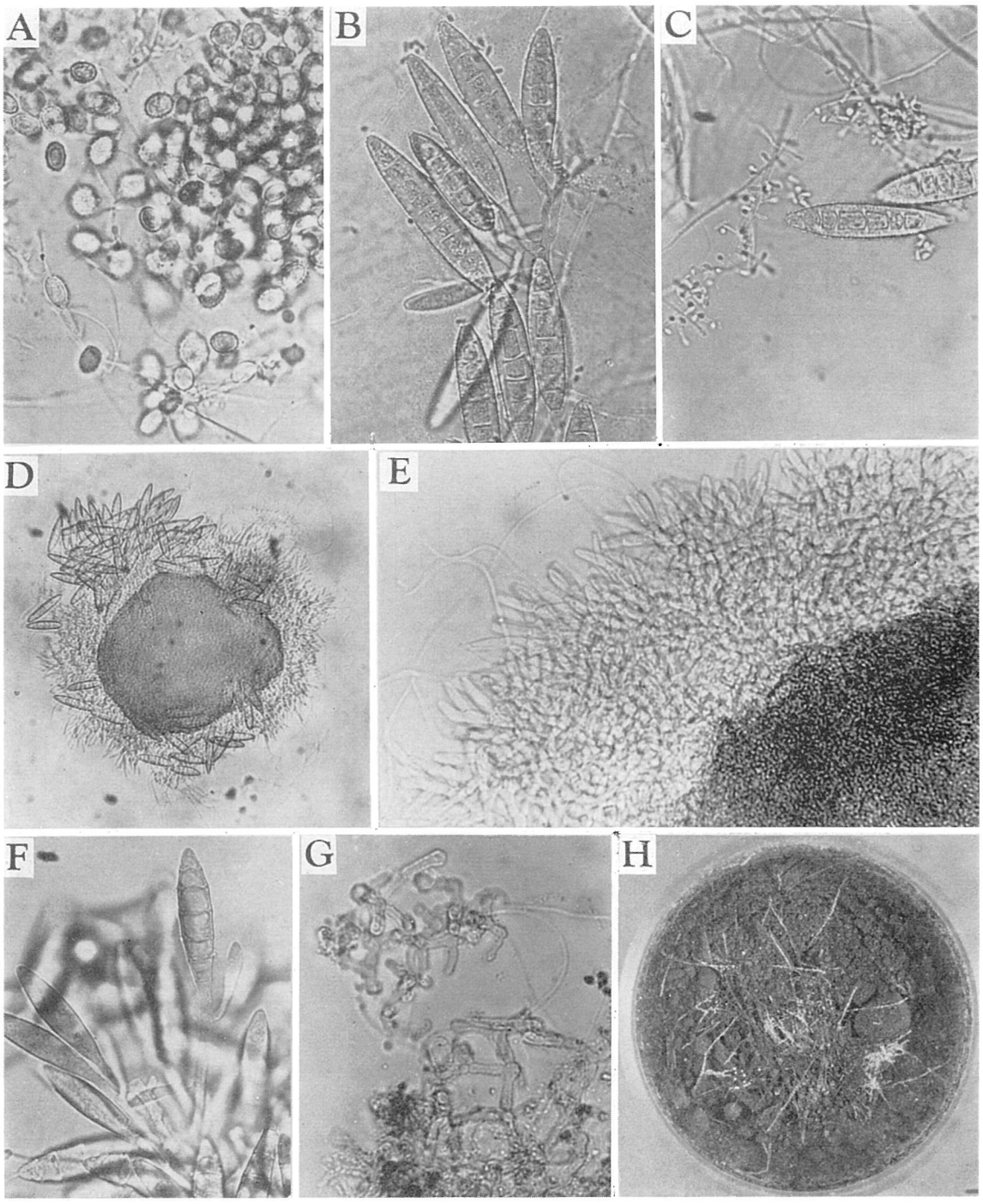

Plate II. **Kertinophilic Fungi** A. *Chrysosporium asperatum* (× ca. 300). B. *Keratinomyces ajelloi* (×100). C. Macro-and microconidia of *Nannizzia cajetana* (=*Microsporum cookei*) (×100). D-E. Cleistothecium of *N. cajetana* (D, ×20) and itw its peridial hyphae (E, ×100). F-G. Macroconidia of *M N. incurvata* (=*Microsporum gypseum*) and its peridial hyphae (G, ×100). H. Hair-baiting soil plate (×0.8).

