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FORWORD

The Institute for Fermentation was established in 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.

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 research 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 microbiology. Since then, the Institute has made an issue of its own periodical that includes original articles and summaries of published papers. It would be a great pleasure for the Institute to seek the advice of acknowledged authorities in all countries on this field of study.

Chairman of the Board of Trustees

CONTENTS

ADMINISTRATIVE REPORT

Ann. Rep. Inst. Fermentation, Osaka
No. 4, p. 1~11, 1969 (February)

PATHOGENICITY OF BACTERIA FOR SILKWORM LARVAE REARED ASEPTICALLY ON AN ARTIFICIAL DIET

Rejiro KODAMA and Yugoro NAKASUJI

For a long time, streptococci were considered only as the secondary invadors in virus diseases of silkworms, *Bombyx mori* (Linn.). Recently Lysenko[3] showed that large doses of streptococci must be used to bring about diseases to silkworm larvae under natural conditions. Ono, Iwanami and Enomoto[5] noted that silkworm larvae died from septicemia at a relatively high rate, following oral inoculation of the strains of the genera *Aeromonas*, *Pseudomonas* and *Serratia*. These studies on the pathogenicity of bacteria for silkworm larvae were made by the use of larvae fed only with mulberry leaves. These larvae are not always suitable for the investigation of the pathogenicity of a special kind of bacteria, because their intestines usually contain various kinds of bacteria at the period used for the test. In this respect silkworm larvae reared aseptically on an artificial diet afford an advantage to studies on pathogenicity of bacteria for the insects.

In this paper the results of pathological studies with some strains of lactic acid bacteria and gram-negative bacteria in gnotobiotic silkworm larvae fed with an artificial diet will be presented.

Materials and methods

The bacteria tested. They included strains isolated from epizootics in populations of silkworms and those maintained at the culture collection in Institute for Fermentation, Osaka.

Bacterial suspension. Each bacterial suspension was prepared as follows: Two loopfuls of 48 hours growth on an agar medium described previously,[2] were suspended in 5 ml of 0.85% saline and centrifuged aseptically. After being washed twice with 0.85% saline, they were resuspended in saline. Then, the resulting suspension was diluted to desired concentration.

The examination of pathogenicity. This was done on healthy silkworm larvae in the 5th instar, which were reared aseptically on an artificial diet according to the method of Matsubara and his associates.[4] The infectivity of these bacterial strains was tested by feeding the organisms to larvae, and also by injection into the hemocoele of larvae.

(a) Feeding experiments were carried out in the following way: each 5 g of artificial diet was autoclaved at 121°C for 40 minutes in a test tube (3.0×19.5 cm), and inoculated with 0.2 ml of the suspension of each strain of bacteria; then a larva

in the 5th instar was placed into each tube; after feeding for 4 hours, each larva was transferred into another tube containing 5 g of sterilized diet; the transfer was repeated every 20 to 24 hours, until spinning of cocoons occurred. For each experiment 10 larvae were used.

(b) In injection experiments a larva was transferred into another test tube immediately after injection, and diet was withheld thereafter to avoid invasion of bacteria in the gut through the diet as much as possible. For each injection experiment 5 larvae were used.

Recovery of viable cells of *Streptococcus faecalis-Streptococcus faecium* intermediate E–5. One drop of the blood exudated after the cercus was cut off and a loopful of the gut contents after the abdominal region was incised, were used for recovery of the streptococci from the blood and the gut contents respectively. These processes were all carried out aseptically.

Results

I. Lactic acid bacteria Only the strains of the genus *Streptococcus* brought

Table 1. Pathogenicity of some strains of lactic acid bacteria by feeding and the maximum pH values for growth. (0.7×10^2 to 3.7×10^3 of viable cells of each strain were added to 5 gm of a diet.)

Strain tested	Lethality	The maximum pH values for growth
Streptococcus		
S. facealis IFO 3181	0%	9.73–10.25
S. faecalis-S. faecium intermediate E–5*	100	10.65–11.24
Leuconostoc		
L. dextranicum IFO 3347	0	8.13–8.75
L. mesenteroides IFO 3832	0	8.75–9.15
Pediococcus		
P. acidilactici IFO 3076	0	8.13–8.75
P. cerevisiae IFO 12229	0	8.13–8.75
Lactobacillus		
L. acidophilus IFO 3831	0	7.21–8.05
L. arabinosus IFO 3070	0	8.60–8.86
L. bulgaricus IFO 3533	0	8.05–8.48
L. casei IFO 3425	0	7.73–8.13
L. brevis IFO 3960	0	7.73–8.13
L. buchneri IFO 3961	0	8.60–8.86
L. fermenti IFO 3071	0	7.21–8.05
Control∫	0	

* Isolated from dead silkworms, 1963.
∫ Instead of bacterial suspension the same amount of 0.85% saline was used.

about disease in larvae by feeding; those of the genera *Lactobacillus*, *Leuconostoc* and *Pediococcus* did not (Table 1). Among the streptococci tested, *Streptococcus alcalophilus* IFO 3531, *Streptococcus faecium* N–42 and the strains which originated from silkworm larvae produced disease. Three strains, E–5, N–3 and 079, which were related to both *Streptococcus faecalis* and *Streptococcus faecium* taxonomically, had the greatest pathogenicity, showing 100% lethality (Table 2). The disease in this case had a chronic character, exhibiting symptoms of diarrhea, loss of appetite and reduction of body weight. Bacteria could not be recovered in any of the healthy pupae in this experiment.

Table 2. Pathogenicity of some strains of the genus *Streptococcus* by feeding and the maximum pH values for their growth. (0.7×10^3 to 2.3×10^3 of viable cells of each strain were added to 5 gm of a diet.)

Strain tested	Lethality	The maximum pH values for growth
S. alcalophilus IFO 3531	30%	11.07–11.20
S. faecalis OUT 8133	0	10.74·11.07
〃 OUT 8177	0	10.74–11.07
〃 OUT 8200	0	10.42–10.74
S. faecium N–42*	20	10.74–11.07
〃 PG–55*	0	10.74–11.07
S. faecalis-S. faecium intermediate E–5	100	10.74–11.07
〃 G–27†	20	10.74–11.07
〃 N–3†	100	10.74–11.07
〃 079‡	100	10.74–11.07
〃 248‡	30	10.74–11.07
S. faecalis var. zymogenes IFO 3989	0	10.42–10.74
S. lactis IFO 12007	0	8.20– 9.05
S. salivarius IFO 3350	0	10.42–10.74
S. thermophilus IFO 3535	0	10.42–10.74
Control∫	0	

* Kindly supplied by Dr. M.E. Sharpe, England.
† Isolated from dead silkworms, 1963.
‡ Kindly supplied by Dr. O. Lysenko, Czechoslovakia.
∫ Instead of bacterial suspension the same amount of 0.85% saline was used.

The pathogenic strains had a tendency to be capable of growing in media of relatively high pH values, compared with the nonpathogenic strains.

II. Gram-negative bacteria Of the gram-negative bacteria tested, the strains belonging to the genera *Proteus* and *Serratia* (except *Serratia plymuthica* IFO 3055) and *Pseudomonas aeruginosa* IFO 3898 were pathogenic for larvae either by feeding or by injection. *Aeromonas hydrophila* IFO 3820 and *Flavobacterium aquatile* IFO 3772

Table 3. Pathogenicity of some strains of gram-negative bacteria by feeding or by injection and the maximum pH values for their growth.

Strain tested	Lethality		The maximum pH values for growth
	by feeding**	by injection††	
Aerobacter aerogenes IFO 3317	0%	0%	8.95–9.10
〃 〃 G–33*	20	0	9.10–9.30
Aerobacter cloacae A_2–4†	20	20	9.10–9.30
Aeromonas hydrophila IFO 3820	0	100	9.30–9.71
Achromobacter liquidum IFO 3084	0	20	8.46–8.95
Erwinia carotovora IFO 3380	10	0	8.46–8.95
Escherichia coli IFO 3806	0	0	8.95-9.10
Flavobacterium aquatile IFO 3772	0	60	8.46-8.95
Klebsiella pneumoniae IFO 3512	0	0	8.46–8.95
Proteus mirabilis IFO 3849	100	100	8.95–9.10
Proteus morganii IFO 3848	40	100	8.95–9.10
Proteus vulgaris IFO 3045	10	100	8.95–9.10
Pseudomonas aeruginosa IFO 3898	40	100	8.95-9.10
Serratia indica IFO 3759	80	100	9.10–9.30
Serratia marcescens IFO 3046	40	100	8.95–9.10
Serratia piscatorum E–15‡	100	100	9.10–9.30
Serratia plymuthica IFO 3055	0	0	8.46–8.95
Control∫	0	0	

* Isolated from dead silkworms, 1962.
† Isolated from dead silkworms, 1965.
‡ Isolated from dead silkworms, 1963.
∫ Instead of bacterial suspension the same amount of 0.85% saline was used.
** Viable cells of each strain within the limists of 1.6×10^6 to 1.9×10^7 were added to 5 gm of a diet.
†† Viable cells of each strain within the limits of 1.2×10^3 to 7.4×10^3 were injected into the hemocoele of larva.

were pathogenic only by injection (Table 3). They produced a septicemia, but no intestinal disease.

III. A synergistic effect in the pathogenicity of *S. faecalis-S. faecium* intermediate E–5 and that of *Serratia piscatorum* E–15.

Further investigation was made on the pathogenicity of bacteria for aseptically reared silkworm larvae. For this purpose, *S. faecalis-S. faecium* intermediate E–5 and *Serratia piscatorum* E–15 were picked out of the stains mentioned above, as they were isolated from dead larvae of silkworms in a cocoonery.

(a) Physiological characters of the strains.

1) *Streptococcus faecalis-Streptococcus faecium* intermediate E–5

The strain grew abundantly in glucose broth. Gram-positive, catalase-negative, and did not reduce nitrate to nitrite. Grew at 10°, but not at 45°. Indole was not

formed. Methyl red test positive, Voges-Proskauer test slightly positive. Sodium citrate was not utilized. Litmus milk was acidified and curdled with reduction of litmus before curdling. Grew in the presence of 0.3% methylene blue, on agar medium containing 0.04% potassium tellurite and in 6.5% sodium chloride. Tetrazolium was reduced within 8 hours. Arginine and esculin were hydrolyzed, but gelatin and sodium hippurate were not. Tyrosine was decarboxylated. Serologically belonged to Lancefield Group D, though the reaction was slow.

Strach, dextrin, raffinose, maltose, sucrose, lactose, melibiose, cellobiose, trehalose, glucose, fructose, mannose, galactose, xylose, salicin, mannitol and sorbitol were fermented. Inulin, melezitose, rhamonose, sorbose, arabinose, α-methyl glucoside, dulcitol, adonitol, inositol and glycerol (anaerobically) were not fermented.

Arginine was utilized as an energy source for the growth, but serine, gluconate, malate and pyruvate were not.

Over 90% of the glucose fermented was converted to lactic acid. Lactic acid produced was the dextro form.

Pantothenate, nicotinic acid, biotin, folic acid, pyridoxal (or pyridoxamine), arginine, glutamic acid, glycine, histidine, isoleucine, leucine, methionine, threonine, tryptophan and valine were essentially, and alanine and cysteine were stimulatorily required for the growth.

From these characters, the strain E–5 was found to be related to both *S. faecalis* Andrewes et Horder and *S. faecium* Orla-Jensen, and also partially to *Streptococcus lactis* Lister (Loehnis) taxonomically.

2) *Serratia piscatorum* E–15

The strain E–15 was short rods. Motile by lateral flagella. Gram-negative, catalase-positive, reduced nitrate to nitrite. Optimum temperature for growth 28°; grew at 37°, but not at 45°. Milk was coagulated and peptonized. Both indole and hydrogen sulfide were not formed. Methyl red test netative, Voges-Proskauer test positive. Sodium citrate was utilized. Cytochrome oxidase test negative. Good growth in 3% Nacl, slight growth in 7% Nacl and no growth in 10% Nacl. Gelatin, esculin, arginine and urea were hydrolyzed, but starch was not. Phenylalanine was not decomposed.

Acid was produced from dextrin, maltose, sucrose, cellobiose, trehalose, glucose, fructose, mannose, galactose, ribose, salicin, mannitol, adonitol, sorbitol and glycerol, but not from starch, inulin, raffinose, melezitose, lactose, melibiose, sorbose, rhamnose, arabinose, xylose, α-methyl glucoside and dulcitol. No gas was formed from any of the carbon sources mentioned above.

A rose-red pigment was produced under some cultural conditions. When mannitol was used as an energy source, the pigment was produced after 3 to 5 days incubation at 20° to 28°, but the pigment formation was never observed even after 3 weeks incubation at 37°

The concentrate of the pigment did not seem soluble in ether, benzene, propanol,

butanol, chloroform, ethylacetate and toluene, but was soluble in methanol, ethanol and water. The adsorption spectrum of the crude pigment in acid ethanol had a band with a maximum at 540 mμ.

From these characters, the strain E–15 was found to be closely related to *Serratia piscatorum* (Lehmann et Neumann) Breed taxonomically.

(b) A synergistic effect. When each strain was inoculated to larvae by feeding, lethality eventually reached 100%. The symptoms, however, varied with the species; larvae infected with *S. faecalis-S. faecium* intermediate E–5 shrinked accompanying diarrhea, while those infected with *Serratia piscatorum* E–15 generally fell down in a swoon during the next several days, although they continued their growth until that time.

Death of larvae was hastened by mixed infection of both strains, exhibiting a synergistic effect in the pathogenicity of *S. faecalis-S. faecium* intermediate E–5 and that of *Serratia piscatorum* E–15 (Table 4).

Table 4. Synergistic effect in the pathogenicity of *S. faecalis-S. faecium* intermediate E–5 and that of *Serratia piscatorum* E–15 by feeding.

Strain tested	Viable cells added to 5 gm of a diet	Number of died silkworms									Lethality
		on indicated day after inoculation								in cocoon	total	
		1	2	3	4	5	6	7	8			
Control*											0	0%
S.faecalis— *S. faecium* intermediate E–5	2.2×10^7				1	2	5	2			10	100
Serratia piscatorum E–15	2.4×10^7		1	2	1		3		1	2	10	100
S. feacalis— *S. faecium* intermediate E–5 + *Serratia piscatorum* E–15	E–5 1.1×10^7 E–15 1.2×10^7	3	2	1	4						10	100

* Instead of bacterial suspension the same amount of 0.85% saline was used.

IV. The relation between inoculum size and lethality by feeding.

In each case of *S. faecalis-S. faecium* intermediate E–5 and *Serratia piscatorum* E–15, the death of larvae occurred sooner with the increasing amounts of viable cells inoculated by feeding. However, a marked difference was observed between the number of viable cells of these two species required for accomplishment of 100% lethality (Table 5).

V. Multiplication of *S. faecalis-S. faecium* intermediate E–5 and *Serratia piscatorum* E–15 in the gut and the hemocoel of larvae. *S. faecalis-S. faecium*

Table 5. Relation between inoculum size and lethality by feeding.

Strain tested	Viable cells added to 5 gm of a diet	Number of died silkworms on indidated day after inoculation 1 2 3 4 5 6 7 8 9 10	Number of died silkworms in cocoon	Number of died silkworms total	Number of healthy pupae	Lethality
S. faecalis — *S. faecium* intermediate E–5	0			0	10	0%
	5.0×10^{1}	5 2 1 2		10	0	100
	5.0×10^{3}	1 1 2 4 1 1		10	0	100
	5.0×10^{5}	1 1 2 3 3		10	0	100
	5.0×10^{7}	3 4 2 1		10	0	100
	5.0×10^{8}	3 4 2 1		10	0	100
Serratia piscatorum E–15	0			0	10	0
	2.4×10^{2}	1 1 1	1	4	6	40
	2.8×10^{4}	1 1 2	2	6	4	60
	2.6×10^{6}	2 1 1 1 1	4	10	0	100
	2.7×10^{8}	1 2 2 2 2 1		10	0	100
	1.2×10^{9}	2 2 1 3 2		10	0	100

Table 6. Multiplication of *S. faecalis*-*S. faecium* intermediate E–5 and *Serratia piscatorum* E–15 in the gut and the hemocoele of larvae which were abstained from a diet after feeding and after injection.

Strain tested	Hours after feeding and after injection	Viable cells per larva* in the gut content after feeding	Viable cells per larva* in 0.01 ml of the blood after injection
S. faecalis — *S. faecium* intermediate E–5	0	less than 1×10^{2}	4.4×10^{2}
	10		6.0×10^{2}
	18	1.4×10^{7}	
	28		4.2×10^{2}
	44	5.6×10^{5}	
Serratia piscatomu E–15	0	less than 1×10^{1}	less than 1
	10		2.1×10^{3}
	18	less than 1×10^{1}	
	28		6.3×10^{7}
	44	less than 1×10^{1}	

* An average of five larvae.

Table 7. Recovery of viable cells of *S. faecalis-S. faecium* intermediate E–5 from the gut contents and the blood of larvae which were fed for 4 hours with a diet infected with the suspension of the organism and starved thereafter.

Hours after abstention from a diet	Number of larvae tested	Recovery of bacteria from		Remarks
		gut content	blood	
0	10	10+*	10−**	
24	10	10+	10−	Larvae tested
48	10	10+	7− 3+	were all alive at these
72	10	10+	1− 9+	periods.

* 10 + indicates that viable cells of the strain were recovered from 10 larvae.
** 10 — indicates that viable cells of the strain were not recovered from 10 larvae.

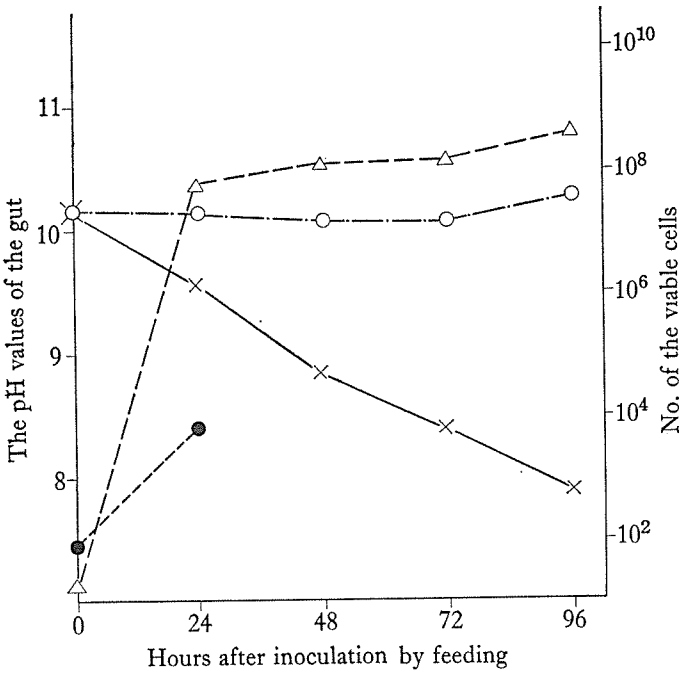

Fig. 1. Change of the pH values of the gut and the number of the viable cells in the gut.

In this feeding experiment, larvae inoculated with *S. faecalis-S. faecium* intermediate E–5 were transferred into another tube containing a freshly prepared aseptic diet, the transfer being continued once a day thereafter. ×——× The pH values of the gut (an average of 5 larvae); △- - -△ Number of the viable cells in the gut (an average of 5 larvae); ○——○ The pH values of the gut of larva, to which 0.85% saline was added instead of the bacterial suspension (an average of 5 larvae). ●----● Number of the viable cells in a diet. It was judged that there was no necessity for counting the viable cells in a diet at and after 48 hours after inoculation, because at that time a diet was contaminated with loose feces in which the bacterial cells of *S. faecealis-S. faecium* intermediate E–5 are excreted.

intermediate E–5 grew rapidly in the gut of larvae within 18 hours after feeding for 4 hours, the diet being withheld from the larvae thereafter (Table 6). The streptococci could not be found in the blood at 24 hours, but within 48 to 72 hours they were recovered (Table 7). At this time the insects were still alive.

When the insects were transferred following inoculation into another tube containing a freshly prepared aseptic diet, and the transfer was repeated every 20 to 24 hours thereafter, the streptococci grew rapidly in the gut, resulting in remarkable lowering of the pH values of the gut contents (Fig. 1).

On the other hand, death of larvae was not hastened by injection of at least 10^3 of viable cells of *S. faecalis-S. faecium* intermediate E–5, although larvae died during the next several days because they had not been fed. Similar results were obtained by injection of 2.8×10^5 viable cells of the same strain.

On the contrary, *Serratia piscatorum* E–15 could not grow in the guts of aseptically reared silkworm larvae which were not fed thereafter (Table 6).

Discussion

The pathogenic streptococci which were isolated from diseased larvae in a cocoonery taxonomically resembled those described by Lysenko[1]. Regarding pathogenic effects, however, some disagreements were found between the results obtained by us and those described by Lysenko. That is, in the experiments carried out with a strain of streptococci, *S. faecalis-S. faecium* intermediate E–5, it was found: (1) by feeding, even with small doses, the lethality always reached 100%; (2) the streptococci were recovered in the blood within 48 to 72 hours after feeding, although the insects were still alive at this time; (3) apparent pathogenicity was not observed by injection of at least 10^3 of viable cells.

The pH of the gut of aseptically reared silkworm larvae in the 5th instar ranges from 10.0 to 10.5. If there are bacteria capable of multiplying in the gut at this pH, they must be of a relatively alkali-resistant character. The strains included in the genus "Enterococcus" fit that condition. In the above experiments, a full parallel was not observed between an alkali-resistant property of the enterococci and their pathogenicity for silkworm larvae. But it is postulated that an alkali-resistant character must be required for exhibiting pathogenicity by the enterococci.

The results obtained with *S. faecalis-S. faecium* intermediate E–5 indicate that the streptococci grow first in the gut of silkworm larvae when ingested, and then invade into the hemocoele, but do not cause septicemia.

On the other hand, the results obtained with *Serratia piscatorum* E–15 indicate that this organism is to be included in a group of "potential pathogens" in the classification by Bucher,[1] because they cannot grow in the gut of silkworm larvae starved after feeding for four hours, but cause septicemia within 24 hours after being injected.

Through what process does the invasion by strains of that group occur? We

must consider the following process as one possible mechanism. Within the range of our experiments, the maximum pH values for growth of the members of "potential pathogens" examined were relatively lower than those of the enterococci. Consequently, it is difficult to infer that they can multiply readily in the gut of silkworm larvae. When the stranis of "potential pathogens" are coexisting with the streptococci in the gut, however, it is conceivable that the streptococci grow first, resulting in a form of dyspepsia. They then invade into the homocoele, affording an opportunity for other bacteria to invade. Lowering of the pH values of the gut as a result of multiplication of the streptococci may prepare an environmental condition favorable for the multiplication of strains of "potential pathogens". This resulted in a considerably increased opportunity for invasion. On the other hand, it had been observed by Ono and Ichikawa[6] that some strains belonging to the genus *Aeromonas* and others have a property capable of dissolving the peritrophic membrane of silkworm larvae. Such a property of these bacteria may accelerate their own invasion into the hemocoele of silkworms. The fact that *Serratia piscatorum* E–15 exhibited a greater pathogenic effect with increasing amounts of viable cells inoculated, seems to support the inference.

Thus, the authors favor the view that in bacterial epizootic of silkworms the pathogenic streptococci cause an intestinal disease of chronic character as primary invadors, and also play an inductive role for the strains of various species of gram-negative and, presumably, gram-positive bacteria as the secondary invadors.

There is no evidence that the pathogenic mechanism of bacteria, as described above, can also be applied to silkworm fed with mulberry leaves. But it is believed that a similar mechanism may be playing a role under natural conditions.

Summary

Investigation was made on pathogenicity of various species of lactic acid bacteria and gram-negative bacteria for healthy silkworm larvae in 5th instar, which were reared aseptically on an artificial diet. The results obtained were:

(1) Among lactic acid bacteria tested, only the strains of the genus *Streptococcus* brought about disease in larvae by feeding; those of the genera *Pediococcus*, *Leuconostoc* and *Lactobacillus* did not cause any disease.

(2) Of the streptococci tested, the strains which originated from silkworms had the greatest pathogenicity by feeding. However, death of larvae was not hastened by injection of at least 10^3 of viable cells of these streptococci.

(3) The pathogenic streptococci isolated were a form taxonomically related both to *Streptococcus faecalis* and *Streptococcus faecium*.

(4) Among gram-negative bacteria tested, the strains belonging to the genera *Proteus*, *Pseudomonas* and *Serratia* (except *Serratia plymuthica*) were pathogenic for larvae either by feeding or by injection.

(5) *S. faecalis-S. faecium* intermediate E–5 and *Serratia piscatorum* E–15 were

picked out of the strains tested for the purpose of studying the pathogenic mechanism of bacteria for the insects.

When each strain was inoculated to larvae by feeding, the death of larvae occurred sooner with the increasing amounts of viable cells inoculated. However, a marked difference was observed between the number of viable cells of these species required for accomplishing of 100% lethality.

Death of larvae was hastened by mixed infection of these two strains, exhibiting a synergistic effect in the pathogenicity of the strain E–5 and that of the strain E–15.

(6) *S. faecalis-S. faecium* intermediate E–5 grew rapidly in the gut of larvae after their feeding for four hours, the diet being withheld from the larvae thereafter. The streptococci could not be found in the blood at 24 hours, but within 48 to 72 hours they were recovered.

Also when the insects were transferred following inoculation into another tube containing a freshly prepared aseptic diet, and the transfer was repeated every 20 to 24 hours thereafter, the streptococci grew rapidly in the gut, resulting in remarkable lowering of the pH values of the gut.

