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REPORT OF THE DIRECTOR

The Institute for Fermentation Osaka (IFO) has been engaged in preservation and distribution of microorganism of importance to science and technology. For these purposes the institute has devoted its energies to basic research in the fields of toxonomy, physiology, biochemistry and genetics.

In the past two years (1975–1976) the institute has added about two hundred of strains to its culture collection, bringing the total number of strains preserved to about nine thousand at the end of 1976. In accordance with the increase in the number of strains preserved, the list of cultures 5th edition (1972) has required constant updating. In February, 1975 a supplement to the 5th edition was published which listed 183 strains of actinomycetes, 166 strains of bacteria, 125 strains of yeasts and 474 strains of fungi. We are aiming to publish a new edition of the list of cultures at the end of 1977. The number of cultures distributed by the institute past two years was about 14,000. Reexamination of characteristics of strains in the culture collection is being actively continued.

The Internationl Streptomyces Project (ISP) started in 1967 with basic philosophy of ensuring world-wide reproducibility and availability of authorized strains of the streptomyces to investigators. The committee for Confirmation of ISP Cultures in Japan conducted a second confirming test for the ISP strains preserved in IFO. All these strains were tested for their viability and some of their characteristics after five years preservation in a lyophilized state. Almost all the ISP strains were confirmed to have the characteristics, specific of the respective species.

In response to the increase in the number of strains for distribution and accession of cultures in IFO, a laboratory manual for acquisition, accession, deacquisition and distribution of cultures as well as for culture data was completed in 1976. This promoted efficiencey in the routine handling of cultures and reduced the time between receiving of orders and shipping of cultures.

In 1975 and 1976 the institute underwent great changes. At the annual meeting of the Board of Trustees in 1976, the members of the Board resolved to reorganize the Board, and nominated the members of new Borad of Trustees and Councilors. Changes in the research staff during the past two years are as follows. Dr. Takezi Hasegawa retired from the position of director after 15 years of service, and was succeeded by Dr. Teiji Iijima. Dr. Koiti Nakazawa retired after many years of his research activity in February, 1976. He contributed toward confirmation of the actinomycetes preserved in the IFO culture collection. Dr. Keisuke Tubaki moved to the Universtiy of Tsukuba as the Professor of the Institute of Biological Science in March, 1976. Dr. Ken-ichi Sasajima joined the staff of the biochemistry and genetics section. The institute now comprises three sections, the bacteriology and yeast science section under Dr. Isao Banno, the mycology section under Dr. Tatsuo Yoko-

yama and the biochemistry and genetics section under the Dr. Ken-ichi Sasajima as well as an administrative office under the treasurer Mr. Ihei Tanida. The main research themes of these sections are as follows. Bacteriology and yeast science section: Tricarboxylic acid transport systems in enteric bacteria, examination of yeast flora in Japan, and preservation of bacteria and yeasts by L-drying. Mycology section: Studies on fungal flora in rice paddy fields, ecological studies on leaf-litter fungi. Biochemistry and genetics section: Studies on carbohydrate metabolism mutants of Bacillus subtilis and on phage typing in *Pseudomonas aeruginosa.*

Drs. Tubaki and Yokoyama have joined a research project organized by the National Food Research Institute, the Ministry of Agriculture and Forestry, Japan, examining the flora of thermophilic fungi of imported feed grains, in 1975–1976.

At the annual Insernational Post-graduate University course in Microbiology in October 1975 and 1976, Drs. Hasegawa, Tubaki and Banno gave lectures for this for this tranining course. At the General Meeting of International Society of Human and Animal Mycology (ISHAM) held in Tokyo in July 1975, Dr. Tubaki presented a paper on arsenic gas production from annellation forming fungi. And at the third International Conference on Culture Collections (ICCC) held in March 1976 in Bombay, India, under the sponsorship of UNESCO, UNEP, ICRO, IUBS, WFCC and University of Bombay, Dr. Hasegawa as the representaive of Japan Federation of Culture Collections (JFCC), read a paper on the activity and organization of the JFCC. During this conference, the WFCC General Meeting was held in Bombay, at which Dr. Hasegawa was elected to Vice president of WFCC.

The chairman of the Board of Trustees, Chobei Takeda was decorated in recognition of his brilliant service to the pharmaceutical industries and to public health, in May 1976. Professor emeritus Kakuo Kitahara, Councilor of the IFO was decorated in recognition of his invaluable contribution to Microbiology and Eduction, in November 1976.

(T. Iijima)

Heartful condolences are offered to

Professor emeritus Toshinobu Asai who passed away on 24th August, 1975.

Professor Koji Ando who passed away on 24th February, 1976.

Profeasor Koichi Ogata who passed away on 4th September, 1976.

They gave great contributions to the establishment and the development of the Institute for Fermentation Osaka.

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3–5, 1977 (March)

THE ACTIVITY AND ORGANIZATION OF THE JFCC*

Takezi HASEGAWA

The maintenance of a culture collection of microorganisms needs much more painful labor and more expensive facilities than that of dried specimens of higher plants, because the collection really consists of many kinds of living cells. The experts in charge, even now, are troubled with spontaneous mutation or death of cultures in spite of recent progress in the methods for keeping cultures.

The Second World War caused serious damage to many local culture collections the world over, and this prompted the establishment of an international system of cooperation in the maintenance and exchange of cultures of microorganisms. The International Federation of Culture Collections of Microorganisms (IFCC) was established at the advice of the International Association of Microbiologists in 1949. This event prompted us to form a national federation, which was founded in 1951 with the support of Japanese Ministry of Education and named The Japan Federation for Culture Collections (JFCC). Although the IFCC had been operated only until 1954, this national federation has continued without interruption.

In 1953, Professor Kiyoshi Kominami (1), President of the JFCC, published under the auspices of Japan Ministry of Education a general catalogue of the microbial cultures preserved throughout Japan including their sources and histories. This catalogue gave very useful material for our subsequent work. Professor Kin'ichiro Sakaguchi, Vice-president, organized an official research team for the purpose of gathering all the listed cultures and confirming their properties. This cooperative research continued for six years from 1954. As a result, "The JFCC Catalogue of Cultures" was issued in 1962, and supplements were published in 1966 and 1968.

In 1962, the Japanese Government proposed a Unesco programme; "Promotion of Research on Microorganisms" from the viewpoint of a contribution to the food problems in the developing world and also of the fostering of an international network of culture collections. This ten-year programme by Unesco began in 1965. The first International Conference on Culture Collections was held in Tokyo by the JFCC and the Japanese National Commission of Unesco. This conference (2) contributed greatly to global knowledge of culture collection management, and in acquainting culture collections the world over with each other. It was a great pleasure to the Japanese people that the foundations of the World Federation for Culture Collections

* This paper was presented at The Third International Conference on Culture Collections in Bombay, 1976 by the auther who is deeply obliged to the UNEP/Unesco/ICRO Panel on Microbiology, the ICCC-III Organizing Committee, and the JFCC for their financial support to his attendance.

and of the World Data Center in Brisbane resulted from the resolutions of this conference.

Since 1973, the Japanese Government has held an international post-graduate university course in microbiology for students in the Asian developing countries at the Osaka University and several other academic establishments in Japan every year. The JFCC sends taxonomists to this course as members of the teaching staff.

A list of the current organization of the JFCC and its member collections is given in the table. The membership is open to individuals as well as culture collections. The federation is now preparing to institute the sustaining membership for financial support. A general assembly is held annually, where the problems confronting each of the member collections are reported together with the results of gathering and distributing cultures. There are significant differences between the member collections in their histories of development, and their present circumstances, are not necessarily directed towards the specialization in the microbiological fields. Many categories need to be further specialized. A successful form of organization of a national federation of culture collections of microorganisms would be a network consisting of

JAPAN FEDERATION FOR CULTURE COLLECTIONS

AGRICULTURAL

National Institute of Agricultural Sciences, Tokyo.

Wood-rotting fungi:

Government Forest Experiment Station, Tokyo.

Veterinary microorganisms:

National Institute of Animal Health, Tokyo.

INDUSTRIAL

The Institute of Applied Microbiology, The University of Tokyo, Tokyo.
Faculty of Agriculture, Hokkaido University, Sapporo.
Faculty of Agriculture, University of Tokyo, Tokyo.
Faculty of Engineering, Osaka University, Osaka.
Faculty of Engineering, Hiroshima University, Hiroshima.

MEDICAL

The Institute of Medical Science, The University of Tokyo, Tokyo.
Research Institute for Microbial Diseases, Osaka University, Osaka.
Faculty of Medicine, University of Tokyo, Tokyo.

SPECIALIZED

Actinomycetes:

Research Institute for Chemobiodynamics, Chiba University, Chiba.

Ascomycetes & Mycotoxin-producing fungi:

National Institute of Hygienic Sciences, Tokyo.

Drug-resistant bacteria:

School of Medicine, Gunma University, Maebashi.

Microorganisms for sake brewing:

National Research Institute of Brewing, Tokyo.

GENERAL

Institute for Fermentation, Osaka.

a small number of center collections, and of a great number of research institutes each having a distinctively specialized culture collection but working in close cooperation with the center collections. I think the goal is still far off.

References

1) Higher Education & Science Bureau, Ministry of Education, Japan. 1953. A General Catalogue of the Cultures of Microorganisms Maintained in the Japanese Collections. 186 pp. Kasai Publ. Print. Co., Tokyo.
2) Iizuka, H. and T. Hasegawa (ed.) 1970. Proceedings of the First International Conference on Culture Collections. 625 pp. University of Tokyo Press, Tokyo.

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7–17, 1977 (March)

ISOLATION OF YEASTS BY ENRICHMENT METHOD

Isao BANNO and Kosaburo MIKATA

Summary

In the isolation of yeasts from natural substrates by enrichment method, the efficiencies of enrichment of four media, orange, malt, nitrate, and ume were examined. The enrichment method allowed isolation of yeasts which could not be isolated by direct plating. The predominant yeasts in the enrichment culture changed with the incubation period. The yeasts isolated from a particular sample varied considerably with the enrichment medium. The efficiencies of the orange, malt, and nitrate media were approximately equal; about 45 % of the yeasts on samples could be isolated with one of these media. The efficiency of the ume medium was very low; only 15 % of the yeasts was isolated. It is suggested that enrichment with two media would allow isolation of, on average, 75 % of yeast strains on a sample. There was not recognized specific relation between the enrichment medium and the kind of yeast isolated.

In survey of yeast flora on substrates of the field, enrichment method is necessary for the isolation of yeasts from substrates in which the population of yeast is minute and filamentous fungi are predominant. A differential medium permitting only yeast to grow while preventing fungal growth has not been devised yet.

Many researchers have reported the isolation of yeasts from natural substrates by enrichment culture in media ordinarily used in the cultivation of yeasts[2]. But there have heen remarkably few studies concerning the extent to which yeasts can be isolated selectively by enrichment method. We have attempted to isolate yeasts from samples of various substrates collected in the forest in order to examine the yeast flora on them. In the process of isolation of yeasts by the enrichment method, the yeast-isolating efficiency of the method was examined. The present paper described the efficienices of the four kinds of enrichment media used. With none of these media, more than a half of yeasts on samples could be isolated. But use of two media, orange and malt allow the isolation of about 75 % of yeasts isolated with the four media.

Materials and Methods

Samples for isolating yeasts included flowers, leaves, decayed leaves, tree bark, soil etc., which were collected in the forest of Mt. Odaigahara, Mt. Daisen and The Yaku-island.

The following 4 media were used for enrichment culture:

1 Orange medium
condensed orange juice (5-fold conc.) 200 ml, glucose 50 g, distilled water 800 ml, pH 3.3.

2 Malt medium
malt extract dehydrated (Difco) 30 g, glucose 70 g, chloramphenicol 10 mg, distilled water 1000 ml, adjusted to pH 4.5.

3 Nitrate medium
KNO_3 2 g, glucose 50 g, KH_2PO_4 1 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, NaCl 0.1 g, $CaCl_2$ 0.1 g, vitamin-mixture (100-fold conc.) 10 ml, chloramphenicol 20 mg, distilled water 1000 ml, adjusted to pH 4.5.

4 Ume medium
Ume-plum* (500 g) was preserved in sugared water (250 g of glucose in 100 ml of distilled water) for two months. The juice exudating from the fruits was separated by filtration with gauze and made up to 1000 ml with distilled water, adjusted to pH 4.5.

The orange medium was chosen from natural media because of its ready availability. The malt medium was used because of its popularity in the yeast research. the synthetic nitrate medium containing potassium nitrate as the sole nitrogen source was used to select nitrate-utilizing yeasts. And the ume medium was used on the basis of the idea that the medium might select novel yeasts since the juice of ume-plum has a weakly antiseptic activity. The orange medium was adjusted to pH 3.3 and an antibiotic, chloramphenicol, was added to the malt and nitrate media to control bacterial growth.

The use of fungistatic agents, propionate and diphenyl, was suggested by Hertz and Levine[4]. But since the two agents were reported to suppress the growth of some yeasts by Buhagiar and Barnett[3], we did not include them in the enrichment cluture.

A piece or a spoonful of samples of the substrates was inoculated into 10 ml of the media in 15 mm ϕ test tubes and incubated at 25 C without shaking. On the 7th and 14th days after inoculation, the fungus-mat which appeared on the surface of the medium was removed and a loopful of the culture liquid was spread on YM agar plates. The plates were incubated at 25 C. On most plates yeast colonies appeared among significant numbers of fungal colonies. The colonies on the plates were inspected with the naked eye and with a dissecting microscope after 2 to 7 days. Yeasts from all of colonies differing in appearance were picked and purified by further plating.

All the isolates were microscopically examined and distinguished on the basis of their micro- and macro-morphology. We regarded as the same strain the yeasts isolated from the same sample which showed the same morphological characteristics. The isolates were determined according to the standard procedures[1,5] in yeast classification.

Results and Discussion

Comparison between yeasts isolated by the direct plating and by the enrichment method

*: Fruits of *Prunus mume* Sieb. & Zucc.

In a preliminary experiment, several samples were added to the malt medium and vigorously agitated to disperse microorganisms. Then a portion of the medium was immediately spread on YM agar plates and the remaining medium was incubated at 25 C for enrichment. Yeasts from the colonies on the directly plated dishes and those spread on the plates after the enrichment culture for 7 days were isolated by the method described above.

Table 1. Comparison of isolates between direct plating and enrichment methods.

Sample	Direct	Enrichment
D10 (F)*	10d1*2 10d2 10d4 10d5 *Sporobolomyces* sp. 10d8	10d2*2 10a3
D11 (F)	11d1 11d2 11d3	11d1 11d3 *Hanseniaspora valbyensis*
D13 (F)	13d1 13d4	*Saccharomyces chevalieri* 13d1
D24 (B)	*Kloeckera* sp. 24bd1 24ba3	*Hanseniaspora uvarum* *Saccharomyces cerevisiae* 24aa12 24ba1
D37 (L)	no	*Kloeckera* sp. *Saccharomyces cerevisiae* *Pichia membranaefaciens*
D38 (L)	no	*Pichia membranaefaciens* 38m2
D29 (T)	no	*Pichia dispora*
D34 (T)	no	*Kluyveromyces veronae* *Saccharomyces cerevisiae* 34a8
D107 (S)	no	*Hanseniaspora occidentalis* *Saccharomyces cerevisiae* *Kleockera* sp. 107a2
D112 (S)	no	*Debaryomyces cantarellii*
D116 (S)	116d1	*Kluyveromyces veronae*

( )*: F, flower; B, berry; T, tree bark; S, soil.
*2: Code of strain isolated.

The yeasts isolated by the direct plating were compared with those isolated after the enrichment. The results obtained with 11 samples are presented in Table 1. Sporogenous strains were completly identified, but asporogenous strains were not specifically identified and will be expressed in strain code below. Yeasts were isolated by direct plating from samples D10, D11, D13 and D24 of flowers and fruit. The enrichment method enable us to isolate different yeasts from the direct isolates from the same samples. But from samples D37, D38, D29, D34, D107, D112 and D116 of decayed leaves, tree bark and soil, yeast could hardly be isolated by the direct plating, because an overwhelming population of fungi covered the plates. However the enrichment method allowed isolation of yeasts from these samples. The enrichment method is necessary for isolation of yeast from the natural substrates such as decayed leaf and soil in which the population of yeast is minute.

Difference in the kinds of yeasts isolated with the period of enrichment

A comparison was made between the yeast strains isolated after 7 days enrichment culture and those isolated after 14 days, using the 4 media and 108 samples in each. Table 2 presents the results of eight representatives of the 108 samples. In samples OD33 and DA208 yeast was not found after 7 days incubation but was found after 14 days. In samples DA203 and DA205, the same strains as those isolated after 7 days enrichment culture and new strains were found after 14 days enrichment culture. In samples OD63 and YA303, some of the isolated after 7 days were not isolated after 14 days when different yeasts were found. In samples DA200 and DA206, yeasts isolated after 14 days differed completely from those isolated after 7 days. These examples indicate that the kinds of yeasts and their population in the enrichment culture change during the period of incubation. Therefore it is desirable to isolate yeast from the enrichment culture at intervals.

Difference in yeasts isolated in the four enrichment media

It was presumed that the differences in composition and physiological conditions of the enrichment media would result in the selection of different kinds of yeasts from the samples. So the yeasts isolated from the same sample by using the 4 media were determined and compared.

The kinds of yeasts isolated from most samples after the enrichment varied with the medium used. Representative results obtained with 12 samples are given in Table 3. In samples YA311, OD117, OD3, YA303, YA305, OD32 and OD116, some of yeasts isolated were common to two or three of the four media while others were restricted to one of the media. In samples OD38, OD104, and DA200, the four media selected different yeasts.

Isolating efficiency of the enrichment medium

In order to evaluate the isolating efficiency of the enrichment medium, we calculated a coefficient:

Table 2. Variation in isolates with period of enrichment culture.

Sample No.	Name or code of isolated strain	7 days	14 days
OD33 (T)*	*Pichia* sp.		+
[O]**	33a2		+
DA208 (L)	*Debaryomyces cantarellii*		+
[M]	*Kluyveromyces veronae*		+
	208m1		+
	208m3		+
DA203 (L)	*Kloeckera* sp.	+	+
[U]	*Saccharomyces uvarum*	+	+
	203u1	+	+
	203m1	+	+
	Debaryomyces hansenii		+
	Kluyveromyces veronae		+
DA205 (L)	205u1	+	+
[U]	*Kloeckera* sp.		+
	Pichia quercuum		+
	Saccharomyces cerevisiae		+
OD63 (A)	*Saccharomyces mrakii*	+	
[O]	63a1	+	+
	Saccharomyces florentinus		+
YA303 (S)	*Saccharomyces florentinus*	+	
[N]	303k4	+	
	Hansenula saturnus	+	+
	Rhodotorula sp.	+	+
	303k3	+	+
	Pichia membranaefaciens		+
DA200 (L)	*Pichia dispora*	+	
[O]	200a1	+	
	Saccharomyces cerevisiae		+
DA206 (L)	206m1	+	
[M]	*Pichia dispora*		+
	Saccharomyces cerevisiae		+
	Saccharomyces kluyveri		+

( )* : A, mushroom; F, fallen leaf; L, decayed leaf; S, soil; T, tree bark.
[ ]**: Enrichment medium used, O, orange medium; M, malt medium; N, nitrate medium; U, ume medium.
+ sign represents isolation of a given strain.

Table 3. Comparison of isolates among the 4 enrichment culture media.

Sample No.	Name or code of isolated strain	Medium			
		O	M	N	U
YA311 (L)*	*Kluyveromyces africanus*	+	+	+	
	Saccharomyces rouxii	+			
	Kloeckera sp.	+			
	Hansenula saturnus		+		
	Kluyveromyces veronae		+		
	311k1			+	
OD117 (S)	*Hansenula saturnus*		+	+	
	Kloeckera sp.	+			
	Pichia dispora		+		
	117m2		+		
	117m4		+		
	117 k1			+	
	117 k2			+	
OD3 (F)	3a4	+			+
	Saccharomyces unisporus	+			
	Saccharomyces cerevisiae		+		
	3k1			+	
	Pichia pinus				+
YA303 (S)	303a4	+			
	303a2	+	+		+
	Hansnula californica		+		
	303m6		+		
	303 k5		+		
	Hansenula saturnus		+	+	
	Rhodotorula sp.		+	+	
	Saccharomyces florentinus.	+		+	
	Pichia membranaefaciens			+	
	303 k3			+	
	303 k4			+	
	Saccharomyces bailii				+
YA305 (S)	*Kluyveromyces africanus*	+			
	Saccharomyces unisporus	+			
	Debaryomyces hansenii	+	+	+	
	Pichia membranaefaciens	+	+		+
	Hansenula saturnus		+	+	
	305m4		+		
	Rhodotorula sp.			+	
OD124 (S)	*Hansenula californica*	+	+	+	

Table 3. (continued)

Sample No.	Name or code of isolated strain	Medium			
		O	M	N	U
OD32 (T)	*Saccharomyces cerevisiae*	+	+	+	+
	Saccharomyces florentinus	+	+	+	+
	Pichia dispora	+			
	Pichia fluxuum		+		+
OD116 (S)	*Hansenula saturnus*	+	+	+	+
	116 a3	+	+		+
	116m2		+	+	+
	Hansenula beijerinckii	+			
	116 a5	+			
OD106 (S)	*Pichia dispora*	+			
OD38 (T)	38 a1	+			
	38 a2	+			
	38m1		+		
	Pichia pinus		+		
OD104 (S)	104 a1	+			
	104m1		+		
	104m2		+		
	Hansenula californica			+	
	104k1			+	
	104k3			+	
DA200 (L)	*Pichia dispora*	+			
	Saccharomyces cerevisiae	+			
	200a1	+			
	Saccharomyces bayanus		+		
	200m2		+		
	200u 1				+

See footnote of Table 2.

$$C_{xy} = \frac{N_{xy}}{N_x}$$

where N_x is the total number of strains isolated from sample x by using all the four media and N_{xy} is the number of strains isolated from the sample x by using only the medium y.

From the 108 samples, yeasts were not isolated by the direct plating but isolated by the enrichment method. The isolating coefficients of the four media were calculated in every cases of isolation of yeast from the 108 samples. The results are given in Table 4. More than one strain was isolated from each of 80 of these samples. In most of the 80 samples, more than one of the four media were necessary

Table 4. The isolating coefficient of the medium in 108 samples.

Sample No.	Medium				Sample No.	Medium			
	O	M	N	U		O	M	N	U
OD1	1/2	1/2	0	0	OD105	2/4	1/4	2/4	0
2	1/3	2/3	1/3	0	106	1/1	0	0	0
3	2/5	1/5	1/5	2/5	107	2/5	2/5	3/5	1/5
4	0	3/4	2/4	0	108	1/2	1/2	0	0
5	1/4	2/4	2/4	0	109	1/2	0	1/2	0
6	2/4	2/4	1/4	1/4	110	2/2	0	0	0
7	1/1	1/1	0	0	111	1/2	0	2/2	0
8	1/1	0	0	0	112	1/1	0	1/1	0
9	1/1	0	1/1	0	113	0	1/1	1/1	0
10	0	0	0	3/3	114	0	0	1/1	0
11	0	1/1	1/1	1/1	116	4/5	3/5	2/5	3/5
12	0	2/2	1/2	0	117	1/7	4/7	3/7	0
13	1/2	1/2	1/2	0	119	1/2	0	1/2	0
14	1/2	2/2	0	1/2	120	0	0	1/1	0
15	1/2	1/2	0	1/2	122	1/2	1/2	1/2	0
16	0	1/1	0	0	123	2/4	1/4	2/4	1/4
18	0	1/1	1/1	0	124	1/1	1/1	1/1	0
21	1/1	0	0	0	125	0	2/2	1/2	0
22	1/1	1/1	0	0	126	2/2	0	0	0
23	0	0	1/1	0	127	1/3	0	2/3	0
25	1/1	0	1/1	0	128	3/7	2/7	3/7	0
26	1/1	0	0	0	129	2/2	0	1/2	0
27	2/2	0	0	0	131	0	0	1/1	0
28	1/1	0	0	0	132	1/4	0	4/4	0
29	2/3	0	1/3	1/3	133	1/2	0	1/2	0
30	1/2	1/2	0	0	134	0	1/1	0	0
32	3/4	3/4	2/4	3/4	135	1/5	2/5	3/5	0
33	2/4	2/4	1/4	0	136	2/4	2/4	2/4	0
34	4/7	2/7	4/7	4/7	137	1/2	0	1/2	0
35	2/2	2/2	0	1/2	138	0	0	1/1	0
36	0	0	1/1	1/1	142	1/2	1/2	1/2	0
37	1/1	0	1/1	0	143	0	1/1	0	0
38	2/4	2/4	0	0	144	2/4	0	2/4	0
39	0	1/1	0	0	145	1/3	2/3	1/3	0
40	0	0	0	1/1	146	0	1/3	3/3	0
41	0	0	1/1	0	DA200	3/6	2/6	0	1/6
42	0	1/1	0	0	201	1/5	1/5	3/5	1/5
45	1/2	1/2	0	0	202	3/4	2/4	1/4	1/4
46	0	1/1	1/1	0	203	3/11	5/11	2/11	6/11
103	2/2	1/2	2/2	0	204	1/4	3/4	0	1/4
104	1/6	2/6	3/6	0	205	1/7	1/7	2/7	4/7

Table 4. (continued)

Sample No.	Medium				Sample No.	Medium			
	O	M	N	U		O	M	N	U
DA206	1/6	4/6	2/6	2/6	YA310	1/1	0	0	0
207	1/5	3/5	1/5	5/5	311	3/6	3/6	2/6	0
208	1/5	4/5	0	1/5	312	2/2	2/2	2/2	0
209	0	4/8	2/8	5/8	313	2/5	2/5	2/5	1/5
YA300	1/6	4/6	4/6	1/6	314	1/1	0	0	0
301	0	2/3	3/3	0	315	3/5	2/5	1/5	0
302	2/5	2/5	4/5	0	316	3/4	2/4	1/4	1/4
303	3/12	6/12	6/12	2/12	317	1/1	0	0	1/1
304	1/5	3/5	3/5	1/5	318	2/3	2/3	1/3	0
305	4/7	4/7	3/7	1/7	319	3/6	3/6	3/6	0
306	1/3	3/3	1/3	0	320	1/1	0	0	0
307	1/4	2/4	2/4	0	321	3/6	2/6	4/6	3/6
308	1/3	1/3	2/3	0					
309	1/3	1/3	3/3	0	mean	0.46	0.42	0.43	0.15

to isolate all strains. No all-purpose enrichment medium for all samples was found among the the four media. The maximum number of strains isolated from a single sample was 12, that was found in sample YA303 of soil.

The mean value of the isolating coefficient of the orange, malt, nitrate, and ume media for the 108 samples were 0.46, 0.42, 0.43 and 0.15 respectively. These mean values indicate that when only one medium is used for enrichment, we can statistically expect to isolate less than a half of the yeasts in samples. The ume medium shows a low value of the coefficient, and is probably not suitable for isolation of yeast. The values of the orange, malt, and nitrate media are approximately equal and none of them is superior in the isolating efficiency. When both the orange and malt media were used for enrichment, the mean value of the coefficient was 0.76. Obviousely a variety of media should be used for the enrichment in order to isolate as many yeasts from the substrate as possible, but even the use of the two media will allow isolation of about 75 % of the yeast strains on substrates.

A total of 334 strains were isolated from the 108 samples by using the four enrichment media. Using only the orange medium 135 isolates were obtained from 83 samples; using the malt medium 146 from 71 samples; using the nitrate medium 139 from 76 samples; using the ume medium 63 from 33 samples.

Relation between the medium and the yeasts isolated

In order to examine whether a particular enrichment medium selectively favors the growth of specific yeasts, the frequency of isolation of the ascosporogenous species from the 108 samples was determined for the four media. The data obtained are given in Table 5.

Table 5. The number of strains of sporogenous Species isolated from 108 samples with each enrichment culture medium.