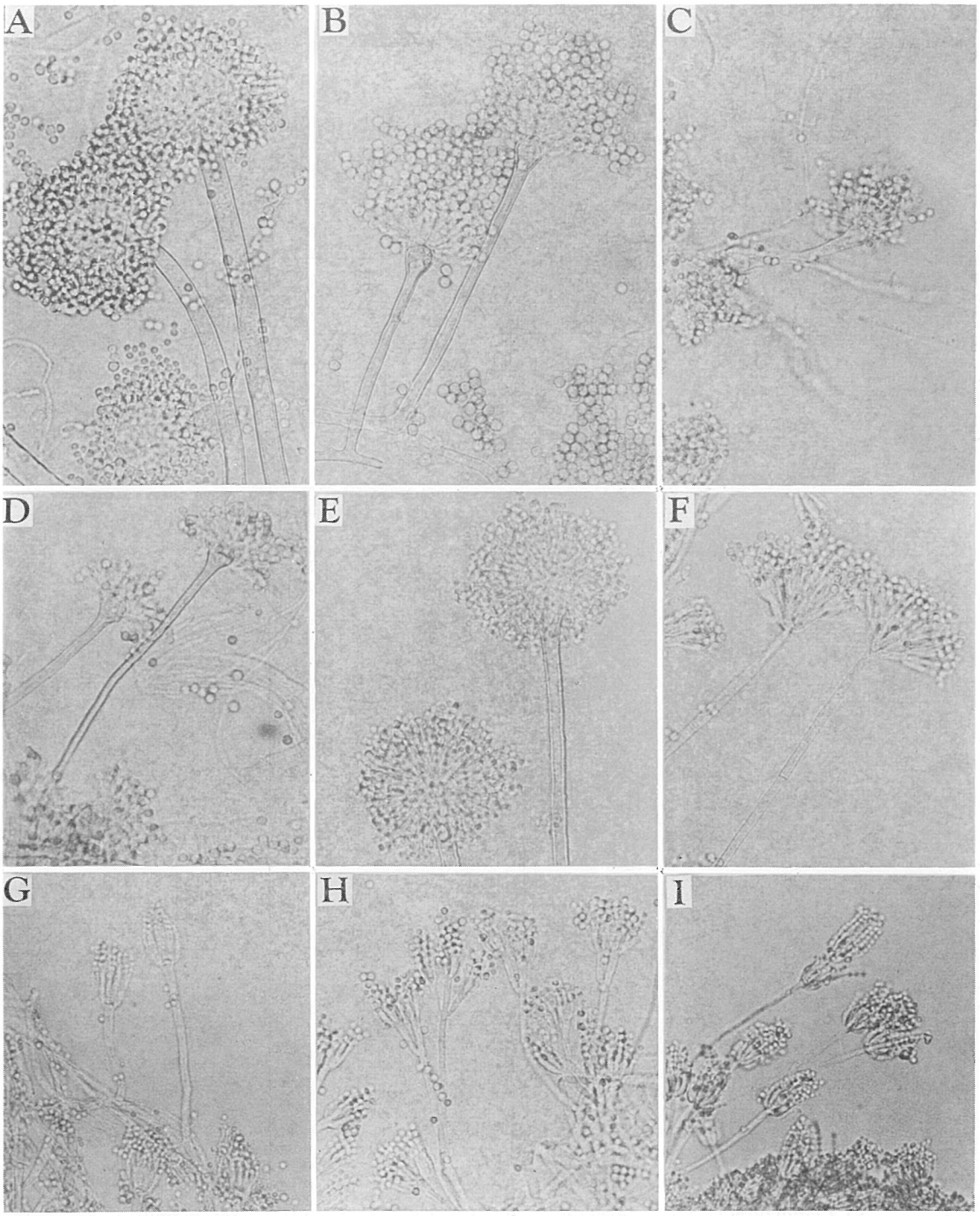

Plate III. ***Aspergillus* and *Penicillium*.** A. *A. cervinus*. B. *A. nidulans*. C. *A. unilateralis* D. *A. ustus* E. *A. versicolor* F. *P. aculeatum* G. *P. adametzi* H. *P. canescens* I. *P. citreo-viride*. (× ca. 400)