(7) *Serratia piscatorum* E–15 could not grow in the gut of larvae that were abstained from a diet after their feeding for four hours, but could grow in the hemocoele rapidly after being injected.

(8) A mechanism of pathogenic effects of bacteria on silkworm larvae was discussed.

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STUDIES ON THE JAPANESE MARINE FUNGI LIGNICOLOUS GROUP (III), ALGICOLOUS GROUP AND A GENERAL CONSIDERATION

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The previous papers given by the present author (1966, 1968) dealt with the presence of the marine fungi, especially of the lignicolous ascomycetous and the hyphomycetous fungi from Japan. Because the papers hitherto reported on the marine fungi in Japan were mostly restricted to the marine Phycomycetes, in the first report (1966), the descriptions of two species of Ascomycetes and four of Fungi Imperfecti from Japanese marine habitats was made about the collecting method adopted in the studies. Toluidine-blue staining of the ascospore-appendage and the condition of ascospore formation of *Ceriosporopsis halima* were described. In the second paper (1968), sixteen additional lignicolous marine fungi were recorded including two new species of *Remispora* and *Sphaerulina*.

In addition to these foregoing studies in the author's laboratory which have dealt with the attack by marine lignicolous species upon wood and other cellulosic material submerged in the sea, the mycological studies on algicolous and foliicolous marine mycota have been carried out together with the lignicolous group. In the present paper, taxonomic data up to the present in this serial studies are summarised and physiological studies are reported with a general discussion on the marine fungi.

The collecting method in the present study is basically the same as that of the previous papers. In most cases, wood panels were made with balsa, *Abies firma*, *Cryptomeria japonica*, *Paulownia tomentosa* and *Phyllostachys pubescens*. Those of balsa and *Phyllostachys* are the most useful for collecting the marine fungi because different species of fungi may develop depending on the substrates.

In addition to seven collecting places previously described, Shimoda in Izu Peninsula was newly chosen as one of the places where the Kuroshio and the Oyashio current meet in the offing (Fig. 1). The present paper is consisted of five major articles and each is divided as follows:

I. Classification
   1. Lignicolous group...summarised data of the foregoing results with additional one species not reported previously
   2. Algicolous and foliicolous group
   3. Fungi in sea mud

II. Occurrence and distribution

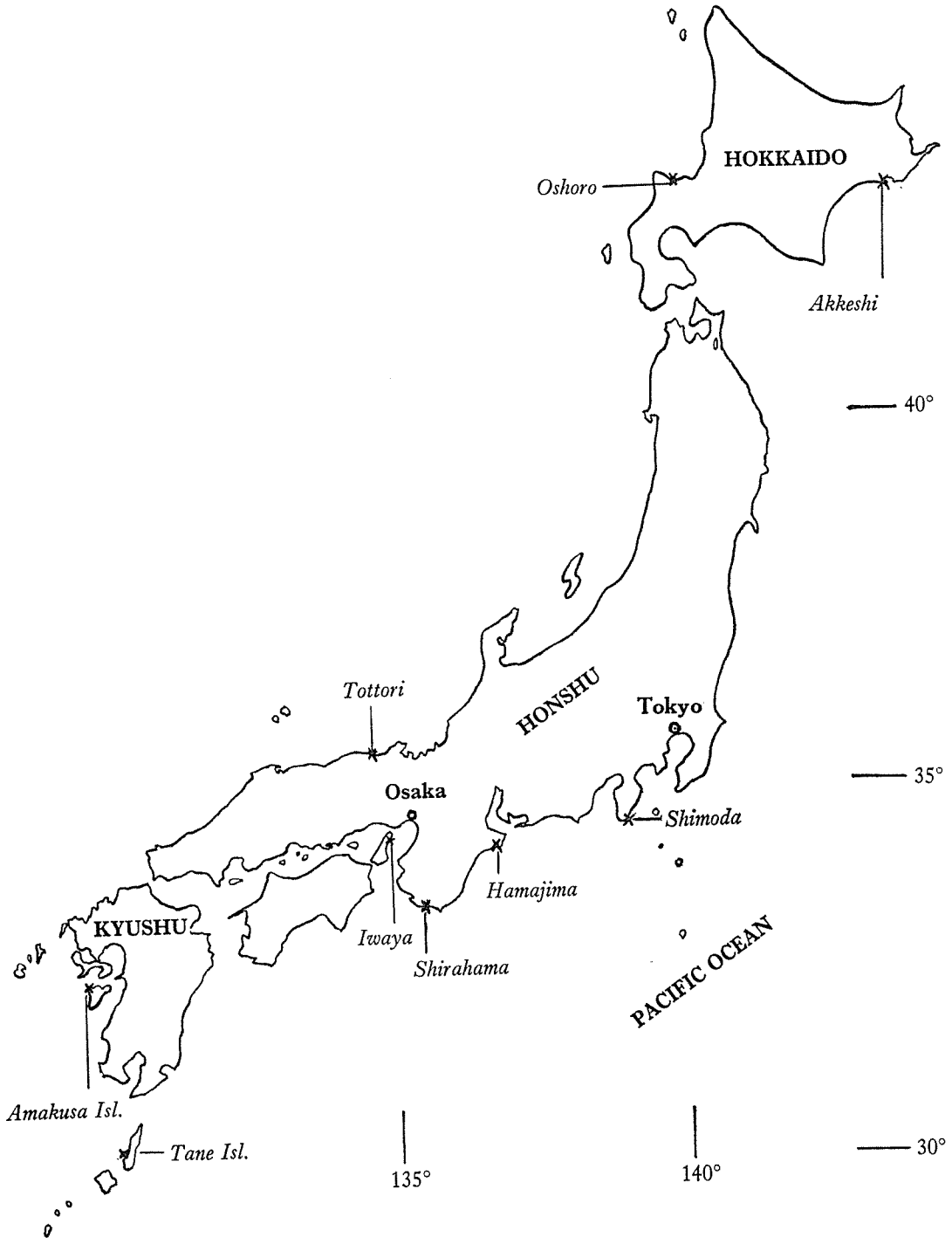

Fig. 1. Location of collection stations in Japan.

III. Physiology

1. Preliminary survey on polysaccharide
2. Effects of vitamin on the growth
3. Effects of inorganic macroelements on the growth
4. Effects of temperature on the growth

IV. Experimental aspects of marine fungi
V. Discussion and conclusion

I. CLASSIFICATION

Lignicolous group

Ascomycetes

Antennospora quadricornuta (Cribb et Cribb) Johnson

Johnson, in Jour. Elisha Mitchel Sci. Soc. **74:** 46 (1958);
Tubaki, in Publ. Seto Mar. Biol. Lab. **15:** 359 (1968).

This species was found only in Tane Island (1968). Main characteristic is: ascospores with paired, non-gelatinous appendage at both ends and these appendages being at right angle to one another.

Ceriosporopsis halima Linder

Barghoorn & Linder, in Farlowia **1:** 409 (1944);
Tubaki, in Trans. Mycol. Soc. Japan **7:** 96 (1966)

This species is most common one and distributed widely in Japan. Main characteristics of this species are: 1) didymous ascospores with gelatinous, broad and elongate appendages at each end. 2) formation of perithecium on unsupplemented seawater agar (SW) medium; in this condition, appendage lacks completely differing from that produced under normal SW medium (Tubaki, 1966). 3) homothallic (Tubaki, 1966). 4) unable to utilize $NaNO_2$ (Tubaki, 1966). 5) undiluted SW is most favourable for the growth, indicating that the present species is a true marine fungi.

Shape of the perithecia are variable. Usually they are globose with centric or eccentric cylindrical necks, but they varied for globose to elongate or oblong elliptical and the neck also varied in number from single to two and often branched. Ascospore-appendages is mainly composed of the acid-polysaccharide substances (Tubaki, 1966), and these appendages may disappear as the spore germinate. The appendage also lacks in the case of ascospores developed on unsupplemented SW medium as described above. These unstable nature of the appendage formation indicate that the sharp definition of the species by the appendage character is unreasonable.

Corollospora maritima Werdermann

Notizl. Bot. Gard. Mus., Berlin-Dahlem **8:** 248 (1922);
Tubaki, in Trans. Mycol. Soc. Japan **7:** 79 (1966)

This species is one of the most common and widespread species like *Cer. halima* in the sea. Main characteristics are: 1) black, carbonaceous and globose perithecia with papilliform or very short cylindrical necks. 2) didymous ascospores with one terminal,

gelatinous appendages at each end and flagellum-like appendages around central septum. 3) undiluted SW is most favourable for the growth indicating that the present species is a true marine fungi. 6) addition of glucose, galactose, starch or cellulose to the SW stimulates the perithecial formation.

Occurrence of the present species on natural substrates can easily be recognized by their black and globose perithecia with a metallic luster. In addition to the immersed wood material which are common substrate for this species, sandy grains or other calcareous materials are also favourable for the production of the perithecia like *Cor. trifurcata* as described below though the vegetative hyphae are attached to nearly wood substratum. Perithecia under the test tube culture are found on the glass-walls of the tubes. From this reason, the present species is called as a arenicolous fungi together with *C. trifurcata.* As regards to the ascospore appendage, toluidine blue staining shows that the both terminal and lateral appendages are mainly composed of the acid-polysaccharide substances, but the terminal appendages are stained dark purple by Azur-A and the lateral ones remained unstained differing from the former (Tubaki, 1966).

Antennospora quadricornuta (Cribb et Cribb) Johnson

Johnson, in Jour. Elisha Mitchel Sci. Soc. **74:** 46 (1958);
Tubaki, in Publ. Seto Mar. Biol. Lab. **15:** 359 (1968)

This species was found only once in Tane Isl. (1958). Main characteristic is: ascospores with paired, non-gelatinous appendage at both ends and these appendages being at right angle to one another.

Corollospora trifurcata (Höhnk) Kohlmeyer

Kohlmeyer, in Ber. Deut. Bot. Ges. **75:** 126 (1962);
Tubaki, in Publ. Seto Mar. Biol. Lab. **15:** 359 (1968)

This species is distributed in Tottori and Hokkaido. Main characteristics are: 1) arenicolous habitat; regularly found on sandy grains or other calcareous materials. 2) black perithecia with finely echinulate wall and attaching to substrate by broad base. 3) ascospores with non-gelatinous three appendages at each end. 4) unable to utilize $NaNO_2$. 5) germinating ability is reduced markedly on an unsupplemented SW medium, but addition of yeast-extract stimulates it greatly. This species is unique in its special arenicolous character and the trifurcate appendage of the ascospores. Like *Cor. maritima,* the vegetative hyphae forming perithecia are attached to the wood substratum. As described above, germinating ability of the ascospores is nearly 60% under 0.1% yeast extract-SW while the ability increases to 80% or more as the concentration of the yeast extract increases to 1%. Arenicolous habitat is a most significant character ecologically (Kohlmeyer, 1966), however, no discussion has been presented on the significance of this affinity to sand or other calcareous materials. Neither of silicate-utilization nor acid-production was recognized among them.

Gnomonia longirostris Cribb et Cribb

Papers Univ. Queensland Dept. Bot. **3:** 101 (1956);
Tubaki, in Publ. Seto Mar. Biol. Lab. **15:** 360 (1968)

This species was found in Tane Isl. and Shirahama. Main characteristics are : 1) subhyaline or pale brown perithecia. 2) apical thickening of matured asci with deliquescent wall. 3) didymous ascospores without appendages.

During the study of marine fungi, such fungi having didymous ascospores without appendage are fairly common. In most cases, they can be assigned to *Gnomonia* or *Lignincola*. Because of uncertain generic differences, only the thick-walled apices of the asci is chosen as a critical characteristic on which to assign the fungus to *Gnomonia*. As described previously (1968), two collections were described under the name of *G. longirostris* because of the thick-walled apices of the immature asci though Kohlmyer (1961) reglected it to synonymy under *Lignincola laevis* (later, in 1964, Kohlmeyer treated *G. longirostris* as a distinct species.).

Leptosphaeria discors (Sacc. et Ellis) Sacc. et Ellis

Michelia **2:** 567 (1879); Tubaki, in Publ. Seto Mar. Biol. Lab. **15:** 361 (1968)

This species is distributed more widely than that described in the previous paper (1968) and is not uncommon on the immersed or drift bamboo materials, especially on the inner surface of them. Main characteristic is : 4–celled ascospores with hyaline end cells. On the inner surface of the bamboo materials, perithecia scattered as black and tiny spots in abundance. Neck is short and basal half of the perithecium is dark brown while upper half is usually paler or subhyaline.

Leptosphaeria orae-maris Linder

Barghoorn & Linder, in Farolwia **1:** 413 (1944);
Tubaki, in Publ. Seto Mar. Biol. Lab. **15:** 361 (1968)

This species is distributed only in Amakusa in my study and was found on the inner surface of the almost bamboo-materials collected. Main characteristic is: ascospores are at first two-celled, then become 4–celled.

Lignincola laevis Höhnk

Veröffentl. Inst. Meeresforsch. Bremerhaven **3:** 216 (1955);
Tubaki, in Publ. Seto Mar. Biol. Lab. **15:** 362 (1968)

This species was found in Amakusa (1968) but distributed more widely around Japan. Main characteristics are: 1) perithecia examined are almost hyaline or subhyaline and sometimes blackish. 2) thin-walled, persistent asci. 3) hyaline, didymous ascospores without appendages.

This species is very similar to foregoing species, *Gnomonia longirostris*, however, persistent asci and not-thickened ascus-apex are dependable nature to distinguish from the latter.

Remispora galerita Tubaki*

Publ. Seto Mar. Biol. Lab. **15:** 362 (1968)

This species was found in Amakusa, Kyushu. Main characteristics are: 1) pale yellow to pale fuscous, globose to subglobose perithecia. 2) didymous ascospores surrounded with gelationous, flexuous sheath. 3) appendages are flexuous sheath-like and skull-cap shape or lobed under staining by toluidine-blue characteristically, striated under staining by trypane-blue. Finely striate appendages attached to both ends of the ascospores are very characteristic and easily differentiated from other species especially under staining by trypane-blue.

Remispora maritima Linder

Barghoorn & Linder, in Farlowia **1:** 410 (1944);
Tubaki, in Publ. Seto Mar. Biol. Lab. **15:** 365 (1968)

Only one collection was made from balsa panel in Hamajima, Mihe, nearly a central part of Japan. The main characteristic is : didymous ascospores provided with a pair of hyaline, stout, tapering, divergent, fairly striate, gelatinous appendages at both ends. As described and illustrated by Johnson & Cavaliere (1963), there are no distinct, regular striation in the appendages and these natures can be observed more clearly under staining by toluidine-blue than under ordinal transmitted light.

Remispora ornata Johnson et Cavaliere

Nova Hedwigia **6:** 188 (1963); Tubaki, in Publ. Seto Mar. Biol. Lab. **15:** 364 (1963)

This species is rather common around Japan. The main characteristic is: ascospores provided with a single, hamate or cornute, flexuous, non-gelatinous appendages at each end, and 2–4 similar, gelatinous equatorial ones.

Remispora quadri-remis (Höhnk) Kohlmeyer

Kohlmyer, in Nova Hedwigia **2:** 332 (1960);
Tubaki, in Pub. Seto Mar. Biol. Lab. **15:** 365 (1968)

This species was found only on the panel of *Crptomeria japonica* in Moroyose, Tottori. The main characteristic is : didymous ascospores provided with a pair or 3–4 in number or semi-rigid, ventricose, gelatinous, divergent, recurved and tapering at each end. The appendages can hardly be detached under the ordinal transmitted light,

* After the present manuscript was submitted in this journal, *R. galerita* appeared to be identical with *Lentescospora submarina* Linder (personal communication from Dr. J. Kohlmeyer).

however, clearly differentiated under the toluidine-blue stain. The appendages are peculiar in these beautiful moustache-shape. This species is more widely distributed than described previously (1966), and has been found in Hamajima, Mihe and Shimoda, Izu.

Sphaerulina albispiculata Tubaki

Publ. Seto Mar. Biol. Lab. **15:** 366 (1968)

This species was found twice on the driftwood in Tane Island. Main characteristics are: 1) thick and white haired neck of the perithecia exposed by the erosion of the wood. 2) 6–celled, hyaline ascospores. Especially the first feature is unique and pure white, writing-brush like neck of the perithecia can easily examined even by the naked eye. Germination of the ascospores is also peculiar. They does not germinate on ordinal seawater or other common media, but can germinate freely on the 0.1% Na-lignosulforic acid SW media containing 0.1% of yeast extract.

Torpedospora radiata Meyers

Mycologia **49:** 496 (1957);
Tubaki, in Pub. Seto Mar. Biol. Lab. **15:** 367 (1968)

This species was found in different three places, Tottori and Shimoda. Main characteristic is: elongate clavate, or cylindrical, 4–8–celld ascospores which are provided with 3–4–gelatinous, acuminate, fragile appendages at one end. Under high power magnification, an appendage-bearing swelling of the ascospores is evident which was not described in the original description. Meyers (1957) reported ascocarp production on SW medium, but Johnson & Sparrow (1961) did not succeeded to produce perithecia by the North Carolina coastal strains on SW medium with the nutrients. In fact, in my work, the first and third mentioned strains with nearly 7–8–celled ascospores does not produce perithecia under culture, while all isolates of the second mentioned strains with 4-5-celled ascospores freely developed perithecia on the SW medium. Such perithecial production may depend to the strains. However, I considered that these differences are not sufficient to warrant separate species.

Fungi Imperfecti

Cirrenalia macrocephala (Kohlmeyer) Meyers et Moore

Amer. Jour. Bot. **47:** 347 (1960); Tubaki, in Trans. Mycol. Soc. Japan **7:** 75 (1966)

This species is one of the widely distributed marine fungi and is very common in the warmer area of Japan. Main characteristic is: 4–6–celled, recurved or bent, yellow brown aleuriospore-type conidia. This species is found most common on the coniferous plant immersed in the seawater.

Clavariopsis bulbosa Anastasiou

Mycologia **53:** 11 (1961); Tubaki, in Publ. Seto Mar. Biol. Lab. **15:** 368 (1968).

This species was found only in Shirahama in addition to Salton Sea of California (Anastasiou, 1961). Star-shaped conidia are characteristic like those of the fresh water fungus, *Clavariopsis*.

Culcitalna achraspora Meyers et Moore

Amer. Jour. Bot. **47:** 349 (1960); Tubaki, in Trans. Mycol. Soc. Japan **7:** 75 (1966)

This species is also one of the very common species. Main characteristic is: broad obpyriform, clavate or ovate, 3–5–celled fuscous, aleuriospore-type conidia.

Humicola alopallonella Meyers et Moore

Amer. Jour. Bot. **47:** 346 (1960); Tubaki, in Publ. Seto Mar. Biol. Lab. **15:** 369 (1968).

This species was found very common in Iwaya and is thought to be distributed more widely. Single celled, globose or elongate, dark colored aleuriospores are characteristic.

Monodictys pelagica (Johnson) Jones

Trans. Brit. Mycol. Soc. **46:** 138 (1963); Tubaki, in Publ. Seto Mar. Biol. Lab. **15:** 369 (1968).

This species was found only in Oshoro Bay. Muriform, obpyriform, opaque aleuriospores are characteristic.

Nia vibrissia Moore et Meyers (Pl. 1, A-C)

Mycologia **51:** 874 (1959)

Pycnidia superficial, scattered or gregarious, subhyaline to depressed subglobose, villose, coriaceous, 1.2–2.0 mm, at first pale yellow, then becoming orange or yellow orange; walls 10–20 (23) μ thick; external hyphae simple, thick walled, straight or curved, often slightly curled apically, up to 250 μ long, (3)4–5 μ in diam., hyaline. Conidiophores filiform, septate, irregularly branched, filling randomly inside of pycnidium. Conidia single, develop on sporogenous teech of conidiophore, one celled, ovoid, ellipsoid or long clavate with a acute end and a flat projection at base, (10) 11–14 (15) $\times$6.7–7.2 μ, with single terminal and three to five similar subterminal hyaline, non-gelatinous appendages; terminal appendages slender, flexible, attenuate, 20–43 μ long,

subterminal appendages radiate, 20–32 μ long.

Growth on SW slow, restrict, tough, with submerged hyphae in abundance, hyaline to pale yellow colored; light colored stroma-like structures developed on surface and few of them developed to orange pycnidia.

Hab. On the immersed test panel of *Fagus crenata*, immersed three months in Oshoro Bay, Hokkaido, Sept., 9, 1967.

Papulaspora halima Anastasiou

Nova Hedwigia **6:** 266 (1963); Tubaki, in Trans. Mycol. Soc. Japan **7:** 75 (1966)

This species was found in Shirahama in addition to Iwaya and Akkeshi, as described previously (1966), and is fairly common. Main characteristics are: 1) pale colored bulbils which scattered on the substrata. 2) brownish pigmentation in the medium under culture. The latter characteristic is peculiar because mostly marine fungi are dark colored with no pigmentation in the medium.

Zalerion maritima (Linder) Anastasiou

Canad. Jour. Bot. **41:** 1136 (1963);
Tubaki, in Trans. Mycol. Soc. Japan **7:** 76 (1966)

This species is more widely distributed in the coastal sea of Japan than that described previously (1966). Main characteristic is: pale-fuscous helicoid conidia which coiled in more than one plane. This identification of *Zalerion*-species depend to that described by Anastasiou (1963).

Zalerion varia Anastasiou

Canad. Jour. Bot. **41:** 1136 (1963);
Tubaki, in Publ. Seto Mar. Biol. Lab. **15:** 370 (1968)

This species was found only from two places, Tomo and Tane Island. Main characteristic is: irregular coiled many colled conidia.

2. Algicolous group

Together with the survey on the lignicolous fungi, attemps have been made to obtain fungi from the necrotic sea grass tissue. In May of 1967, debris or sandy beaches containing *Sargassum* sp. were collected at Shimoda, Izu Peninsula, and these pieces of *Sargassum* were incubated on sterilized filter paper in perti dishes. After three weeks at the room temperature, two kinds of blackish perithecia were found scattered on the filter paper around the algal pieces and also on *Sargassum* themselves. The studies on the perithecia and the ascogenous contents of these fungi have shown that they are species of *Lindra* Wilson (1956) and of *Corollospora* Wedermann (1922). The former

has characteristic structure of the ascospore in U- or S-shaped and multiseptated. The genus *Lindra* includes two species, namely *Lindra inflata* Wilson (type species, 1962) and *L. thalassiae* Orpurt et al (1964). The present fungus somewhat differs from the description of *L. thalassiae* given by Orpurt et al., but can be included in this species because such differences are not sufficient enough to make separate species. Three and four months later, in August and September of the same year, two strains of a deuteromycete species were isolated from *Hyponea* and *Zostera*, collected at Iwaya, Awaji Island. This species is *Varicosporina ramulosa* Meyers et Kohlmeyer.

Lindra thalassiae Orpurt, Meyers, Boral et Sims (Pl. 1, D-F)

Bull. Miami Sci. Gulf & Caribb. **14:** 406 (1964)

Perithecia scattered, subglobose or ovate, membranaceous, dark brown to black, 160–200 × 120–200 μ (excluding neck); necks papilliform or long cylindrical, 40–150 μ or more in length by 26–50 μ wide, often over 500 μ long under culture; aparaphysate; asci long cylindrical, 65–67 × 6–7 μ, early deliquescent. Ascospores filiform, multiseptate (15–26 septa in number), with slight constriction at septa, usually U- or S-shaped, tapering gradually, with or without minute appendages at one end, 220–350 (400) × 3–4(5) μ, hyaline. The venter wall structure varies in thickness. The wall, composed of homogenously thin walled cells with large lumina: Medusa-type (sensu Cavaliere, 1966).

Hab. On *Sargassum* sp., Shimoda, Izu Peninsula, May, 1967.

Isolation was made from the matured ascospores of the perithecia developed on the filter paper on which *Sargassum* was deposited.

Growth on SW rapid, composed of hyaline to pale olive mycelium, with moderate production of perithecia. Most dense perithecial development occurred in Laminarin-agar (pl. 1F), indicating the natural occurrense of this fungus on the phaeophytous algae as described later. Appendages of the ascospores were described by Orpurt et al as small, often inconspicuous conoid and slightly swollen, and, in the case of the present fungi, the appendages are usually inconspicuous and remained unstained by toluidine-blue.

Corollospora maritima Werdermann. Strain 154–12–1 (Pl. 2, A-B)

In addition to the already described lignicolous strains, the present isolate exhibited different morphological and physiological characteristics. This isolate was made from a frond of *Sargassum* sp. The perithecia developed in abundance on calcareous shell surface, sand grains or on surface of the frond. On SW medium, the isolate produces whitish mycelial growth in which abundant perithecia developed. Such superficial growth clearly different from that of the common isolates of *C. maritima* which develop dask colored mycelium. Comparative growth of 154–12–1 and a typical *C. maritima* was examined on four kinds of agar media: glucose (1%)-SW, laminarin-SW, carrageenin-SW and malt extract agar (pl. 2 A). A typical isolate developes perithecia

on the exposed glass surface of the petri-dish and within the agar. On the contrary, it is interesting that 154–12–1 regularly produces perithecia on or in the laminarin-SW most abundantly and fairly abundantly in the agar of the carrageenin-SW and on or in the usual SW medium; perithecia does not develop on the glass surface. The ascospores of 154–12–1 are similar to the common isolate, but are narrower than those described by Kohlmeyer (1964), Johnson & Sparrow (1961) or Cavaliere (1966), 30–36 × 3.8–4(4.5) μ (pl. 2 B). Perithecia also developed in abundance on the agar of cellolose-SW medium. However, it is not impossible to separate the present isolate from *C. maritima* because ascospore-variability is common among the marine Ascomycetes. The striking dissimilarities in growth and reporductive characteristics may suggest that many physiological groups may represent in the genus *Corollospora* as recently discussed by Meyers in two isolates of the genus (1968). It can be speculated that the present isolate is not a lignicolous but a algicolous because it assimilates well both laminarin and carrageenin with producing perithecia.