Species	Orange	Malt	NO_3	Ume
Debaryomyces				
cantarellii		○○		
hansenii	○	○○	○	○○
vanriji	○			
Hanseniaspora				
uvarum		○		
Hansnula				
anomala	○	○	○○	
beijerinckii	○			
californica	○○	○○○	○○○○○	○
capsulata	○	○		○
saturnus	○○○	○○○○○○○○○	○○○○○○○○○	○○
Kluyveromyces				
africanus	○○○○○○○○○	○○○○○○○	○○○○○	
veronae	○○○	○○○○	○	○○
Pichia				
dispora	○○○○○○○○○	○○○○○	○○○○	○○
fluxuum		○○		○
membranaefaciens	○	○○	○○	○○○○
pinus		○○	○	○○
quercuum				○
saitoi	○			
sp. n.	○			
Saccharomyces				
bailii				○
bayanus	○○○	○○○	○○	
cerevisiae	○○○	○○○○○○○○○	○○○○○○	○○○○
florentinus	○○○○	○○○	○○○	○○○○
globosus		○○	○	
kluyveri		○○		○
mrakii	○			
rosei				○
rouxii	○			
unisporus	○○○○	○	○	○○
uvarum	○○○	○	○	○○○
sp. n.	○			

The number of strains is expressed by the number of circle

With the ume medium all species were isolated with lower frequency. The nitrate medium selected not only nitrate-utilizing yeasts but also nonutilizing yeasts. The latter seemed probably to grow by assimilating N-compound such as ammonium that nitrate-utilizing organisms produced from nitrate. *Hansenula saturnus* and *Saccharomyces cerevisiae* tended to be easier to isolate with the malt or the nitrate media, and *Pichia dispora* with the orange medium. One strain each of *Debaryomyces vanriji, Hansenula beijerinkii, Pichia saitoi,* a new species of *Pichia, Saccharomyces mrakii, S. rouxii* and a new species of *Saccharomyces* were isolated only with the orange medium. One strain each of *Debaryomyces cantarellii* and *Hanseniaspora uvarum* were isolated only with the malt medium, and *Pichia quercuum, Saccharomyces bailii* and *S, rosei* were isolated only with the ume medium. Nevertheless it seems unlikely that there is specific relation between the enrichment medium used and yeast species isolated.

Of 30 species of the ascosporogenous yeasts, 27 spesies could be isolated by using the two media, orange and malt.

It has been found in the present examination that in most substrates, the predominant yeast in enrichment culture changed with the incubation period, and the yeasts isolated varied with the medium used for enrichment. The results suggested that use of the two enrichment media, orange and malt, and making isolation of yeast at intervals during enrichment culture would make it possible to obtain over 75% of yeasts on various substrates.

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SUCCESSIVE FUNGAL FLORA ON STERILIZED LEAVES IN THE LITTER OF FOREST. V.

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Summary

Successive fungal colonization on embedded sterilized leaves of *Castanopsis cuspidata* and *Quercus phillyraeoides* incubated for one to six months in the litter layer of selected forests was investigated in four stations situated in Sata-cho, near Cape Sata, Oosumi Peninsula, Kagoshima Pref. Fifty-eight, fifty-two, sixty-eight and seventy-one species of fungi were found to colonize on the leaves in the Stations A, B, C and D, respectively, during the periods of July, 1975 through May, 1976. The total number of fungal species encountered in this experiment was at least one hundred and five: one of Zygomycotina, four of Ascomycotina, one of Basidiomycotina and ninety-nine of Deuteromycotina.

The fungal flora and population, autoecology of an individual species and seasonal fluctuation of the occurrence of these fungi are discussed. Unexpected rich and dominant colonization by the so-called leaf-litter fungi was observed in forests solely of *Lithocarpus edulis*. Characteristic fungi in *Lithocarpus* forests were *Beltraniella japonica, Chaetendophragmia triangularia* and *Menisporopsis novae-zelandiae*. In contrast, colonization of the litter fungi in *Castanopsis* and *Quercus* forests was less marked, particularly in summer. This phenomenon was considered to reflect the dry summer climate in this locality. Characteristic fungi in these forests were *Candelabrum brocchiatum, Subulispora procurvata* and *S. rectilineata*. Dominant colonization of the fungi was more prominent in winter than in summer.

The fungal flora and population and their seasonal occurrence were also compared with those observed previously in other locations.

Annual occurrence of the macrofungi, particularly of the agarics, in the forests of three stations excepting Station A was also listed and briefly noted.

Following previous investigations (10, 11, 12, 14) into successive colonization of the litter fungi on the embedded leaves of *Castanopsis cuspidata* Schottky and *Quercus phillyraeoides* A. Gray in the evergreen oak forests of Japan, the successive colonization of these fungi was examined under different conditions, e.g., dry local conditions, and in different vegetation, e.g., the forest of *Lithocarpus edulis* Nakai.

This paper describes the fungal flora and population in these situations and the autoecology of individual fungi, in addition to the seasonal fluctuation in the occurrence of these fungi.

We have also extended the consideration and discussion of the successive fungal colonization in comparison with those observed in the previous works (10, 11, 12, 14).

Taxonomic treatment of several noteworthy fungi and of unidentified species will be reported elsewhere.

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Materials and Methods

Stations. The forests in which the sterilized leaves were embedded are near Oodomari, Sata-cho, Oosumi Peninsula, Kagoshima Prefecture, a little north of Cape Sata, the southernmost tip of the Oosumi Peninsula and also of Kyushu Island. The latitude is ca. 30°59′ N and longitude of ca. 130°40′ E, and the annual average temperature is ca. 19 C. The annual rain-fall in this area does not exceed 1,500 mm, and the area is generally recognized to have dry local conditions. In this area, we made the following leaf-litter plots in four natural forests (Fig. 1 & Pl. 1).

Station A is located at Shimadomari. The forest is on the slope of a hill ca. 1.5 Km east of the west coast facing the South China Sea and consists exclusively of *Castanopsis cuspidata* Schottky.

Station B is located at Oodomari. The forest is on an inland hill about 0.5 Km north of the coast. Vegetation comprises mostly *Quercus phillyraeoides* A. Gray.

Station C is located at Tanzakibana, Oodomari. The forest adjoins the beach and consists dominantly of *Lithocarpus edulis* Nakai (=*Pasania edulis* Makino) which is natural vegetation characteristic of the Southern Kyushu district.

Station D is located at Yaneda, Oodomari. The forest is about 1 Km north of the Oodomari beach and consists exclusively of *L. edulis*.

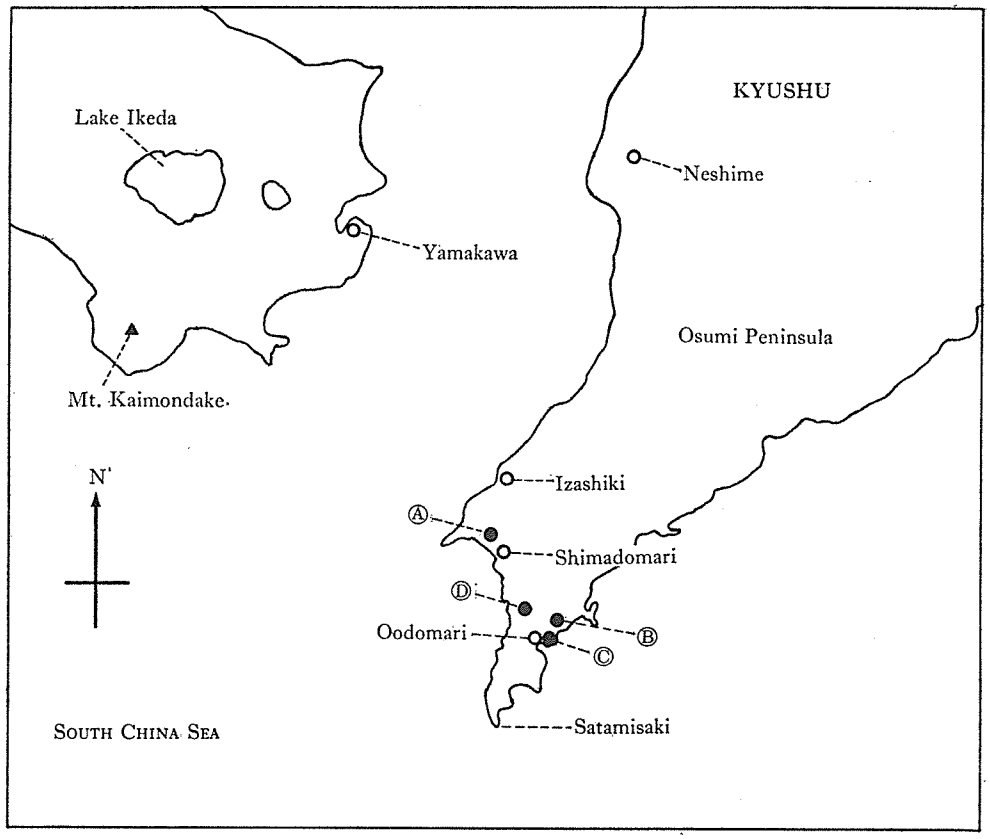

Fig. 1. Locations of the stations where sterilized leaves were embedded.
Ⓐ—Ⓓ: Stations A—D

Experimental technique. The experimental procedure is as described in the previous papers (10, 11, 12). Traps comprising ten sterilized leaves each of *Castanopsis cuspidata* and *Quercus phillyraeoides* were embedded in the forest litter layer.

The experiment was repeated in two different seasons. The first experiment was started on 11th June, 1975, by embedding six leaf-traps in each of the Stations A-D. In each successive month, that is, on 12th July, 20th Aug., 10th Sept., and 11th Nov., 1975, one trap was taken to the laboratory. Unfortunately, embedded traps of five months decay at both Stations C and D were not recovered. These may have been spoiled by wild animals, possibly weasels. The second experiment was started by embedding traps in the same stations on 11th November, 1975, followed by sampling once a month, on 14th Dec., 1975, 15th Jan., 14th Feb., 15th Mar., 15th Apr., and 13th May, 1976. Traps of four to six months decay at Station B and of one month decay at Station D could not be recovered. These samples may also have been spoiled by weasels.

After the given incubation period, traps were removed from the litter layer in the forest, put into gas-sterilized polyethylene bags as aseptically as possible and taken to the laboratory as soon as possible. All leaves from the traps and ten naturally fallen leaves from around the traps were placed individually into sterilized petri-dishes of 9 cm in diameter with a small amount of sterilized deionized water. All fungi colonized on the surface of leaves during incubation at room temperature (21–24 C) for more than one month were isolated and cultured on slants.

Most of these isolates were successfully identified as described in the previous paper (10). Adopted scientific names of the fungi in this paper are based mainly on recent taxonomical treatises on microfungi and on monographs of, for example, Ellis (1,2), Matsushima (4,5), Subramanian (7), C.M.I. Mycological Papers, Studies in Mycology, CBS and others.

Results and Discussion

Difference in the fungal flora of the four stations examined

Genera or species of the fungi appearing in these experiments and the stations from which they were isolated are listed in Table 1. Fungi found to colonize only on the naturally decayed leaf-litter are also listed and indicated by asterisk (*). If such fungi were encountered only in a particular station where they were not colonized on the embedded sterilized leaves, the location is indicated by (A)-(D).

The fungal flora of the four experimental stations were essentially similar. Dominant fungi common to the four stations were *Beltrania rhombica, Centrospora gracilis, Cladosporium inaequiseptatum, Clonostachys cylindrospora, Codinaea simplex, Endophragmia uniseptata, Helicosporium lumbricoides, Monacrosporium ellipsosporum, Paecilomyces elegans, Penicillium citrinum, Pleurophragmium cylindrosporum, Ramularia fusisaprophytica, Thozetella cristata, Volutina concentrica.* Species of *Cephalosporium, Chalara, Dactylaria, Penicillium, Trichoderma, Verticillium* were also dominant fungi

Table 1. List of fungi encountered in four stations examined.

Zygomycotina				
Mortierella sp.	A	B	C	D
Ascomycotina				
Ascomycete			C	
Discomycetes*	(A)	(B)		
Chaetomium globosum Kunze ex Fries		B		
Nectria sp.	A			
Basidiomycotina				
Basidiomycetes	A	B	C	D
Deuteromycotina				
Acrodontium crateriforme (van Beyma) de Hoog	A	B	C	D
Alternaria alternata (Fr.) Keissler	A			
Arthrinium sp.		B	C	
Arthrobotrys oligospora Fresenius*				(D)
Beltrania rhombica Penzig	A	B	C	D
Beltraniella japonica Matsushima			C	
Blastophorum truncatum Matsushima	A		C	D
Calcarisporium arbuscula Preuss*		(B)		
Candelabrum brocchiatum Tubaki	A	B		
Catenularia sp.	A		C	D
Centrospora gracilis Matsushima	A	(B)	C	D
Cephalosporium sp.	A	B	C	D
Chaetochalara sp.*		(B)		
Chaetendophragmia triangularia Matsushima			C	
Chaetopsina fulva Rambelli			C	D
Chalara sp.	A	B	C	D
Chloridium chlamydosporis (van Beyma) Hughes	A	(B)		(D)
C. laeense Matsushima*				(D)
Chloridium sp.	A			
Circinotrichum maculiforme C.G. Nees ex Persoon		B	C	D
Cladosporium cladosporioides (Fresenius) de Vries	A	B	C	D
C. inaequiseptatum Matsushima	A	(B)	C	(D)
C. oxysporum Berk. & Curt.	A		C	D
Cladosporium spp.	A	B	C	D
Clonostachys cylindrospora Arnaud	A	B	C	D
Codinaea simplex Hughes & Kendrick	A	B	C	D
Coronospora uniseptata Matsushima		B		
Cryptophiale guadalcanalense Matsushima	A			
C. udagawae Pirozynski & Ichinoe apud Pir.	A	B		(D)
Cylindrocarpon sp.		B		
Cylindrocladium parvum Anderson (multiplex)				D
Cylindrocladium sp.			C	D
Dactylaria fusiformis Shearer & Crane	(A)	B	C	D
Dactylaria sp.	A	B	C	D

Table 1. (continued)

Dactylella ramosa Matsushima				D
Dactylella sp.	A		(C)	
Dictyosporium elegans Corda	A			
Ellisiopsis gallesiae Batista & Nascimento		B	C	D
Endophragmia uniseptata M. B. Ellis	A	B	C	D
Fusicoccum sp.	A			
Gliocladium roseum (Link) Bainier	A		C	D
Gliocladium sp.			C	
Gliomastix sp.			C	(D)
Gyrothrix circinata (Berk. & Curt.) Hughes			C	
G. podosperma (Corda) Rabenhorst		B	C	
Hansfordia pulvinata (Berk. & Curt.) Hughes				D
Helicomyces roseus Link*		(B)		(D)
Helicosporium lumbricoides Sacc.	A	B	(C)	D
Helicosporium sp.	A			D
Helminthosporium velutinum Link ex Fr.*			(C)	
Idriella fertilis (Pirozynski & Hodges) Matsushima	(A)		C	D
Idriella sp.	A	B	C	D
Kramasamuha sibika Subramanian & Vittal				D
Menisporopsis novae-zelandiae Hughes & Kendrick			C	D
Monacrosporium ellipsosporum (Grove) Cooke & Dickinson	A	B	C	D
Myrothecium leucotrichum (Peck) Tulloch				D
M. roridum Tode ex Fr.*				(D)
Nakataea fusispora (Matsushima) Matsushima		B	C	
Paecilomyces elegans (Corda) Mason & Hughes	A	B	C	D
P. terricola (Miller, Giddens & Foster) Onions & Barron			C	
Penicillium citrinum Thom	A	B	C	D
P. funiculosum Thom			C	D
P. implicatum Biourge	A		C	D
P. multicolor Grigorieva-Manoilova & Poradielova				D
Penicillium spp.	A	B	C	D
Periconia byssoides Pers. ex Mérat				D
Pestalotia spp.	A	B	C	D
Phialophora sp.*	(A)			
Pleurophragmium cylindrosporum Matsushima	A	(B)	(C)	(D)
P. bicolor Matsushima*			(C)	
P. simplex (Berk. & Br.) Hughes*	(A)			
Polyscytalum sp.		B		D
Pseudobotrytis terrestris (Timonin) Subramanian	A		C	D
Pseudofusarium semitectum (Berk. & Rav.) Matsushima			C	
Ramularia fusisaprophytica Matsushima	A	B	C	D
Rhinocladiella rigidiphora Matsushima*				(D)
Scolecobasidium cateniphorum Matsushima	A		C	D

Table 1. (continued)

S. constrictum Abbott			C	
S. variabile Barron & Busch*	(A)			
S. humicola Barron & Busch			C	(D)
Selenosporella curvispora Arnaud	(A)	B	C	D
Solosympodiella clavata Matsushima	A		(C)	D
S. cylindrospora Matsushima*				(D)
S. rigididentata Matsushima*			(C)	
Speiropsis pedatospora Tubaki				D
Stachybotrys cylindrospora Jensen*				(D)
Subulispora procurvata Tubaki apud Tubaki & Yokoyama	A	B		
S. rectilineata Tubaki apud Tubaki & Yokoyama	A	B		
Sympodiella laxa Subramanian & Vittal		B	C	(D)
Thozetella cristata Pirozynski & Hodges	A	B	C	D
T. tocklaiensis (Agnih.) Pirozynski & Hodges		B	C	D
Trichoderma spp.	A	B	C	D
Uberispora simplex (Ichinoe) Pirozynski & Hodges			C	D
Verticillium sp.	A	B	(C)	D
Volutella sp.	(A)	(B)	C	D
Volutina concentrica Penzig & Sacc.	A	B	C	D
Wiesneriomyces javanicus Koorders	(A)		C	
Zygosporium gibbum (Sacc., Rouss. & Bomm.) Hughes	A	B	C	D
Z. masonii Hughes		B	C	D

Fungi indicated by asterisk (*) are those found to colonize only on the naturally decayed fallen leaves. A-D indicate the Stations A-D in which the respective fungi were detected and those in parentheses indicate that these fungi were found to colonize only on the naturally decayed fallen leaves selectively in the respective stations.

common to all stations.

Candelabrum brocchiatum and *Subulispora procurvata* were found to colonize predominantly on the traps of Stations A and B, and were not isolated from Stations C and D. Unidentified discomyceteous fungi appeared only in Stations A and B.

On the other hand, *Cylindrocladium parvum, Chaetopsina fulva, Menisporopsis novae-zelandiae, Penicillium funiculosum, Scolecobasidium humicola, Uberispora simplex* were found in Stations C and D where *L. edulis* is dominant. In these stations, an unidentified species of ascomyceteous genus and of *Cylindrocladium* were also isolated.

Detailed discussion of the differences in the distribution of the recognized species or genera will appear later. However, the number of fungi appearing and their frequency is somewhat larger in Stations C and D than in Stations A and B. This does not necessarily result from differences in location, but may reflect differences in vegetation.

Fungal flora and seasonal fluctuation of the occurrence of the fungi appearing in Stations A-D

Table 2. Seasonal occurrence of fungi on the leaves* in four stations (A-D) during the periods July through November and of December through May. The occurrence of fungi was shown by ○ (■, No leaves available).

Station A

Fungus	July			Aug.			Sept.			Oct.			Nov.			Dec.			Jan.			Feb.			Mar.			Apr.			May		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Mortierella sp.											○			○															○		○	○	
Discomycete																											○						
Nectria sp.	○																																
Basidiomycetes					○					○																				○			
Acrodontium crateriforme																			○	○			○										
Alternaria alternata																											○						
Beltrania rhombica	○	○	○	○	○		○	○	○	○	○		○	○														○		○			○
Blastophorum truncatum							○	○			○		○	○														○	○	○	○	○	
Candelabrum brocchiatum													○	○																			
Catenularia sp.					○	○																											
Centrospora gracilis																					○		○		○	○	○			○			
Cephalosporium sp.				○												○			○			○	○			○					○	○	
Chalara sp.				○	○	○	○	○			○	○	○		○	○	○	○		○	○	○	○	○	○	○		○	○	○	○	○	
Chloridium chlamydosporis															○	○	○	○			○	○	○						○		○	○	○
Chloridium sp.		○																							○	○	○						
Cladosporium cladosporioides																			○			○											
C. inaequiseptatum																														○		○	
C. oxysporum																												○					
Cladosporium sp.										○		○		○	○	○		○			○			○			○						
Clonostachys cylindrospora	○															○						○						○	○				
Codinaea simplex	○			○	○		○	○	○	○	○	○	○	○		○	○	○	○	○	○	○	○	○	○	○	○	○		○	○	○	○
Cryptophiale guadalcanalense	○					○	○	○	○	○		○																		○			
C. udagawae					○									○	○												○				○		
Dactylaria fusiformis															○			○			○												
Dactylaria sp.				○		○					○			○							○												

Station A (continued)

Fungus	July			Aug.			Sept.			Oct.			Nov.			Dec.			Jan.			Feb.			Mar.			Apr.			May		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Dactylella sp.																						○											
Dictyosporium elegans														○																			
Endophragmia uniseptata					○		○	○		○	○	○	○	⊙									○						○		○	○	
Fusicoccum sp.														○																			
Gliocladium roseum	○																																
Helicosporium lumbricoides		○							○																							○	
Helicosporium sp.														○																			
Idriella fertilis																								○									
Idriella sp.	○	○	○	○	○	○		○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○		○	○	○	○		○	○		○
Monacrosporium ellipsosporum							○	○			○	○		○							○			○					○	○		○	
Paecilomyces elegans	○	○									○					○	○					○	○		○	○		○	○		○	○	
Penicillium citrinum																○	○		○	○		○	○			○							
P. implicatum																									○								
Penicillium sp.		○																										○					
Pestalotia sp.																○											○						
Phialophora sp.												○																					
Pleurophragmium cylindrosporum					○													○															
P. simplex																								○									
Pseudobotrytis terrestris											○																						
Ramularia fusisaprophytica						○							○		○						○			○	○		○	○		○			○
Scolecobasidium cateniphorum										○		○										○					○	○		○	○		
S. variabile																								○									
Selenosporella curvispora												○																					
Solosympodiella clavata																																○	
Subulispora procurvata	○	○	○			○		○	○	○	○	○		○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○

Station A (continued)

Fungus	July			Aug.			Sept.			Oct.			Nov.			Dec.			Jan.			Feb.			Mar.			Apr.			May		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
S. rectilineata																									○		○			○			
Thozetella cristata								○		⊙	○													○									○
Trichoderma sp.	○	○	○			○		○			○	○	○			○	○		○	○		○	○		○	○			○		○	○	○
Verticillium sp.														○		○						○									○	○	
Volutella sp.			○						⊙			⊙												○									○
Volutina concentrica	○						⊙		○				○								○	○										○	
Wiesneriomyces javanicus									○																								
Zygosporium gibbum															○				○	○	○				○		○						

* Sterilized embedded leaves of *Castanopsis cuspidata* (1) and *Quercus phillyraeoides* (2) and naturally decayed fallen leaves of *C. cuspidata* (3).

Station B

Fungus	July			Aug.			Sept.			Oct.			Nov.			Dec.			Jan.			Feb.			Mar.			Apr.			May		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Mortierella sp.					○									○	○																		
Discomycetes																					○			○			○						
Chaetomium globosum										○																							
Basidiomycetes							○																	○									
Acrodontium crateriforme																	○																
Arthrinium sp.																○											○						
Beltrania rhombica	○	○	○	⊙	○	○	○	○	○	○	○	○	○	○	○		○				○	○	○	○						○			○
Calcarisporium arbuscula																								○									
Candelabrum brocchiatum													○																				
Centrospora gracilis																					○			○			○						
Cephalosporium sp.							○																										

Station B (continued)

Fungus	July			Aug.			Sept.			Oct.			Nov.			Dec.			Jan.			Feb.			Mar.			Apr.			May		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Chaetochalara sp.			○												○			○															
Chalara sp.				○		○			○			○	○		○	○					○	○	○				○						
Chloridium chlamydosporis																		○															
Circinotrichum maculiforme																○		○			○			○			○			○			○
Cladosporium cladosporioides																○																	
C. inaequiseptatum																														○			
Cladosporium sp.	○					○				○	○	○		○	○			○			○	○		○			○						
Clonostachys cylindrospora																			○			○											
Codinaea simplex	○			○	○		○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○			○			○			○
Coronospora uniseptata																		○															
Cryptophiale udagawae				○	○			○	○			○			○																		
Cylindrocarpon sp.										○																							
Dactylaria fusiformis								○	○		○										○												
Dactylaria sp.							○	○		○	○	○	○	○	○							○											
Ellisiopsis gallesiae										○						○	○							○			○						
Endophragmia uniseptata							○			○			○																				
Gyrothrix podospermum																							○	○									
Helicomyces roseus												○																					
Helicosporium lumbricoides						○	○					○																					
Idriella sp.	○			○						○		○				○	○	○	○	○			○								○		○
Monacrosporium ellipsosporum							○	○	○	○	○	○	○	○										○							○		
Nakataea fusispora											○	○																					
Paecilomyces elegans	○	○		○	○		○	○		○		○	○				○		○	○			○										○
Penicillium citrinum																○	○		○	○		○	○										
Penicillium sp.		○																															

Station B (continued)

Fungus	July			Aug.			Sept.			Oct.			Nov.			Dec.			Jan.			Feb.			Mar.			Apr.			May		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Pestalotia sp.																				○													
Pleurophragmium cylindrosporum			○			○			○						○			○			○			○			○			○			○
Polyscytalum sp.								○	○																								
Ramularia fusisaprophytica	○			○		○	○	○	○			○			○	○	○	○		○	○	○	○	○			○			○			○
Selenosporella curvispora										○																	○						
Subulispora procurvata	○		○														○			○													
S. rectilineata																			○	○	○									○			
Sympodiella laxa																			○		○	○		○			○			○			○
Thozetella cristata		○		○	○	○				○		○						○												○			
T. tocklaiensis	○	○	○	○	○		○	○	○	○	○	○	○	○				○			○												
Trichoderma sp.	○	○								○	○			○			○		○	○		○	○										
Verticillium sp.														○			○		○														
Volutella sp.																																	○
Volutina concentrica	○			○			○			○		○																					
Zygosporium gibbum																			○	○			○										
Z. masonii																○			○														

* Sterilized embedded leaves of *Castanopsis cuspidata* (1) and *Quercus phillyraeoides* (2) and naturally decayed fallen leaves of *Q. phillyraeoides* (3).

Station C

Fungus	July			Aug.			Sept.			Oct.			Nov.			Dec.			Jan.			Feb.			Mar.			Apr.			May		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Mortierella sp.										○	○																		○				
Ascomycete																									○								
Basidiomycetes							○								○												○	○					

Station C (continued)

Fungus	July			Aug.			Sept.			Oct.			Nov.			Dec.			Jan.			Feb.			Mar.			Apr.			May		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Acrodontium crateriforme																			○	○													
Arthrinium sp.																○						○	○										
Beltrania rhombica	○	○	○	○	○	○		○	○	○	○	○				○	○	○	○	○	○	○	○	○	○		○	○	○		○	○	○
Beltraniella japonica		○						○																									
Blastophorum truncatum							○	○		○	○										○										○	○	
Catenularia sp.					○																												
Centrospora gracilis																					○		○										
Cephalosporium sp.				○			○		○						○			○	○	○	○	○	○	○	○		○	○			○	○	
Chaetendophragmia triangularia												○						○			○	○		○			○	○		○			
Chaetopsina fulva	○	○	○	○		○	○	○	○			○			○	○		○				○		○			○	○		○			○
Chalara sp.					○		○	○		○	○														○			○	○		○	○	
Circinotrichum maculiforme									○			○			○	○		○			○			○			○			○			
Cladosporium cladosporioides							○									○	⊙		○	○		○	○		○			○	○				
C. inaequiseptatum																															○		
C. oxysporum																												○			○		
Cladosporium sp.	○	○		○							○							○			○						○						
Clonostachys cylindrospora	○	○	○	○	○	○	○	○	○	○	○	○			○				○	○	○	○	○	○			○			○			○
Codinaea simplex																		○		○					○		⊙	○		○	○	○	
Cylindrocladium sp.																	○	○															
Dactylaria fusiformis							○	○			○							○															
Dactylaria sp.										○	○	○			○	○		○			○												
Dactylella sp.																														○			
Ellisiopsis gallesiae												○				○				○		○	○	○	○	○	○	○		○	○	○	○
Endophragmia uniseptata							○			○																							
Gliocladium roseum	○	○																															

Station C (continued)

Fungus	July			Aug.			Sept.			Oct.			Nov.			Dec.			Jan.			Feb.			Mar.			Apr.			May		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Gliocladium sp.																	○																
Gliomastix sp.																							○										
Gyrothrix circinata																												○					
G. podosperma																				○													
Helicosporium lumbricoides												○																					
Helminthosporium velutinum																					○												
Idriella fertilis																						○	○	○								○	
Idriella sp.									○						○					○			○										
Menisporopsis novae-zelandiae	○	○	○	○		○	○		○			○			○	○		○	○	○	○	○	○	○	○		○	○	○	○		○	○
Monacrosporium ellipsosporum							○	○		○	○																						
Nakataea fusispora																							○										
Paecilomyces elegans					○																		○							○			
P. terricola					○																												
Penicillium citrinum																○	○		○	○		○	○		○	○							
P. funiculosum																						○				○							
P. implicatum																									○	○							
Penicillium sp.	○	○																										○	○		○	○	
Pestalotia sp.																									○	○							
Pleurophragmium bicolor																											○						
P. cylindrosporum															○			○												○			
Pseudobotrytis terrestris							○	○		○	○																				○		
Pseudofusarium semitectum																						○											
Ramularia fusisaprophytica	○															○		○		○				○						○		○	○
Scolecobasidium cateniphorum							○		○													○		○			○		○		○		○
S. constrictum								○		○																		○		○			

Station C (continued)

Fungus	July			Aug.			Sept.			Oct.			Nov.			Dec.			Jan.			Feb.			Mar.			Apr.			May		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
S. humicola					○																												
Selenosporella curvispora								○																									
Solosympodiella clavata									○									○			○			○						○			
S. rigididentata																											○						
Sympodiella laxa																					○	○		○			○	○		○			
Thozetella cristata		○		○	○	○	○	○		○	○					○					○	○		○				○	○	○	○	○	
T. tocklaiensis	○	○	○		○					○	○																				○		
Trichoderma sp.	○	○		○	○		○	○								○	○		○	○		○	○					○	○		○		
Uberispora simplex					○					○																		○			○	○	
Verticillium sp.			○																														
Volutella sp.	○		○				○		○																								○
Volutina concentrica	○				○	○						○			○			○						○						○	○		○
Wiesneriomyces javanicus				○											○																		
Zygosporium gibbum															○										○	○			○				
Z. masonii																									○						○		

* Sterilized embedded leaves of *Castanopsis cuspidata* (1) and *Quercus phillyraeoides* (2) and naturally decayed fallen leaves of *Lithocarpus edulis* (3).