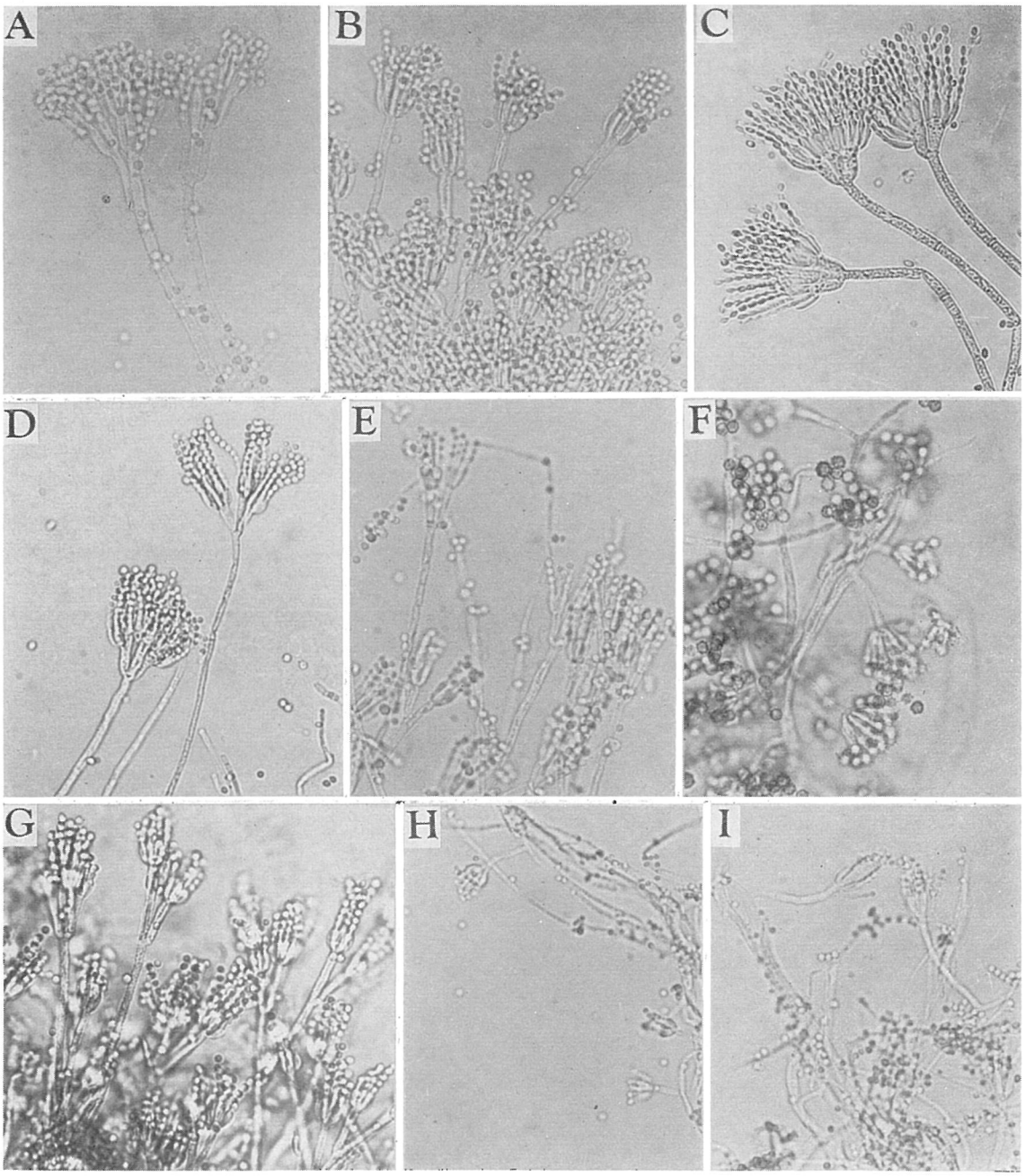

Plate IV. A. *P. cyclopium* B. *P. frequentans* C. *P. herquei* D. *P. miczynskii* E. *P. janthinellum* F. *P. pinetorum* G. *P. radulatum* H. *P. restrictum* I. *P. roseo-purpureum.*