Varicosporina ramulosa Meyers et Kohlmeyer (Pl. 2, C-D)

Canad. Jour. Bot. **43:** 916 (1965)

Conidiophores simple or rarely branched, cylindrical, septate, short, 10–20 μ, 2.5–3 μ wide, hyaline; typically straight, but geniculated because of repeated growth. Conidia are of the aleuriospore-type, arise singly at ends of short conidiophores, and after the first conidium developed, the second one arises on new teeth-like apex of conidiophore pushing asideways the first one and the process repeates several times; each conidium detached readily. Conidia usually consists of one main axis, 35–66(75) μ long, 2–3 μ wide at base, 3–4 μ at apex, and of two side branches, 24–30 × 3.5–5 μ, multiguttulate, septate, with or without slight constriction at septa, hyaline; each side branch arises from main axis or first branch at almost right angle, so that a ramulose, irregularly staurospore is ultimately formed; often unbranched, 7–8–celled, long clavate conidia developed, measuring 72–90× 3.5–4.0 μ, hyaline.

Hab. On *Hypnea charoides*, Iwaya, Awaji Island, Aug. 1967; on fallen leaves of *Zostera marina*, Iwaya, Awaji Island, Sept., 1967.

Growth on SW spreading, velvety, powdery and white as conidia developed, showing concentric zones; reverse and agar dark olive green to almost black. On malt agar prepared by disitlled water, conidial production scanty.

3. Fungi in the sea mud

Since no systematic survey of the sea mud has yet been made in the present study, isolation of fungi from coastal mud-samples was carried out. Collection was made at coastal places, encompassing an inshore area of Hatsushima, Arita City, Wakayama Pref. where many industrial factories located. The sample sites, 22 in number, were selected to include a variety of shore environments. A total of 77 separate mud collections were

made using the Snapper Bottum Sampler*. The location of the collection areas and the depth were shown in fig. 2 and table 1 respectively; each site was located about 200–500 meters off shore. The mud samples were collected nearly once a month from June, 1967 to March, 1968 in a 50 ml polyethylene-jars with screw caps previously alcohol-sterilized. Each month, 5 to 12 samples were collected from the various sites. Immediately following collections, the jars were stored in the refrigirator (4–5°C) until the subsequent isolation in the laboratory. For the isolation, approximately 2.5 g of each wet mud was aseptically transferred to 5 ml of sterile seawater and 0.5 ml of the mud-suspension was

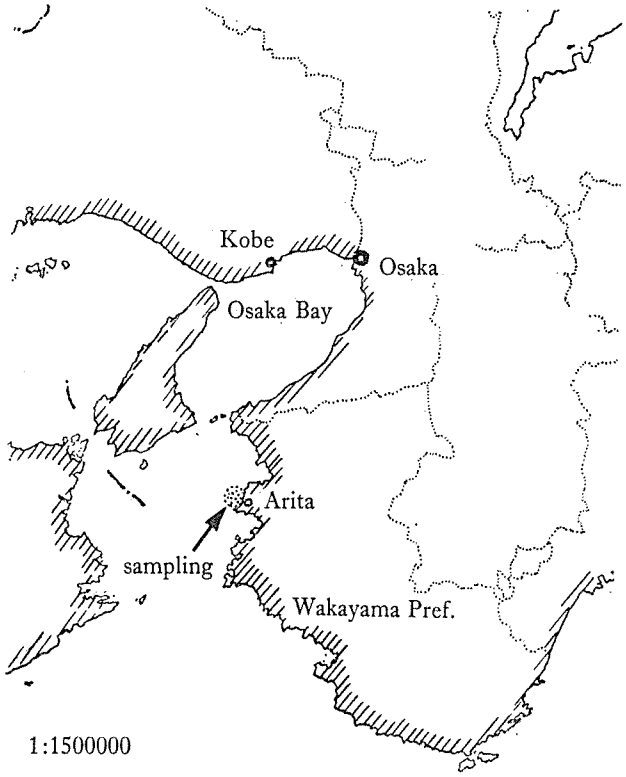

Fig. 2. Location of the collection areas.

diluted to 1:10. The inoculum comprised 0.3 ml of both suspension under the pour plate method. Because the lower population of the fungi was known in a previous examination, each mud sample of 0.3 g was smeared directly on an agar plate. All media used in the incubation and isolation procedures were prepared with seawater. During the isolation, bacterial growth was contained by the incorporation 50 γ of tetracycline per ml of the medium. Fungal colonies which developed on the surface of the agar plates within the 7-day incubation period at 24°C were counted and some fungi were identified directly from the plates. The results were shown in the table 1 and 2.

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* The collection was kindly made by the staff of the Hygienic Laboratory of Wakayama Prefecture to whom I owe many thanks.

Table 1. Number of colonies of the mud fungi developed on the agar plates.

Date of collection		No. of sites & depth (m) 1 (6)	2	3 (14)	4	5	6	7	8 (16)	9 (27)	10	11 (4.5)	14 (5)	15 (18)	16	18	19 (4)	22
June 8, 1967	S	0	–	0	–	–	–	1	0	0	–	2	–	–	1	1	0	–
	P																	
July 7, 〃	S	–	–	1	–	–	–	–	1	0	–	0	3	–	–	–	0	–
	P	–	–	1	–	–	–	–	2	1	–	1	2	–	–	–	–	1
Aug. 8, 〃	S	6	–	13	–	–	–	–	9	0	–	6	6	6	–	–	0	–
	P	4	–	15	–	–	–	–	6	3	–	4	9	1	–	–	0	–
Sept. 4, 〃	S	15	–	18	–	–	–	–	13	1	–	1	14	1	–	–	3	–
	P	22	–	0	–	–	–	–	0	0	–	6	0	4	–	–	2	–
Oct. 2, 〃	S	6	–	10	–	–	–	–	2	1	–	–	0	–	–	–	1	–
	P	6	–	16	–	–	–	–	7	1	–	–	0	–	–	–	4	1
Nov. 6, 〃	S	–	–	–	–	–	–	–	–	0	–	–	6	–	–	4	2	–
	P	–	–	–	–	–	–	–	–	3	–	–	0	–	–	6	3	–
Jan. 22, 1968	S	0	4	4	5	–	–	1	5	–	–	–	–	–	–	–	–	–
	P	0	5	4	6	–	–	7	0	–	–	–	–	–	–	–	–	–
Feb. 1, 〃	S	–	–	–	–	6	–	–	–	1	–	–	1	2	–	–	0	–
	P	–	–	–	–	2	–	–	–	0	–	–	3	2	–	–	0	–
Feb. 23, 〃	S	1	30**	0	–	0	0	0	2	2	–	1	0	–	–	1	0	–
	P	0	14	1	–	0	0	0	0	0	–	0	0	–	–	0	0	–
March 11, 〃	S	11	–	6	–	–	–	–	50	4	–	0	2	2	7	–	3	8
	P	7	–	6	–	–	–	–	3	2	–	3	0	2	3	–	1	3

S ··· direct smear; P··· pour plate; –··· no sample was obtained
**··· mostly of *Emericellopsis*-species

Table 2. Genera isolated from the sea mud

name	number of isolates	name	number of isolates
Phycomycetes			
Absidia	3	*Geotrichum*	10
Mucor	3	*Humicola*	1
Ascomycetes		*Monosporium*	1
Chaetomium	2	*Nigrospora*	2
Emericellopsis	25	*Pachybasium*	2
Talaromyces	3	*Paecilomyces*	32
Deuteromycetes		*Penicillium*	52
Sphaeropsidales	2	*Pestalotia*	1
Acremonium	1	*Sclerotium*	1
Aspergillus	32	*Trichoderma*	25
Cephalosporium	18	unidentified	12
Cladosporium	1	*Streptomyces*	2
Curvularia	1	total	236 strains
Fusarium	1		

As expected, many fungi known as non-marine were isolated and members of the Hyphales were common. Eventually, from 139 mud samples collected from the total number of 17 sites, species of fungi representing 22 genera were recovered (Table 2). The most striking result was the isolation of *Emericellopsis*-species from eight mud samples of six sites together with three species of *Talaromyces*.

Isolation of *Emericellopsis*: As already known to my knowledge, many marine Ascomycetes have been reported from different areas of the world and all of these true marine Ascomycetes are members of the Pyrenomycetes, the "perithecial fungi". Members of the Plectomycetes, the "cleistothecial fungi", also appear on marine habitats, but these fungi are not included in the true marine fungi by the sense of Meyers (1957) or Kohlmeyer (1964) though a clear separation of marine and limnic is nearly impossible.

The present isolations, typically of the Eurotiales of the Plectomycetes, develop numerous cleistothecia with a transparent peridium and the ascospores are one-celled, ellipsoid, dark walled and provided with longitudinal wings. Most of them have a *Cephalosporium*-state. They are eventually determined to be two species of the *Emericellopsis*. The total number of the *Emericellopsis*-isolates is 25 and they were found scattered in six stations. All isolates of *Emericellopsis* belong to the small-spored section of the genus (sensu Backus & Orpurt, 1961) and were separated into two species, namely *E. humicola* and probably *E. microspora*. It is still uncertain why such *Emericellopsis*-species which have been known as terrestrial originally, appeared on the marine mud in abundance. None of these species have been reported from the marine environments so far.

Emericellopsis humicola (Cain) Gilman: The isolates were obtained from the mud samples collected in February, September and October. In a February-collection, as many as 21 colonies developed in a agar plate. Ascocarps are (80)110–220 μ in diameter and ascospores are broadly ellipsoid to ovate, provided with delicate hyaline longitudinal wings, 5.0–6.2 $\times$2.5–3.5 μ, olive colored. Conidial state is the *Cephalosporium*-type. Growth on oatmeal agar fast growing, more or less folded, with tough mycelial mat, white to pinkish white to greyish white, eventually darker as a result of profuse development. On SW growth is moderate, somewhat restrict, with ascocarp-development in fairly abundance (pl. 2, E 1, 2).

Emericellopsis microspora Backus et Orpurt (?): Three isolates were obtained from the mud sample collected in January, February and March. Ascocarps are dark to almost black and characteristically small, 63–130 μ in diam., and ascospores are broadly ellipsoid to ovate, provided with three delicate longitudinal wings, 5.0–6.0 $\times$ 3.0–3.5μ. These isolates were compared with a typical strain of *E. microspora* IFO–8614 (Univ. Toronto, isolated by Udagawa & Cain) and were eventually determined to be included in or very closely related to the present species though there are some differences between them in the ascocarp dimension. Ascocarps of the typical *E. microspora* are apparently smaller (35–80 μ to my measurement) than those of the present isolates and somewhat

appreciable difference is present between the typical and the present isolates. However, no hitherto known species of *Emericellopsis* is assigned closely to the present isolates. Further taxonomical studies on this peculiar group of fungi will be done together with other terrestrial isolates in the near future (pl. 2, E 3, 4).

Talaromyces spiculisporus (Lehman) Benjamin: One strain was isolated from the mud sample collected in October. On malt agar, growth is moderate, thin, with loose aerial mycelium, cream to pale yellow, producing ascocarps in abundance. Ascocarps globose to oval, 300–400 μ or more in diameter, white to dull cream colored; ascospores are elliptical, spinulose, 3.0–3.7 $\times$ 2.0–2.8 μ. Conidial state is of the *Penicillium*-type: biverticillata-symmetrical.

Talaromyces ucrainicus (Panasenko) Udagawa: One starin was isolated from mud sample collected in March. On malt agar growth is rapid, with thin, loose-textured mycelial mat, white to bright yellow as the ascocarps develop in abundance. Ascocarps are globose, 100–400 μ in diam. usually, bright yellow; ascospores are elliptical, spinulose over entire surface, showing characteristic equatorial furrow with two ridges; the ridges are obliquely curved very often and sometimes are nearly transverse. Conidial state is less developed and of the *Penicillium*-type; biverticillata-symmetrical. The ridges of the ascospores are peculiar and can be differentiated from other species. The present species was originally described as *Penicillium ucrainicum* by Panasenko and was transferred to *Talaromyces* by Udagawa (1966).

Talaromyces vermiculatus (Dangeard) Benjamin: One strain was isolated from mud sample collcted in March. On malt agar growth is rapid, spreading with close-texured and floccose mycelium, yellow to orange yellow; reddish in reverse of the colony and in agar. Ascocarps developed in abundance, rather soft, surrounded by loose networks of pigmented hyphae, 150–400 μ in diameter; ascospores elliptical, conspicuously echinulate, 4.0–5.0 $\times$ 3.6 μ, slightly yellow. Conidial state is of the *Penicillium*-type: biverticillata symmetrical.

II. OCCURRENCE AND DISTRIBUTION IN JAPANESE MARINE LOCALITIES

Within the past four years many information has accumulated on the presense of the Japanese marine fungi, and the uncompleted distribution map of them was presented previously (Tubaki, 1968). The map presented here (fig. 3) is a continuation of the information on the occurrence of them. As generally known, the Japanese archipelago is one of the several island arcs that stretches north and south in the western pacific between the southern tip of Kamchataka peninsula in Siberia and southeast Asia. The Japanese coast line is noted for its length and variety; offshore waters are the meeting ground of warm currents, the Kuroshio, moving northward from equatorial areas and cold currents, the Oyashio, bearing southward from the polar area. In addition, an offshoot of the Kuroshio, the Tsushima current, makes its way through Tsushima strait into the Japan sea, where it parallels the Japanese coast. Thus, Japan has a latitudinal

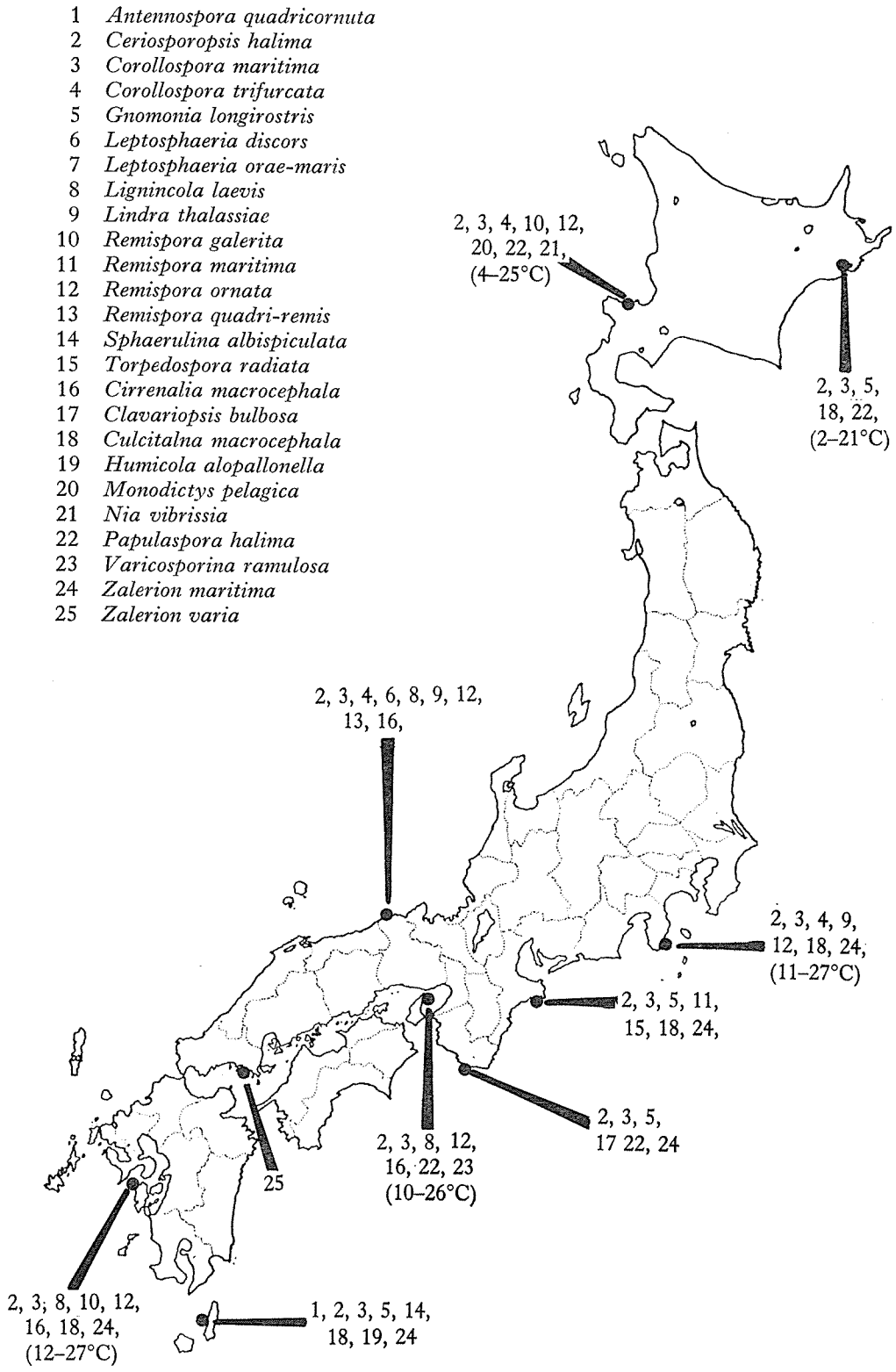


Fig. 3. Geographical distribution of marine fungi in Japan.

span of 15°, and, therefore, there are significant regional differences in temperature between the extremes of subtropical Kyushu district and continental Hokkaido. Surrounding oceanic bodies exert a modifying influence on seasonal temperatures of the coastal seawater. Maximum and minimum temperatures of the seawater roughly estimated of some stations where the fungi were collected in this study are shown in the fig. 3.

III. PHYSIOLOGY

1. Preliminary survey on the polysaccharide utilization

As described by many investigators, the marine fungi are adopted to the sea physiologically. The lignicolous marine fungi developed on wood or fibers have cellulolytic activities. Their activities have easily been demonstrated by microscopic or enzymatic observations and the type of wood destruction was called "soft rot" (Kohlmeyer, 1963). On the other hand, little was known about the physiological adaptation of the algicolous and foliicolous marine fungi to the sea. Although some attemps have been made (Meyers & Hoyo, 1965), investigations which hitherto reported on such groups of the marine fungi have not been sufficiently in detail or extensive to support claims of correlation between these fungi growing on seawater algae or the herbaceous flowering plant, Angiosperma, and nutritional activities. Therefore the present investigation provides prerequisite information for determining the existence and the nature of the relationships between the environments and nutritional activities of the algicolous and the foliicolous marine fungi obtained.

The following fungi were employed as members of the algicolous and the foliicolous fungi: *Dendryphiella arenaria* (S–43–1); *Varicosporina ramulosa* (IBA; 160–1); *Lindra thalassiae* (154–12–2). *Lindra thalassiae* is known to develop perithecia on gas-sterilized leaves of *Thalassia* but failed to reproduce on balsa wood (Meyers, 1965). Leaves of the host plant, *Thalassia*, may be considered to supply necessary nutrients for the fungus under natural condition. Although chemical components of the *Thalassia* are not clearly known, in the case of the marine algae, three kinds of polysaccharide were rather common and served at present as carbon sources for the marine algicolous and foliicolous fungi. Polysaccharides of the algae are generally considered to be chemical components of the cell wall and are used by the algae as their structural elements rather than cellulose. These algal polysaccharides make up the exterior wall and cover all extension surface of the plants causing them to have a slippery, mucilaginous feel. Among these substances, following three compounds are famous: laminarin as non cell-wall constituents, carrageenin and alginic acid as principally cell-wall constituents. Laminarin, a water soluble polymer of D-glucose, is found in the greatest amount in the frond of the *Laminaria* and is hydrolysed by the enzyme to glucose. Carrageenin, a sulfated polysaccharide, constitutes about 80% of the dry weight of *Chondrus crispus* (Irish moss) and is the principal cell-wall structural element.

Alginic acid appear, probably as the calcium and magnesium salts, in the cell-wall of most brown algae.

1% of each one of the following polysaccharides is added to the 0.1% yeast extract SW medium: Laminarin (K & K Lab.), Carrageenin (Pence Gel Co.), Na-alginate (Wako Pure Chem. Ind.). As a controlled study, 1% each of glucose and celluose powders were added to the basal medium. The growth of the fungi was measured by the radial growth on the surface of the media after 14 days at 24°C. The result was shown in the table 3.

Table 3. Utilization of polysaccharides

species \ compounds	laminarin	carrageenin	Na-alginate	cellulose	SW
Dendryphiella arenaria	+++	+	+	±	+++
Varicosporina ramulosa	+++*	++	±	+	++
Lindra thalassiae	+++	+	±	++	+++

Growth was estmated by— (no growth), ± (scanty), + (moderate), ++ (good) and +++ (luxuriant).

From the above table, it is evident that laminarin seems adequate as a carbon source comparing favourably with glucose except for four species indicated by* in which the growth was slightly reduced when the Zeiss-filtered laminarin was added. Laminarin can be utilized not only for the algicolous group but those of the lignicolous group probably because of its simple repeating polymer unit of a single sugar, D-glucose. Carrageenin is not favourable as laminarin for most species but is satisfactory as nearly the same degree as glucose or it helped the growth less than did glucose. Extensive enzymatic activity of the marine fungi to decompose the polysaccharides should be revealed in future.

2. Influence of vitamin on the growth

The following experiment was carried out in order to examine the influence of the vitamin on the radial growth of the marine fungi. Influence of the vitamin on the growth was estimated by the following method. Vitamin-solution** was substituted for the yeast extract, and as a control, vitamin was completely ommited dealing with influence

** Vitamin solution: Biotin 2 γ, Clacium pantothenate 400 γ, Folic acid 2 γ, Inositol 2000 γ, Niacin 400 γ, p-aminobenzoic acid 200 γ, Pyridoxine hydrochloride 400 γ, Riboflavin 200 γ, Thiamin hydrochloride 400 γ, Cholin 1000 γ, water 10 ml. 0.1 ml of this solution was added to 10 ml of the medium.

of it on the growth.

For the test, 38 strains of the marine fungi used in the polysaccharide utilization procedure were also employed. As a result, the growth was reduced markedly in the following species: *Clavariopsis bulbosa*, *Cirr. macrocephala*, *Culcitalna achraspora*, *Humicola alopallonella*, *Monodictys pelagica*, *Zalerion maritima*, *Z. varia*, *Antennospora quadricornuta*, *Ceriosporopsis halima*, *Corollospora maritima*, *Leptosphaeria discors*, *L. orae-maris*, *Lignincola laevis*, *Remispora galerita*, *R. maritima*, *R. ornata*, *R. quadri-remis*, *Lindra thalassiae* and *Torpedospora radiata*. Further examination by the Burkholder's method on influence of each vitamin on the growth of these species showed that the majority of them require external supply of thiamin. Because many species were affected by the presence of thiamin in the medium on the resultant vegetative growth, these thiamin-deficient marine fungi were tested for thiamin or thiamin-moiety requirement to know whether they differ in their ability to utilize or synthesize the moieties of thiamin. As generally known, organisms may be placed in the following four groups according to their thiamin requirement: (1) those fungi which require the intact molecule of thiamin, (2) those fungi which are able to synthesize thiamin when furnished with a mixture of the two thiamin-moieties, (3) those fungi which are able to synthesize the thiazol moiety and combine it with the addition of the pyrimidine moiety to make thiamin, (4) those fungi which are able to synthesize the pyrimidine moiety and complete the synthesis of thiamin when furnished with the thiazol moiety. 21 species above which were investigated to be thiamin-deficients were grown on the Czapek solution agar medium to which 100 γ/ml each of the thiazol moiety (4–methyl–5–β-hydroxy ethylthiazol), the pyrimidine moiety (2–methyl–4–amino–5–methylpyrimidine) and thiamin hydrochloride had been added. Inoculum was made by pre-culturing of these species which succeed-

Table 4. Response to thiamin and its moieties.

species \ substances	thiamin	thiazol	pyrimidine	thiazol + pyrimidine	0	*
Ant. quadricornuta	++	—	+	++	—	3
Cer. halima	++	—	+	++	—	3
Cor. trifurcata	++	—	+	++	—	3
Lept. oraemaris	++	—	+	++	—	3
Rem. maritima	++	—	+	++	—	3
Rem. quadri-remis	++	—	+	++	—	3
Cirr. macrocephala	++	—	+	++	—	3
Clav. bulbosa	++	—	+	++	—	3
Hum. alopallonella	++	—	+	++	—	3
Mon. pelagica	++	—	+	++	—	3
Pap. halima	++	—	+	++	—	3
Zal. maritima	++	—	+	++	—	3
Zal. varia	++	—	+	++	—	3

* Grouping of the fungi according to their thiamin requirements.

ed two times in a vitamin-free Czapek solution. After the growth at 24°C for three weeks, the radial growth was examined. The data are collected in the table 4.

From the table, it is assumed that the most of thiamin-deficient marine fungi were included in the group (3) previously described, and none of the group (1), (2) and (4) was found in the present study. *Gnomonia longirostris*, *Remispora galerita*, *R. ornata*, *Sphaerulina albispiculata*, *Torpedospora radiata*, *Lindra thalassiae* and *Nia vibrissia* are uncertain in the grouping. Probably some other supplies are needed for the growth.

It is interesting to know that every species requiring the thiamin moiety is restricted to those that are able to synthesize only the thiazol moiety. The reason is not clear at present.