Station D

Fungus	July			Aug.			Sept.			Oct.			Nov.			Dec.			Jan.			Feb.			Mar.			Apr.			May		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Mortierella sp.										○																							
Basidiomycetes								○		○	○																						
Acrodontium crateriforme																						○	○										
Arthrobotrys oligospora															○																		

Station D (continued)

Fungus	July			Aug.			Sept.			Oct.			Nov.			Dec.			Jan.			Feb.			Mar.			Apr.			May		
	1	2	3	1	2	3	1	2	.3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Beltrania rhombica	○	○	○	○	○	○	○	○	○	○	○	○							○	○	○	○	○	○	○	○		○	○		○		○
Blastophorum truncatum							○		○	○																					○	○	
Catenularia sp.				○																													
Centrospora gracilis																			○	○	○			○			○		○		○		
Cephalosporium sp.				○	⊙		○												○	○	○	○	○	○	○	○	○	○	○		○		○
Chaetopsina fulva	○	○	○	○	○	○	○	○	○	○		○			○				○	○	○	○	○	○	○		○	○	○	○			○
Chalara sp.					○			○													○		○				○	○	○		○		
Chloridium chlamydosporis																																	○
C. laeense																					○												
Circinotrichum maculiforme																								○				○					
Cladosporium cladosporioides																			○	○		○	○		○			○			○	○	
C. inaequiseptatum																														○			
C. oxysporum																												○			○		○
Cladosporium sp.				○	○					○	○								○					○									
Clonostachys cylindrospora	○	○	○	○	○	○	○		○	○	○	○			○				○	○	○	○			○		○			○	○	○	○
Codinaea simplex																				○	○	○		○		○	○		○	○	○	○	○
Cryptophiale udagawae						⊙																											
Cylindrocladium parvum																									○	○	○						
Cylindrocladium sp.	○		○												○																		
Dactylaria fusiformis				○			○			○	○	○																					
Dactylaria sp.								○																									
Dactylella ramosa					○																												
Ellisiopsis gallesiae													○						○			○	○	○	○	○		○	○	○	○		○
Endophragmia uniseptata								○		○	○																	○	○				
Gliocladium roseum	○		○												○																		

Station D (continued)

Fungus	July			Aug.			Sept.			Oct.			Nov.			Dec.			Jan.			Feb.			Mar.			Apr.			May		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Gliomastix sp.																					○												
Hansfordia pulvinata									○						○							○											
Helicomyces roseus																														○			
Helicosporium lumbricoides				○	○		○	○	○	○		○			○						○										○	
Helicosporium sp.								○																									
Idriella fertilis																			○	○	○	○		○	○			○					
Idriella sp..				○								○			○			○	○	○			○				○	○					
Kramasamuha sibika																									○								
Menisporopsis novae-zelandiae	○	○	○	○		○	○		○			○			○			○	○	○	○	○	○	○	○		○	○	○	○	○	○	○
Monacrosporium ellipsosporum							○	○		○	○																						
Myrothecium leucotrichum	○					○	○		○																								○
M. roridum																					○												
Paecilomyces elegans								○											○				○						○				
Penicillium citrinum																			○	○		○	○			○							
P. funiculosum																							○										
P. implicatum					○																					○					○		
P. multicolor																													○				
Penicillium sp.		○																							○								
Periconia byssoides																						○		○									
Pestalotia sp.																							○			○							
Pleurophragmium cylindrosporum															○																		
Polyscytalum sp.					○																												
Pseudobotrytis terrestris				○																													
Ramularia fusisaprophytica			○		○													○		○				○									○
Rhinocladiella rigidiphora																								○									

Station D (continued)

Fungus	July			Aug.			Sept.			Oct.			Nov.			Dec.			Jan.			Feb.			Mar.			Apr.			May		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Scolecobasidium cateniphorum																					○	○	○				○	○	○		○	○	
S. humicola																								○									
Selenosporella curvispora																			○	○	○												
Solosympodiella clavata						○												○		○	○									○			
S. cylindrica																		○															
Speiropsis pedatospora			○				○																										
Stachybotrys cylindrospora																					○												
Sympodiella laxa																								○									
Thozetella cristata				○	○			○															○							○		○	
T. tocklaiensis		○									○																						
Trichoderma sp.	○	○		○	○		○	○			○	○			○			○	○	○	○	○	○	○	○	○	○		○		○	○	
Uberispora simplex																													○		○	○	
Verticillium sp.		○																															
Volutella sp.																					○					○							○
Volutina concentrica	○	○	○	○	○		○		○						○			○	○	○	○	○		○	○		○	○	○		○		○
Zygosporium gibbum																			○			○	○				○		○				
Z. masonii																						○											

* Sterilized embedded leaves of *Castanopsis cuspidata* (1) and *Quercus phillyraeoides* (2) and naturally decayed fallen leaves of *Lithocarpus edulis* (3).

Fungi appearing both on the embedded sterilized leaves and on the naturally fallen leaves around them are listed in Table 2 for each of the stations. Fungi characteristic in their dominant colonization in both experiments in each station are listed in Tables 3-6.

Generally, the decomposition of the embedded leaves in these areas seemed to progress fairly slowly. Contrary to expectations, the embedded leaves were not markedly degraded in shape even after five to six months in the summer season under high temperatures. This is one of the significant differences from the experimental results obtained in both Yakushima and Tanegashima areas, Kagoshima Pref., as reported previously (11,12). This phenomenon may be caused by the lower amount of rain-fall and the lower humidity in the areas tested in this study.

Comparison of the relative degrees of the leaf-decomposition during incubation period in both experiments and in each station are shown in Plates 2–9.

Station A. Forty-two species were isolated in the first experiment and forty-eight in the second experiment. The total number of fungal species encountered in this station was fifty-eight, of which thirty-two species were found to colonize on the leaves in both experiments.

In this forest, *Codinaea simplex, Monacrosporium ellipsosporum, Subulispora procurvata,* species of *Chalara, Cladosporium, Idriella* and *Trichoderma* were most dominant. *Beltrania rhombica, Cryptophiale guadalcanalense* and *Endophragmia uniseptata* were found to colonize dominantly in the first experiment (July to November), but less dominantly or not at all in the second experiment (December to May). These fungi could not be isolated during the winter season. On the other hand, *Centrospora gracilis, Cladosporium cladosporioides* and *Penicillium citrinum* were found to colonize predominantly only in the second experiment.

It is noteworthy that *Cryptophiale guadalcanalense* colonized dominantly and exclusively in this station and *Alternaria alternata, Dictyosporium elegans, Pleurophragmium simplex, Scolecobasidium variabile* and species of *Chloridium, Fusicoccum, Phialophora* and *Nectria* were also found only in this station. However, *Circinotrichum maculiforme, Ellisiopsis gallesiae, Sympodiella laxa, Thozetella tocklaiensis, Zygosporium masonii* which are fairly dominant fungi in the other three forests tested and are also known to occur commonly in the evergreen oak forests in Japan have not yet been isolated in Station A. Furthermore, *Dactylaria fusiformis* and *Selenosporella curvispora* which were found to colonize on the embedded leaves in this station were not isolated from embedded sterilized leaves in this station.

Pleurophragmium cylindrosporum was found, though in only one instance, to colonize on the embedded *Quercus* leaves. This fungus was usually colonized on the naturally decayed leaves in all the forests tested.

Seasonal fluctuation of the occurrence of fungi is difficult to explain. However, *Beltrania rhombica, Cryptophiale guadalcanalense, C. udagawae, Endophragmia uni-*

Table 3. Fungi characteristic in their dominant colonization in Station A.

Dominant in the first experiment (July-November)	Dominant in the second experiment (December-May)
Beltrania rhombica	*Acrodontium crateriforme**
Chalara sp.	***Chalara*** sp.
Codinaea simplex	*Centrospora gracilis*
Cryptophiale guadalcanalense	*Chloridium chlamydosporis*
Dactylaria sp.*	*Cladosporium cladosporioides**
Endophragmia uniseptata	***Codinaea simplex***
Idriella sp.	***Idriella*** sp.
Subulispora procurvata	*Paecilomyces elegans*
Trichoderma sp.	*Penicillium citrinum*
	Ramularia fusisaprophytica
	*Scolecobasidium cateniphorum**
	Subulispora procurvata
	*S. rectilineata**
	Trichoderma sp.
	Verticillium sp.*
	Zygosporium gibbum

* less dominant.
Fungi in bold face are dominant ones common to both experiments.

septata which were dominant colonizers in the summer season, were less dominant or absent in the winter season. In contrast, *Acrodontium crateriforme, Chloridium chlamydosporis, Dactylaria fusiformis, Penicillium citrinum, Zygosporium masonii* and species of *Cladosporium* which were less dominant or absent in the summer season were found to colonize selectively in the winter season. *Codinaea simplex* was also found to be a dominant colonizer in the winter season rather than in the summer season. *Blastophorum truncatum, Endophragmia uniseptata, Monacrosporium ellipsosporum, Ramularia fusisaprophytica, Scolecobasidium cateniphorum, Thozetella cristata* were found on the leaves of more than two to three months decay. On the other hand, *Beltrania rhombica, Codinaea simplex, Subulispora procurvata,* species of *Idriella* and *Trichoderma* were very common on the leaves of early decay and also dominant later on, at least in the summer season. The occurrence of *Alternaria alternata, Cladosporium oxysporum, Dictyosporium elegans, Gliocladium roseum, Idriella fertilis, Pleurophragmium simplex, Scolecobasidium variabile, Selenosporella curvispora, Wiesneriomyces javanicus* and some other unidentified species were the only instances of isolation out of the many fungi detected in this station during the study.

The occurrence of fruit bodies of the macrofungi in the forest of this station was not investigated at all.

Station B. In Station B, where *Quercus phillyraeoides* grows exclusively, the total numbers of fungal species encountered in the first and second experiments were thirty-

three and thirty-nine, respectively, of which twenty species were common to both experiments. A total of fifty-two species of fungi was isolated in this station.

Unfortunately, three sets of the sterilized traps embedded for the second experiment had disappeared before the sampling of five months decay, as shown in Plate 5. Of the fungi encountered, *Beltrania rhombica, Codinaea simplex, Paecilomyces elegans, Ramularia fusisaprophytica, Thozetella tocklaiensis,* and unidentified species of *Chalara, Cladosporium, Idriella* and *Trichoderma* were predominant, and *B. rhombica, P. elegans* and *T. tocklaiensis* were more predominant in the first experiment than in the second.

Monacrosporium ellipsosporum and species of *Dactylaria* were also more frequently isolated in the first experiment than in the second. However, *Cryptophiale udagawae, Endophragmia uniseptata, Helicosporium lumbricoides, Volutina concentrica,* which were fairly common in the first experiment, could not detected in the second. On the other hand, *Circinotrichum maculiforme, Penicillium citrinum, Subulispora rectilineata, Sympodiella laxa, Zygosporium gibbum* which were not uncommon in the second experiment were not found in the first experiment.

Characteristic fungi limited to this station were *Calcalisporium arbuscula, Chaetomium globosum, Coronospora uniseptata* and species of *Chaetochalara* and *Cylindrocarpon.* Of these, *C. arbuscula* and species of *Chaetochalara* were found only on the naturally decayed leaves.

Fungi common to Stations A and B were *Candelabrum brocchiatum, Subulispora procurvata, S. rectilineata* and species of *Cladosporium;* those common to Stations B and C were *Gyrothrix podosperma, Nakataea fusispora* and species of *Arthrinium;* and those common to Stations B, C and D were *Circinotrichum maculiforme, Ellisiopsis gallesiae, Sympodiella laxa, Thozetella tocklaiensis* and *Zygosporium masonii. Dactylaria fusiformis* and *Selenosporella curvispora* were also common to Stations B, C and D, but were also isolated on the naturally decayed leaves in Station A.

Some fungi were found to colonize fairly commonly in other stations and were absent from Station B: *Blastophorum truncatum, Cladosporium oxysporum, Gliocladium roseum, Penicillium implicatum, Scolecobasidium cateniphorum* and *Solosympodiella clavata.*

Seasonal fluctuation of the occurrence of the fungi in the Station B is not so clear, particularly because of lack of the later half of the experimental data. It is supposed, however, that *Beltrania rhombica, Cryptophiale udagawae, Thozetella tocklaiensis* and *Volutina concentrica* were predominant in the summer season and less dominant or absent in the winter season. On the other hand, *Centrospora gracilis, Circinotrichum maculiforme, Penicillium citrinum, Subulispora rectilineata, Sympodiella laxa, Zygosporium gibbum* and *Z. masonii,* and species of *Idriella* and *Verticillium* were predominant in the winter season.

Beltrania rhombica, Codinaea simplex, Paecilomyces elegans, Ramularia fusisaprophytica, Thozetella tocklaiensis, and species of *Chalara* and *Idriella* were thought to be evenly dominant colonizers throughout the process of the decay, at least in the warm

season. On the other hand, *Subulispora procurvata* was predominant only at an early stage of decay and then decreased. *Monacrosporium ellipsosporum, Helicosporium lumbricoides* and species of *Dactylaria* were supposed to colonize after two to three months decay.

A total of ten species of the macrofungi was found to make their fruit bodies during the investigation in the forest of this station (Table 7). They are, for example, *Tricholoma flavobrunneum, Oudemansiella longipes, Amanita pantherina, A. fuliginea, Pulveroboletus retipes, Boletus edulis, Cantharellus cibarius*, etc.

Table 4. Fungi characteristic in their dominant colonization in Station B.

Dominant in the first experiment (July-November)	Dominant in the second experiment (December-May)
Beltrania rhombica	*Centrospora gracilis**
Chalara sp.	***Chalara*** sp.
Cladosporium sp.	*Circinotrichum maculiforme*
Codinaea simplex	***Cladosporium*** sp.
Cryptophiale udagawae	***Codinaea simplex***
Dactylaria fusiformis	*Ellisiopsis gallesiae**
Dactylaria sp.	***Idriella*** sp.*
*Endophragmia uniseptata**	***Paecilomyces elegans***
Idriella sp.*	*Penicillium citrinum*
Monacrosporium ellipsosporum	***Pleurophragmium cylindrosporum****
Paecilomyces elegans	***Ramularia fusisaprophytica***
Pleurophragmium cylindrosporum*	*Subulispora rectilineata**
Ramularia fusisaprophytica	*Sympodiella laxa*
*Thozetella cristata**	*Zygosporium gibbum**
*T. tocklaiensis**	*Z. masonii**

* less dominant.
Fungi in bold face are dominant ones common to both experiments.

Station C. A greater number of species was found in Station C in both seasons than in Stations A and B. During the first and second incubation periods, forty-three and fifty-seven species, respectively were found to colonize on the leaves. Thirty-two species were common to both experiments, and a total of sixty-eight species was isolated.

In the first experiment, the trap containing the sterilized leaves embedded for five months was lost, possibly destroyed by weasels, as shown in Plate 6.

In this station, the forest consists of *L. edulis* and therefore the naturally fallen decaying leaves of *L. edulis* surrounding the embedded traps were used as a control instead of *C. cuspidata* as in the case of Station A and *Q. phillyraeoides* as in the case of Station B.

The most predominant fungi in this station were *Beltrania rhombica, Chaetopsina fulva, Clonostachys cylindrospora, Menisporopsis novae-zelandiae, Thozetella cristata* and species of *Trichoderma. Chaetendophragmia triangularia, Circinotrichum maculiforme, Cladosporium cladosporioides, Ellisiopsis gallesiae, Ramularia fusisaprophytica*

and *Volutina concentrica* were also not uncommon.

It is worthwhile noting that *Beltraniella japonica, Chaetendophragmia triangularia, Gyrothrix circinata, Paecilomyces terricola, Pseudofusarium semitectum* and *Scolecobasidium constrictum* which colonized on the embedded leaves and *Helminthosporium velutinum, Pleurophragmium bicolor* and *Solosympodiella rigididentata* which occurred only on the naturally fallen leaves used as control, were all isolated only in this station.

Scolecobasidium humicola, Wiesneriomyces javanicus and species of *Gliomastix* were found to colonize on the embedded leaves exclusively in this station, though these fungi were also found on the naturally decayed leaves in other stations.

Chaetopsina fulva, Idriella fertilis, Menisporopsis novae-zelandiae, Penicillium funiculosum, Scolecobasidium humicola, Uberispora simplex, species of *Cylindrocladium* and *Volutella,* and an unidentified Ascomycete which colonized on the embedded leaves were common to Stations C and D, while *I. fertilis* was also found on the naturally decayed leaves in Station A.

Although *Beltrania rhombica* was less dominant in the winter season in other stations, in Station C it was significantly dominant throughout the year. Moreover, *Chaetendophragmia triangularia, Circinotrichum maculiforme, Cladosporium cladosporioides, Penicillium citrinum, Ramularia fusisaprophytica, Solosympodiella clavata,* species of *Cephalosporium* and *Dactylaria* were more abundant in winter than in summer. However, *Circinotrichum maculiforme, Ramularia fusisaprophytica* and *Solosympodiella clavata* hardly colonized on the embedded leaves despite the fact that they were easily isolated from the naturally developed leaf-litter.

On the other hand, *Chaetopsina fulva, Thozetella cristata, T. tocklaiensis, Volutina concentrica,* species of *Volutella* were not uncommon in summer, but were less common or absent in winter. *Cladosporium cladosporioides, Codinaea simplex, Ellisiopsis gallesiae, Ramularia fusisaprophytica* which were not uncommon in the second experiment from November through May, next year, were not found to colonize on the embedded leaves in the first experiment from June to November, with one each exception. The reason for this is not certain.

Solosympodiella clavata did not colonize on the embedded leaves at all, although it was observed almost all the year round on the naturally fallen leaves. *Blastophorum truncatum, Pseudobotrytis terrestris, Scolecobasidium cateniphorum, Uberispora simplex* and species of *Chalara* were not found on the leaves of early decay, but seemed to colonize on the leaves of later decay. *U. simplex* is an unique fungus which colonized selectively on the embedded leaves of *Castanopsis cuspidata* and *Quercus phillyraeoides,* but was not found on the naturally developed leaf-litter of *Lithocarpus edulis. Chaetendophragmia triangularia* is also a unique fungus which appeared only in this station. This fungus usually developed on the naturally fallen leaves, but was found on the embedded leaves twice in the second experiment.

Subulispora procurvata, S. rectilineata and *Cryptophiale udagawae* which are not uncommon in the *Castanopsis* forests in Japan have so far not been isolated in this station. *Candelabrum brocchiatum* was also absent in this station.

Table 5. Fungi characteristic in their dominant colonization in Station C.

Dominant in the first experiment (July-November)	Dominant in the second experiment (December-May)
Beltrania rhombica	*Acrodontium crateriforme**
*Blastophorum truncatum**	***Beltrania rhombica***
Chaetopsina fulva	*Cephalosporium* sp.
Chalara sp.*	*Chaetendophragmia triangularia*
Clonostachys cylindrospora	***Chaetopsina fulva***
Dactylaria fusiformis	***Chalara*** sp.*
Menisporopsis novae-zelandiae	*Circinotrichum maculiforme**
Mortierella sp.*	*Cladosporium cladosporioides*
*Pseudobotrytis terrestris**	***Clonostachys cylindrospora***
Thozetella cristata	*Codinaea simplex*
T. tocklaiensis	*Ellisiopsis gallesiae*
Trichoderma sp.	*Idriella fertilis**
	Menisporopsis novae-zelandiae
	Penicillium citrium
	Ramularia fusisaprophytica
	Scolecobasidium cateniphorum
	Solosympodiella clavata
	Sympodiella laxa
	Thozetella cristata
	Trichoderma sp.
	*Zygosporium gibbum**
	*Z. masonii**

* less dominant.
Fungi in bold face are dominant ones common to both experiments.

Formation of the fruit bodies of several mushrooms was recorded during the period of the present study in this station (Table 7). A total of thirteen species was found to occur and they are, for example, *Tricholoma ustale, Marasmius purpureostriatus, Amanita virosa, A. pseudoporphyria, Tylopilus virens, Russula senecis, R. lepida, Lactarius hygrophoroides, L. piperatus, Cantharellus cibarius*, etc.

Station D. Unfortunately, the set of sterilized leaves for five months decay in the first experiment was lost, possibly due to weasels. Therefore, isolation of the fungi in November, 1975 was done only from the naturally fallen leaves of *L. edulis* in the station which was shown in Plate 8. Similarly, isolation of the one month sample of sterilized embedded leaves in the second experiment was also impossible because of an accident. This is shown in Plate 9.

In this station, as in Station C, the forest consits exclusively of *L. edulis*. During the first and second incubation periods, forty and fifty-three species of fungi, respectively, were found to colonize on the leaves. Seventy-one fungal species in total were isolated of which only twenty-two were common to both experiments. This is an example of a large fluctuation in the species of fungi appearing under different conditions. As also seen in Station C, fungi colonized on the embedded leaves in the

forest of *L. edulis* in the summer season were fewer and less vigorous in growth.

In this station, *Beltrania rhombica, Chaetopsina fulva, Clonostachys cylindrospora, Menisporopsis novae-zelandiae, Volutina concentrica* and species of *Trichoderma* were the most dominant colonizers in both experiments. A species of *Cephalosporium* was also predominant in the second experiment.

Ellisiopsis gallesiae, Helicosporium lumbricoides and unidentified species of *Idriella* were also not uncommon in this forest.

Noteworthy fungi in this station were *Cylindrocladium parvum, Dactylella ramosa, Hansfordia pulvinata, Kramasamuha sibika, Penicillium multicolor, Myrothecium leucotrichum* and *Speiropsis pedatospora* which were found to colonize on the embedded leaves only in this forest.

Arthrobotrys oligospora, Solosympodiella cylindrospora and *Stachybotrys cylindrospora* were also isolated on the naturally fallen leaves of *L. edulis*, but not on the embedded leaves of *C. cuspidata* and *Q. phillyraeoides*, only in this station.

Chaetopsina fulva, Idriella fertilis, Menisporopsis novae-zelandiae, Penicillium funiculosum, Scolecobasidium humicola, Uberispora simplex, unidentified species of Ascomycetes, *Cylindrocladium* and *Gliomastix* which were not found in Stations A and B were common to Stations C and D. Of these, *U. simplex* is characterized by its selective and vigorous colonization on the embedded leaves of *C. cuspidata* and *Q. phillyraeoides* after five months decay.

On the other hand, fungi such as *Beltraniella japonica, Chaetendophragmia triangularia, Gyrothrix circinata, Helminthosporium velutinum, Paecilomyces terricola, Pleurophragmium bicolor, Pseudofusarium semitectum, Scolecobasidium constrictum* and *Solosympodiella rigididentata,* which were found to colonize on the leaves in Station C, have not yet been found in Station D.

Furthermore, *Gyrothrix podosperma* and *Nakataea fusispora,* which were found in Stations B and C, and *Wiesneriomyces javanicus,* which was found in Stations A and C, have not yet been detected in this station.

It is noteworthy that *Subulispora procurvata* and *S. rectilineata,* which were dominant fungi in both *Castanopsis* and *Quercus* forests, were not detected in this station or in Station C.

In the first experiment, *Dactylaria fusiformis* and *Monacrosporium ellipsosporum* were moderately common after three months decay. However, they were not detected in the second experiment at all. *Blastophorum truncatum, Helicosporium lumbricoides* and *Myrothecium leucotrichum* were somewhat more abundant in the first experiment than in the second.

In contrast, some fungi not found in the first experiment were found to colonize on the leaves in the second. These were *Centrospora gracilis, Cladosporium cladosporioides, Codinaea simplex, Idriella fertilis, Penicillium citrinum, Scolecobasidium cateniphorum* and *Zygosporium gibbum.* These results agreed well with those obtained in Station C and may reflect seasonal fluctuation in the occurrence of these litter fungi.

Ellisiopsis gallesiae which was detected once on the naturally decayed leaves was

also found to colonize dominantly on the leaves in the second experiment, particularly after three months decay.

In Station D in the summer, *Blastophorum truncatum, Helicosporium lumbricoides, Dactylaria fusiformis, Monacrosporium ellipsosporum, Myrothecium leucotrichum,* species of *Thozetella* and Basidiomycetes were dominant colonizers, while *Centrospora gracilis, Cladosporium cladosporioides, Codinaea simplex, Ellisiopsis gallesiae, Idriella fertilis, Penicillium citrinum, Scolecobasidium cateniphorum, Uberispora simplex, Zygosporium gibbum,* species of *Chalara, Cephalosporium* and *Idriella* were predominant colonizers in the winter.

Ramularia fusisaprophytica and *Idriella fertilis* were detected on the embedded leaves at an early stage of decay, while *Blastophorum truncatum, Ellisiopsis gallesiae, Endophragmia uniseptata, Monacrosporium ellipsosporum* and *Scolecobasidium cateniphorum* were found to colonize on the leaves at the later stage.

During the incubation period in Station D where *L. edulis* grows solely, a greater number of species of the macrofungi was detected when they have appeared as fruit body (Table 7). They are, for example, *Hygrophorus cuspidatus, Tricholoma ustale,*

Table 6. Fungi characteristic in their dominant colonization in Station D.

Dominant in the first experiment (July-November)	Dominant in the second experiment (December-May)
Beltrania rhombica	*Acrodontium crateriforme**
*Blastophorum truncatum**	***Beltrania rhombica***
Chaetopsina fulva	*Centrospora gracilis*
Clonostachys cylindrospora	*Cephalosporium* sp.
*Dactylaria fusiformis**	***Chaetopsina fulva***
Helicosporium lumbricoides	*Chalara* sp.
Menisporopsis novae-zelandiae	*Cladosporium cladosporioides*
*Monacrosporium ellipsosporum**	***Clonostachys cylindrospora***
*Myrothecium leucotrichum**	*Codinaea simplex*
*Thozetella cristata**	*Ellisiopsis gallesiae*
*T. tocklaiensis**	*Idriella fertilis*
Trichoderma sp.	*Idriella* sp.
Volutina concentrica	***Menisporopsis novae-zelandiae***
	Penicillium citrinum
	*Ramularia fusisaprophytica**
	Scolecobasidium cateniphorum
	*Solosympodiella clavata**
	Trichoderma sp.
	Volutina concentrica
	Zygosporium gibbum

* less dominant.
Fungi in bold face are dominant ones common to both experiments.

Table 7. List of macrofungi appeared as fruit bodies in three stations examined.

Fungus	Station		
	B	C	D
Basidiomycotina			
Agaricales			
Hygrophorus cuspidatus Peck	−	−	+
Tricholoma ustale (Fr.) Kummer	−	+	+
T. flavobrunneum (Fr.) Quél.	+	−	−
Oudemansiella longipes (St. Amans) Moser	+	−	−
Marasmius purpureostriatus Hongo	−	+	−
Mycena rorida (Fr.) Quél.	−	−	+
Amanita vaginata (Fr.) Vitt.	−	−	+
A. pantherina (Fr.) Secr.	+	−	+
A. virosa Secr.	−	+	+
A. pseudoporphyria Hongo	−	+	−
A. caesarea (Fr.) Quél.	−	−	+
A. fuliginea Hongo	+	−	−
A. spissa (Fr.) Kummer	−	−	+
A. spissacea Imai	−	−	+
Tylopilus eximius (Peck) Sing.	−	+	−
T. virens (Chiu) Hongo	−	+	−
Pulveroboletus retipes (Berk. & Curt.) Sing.	+	−	−
Boletus edulis Fr.	+	−	−
Strobilomyces floccopus (Fr.) Karst.	−	−	+
Russula compacta Frost & Pk.	+	−	−
R. senecis Imai	−	+	−
R. cyanoxantha (Secr.) Fr.	−	+	−
R. amoena Quél.	+	−	−
R. bella Hongo	−	−	+
R. virescens (Zanted.) Fr.	−	−	+
R. lepida Fr.	−	+	−
R. aurata Fr.	+	+	−
Lactarius hygrophoroides Berk. & Curt.	−	+	−
L. piperatus (Fr.) S. F. Gray	−	+	−
L. subzonarius Hongo	−	−	+
Aphyllophorales			
Clavaria rosea Fr.	−	−	+
C. vermicularis Fr.	−	−	+
Cantharellus cibarius Fr.	+	+	−
Gasteromycetales			
Mutinus bambusinus (Zoll.) Fisch.	−	−	+
Calvatia craniformis (Schw.) Fr.	−	−	+
Scleroderma verrucosum (Vaillant) Pers.	−	−	+
Total number of species	10	13	18

Amanita pantherina, A. virosa, A. caesarea, A. spissa, A. spissacea, Strobilomyces floccopus, Russula bella, Lactarius subzonarius, Clavaria rosea, C. vermicularis, Mutinus bambusinus, Scleroderma verrucosum and *Calvatia craniformis.*

In Station D, the occurrence of species of *Amanita* seems to be predominant than in Stations B and C.