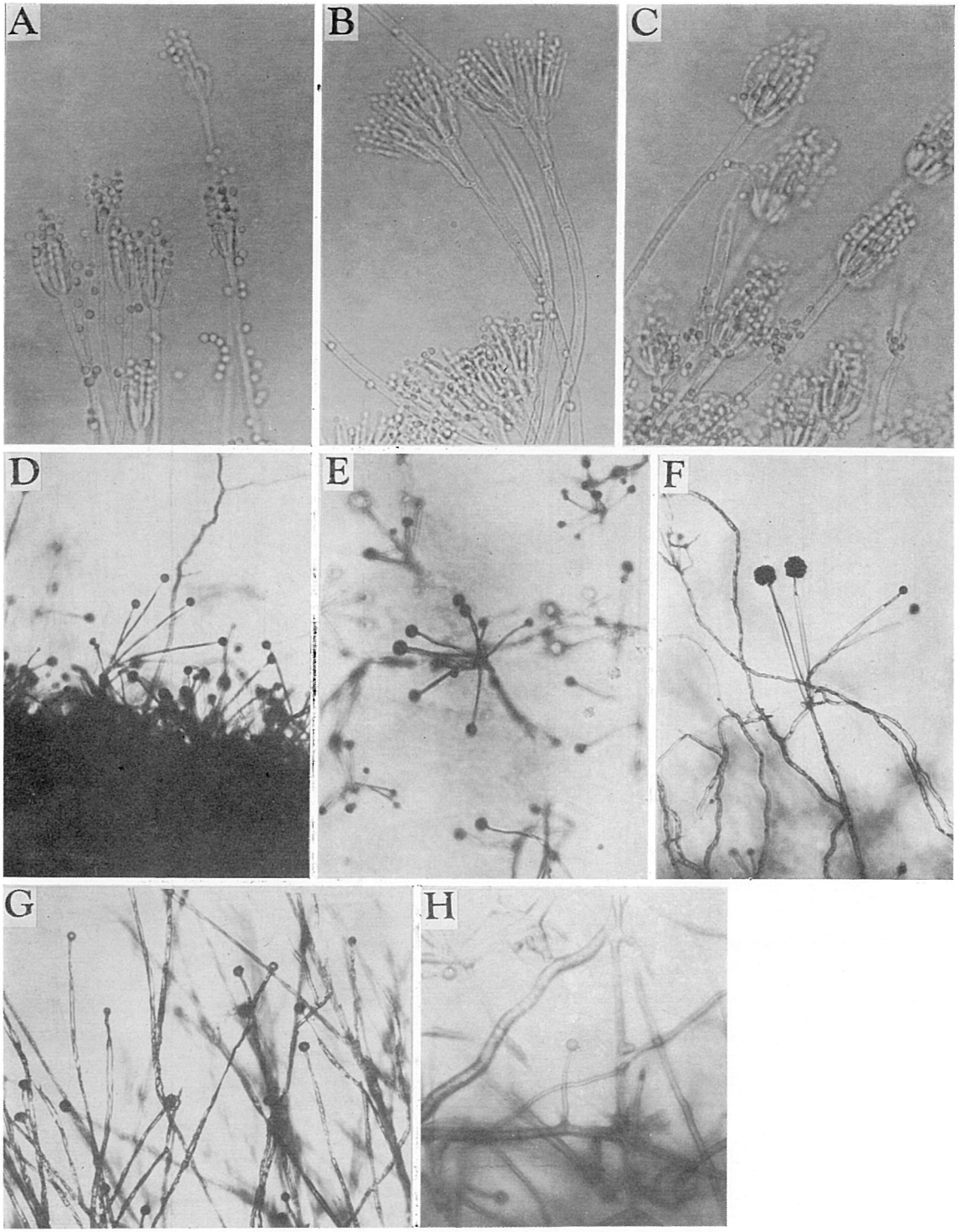

Plate V. A. *P. spinulosum* B. *P. stoloniferum* C. *P. thomii* D. *Mortierella isabellina* (×100). E. *Mort. ramanniana* var. *angulispora* (×100). F. *Mort. humicola* (×150). G. *Mort. longicollis* (×100). H. *Mort. nana* (×250).