Table 5. Growth response to the salts

species \ salts	NaCl (%)					KCl (%)					$MgCl_2$ (%)				
	2	3	4	5	7	2	3	4	5	7	2	3	4	5	7
Ant. quadricornuta	###	###	##	+	±	###	###	##	+	—	###	###	###	###	##
Cer. halima	###	##	##	+	—	###	###	##	+	±	###	###	###	###	##
Cor. maritima	##	##	##	+	—	###	###	###	###	###	###	###	###	###	###
Cor. trifurcata	###	##	##	+	—	###	###	###	##	+	###	###	###	##	##
G. longirostirs	###	##	##	+	—	###	###	##	##	—	###	###	###	###	###
Lept. discors	###	###	###	##	+	###	###	###	##	##	###	###	###	###	##
Lept. orae-maris	###	###	###	##	##	###	###	###	###	##	###	###	###	###	###
Lig. laevis	##	##	+	—	—	+	+	+	±	—	###	###	###	##	+
Lind. thalassiae	+	±	—	—	—	###	###	###	##	+	###	###	###	##	##
Rem. galerita	##	##	+	+	—	###	###	##	##	+	###	###	###	###	###
Rem. maritima	###	##	+	±	—	###	##	##	+	±	###	###	###	###	##
Rem. ornata	+	—	—	—	—	+	—	—	—	—	###	###	##	+	—
Rem. quadri-remis	##	##	+	—	—	###	##	##	—	—	###	###	###	###	##
Sph. albispiculata	##	+	—	—	—	##	+	+	—	—	###	###	##	+	+
Torp. radiata	###	###	###	##	+	###	###	##	##	+	###	###	##	##	##
Cirr. macrocephala	##	##	+	±	—	###	##	##	±	—	###	###	###	##	##
Clav. bulbosa	###	###	###	##	+	###	###	##	##	##	###	###	###	##	##
Cul. achraspora	###	##	+	+	—	###	###	##	##	+	###	###	###	##	##
Dend. salina	###	###	###	###	###	###	###	###	###	###	###	###	###	###	###
Hum. alopallonella	###	##	##	+	+	###	##	##	##	+	###	###	###	##	##
Mon. pelagica	###	##	##	+	—	###	###	###	+	+	###	###	###	##	##
Nia vibrissia	###	###	##	+	±	###	###	###	##	+	###	###	###	##	##
Pap. halima	##	##	+	+	—	###	###	##	##	+	###	###	###	##	##
Varic. ramulosa	###	##	##	+	±	###	###	###	###	##	###	###	###	##	##
Zal. maritima	##	##	##	+	+	###	###	##	##	##	###	###	###	##	##
Zal. varia	##	##	+	+	—	###	##	##	##	—	###	###	###	###	##
Emer. humicola	###	###	###	###	##	###	###	###	###	###	###	###	###	###	###
Emer. microspora	###	###	###	###	##	###	###	###	###	##	###	###	###	###	###

3. Effect of inorganic macroelements to the growth

Fungus growth responses to seawater inorganic macroelements were reported by Vishiniac (1955), Goldstein (1962) and Sguros & Simms (1964) on marine Myxomycetes, Phycomyeetes and Ascomycetes & Deuteromycetes respectively. Three of myxomycete were obligately marine requiring NaCl and KCl as well as Mg or Ca, wheres the pigmented phycomycete irrplaceably required NaCl. Sguros reported that only K and Ca appeared nontoxic at seawater concentrations, but Na antagosism by Mg presented. In the case of marine bacteria, effect of various cations was shown recently by Stanley & Morita (1968): the order of effectiveness of cations in restoring the normal maximal growth temperature, when added to dilute seawater, was $Na^+ > Li^+ > Mg^{++} > K^+ > Rb^+ > NH_4^+$.

In the present study, a response of the growth of 29 marine fungi to changing salinity with NaCl, KCl and $MgCl_2$ were investigated. These salts were added to the basal medium to determine whether any of them inhibit growth at the concentrations of 2 to 7%. The basal medium was composed of glucose 30 g; peptone 1 g; $MgSO_4$ 7aq. 0.5 g; $FeSO_4$ 7 aq. 0.01 g; K_2HPO_4 1 g; yeast-extract 0.5 g; agar 15 g; distilled water to a total volume of 1,000 ml. The pH was adjasted to 7.0 with KOH. Growth was estimated by diameter of the colonies developed at 25°C for 14 days. The result was shown in table 5.

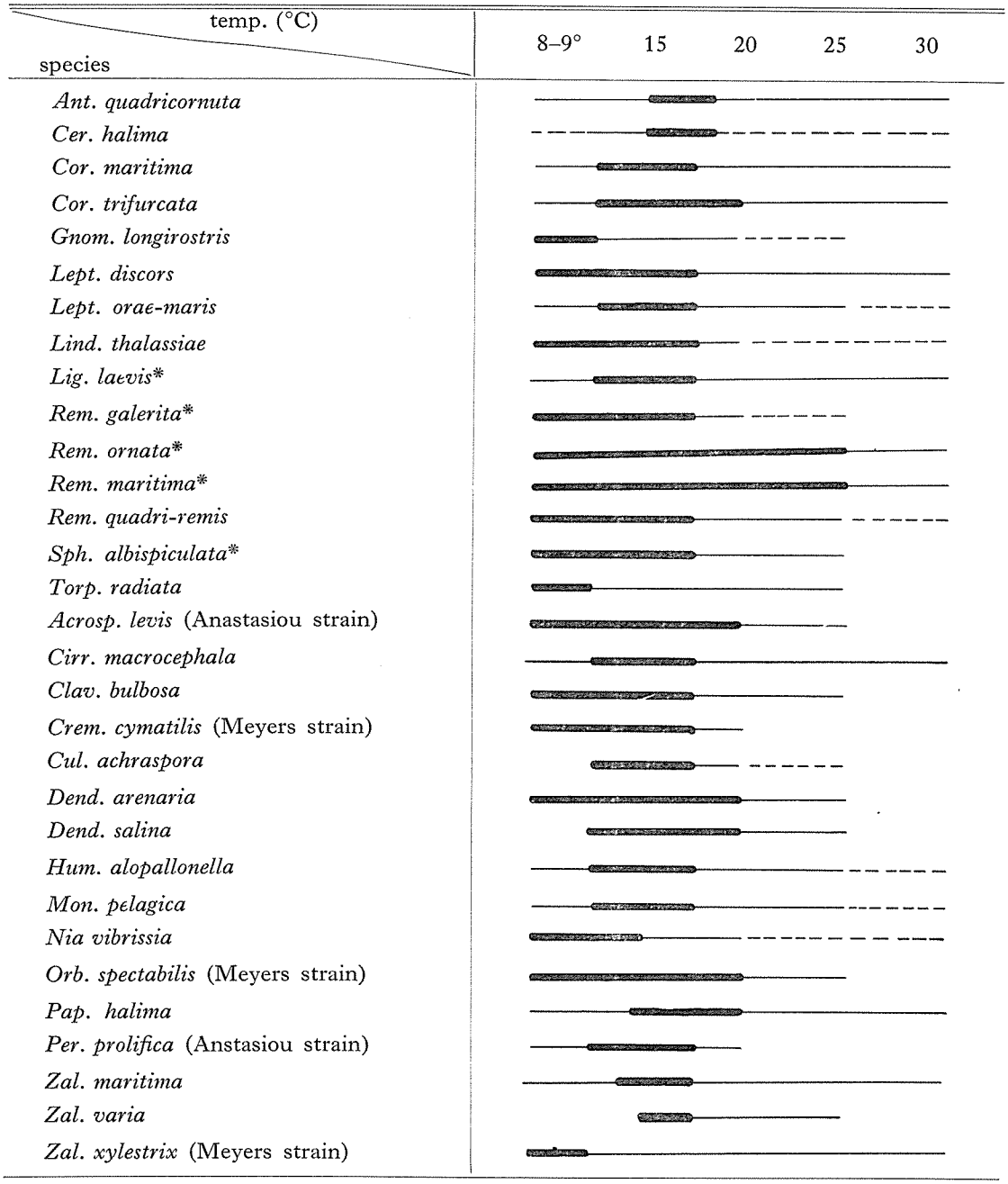
From the above table, it appears that growth of the marine fungi at various concentrations of NaCl are less than those of each concentrations of KCl or $MgCl_2$. Though NaCl is an essential salt for the marine fungi, the growth is prevented in the medium containing only NaCl as the sole source of mineral salt and the mixtures of all salts may be needed for promoting the marine fungi as in the case of the marine bacteria (Hidaka & Sakai, 1968). In contrast to that the luxuriant growth occurred at 2–3% of NaCl in most species and was prevented at 5–7%, the growth was not influenced by $MgCl_2$ even at the concentrations of 4–5%, sometimes 7%.

4. Effects of temperature on the growth

Because temperature has a marked effect on the fungus growth, Barghoorn (Barghoorn & Linder, 1944) investigated extensively on temperate tolerance of the marine fungi and propageted that the optimum temperature for the growth is ranged from 22 to 25°C in many ones. Four years later, Ritchie has shown the combined effects of temperature and salinity. In the case of marine bacteria, temperature in which the maximal growth occur was found to be dependent on the salinity of the growth medium (Stanley & Morita, 1968).

In the present study, optimum temperature for the radial growth was investigated using 31 species. Temperature tests were run at 8–9, 15, 20, 25 and 30°C on the seawater agar medium with 0.1% yeast extract. After inoculation, two sets of the agar plates were maintained at the above mentioned five temperatures. The grwoth was examined for three weeks and the experiment repeated twice. Result was shown in the

Table 6. Growth response to the temperature



– – –…very weak growth; ——…moderate growth; ——…luxuriant radial growth

table 6. Optimum radial growth of each fungi is indicated by a thick black line.

From the above table, it should be emphasized that all fungi indicated by the sign of * were found from the rather warmer area of Japan, Kagoshima, Amakusa, Shirahama, Mihe, Tane Island and Seto. Generally the optimum temperature for the radial growth of the marine fungi are not specielly lower than those of the terrestrial fungi.

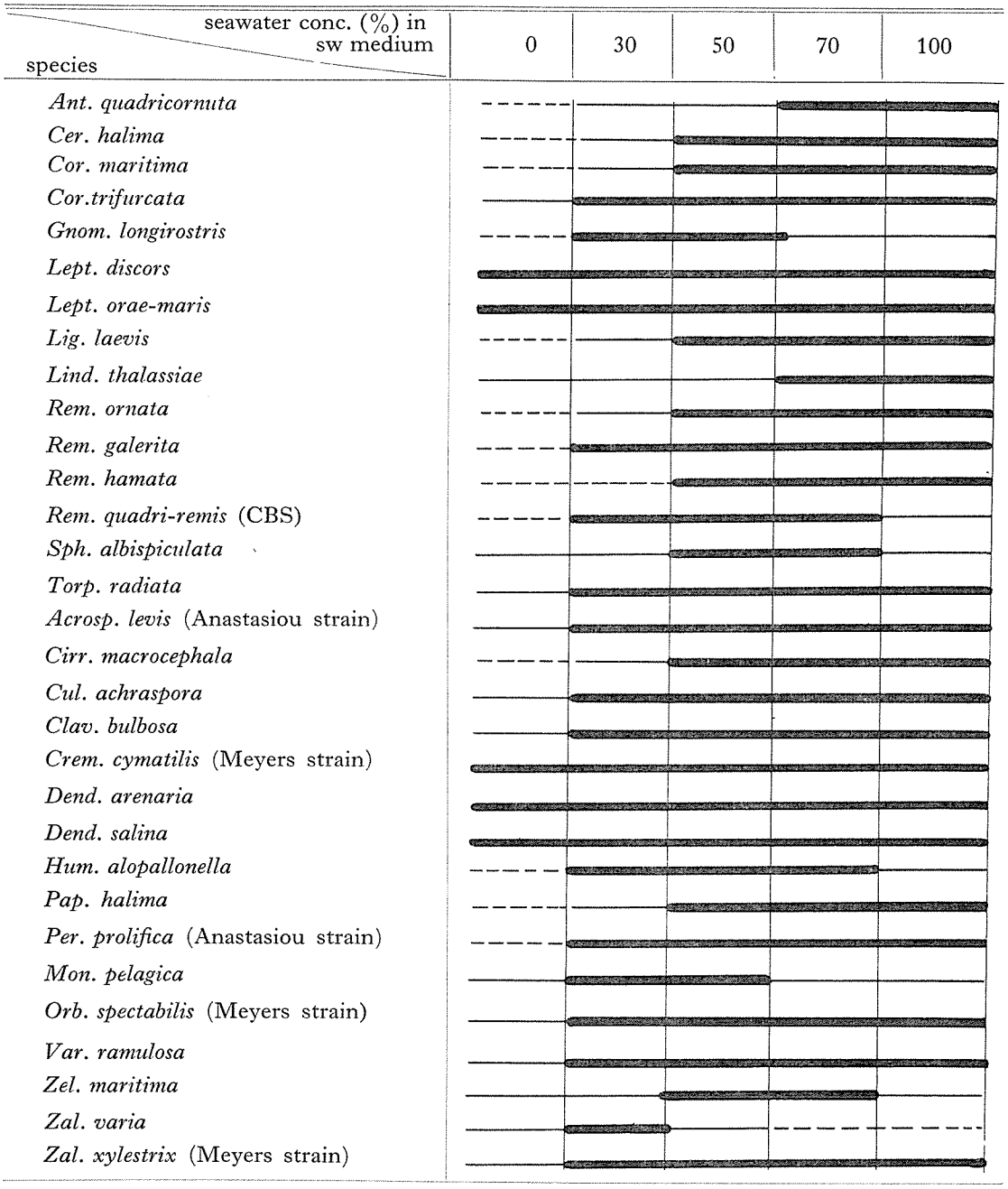
5. Experimental aspects of marine fungi

Physiological adaptation of the marine fungi to the life in the sea can generally be summarised as follows: A) better growth and reproduction on substrata with normal seawater concentrations of salts than those with fresh water, and B) distinct adaptation to the alkaline habitat in terms of germinating ability of spores and growth. Many reports have been made on the first and the same has been discussed by Barghoorn & Linder, Gustaffson & Fries, Meyers & Reynolds, Nicot, Ritchie, Gold, Kohlmeyer and Meyers. In some cases, the best growth was obtained only on the medium made up with seawater, while, in other cases, similar growth was obtained on the media with distilled water. These results seem to be contradictory at the first glance, but they are true from the view point of marine fungus distribution. Delimitation of the marine fungi is discussed by many scientists such as Meyers (1957), Kohlmeyer (1964), etc., but any definition of a "marine" should be flexible as claimed by Meyers. At this time, it should be recognized that such delimiation of the marine fungi is influenced greatly by the condition of the sea itself. What is the sea where the marine fungi are living, then? The "sea" that we call in the study of marine fungi is not restricted to the ocean but it ranges from a brackish water sector of the estuary to the ocean; in other words, from waters having a salinity less than 15‰ to waters of a "normal" seawater salinity. From the result shown by Gold (1959), five species of Deuteromycetes and eighteen of Ascomycetes, collected in an estuary, were divided into three groups with regard to salinity; namely, 1) fresh water fungi, represented by *Cer. hamata*, etc. 2) sea water fungi, represented by *Cer. halima* 3) fresh, brackish and seawater fungi, represented by *Cer. cambrensis*. Naturally such salinity-tolerance of the fungi is greatly influenced by water temperature, but it should be noted that some fungi, *Leptosphaeria discors*, *Lignincola laevis* and *Torpedospora radiata*, for eample, are found both in the fresh water and seawater sector of an estuary.

Therefore the first point, described previously as (A), must be re-described as "better growth and reproductive occurrence on substrates with wide range of salinity than those with fresh water, or growth of nearly the same degree on substrate with salinity of sea water and with fresh water". For the purpose to confirm the above definition, the salinity response of 31 marine species was investigated under culture. The result shown in table 7 can be explained to some extent by saying that the four groups above show a distributional propensity for the following four categories and the fungi tested can be placed into one of the four groups.

1) Seawater fungi, usually described as "marine", comprise the first group ..... *Antennospora quadricornuta, Ceriosporopsis halima, Corollospora maritima, Lignincola laevis, Remispora ornata, R. hamata, Cirrenalia macrocephala* and *Papulaspora halima*. Exceptionally, *A. quadricornuta* exhibited better reproduction at 70% seawater medium; *R. ornata* and *Cirr. macrocephala* exhibited better growth at 50–70%. Although those of this group show a fairly broad salt tolerance, notable characteristic of them is a poor growth in the 30% seawater.

Table 7. Growth response to the seawater concentration



– – –…very weak growth, ——…moderate growth; ——…luxuriant growth

2) Brackish and seawater fungi, also called as "marine", comprises the second group ..... *Corollospora trifurcata, Remispora galerita, Torpedospora radiata, Acrospeira levis, Culcitalna achraspora, Clavariopsis bulbosa, Periconia prolifera* and *Zalerion xylestrix*. Among this group, *Torp. radiata, Acr. levis, Cul. achraspora, Clav. bulbosa* and *Per. prolifica* exhibited poor but distinct growth at 0% seawater agar medium.

3) Brackish water fungi or those found in seawater sectors of estuary, usually

described as "coastal", comprises the third group ..... *Gnomonia longirostris, Remispora quadri-remis, Sphaerulina albispiculata, Humicola alopallonella, Monodictys pelagica, Zalerion maritima* and *Z. varia.*

4) Fresh, brackish and seawater fungi, comprises the fourth group ..... *Leptosphaeria discors, L. orae-maris* and *Cremasteria cymatilis.*

The result above approaches fairly closely to the experiment reported by Gold (1959), especially in the case of *Cer. halima* and others. However, as already pointed out by some scientists (Gold, 1959; Ritchie, 1954), distributional patterns are affected by temperature of the sea.

V. DISCUSSION AND CONCLUSION

The first comprehensive work dealing with higher marine fungi is thought to be started by Linder (in Barghoorn & Linder, 1944). Since then, a considerable interest has been paid to marine fungi as well as to many other adjacent fields in seawater. The most characteristic aspect on this recently progressed field of mycology is considered to be that the taxonomy of these marine inhabiting fungi has been investigated closely, followed only by or even it runs parallel to, the physiological and ecological studies on them, differing from the case of terrestrial fungi in which the taxonomical studies started much earlier. From the literatures and also from the results done in the present study, it appeared that the following four aspects have received considerable emphasis.

Taxonomically the majority of marine Ascomycetes are members of the Pyrenomycetes. To explain this, it should be necessary to make the criteria in the origin of the Pyrenomycetes, if the Ascomycetes are considered to be derived from Floridean ancestors. Denison & Carroll said in their creative hypothesis that the ancestral marine Ascomycetes was a pyrenomycete. As they claimed, the perithecia of most marine Pyrenomycetes are dark colored and membranaceous to carbonaceous; monoecism was found in two most common species, *Ceriosporopsis halima* and *Corollospora maritima* (Tubaki, 1966); ascus-wall is mostly deliquescent; conidial states lacked in all cases as discussed below. If their hypothesis had been correct and the marine Pyrenomycetes is an ancestor of the Ascomycetes, a phylogenetical arrangement from the marine Ascomycetes to the fresh water's group or directly to the terrestrial group should be drawn; but no satisfactroy answer is provided in the field.

Next to these species of the Pyrenomycetes, a few restricted species of the Eurotiales were often found in the maritime environments as described previously. They are not true marine fungi but considered to be the estuary fungi: *Heleococcum* (Tubaki, 1967), *Emericellopsis, Talaromyces,* etc. It is interesting from the veiw point of phylogeny that these fungi can survive and develop both in the seawater and in the terrestrial condition.

Not only the perithecia of the marine Ascomycetes but mycelial growth of the Fungi Imperfecti is dark colored in most species.

Exceptional fungi are *Sphaerulina albispiculata, Clavariopsis bulbosa, Papulaspora*

halima, Nia vibrissia and *Varicosporina ramulosa.* Two ascomycetes in this study possessing light colored ascocarps are *Heleococcum japonense* and *Emericellopsis,* all of which are considered to be brackish water fungi, not true marine fungi. To resolve this problem, fundamental biochemical activities of these dark colored pigment should be made more clear.

The marine Ascomycetes are distinguished by a paucity of conidia.

As described by Johnson & Sparrow (1961) and later by Denison & Carroll (1966), the marine ascomycetous fungi almost lack the conidial states. In fact, this problem is also unsolved in the present author's knowledge. In a report of the author (1958), a number of examples of the relationships between the perfect and imperfect states were described in the case of terrestrial pyrenomycetes. In the case of fresh water pyrenomycetes, two connections were made only by Ranzoni (1956) and Webster (1959) between *Nectria* and their imperfect states, *Flagellospora* and *Heliscus.* Other connections in fresh water fungi were found in two cases of discomycetes, *Mollisia* vers. *Anguillospora* (Webster, 1961) and *Hymenoscyphus* vers. *Varicosporium* (Tubaki, 1966). On the contrary, in the case of marine pyrenomycetes, nothing is known about such a connection. Some undescribed fundamental difference is believed to be present between asexual states of the marine fungi and of the terrestrial fungi. Denison & Carroll described that the primitive ascomycetes probably had either poorly developed conidial states or lacked them altogether. But what is the meaning of the "poor"? As described already (1958), the progress of the method of conidial development does not always means the evolution of fungi themselves. Often imperfect states showing a primitive conidial development can be found in fairly well differentiated fungi of higher ascomycetes, the members of the Pezizales, for instance. Progress in the method of conidial development in the sense of ontogeny does not parallel to differentiation of the gross morphology of the conidial apparatus. It can be speculated that ascospores of the marine ascomycetes may act both as that of sexual reproduction and dispersal with the aid of appendages. In the case of terrestrial ascomycetes, it is known that no fungi having ascospore-appendages develop a conidial state furthermore. This problem is a significant one in the case of marine fungi so far as a correlation between taxonomy and ecology is concerned.

Conidial types of the marine Fungi Imperfecti are characteristically unifirm.

As usually known, various conidial types have been found among the terrestrial fungi. They are the types of blastospore, radulaspore, phialospore, aleuriospore, porospore, arthrospore, etc. In the case of the marine Fungi Imperfecti, especially of Hyphomycetes, the conidia are mostly restricted to the aleuriospore-type. Conidia of *Cirrenalia, Clavariopsis, Cremasteria, Culcitalna, Humicola, Monodictys, Orbimyces, Varicosporina* and *Zalerion* arise solitarily as a blown-out end of a hyphal tip or as a lateral protrusion, which is cut off by septum. Exceptions are those of *Dendryphiella* and *Nia.* Conidia of the former, though the fungus is not a true marine fungus, are

of the radulaspore-type, and those of the latter are of the pycnidiospore. No conidial type of the blastospore, phialospore, radulaspore, porospore or arthrospore has ever been found in those of the true marine fungi. To my knowledge, the single aleuriospore-formation is the most significant in the conidial state of the Gymnoascaceae (e.g. *Arachniotus*, *Arthroderma*, *Gymnoascus*, *Nannizia*, *Shanorella*, etc.); ascocarps are cleistothecial and non-stromatic. In the case of the perithecia-forming typical pyrenomycetes, such imperfect state having the solitary aleuriospore-type conidia is scanty; *Eriosphaeria aggregata* Müller et Munk (conid. st.: *Sporidesmium scutellare* Berk. et Br.) is one of the examples. Consequently, the author believes that the marine Pyrenomycetes and the marine Fungi Imperfecti which have the aleuriospore-type conidial state are quite different in their evolutionary line.

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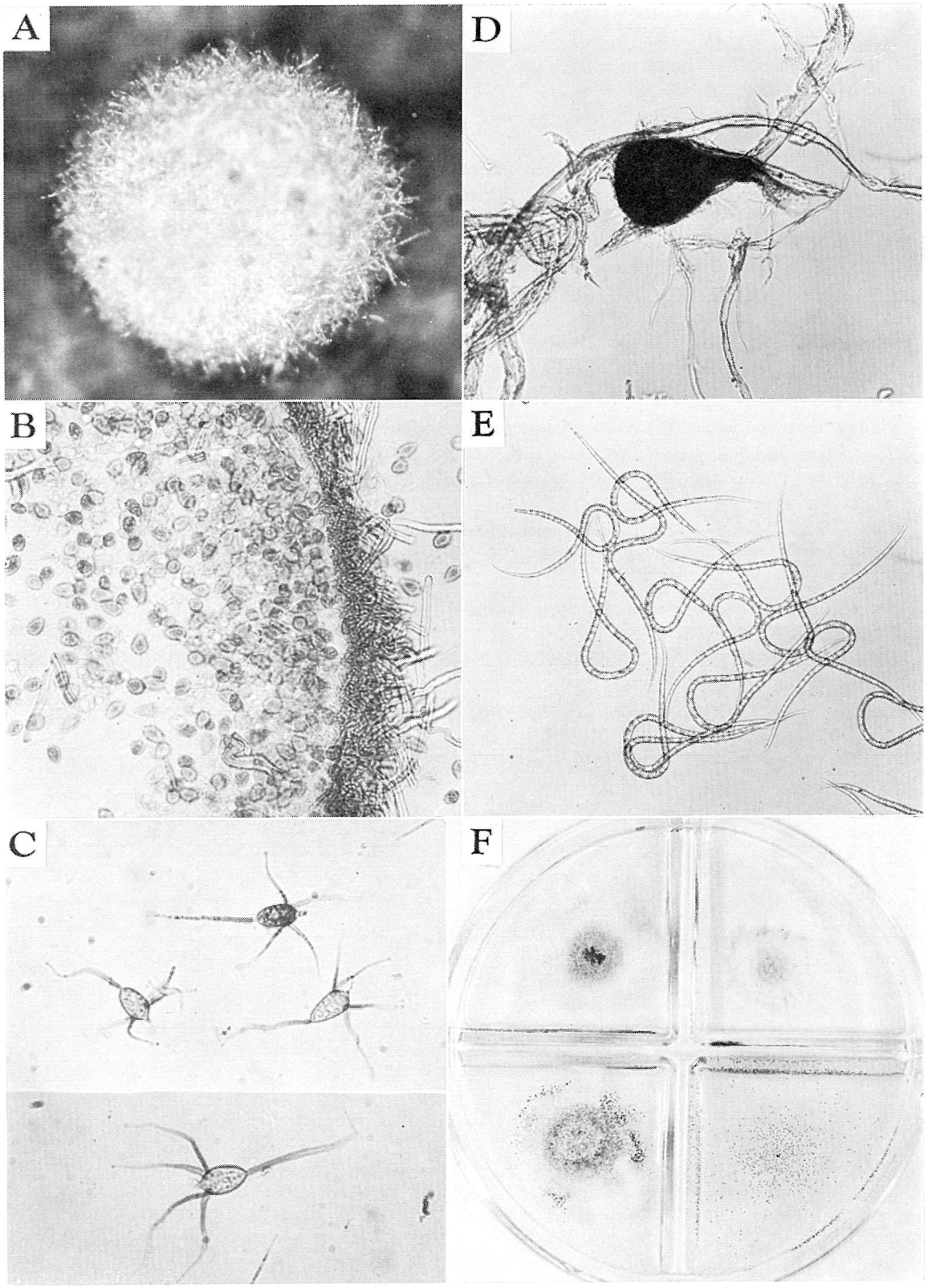

Plate 1. A–C. *Nia vibrissia* A. Pycnidium on the panel (×38). B. Section of pycnidium (×250). C. Conidia (×400). D–F. *Lindra thalassiae*. D. Perithecium (×100). E. Ascospores (×100). F. Comparative growth on different media (×0.7). Upper left, SW; upper right, malt agar; lower left, laminarin agar; lower right, carrageenin agar.