Autoecology and brief notes on the species encountered

Some consideration of the autoecology of the species and genera found on both the sterilized embedded leaves and naturally fallen leaves surrounding them was given in our previous papers (10, 11, 12, 14). In addition, several characteristic habitats or occurrence of particular fungi were also mentioned above. We extend our consideration of the autoecology of the individual fungi by comparing the data obtained in Stations A-D.

Zygomycotina

Generally, the fungi belonging to Mucorales are believed to be abundantly distributed in the soil of the forest and may develop on the surface of the sterilized embedded leaves in the comparatively early stage of decay, particularly at high humidity. However, few were found to colonize on the leaves in the present experiment. Only one species of *Mortierella* was found on the sterilized embedded and naturally fallen leaves.

Ascomycotina

Chaetomium globosum was found once on the embedded leaves of *C. cuspidata* in Station B. An unidentified species of *Nectria* also occurred once on the embedded leaves of *C. cuspidata* in Station A.

Unidentified discomyceteous fungi were found to colonize on the naturally fallen leaves of *C. cuspidata* and *Q. phillyraeoides,* respectively, at four months decay in Station A and two to four months decay in Station B. Moreover, in Station C, an unidentified Ascomycete was found once on the embedded leaves of *C. cuspidata* of four months decay.

Basidiomycotina

Basidiomyceteous fungi, particularly agarics, have been frequently found on the litter of the evergreen oak forests, as previously pointed out (10, 11, 12, 14). However, in this experiment, isolation of basidiomyceteous fungi was less frequent. Some unidentified species of mycelial form were found on both the sterilized embedded and naturally fallen leaves in all the stations. In no instance has a basidiomyceteous fungus with *Rhizoctonia* state been isolated so far. These fungi were not uncommon on the leaves in the evergreen oak forests in both Yakushima and Tanegashima, Kagoshima Pref., as reported previously (12).

Deuteromycotina

Acrodontium crateriforme was found to colonize only on the sterilized embedded leaves of *C. cuspidata* and *Q. phillyraeoides* in all the stations and selectively in

December to February. This species tends to develop during the winter season.

Beltrania rhombica was one of the most frequently encountered fungi in this experiment. It was very common and grew rapidly on the surface of the leaves throughout the summer season in all the stations; it was not uncommon in winter and spring in Stations C and D, but was less common or not detected in winter in Stations A and B.

Beltraniella japonica, which is characteristic in its conidial formation on the fertile terminal of long rigid seta and smaller size of conidia, was found twice on the embedded leaves of *Q. phillyraeoides* in Station C. Habitat and manner of colonization are not clear. This fungus was originally described by Matsushima (5) based on the isolates from Kishiwada, Osaka Pref. and Yakushima, Kagoshima Pref., but seems to be not uncommon in the evergreen oak forests in Japan.

Blastophorum truncatum (4, 5, 13) was not uncommon on the embedded leaves of two to three months decay and less common on the naturally decayed leaves. This fungus has not yet been isolated in Station B, though it was found fairly commonly in the evergreen oak forests.

Candelabrum brocchiatum was found to colonize on the sterilized embedded leaves of *C. cuspidata* and *Q. phillyraeoides* in Stations A and B, all of five months decay in the summer season.

Catenularia was a rare fungus in this experiment and the species has not yet been identified.

Centrospora gracilis was reported recently (5) and is not uncommon on the leaves of two to three months decay. This species was found in all the stations and was considered to be a predominant colonizer in the winter season.

Chaetendophragmia triangularia was originally described from New Guinea by Matsushima in 1971 (4) and is unique in the characteristic shape of the conidia which have two to three long appendages downwards on the forth cell from the base. This fungus was found on the embedded leaves of *C. cuspidata* and more frequently on naturally fallen leaves of *L. edulis* in Station C. This fungus appeared from October, 1975 through April, 1976.

Chaetopsina fulva was found only in Stations C and D where *L. edulis* grows exclusively. This fungus develops in abundance throughout the year, but tends to more or less decrease at a later stage of decay.

Chalara sp. was not uncommon in all the stations and develops on the whole surface of the leaves throughout the year.

Chloridium chlamydosporis was very common in Station A. In Stations B and D, this fungus was found once on the naturally decayed leaves.

Chloridium laeense was isolated once on the naturally decayed leaves of *L. edulis* in Station C. This fungus was originally reported by Matsushima on decayed leaves of unidentified broad-leaved tree in Lae, New Guinea (4).

Circinotrichum maculiforme was found in Stations B, C and D and was not uncommon particularly on the naturally decayed leaves in Stations B and C. This fungus is thought to be unlikely to colonize on the embedded leaves, at least of more or less

early decay.

Cladosporium cladosporioides was not uncommon, except in summer, in Stations C and D where it occurred exclusively on the embedded leaves, and was less common in Stations A and B where it was found exclusively on the embedded leaves of *C. cuspidata*. This fungus is one of the most common saprophytes in the world.

C. inaequiseptatum was less common and selectively colonized on the naturally fallen leaves in all the stations with one exception, the embedded leaves of *Q. phillyraeoides* in Station A. This fungus was found to develop only in April and May. This fungus was described by Matsushima based on the fungus on dead leaves of *Q. phillyraeoides* in Hiroshima, Japan (5).

C. oxysporum is known to occur commonly in the tropics on dead leaves and stems of both herbaceous and woody plants (1). This fungus was found to colonize mainly on the embedded leaves of *C. cuspidata* after five months decay in the second experiment in Stations A, C and D. In Station D, this fungus was also isolated from the naturally fallen leaves of *L. edulis* in May, 1976. In no instance was this detected in the first experiment.

Clonostachys cylindrospora, though less common in Stations A and B where it was found to colonize selectively on the embedded leaves of *C. cuspidata,* was one of the most dominant fungi in Stations C and D where it was found to colonize very vigorously throughout the year on all kinds of leaves so far tested.

Codinaea simplex was also one of the most common fungi in all the stations and developed rapidly on the surface of the leaves within one month. However, it was not found in July through November in Stations C and D.

Cryptophiale guadalcanalense was found only in Station A where it was not uncommon except in winter. This fungus has been found to colonize on the leaves in the evergreen oak forests in Kagoshima Pref., and Okinawa Pref., but less common than *C. udagawae* (5, 13).

C. udagawae was fairly common in Stations A and B, except in winter, and developed on the leaves after two months decay. In Stations C and D, this fungus did not colonize on the embedded leaves at all, though was once isolated on the naturally fallen leaves in Station D. This fungus is one of the most common litter fungi in the evergreen oak forests in Japan and also in the countries of South Asia and Oceania.

Cylindrocladium parvum appeared only twice in Station D, where it was found to colonize on the leaves of four and five months decay in the second experiment. An unidentified species of *Cylindrocladium* was also encountered in both Stations C and D, but seemed sporadic in colonization.

Cylindrocladium state of *Calonectria kyotensis* with its perithecial state was not isolated in this work, though it was reported in our previous paper to be fairly common in an early stage of decay in the stations of Yakushima and Tanegashima (12).

Dactylaria fusiformis was not common, but was found to colonize on the leaves of three to four months decay only in the summer season. It has not been found to

colonize in the winter season, even if it was found growing on the naturally decayed leaves. *Mirandina typica* Matsushima is synonymous with this species. An unidentified species of *Dactylaria* was fairly common in Stations A and B and developed after two to three months decay in the summer season. In Stations C and D, it was less common.

Ellisiopsis gallesiae, the comparative name of which is *Beltraniella portoricensis,* was not uncommon in Stations C and D, less common in Station B, but was not found in Station A. This fungus was found to colonize dominantly particularly after three months decay in winter season. This is one of the most predominant litter fungi in the evergreen oak forests in Japan.

Endophragmia uniseptata was fairly common in all the stations and developed after two to three months decay. This fungus appeared to colonize preferentially on the embedded leaves of *C. cuspidata* in the summer season. However it was also found to colonize on the embedded leaves of *Q. phillyraeoides* in Stations A and D. In no instance has this fungus been isolated from the naturally decayed fallen leaves.

Hansfordia pulvinata was detected only in Station D, but was rare in its occurrence and less in its colonization.

Idriella fertilis is the proposed new combination name for *Circinotrichum fertile* Pirozynski & Hodges including *I. variabilis* Matsushima as synonyms (4, 5). It was common in Stations C and D, though rare or absent in Stations A and B, and appeared on the leaves of after two to three months decay. An unidentified species of *Idriella* was very common particularly in Stations A and B, and developed dominantly on the leaves after one month decay. In Stations C and D, however, this fungus developed after two to three months.

Kramasamuha sibika (8) is a rare fungus which was found once on the embedded leaves of *Q. phillyraeoides* of four months decay in Station D. This fungus was reported to have been isolated twice in Japan, Tokyo and Mie (5).

Menisporopsis novae-zelandiae was one of the most predominantly encountered fungi and was appeared exclusively in Stations C and D where it was developed even after one month decay. This fungus, which seems to be not uncommon in the subtropical and warm-temperate evergreen oak forests (9), was not found in our previous experiments (10, 11, 12, 14) or in Stations A and B in this experiment. Another species of the genus, *M. theobromae* (9) was found on the embedded leaves of *Q. phillyraeoides* in Kirishima, Kagoshima Pref., Kyushu, as reported previously (13), but was not found in this experiment.

Monacrosporium ellipsosporum is fairly common and appeared after three months in summer. This fungus was found infrequently on the embedded leaves in the winter season.

Paecilomyces elegans was not uncommon, particularly in Station B, and developed after one month.

Penicillium citrinum is characteristic in its seasonal occurrence and substrate speci-

ficity. This fungus appeared only in the winter season and was found to colonize only on the embedded leaves of *C. cuspidata* and *Q. phillyraeoides*; it was not isolated from the naturally decayed leaves used in this experiment.

Penicillium funiculosum was found only twice, in Stations C and D.

P. implicatum, the dominant fungus colonized on the embedded leaves in the previous experiments (10, 11, 12, 14), was hardly detected in this experiment.

P. thomii was also a dominant colonizer on the embedded leaves in the previous experiments (10, 11, 12, 14), but was not encountered in this experiment.

Pleurophragmium cylindrosporum was not uncommon in all the stations and was particularly dominant in Station A all the year round. However, this fungus, which usually colonized on the naturally developed leaf-litter, never colonized on the embedded sterilized leaves. Only one exception was found, on the embedded leaves of *Q. phillyraeoides* of two months decay in Station A.

P. bicolor and *P. simplex* also appeared rarely. Both fungi were found only on the naturally decayed leaves.

Pseudobotrytis terrestris was rare and appeared only on the embedded sterilized leaves in Stations A, C and D.

Ramularia fusisaprophytica was very common among the fungi encountered in Station B and was found to colonize dominantly after one month. This fungus was also not uncommon, particularly in winter, in the other three stations.

R. rhombica Matsushima (5) was reported on the dead leaves of *Quercus glauca*, *C. cuspidata* and *C. cuspidata* var. *sieboldii* in Japan. This fungus has been treated as *Ramularia* sp. in our previous report (10) which appeared significantly on the sterilized green leaves throughout decay and grown very luxuriantly. In no instance, however, was this fungus encountered in this experiment.

Scolecobasidium cateniphorum was not uncommon, except in Station B where it was not detected at all, and developed on the embedded leaves of after three months decay predominantly in the winter season.

Selenosporella curvispora was rare and sporadic in its occurrence. We failed to isolate into pure culture.

Speiropsis pedatospora was found twice in Station D, once on the naturally fallen leaves and once on the embedded leaves of *C. cuspidata* of three months decay.

Solosympodiella clavata (4, 5, 13) was found once on the embedded leaves of *Q. phillyraeoides* of six months decay in Station A, and was absent in Station B. On the other hand, this fungus was found on the naturally decayed leaves of *L. edulis* in Stations C and D, but was found once on the embedded leaves of *Q. phillyraeoides* of two months decay in the Station D.

Sympodiella laxa was fairly common in Stations B and C. This fungus appeared selectively on the embedded leaves of two to three months decay exclusively in the winter season in addition to on the naturally decayed leaves.

Subulispora procurvata was very common in Station A throughout the year and

was also found to colonize at an early stage of decay in Station B. Moreover, *S. rectilineata* was not uncommon in both stations, appearing on the leaves of four months decay in Station A and of two months decay in Station B. Neither species was found in Stations C and D where *L. edulis* grows exclusively.

Thozetella cristata (6, 13), the old name of which was *Neottiosporella* spp. or partly *N. radicata* in the previous reports (11, 12, 14), was not uncommon in all the stations, and developed on the leaves after one month decay, particularly in summer and more frequently in Stations B and C.

T. tocklaiensis was also predominant in summer in Stations B and C.

Trichoderma was also very common in all the stations tested and grew vigorously on the surface of the leaves after one month. Most isolates belong to *T. viride* aggr.

Uberispora simplex (6), *Arachnophora simplex* Ichinoe (3) is a basionym, is unique in the shape of its conidia which have characteristic, hyaline, peripheral, napiform satellite cells and was not uncommon in its occurrence in Stations C and D. This fungus was found to colonize only on the embedded leaves of *C. cuspidata* and *Q. phillyraeoides*, of two and four months decay in summer in Station C and of five months decay in winter in Stations C and D. This is the first record of this fungus in this series of work.

Volutina concentrica was not uncommon, particularly in summer and in Station D, and colonized mainly on the embedded leaves of *C. cuspidata* throughout the year.

Wiesneriomyces javanicus, which is very common in the warm temperate evergreen oak forests in Japan, was hard to detect in this experiment. The reason for this failure in isolation or detection is uncertain.

Zygosporium gibbum was not rare, but found to colonize on the leaves exclusively in winter. This fungus tends to occur at a later stage of decay.

Z. masonii was also rare and was not found in summer. This fungus was found to colonize in the winter season, selectively on the embedded sterilized leaves of *C. cuspidata* in Stations B, C and D. Colonies of this fungus, however, did not extend over the surface of the leaves, but were restricted to a small area.

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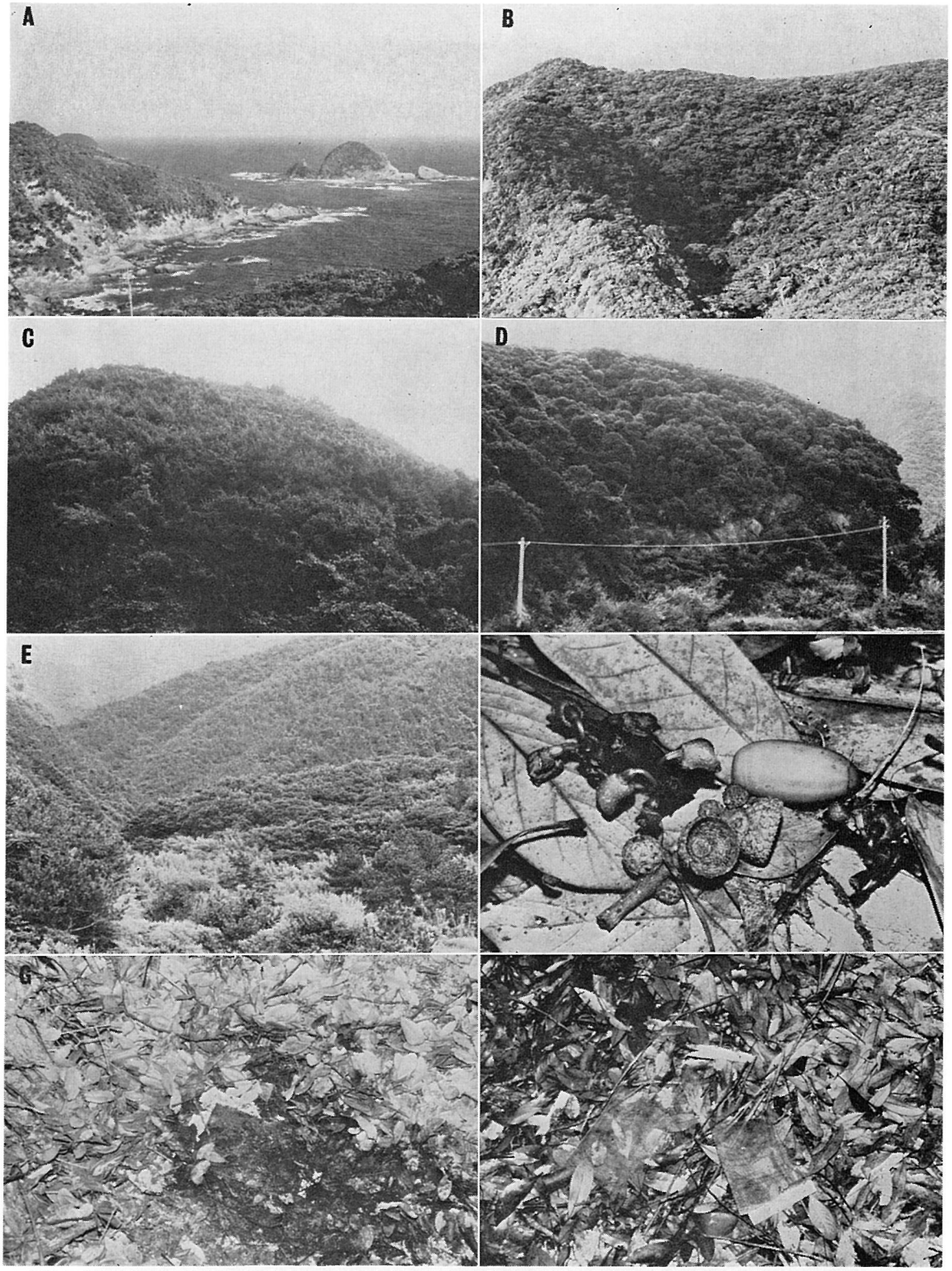

Plate 1. General views of the natural forests on Oosumi Peninsula tested. **A**. Southernmost part of Oosumi Peninsula (Cape Sata) facing the South China Sea. **B**. Forest of *Castanopsis cuspidata* in Station A near Shimadomari. **C**. Forest of *Quercus phillyraeoides* in Station B near Oodomari. **D**. Forest of *Lithocarpus edulis* in Station C at Yaneda, near Oodomari. **E**. Forest of *L. edulis* in Station D at Oodomari. **F**. Fallen leaves, nuts and cupules of *L. edulis* in Station C. **G**. Sample of five months' decay at the experiment site in Station B. **H**. Destroyed samples at the experiment site in Station D.

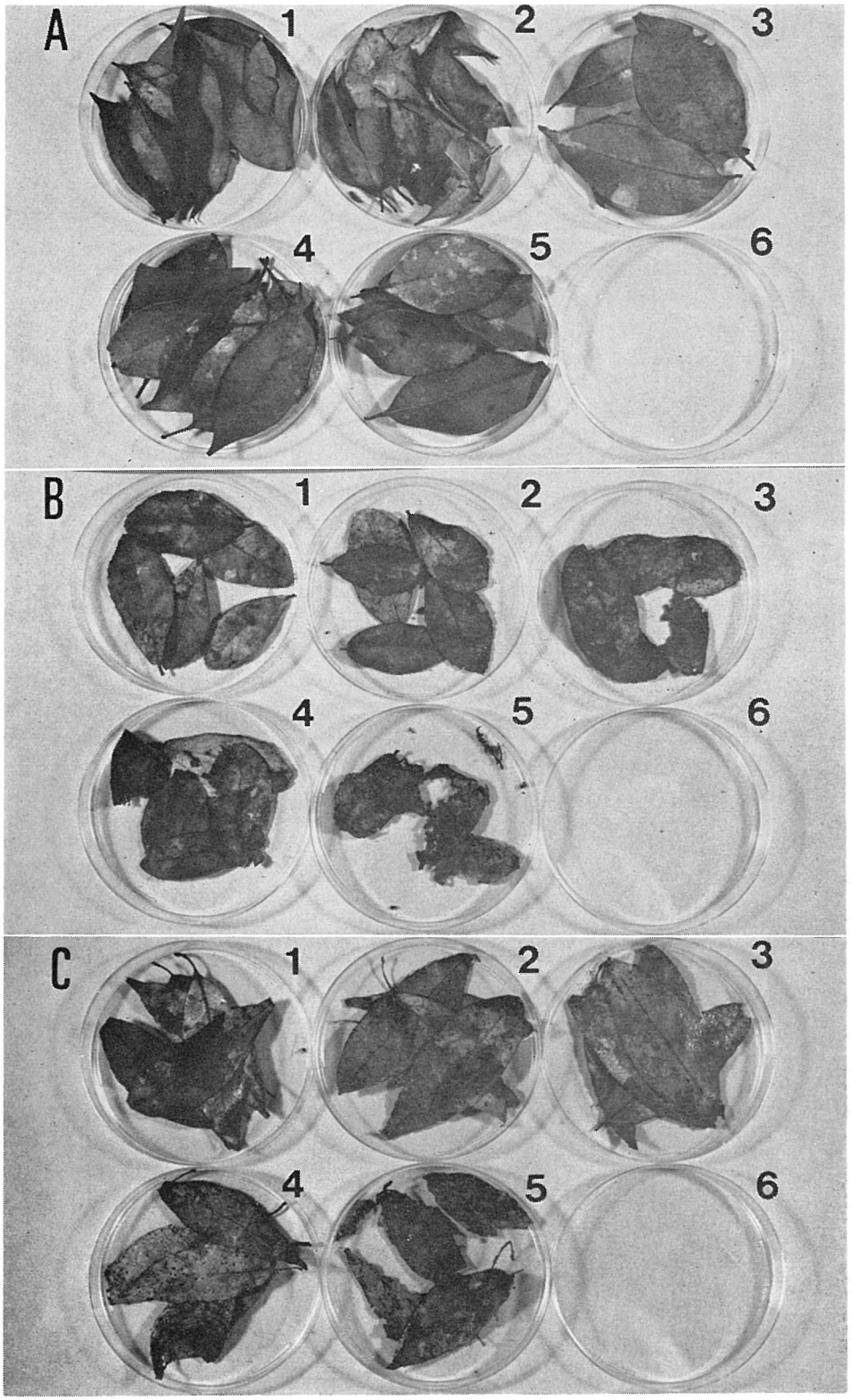

Plate 2. Comparison of the relative degree of the leaf-decomposition in the Station A, during five months (1–5) from June through November. A. Embedded leaves of *Castanopsis cuspidata*; B. Embedded leaves of *Quercus phillyraeoides*; C. Naturally fallen leaves of *C. cuspidata*.

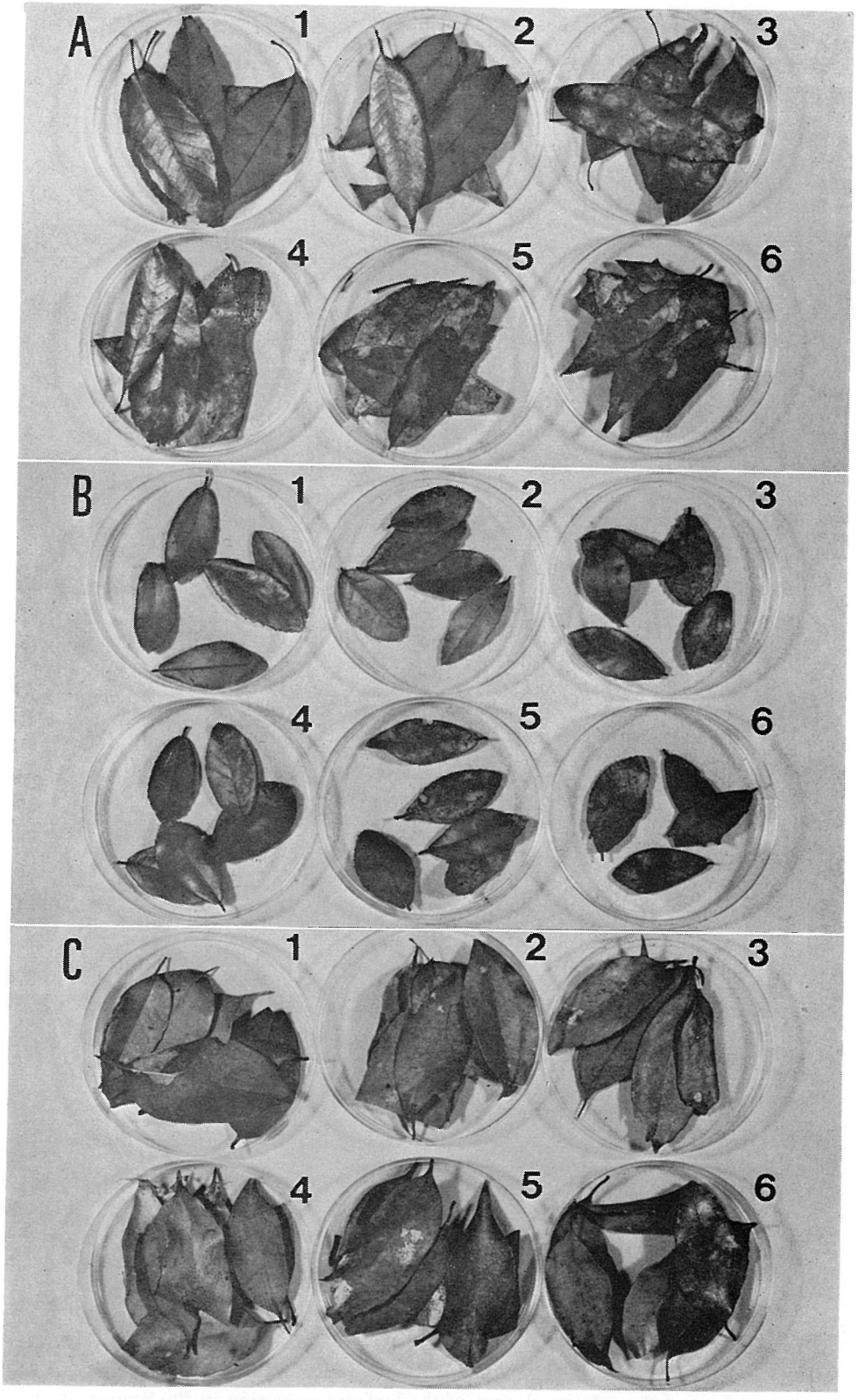

Plate 3. Comparison of the relative degree of the leaf-decomposition in the Station A, during six months (1–6) from November through May. A-C. Same as those in Plate 2.