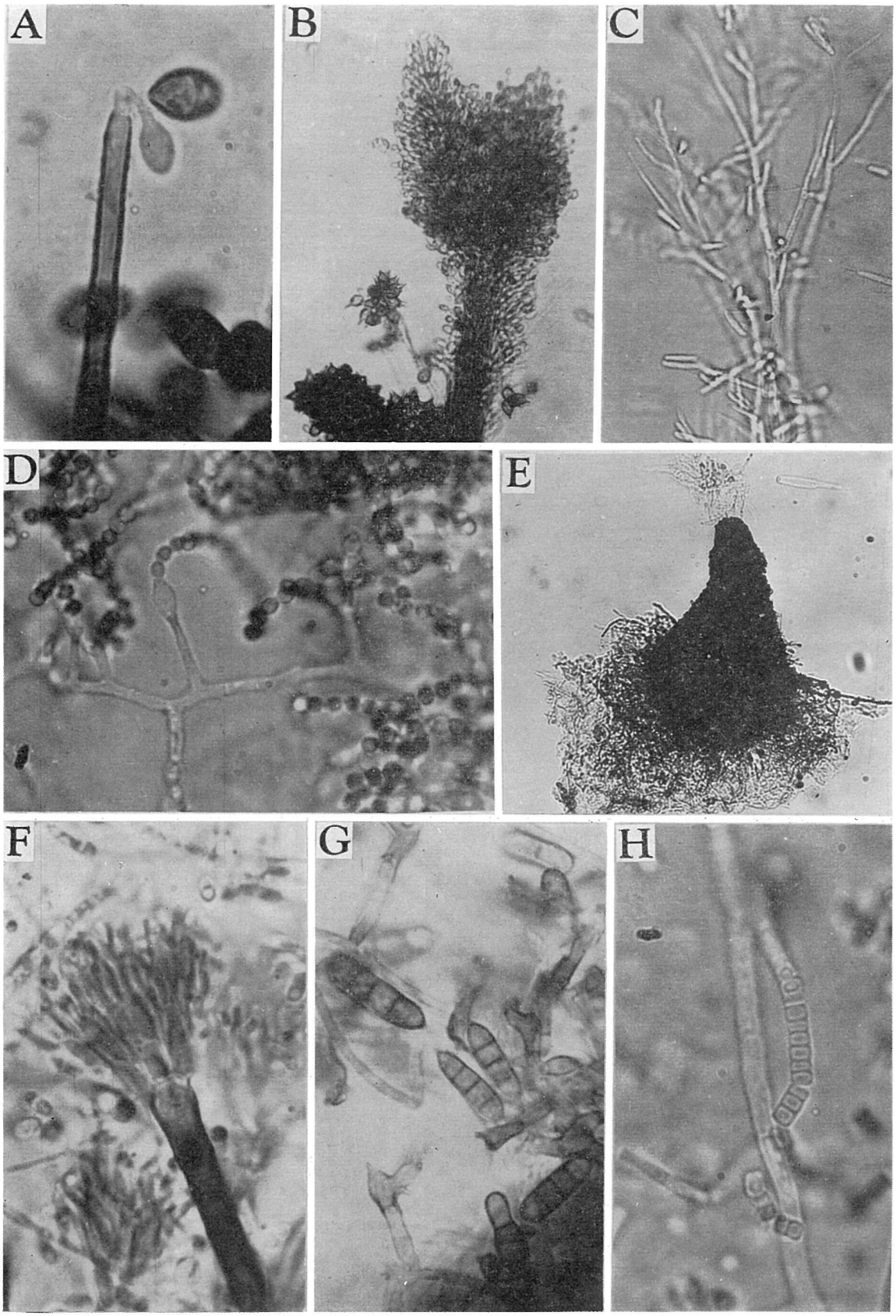

Plate VI. A. *Cordana pauciseptata* (×100). B. *Doratomyces stemonitis*, showing *Stysanus*-and *Echinobotryum*-stages (×40). C. *Hyaloflorae namosa* (×40). D. *Monocillium humicola* (×100). E. *Sordaria fimicola* (×40). F. *Verticicladiella serpens* (×100). G. *Scolecobasidium variabile* (×100). H. *Sporendonema* sp. (×100).

ADMINISTRATIVE REPORT

REPORT OF THE DIRECTOR

In the beginning of April this year we had a new enlarged establishment in which installations of research laboratories and facilities for the culture collection were completed as previously arranged. The present staff is composed of twenty three members including a few clerks. The organization of new laboratories consists of three sections majoring in branches of the microbiology—mycology, bacteriology and microbial genetics.

This report covers the activities of the Institute during the years, 1963 and 1964. For these two years researches were carried on into taxonomical and ecological studies on fungi and bacteria, and into genetic studies on temperate phages. Towards the end of 1963 Dr. Keisuke Tubaki made a mycological expedition to Australia and New Zealand with Dr. Yosio Kobayasi, Chief Curator of the Division of Cryptogams of the National Science Museum, and Mr. Daisuke Shimizu, Member of the Yonezawa Municipal Museum. You can see the fruit obtained through their expedition in Dr. Tubaki's article published in the present issue.

Concerning the activities of the culture collection, 622 strains of fungi and bacteria were obtained from natural sources and collected from other research organizations. As the result a total of 5732 cultures were maintained in the collection at the end of 1964. We also distributed 5312 cultures to domestic and foreign organizations during the same period.

It is a pleasure for the Institute to have the second publication of its annual report. Four research communications given in this report are all originals, written in elucidation of laboratory subjects and for publishing research results thereof.