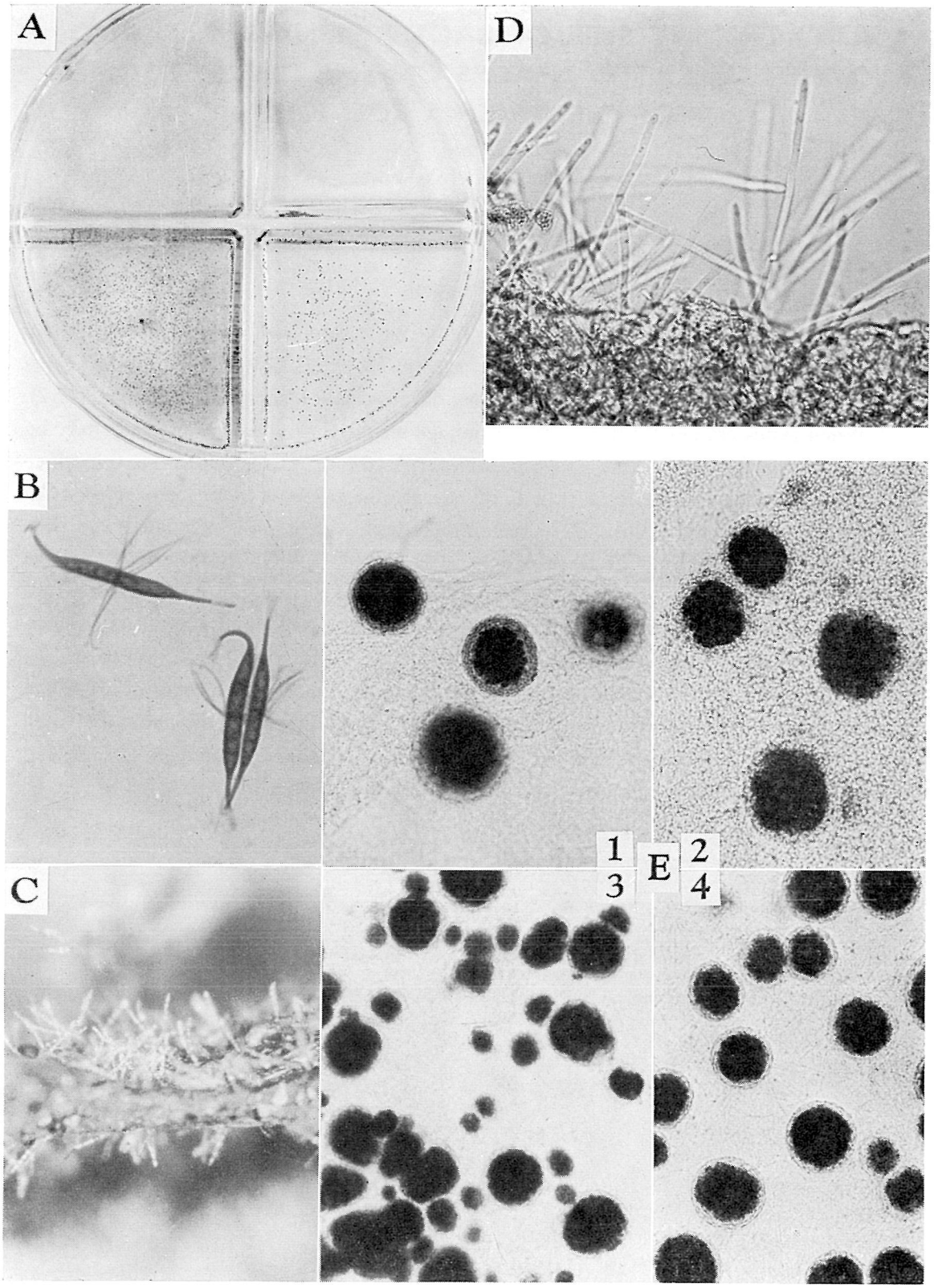

Plate 2. A–B. *Corollospora maritima*. Strain 154–12–1. A. Comparative growth on different media (×0.7, same with pl. 1 F). B. Ascospores, stained by toluidine blue (×500). C–D. *Varicosporina ramulosa*, habit on *Hypnea charoides* (C, ×63; D, ×250). E. Ascocarps of *Emericellopsis*-species (×100). 1–2. *E. humicola*, IFO–8518 (Univ. Toronto) and a marine isolate, VI–18. 3–4. *E. microspora*, IFO–8614 (Univ. Toronto) and a marine isolate, IX–2.

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ATTACHMENT SITE OF BACTERIOPHAGE φ170 AND OTHER λ-RELATED PHAGES ON THE CHROMOSOME OF *ESCHERICHIA COLI* K 12.

Teiji IIJIMA and Yutaka SAKAMOTO

Introduction

Temperate phages are able to establish lysogenic systems when they infect sensitive cells. Different types of temperate phage of *Escherichia coli* K 12 have different locations each one occupying a specific site of the host chromosome (Jacob and Wollman 1957).

When genes are closely linked to a prophage on the host chromosome they may be transduced together with these phage particles. As the prophage φ170 can transduce the *Gal* markers among the strains of K 12, the prophage seems to locate near the *Gal* loci (Iijima 1963, Iijima 1966). φ170 recombines with λ at a high frequency, and produces recombinant types φ170 hy ($i^{\lambda 170} h^{\lambda}$) and λ hy ($i^{\lambda} h^{\phi 170}$). On the other hand, phage φ80 which lysogenizes near the *Trp* loci (Matsushiro 1961), recombines with λ (or φ170) at a low frequency. Moreover, double lysogens for λ and φ170 are unstable and segregate monolysogens, however, double lysogens for φ80 and λ-related phages are stable.

This paper represents investigations to elucidate the exact genetic locus of prophage φ170 in relation to λ (and λ-related phages), and to know the realtionship between unstability of double lysogen and genetic loci of its prophages. Analysis of λ-related phages is also significant to inquire the interrelation and the origin of lambloids.

Materials and Methods

Bacteriophages: Bacteriophages used in these experiments are shown in Table 1.

Table 1. Characteristics of phages used

indicator	characteristics	φ170	434	82	λ	φ80	φ170hy	434hy	82hy	λhy	λ_J
F1	W3110	+	+	+	+	+	+	+	+	+	(+)
F1539	F1/λ	+	+	+	−	+	−	−	−	+	−
F1467	F1/φ170	−	−	--	+	+	+	+	+	−	(+)
F1541	F1/φ80	+	+	+	+	−	+	+	+	+	(+)
F563	F1(φ170)	−	+	+	+	+	−	+	+	+	+
F1018	F1(434)	+	−	+	+	+	+	−	+	+	+
F1381	F1(82)	+	+	−	+	+	+	+	−	+	+
F1192	F1(λ)	+	+	+	−	+	+	+	+	−	−
F1039	F1(φ80)	+	+	+	+	−	+	+	+	+	(−)

φ170 hy: $i^{\phi 170} h^{\lambda}$, 434 hy: $i^{434} h^{\lambda}$, 82 hy: $i^{82} h^{\lambda}$, λhy: $i^{\lambda} h^{\phi 170}$
λ_J: mutant in J cistron +: Lytic zone appeared −: no lytic zone.

Bacterial strains: Bacterial strains are also shown in Table 2.

Table 2. Bacterial strains used

Strain	origin	properties
F1	W3110	F$^-$ nonlysogenic
F563	F1	F1 (ϕ170) : ϕ170 lysogenic
F918		F1 (ϕ170 hy): ϕ170 hy lysogenic
F1018		F1 (434): 434 lysogenic
F1039		F1 (ϕ80): ϕ80 lysogenic
F1192		F1 (λ) : λ lysogenic
F1381		F1 (82) : 82 lysogenic
F1539	F1	F1/λ : resistant to λ
F1467		F1/ϕ170 : resistant to ϕ170
F1541		F1/ϕ80: resistant to ϕ80
F1002	W3350	F$^-$ $Gal^-_{1,2}$ nonlysogenic
F1444	F1002	F1002 (ϕ170d): *Gal*$^-$ derivative from (ϕ170dg)
F1457		F1002 (λ d): *Gal*$^-$ derivative from (λdg)
F1810		F1002 F$_8$′-gal (ϕ170)
F1811		F1002 F$_8$′-gal (λ)
F1815		F1002 (ϕ170 d)/λ
F1819		F1002 (λd)/ϕ170
F2379		F1002 F$_1$′-gal (ϕ170)
F2385		F1002 F$_1$′-gal (λ)
F2405		F1002 (λ_J)/λ (ϕ170)/ϕ170
F2461	F563	F1 (ϕ170)/ϕ170 (λ)/λ
F2462		F1 (ϕ170)/ϕ170 (λ)/λ
F2463	F1192	F1 (λ)/λ (ϕ170)/ϕ170
F2464		F1 (λ)/λ (ϕ170)/ϕ170
F2619	F1002	F1002 (82)/ϕ170 (λ)/λ
F2621		F1002 (ϕ80)/ϕ80 (λ)/λ
F2623		F1002 (ϕ80)/ϕ80 (ϕ170)/ϕ170
F2625		F1002 (ϕ170)/ϕ170 (λ)/λ
F2627		F1002 (ϕ170)/ϕ170 (λ)/λ
F2631		F1002 (434)/ϕ170 (λ)/λ
F2632		F1002 (434)/ϕ170 (λ)/λ
F2633		F1002 (434)/ϕ170 (λ)/λ
F2278	W3623	F$^-$ rec^-_{76} Gal^-_6 Ind^-_2 Spec^r nonlysogenic
F2529		F2278 (ϕ170)/ϕ170 (λ)
F2615		F2278 (ϕ170)/ϕ170 (λ)

Test for zygotic inductions: Indicator strain B, such as F1444, (ϕ170 d)*, were seeded with soft agar on a nutrient agar plate. Then another indicator A, such as F1819,

* Gal$^-$ strain having a defective prophage derived from ϕ170dg or λ dg

(λd)/ϕ170*, were spread on the surface of the agar layer of the indicator B. Then a loopful of culture of a donor having F′-gal were spotted and incubated at 37°C for 18 hours. If a prophage which locates on the F′-gal is transferred into the recipient cell (indicator A), by F′-duction (Hirota and Sneath 1961), the prophage is induced by zygotic induction and multiplies in the recipient cell. The phage particles recombine with heteroimmune prophage which is carried in the strain A, and produce recombinant type particles. The recombinant type phages are liberated from indicator A, infect cells of indicator B and lyse them. Thus the lytic zone will be formed on the indicators. Indicator A is resistant to the phage which is carried in donor strain, so as to eliminate the external infection of free phage. Indicator B is immune to the phage which is carried in donor strain. So the lytic zone is formed only by recombinant phage between prophage on the F′ episome in the donor and prophage of strain A.

Preparation of double lysogens: Doubly lysogenic strains were prepared by the following procedures. Nonlysogenic strain was infected by one phage, for example λ, and then select a resistant strain to this phage by cross-brushing with a virulent mutant or a virulent recombinant. After superinfection of another phage, for example ϕ170, resistant marker to the second phage was added. The double resistant markers to the both phages are important to exclude the external infection of free phages to segregants. These double lysogens designated as follows; prophages are designated by phage symbols in parenthesis arranging from left to right according to the order of lysogenization.

Analysis of segregants from double lysogens: Single colonies of a double lysogen were suspended in Davis medium (Davis and Mingioli 1950) and plated on nutrient agar plates after appropriate dilutions. Colonies appeared on the plates are picked with sterile tooth-picks to fresh nutrient plates. After overnight incubation these master plates were replica plated onto various indicators which were previously seeded with soft agar on the surface of agar plates then were induced by UV irradiation.

Doubly lysogenic strains of rec^-_{76}: Recombination-deficient mutants of K12, rec^-_{76}, which was originally isolated by Dr. Ogawa in the National Institute of Health of Japan, was kindly supplied from Dr. Matsushiro in the Institute of Microbial Diseases of Oasaka University. This strain has gained an increased sensitivity to UV irradiation in accordance with an inability to form a conjugal recombinant.

Double lysogens of rec^-_{76} were isolated by the following procedures.

$rec^-_{76}(\lambda) \rightarrow rec^-_{76}(\lambda)/\lambda \rightarrow rec^-_{76}(\lambda)/\lambda\ (\phi170)$.

$rec^-_{76}(\phi170) \rightarrow rec^-_{76}(\phi170)/\phi170 \rightarrow rec^-_{76}(\phi170)/\phi170\ (\lambda)$.

However monolysogens of rec^-_{76} strains liberated few phage, these double lysogens gained the abilities to liberate free phages. Unexpectedly, phage liberation from double phage-resistant and double lysogenic strains, $rec^-_{76}(\lambda)/\lambda(\phi170)/\phi170$ and $rec^-_{76}(\phi170)/\phi170\ (\lambda)/\lambda$, reduced in compared with $rec^-_{76}(\lambda)/\lambda\ (\phi170)$ and

rec$^-_{76}$(ϕ170)/ϕ170 (λ). So it is difficult to differentiate monolysogenic segregants from the parental strain by replica plating method. Segregational patterns of double lysogen of *rec*$^-_{76}$ were tested by using these single resistant strains. Lysogeny of segregants were confirmed by cross-brushing to phages, $i^{\lambda}\,h^{\phi 80}$ and $i^{\phi 170}\,h^{\phi 80}$, because of resistant markers for ϕ170 (or λ).

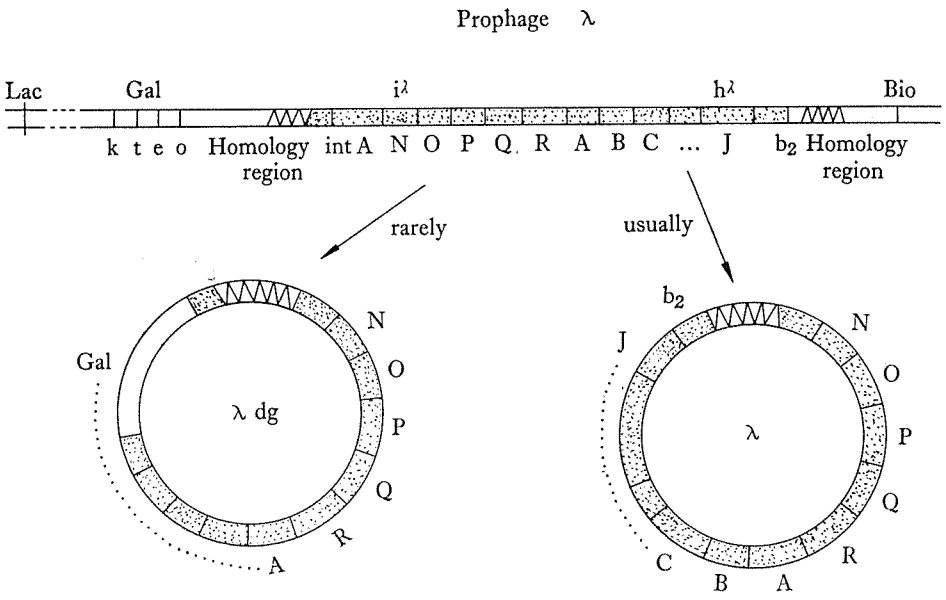
Results and Discussions

Genetic structure of ϕ170 dg: Immunity specificity of ϕ170 is different from those of 82,434 and ϕ80 and the host range of ϕ170 is different from those of λ and ϕ80 (Table 1). Defective regions of ϕ170 dgs in independent transductants are tested by complementation test with λ *sus* (Iijima and Sakamoto 1968). A culture of a defective lysogen to be tested is mixed in soft agar and layered on a nutrient plate. Samples of various λ *sus* mutant phages are spotted on the surface of soft agar containing *Pm*$^-$ (ϕ170 dg). In *Pm*$^-$, non-permisive strain, each of the λ *sus* mutants does not mature because of blocking of some essential functions. If a prophage of heteroimmune specificity confer the blocked function, some of the λ *sus* mutants will grow in the strain. Table 3 show that most of the λ *sus* phages, except mutants in N and O cistroins, can grow in *Pm*$^-$ (ϕ170) cell, while they cannot grow alone in nonlysogenic *Pm*$^-$ cell.

Table 3. Complementation test with λ *sus*

	λ *sus* phages														No. of ϕ170 dg
	N	O	P	Q	R	A	B	C	E	G	M	L	I	J	
Pm$^-$(ϕ170 dg-a)	−	−	+	+	+	−	−	−	−	−	−	−	−	−	4
Pm$^-$(ϕ170 dg-b)	−	−	+	+	+	+	−	−	−	−	−	−	−	−	6
Pm$^-$(ϕ170 dg-c)	−	−	+	+	+	+	+	+	−	−	−	−	−	−	14
Pm$^-$(ϕ170 dg-d)	−	−	+	+	+	+	+	+	+	−	−	−	−	−	1
Pm$^-$(ϕ170)	−	−	+	+	+	+	+	+	+	+	+	+	+	+	

Twenty five independent defective ϕ170 strains were tested and 4 strains complement with cistrons of λ from P to R, 6 strains complement from P to A, 14 complement from P to C and one strain complements from P to E. This result shows that the defective region of ϕ170 dg-a is from A cistron to J, and so on. This would mean that the arrangements of cistrons and the functions are similar in these two related phages and the defective region of ϕ170 dg is correspond to that of λ dg. These data also suggest that the mechanism of formation of ϕ170 dg from ϕ170 lysogenic strain is similar to that of λ dg. Mechanism of formation of λ dg from lysogenic bacteria (Cambell 1962, Hogness 1966) is shown in Fig. 1. If the mechanism can be applied for the formation of ϕ170 dg, still two possible locations may be considered: Prophage ϕ170 locates between the *Gal* and the *Bio* loci in the order of *Lac...Gal-*(*N O P Q R A B...J*)*-Bio* or between the *Lac* and the *Gal* loci in the order of *Lac...*(*J...B A R Q P O N*)*-Gal...Bio*,



A～J cistron of λ
Gal Gal maker of the host bacteria
Bio Biotin marker of the host bacteria
i Immunity marker of phage
h Host range marker of phage

Fig. 1. Formation of λ dg from λ lysogenic bacteria (Campbell 1962, Hogness 1966)

Table 4. Zygotic induction by F-gal

1. Test for *att* φ170
   Indicator A: F1819 (λ d)/φ170
   Indicator B: F1444 (φ170 d)

Donor	F1810 F_8'-gal (φ170)	F2379 F_1'-gal (φ170)	F563 F^-(φ170)	F918 F^-(φ170 hy)
Lysis zone	--	++	--	++

2. Test for *attλ*
   Indicator A: F1815 (φ170 d)/λ
   Indicator B: F1457 (λ d)

Donor	F1811 F_8'-gal (λ)	F2385 F_1'-gal (λ)	F1192 F^-(λ)	F1103 F^-(λ hy)
Lysis zone	--	++	--	++

Zygotic induction: To determine the exact location of prophage ϕ170, zygotic induction by two F′-gal episomes, F_1′-gal and F_8′-gal, was tested. F_1′-gal episome possesses a wide range of host chromosome from the *Gal* to the *Bio* loci containing *att* λ. From Table 4, zygotic induction was occured by F_1′-gal but not by F_8′-gal which has a shorter region neighbouring the *Gal* loci. Control experiment shows that prophage enters into indicators only by F_1′-gal as a vector. A possibility that the zygotic induction would occured by an Hfr strain which was derived from the *F*′-gal, does not exclude in this experiment. Some experiments to test this alternative are carried out but the clear result could not be obtained.

The result of zygotic induction shows that ϕ170 can lysogenize on F_1′-gal episome presumably at *att* λ or in the region neighbouring *att* λ.

Segregational pattern of double lysogen: As reported earlier, double lysogen for both λ and ϕ170 was unstable and segregates stable monolysogens (Iijima 1966). Examining the host range characters of prophages in each segregant, it was noticed that the recombinant type prophages, such as λ hy ($i^{\lambda}h^{\phi 170}$) or ϕ170 hy ($i^{\phi 170}h^{\lambda}$) were involved in these segregants, along with parental type prophages, even if the double lysogens have resistant markers to prevent the superinfection of free phages.

Another fact is that nonlysogenic and monolysogenic strain for the superinfected phage (exchanged type) are found in the survivors (heteroimmune curing), when a monolysogenic strain is superinfected with another heteroimmune phage. These phenomena

Table 5. Segregational pattern of double lysogens

Strains used (Double lysogens)	No. of colonies tested	No. of double lysogen	No. of segregants	Type of segregation			% of segregants	type of integration
				α'	β'	γ'		
F2405: (λ_J)/λ(ϕ170)/ϕ170	9752	9635	117	68	7	42	1.2	α
F2405	11660	11533	127	103	11	13	1.1	α
F2405	12932	12640	292	249	3	40	2.3	α
F2463: (λ)/λ(ϕ170)/ϕ170	2279	2216	63	50	3	10	2.8	α
F2464: (λ)/λ(ϕ170)/ϕ170	424	410	14	8	3	3	3.3	α
F2625: (ϕ170)/ϕ170(λ)/λ	1484	958	526	492	28	6	35	α
F2627:	1484	1156	328	277	49	2	22	α
F2461:	1233	530	703	582	115	6	57	α
F2462:	259	142	117	78	34	5	45	α
F2619: (82)/ϕ170(λ)/λ	3180	2620	560	338	193	29	17.6	α
F2631: (434)/ϕ170(λ)/λ	1484	1402	82	74	6	2	5.5	α
F2631	636	599	37	34	2	1	5.8	α
F2631	848	800	48	38	4	6	5.7	α
F2633: (434)/ϕ170(λ)/λ	636	603	33	29	2	2	5.2	α
F2632: (434)/ϕ170(λ)/λ	6572	6494	78	51	27	0	1.2	γ
F2632	848	842	6	3	2	1	0.7	γ
F2621: (ϕ80)/ϕ80(λ)/λ	848	848	0	0	0	0	0	
F2623: (ϕ80)/ϕ80(λ)/λ	1060	1060	0	0	0	0	0	

suggest that strong inteference between λ and λ-related phage for the same attachment site.

In a genetic mapping of prophage in double lysogen of λ (Calef 1967), it was shown that the structure of the double lysogen was deduced by examining their monolysogenic segregants and that the structure of the double lysogen is linearly arranged in tandem structure.