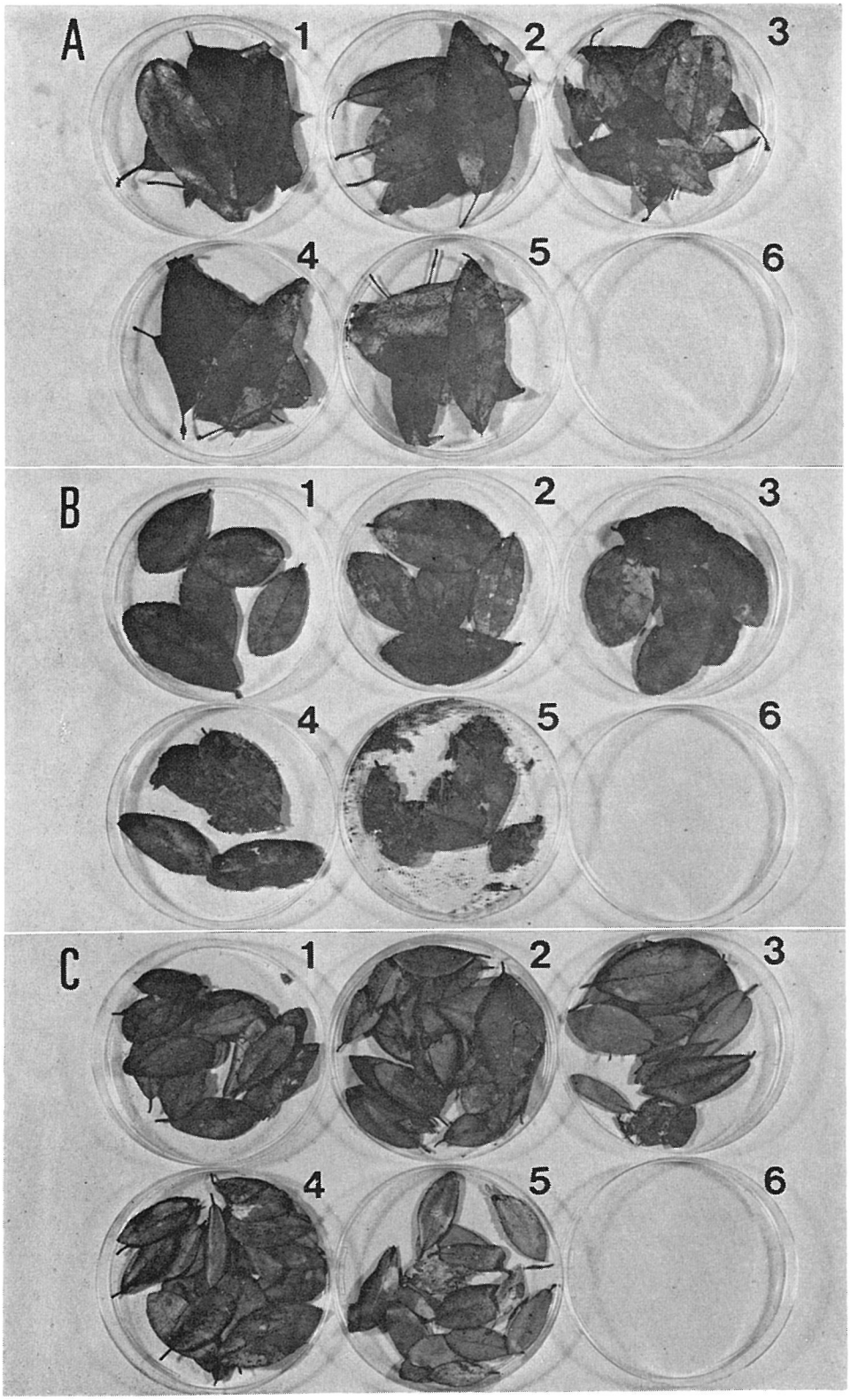

Plate 4. Comparison of the relative degree of the leaf-decompsition in the Station B, during five months (1–5) from June through November. A and B. Same as those in Plate 2; C. Naturally fallen leaves of *Q. phillyraeoides*.

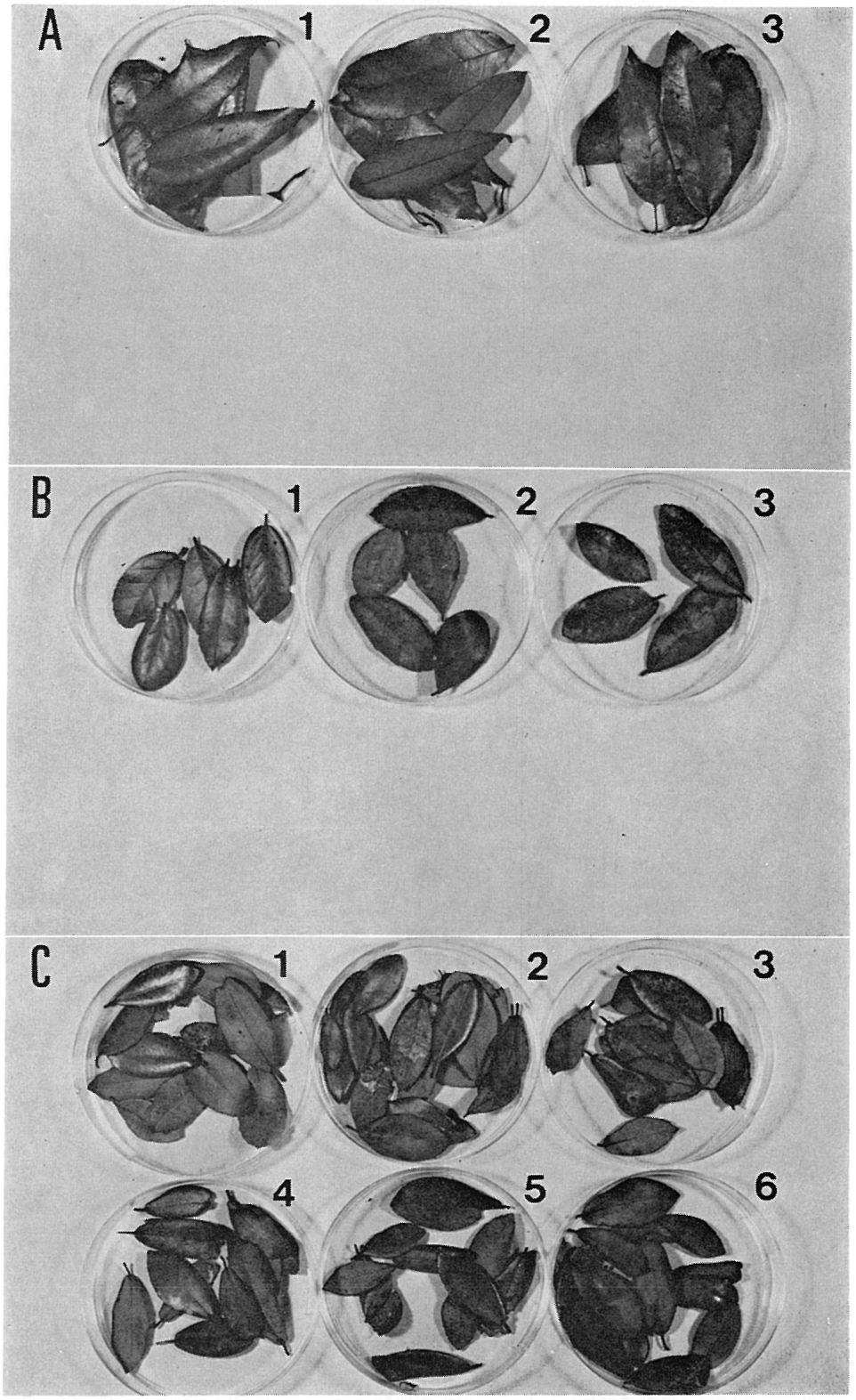

Plate 5. Comparison of the relative degree of the leaf-decomposition in the Station B, during six months (1–6) from November through May. Embedded leaves after four months were not recovered. A-C. Same as those in Plate 4.

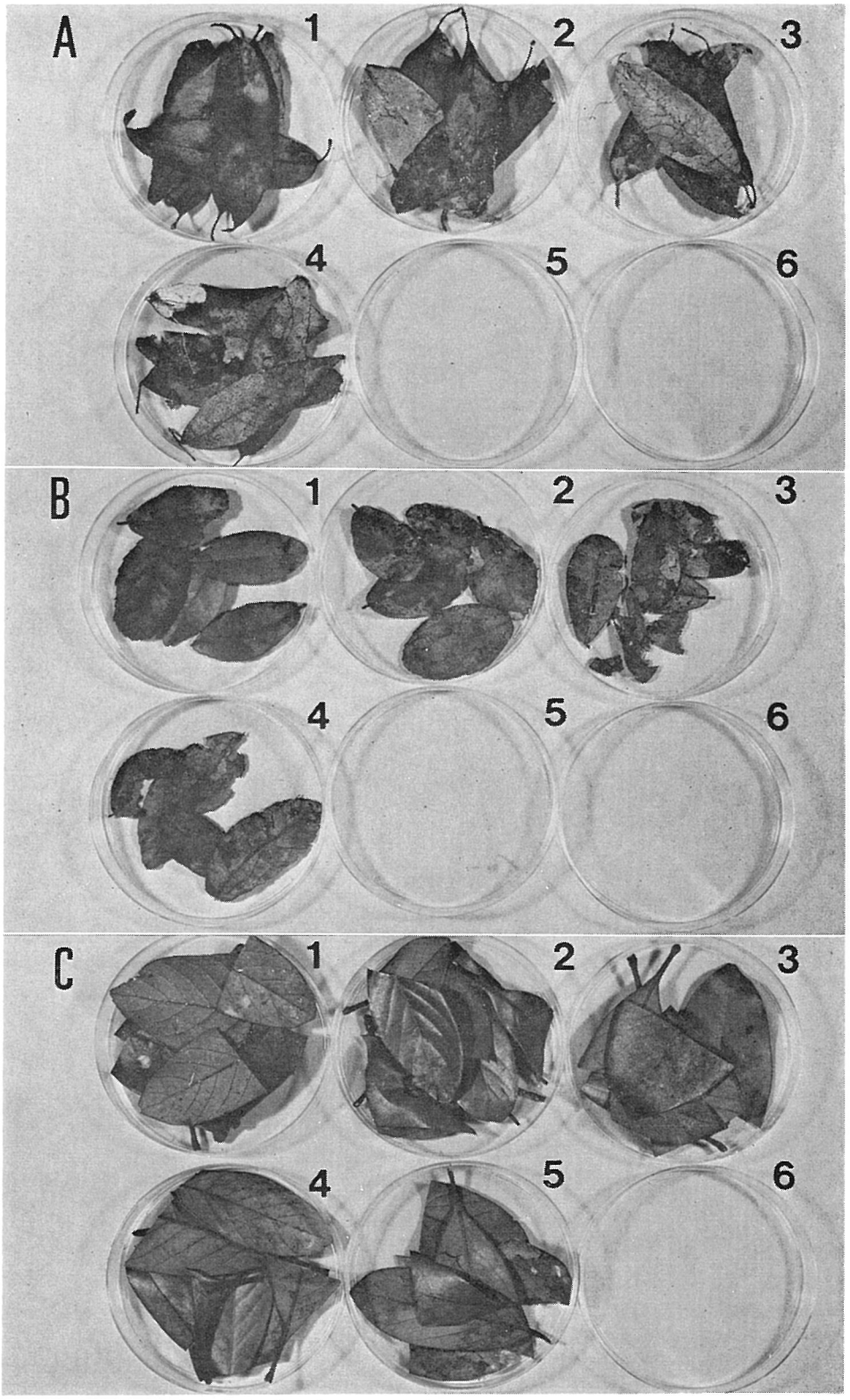

Plate 6. Comparison of the relative degree of the leaf-decomposition in the Station C, during five months (1–5) from June through November. Embedded leaves after five months were not recovered. A and B. Same as those in Plate 2; C. Naturally fallen leaves of *Lithocarpus edulis*.

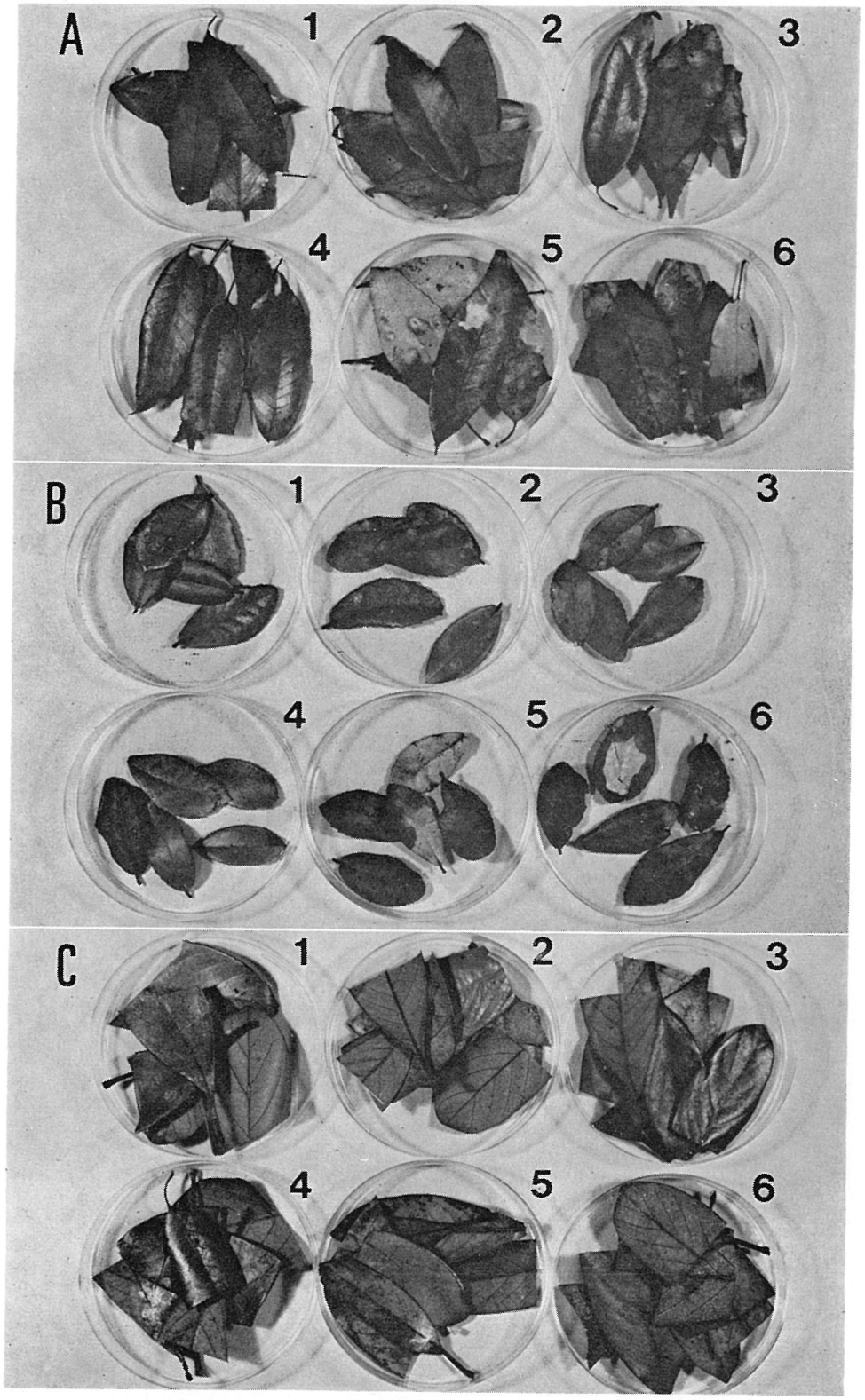

Plate 7. Comparison of the relative degree of the leaf-decomposition in the Station C, during six months (1–6) from November through May. A-C. Same as those in Plate 6.

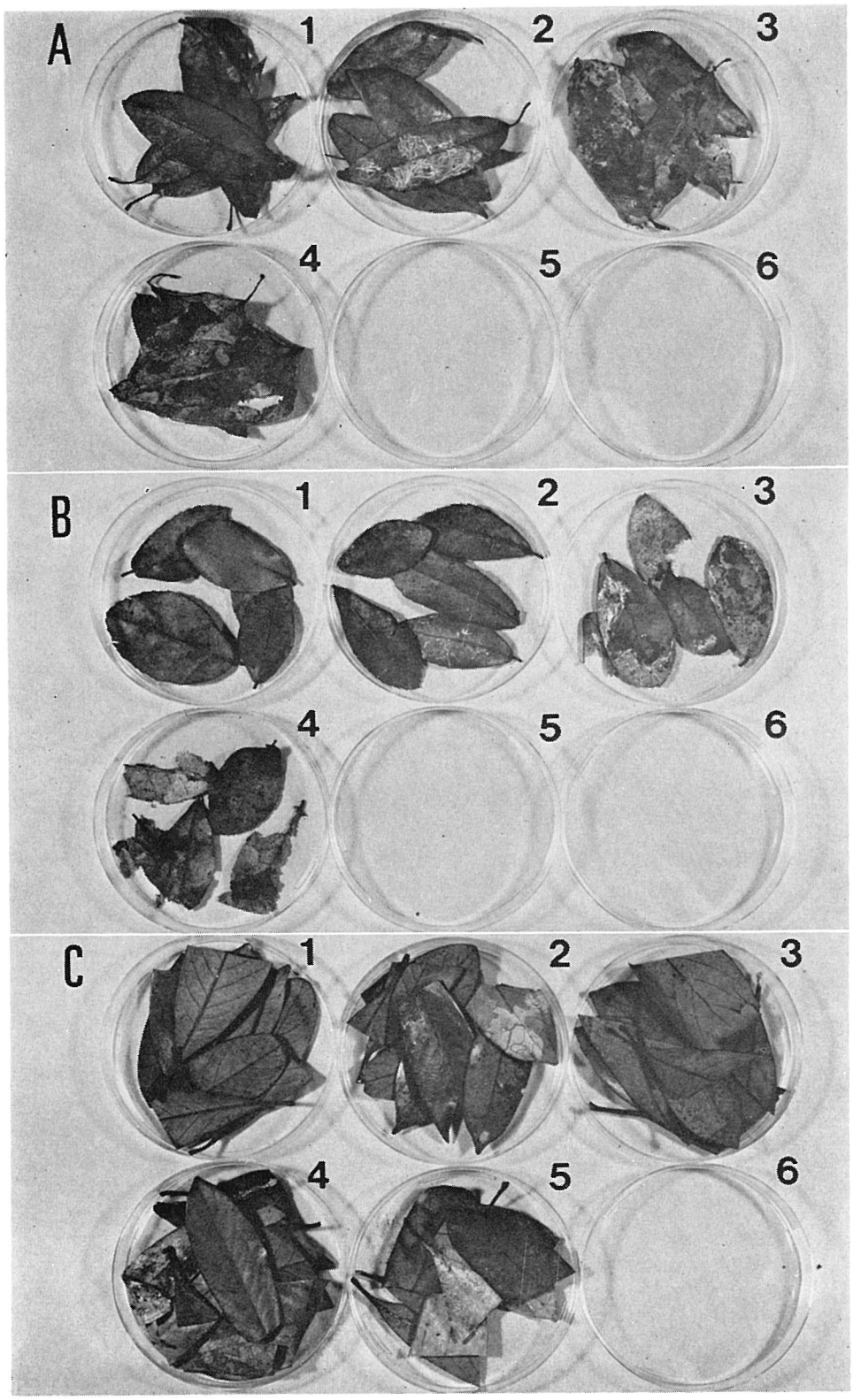

Plate 8. Comparison of the relative degree of the leaf-decomposition in the Station D, during five months (1–5) from June through November. Embedded leaves after five months were not recovered. A-C. Same as those in Plate 6.

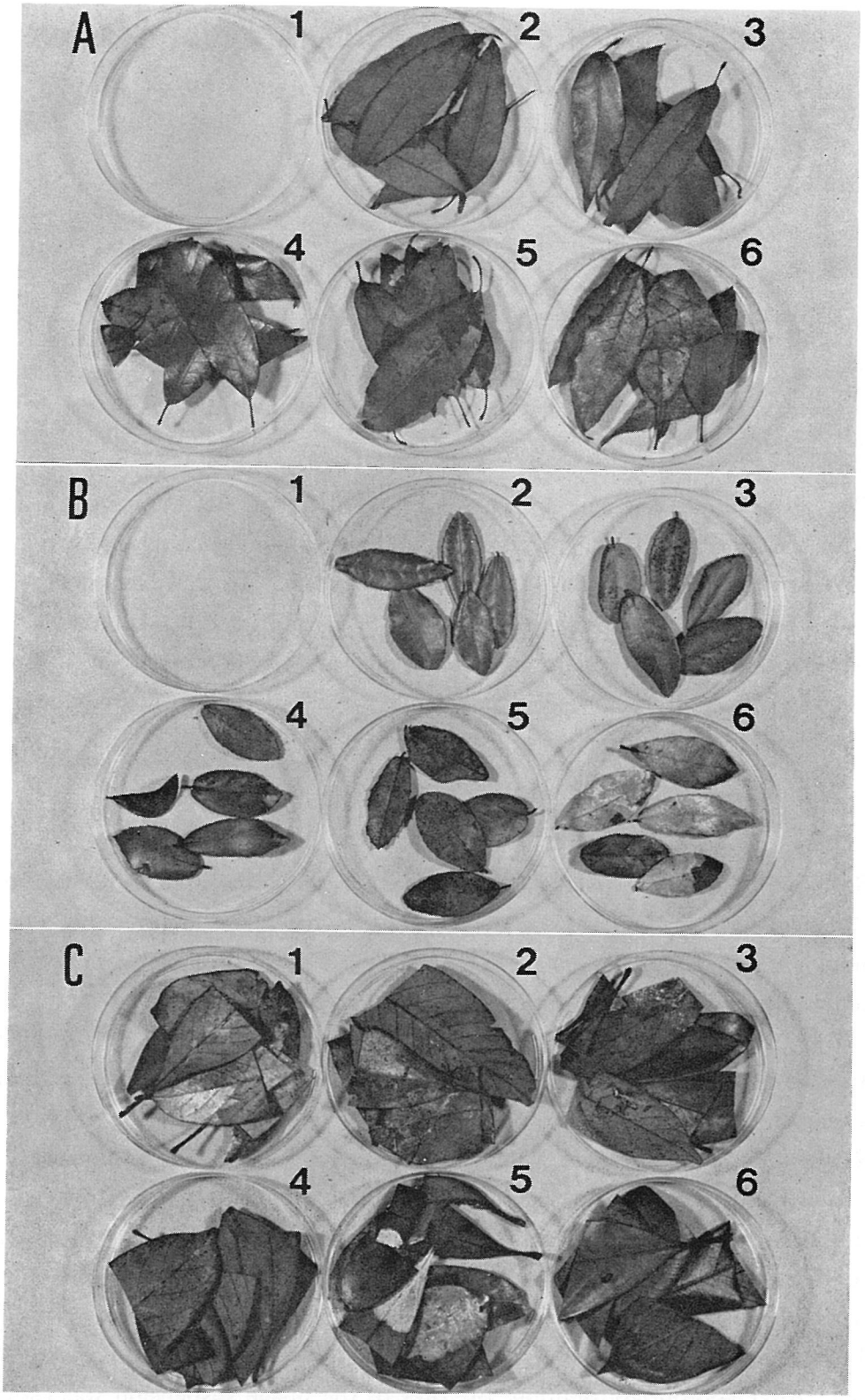

Plate 9. Comparison of the relative degree of the leaf-decomposition in the Station D, during six months (1–6) from November through May. Embedded leaves of one month decay were not recovered. A-C. Same as those in Plate 6.

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PRESERVATION OF *ESCHERICHIA COLI* PHAGES BY L-DRYING

Teiji IIJIMA and Takeshi SAKANE

Summary

Bacteriophage samples prepared by L-drying have been preserved for five years. The loss in titer of the phages on preservation was examined after rehydration of the samples. The results showed that the method was efficient for preserving phages over a long term without loss of titer.

The preservation of bacteriophages is achieved by various methods, i.e. storing lysates in a refrigerator, storing lysates at ultralow temperature such as in liquid nitrogen, or storing phage particles in the lyophilized state. In this laboratory bacteria and yeasts have been preserved by L-drying (3,5), as proposed by Annear (1). This method was also applicable to preservation of phages (4). The results in the previous paper showed the titers of phages immediately after L-drying. In this communication, we present the results after five years preservation.

Materials and Methods

Bacteria and phages. *Escherichia coli* and its phages were used as test organisms.

Phage lysates. Phage lysates were prepared by the soft agar layer method or by UV-induction, when the phages were temperate. The resulting phage lysates were poured into tightly stoppered tubes and stored in a refrigerator.

L-drying. For long term preservation the lysate was dispensed into samll tubes and dried *in vacuo* as described in the previous paper (4). The samples were stored in a refrigerator at 5 C.

Rehydration of the specimen. Before opening ampoules the maintenance of vacuum in the ampoule was checked with a High Frequency Tester (Edwared High Vacuum Ltd. Model T2). A small file mark was made on the ampoule at about the middle of the cotton plug, and a red-hot glass rod was applied to crack the ampoule. Half a ml of broth (peptone 1%, yeast extract 0.2% and NaCl 0.2% pH 7.2) was added to the ampoule and the contents were stirred gently to give a homogeneous suspension. After appropriate dilution, the titer of the phage suspension was estimated by the soft agar layer method on nutrient agar plates with an appropriate indicator strain.

Results and Discussion

The titer of the phages was checked by the method described in the previous section. Table 1 gives the results after five years preservation.

The loss in titer on preservation of L-dried specimens at 5 C was small, not exceeding 1.5 on a log scale except of T2 and T6. These two phages are sensitive to freezing or freeze-drying because of their complex morphology and of their sensitivity to osmotic shock (2). Table 1 also shows which process, L-drying or preservation, caused greater inactivation of phage particles. The result in Table 1 shows that reduction of phage titer was more noticeable on L-drying than on preservation, that is, the reduction of phage titer on the preservation was less than 1.0 on log scale.

In L-drying method, phage lysates were diluted two fold with phosphate buffer containing 3 % sodium glutamate and dried *in vacuo*. The composition of the suspending medium and drying condition were practically the same throughout the experiments. If we can enhance the number of phage survivors of the L-drying procedure by improving suspending medium or conditions of drying the method may be practically useful even for the extremely sensitive phages. Small phages such as ϕX 174, Qβ and MS2 were stable on L-drying.

Table 1. Loss in phage titer during L-drying and preservation.

phages	phage titer (log PFU*/ampoule)		
	before	L-drying immediately after	After preservation for 5 years
T1	9.3	9.1	8.9
T2	9.4	7.9	7.2
T3	8.2	8.2	7.4
T4	10.1	9.1	8.7
T5	9.7	9.0	8.5
T6	9.4	7.0	6.5
T7	9.4	9.0	9.1
lambda	8.5	7.2	7.2
lambda vir	9.8	9.5	9.5
ϕ 170	8.7	7.7	7.5
ϕ 170 vir	8.9	8.1	7.9
Qβ	9.4	9.4	9.1
f1	9.3	9.3	8.2
f2	9.1	9.4	9.1
M 12	9.2	9.2	8.9
MS2	9.3	9.0	8.6
ϕX 174	9.4	9.4	9.2
ATCC 11984B**	9.4	9.1	9.4

* PFU: plaque forming unit
** *Streptomyces griseus* phage

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LOCATION OF *tct* (TRICARBOXYLIC ACID TRANSPORT) GENES ON THE CHROMOSOME OF *SALMONELLA TYPHIMURIUM*

Ko IMAI, Teiji IIJIMA, and Isao BANNO

Summary

The mapping of *tct* (tricarboxylic acid transport) genes in *Salmonella typhimurium* was carried out by interrupted conjugation crosses. It was found that the *tctI* gene governing the first system, which is induced by citrate, isocitrate or *cis*-aconitate and transports citric and isocitric acids, is approximately 24 min clockwise from *hisF*, and the *tctII* gene governing the second system, which is induced by the same acids as in the first system and carries *cis*-aconitic acid, is also located at 24 min after the *hisF* marker. Both systems may be controlled under the same operon. The *tctIII* gene governing the third system, which is induced by tricarballylate and transports tricarballylic, citric and *cis*-aconitic acids, was located at about 3 to 4 min after the origin of transfer of HfrK4. The genes affecting the metabolism and/or transport of tricarballylic acid were found to cluster in this region.

Utilization of citric acid is an important characteristic for the identification of the species belonging to Enterobacteriaceae. Most of bacteria which are capable of growing in aerobic conditions should have the enzymes of the Krebs cycle, so the utilization of the intermediates of this cycle by the bacteria must depend on the existence of permeation systems for these acids. Previously we reported that *Salmonella typhimurium* utilizes citric, isocitric, *cis*-aconitic and tricarballylic acids and possesses four inducible transport systems for these tricarboxylic acids (2, 3). The first system is induced by citrate, isocitrate or *cis*-aconitate and transports citric and isocitric acids. The second system, induced by the same acids, carries *cis*-aconitic acid. The third system is induced by tricarballylate and carries tricarballylic, citric and *cis*-aconitic acids. The fourth system is induced by citrate and carries citric acid.

It is the purpose of this communication to report the mapping of the genes governing these transport systems.

Materials and Methods

Strains used. Table 1 lists the genetic stocks of *S. typhimurium* employed in this study. Isolation method and partial characterization for the *tct* (tricarboxylic acid transport) mutants have been reported previously (2). Two Hfr strains, SU418 (HfrB2) and SA534 (HfrK4), were obtained from the Salmonella Genetic Stock Centre, maintained at the University of Calgary, through the kindness of Dr. Sanderson. The positions of origins of transfer of the Hfr strains and auxotrophic markers of the

Table 1. Bacterial strains used.