December, 1965.

Takezi HASEGAWA

DIRECTION COMMITTEE *alphabetical*

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Kikiti	SATO	ex-Director, the Institute for Fermentation
Sueo	TATSUOKA	General Manager, Research and Development Division, Takeda Chemical Industries Ltd.

RESEARCH PROGRAM FOR 1963-1964

1. Studies on *Rhodotorula* yeasts
   Persons in charge: Takezi HASEGAWA, Dr. Ag., Isao BANNO, B. Ag.
2. Lactic acid bacteria and their application
   Person in charge: Reijiro KODAMA, Dr. Ag.
3. Insect and acid-producing bacteria
   Person in charge: Reijiro KODAMA, Dr. Ag.
4. Taxonomic studies on micro-fungi
   Person in charge: Keisuke TUBAKI, Dr. Sc.
5. Genetic studies on temperate phage
   Person in charge: Teiji IIJIMA, Dr. Sc.

JOINT RESEARCHES SUPPORTED BY A GRANT-IN-AID FROM THE MINISTRY OF EDUCATION

1. Studies on the taxonomic basis of microorganisms (1960—1963)
   Chief: Toshinobu ASAI, Director of The Brewing Science Research Institute
   Personnel: Keisuke TUBAKI
2. Studies on the Antarctic organisms (1961—1964)
   Chief: Denzaburo MIYADI, Professor of Kyoto University
   Personnel: Keisuke TUBAKI
3. Fundamental studies on the application of lactic acid bacteria (1962—1965)
   Chief: Kakuo KITAHARA, Professor of the University of Tokyo
   Personnel: Reijiro KODAMA

ABSTRACTS OF SCIENTIFIC PAPERS

Tokuya HARADA*, Toshio FUKUI*, Ziro NIKUNI*, Isao BANNO and Takezi HASEGAWA

The Composition of Extracellular Polysaccharides of *Rhodotorula*

Nippon Nôgeikagaku Kaishi 37(4): 226-230 (1963)

Polysaccharides were obtained from culture media inoculated with six different strains of Rhodotorula. Three of them belong to the subgenus Flavotorula and other three strains to the subgenus Rubrotorula. All Flavotorula strains tested produced extracellularly amylose-like polysaccharides which stained blue with iodine, whereas polysaccharides produced by the Rubrotorula strains gave no blue color reaction with iodine.