In the similar way the structure of heteroimmune double lysogen of λ and λ-related phages would be deduced by examining the segregational pattern. The results in Table 5 shows that segregants from one double lysogen consist of three definite types including recombinant type (or types) even in its progenies (*cf.* F2405, F2631 and F2632 in Table 5.). If recombinant type phages might be resulted by a recombination in vegetative development of two phages or internal pairing and crossing over of two prophages (Campbell 1963) in the host cell, four possible types (two parental and two recombinant types) should be formed. The results in Table 5 exclude an assumption of secondary recombination as the mechanism of forming recombinant type phages. In addition, the assumption of secondary recombination in vegetative development cannot explain a difficulty of reintegration of recombinant type phage into

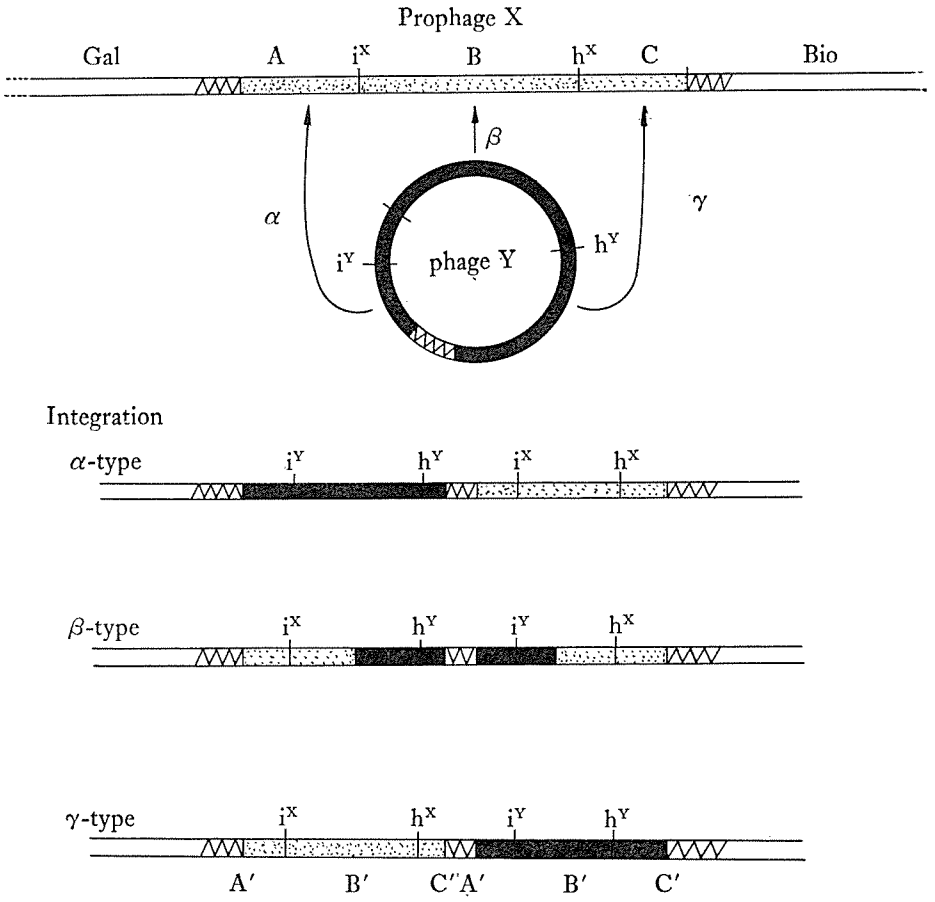


Fig. 2. Model of integration and excision crossover

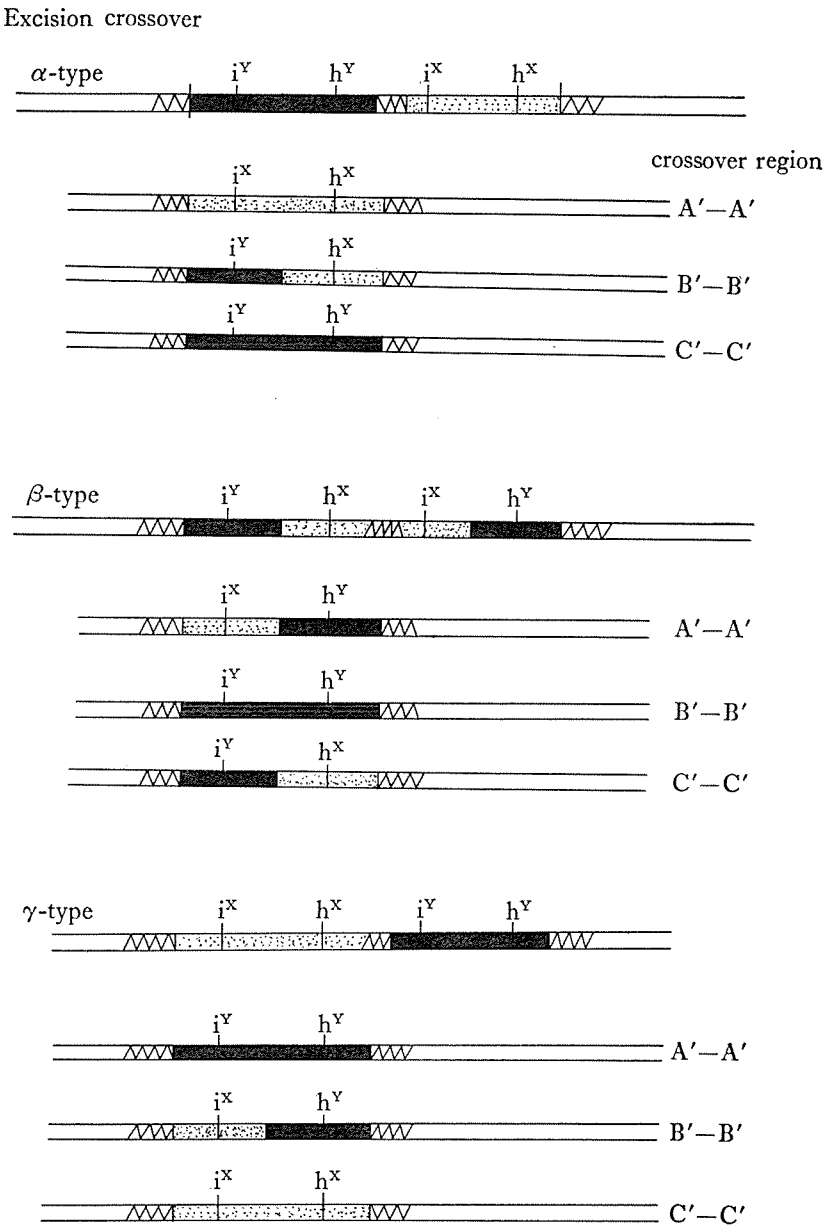


Fig. 2 (Continued)

the host chromosome without lysis of host cell. The results shown in Table 5 may be explained by the assumption that λ and λ-related phages lysogenize on the same locus, arranging linearly in tandem structure. As mentioned above, arrangement of cistrons is similar in two phage, λ and ϕ170, then the phage genome can be devided into three parts A, B and C by two markers, immunity *i* and host range *h*, which are available to this analysis. If, for example, λ-lysogenic strain is superinfected with ϕ170, the phage can lysogenize in those three possible regions. If the ϕ170 recombines with the prophage λ in A region by single crossover and genome of ϕ170 phage is linearly inserted into the genome of prophage we may obtain an α-type double lysogen. In a similar way β- or γ-type double lysogens will be obtained.

Table 6. Segregational patterns of prophages in monolysogens from the model of integration and excision crossover.

type of integration crossover	α			β			γ		
Type of excision crossover	α'	β'	γ'	α'	β'	γ'	α'	β'	γ'
Type of prophages *	1	4	2	4	1	3	2	3	1
Double lysogens									
(λ)/λ (ϕ170)/ϕ170	λ	ϕ170hy	ϕ170	ϕ170hy	λ	λhy	ϕ170	λhy	λ
(ϕ170)/ϕ170 (λ)/λ	ϕ170	λhy	λ	λhy	ϕ170	ϕ170hy	λ	ϕ170hy	ϕ170
(λ)/λ (434)/ϕ170 **	λ	434hy	434	434hy	λ	λhy	434	λhy	λ
(434)/ϕ170 (λ)/λ	434	λhy	λ	λhy	434	434hy	λ	434hy	434
(λ)/λ (82)/ϕ170 **	λ	82hy	82	82hy	λ	λhy	82	λhy	λ
(82)/ϕ170 (λ)/λ	82	λhy	λ	λhy	82	82hy	λ	82hy	82

* Type of prophages 1: $i^X h^X$ 2: $i^Y h^Y$ 3: $i^X h^Y$ 4: $i^Y h^X$ (see also Fig. 2)
** Analysis of these double lysogens were not undergone

As the segregation proceeds in the reciprocal way of integration, three types (α', β' and γ') of circularization and excision of one complete set of phage genome may be possible, following rules imposed by the type of integration. According to this model, from an α-type double lysogen, for example (λ)/λ (ϕ170)/ϕ170, we expect to find three definite types of monolysogen, (λ), (ϕ170 hy) and (ϕ170) strains as segregants (Table 6). Conversely we can assume the integration type of double lysogen by examining the prophages of monolysogenic segregants. From Table 5, it is noticed that the most of the double lysogens were α-type irrespective of the starting monolysogen, and that the segregation proceeded most frequently, so as to remain an α-type monolysogen. If a starting monolysogen is λ-lysogenic, for example, the resulted double lysogen is mostly α-type, and the double lysogen segregates λ-lysogenic cell most frequently (α'-type). This result suggest that the superinfecting phage integrates into the host chromosome between the *Gal* and the *i* locus of the original prophage (near Homology region of the *Gal* side), and that the segregation occurs most frequently in the same region irrespective of the type of double lysogen.

In the case of double lysogens for both ϕ80 and λ (or ϕ170), they were stable and no segregants are found under the experimental conditions (Table 5). It is well-known that ϕ80 lysogenize at a different locus from that of λ.

Segregation of rec⁻ double lysogens: When a temperate phage is linearly inserted into the chromosome of the host, a site specific recombination enzyme (product of phage genome *int*) of phage and a specific sites (Homology regions) of the phage and bacterial chromosome concerned in this process (Gottesman and Yarmolinsky 1968). According to the model shown above the *int* enzyme may play a part in segregation process, because of the segregation is the reciprocal process of integration. β'-type of segregation might involve another enzyme, because the sites of β'-type excision are quite different from those of α'- and γ'-type excision. To test the participation of recombination

enzyme (product of the *rec* gene) of bacteria to the segregation of double lysogen, recombination-deficient mutant (*rec*$^-$) of the host was used. Table 7 shows the segregational pattern of rec_{76}^- double lysogens, F2529 AA and F2615.

Table 7. Segregational pattern from rec_{76}^- double lysogens

Strains	No. of colonies tested	single lysogen	Type of segregation α' (ϕ170)	β' (λhy)	γ' (λ)	(ϕ170hy)
F2529 AA	425	64	41	0	23	0
	425	32	25	0	7	0
F2615	1378	175	136	0	39	0
	595	168	155	0	13	0

F2529 AA: rec_{76}^- (ϕ170)/ϕ170 (λ)/λ
F1615 : rec_{76}^- (ϕ170)/ϕ170 (λ)/λ

It is shown that only parental prophages were detected in the segregants in this case. According to the segregational pattern shown in Table 6, the pattern shows that the rec_{76}^- double lysogen belong to α-type or γ-type. Segregation of β'-type was not detected among about 300 monolysogenic segregants. This may be enough number to detect β'-type segregants in the case of *rec*$^+$ double lysogen. This suggest that rec_{76}^- gene contribute β'-type segregation, that is, β'-type segregation partly proceeds by *rec* enzyme of the host.

In conclusion; from the data of unstability of double lysogens, and of hetero-immune curing, it was shown that λ-related phage lysogenized at the same locus when a double lysogen was isolated by superinfection. Segregation of the double lysogens of λ-related phages proceeds the reciprocal process of integration; circularization and excision of one complete phage genome from two sets of phage genome which arranged tandem in linear structure. Enzyme (or enzymes) controlled by gene rec_{76} participate the segregation in partly, presumably in β'-type segregation.

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Ann. Rep. Inst. Fermentation, Osaka
No. 4, p. 53～59, 1969 (February)

OCCURRENCE OF *RHODOSPORIDIUM TORULOIDES* AND *RHODOTORULA* YEASTS IN A FOREST

Isao BANNO and Kōzaburō MIKATA

A sexual stage has been found among 5 strains of *Rhodotorula glutinis*. Yeast cells of arbitrary two strains different in mating type conjugated and produced a dikaryotic mycelium like basidiomycetes. Furthermore, this fungus was found to perform *in vitro* a life cycle simillar to that of Ustilaginaceae. Consequently this was regarded as a new organism belonging to heterobasidiomycetes and was named *Rhodosporidium toruloides* (Banno 1963, 1967). The present work was attempted to look for the existence of mycelial stage of this fungus in the nature. Although *Rhodotorula* yeasts have been isolated from various sources, their occurrence seems to be more frequent in the air and on the plant. The basidiomycetous fungi were mostly found on plants. From this reason, plants and atmosphere in a forest were examined.

Methods

Microbial samples were collected in a forest, 20 km North from Osaka city, on the slope of a hill by the river Yonogawa (Nishiyamaguchi, Minoo city) (Fig 1). This forest

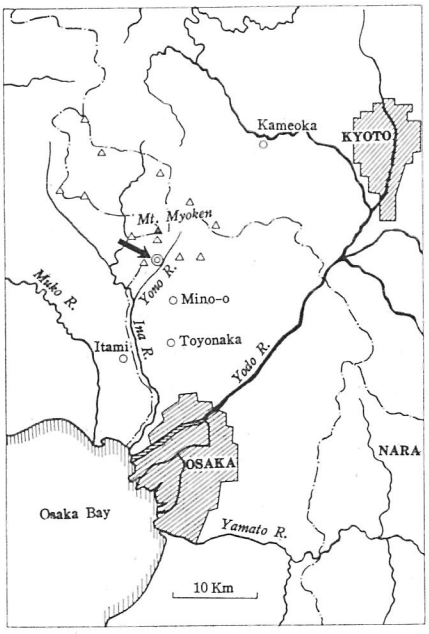


Fig. 1. The map of KINKI area. Arrow indicates the sampling location.

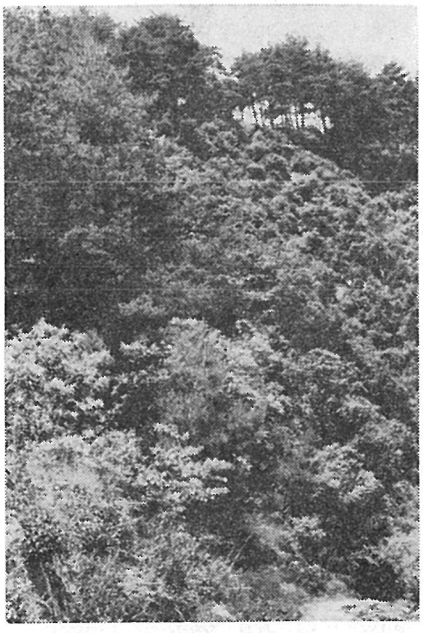


Fig. 2. The circumstances of sampling location.

constists of *Pinus densiflora, Castanea crenata, Quercus dentata, Chamaecyparis obtusa, Alnus japonica, Rhododendron kaempferi* etc. (Fig. 2). Samples were collected twice a month during the period of May 15th to Nov. 28th 1964. Sixty dishes were exposed to the air at intervals of 2 m on the ground and a total of 40 various leaves were collected in an examination.

Medium: PYG agar was used. It contains a decoction of 200 g potato, 10 g yeast extract, 30 g glucose, 10 mg tetracycline, 25 mg actidion and 15 g agar per 1 liter, pH 5.6.

Isolation of the organisms from the air: Petri dishes (90 mm) containing 20 ml of PYG agar were opened and exposed to the air for 15 min. The dishes were incubated at 25 C.

Isolation of the organisms from plant leaves: The leaves of various plants collected in the forest were treated in laboratory within 5 hours. A leaf was pressed on PYG agar in a petri dish for 30 sec, and then the dish was incubated at 25 C. After 3 to 5 days, the pink to red and the purplish brown colonies appeared on the agar plates were counted and transferred to the PYG agar slants. After being reisolated on the agar plate, the established isolates were subjected to detailed analyses. Morphological and physiolosical properties of the isolates were examined according to the methods described previously (Hasegawa et al. 1960a, b, Banno 1967).

Conjugation test was performed as follows. The equal amounts of yeast cells of an appropriate couple of the isolates were mixed together on the PYG agar and incubated overnight at 25 C. One part of this mixture was examined microscopically for conjugation of cells and the rest was transferred onto the honey agar to enhance the mycelial growth of conjugated cells.

Results

The number of *Rhodotorula* yeasts isolated in each sampling is presented in histogram of Fig. 3. *Rhodotorula* yeasts occurred most numerously in June followed by those recognized in the middle of August. Decrease in the number of isolates in July might be due to seldom rain and low humidity in the early summer of this year. It is probable that an appreciable number of *Rhodotorula* yeasts inhabits on the leaf and their cells are floating in the atmosphere of forest. The number of isolates from the air were smaller in the dry season of September to November.

On a total of 321 isolates, carbon and nitrogen assimilation and vitamin requirements were examined. As a result, they were classified into 7 metabolic groups (A to G) by means of the physiolosical criteria. Their taxonomic designation and properties are presented in Table 1. Group A corresponds to *Rhodotorula glutinis* (Fres.) Harrison; B, *Rh. graminis* di Menna; C, *Rh. rubra* (Demme) Lodder emend. Hassegawa; D, *Rh. lactosa* Hasegawa; E, *Rh. texensis* Phaff, Mrak et Williams and G, *Rhodosporidium toluloides* Banno. Besides, a strain of group F is regarded as a hitherto undescribed species, because of its ability to assimilate inositol and soluble

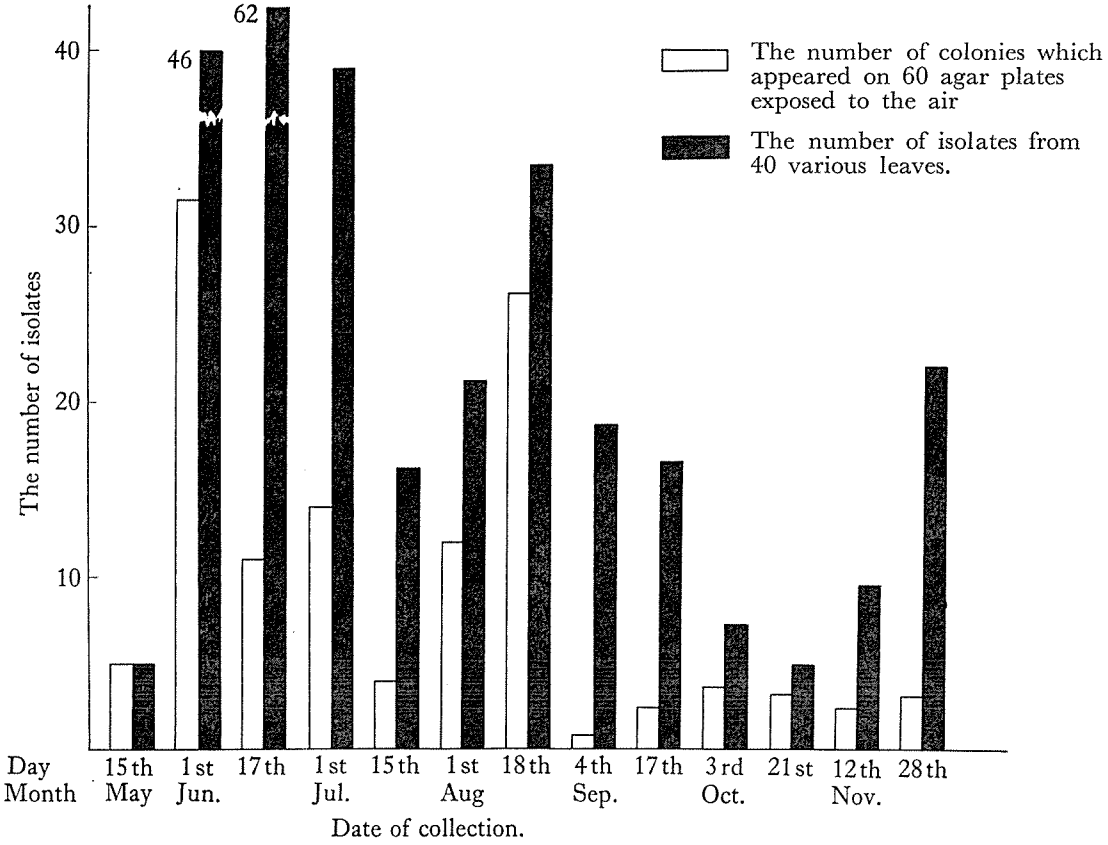


Fig. 3. Seasonal distribution of *Rhodotorula* yeasts isolated from the leaves and the atmosphere of a forest.

Table 1. Physiological properties of the isolates

Group	Designation	The number of isolates	Assimilation tests								Vitamin requirements		
			Galactose	Maltose	Sucrose	Lactose	Raffinose	Inositol	KNO_3	Urea	Thiamine	pABA	Biotin
A	*Rhodotorula glutinis*	225	+	+	+	−	+	−	+	+	no, S	no	no
B	*Rh. graminis*	8	+	−	+	−	+	−	+	+	no	no	no
C	*Rh. rubra*	68	+	+	+	−	+	−	−	+	E	no	no
D	*Rh. lactosa*	14	W	+	+	+	+	−	+	+	S	E	no
E	*Rh. texensis*	1	W	−	+	+	W	−	−	+	E	E	no
F	*Rh.* sp.	1	+	+	+	−	W	+	+	+	S	no	no
G	*Rhodosporidium*	2	+	+	+	−	+	−	+	+	no	no	no

+ ; Assimilable W; Weakly assimilable − ; No assimilation
no ; Independent S ; Stimulative E ; Essential

starch. The details of this strain will be published elswhere.

Rhodotorula glutinis was most predominant followed by *Rh. rubra*. *Rhodotorula texensis* and an unknown species mentioned above were isolated only once on August 1st and 18th respectively.

Seasonal distributions of the strains of *Rh. glutinis*, *Rh. graminis*, *Rh. rubra* and *Rh. lactosa* were presented in Fig. 4. The strains of *Rh. glutinis* were isolated in all the seasons examined and especially abundantly in June and July. The *Rh. graminis*, however, occurred occasionally. *Rhodotorula rubra* was observed appreciably after July. The number of this yeast was the greatest in the middle of August. It appears conceivable that the seasonal succession from *Rh. glutinis* to *Rh. rubra* takes place on the leaves. The strains of *Rh. lactosa* were isolated exclusively from the air on August 18th. *Rhodotorula glutinis*, *Rh. rubra* and *Rh. graminis* were obtained more frequently from the leaves than from the air.

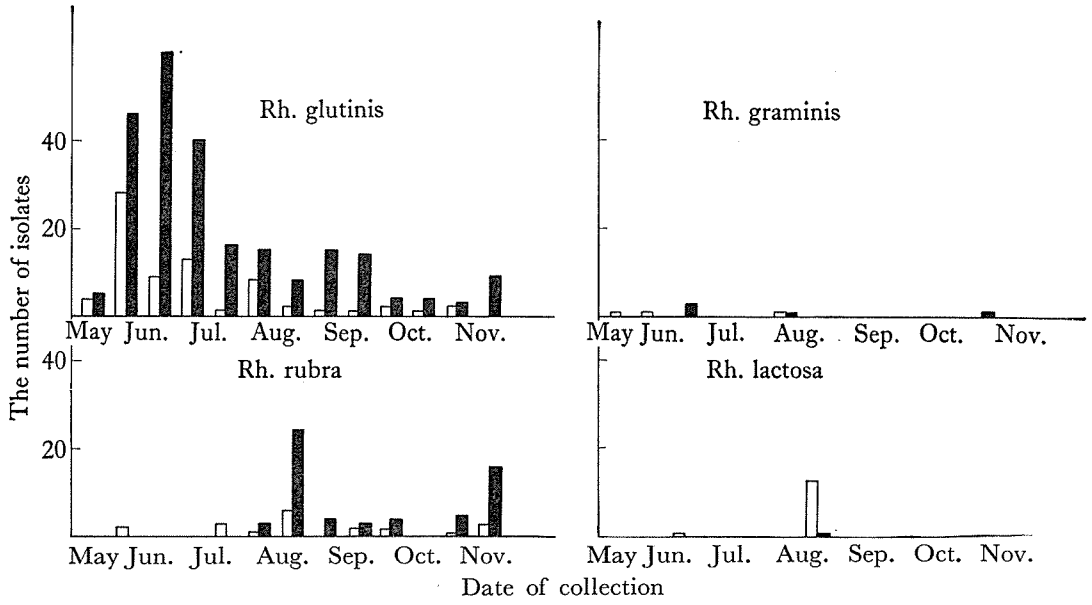
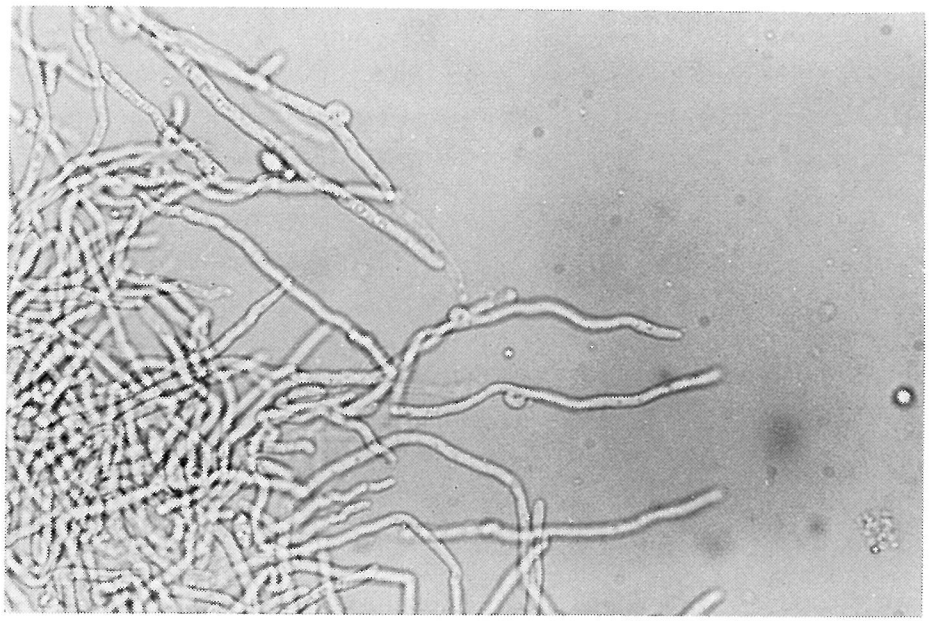


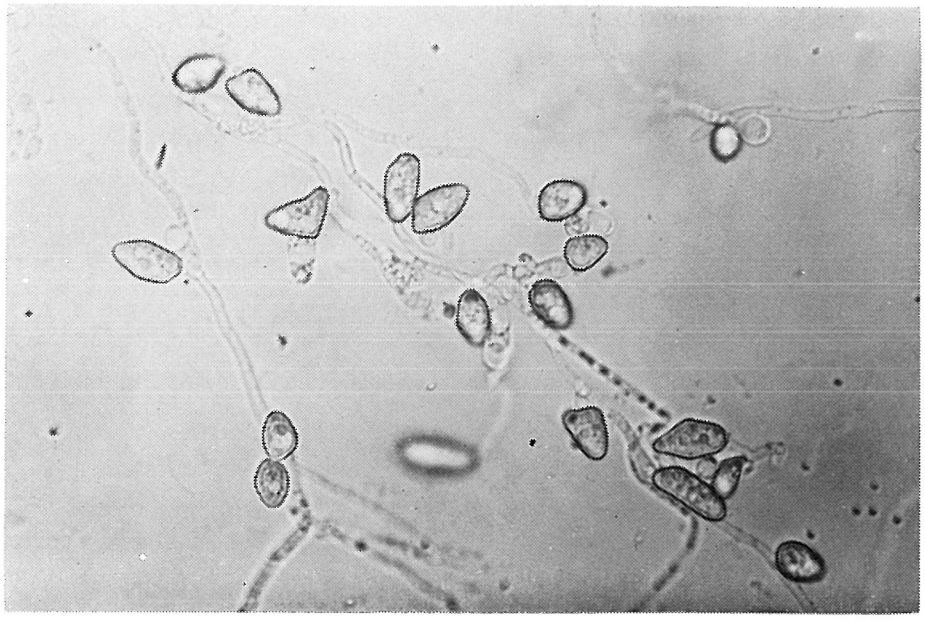
Fig. 4. Seasonal distribution of the species of *Rhodotorula* isolated. (see note of Fig. 3)

The two strains of *Rhodosporidium toruloides* isolated from the air on June 17th developed a true mycelium on the ordinary agar media. The morphological and physiological characteristics of the mycelium were identical with those of *Rhodosporidium toruloides* (Banno 1967). The mycelia had septa randomly with clamp-connection and formed pear-shaped resting spores with clamp-like structure at base (Figs. 4 and 5). The spores, when being transplanted onto a fresh PYG agar, germinated and produced promycelia, but formed no sporidia. These two fungi developed as a yeast state in liquid medium. This yeast state cell produced again the same dikaryon mycelia on a honey agar and, therefore, must be diploid as reported by Banno (1967).