Strain	Sex	Genotype[a)]	Utilization of the tricarboxylic acids[b)]
M72	F^-	*hisF*$^-$, *metA*$^-$, *trpB*$^-$, *xyl*$^-$, *strA*, *tctI*$^-$	CIT$^+$, ISO$^-$, C-A$^-$, TRI$^+$
M189	F^-	*hisF*$^-$, *metA*$^-$, *trpB*$^-$, *xyl*$^-$, *strA*, *tctII*$^-$	CIT$^+$, ISO$^+$, C-A$^-$, TRI$^+$
M272	F^-	*hisF*$^-$, *metA*$^-$, *trpB*$^-$, *xyl*$^-$, *strA*, *triR*$^-$	CIT$^+$, ISO$^+$, C-A$^+$, TRI$^-$
PT-1	F^-	*ilvA*$^-$, *tctI*$^-$, *tctIII*$^-$, (P22)$^+$	CIT$^+$, ISO$^-$, C-A$^-$, TRI$^-$
PT-5	F^-	*ilvA*$^-$, *triM*$^-$, (P22)$^+$	CIT$^+$, ISO$^+$, C-A$^+$, TRI$^-$
SU418	HfrB2	*proA*$^-$, (P22)$^+$	CIT$^+$, ISO$^+$, C-A$^+$, TRI$^+$
SA534	HfrK4	*serA*$^-$	CIT$^+$, ISO$^+$, C-A$^+$, TRI$^+$

a). Genetic symbols *tctI*, *tctII*, *tctIII*, *triM*, and *triR* designate the genes governing the first system, the second system, the thord system, the metabolism for tricarballylic acid, and the metabolism and transport for tricarballylic acid, respectively. Other symbols were described by Sanderson (4).

b). Phenotypic symbols + and − for the utilization of the tricarboxylic acids denote respectively the ability and inability to utilize citrate (CIT), isocitrate (ISO), *cis*-aconitate (C-A), or tricarballylate (TRI).

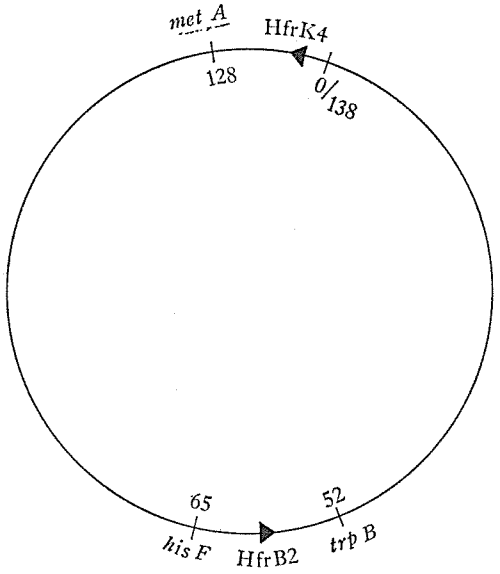

Fig. 1. Genetic linkage map of *S. typhimurium*, based on that of Sanderson (4), showing positions of relevant markers and origins of transfer of Hfr starins.

tct mutants are shown in Fig. 1.

Media. Nutrient broth (Difco) was used for liquid cultures or solidified with 0.8 % agar (nutrient soft agar) or 1.5 % agar (nutrient agar). The basal medium consisted of 7 g of K_2HPO_4, 3 g of KH_2PO_4, 1 g of $(NH_4)_2SO_4$, 1 g of NaCl, 0.1 g of $MgSO_4 \cdot 7H_2O$, and 1,000 ml of distilled water. Basal soft agar medium contained the constituents of the basal medium, 0.1 % streptomycin, and 0.8 % agar. The basal agar medium (the basal medium plus 1.5 % agar) supplemented with amino acids and 0.3 % one of the carbon sources, *i. e.*, isocitrate, *cis*-aconitate, tricarballylate, or succi-

nate, was used for the detection of the recombinants or the transductants. The concentration of each amino acid supplemented was 50 μg/ml.

Genetic techniques and procedures. Mating and kinetic analysis of the chromosome transfer were carried out by the method described by Sanderson and Demerec (5). The P22-mediated transduction was performed according to the method of Sanderson and Hall (6).

Results and Discussion

The locus of the tctI gene

In the previous IFO Research Communications (1), we reported that the *tctI*$^-$ gene of M72 is located between *hisF* and *thyA*. A kinetic analysis of transfer to M72 of *hisF*$^+$ and *tctI*$^+$ markers by SU418 was carried out by interrupted conjugation cross (Fig. 2). The recombinants for ISO$^+$ and C-A$^+$ were independently selected, since the function of the second system which carries *cis*-aconitic acid depends on the first system, and all mutants deficient in the first system do not utilize *cis*-aconitic acid (2). The result in Fig. 2 showed that the *tctI*$^+$ character was transferred approximately 24 min after the *hisF*$^+$ marker.

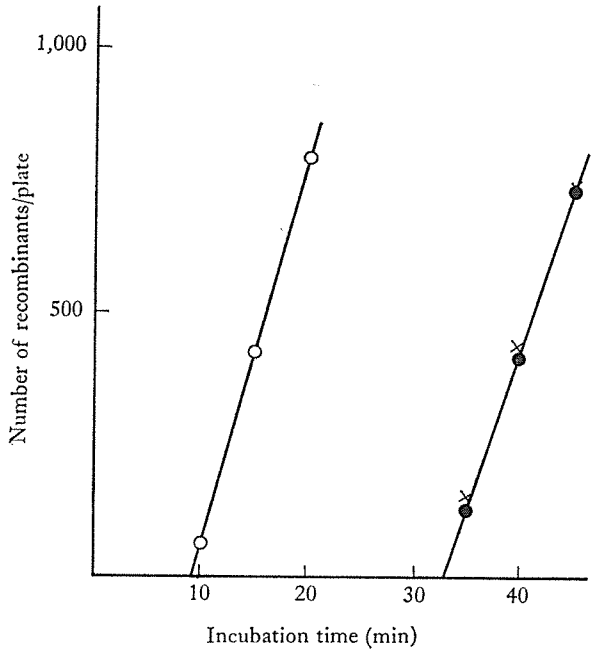

Fig. 2. Kinetics of transfer at 37 C to M72 of *hisF*$^+$ (—○—), and *tctI*$^+$ (ISO$^+$, —×— or C-A$^+$, —●—) markers by SU418.

The locus of the tctII gene

In a preliminary experiment, the *tctII*$^-$ gene of M189 was found to be located between *hisF* and *metA* (Table 2). A kinetic analysis by interrupted conjugation cross with SU418 and M189 showed that the *tctII* gene was transferred about 24 min

Table 2. Transfer of various markers in the crosses.

Cross	Number of recombinants/plate			
	trpB$^+$	*hisF*$^+$	*metA*$^+$	*tct*$^+$
SU418×M189 (P22)$^+$	39	9,240	82	1,230
	28	8,950	94	1,540
SA534×M189	39	24	0	5
	45	31	2	4
SU418×M189 (P22)$^+$	15	11,530	128	55
	16	8,340	136	38
SA534×M272	28	7	0	261
	24	11	3	235

About 2×10^8 donor cells and 10^9 recipient cells were mixed and collected on a Millipore filter. The filter was incubated on a plate of nutrient soft agar at 37 C for 5 min and placed in 16 ml of fresh nutrient broth for 3 hr at 37 C. The mating mixture were diluted in the basal medium, and the mated pairs were separated by vigorous shaking. One tenth ml of diluted samples was pipetted into 5 ml of basal soft agar maintained at 45 C and then poured onto each selective medium.

after the *hisF* marker as in the case of the *tctI* gene (Fig. 3). The first and the second systems for the tricarboxylic acids of the Krebs cycle in *S. typhimurium* are induced by the same acids (3), so the genes governing both systems may be involved in the same operon. We have also suggested that the function of the second system depends on the first system at the protein level (2). These findings may suggest that the first

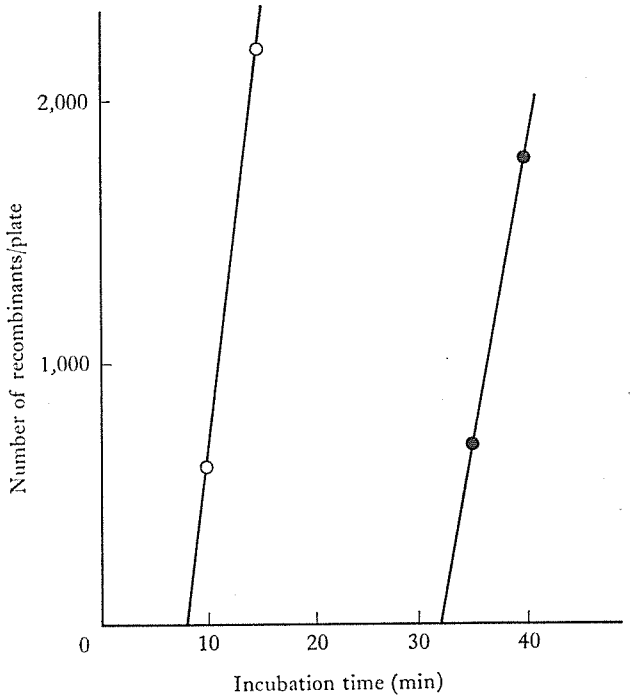

Fig. 3. Kinetics of transfer at 37 C to M189 of *hisF*$^+$ (—○—), and *tctII*$^+$ (C-A$^+$, —●—) markers by SU418.

and the second systems have evolved correlatively from their origins.

The locus of the tctIII gene

The result shown in Table 2 suggested that *triR*$^-$ gene of M272 is located between the origin of the transfer of HfrK4 and *trpB*. An interrupted conjugation cross with HfrK4 and M272 showed the TRI$^+$ recombinants appeared about 3 to 4 min after the onset of mating (Fig. 4).

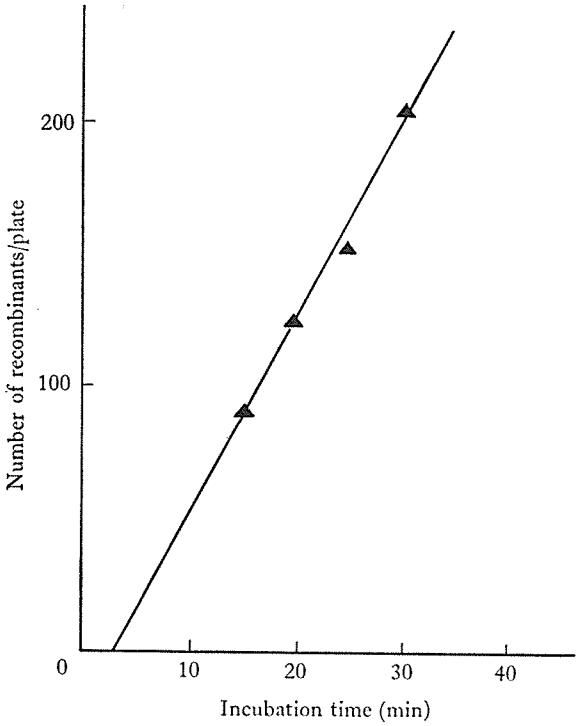

Fig. 4. Kinetics of transfer at 37 C to M272 of *triR*$^+$ (TRI,$^+$ —▲—) marker by SA534.

The general transducing phage P22, propagated on M272, was added to overnight broth cultures of PT-1 deficient in the third system (*tctIII*$^-$) and of PT-5 deficient in the metabolism of tricarballylic acid (*triM*$^-$) at the m. o. i. 5, and the TRI$^+$ and *ilvA*$^+$ trandsuctants were independently selected. The number of the *livA*$^+$ transductants of PT-1 and PT-5 was about 250 to 300 per plate inoculated with 2×10^8 recipient cells, while the TRI$^+$ trandsuctants were few. This result indicates that the genes, *tctIII*, *triM*, and *triR*, may form a cluster in or near the 2 to 3 min region on the map of *S. typhimurium* chromosome.

The gene governing the fourth system which is induced by citrate and carries citric acid has not yet been examined.

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A PLEIOTROPY IN CARBOHYDRATE METABOLISM OF *BACILLUS SUBTILIS* MUTANT LACKING TRANSKETOLASE

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Summary

A transketolase mutant of *Bacillus subtilis* showed the following pleiotropic phenotypes in carbohydrate metabolism. (i) It could not assimilate carbohydrates such as D-gluconate, D-xylose, L-arabinose and D-ribose which are metabolized initially through the pentose phosphate pathway. (ii) It scarecely grew on carbohydrates such as D-glucose, D-mannose, maltose, saccharose, trehalose and pyruvate. (iii) The assimilation of D-fructose, D-mannitol or glycerol was inhibited by D-glucose, D-gluconate, D-xylose or L-arabinose. (iv) It required for its growth aromatic amino acids such as L-tryptophan, L-tyrosine and L-phenylalanine. (i) and (iv) are readily explained by the deficiency in transketolase. But (ii) and (iii) are not accounted for. The mechanism of the pleiotropy is discussed.

Accumulation of a large amount of D-ribose by transketolase mutants of *Bacillus* species in the culture medium was reported in a previous paper (14). During the course of the study, it was also revealed that the transketolase mutants had a pleiotropy in carbohydrate metabolism (16), *i.e.* the mutant could not assimilate carbohydrates such as D-gluconate and D-ribose, scarecely grew on D-glucose and could not synthesize aromatic compounds such as L-tryptophan, L-tyrosine and L-phenylalanine. The deficiency in assimilation of carbohydrates related to the pentose phosphate pathway and in synthesis of aromatic compounds could be readily explained by the deficiency in transketolase which is a common key enzyme in both pathways (Fig. 1). But the malutilization of D-glucose by the transketolase mutant could not be accounted for. This investigation aimed initially to solve this problem and resulted in the finding of other pleiotropic phenotypes.

This paper deals with the pleiotropic phenotypes of the transketolase mutants of *Bacillus subtilis* which have been found so far. The mechanism of the pleiotropy is also discussed.

Materials and Methods

Bacterial strains. *Bacillus subtilis* IFO 12114 and its transketolase mutant BG 2607, which was isolated as a shikimic acid-requiring strain according to the method described previously (13), were used.

Assay of transketolase activity. Preparation of cell extracts and assay of transketolase activity were done according to the methods described previously (15), with

the following slight modification of the transketolase assay. D-Ribose 5-phosphate ketol-isomerase and D-ribulose 5-phosphate 3-epimerase, which have recently become available commercially, were used in this study. These enzyme preparations, as well as α-glycerophosphate dehydrogenase and triosephosphate isomerase mixture, contain a small amount of transketolase, and this activity, together with NADH oxidase activity, was subtracted from the apparent activity.

Growth test. (a) The short-time growth test for carbohydrate assimilation was carried out according to the method described previously (16). (b) The long-time growth test for the requirement of aromatic amino acids was done as follows. The cells of an overnight culture of the parent or the mutant strain in the same medium as that used for the short-time growth test were washed with 0.12M phosphate buffer, and suspended in the buffer to give an absorbance of 1.0 at 650 nm with a glass cell of 1-cm light path. Half a milliliter of the cell suspension was inoculated into 4.5 ml of the same medium containing 1.0% of sorbitol as the carbon source in a test tube (17-mm diameter). Shikimic acid, L-tryptophan, L-tyrosine or L-phenylalanine was added at the concentration of 0.5 mM. The test tubes were incubated at 37C on a reciprocal shaker. At given intervals, the absorbance at 650 nm of each culture was measured with Shimazu-Baush & Lomb Spectronic 20.

In both growth tests, 0.5% of sorbitol and 0.5% of L-glutamate were used as the carbon source in the seed cultures.

Chemicals. D-Ribose 5-phosphate ketol-isomerase, D-ribulose 5-phosphate 3-epimerase and α-glycerophosphate dehydrogenase and triosephosphate isomerase mixture were purchased from Sigma Chemical Co. (St. Louis, U.S.A.). D-Ribose 5-phosphate and NADH were products of Boehringer Mannheim Corp. (Tokyo, Japan). Shikimic acid and thiamine pyrophosphate were purchased from Wako Pure Chemical Ind. Ltd. (Tokyo, Japan) and Tokyo Kasei Kogyo, Ltd. (Tokyo, Japan), respectively.

Results

Isolation of transketolase mutant of B. subtilis IFO 12114

Transketolase is one of the key enzymes in pentose assimilation and is also an important enzyme in aromatic biosynthesis: it catalizes the reaction producing D-erythrose 4-phosphate which is a precursor moiety of aromatic compounds (Fig. 1). Therefore, the mutant can be isolated as a mutant requiring shikimic acid for its growth, which is an intermediate of aromatic biosynthesis. Strain BG2607 was isolated as a shikimic acid-requiring mutant. Determination of transketolase activity of strain BG2607 revealed that it lacked the enzyme (Table 1).

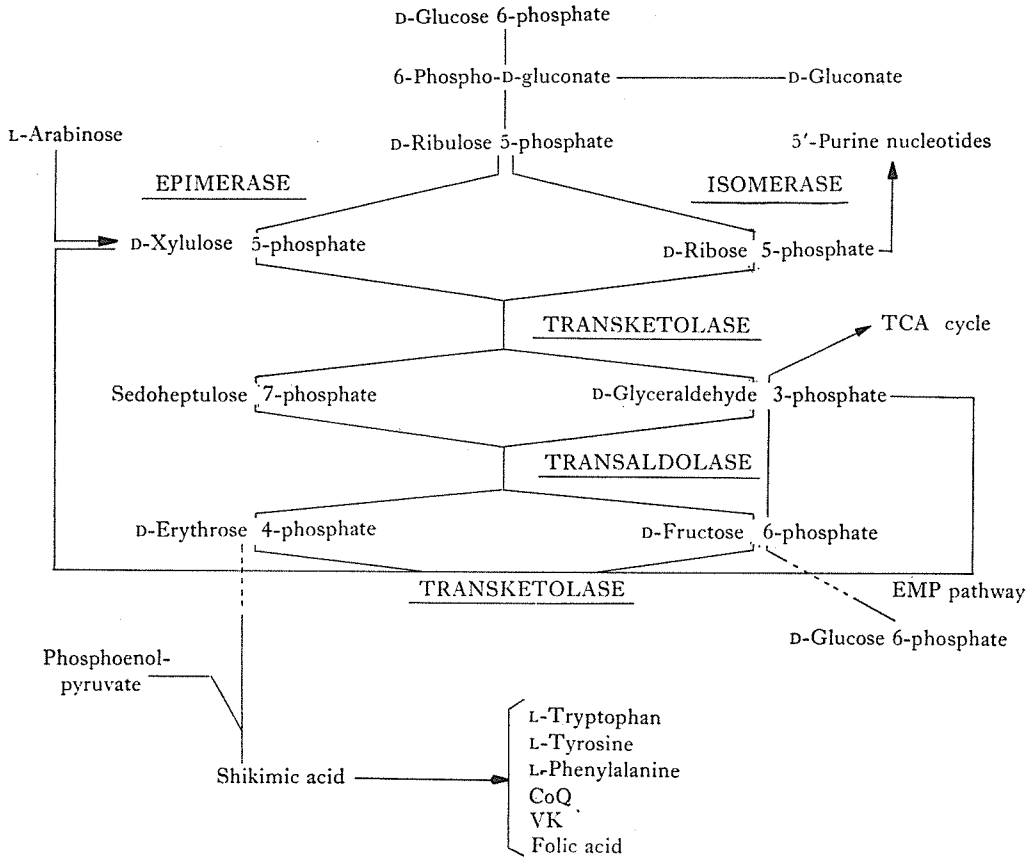

Fig. 1. The pentose phosphate pathway and related pathways.

Table 1. Transketolase activity in the parent and the mutant strains.

Strain	Transketolase activity (μ mol/min/mg of protein)
IFO 12114	0.19
BG 2607	0.00

The parent and the mutant strains were cultivated in the medium containing 1.0 % of sorbitol as the carbon source.

Pleiotropic phenotypes of the transketolase mutant

Defect in the pentose phosphate pathway. As shown in Figure 2, strain BG2607 could not assimilate D-xylose, L-arabinose and D-ribose which are catabolized through the pentose phosphate pathway. D-Gluconate can be assimilated usually through both the pentose phosphate pathway and the Entner-Doudoroff pathway (5). The transketolase mutant of *B. subtilis* could not assimilate D-gluconate because the Entner-Doudoroff pathway is lacking in *B. subtilis* (11).

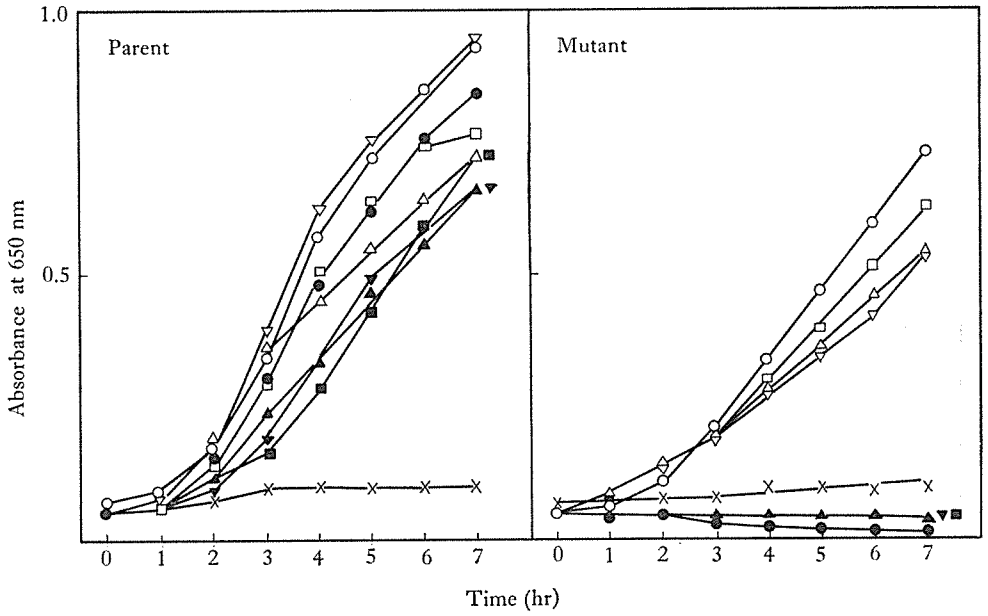

Fig. 2. Deficiency in assimilation of D-gluconate, D-xylose, L-arabinose or D-ribose by the transketolase mutant of *B. subtilis*.
The sugars were present at the concentration of 1.0 %. Blank, ×—×; D-fructose, ○—○; sorbitol, △—△; D-mannitol, ▽—▽; glycerol, □—□; D-gluconate, ●—●; D-xylose, ▲—▲; L-arabinose, ▼—▼; D-ribose, ■—■.

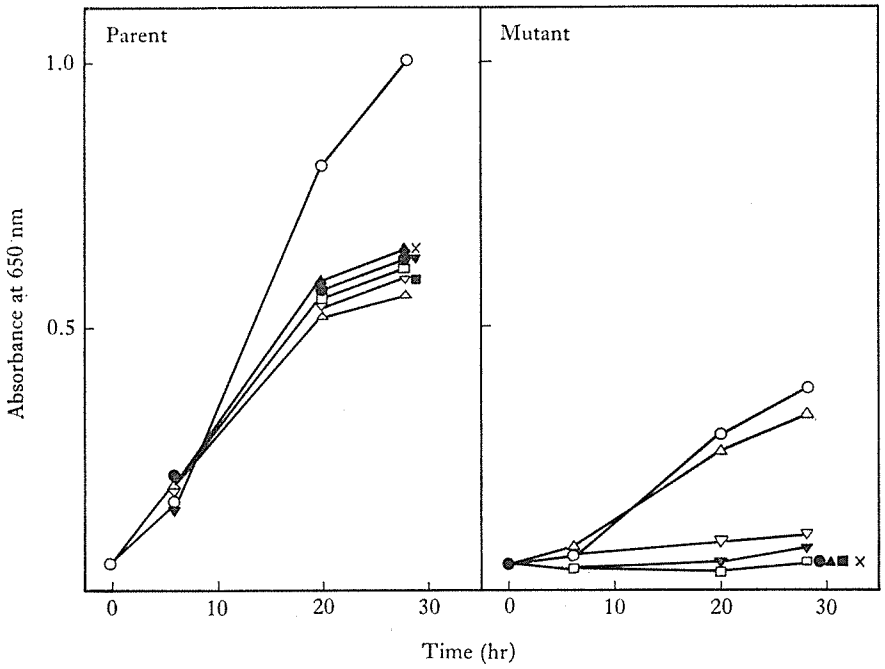

Fig. 3. Requirement of aromatic amino acids by the transketolase mutant of *B. subtilis* for its growth.
Sorbitol was used as the carbon source. Blank, ×—×; shikimic acid, ○—○; L-tryptophan, L-tyrosine and L-phenylalanine, △—△; L-tryptophan and L-tyrosine, ▽—▽; L-tryptophan and L-phenylalanine, □—□; L-tyrosine and L-phenylalanine, ●—●; L-tryptophan, ▲—▲; L-tyrosine, ▼—▼; L-phenylalanine, ■—■.

Defect in the aromatic biosynthesis. As strain BG2607 was isolated as a shikimic acid-requiring mutant, it is self-evident that either a mixture of L-tryptophan, L-tyrosine and L-phenylalanine or shikimic acid is essential for its growth as shown in Figure 3. Folic acid, vitamin K and coenzyme Q, which are also products of the aromatic biosynthesis, might be formed from L-tryptophan, L-tyrosine and/or L-phenylalanine because omission of these vitamins had no effect on its growth. Of course, shikimic acid was sufficient for all the requirements.

Malutilization of D-*glucose,* D-*mannose, maltose, saccharose, trehalose and pyruvate.* The transketolase mutant scarecely grew on D-glucose, D-mannose, maltose, saccharose, trehalose and pyruvate (Fig. 4). This phenotype can not be explained in terms of current knowledge of the role of transketolase in carbohydrate metabolism.

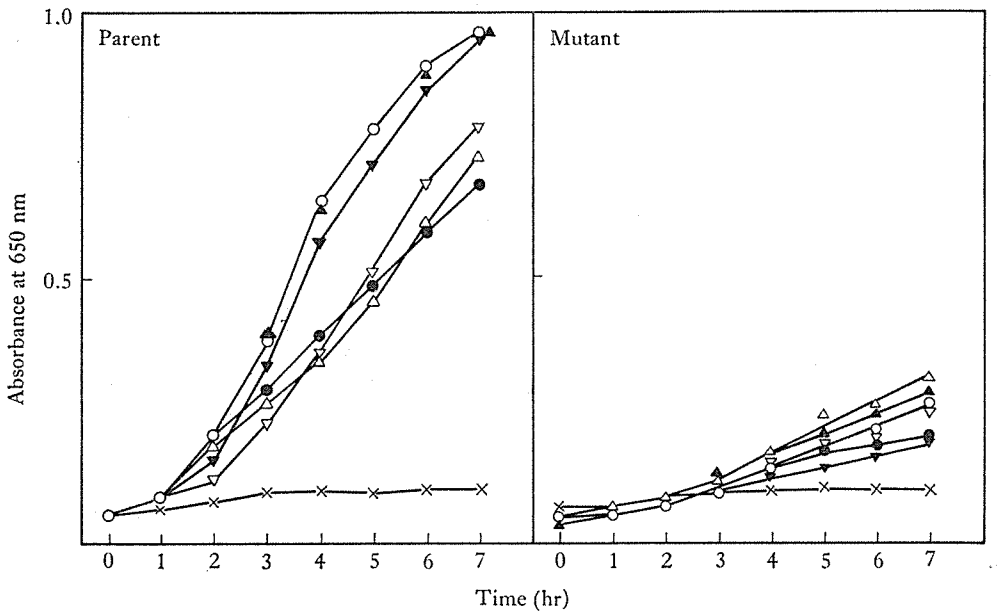

Fig. 4. Malutilization of D-glucose, D-mannose, maltose, saccharose, trehalose or pyruvate by the transketolase mutant of *B. subtilis.*
The sugars were present at the concentration of 1.0 %. Blank, ×—×; D-glucose, ○—○; D-mannose, △—△; pyruvate, ▽—△; maltose, ●—●; saccharose, ▲—▲; trehalose, ▼—▼.

Inhibition of the assimilation of D-*fructose,* D-*mannitol or glycerol by* D-*glucose,* D-*gluconate,* D-*xylose or* L-*arabinose.* In strain BG2607, the assimilation of D-fructose, D-mannitol, or glycerol was inhibited by D-glucose, D-gluconate, D-xylose or L-arabinose (Fig. 4). Sorbitol utilization appeared to be inhibited only weakly. D-Ribose scarecely inhibited the assimilation of these carbohydrates. This phenotype can also not be explained by transketolase deficiency. Very low concentration of D-glucose was enough to inhibit D-fructose assimilation (Fig. 5).