These polysaccharides were hydrolysed with sulphuric acid, and their constitutional monosaccharides were identified by paper chromatography. Polysaccharides produced by Flavotorula strains were principally composed of xylose, mannose, glucose and galactose (galactose could not be found in one exceptional case); polysaccharides produced by Rubrotorula strains were composed of mannose, glucose and galactose. Xylose could not be detected in the latter case. [In Japanese]

* The Institute of Scientific and Industrial Research, Osaka University.

Takezi HASEGAWA and Isao BANNO

Vitamin Requirement as A Taxonomic Key for *Rhodotorula* spp.

J. Gen. Appl. Microbiol. 9(3): 279-285 (1963)

Using several strains of *Rhodotorula*, the stability of the requirement of thiamine and para-aminobenzoic acid was reexamined. Each of the tested organisms had been maintained on two different media. Besides, the tests were performed in comparison between two methods of incubation. It was found that the degree of requirement for thiamine and for its two pyrimidine and thiazole moieties considerably varied with cultural conditions, while that of para-aminobenzoic acid by some strains did not vary with conditions of preservation and incubation, indicating its essentiality in accordance with the previous results. Discussions were made on the taxonomic significance of the vitamin requirement for the genus *Rhodotorula*.

Isao BANNO

Preliminary Report on Cell Conjugation and Mycelial Stage in *Rhodotorula* Yeasts

J. Gen. Appl. Microbil. 9(2): 249-251 (1963)

A phenomenon which is considered to be sexual is described.

A conjugation was observed between two cells of *Rhodotorula glutinis* IFO 0559 and 0880. A mycelium developed from the conjugated cells, forming true mycelial colony on agar medium. The mycelium septates with clamp connections. Resting spores, pouch-shaped brownish purple, thick walled, were produced on short stalks of the mycelium. The resting spore germinated a promycelium, from which sporidia were budded off and the sporidium continued to bud producing a yeast-colony. The mycelium is dikaryon, the resting spore is diploid and the sporidia or yeast cells are haploid. The life cycle of the microorganism is discussed.

Takezi HASEGAWA and Isao BANNO

Rhodotorula infirmo-miniata (Okunuki) comb. nov.

Antonie van Leeuwenhoek 30: 65-67 (1964)

Torula infirmo-miniata Okunuki was taxonomically compared with *Rhodotorula macerans* Sonne-Frederiksen. As these two yeasts proved to be very closely related, it is proposed to incorporate both in a new combination, *Rhodotorula infirmo-miniata.*

Keisuke TUBAKI and Isamu ASANO

Talaromyces rotundus (Raper et Fennell) C. B. Benjamin, Newly Found in Japan

Trans. Mycol. Soc. Japan 4: 59-60 (1963)

Talaromyces rotundus, a perfect stage of *Penicillium rotundum*, was isolated from the burnt grass in National Hygienic Laboratory, Shimokamo. This is a first record from Japan.

Keisuke TUBAKI

Notes on the Japanese Hyphomycetes. I.

Trans. Mycol. Soc. Japan 4: 83-90 (1963)

Six species of Hyphomycetes are described in which *Stachybotrys reniformis* is described as a new Hyphomycetes to be distinct from the hitherto known species of the genus in its peculiar reniform and warted conidia. *Chloridium chlamydosporis* (v. Beyma) Hughes, *Clonostachys cylindrospora* Arnoud, *Isthmospora trichophila* (Atk.) Damon, *Pseudobotrytis terrestris* (Timonin) Sub. and *Stephanoma strigosum* Wallr. are described as the first records from Japanese flora.