The conjugation test was performed between every isolate and either of the



A



B

Fig. 5. The morphology of a isolated *Rhodosporidium toruloides*, strain 3–75.
A: Dikaryon mycelium with clamp-connections.
B: Resting spores produced terminally on mycelium.

following standard strains of mating types; IFO. 0559 *A* and IFO. 0880 *a*, and in all the pair-wise combinations among isolates of each group. As a result, only two isolates of *Rh. glutinis* were found to be able to mate with IFO. 0880 (mating type *a*) and to develop true mycelia identical with *Rhodosporidium toruloides*. One was an isolate from the air and the other from the leaf on August 1st. In none of other combination tested, there was observed any reaction.

Discussion

Predominant species of *Rhodotolula* on leaves and in atmosphere of the forest examined by us were *Rh. glutinis* and *Rh. rubra*. The two species were the most frequently isolated species of the genus from terrestrial sources (Huxley & Hurd 1956, Di Menna 1959) and also have been found to be common yeasts found abundantly in fresh waters, esteurian, coastal waters and ocean (Fell & van Uden 1963, Ahearn et al. 1968). From these observations it is clear that these two species are the most widespread yeasts in the world. *Rh. graminis*, which were isolated in small number in the present study, have been also observed in terrestrial and marine sources (Di Menna 1959, Ahean et al. 1962, 1968). Furthermore Ahearn et al. (1962) reported that no distinctive metabolic difference was observed between marine and terrestrial isolates of *Rhodotorula*. These *Rhodotorula* yeasts might not seriously require any environmental condition and could propagate easily in any circumstances which would provide them with appropriate nutrients.

In the present work, mycelial culture and haploid yeast (mating type *A*) of *Rhodosporidium* were obtained from the natural environment of the forest. While this paper was under preparation, it was reported that mycelial cultures of *Rhodosporidium* were also isolated from coastal waters (Ahearn et al. 1968). These facts strongly indicate the existence of the life cycle of this fungus in the nature, which had been found *in vitro* with the conjugation of *Rhodotorula* yeasts.

The existence of the fungus in a forest and in sea water might lead us to thinking that the perfect life cycle might be carried out in various places. But this seems unlikely. The life cycle may probably completed on a special habitat.

However, no strain of mating type *a* was isolated in this work. And with the isolated two strains of *Rhodosporidium toruloides*, resting spores failed to produce sporidia. Probably the spore requires a special condition for its normal germination. In these respects further investigation must be necessary.

Summery

1. Occurrence of *Rhodosporidium* and *Rhodotorula* in the leaves and atmosphere of a forest was investigated.

2. The most predominant species were *Rhodotorula glutinis* and *Rh. rubra*.

Rhodotorula graminis, Rh. lactosa and *Rh. texensis* were not frequently isolated. The number of isolates varied with season. *Rhodotorula glutinis* was more abundant in early summer and *Rh. rubra* and *Rh. lactosa* occurred mainly after the midsummer.

3. Two strains of *Rhodosporidium* were obtained from atmosphere in early June and two haploid strains of its mating type *A* were isolated from a leaf and from the atmosphere in early August.

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DESCRIPTIVE CATALOGUE OF I.F.O. FUNGUS COLLECTION

In the routine work of determination of fungi, especially in consulting the list of fungi preserved in the IFO-collection, many species have been appeared for the first records from Japan. The object of this series of paper is to provide descriptions of fungi received in the IFO-collection from various parts of Japan which have never been recorded from this country and seem worthy to constitute towards the knowledge of the fungus flora of Japan. Fungi presented in this serial work are restricted to that not submitted to original papers. New genera or new species will be described in other mycological periodicals. Every description will bring plates and will be provided by names who collected, isolated and determinated the fungi.

(K. Tubaki)

1. ***Dendryphiopsis atra*** (Corda) Hughes (Pl. 1 A) Hyphomycetes

Hughes, in Canad. Jour. Bot. **31:** 655 (1953).

On malt agar, growth rapid, broadly spreading, almost velvety to woolly, with dense aerial mycelium, dark olive to almost black; reverse same colored. Conidiophores developed from aerial hyphae, stout, upright, septate, dendritically branched at upper part, 5–7 μ in diam., olive to dark brown colored. Each branch arises in turn, bursting through outer wall of conidiophore or basal branch, straight or curved, constricted at base, rounded at above, olive to dark brown; ultimate branches, 20–34$\times$6–7 μ, developed apical conidia, thus showing a dichasium-like conidial fructification. Conidia are of the porospore-type, 3–5–septate (4–septate is common), thick walled, cylindrical, straight or slightly curved, at first olive, then darker: 3–septate, 45–50$\times$ 14–15 μ; 4–septate, (50) 56–70 (72)$\times$15–18 μ; 5–septate, 68–72$\times$14–18 μ.

Hab.: On the decayed wood, Experimental Forest of Mie Univ., Mie Pref., July, 1967.

Strain preserved: IFO–8839

Coll. K. Tubaki
Isol. T. Ito
Det. K. Tubaki

2. ***Dictyosporium elegans*** Corda (Pl. 1 B) Hyphomycetes

Weitenweker's Beiträge **1:** 87 (1836); Damon, in Lloydia **15:** 113 (1952).

On malt agar, growth moderate, more or less felty or closely floccose, round, pale gray to pale olive; reverse and agar pale yellow to dark yellow brown. Sterile hyphae effuse, branched irregularly on which conidia develop, 1.5–2 μ in diam., hyaline to pale yellow; branches straight or sinuous. Conidia are of the aleuriospore-type, U- or conical shaped, mostly 5–7–branched, branches consist of parallel rows of

cells regularly, multiseptate, constricted at septa; tips of branches slightly curved rarely; dark brown; 62–68×26–32(36) μ.

Hab.: Isolated from drift wood, Tomioka, Amakusa, Kyushu, June, 1966.

Strain preserved: IFO–8932 Coll. K. Tubaki
Isol. 〃
Det. 〃

The conidia of the present species are characteristic in their typically flattened shape in front view and not separating branches. *D. toruloides* nears the present species, but differs in not flattened conidia and distinctly separated branches.

3. ***Hyalopycnis blepharistoma*** (Berk.) Seeler (Pl. 1 C, D) Hyphomycetes

Seeler, in Farlowia **1:** 124 (1943); Petch, in Trans. Brit. Mycol. Soc. **26:** 56 (1943).

On malt agar, growth moderate, rather restrict, with a close mat of mycelium, cartilaginous, white to pale cream; reverse same colored. Pycnidia not developed. Thread-like conidia developed from apices of short aerial hyphae, solitary, variable in length, usually 20–40 μ long, 4–5 μ wide, long ellipsoid, straight or curved, hyaline. Pycnidia on PCA superficial, at first solitary to scattered, then growing close to gather to gregarious, with a globose or subglobose basal swelling measuring 150–350μ width, tapering gradually or abruptly into a long subcylindrical neck which measuring 550–650 μ long, 50–70 μ in diam. and fimbriate at apex by flaring delicate hyphae, 5–6 μ in diam., soft and membraneous, white or creamy white colored, producing milky opaque spore–mass at head; venter wall hyaline, consisting of 2–several parallel hyphal layers, 4–10 μ in diam. Pycnophores developed from base of pycnidial cavity, simple or branched in dendroid manner, producing pycnospores acrogenously, 2.5–3 μ in diam. Pycnospores 1–celled, cylindrical, oblong-oval, long oval or irregular, (9) 10–15(18)×3–4μ, hyaline; often bud repeatedly at one end forming cruciform clusters of cells.

Pycnidia developed in abundance on the Langeron's weak potato carrot agar medium (PCA) and also propylene-oxide sterilized leaves of *Cyclobalanopsis glauca, Prunus* sp. and *Populus* sp. after two weeks at 24°C.

Hab.: On the squash (*Cucurbita pepo*), Hokkaido, Oct., 1967.

Strain preserved: IFO–8906 Isol. T. Yokoyama
Det. 〃

At nearly the same time when Seeler published the present new combination, Petch also described a same fungus from England under the name of *H. blepharistoma* (Berk.) Hohnel (1943). However, after Seeler (1943), v. Hohnel established *Hyalopycnis vitrea* saying differs from *Sphaeronaema blepharistoma* and no new combination, *H. blepharistoma* (Berk.) Hohnel, was validly published, therefore, Seeler's proposal was accepted at present as a valid publication of new combination.

The morphological structure of the present isolate is same with that given by

Dr. C.T. Rogerson, New York Botanical Garden, 66–90 & 67–71, isolated from the edcayed mushroom.

4. ***Monotosporella sphaerocephala*** (Berk. et Br.) Hughes (Pl. 2 A) Hyphomycetes

Hughes, in Canad. Jour. Bot. **31:** 654 (1953).

syn. *Monotospora sphaerocephala* Berk. & Br., in Ann. Mag. Nat. Hist. **1859:** 6 (1859).

Growth on malt agar, restrict, flat, moist and mucoid, with aerial mycelium at central area, dark brown-black with hyaline submerged marginal hyphae; reverse dark olive, dark brown to almost black. Conidiophores erect nearly perpendicular from ascending hyphae, straight, thick walled, septate, simple, bearing a first conidium terminally, 195–320 μ long, 6–7 μ wide at base, 4 μ wide at apex, dark brown; subsequent conidia develop by proliferation of conidiophore through conidial scar left by previous conidium so that conidiophore becomes annellate. Conidia are of the aleuriospores-type, developed terminally and subsequent conidia often still attached laterally to conidiophore, thick walled, globose or sometimes oval, with flattened bases encircled by minute but conspicuous frill, 19–26 μ in diam., with many granular contents, dark brown.

Hab.: On decayed wood, Shimoda, Shizuoka Pref., Oct., 1967.

Strain preserved: IFO–8934 Coll. K. Tubaki
Isol. T. Ito
Det. K. Tubaki

5. ***Oedemium didymum*** (Schw.) Hughes (Pl. 2 B) Hyphomycetes

Hughes, in Canad. Jour. Bot. **36:** 790 (1958); **41:** 774 (1963).

Batista *et al,* in Pub. Inst. Microb. Univ. Recife **283:** 15 (1960).

Colonies on the substratum black, dry, pulvinate and powdery. Conidiophores develop as erect branches of creeping hyphae, not well differentiated, scattered or in a loosely crowded group, septate, sparingly branched, brown to dark brown, tapering towards apex, 4.5–5 μ wide at lower part, 4–5 μ wide at upper part developing spherical swelling measuring 8–12 μ in diam.; swellings scattered along conidiophores occurring towards distal end of each cell, and apical swelling bears conidia. Conidia are of the blastospore-type, develop from apical globose or pyriform swelling of conidiophore which measuring 10–12$\times$8–10 μ, in a acropetal manner forming a chain; at first minute and hyaline protrusion, then swells into a globose, nonseptate to didymous typical structure; when matured, conidia are one-septate, more or less oblong, narrowed in middle, thick and smooth walled, 14–17$\times$9–12 μ, 8 μ wide at middle, dark brown and subhyaline at both ends, often with hyaline scars.

Hab.: On decayed fruit-body of *Hypoxylon* sp., in association with a fungi (*Acrostaphylus* sp.), Matsunoyama, Niigata Pref., Sept., 1964; did not cultivate.

Dried Specimen preserved: Herb. IFO. H–10881. Coll. K. Tubaki
Det. K. Tubaki

6. ***Speiropsis hyalospora*** Subramanian et Lodha (Pl. 2 C) Hyphomycetes

Canad. Jour. Bot. **42:** 1062 (1964).

On malt agar, growth moderate, compact woolly, with well developed aerial hyphae, dark olive brown to olive gray; reverse dark olive to almost black. Aerial hyphae septate, branched, 2–3 μ in diam., hyaline or subhyaline. Conidiophores dark brown, arise laterally from vegetative hyphae, erect, septate, 50–120 μ long, 5.5–6 μ wide at base, gradually tapering producing an apical cluster of sporogenous cells; sporogenous cells variable in size, bearing conidia, pale olive brown. Conidia consist of a main axis and usually two side arms; main axis composed of six to eight hyaline cells connecting each other by a narrow isthmus, bearing first arm on basal cell laterally and second arm on second cell from base oppositely to first one, 55–70 μ long; first arm, 44–60 μ long; second arm, 38–42 μ long; each cell measuring 9–11$\times$4–5(6) μ except for terminal cell.

Short, irregular shaped, thick walled, knob-like cells may develop directly on aerial hyphae.

Hab.: On dead leaves, Iriomote, Okinawa, collected by Dr. K. Aoshima, Forest. Exp. St., Jan., 1968.

Strain preserved: IFO–9081 Isol. T. Ito
Det. K. Tubaki

7. ***Sympodiella acicola*** Kendrick (Pl. 2 D) Hyphomycetes

Trans. Brit. Mycol. Soc. **41:** 519 (1958).

On malt agar, growth slow and restrict, only few mm in two weeks, compact woolly, raised at center, with sterile dark marginal hyphae, olive gray colored; reverse almost black. Conidiophores solitary, simple, septate, 62–200 μ long, 2.5–3.5 μ wide at base, 2.2–2.4 μ wide at apex, dark colored throughout except for paler apex; conidiophore increases in length with a number of scars as conidia develop. Conidia are of the radulaspore-type, developed acropleurogenously, in a dry and unbranched chain, cylindrical, 9–13$\times$1.8–2.2 μ, hyaline; a new growing point develops first behind a previous one and this process repeat many times resulting a sympodial increasing of a conidiophore. Secondary conidia developed from primary conidia acropetally by repeated extension and abstriction, then dry chain of conidia develop.

Hab.: On fallen leaves of *Pinus densiflora*, Kisaichi, Osaka, Feb., 1968.

Strain preserved: IFO–9082 Coll. K. Tubaki
Isol. T. Ito
Det. K. Tubaki

As described above, the primary conidia are of the radulaspore-type, however, those of secondary or tertiary develop by the manner of the arthrospore-type cutting off from the primary or secondary conidia respectively.

8. ***Thysanophora longispora*** Kendrick (Pl. 2 E) Hyphomycetes

Canad. Jour. Bot. **39:** 826 (1961).

On malt agar, growth rapid, spreading, thin and soft, almost velvety or woolly, with development of conidia at central area; aerial mycelium gray to green gray, yellowish gray at center as conidia develop; reverse olive to dark olive or almost black. Conidiophores solitary or sometimes clustered, with a single stipe bearing penicilli, up to 500 μ in height; stipes erect, simple, sometimes geniculate, often producing simple proliferations apically and repeatedly, 7–8 μ in diam. at base, 4–5 μ at apex, dark brown and paler above. Penicilli develop apically or laterally as stipes proliferate, consisting of metulae and phialides, subhyaline to pale brown; metulae 1–4 in number, 14–25 $\times$ 3–5.5 μ; phialides ampulliform, with a narrow-neck, 16–18 $\times$ 4–4.5 μ. Conidia are of the phialospore-type, 1–celled, long, slender, develop in a chain, 14–15 $\times$ 2.3–3.6 μ, subhyaline to pale brown.

Hab.: On a decaying fruit-body of *Russula xerampelina*, Experimental Forest of Mie Univ., Mie Pref., July, 1967.

Strain preserved: IFO–8842 Coll. K. Tubaki
Isol. T. Ito
Det. K. Tubaki

This species was originally found on needles of *Tsuga canadensis* by Kendrick (1961) and the present isolate was obtained on the decaying mushroom, however, the host mushroom was found in the forest where *Pinus* was growing in a mixed stand with *Tsuga*.

9. ***Tricellula curvata*** Haskins (Pl. 3 A) Hyphomycetes

Canad. Jour. Microbiol. **4:** 279 (1958); Petersen, in Bull. Torrey Bot. Club. **89:** 287 (1962).

On malt agar, growth moderate, somewhat restrict, flat, with inverted folds radiating from central raised portion, pale yellow cream to pale orange; reverse same colored and wrinkled. Conidiophores develop from hyphae, simple or sometimes compound, short, producing groups of branches, 5–13 μ long, 3.5–5 μ wide, hyaline. Conidia are of the blastospore-type, composed of three cells, one cell basal, one apical and third lateral; apical and lateral cells somewhat pointed at free-ends, slightly curved, measuring about 4.0–6.5 $\times$ 2.3–2.7 μ; basal cell more or less long oval or cylindrical, connecting by narrow protoplasmic isthmuses to other two cells, measuring 4–5 $\times$ 2–3 μ.

Hab.: Isolated from air, Nishiyamaguchi, Minoo, Osaka, Aug., 1964.

Strain preserved: IFO–8935 Isol. K. Mikata
Det. K. Tubaki

Conidia of the present fungus approaches to those of *Tricellula inaequalis* v. Beverwijk, but lateral cells of the former conidia arranged at nearly right angles to other two cells like in the description given by Haskins (1958) and the mode of germination of the present fungus is identical to that of the description. Colony color of the present fungus is orange differing from the description, however, distinctly paler than the culture of *T. inaequalis* (CBS-strain).

10. ***Trichurus spiralis*** Hasselbring (Pl. 3 B, C) Hyphomycetes

Bot. Gaz. **29:** 321 (1900); Lodha, in Jour. Indian Bot. Soc. **42:** 135 (1963); Morris, in Ser. Biol. Sci. West Illin. Univ. **3:** 137 (1963).

On malt agar, growth moderate, velvety, with producing synnemata at central one-third of a colony, dark olive to gray-olive; sterile margin wide; reverse gray-green. Synnemata erect, 0.8–2 mm in height, capitate, simple or rarely branched, consist of parallel dark brown hyphae measuring 4–4.5 μ in diam., usually upper half or one third fertile; hyphae of synnemata terminated by conidiophores which branched one to two (or more) times in compact manner. Terminal branch of conidiophore, cylindrical bearing two to four annellophores and hairs, slightly enlarged at apex, 5–9$\times$4 μ. Annellophores short flask-shaped, annellate at upper necks, 4.7–7.5$\times$ 2.0–2.5 μ, hyaline to pale brown. Conidia are of the aleuriospore-type, one-celled, oval, flattened at base, lemon-shaped at apical region, in a short chain, 4.0–5.0$\times$ 2.5–3.7 μ, pale brown. Hairs developed from a corner of terminal branch, once or twice spirally coiled or curved, long, 3.8–4.2 μ wide at base, gradually attenuated to 1–1.5 μ at tips, septate, dark brown and paler above.

Hab.: Isolated from sweet potato, Ibaraki, Osaka, Decm., 1967.

Strain preserved: IFO–8936 Isol. T. Yokoyama

Det. 〃

11. ***Volutina concentrica*** Penzig et Saccardo (Pl. 3 D, E) Hyphomycetes

Malpighia p. 257 (1901); Icon. Fung. Jawa p. 114 (1904); Subramanian, in Jour. Ind. Bot. Soc. **33:** 42 (1954).

Sporodochia scattered, variable in shape and size, mostly clavate, oval, oblong, pyriform or hemi-spherical, constricted at base, typically provided with several conspicuous setae, 136–400 μ broad, 123–300 μ in height, commonly 215$\times$150 μ, at first white, then creamy to yellowish white; setae straight, rounded at apex, more or less narrowed at base, rather thick walled, minutely punctate at apex, 125–400$\times$5–6 μ, hyaline. Hyphae in sporodochia radiate, giving concentric strata. Conidiophores developed from hyphae, compacted together, 1– to 2–times branched in penicillate-manner, 25–50$\times$2.0–2.5 μ, hyaline; phialides long, 7.5–25$\times$2–3 μ, hyaline. Conidia are of the phialospore-type, cylindrical, 4.5–5.5$\times$1.5–2.0 μ, hyaline. *Cephalosporium*-type conidial apparatus may occur directly from aerial hyphae under culture. Sporodochia well developed on malt agar or PDA, but larger than that in nature.

Hab.: On the squash (*Curcubita pepo*), Hokkaido, Oct., 1967.

Strain preserved: IFO–9083 Isol. T. Yokoyama

Det. 〃

Two additional collections were obtained from the sweet potato (*Ipomoea batatas* var. *edulis*), Ibaraki, Osaka, Nov., 1967, and from the dead root & stem of *Scutellaria baicalensis*, Fukuchiyama, Kyoto, June, 1967.

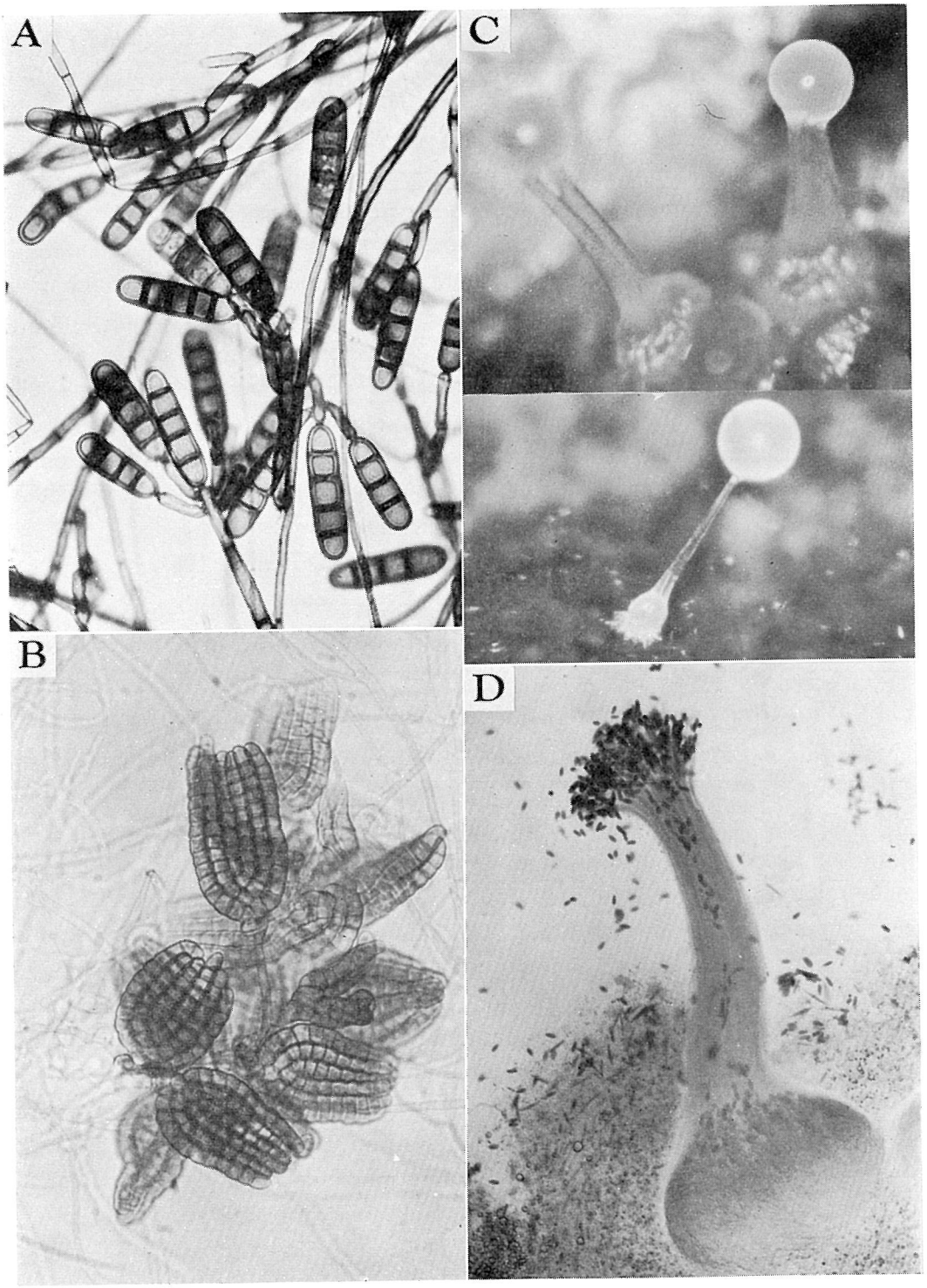

Plate 1. A. *Dendryphiopsis atra* (×250), B. *Dictyosporium elegans* (×250), C–D. *Hyalopycnis blepharistoma* Habit of pycnidia on the squash (Ultropak, ×38) and a crushed pycnidium (×100, stanied by Acid Fuchsin).