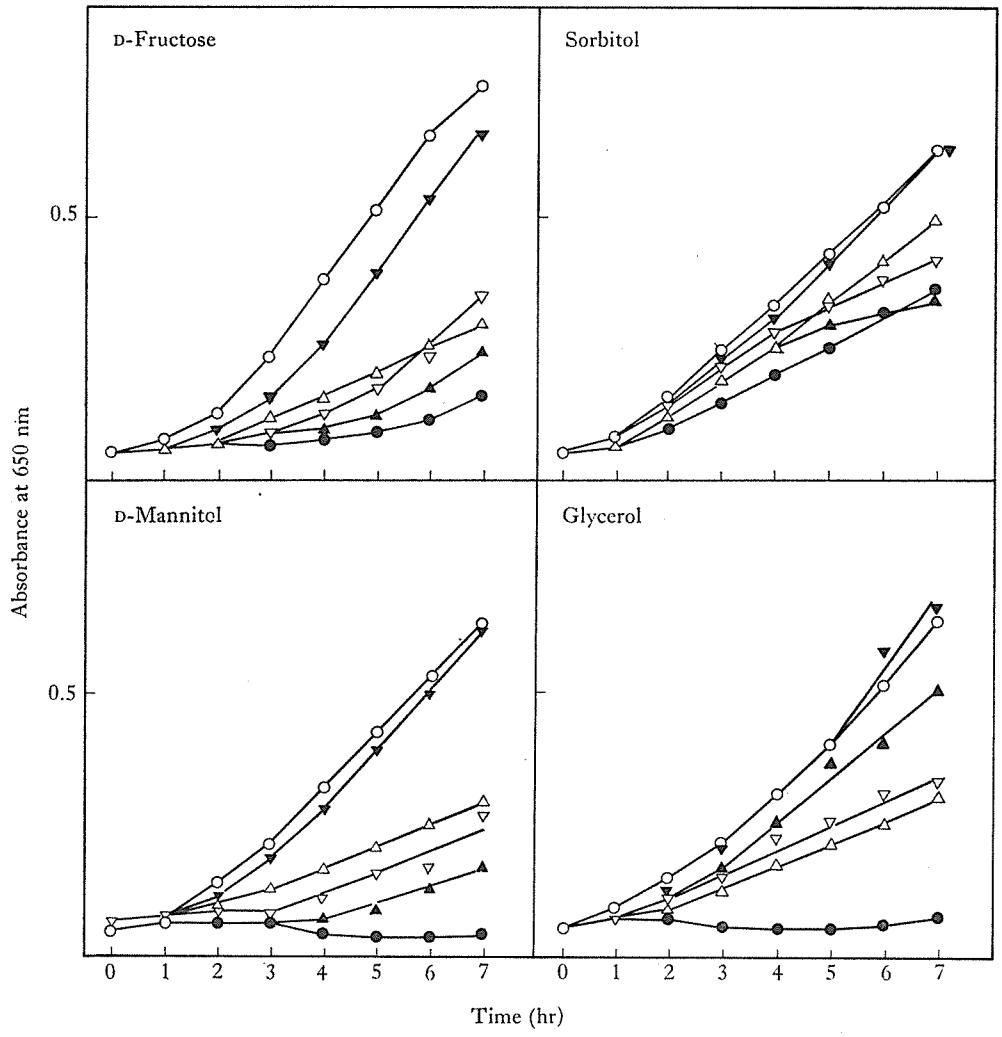

Fig. 5. Inhibition of the assimilation of D-fructose, D-mannitol, sorbitol or glycerol by D-glucose, D-gluconate, D-xylose or L-arabinose in the transketolase mutant of *B. subtilis*.
Sugars were present at the concentration of 1.0 % in control cultures. Other cultures contained 0.5 % of sugars for growth and 0.5 % of inhibitory sugars. Control, ○—○; D-glucose, △—△; D-gluconate, ▽—▽; D-xylose, ●—●; L-arabinose, ▲—▲; D-ribose, ▼—▼.

Discussion

Transketolase mutants have been isolated from *Escherichia coli* (8), *Bacillus* species (13) and *Salmonella typhimurium* (2). Those of *E. coli* can still grow on D-gluconate because *E. coli* is known to have the Entner-Doudoroff pathway (3). However, those of *Bacillus* species can not assimilate D-gluconate, probably because the Entner-Doudoroff pathway is lacking in these *Bacillus* species. *B. subtilis* and *B. pumilus* have been found not to have the pathway (11), while *B. larvae* has the pathway (10).

The transketolase mutant of *B. stubtilis* showed a pleiotropy of carbohydrate metabolism in that it scarecely grew on D-glucose, D-mannose, maltose, saccharose,

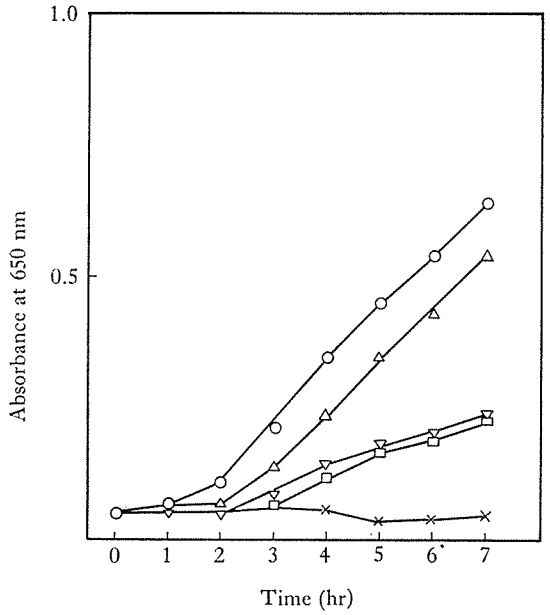

Fig. 6. Effect of D-glucose on D-fructose assimilation.
D-Fructose was present at the concentration of 1.0 %. Control, ○—○; 5.6×10^{-5}M D-glucose, △—△; 5.6×10^{-4}M D-glucose, ▽—▽; 5.6×10^{-3}M D-glucose, □—□; no carbon source, ×—×.

trehalose and pyruvate and in that the assimilation of D-fructose, D-mannitol or glycerol was inhibited by D-glucose, D-gluconate, D-xylose or L-arabinose. Malutilization of D-glucose in the transketolase mutant of *Bacillus* species has been also described in a previous paper (16). The transketolase mutants of *E. coli* grew on D-glucose as well as the parent strain (8). So this phenotype seems to be particular in those of *Bacillus* species.

The relationship between the malutilization of D-glucose, D-mannose, maltose, saccharose, trehalose or pyruvate and transketolase deficiency is not yet clear. However, it is presumed that the phenotype may arise through one of the following possible mechanisms; (i) some intermediate accumulated intracellularly may affect the assimilation of these carbohydrates, (ii) transketolase mutation may affect synthesis of polysaccharide such as lipopolysaccharide containing heptoses, ribitol teichoic acid and the defective synthesis of the polysaccharide may alter the cell surface, resulting in inadequate function of proteins in the membrane or (iii) transketolase itself may have a regulatory effect on the synthesis of inducible degrading enzymes of these carbohydrates. Defective synthesis of lipopolysaccharide in transketolase mutant of *S. typhimurium* was described by Eidels and Osborn (2). Koplow and Goldfine (12) and Bayer et al (1) descibed that integration of protein in the outer membrane was defective in a lipopolysaccharide mutant of *E. coli.*

Josephson and Fraenkel described that xylose and arabinose inhibited the assimilation of mannitol, gluconate, succinate or glycerol (9). Because growth stasis by an intermediate in various carbohydrate metabolism mutants had been described (4, 6, 7),

they thought that the inhibition might be caused through pentose phosphates accumulated intracellularly. However, abnormal high levels of pentose phosphates were not detected. The inhibition of the utilization of D-fructose, D-mannitol or glycerol by D-glucose, D-gluconate, D-xylose or L-arabonose in the transketolase mutant of *B. subtilis* described in this paper seems to be a similar phenomenon. The mechanism is not clear, either. If there is no inhibition by any intermediate accumulated intracellularly, it may be probable that repression of the synthesis of inducible degrading enzymes of D-fructose, D-mannitol or glycerol by D-glucose, D-gluconate, D-xylose or L-arabinose is involved in the growth stasis.

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IFO Res. Comm. 8,
79–89, 1977 (March)

DESCRIPTIVE CATALOGUE OF IFO FUNGUS COLLECTION V.

In the routine work of identification of fungi newly isolated in Japan and in checking the list of fungi preserved in the IFO culture collection for their published records of the occurrence in Japan, many fungi have been found to be a taxon new to Japan and, if not, obscurely or insufficiently described. Sometimes, the first record of the occurrence of particular fungi in Japan have been made solely by referring a name of taxon, without an adequate description of the species concerned. The object of this series of paper is to provide descriptions of fungi preserved or newly deposited in the IFO culture collection or/and in the herbarium of IFO and to contribute to the knowledge of the fungal flora of Japan. New taxa will be described in original papers or submitted to other mycological journals. Author(s) of respective taxon is shown in the bracket.

62. Kickxella alabastrina Coemans (Pl. 1, A-F) Zygomycetes
Bull. Soc. Roy. Bot. Belg. **1.** 155 (1862), Linder, Farlowia **1:** 57 (1943)
Syn. *Coronella nivea* Crouan & Crouan (1867), *Coemansiella alabastrina* (Coemans) Sacc. (1883)

Sporangiophores simple or cymosely branched, straight or flexuous, erect, cylindrical, hyaline, smooth, 1–4 septate, up to 2 mm high, commonly 150–450 μm high in simple ones, 12–20 μm wide, broader at the base measuring 30–35 μm, apex inflated to make sphaerical head of 20–25 μm diameter, very finely echinulate. Sporocladia corniform to uncinulate, borne in a whorl around the upper periphery of swollen apex, 8–12 in number, mostly 2–3 septate, 60–70 $\times$ 8–12 μm, finely echinulate when young, basal cells fertile with many phialides in rows on upper, concave side, distal cell or cells sterile, attenuated to rounded tip, often forked. Phialides ovoid to obpyriform, hyaline, 4–6 $\times$ 3–4 μm. Sporangioles (conidia) spindle form, hyaline, smooth, most commonly with a pseudoseptum or internal annular ring near the central part, 10–12 $\times$ 4–5 μm. Zygotes sphaerical, thick-walled, pale brown, smooth, 30–40 (–50) μm in diameter, wall 6–8 μm thick, homothallic.

Growth on malt agar seen below 10 C but very slow, immersed and aerial hyphae cream yellow to lemon yellow, sporulation fairly well. Growth on PSA seen below 10 C but more restricted, aerial hyphae hardly visible, lemon yellow to sulfer yellow, sporulation very few. Growth on IFO preservation medium for bacterial cultures moderate, but much favorable below 10 C, finely floccose or felty, grayish white to grayish cream-yellow, sporulation abundant.

Hab. On deer dung, Nara Park, Nara, Nara Pref., coll. R. Marumoto, March 13, 1976, isol. & ident. T. Yokoyama 5104–15–1 (IFO 30215 and IFO H–11705)

This fungus has been recorded from Belgium, France, England, United States and U.S.S.R., commonly on horse dung. The present isolate, however, was found to occur on deer dung in Japan. Unpublished records by several Japanese mycologists suggest that this fungus seems not uncommon in Japan.

Growth of this fungus on the commonest media used in mycological laboratory such as malt agar, PSA, cornmeal agar, oatmeal agar, Czapek agar, V-8 juice agar, etc., are very poor, especially above 15 C.

[T. Yokoyama]

63. Mycovellosiella nattrassii Deighton (Pl. 2, A-C) Hyphomycetes
Mycol. Pap. CMI, **137:** 17 (1974); Saito, Shokubutsu Boeki **29:** 243 (1975)

Colonies on leaves mostly hypophyllous, effuse, sooty brown, 3–8 mm in diameter, necrotic spot absent, discolorations on the upper surface of the leaves yellowish to pale brown without definite margin, orbicular. Intercellular hyphae almost colorless. Stroma none. Conidiophores borne on the yellowish brown, septate mycelial hyphae emerging through the stomata and very often forming ropes or loose bundles of three to ten hyphae, terminally or laterally, simple or rarely branched, continuous or rarely 1 septate, cylindrical, more or less tapering towards the distal end, sometimes irregularly swollen and geniculate around the conidiogenous upper portion, very short and mostly 4–6 μm long, 3–5 μm wide, rarely up to 25 μm long and up to 8 μm wide. Conidial scars conspicuous, thickened. Conidia cylindrical, narrowly obclavate, straight, 1–6 septate, subhyaline, pale olivaceous to very pale yellowish brown, catenate, smooth, easily anastomosing each together, 15–60 $\times$ 6–8 μm.

Growth on malt agar moderate, finely floccose, mostly velvety, compact, whitish to pale grayish, sporulating surface olive brown, reverse dark olive brown. Growth on PSA almost the same as on malt agar, but darker in color.

Hab. On living leaves of *Solanum melongena* L. Bettsui, Tondabayashi, Osaka Pref., July 2, 1976, coll. T. Hara, isol. & ident. T. Yokoyama 5107–5–1 (IFO 30262, single conidium isolate and IFO H–11706)

This fungus was described by Deighton (1974) based on an isolate on *Solanum indicum* L, collected by Nattrass in Kenya, 1965, as a type, including a Japanese isolate collected by Fujita in Kochi, 1972. The occurrence of this fungus in Japan, exclusively on the leaves of the egg plants in the greenhouse, has been fully reviewed by Saito (1975) with a description of the symptom and morphology as well as disease control under the name temporally identified as *Mycovellosiella* sp. This fungus has also been found to occur in Sabah, Nepal and China, all on *S. melongena.*

[T. Yokoyama]

64. Ascochyta deutziae Bres. (Pl. 2, D) Coelomycetes
Hedwig. 326 (1900), Sacc., Syll. Fung. **16:** 927 (1897)

Spots on leaves indistinct, yellowish brown, without darker margin. Pycnidia

epiphyllous, scattered, gregarious near the margin, globose, blackish brown, 60–100 μm, often up to 150 μm in diameter, with a distinct pore of 10–15 μm wide. Conidia cylindrical, fusiform to obclavate, smooth, hyaline, straight, rarely curved, 1 septate, 12–20 × 3–4 μm.

Growth on malt agar moderate, finely floccose, chocolate brown, reverse concolor. Growth on PSA quite the same as on malt agar.

Hab. On leaves of *Deutzia crenata* Sieb. & Zucc., Mt. Baijyodake, Fukui Pref., Oct. 28, 1973, T. Yokoyama 4810–28–1 (IFO 9886 and IFO H–11707)

[T. Yokoyama]

65. Macrophoma commelinae Togashi (Pl. 2, E & F) Coelomycetes

Bull. Imp. Coll. Agr. For. Morioka **9:** 25 (1926) (without latin diagnosis)

Spots on the leaves circular or irregular, often confluent, dingy yellow to ochraceous, with a dark brown margin, 2–6 mm, rarely up to 10 mm in diameter. Pycnidia amphigenous, mostly epiphyllous, scattered, globose to subglobose, pale brown, 80–160 μm, mostly 100–150 μm in diameter, with a distinct pore. Conidia cylindrical to obclavate, straight or curved, rounded at both ends, continuous, hyaline, 10–22 × 3–5 μm.

Growth on malt agar rapid, floccose, dirty white to gray at first, finally grayish brown to pale blackish brown, reverse concolor. Growth on PSA the same as on malt agar.

Hab. On leaves of *Commelina communis* L. Ishiyama-Hiratsu-cho, Otsu, Shiga Pref., July 18, 1969, T. Yokoyama 4407–18–1 (IFO 9569 and IFO H–11708), Otsu, Shiga Pref., July 1, 1971, T. Yokoyama 4607–1–6 (IFO 9570 and IFO H–11709), Mt. Rokko, Kobe, Hyogo Pref., Sept. 12, 1971, T. Yokoyama 4609-12-3 (IFO 9571 and IFO H–11710), Sakyo-ku, Kyoto, Kyoto Pref., July 5, 1973, T. Yokoyama 4807–5–2 (IFO 9773, single conidium isolate and IFO H–11711).

This species was described by Togashi in 1926 for the fungus which differs from *Phyllosticta commelinicola* Young parasiting on the leaves of *C. nudiflora* L. by the longer conidia and the nature of the leaf spots. This fungus seems not uncommon on *C. communis* in Japan and found to occur widely from Hokkaido through Kagoshima Pref.

[T. Yokoyama]

66. Phyllosticta ampelicida (Engelman) van der Aa

(Pl. 2, G & H; Pl. 3, A) Coelomycetes

Studies in Mycology, CBS, **5:** 28 (1973)

Spots on leaves circular or angular, solitary or sometimes coalescent, brown or reddish brown, then fading to yellowish brown with a dark purple brown margin, very often with a pale yellowish brown ring just inside the margin, reaching a diameter

of 8 mm. Pycnidia epiphyllous, solitary or scattered, not in a group, unilocular, (80–) 120–180(–200) μm, mostly about 100 μm in diameter, sphaerical to subsphaerical, sometimes depressed, with a distinct circular pore of 10–15 μm in diameter, non ostiolate nor papillate. Pycnidial wall 1–3 cells thick; outer layer composed of thin-walled, isodiametrical, brown cells 5–12 μm in diameter and of smaller and thicker walled cells around the pore giving darker brown appearance on the pycnidia; inner layer of isodiametrical, hyaline cells. Innermost cells functioning as phialides. Phialides cylindrical to conical, 5–6 × 2–3 μm. Conidia subglobose, ellipsoidal, amygdaliform, piriform, often truncated at the base, hyaline to pale yellowish brown, one celled, (5–) 8–10(–20) × (4–)5–7(–8) μm, averaging 5 × 10 μm, surrounded by a hyaline mucilaginous layer, with an apical appendage of 5–10 μm long.

Growth on malt agar moderate to rapid, floccose, dark grayish brown to almost blackish brown, sporulating surface more or less compact, coriaceous or carbonaceous, reverse concolor. Growth on PSA rapid, coarsely floccose, grayish brown to almost blackish brown, sporulating surface compact, coriaceous or carbonaceous, irregularly woven and warty, reverse concolor.

Hab. On young living leaves of *Parthenocissus tricuspidata* (Sieb. & Zucc.) Planch, Mt. Rokko, Kobe, Hyogo Pref., May 22, 1971, T. Yokoyama 4605–22–1 (IFO 9466 and IFO H–11712), Ujina, Hiroshima, Hiroshima Pref., May 25, 1972, T. Yokoyama 4705–25–1 (IFO 9903, single conidium isolate and IFO H–11713), Seta, Otsu, Shiga Pref., May 5, 1973, T. Yokoyama 4805–5–1 (IFO 9757, single conidium isolate and IFO H–11714), Nose, Osaka Pref., June 1, 1976, T. Yokoyama 5106–23–4 (IFO H–11715), Suzurandai, Kobe, Hyogo Pref., June 25, 1976, coll. I. Asano, isol. & ident. T. Yokoyama 5106–25–1 (IFO H–11716).

This fungus is not uncommon in the western district of Japan and usually appears in the early summer, from the end of April through late June, on young, expanding leaves of *P. tricuspidata*. Ascigerous state, *Guignardia bidwellii* (Ellis) Viala & Ravaz, has been recorded, but not yet found in Japan. *Leptodothiorella* state is also present in some collections and is considered as spermatial state.

This species is known to attack the grape vines widely in many countries in Europe and North America. According to Aa (1973), there is a strain of culture which is atypical and more than twenty years old but only one available for cultural study. The in vitro characteristics of this fungus were also reported by some investigators before Caltrider (Phytopath. **51**: 860–863, 1961).

[T. Yokoyama]

67. Septoria centellae Wint. (Pl. 3, B & C) Coelomycetes

Grevillea **15:** 92 (1887), Sacc., Syll. Fung. **10:** 367 (1892), Togashi, Bull. Imp. Coll. Agr. For. Morioka **9:** 26 (1926) (without latin diagnosis)

Spots on leaves distinct, circular or irregular, pale yellowish brown with a distinct

brownish margin, often limited by veins or coalescent, 3–5 mm in diameter. Pycnidia amphigenous, mostly epiphyllous, scattered, globose, brownish, 60–100 μm in diameter, with a distinct pore. Conidia filiform, straight or curved, 0–3 septate, hyaline, 25–45 × 1.5–2 μm.

Growth on malt agar moderate, compact, more or less velvety or minutely floccose, pale lavender gray to pale mouse gray, reverse concolor or almost blackish. Growth on PSA moderate, velvety, purplish gray to mouse gray, reverse fuscous black. Hab. On leaves of *Centella asiatica* Urb., Ishiyama-Hiratsu-cho, Otsu, Shiga Pref., July 18, 1969, T. Yokoyama 4407–18–5 (IFO 9473 and IFO H–11717), June 27, 1976, T. Yokoyama 5106–27–2 (IFO 30270, single conidium isolate and IFO H–11718).

This species was first recorded in 1924 from Shimane Pref. and Kagoshima Pref. as new to Japan. In the suburb of Otsu, this fungus is usually associated with its hosts in the autumn.

[T. Yokoyama]

68. Septoria cercosporoides Trail (Pl. 3, D) Coelomycetes
Scot. Nat. **9:** 89 (1887)

Spots on leaves circular or irregular, dark brown, with blackish brown distinct margin, 3–5 mm in diameter. Pycnidia epiphyllous, scattered, globose, 100–250 μm in diameter, with a distinct pore of 10–20 μm wide. Conidia obclavulate, broader at lower half with distinctly truncated base, attenuated at upper half, straight or slightly curved, with 5 to 8 distinct septa, subhyaline to very pale yellow, thus resembling the conidia of *Cercospora,* 60–90 × 2–4 μm, mostly 70–80 × 3–4 μm.

Growth on malt agar moderate, compact, covered with numerous pycnidia on the surface of more or less stromatic mycelial strands, dark brown to blackish brown, reverse concolor. Growth on PSA the same as on malt agar, dark brown.
Hab. On leaves of *Chrysanthemum morifolium* Ramat. (cultvar. Azumahikari), Habikino, Osaka Pref., November 19, 1973, T. Yokoyama 4811–19–5 (IFO 9906 and IFO H–11719), 4811–19–8 (IFO 9907 and IFO H–11720).

The conidia of this species resemble those of *Cercospora,* thus easily distinguishable from *S. chrysanthemi* Allesch. (syn. *S. chrysanthemella* Sacc.) which has filiform conidia of less than 60 μm in length. *S. chrysanthemella* was said to occur also on the leaves of *C. morifolium* in Japan, though it has never been confirmed by the present author.

[T. Yokoyama]

69. Septoria lysimachiae Westend. (Pl. 3, E & F) Coelomycetes
Bull. Acad. Bruxelles **3:** 120 (1852), Sacc., Syll. Fung. **3:** 533 (1884), Cejp & Jechova, Acta Mus. Nat. Pragae **23** B: 114 (1967)

Spots on leaves circular or irregular, very often indefinitely confluent, ochraceous brown to brown. Pycnidia epiphyllous, solitary or scattered, immersed, sphaerical,

blackish brown, 60–100 μm in diameter, with a distinct pore. Conidia filiform, very slender, straight or flexuous, often curved or sigmoid, 5–7 septate, hyaline, smooth, 30–80(–100)×1.5–2.5 μm.

Growth on malt agar moderate, minutely floccose, wrinkled, dark purplish brown, reverse concolor. Growth on PSA the same as on malt agar, floccose, pale vinaceous brown to purplish brown, reverse concolor.

Hab. On leaves of *Lysimachia clethroides* Duby, Mt. Yamatokatsuragi, Nara Pref., June 11, 1972, T. Yokoyama 4706–11–10 (IFO 9635 and IFO H–11721), Mt. Daimonji, Kyoto, Kyoto Pref., Sept. 26, 1976, T. Yokoyama 5109–28–4 (IFO H–11722)

[T. Yokoyama]

70. Septoria nolitangere Ger. (Pl. 3, G) Coelomycetes

Bull. Torrey bot. Club. **4:** 64 (1873–74), Cejp & Jechova, Acta Mus. Nat. Pragae **23** B: 115 (1967)

Spots on leaves circular or irregular, pale yellowish brown, with a distinct reddish to purplish brown margin, 2–5 mm in diameter. Pycnidia amphigenous, mostly epiphyllous, solitary or scattered, globose, blackish brown, 60–120 μm in diameter. Conidia filiform, indistinctly septate, hyaline, 20–40×1.5–2 μm.

Growth on malt agar more or less restricted, blackish brown, reverse concolor. Growth on PSA moderate, dirty white to gray, reverse dark brownish black.

Hab. On leaves of *Impatiens textori* Miq., Sannosawa, Mt. Daisen, Tottori Pref., July 31, 1974, T. Yokoyama 4907–31–10 (IFO H–11723), Shiroyama, Kisofukushima, Nagano Pref., Aug. 28, 1976, T. Yokoyama 5108–30–7 (IFO 30271 and IFO H–11724).

This species seems not uncommon fungus in Japan.

[T. Yokoyama]

71. Septoria solidaginicola Peck (Pl. 3, H; Pl. 4, A-D) Coelomycetes

N.Y. State Mus. Rept. **40:** 60 (1887), Sacc., Syll. Fung. **10:** 372 (1892), Cejp & Jechova, Acta Mus. Nat. Pragae **23** B: 120 (1967)

Spots on leaves circular or irregular, grayish ochraceous, with a reddish brown distinct margin, 3–6 mm in diameter. Pycnidia epiphyllous, immersed, solitary or scattered, sphaerical to subsphaerical, often with conical ostiole, 50–120 μm, mostly 60–80 μm in diameter, 80–150 μm in height, with a distinct circular pore of 10–20 μm wide, outer wall composed of rectangular, yellowish brown, thick-walled, pseudoparenchymatous cells, inner wall hyaline, thin-walled. Phialides ampuliform, hyaline. Conidia filiform, flexuous or variously bent, hyaline, smooth, indistinctly several septate, 50–70×2 μm.

Growth on malt agar moderate, immersed mycelium grayish brown, aerial mycelium floccose, dirty white with pale salmon tint, reverse blackish brown. Growth

on PSA moderate to more or less rapid, immersed mycelium vinaceous brown, aerial mycelium finely floccose, pale smoke gray to pale olivaceous buff, reverse vinaceous brown.

Hab. On leaves of *Solidago canadensis* L. The Ishiyama Temple, Otsu, Shiga Pref., Sept. 2, 1970, T. Yokoyama 4509–2–1 (IFO H–11725), Tanakami, Otsu, Shiga Pref., Sept. 21, 1971, T. Yokoyama 4609–22–5 (IFO H–11726), Senri Central Park, Toyonaka, Osaka Pref., Sept. 18, 1976, T. Yokoyama 5109–20–3 (IFO 30272, single conidium isolate and IFO H–11727); on the leaves of *S. virga-aurea* L., Ishiyama, Otsu, Shiga Pref., Nov. 6, 1970, T. Yokoyama 4511–6–7 (IFO H–11728).

This fungus occurs commonly in the early autumn.

[T. Yokoyama]

72. Lasiosphaeria ovina (Pers.) Ces. & de Not. (Pl. 4, E-H) Pyrenomycetes
Comm. Soc. Crittogamol. ital. **1:** 229 (1863)

Saprophytic. Perithecia scattered or gregarious, superficial, free, globose, with a small ostiole, black, covered with thick, white, felty sheath when young, which often cracks and becomes easily separable, 400–500 μm in diameter. Ascus cylindrical to elongated ventricose, with a long pedicel, 140–200 $\times$ 12–17 μm, 8-spored. Paraphyses numerous, filiform. Ascospores cylindrical, sigmoid, corniform or hamate, continous, 35–55 $\times$ 3–4 μm, hyaline, smooth.

Growth on malt agar moderate, smooth, pale brown, reverse concolor. Growth on PSA the same as on malt agar, very pale yellowish brown, reverse concolor.