Keisuke TUBAKI

Notes on the Japanese Hyphomycetes. II. Helicosporous Group

Trans. Mycol. Soc. Japan 5: 1-5 (1964)

Following helicosporous Hyphomycetes are described: *Everhartia lignitalis* Thaxter, *Helicoma perelegans* Thaxter, *Helicosporium citreoviride* Tubaki and *Spirosphaera floriforme* v. Beverwijk. *Everhartia lignitalis*, *Helicoma perelegans* and *Spirosphaera floriforme* are the first records from Japanese flora. *Helicosporium citreoviride* is a member of a yellowish *Helicosporium*, differing from *H. aureum* and *H. guianensis* in unbranched conidiophores and larger conidia, and was established as a new species.

H. H. KUEHN, K. TUBAKI and G.F. ORR

Arachniotus aureus

Mycologia 56: 863-872 (1964)

Re-isolation of *Arachniotus aureus* (Eidam) Schroeter is reported and illustrated of this species are given for the first time. This species may be distinguished from other species on the genus since it forms sinuate peridial hyphae; hyaline to pale yellow, echinulate-reticulate ascospores; yellow ascocarps measuring up to 1 mm diam.; and large roughened aleuriospores representative of *Chrysosporium* species.

MISCELLANEOUS SCIENTIFIC PAPERS

1. Takezi Hasegawa 1963. TYPE and Type Culture. Japan. J. Med. Mycol. 4(3): 121-123 [In Japanese]
2. Takezi Hasegawa 1964. Preservation Methods for Microorganisms. Kagaku To Seibutsu 2(3): 157-162 [In Japanese]

PRESENTATION OF PAPERS AT SCIENTIFIC MEETINGS, 1963-1964

Author (s)	Title	Scientific Meeting
T. Hasegawa	TYPE and type culture	General Meeting of the Japanese Society for Medical Mycology in Osaka (March, 1963)
K. Tubaki	Studies on helicosporous Hyphomycetes	General Meeting of the Agricultural Chemical Society of Japan in Tokyo (April, 1963)
I. Banno	Mycelial stage of *Rhodotorula* (1)	*The same as above*
R. Kodama	Acid-producing bacteria isolated from the silk worm and its related materials	General Meeting of the Society of Sericultural Science of Japan in Tokyo (April, 1963)
K. Tubaki	Relationship between *Tremella* and yeast	General Meeting of the Botanical Socienty of Japan in Okayama (September, 1963)
T. Iijima and M. Eguchi†	Isolation of Temperate Phages from natural source	General Meeting of the Genetics Society of Japan (October, 1963)
R. Kodama	Microbiological activities of thiamine and its related compounds for various lactic acid bacteria	Regular Meeting of Kansai subdivision of the Agricultural Chemical Society of Japan in Hiroshima (October, 1963)
K. Tubaki	Mycological expedition to Australia and New Zaeland	Regular Meeting of the Mycological Society of Japan in Tokyo (February, 1964)
K. Tubaki	Fungi collected from Australia and New Zealand	General Meeting of the Mycological Society of Japan in Tokyo (April, 1964)
R. Kodama F. Matsubara† and M. Kato††	Pathogenicity of several strains of bacteria for silk worm caterpillars aseptically reared on artificial diet	General Meeting of the Society of Sericultural Science of Japan in Tokyo (April, 1964)
R. Kodama	A motile lactic cocci isolated from silk worm and its related materials	General Meeting of the Agricultural Chemical Society of Japan in Sapporo (July, 1964)
I. Banno	Mycelial stage of *Rhodotorula* (2)	*The same as above*
K. Tubaki	Criteria for the classification of Hyphomycetes	X International Botanical Congress in Edinburgh (August, 1964)
T. Iijima	A study of a temperate phage, ϕ170	General Meeting of the Genetics Society of Japan in Matsuyama (October, 1964)

† Kyoto University of Industrial Art and Textile Fibers
†† Faculty of Science, Kyoto University

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Review

Communications

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