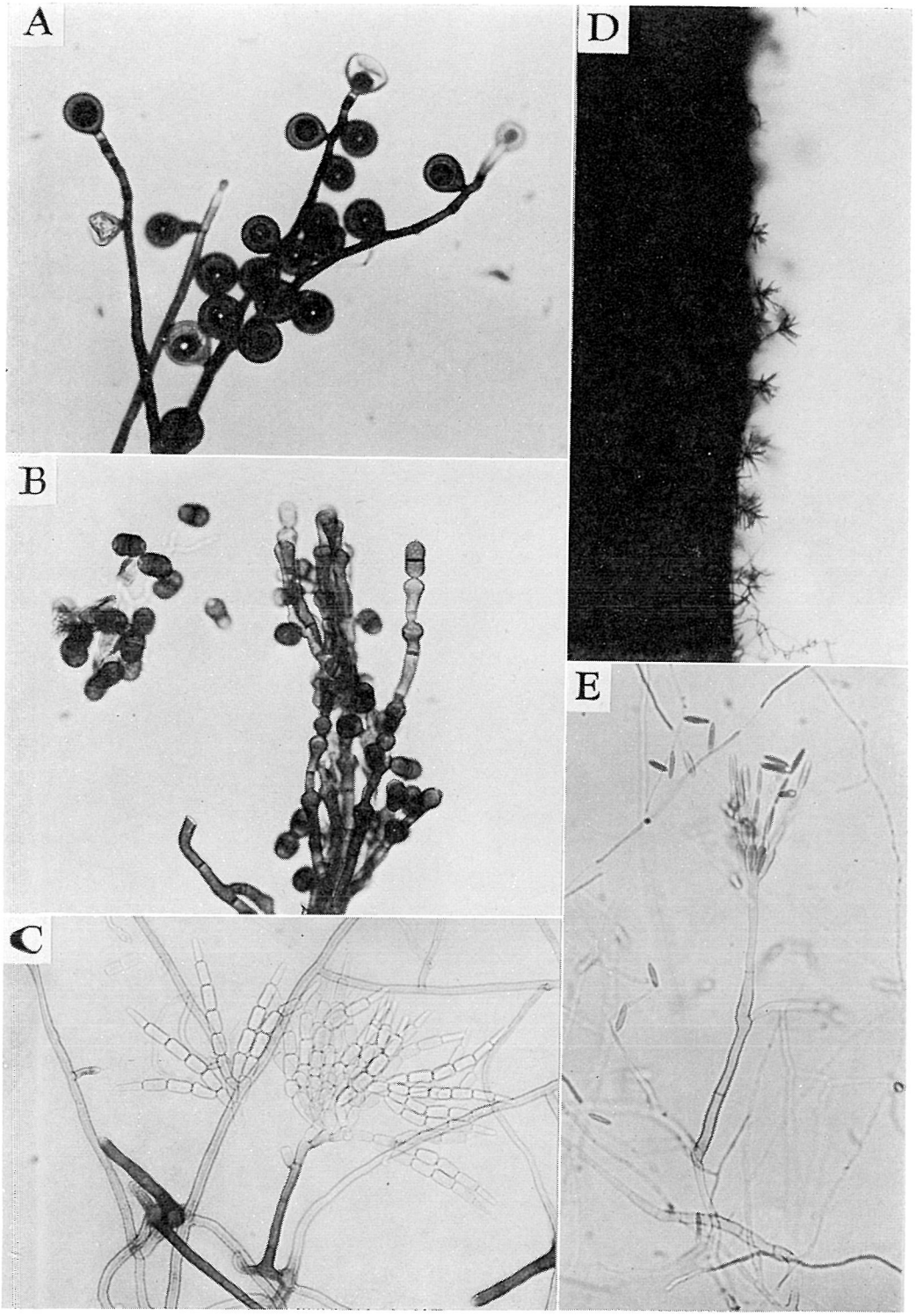

Plate 2. A. *Monotosporella sphaerocephala* (×250), B. *Oedemium didymum* (×250), C. *Speiropsis hyalospora* (×250), D. *Sympodiella acicola*, on the pine needle (×100), E. *Thysanophora longispora* (×250).

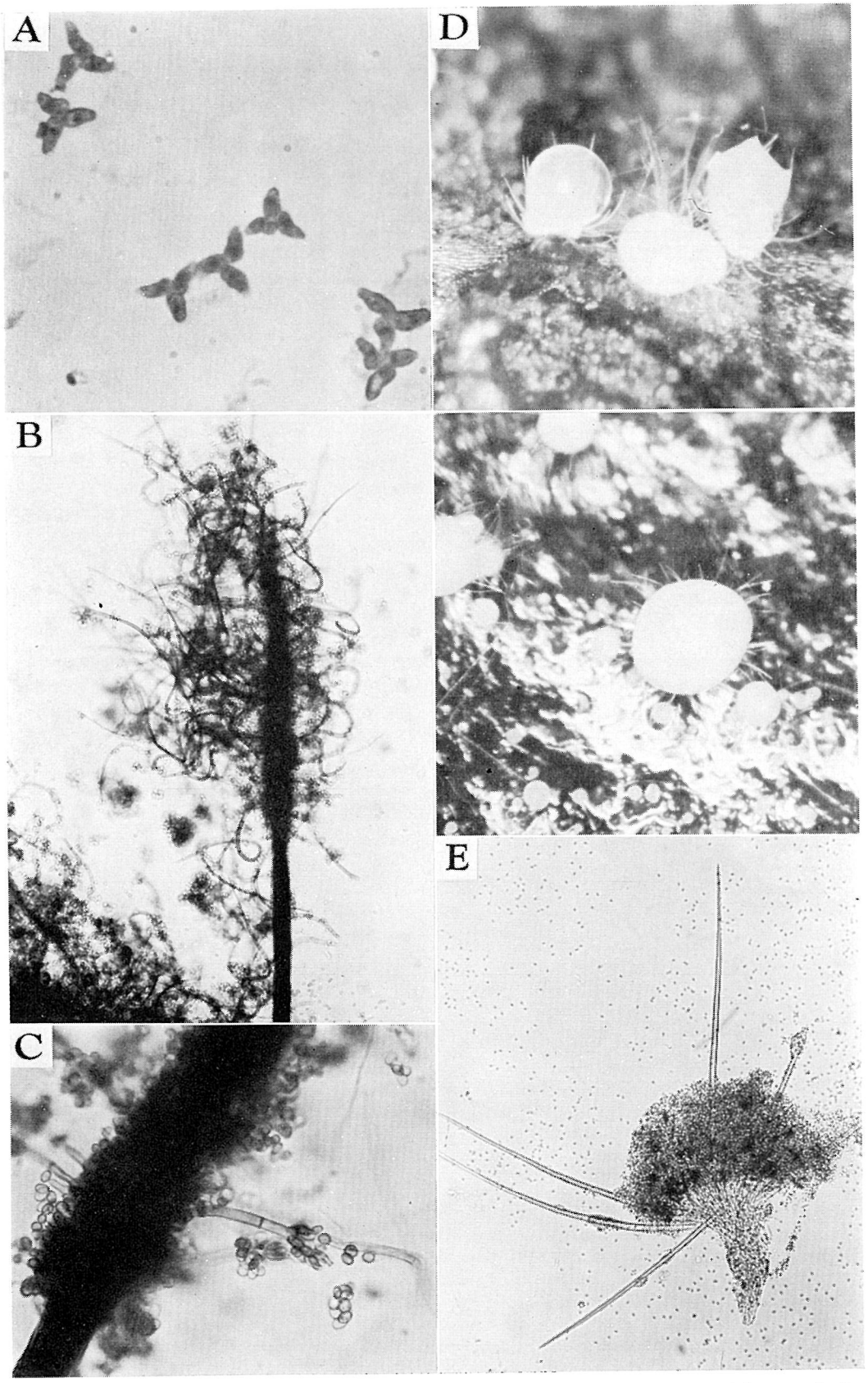

Plate 3. A. *Tricellula curvata* (×1000, stained by Acid Fuchsin), B–C. *Trichurus spiralis* (×100, ×400), D–E. *Volutina concentrica* D. Various shapes of sporodochia developed on the sterilized leaves of *Populus* sp. (upper) and *Quercus glauca* (lower) (Ultropak, ×38). E. A crushed sporodochium.

ADMINISTRATIVE REPORT

REPORT OF THE DIRECTOR

During the years of 1967 and 1968, there took place in Japan three international scientific meetings with which members of the research staff at the Institute had contacts as contributors. The International Symposium on Germfree Life Research entitled *Gnotobiotic Life in Medical and Biological Research* was held in Nagoya and at Inuyama on April 6–9, 1967. Dr. Kodama contributed to the Symposium a paper on a bacteriological study of diseases in silkworm by a gnotobiological method. The Twelfth International Congress of Genetics was held in Tokyo on August 19–28, 1968. Dr. Iijima presented a paper on a temperate phage ϕ170 at the Congress.

Under the sponsorship of UNESCO, the International Conference on Culture Collections was held in Tokyo in October, 1968, jointly by the Japanese National Commission for UNESCO and the Japanese Federation of Culture Collections, in cooperation with the International Association of Microbiological Societies. The organizing commission including myself for the Conference was constituted in March of the year. The Conference began on October 7 and was kept in session for five days. Dr. Tubaki introduced a long history of the mycological research in Japan going back to the early nineteenth century. Adopting resolutions to organize an *ad hoc* committee for the new International Federation of Culture Collections and to hold the next conference in 1972 in Czechoslovakia, the Conference closed with success on October 11. It was a great pleasure for the Institute to have received visits of many participants from abroad on the way of their post-conference tours.

The fourth edition of the IFO List of Culture was brought out in January last year. It contained 4508 names of mold, yeast and bacterial strains and 21 names of bacteriophages. Names of species in the items were given with the cooperation of the following mycologists to whom the director wishes to express his appreciation: Dr. K. Aoshima, Gov. Forest. Exp. St. (Order Aphyllophorales), Dr. T. Hongo, Shiga Univ. (Order Agaricales), Dr. T. Tominaga, Nat. Inst. Agr. Sci. (Plant pathogenic fungi) and Dr. S. Udagawa, Nat. Inst. Hyg. Sci. (Family Sordariaceae). With regard to activities of the Collection during the period under review, 791 strains of fungi and bacteria were obtained from either natural sources or through the courtesy of other organizations. With these additions the number of cultures in the Collection ran into 6954 at the end of 1968. Out of them 6049 subcultures were distributed among research organizations in Japan and abroad during the period of these two years.

February, 1969

Takezi HASEGAWA

DIRECTION COMMITTEE *alphabetical*

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Heartfelt condolences have to be offered to the late Professor Arao Imamura, Trustee of the Institute, who passed away on June 13, 1967.

RESEARCH PROGRAM FOR 1967-1968

1. Insect and acid-producing bacteria.
2. Taxonomic studies on Bacteria.
3. Studies on the marine bacteria.
4. Studies on *Rhodotorula* yeasts.
5. Taxonomic studies on microfungi.
6. Studies on the marine fungi.
7. Studies on phytopathogenic fungi.
8. Genetic studies on temperate phages.
9. Genetic change in bacterial metabolism.

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

1. Studies on the molecular basis of heredity. 1966–1968.
   Chief: Dr. Tetsuo IINO, National Institute of Genetics
   Personnel: Dr. Teiji IIJIMA

ABSTRACTS OF SCIENTIFIC PAPERS

Isao BANNO

Studies on the Sexuality of *Rhodotorula*

J. Gen. Appl. Microbiol., **13**: 167–196 (1967)

A sexual interaction was discovered between two strains of *Rhodotorula glutinis* considered as haploid. The nutritionally prototrophic mycelial colony was obtained by the conjugation and plasmogamy of cells of their auxotrophic mutants. The mycelial development was also found in the couple of their wild type cells. The mycelium has septa with clamp-connections, and consists of dikaryotic cells. It produces terminally brown pear-shaped resting spores which are regarded as diploid. The resting spore germinates to form a promycelium, on which haploid sporidia bud off laterally. Every sporidium propagates by budding and develops to the same haploid yeast as that of the original *Rhodotorula* strains. Among monosporidial yeasts, there are two mating types. The same mycelial stage appears again by conjugation of the two mating types. For the perfect stage of these strains of *Rhodotorula*, a life history is proposed schematically. It was concluded that this organism was related to the order Ustilaginales of Basidiomycetes. After the examination for sexual interaction in many couples of the authentic strains of *Rhodotorula,* the same mating reactions were found between IFO. 0559, IFO. 0413, or IFO. 0871 and IFO. 0880 or IFO. 1236. The mating types of the former three and the latter two were designated by symbols *A* and *a* respectively.

A monotypic new genus, *Rhodosporidium,* was proposed and a new species, *Rhodosporidium toruloides*, was given to the perfect stage of these *Rhodotorula* strains.

Teiji IIJIMA

High Frequency Transduction of Galactose Markers by Phage ϕ170

Japan. J. Genetics **42** (1): 1–10 (1967)

High frequency of transduction of the *Gal* markers was obsarved with lysates prepared from strains, *Gal*$^-$ (ϕ170 dg) (ϕ170) and *Gal*$^-$ (ϕ170) (ϕ170 dg). The former was obtained by superinfecting immune syngenote *Gal*$^-$ (ϕ170 dg) with ϕ170, the latter by transduction with *Gal*$^-$ (ϕ170) as a recipient. The two strains produced active

ϕ170 and showed the same immunity and segregational pattern.

Doubly lysogenic strains such as *Gal*$^-$ (ϕ170 dg) (λ), *Gal*$^-$ (ϕ170 dg) (434) and *Gal*$^-$ (ϕ170 dg) (ϕ80) were isolated by superinfecting immune syngenote *Gal*$^-$ (ϕ170 dg) with active phages. These strains liberated "active ϕ170" phage after UV induction. The proportion of "active ϕ170" in these lysates varied considerably and appeared to be determined by the superinfecting phages. Induction of *Gal*$^-$ (ϕ170 dg) (λ) yielded an Hft lysate.

Keisuke TUBAKI

An Undescribed Species of *Heleococcum* from Japan.

Trans. Mycol. Soc. Japan **8** (1) : 5–10 (1967)

From a wood panel submerged in seawater at Oshoro Bay, Hokkaido, an undescribed *Heleococcum* species, Eurotiaceae, was isolated and a new species, *Heleococcum japonense,* was proposed. The fungus differs from the type species, *H. aurantiacum* Jörg., in the smaller ascospores. A peculiar method of the conidial development was also described.

Keisuke TUBAKI

Studies on the Japanese Marine Fungi Lignicolous Group II

Pub. Seto Mar. Biol. Lab. **15**(5): 357–372 (1967)

Following to the previous paper (1966), sixteen lignicolous marine fungi were recorded from the sea around Japan for the first records of Japanese flora, including species of *Antennospora, Corollospora, Leptosphaeria, Lignincola, Remispora, Sphaerulina, Torpedospora, Clavariopsis, Humicola, Monodictys* and *Zalerion.* On the basis of taxonomic studies, the following two new species were described: *Remispora galerita* and *Sphaerulina albispiculata.*

Chihiro AYUZAWA*, Yoji FURUTA*, Reijiro KODAMA and Yugoro NAKASUJI

On the Synergism between the Virus and the Bacteria in the Development of Flacherie of the Silkworm, *Bombyx mori* L.

J. Sericult. Sci. Japan **37** (5): 395–402 (1968)

The present experiments were carried out to clarify the synergism between the viruses and the bacteria in the development of flacherie of the silkworm, *Bombyx mori* L. by aseptic and individual rearing.

The pathogens used were infectious flacherie virus and *Streptococcus faecalis-Str. faecium intermediate* G–27. The reasons why this bacterial strain was used were as follows: 1) this strain was isolated from the diseased silkworm; 2) this strain was supposed to be spread widely in Japan; 3) the pathogenicity of this strain for the silkworm was relatively low; 4) much bacteriological information on this strain was available; 5) this strain was not a pathogen of the septicemia in the silkworm. To avoid the propagation of the strain tested in a diet, the time of the inoculation by the diet infected with the bacteria was limited to 4 hours and the larvae were transferred into new tubes containing aseptic diet, and the transfer was repeated daily. Inoculation of the virus was carried out at the 3rd, 4th and 5th instars, with the bacteria at the 4th and 5th instars, and with the pathogens in both of these combinations.

The mortality ranged from zero to 20% when single pathogen was used, but when challenged with both pathogens, the mortality became much higher than those obtained with single pathogen, ranging as high as from 60 to 100%. Especially, the mortality increased when the larvae were challenged with the virus in the 3rd or 4th instar and subsequently with the bacterium at the 5th instar. As far as the present study was concerned, the synergistic association between the virus and the bacteria was clearly observed. The multiplication or propagation of the pathogens in the diseased larvae was investigated by bioassay or colony counting method. The number of cells of the bacteria per larva was over 10^8, on the other hand, infective titer of this virus was not neccesarily high.

* *Sericultural Experiment Station, Tokyo*

Teiji IIJIMA and Yutaka SAKAMOTO

Recombination Between Bacteriophage ϕ170 and Other Related Phages

Japan. J. Genetics 43(1): 43–48 (1968)

Defective phages ϕ170 dg recombined with the related phages λ, 434, 82 or ϕ80 when one of the related phages was superinfected to a defective lysogenic strain ϕ170 dg, or when a doubly lysogenic strain (ϕ170 dg) (ϕ) was induced with UV irradiation. The resulted particles having the immune specificity of ϕ170 always have the host range of the colysogenizing phage, that is, ϕ170 hy type.

When a defective lysogenic strain was spotted on a hetero-immune indicator, the recombinant type phage is responsible to form a lytic zone. The recombination was also confirmed by using various λ *sus* mutants. In lysates prepared from doubly lysogenic strain (ϕ170 dg) (λ *sus*) contained both ϕ170 hy and ϕ170 hy *sus*, or ϕ170 hy *sus* alone according to the *sus* markers used. From the recovery of the *sus* markers among ϕ170 hy particles, we may assume the defective region of ϕ170 dg.

Defective region of a given ϕ170 dg was also assumed by complementation test, using Pm^- (ϕ170 dg) and various λ *sus* mutants. From the results obtained by these methods, similarity of arrangement of genetic markers on the chromosome of λ and ϕ170 was assumed.

Reijiro KODAMA and Yugoro NAKASUJI

Application of Gnotobiotic Silkworm Larvae to Studies on Pathogenicity of Bacteria for the Insects.

M. Miyakawa and T.D. Luckey, Advance in germfree research and gnotobiology, CRC Press, Cleveland 417–423 (1968)

Investigation was made on the pathogenicity of some strains of lactic acid bacteria and Gram-negative bacteria, employing silkworm larvae reared aseptically on an artificial diet.

Of lactic acid bacteria examined, only certain strains of the genus *Streptococcus* brought about disease in larvae by feeding. Among the streptococci tested, only those strains which originated in silkworms had great pathogenicity. The pathogenic strains had a slight tendency to be capable of growing in media of relatively higher pH values than the nonpathogenic strains. However, not all of the streptococci with this ability caused disease. On the other hand, death of larvae was not hastened by injection of at least 10^3 of living cells of these streptococci. The pathogenic streptococci isolated were a group related to both *S. faecalis* and *S. faecium* taxonomically. One strain of them, E–5, grew rapidly in the gut of larvae within eighteen hours after food was withheld. Bacteria were found in the blood within 48 to 72 hours. Of the Gram-

negative bacteria tested, the strains belonging to the genera *Proteus, Pseudomonas* and *Serratia* except *S. plymuthica,* were pathogenic for larvae either by feeding or by injection. Strains of *Aeromonas hydrophila* and *Flavobacterium aquatile* were pathogenic only by injection. A mechanism of pathogenic effects of bacteria for silkworm larvae was discussed.

Ikuo NOGAMI*, Makoto KIDA*, Teiji IIJIMA and Masahiko YONEDA*

Studies on the Fermentative Production of Purine Derivatives.

Part I. Derivation of Guanosine and Inosine-Producing Mutants from a *Bacillus* Strain.

Agr. Biol. Chem. **32**(2): 144–152 (1968)

By the application of various genetical techniques, a number of guanosine and inosine-producing mutants were obtained from a *Bacillus* strain. The indispensable genetic characters of the mutants concerning with guanosine productivity were adenine requirement, lack of GMP-reductase and mutation to adenine and adenosine resistance from adenine and adenosine sensitiveness. Main products of these mutants were guanosine and inosine. Their culture broths also contained hypoxanthine, xanthine and sometimes a little guanine and xanthosine. Furthermore a few unknown nucleosides were also detected. The yields of guanosine were about 5 mg/ml in 4-day culture broths.

* *Microbiological Research Laboratories, Reseach and Development Division, Takeda Chemical Industries, Ltd., Osaka*

Keisuke TUBAKI

On Japanese Lignicolous Marine Fungi

Bull. Misaki Mar. Biol. Inst. Kyoto Univ. **12:** 195–202 (1968)

Methods of collection and examination of the Japanese marine fungi were described and a distribution map in Japan was presented.

Keisuke TUBAKI, Yosio KOBAYASI* and Masami SONEDA**

Enumeration of the Higher Fungi, Molds and Yeasts of Spitsbergen

Bull. Nat. Sci. Mus. **11**: 33–76 (1968)

From the Spitsbergen materials collected by Kobayasi in 1966, sixty-seven species including varieties were described. The molds, twenty eight species of *Mucor*, *Mortierella*, *Aspergillus*, *Beauveria*, *Catinula*, *Chrysosporium*, *Cephalosporium*, *Cylindrocarpon*, *Oidiodendron*, *Pachybasium*, *Penicillium*, *Phialophora* & *Cladosporium*, and the yeasts, six species of *Cryptococcus* & *Rhodotorula*, were reported for the first time from Spitsbergen.

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MISCELLANEOUS SCIENTIFIC PAPERS

1. Takezi HASEGAWA 1967. Japanese culture collections of microorganisms in the field of industry—Their Histories and actual state. Bulletin d'Information de la Centre International de Distribution des Souches et d'Information sur les Types Microbiens **2**(3): 43–49.

2. Takezi HASEGAWA 1967. An introduction to the yeast taxonomy (p. 3–9), Taxonomy of the anascosporogenous yeasts (p. 57–99). In Yoshitaka TAKAHASHI: "Science of the yeasts". Iwanami Shoten, Publishers, Tokyo. [in Japanese]

3. Keisuke TUBAKI 1967. Ecology of yeasts (p. 136–151). In Yoshitaka TAKAHASHI: "Science of the yeasts". Iwanami Shoten, Publishers, Tokyo. [In Japanese]

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PRESENTATION OF PAPERS AT SCIENTIFIC MEETINGS, 1967-1968

Author(s)	Title	Scientific Meeting of
T. IIJIMA & Y. SAKAMOTO	Transduction and genetic interaction of temperate phage ϕ170 in *Escherichia coli* K12.	Agricultural Chemical Society of Japan, Meeting in Tokyo (April, 1967)
R. KODAMA & Y. NAKASUJI	Application of gnotobiotic silkworm larvae to studies on pathogenicity of bacteria for the insects.	International Symposium on Germfree Life Research in Nagoya (April, 1967)
Y. NAKASUJI & R. KODAMA	Contributions to studies of the pathogenicity of *Streptococcus faecalis-Streptococcus faecium* intermediate E–5 and *Serratia piscatorum* E–15 for aseptically reared silkworm larvae.	Society of Sericultural Sciences of Japan, Meeting in Tokyo (April, 1967)
K. TUBAKI	On Japanese lignicolous marine fungi II	Botanical Society of Japan, Meeting in Kobe (October, 1967)
T. IIJIMA	Formation of lytic zone by defective lysogen.	Genetics Society of Japan, Meeting in Kobe (October, 1967)
T. IIJIMA & Y. SAKAMOTO	Genetic studies on a temperate phage ϕ170.	12th International Congress of Genetics in Tokyo (August, 1968)
T. IIJIMA & Y. SAKAMOTO	Formation and segregation of double lysogens of lambloid.	Genetics Society of Japan, Meeting in Hiroshima (October, 1968)
T. IIJIMA & Y. SAKAMOTO	Segregational pattern of a *rec*$^-$ double lysogen in *Escherichia coli* K12	*The same as above*
K. TUBAKI	Historical survey of the studies on microfungi in Japan.	International Conference on Culture Collections in Tokyo (October, 1968)
K. TUBAKI	On Japanese marine fungi III. Algicolous and foliicolous group.	Botanical Society of Japan, Meeting in Kumamoto (November, 1968)
Y. NAKASUJI & R. KODAMA	A study of the pathogenic process of bacterial diseases in aseptically reared silkworm larvae.	Society of Sericultural Sciences of Japan, Meeting in Kobe (November, 1968)

TABLE OF CONTENTS

No. 1, 1963

No. 2, 1965

No. 3, 1967

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