Hab. On rotten trunk of *Castanopsis cuspidata* Schottky, Daigo, Kyoto, Kyoto Pref., May 30, 1971, T. Yokoyama 4605–30–6 (IFO 9465 and IFO H–11729)

[T. Yokoyama]

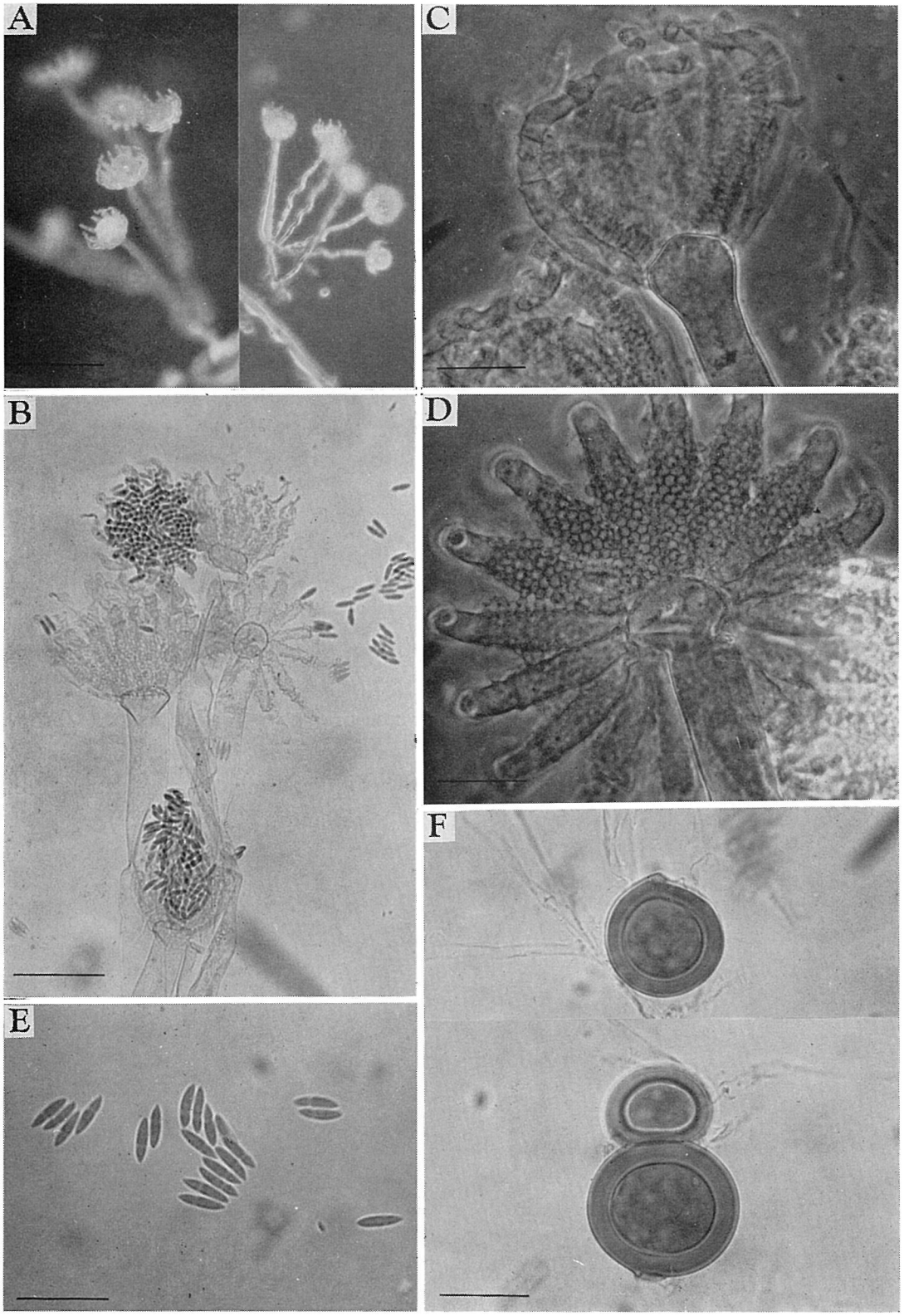

Plate 1. *Kickxella alabastrina* 5104-15-1. A. Habit of the fungus (×68), B. Sporangiophores (×360), C. Side view of sporocladia and phialides (×720), D. Top view of sporocladia and phialides (×720), E. Sporangioles (conidia) (×720), F. Chlamydospores (×720). ——: A=500 μm, B=50 μm, C-F=25 μm

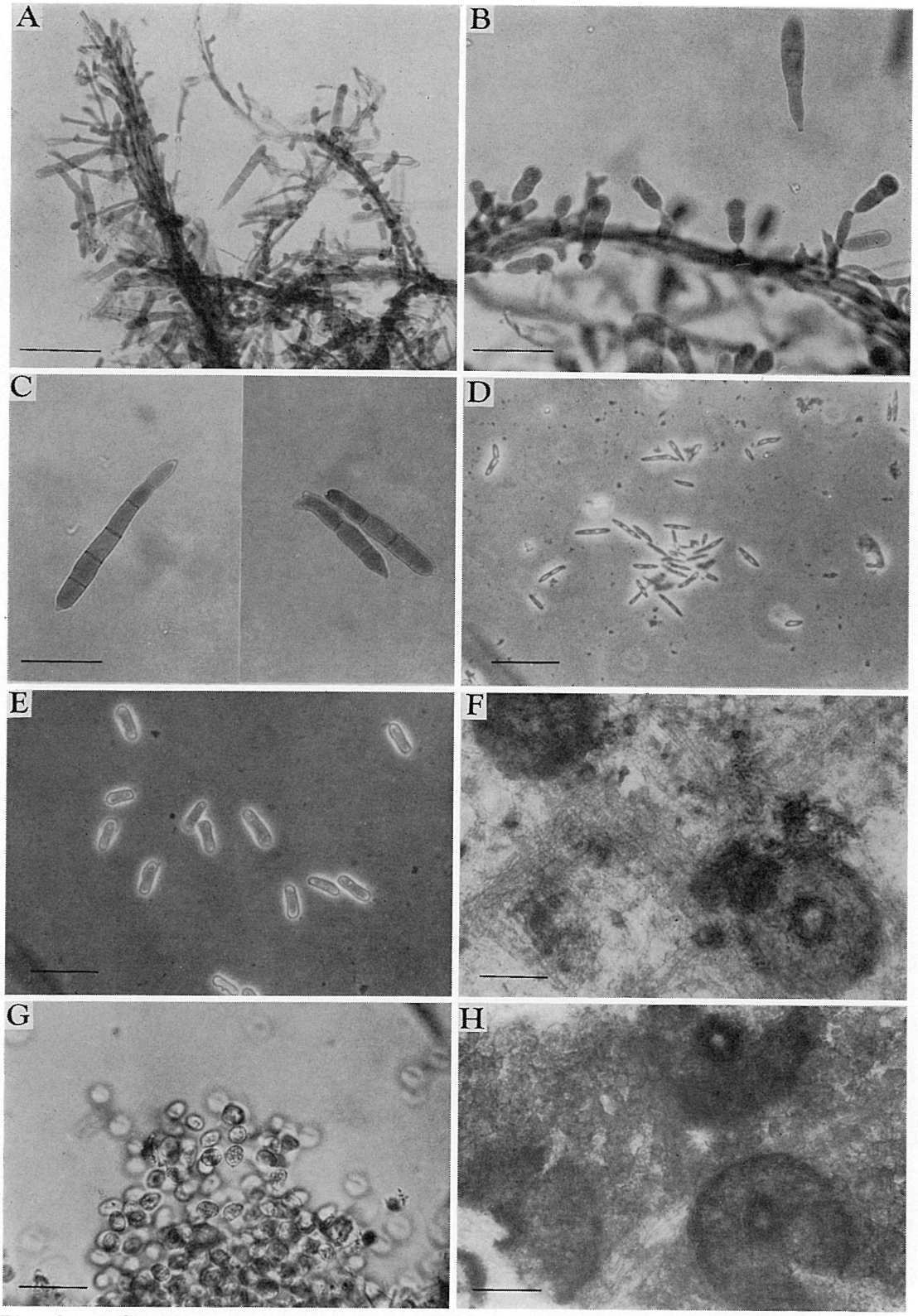

Plate 2. A-C. *Mycovellosiella nattrassii* 5107–5–1, on *Solanum melongena*. A. Conidial structures (×720), B. Conidiophores and young conidia (×720), C. Mature conidia (×720), D. *Ascochyta deutziae* 4810-28-1, on *Deutzia crenata*, conidia (×600), E & F. *Macrophoma commelinae*, on *Commelina communis*. E. Conidia, 4807-5-2(×600), F. Pycnidia immersed in the host tissue, 4609-12-3 (×300), G & H. *Phyllosticta ampelicida*, on *Parthenocissus tricuspidata*. G. Conidia, 4705-25-1 (×600), H. Pycnidia immersed in the host tissue, 4605-22-1 (×300).
——: A=50 μm, B-E, G=25 μm, F & H=50 μm

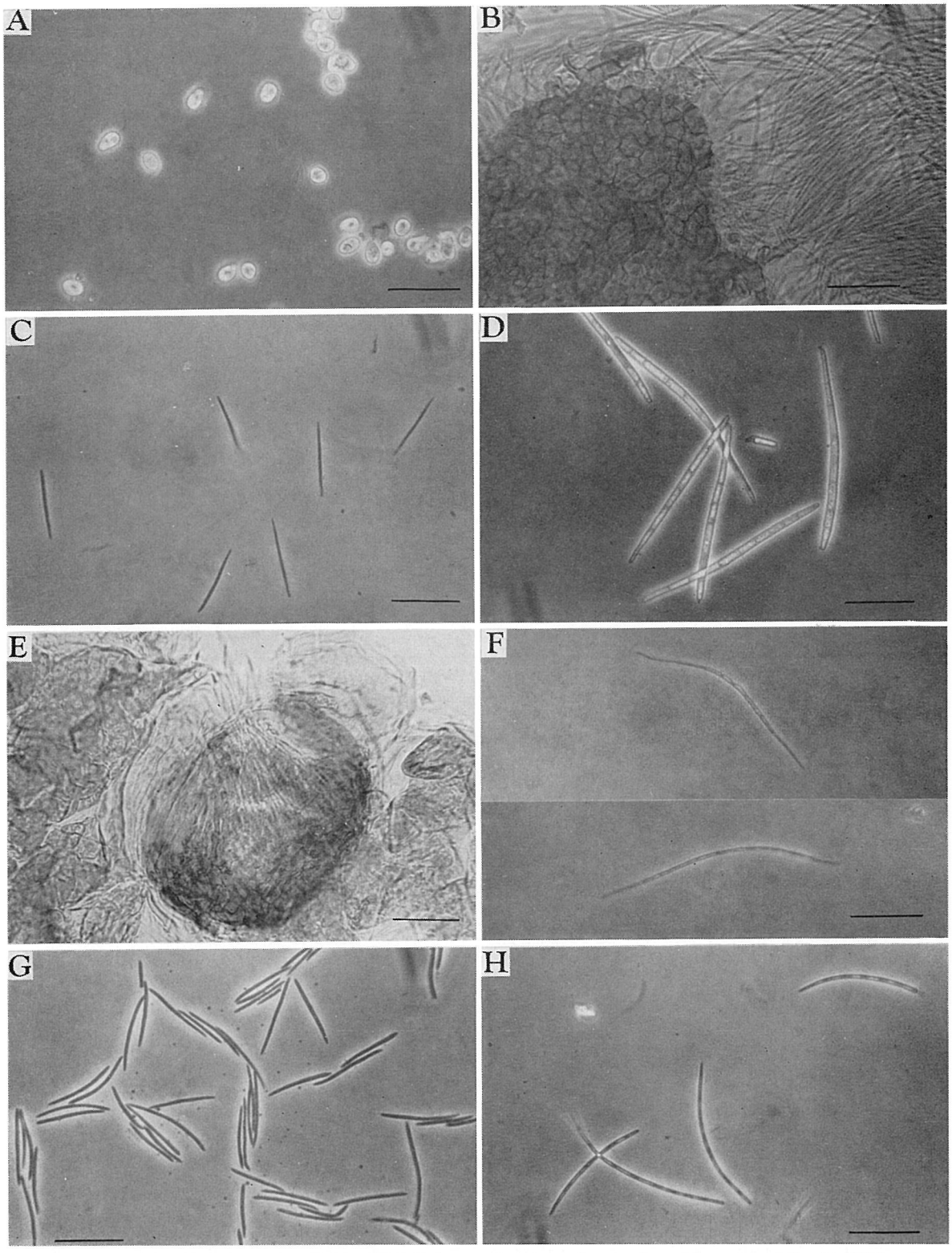

Plate 3. A. *Phyllosticta ampelicida* 4805-5-1, on *Parthenocissus tricuspidata,* conidia (×600), B & C. *Septoria centellae,* on *Centella asiatica.* B. Part of the pycnidial wall, phialides and conidia, 4407-18-5 (×600), D. *Septoria cercosporoides* 4811-19-5, on *Chrysanthemum morifolium,* conidia (×600), E & F. *Septoria lysimachiae* 4706-11-10, on *Lysimachia clethroides.* E. Pycnidia immersed in the host tissue (×300), F. Conidia (×600), G. *Septoria nolitangere* 5108-30-7, on *Impatiens textori,* conidia (×600), H. *Septoria solidaginicola* 4509-2-1, on *Solidago canadensis,* conidia (×600).
——: A-D, F-H=25 μm, E=50 μm

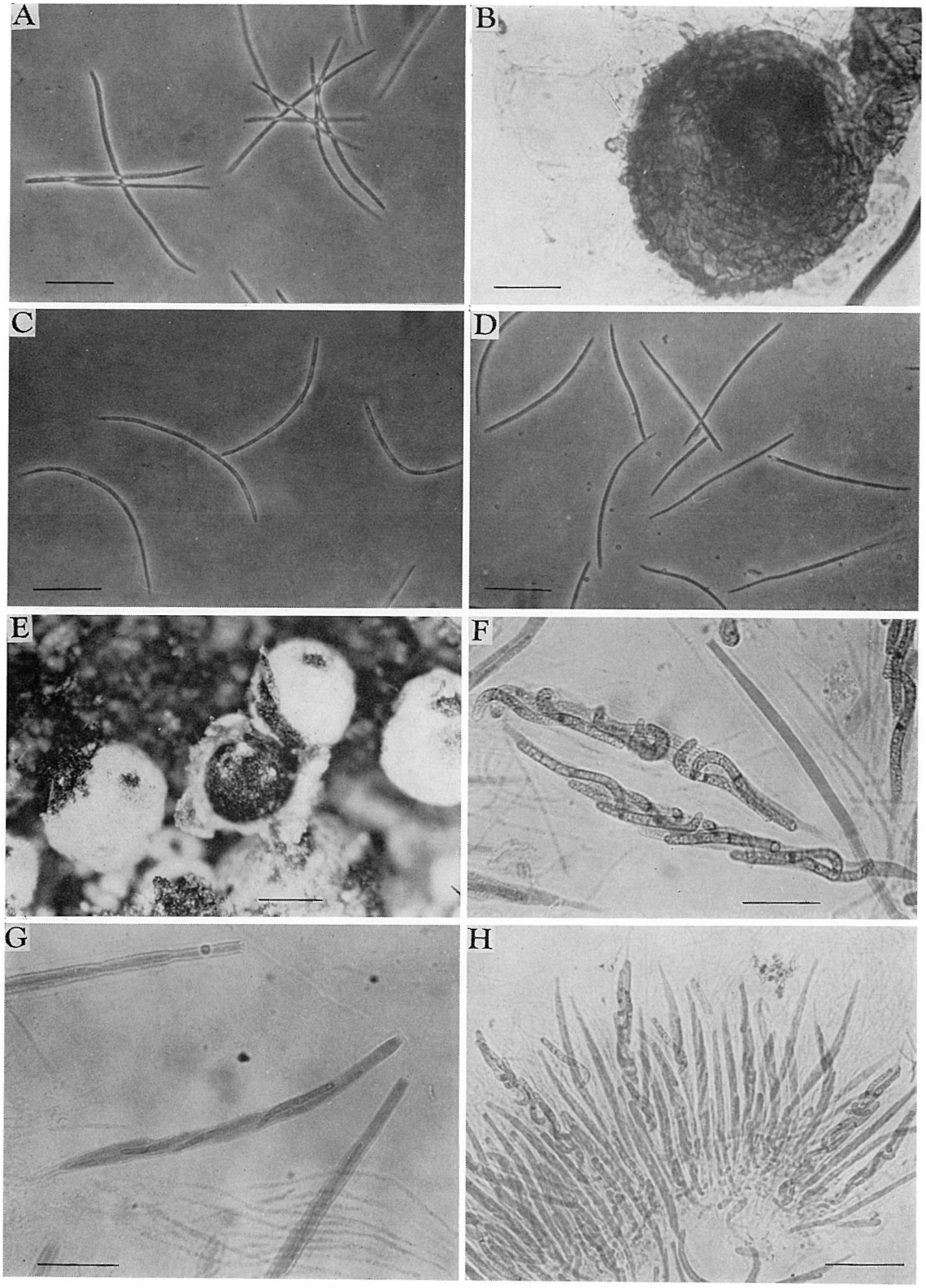

Plate 4. A-D. *Septoria solidaginicola*. A. Conidia, on *Solidago virga-aurea*, 4511-6-7 (×600), B. Pycnidium immersed in the host tissue, 4609-22-5 (×300), C. Conidia, on *Solidago canadensis*, 4609-22-5 (×600), D. Conidia, on *S. canadensis*, 5109-20-3 (×600), E-H. *Lasiosphaeria ovina* 4605-30-6, on natural substrate, E. Habit of the fungus (×78), F. Asci with mature ascospores (×600), G. Young asci with immature ascospores (×600), H. A cluster of asci and paraphyses (×300).
——: A, C, D, F & G=25 μm, B & H=50 μm, E=250 μm

IFO Res. Comm. 8,
91–94, 1977 (March)

DESCRIPTIVE CATALOGUE OF IFO YEAST COLLECTION 1

In the routine work of identifying strains and updating data of strains in the collection, interesting strains worthy of record have been found. The object of this catalogue is to provide descriptions of those strains which have not been reported yet or have only insufficiently been described in Japan. New taxa will be described in original papers. The followiog descriptions are arranged in alphabetical order of the sci entific name. Authors of respective species is indicated in brackets.

1 and 2. **Debaryomyces cantarellii** Capriotti

Capriotti, A. 1961. Arch. Mikrobiol. **39**: 123.

Kreger-van Rij, N.J.W. 1970. *In* The Yeasts, a Taxonomic Study, ed. by J. Lodder, North-Holland Publ. Co., Amsterdam, p. 134.

IFO 1716 and IFO 1717

Growth in glucose-yeast extract-peptone water: After 3 days at 25 C the cells are oval to long oval, 2.3–4.6 × 2.3–8.5 μm; single or in pairs.

After one month at 20 C a sediment and a pellicle are present.

Growth on YM agar: After one month at 20 C the streak culture is yellowish, slightly shiny, smooth. The margin is finely sinuous.

Dalmau plate cultures on corn meal agar: No pseudomycelium is formed. But short chains of cell are present.

Formation of ascospores on YM agar: Conjugation between mother cell and bud precedes ascus formation. The spore are spherical with a warty wall and an oil drop inside. One and occasionally two spores are formed per ascus.

Physiological characteristics are presented in Table 1.

IFO 1716 was isolated from the bark of *Fagus crenata* collected from Mt. Daisen, Tottori Pref., on Aug. 3, 1974. (strain No. D-112a2)

IFO 1717 was isolated from the soil collected from Mt. Odaigahara, Nara Pref., on Aug. 27, 1973. (strain No. O-140a1)

IFO 1717 is the first isolate of the species in Japan.

[K. Mikata & I. Banno]

3. **Hanseniaspora occidentalis** Smith

Smith, M. Th. 1974. Antonie van Leeuwenhoek **40**: 441.

IFO 1718

Growth in glucose-yeast extract-peptone water: After 3 days at 25 C the cells are lemon-shaped, oval or long oval, 2.7–6.6 × 3.5–12.3 μm; single or in pairs. The cell reproduces vegatatively by bipolar budding. A sediment is formed.

Table 1 Physiological characteristics

	D. cantarellii IFO 1716 IFO 1717	*H'spora occidentalis* IFO 1718	*P. quercuum* IFO 1719	*P. stipitis* IFO 1720
Fermentation				
Glucose	+	+	vw	+
Galactose	w	−	−	+
Sucrose	+	+	−	−
Maltose	s	−	−	+
Trehalose	vw	−	−	+
Lactose	−	−	−	−
Raffinose	+	−	−	−
Inulin	+	−	−	−
Soluble starch	−	−	−	+
α-Methyl-D-glucoside	ws	−	−	s
Assimilation of cabon compounds				
Glucose	+	+	+	+
Galactose	+	−	−	+
L-Sorbose	+	−	−	−
Sucrose	+	+	−	+
Maltose	+	−	−	+
Cellobiose	+	+	+	+
Trehalose	+	−	−	+
Lactose	+	−	−	s
Melibiose	+	−	−	−
Raffinose	+	−	−	−
Melezitose	+	−	−	+
Inulin	+	−	−	−
Soluble starch	+	−	−	+
D-Xylose	+	−	s	+
L-Arabinose	+	−	−	s
D-Arabinose	−	−	−	+
D-Ribose	+	−	−	s
L-Rhamnose	−	−	−	+
Ethanol	+	−	+	+
Glycerol	+	vw	s	+
Erythritol	+	−	−	+
Ribitol	+	−	−	+
Galactitol	+	−	−	−
D-Mannitol	+	−	+	+
D-Glucitol	+	−	s	+
α-Methyl-D-glucoside	+	−	−	+
Salicin	+	+	+	+
DL-Lactic acid	w	−	vw	w
Succinic acid	ws	−	s	+
Citric acid	s	−	s	+
Inositol	−	−	−	−
Splitting of arbutin	+	+	+	+
Assimilation of potassium nitrate	−	−	−	−
Growth in vitamin-free medium	+	−	−	−
Growth at 37 C	−	−	−	−

vw=very weak, w=weak, s=slow.

After one month at 20 C a sediment and a thin ring is formed.

Growth on YM agar: After one month at 20 C the streak culture is greyish-cream-colored, smooth, glossy, umbonate to flat, and the margin is entire.

Dalmau plate cultures on corn meal agar: No pseudomycelium is formed.

Formation of ascospores on YM agar: Single diploid cells are directly trans-

formed into asci. One and occasionally two spheroidal ascospores are formed per ascus. The spores are approximately 2.6–3.2 μm in diameter. Spores are not liberated from the ascus.

Physiological characteristics are presented in Table 1.

IFO 1718 was isolated from the soil collected from Mt. Daisen, Tottori Pref., on July 30, 1974. (strain No. D-107a1)

This is the first isolate of the species in Japan.

[K. Mikata & I. Banno]

4. Pichia quercuum Phaff & Knapp

Phaff, H.J., & E.P. Knapp. 1956. Antonie van Leeuwenhoek **22**: 117.

Kreger-van Rij, N.J.W. 1970. *In* The Yeasts, a Texonomic Study, ed. by J. Lodder, North-Holland Publ. Co., Amsterdam, p. 523.

IFO 1719

Growth in glucose-yeast extract-peptone water: After 3 days at 25 C the cells are spherical to short-oval, 2.3–3.8 $\times$ 2.3–5.4 μm; single or in pairs.

After one month at 20 C a sediment is present.

Growth on YM agar: After one month at 20 C the streak culture is yellowish-brown, soft, smooth and shiny. The margin is entire.

Dalmau plate cultures on corn meal agar: No pseudomycelium is formed.

Formation of ascospores on YM agar and corn meal agar: Isogamous conjugation precedes ascus formation, and occasionally single vegatative cells are directly transformed into asci. The spores are hat-shaped; two to four are formed per ascus. They are liberated from the mature ascus.

Physiological characteristics are presented in Table 1.

IFO 1719 was isolated from litter-leaf of *Fagus crenata* collected from Mt. Daisen, Tottori Pref., on Aug. 7, 1975. (strain No. Di-205u5)

This is the first isolate of the species in Japan.

[K. Mikata & I. Banno]

5. Pichia stipitis Pignal

Pignal, M.C. 1967. Bull. Mens. Soc. Linnéenne Lyon **36**: 163.

Kreger-van Rij, N.J.W. 1970. *In* The Yeasts, a Taxonomic Study, ed. by J. Lodder, North-Holland Publ. Co., Amsterdam, p. 533.

IFO 1720

Growth in glucose-yeast extract-peptone water: After 3 days at 25 C the cells are spherical to oval, 3.5–4.6 $\times$ 3.5–6.1 μm; single or in pairs. Occasionally elongated cells occur.

After one month at 20 C a sediment and a dull, smooth pellicle are present.

Growth on YM agar: After one month at 20 C the streak culture is cream-colored,

soft, smooth, semi-dull and slightly folded at right angle to center line. The margin is nearly entire.

Dalmau plate cultures on corn meal agar: Much ramified pseudomycelia consisting of long pseudomycelial cells with ovoid blastspores are abundantly formed.

Formation of ascospores on YM agar: Conjugation between mother cell and bud, and between two single cells precedes ascus formation. The cells form protuberances of various lengths. The spores are hat-shaped; one to two spores are formed per ascus.

Physiological characteristics are presented in Table 1.

IFO 1720 was isolated from soil collected from Mt. Daisen, Tottori Pref., on July 30, 1974. (strain No. D-117a2)

This is the first isolate of the species in Japan.

[K. Mikata & I. Banno]

IFO Res. Comm. 8,
95–103, 1977, (March)

DESCRIPTIVE CATALOGUE OF IFO BACTERIAL COLLECTION 3

Some of the strains newly isolated at the institute and some of the strains used in various researches were identified as known species. However, in most cases of these strains, descriptions of their characteristics in published papers are incomplete. In the routine work of updating the data of strains in the collection, a few of the strains obtained from other organizations were reidentified as different species. The object of this catalogue is to provide the description of these strains. Each description is arranged below in alphabetical order of the scientific name and followed by the name of authors in brackets.

17 and **18. Acinetobacter calcoaceticus** (Beijerinck) Baumann, Doudoroff & Stanier 1968
IFO 12552 and 13006

	IFO 12552	IFO 13006
Cells: Width, μm	0.7–0.9	0.8–0.9
Length, μm	0.7–0.9	0.8–1.0
Gram-reaction	−	−
Sporulation	−	−
Motility	−	−
Catalase	+	+
Oxidase	−	−
Urease	+	−
Reduction of NO_3 to NO_2	−	−
Phosphatase	+	+
Deamination of phenylalanine	−	−
Arginine dihydrolase	−	−
Sensitivity to penicillin	R	R
Hydrolysis of		
Casein	−	−
Starch	−	−
Gelatin	−	−
Esculin	−	−
Tween 80	+	+
Litmus milk	Ac, Co	Ac, Co
OF test	O	O
Methyl red test	+	+
V-P test	−	−
Production of indole	−	−

(Continued)

Growth in a simple mineral medium with ammonium salts and energy source	+	+
Acid production from		
Glucose	+	+
Xylose	+	+
Galactose	+	+
Rhamnose	−	−
Mannitol	−	−
Inositol	−	−
Temperature range for growth, C.	8–38	8–38

Abbreviations: R, resistant; Ac, acidic; Co, coagulated; and O, oxidative

The strain IFO 12552 was obtained from the American Type Culture Collection in 1967, under the name of *Micrococcus cerificans* Finnerty *et al.* The strain IFO 13006 has been entered in the IFO List of Cultures, 5th edition, as *Micrococcus freudenreichii* Guillebeau.

[T. Sakane & I. Banno]

19 and **20. Agrobacterium radiobacter** (Beijerinck & van Delden) Conn 1942 IFO 13258 and 13259

	IFO 13258	IFO 13259
Cells: Width, μm	0.4–0.5	0.3–0.5
Length, μm	1.4–2.2	1.2–2.2
Gram-reaction	−	−
Motility	−	+*
Catalase	+	+
Oxidase	+	+
Reduction of NO_3 to NO_2	+	−
Deamination of phenylalanine	w	+
Oxidation of malonate	−	−
Production of indole	−	−
Production of 3-ketolactose	+	−
Production of dihydroxyacetone	−	−
Hydrolysis of		
Gelatin	−	−
Casein	−	−
Esculin	+	−
Tween 80	−	−
Hippurate	w	w
Litmus milk	Al, Re, Se	Al, Re, Se
Growth in a simple mineral medium with ammonium salts and glucose	+	+

(Continued)

Growth on calcium glycerophosphate, mannitol, nitrate medium	+	+
Aniline blue, mannitol medium		
Growth	+	+
Dye absorption	+	+
Utilization as sole source of carbon		
Glycerol	+	+
Asparagine	+	+
Acetate	+	+
Malonate	−	−
Citrate	+	+
Utilization as sole source of nitrogen		
Ammonium sulfate	+	+
Potassium nitrate	+	+
Asparagine	+	+
Histidine	+	+
Acid production from		
Glucose	+	w
Arabinose	+	+
Sucrose	+	−
Lactose	−	−
Ethanol	+	+
Temperature range for growth, C.	8–37	8–37

*: Motile by peritrichous flagella
Abbreviation: w, weakly positive; Al, alkaline; Re, reduced; and Se, serum zone formed

These strains were obtained from the American Type Culture Collection, in 1971.

[T. Sakane & I. Banno]

21. Bacillus cereus Frankland & Frankland 1887
IFO 3003

Young cells: Gram-positive rods, 0.8–1.0×2.0–3.2 μm; motile by peritrichous flagella.
Spores: Ellipsoidal; central or paracentral; sporangia not swollen.
Catalase: Positive.
*Anaerobic growth: Positive.
V-P test: Positive.
pH in V-P broth: 4.8
Temperature for growth: Maximum, 46 C; minimum, 4 C.
*Egg-yolk reaction: Positive.

*: Gordon, R.E., W.C. Haynes and C. H-N. Pang. 1973. The genus Bacillus. U.S. Dept. Agr. Handbook No. 427, Washington, D.C.

Growth at pH 5.7: Positive.
Growth in 7 % NaCl broth: Negative.
Hydrolysis of starch: Positive.
Hydrolysis of casein: Positive.
Decomposition of tyrosine: Negative.
Utilization of citrate: Negative.
Utilization of propionate: Positive.
Reduction of NO_3 to NO_2: Negative.
Deamination of phenylalanine: Negative.
Acid but no gas from glucose. No acid and no gas from arabinose, xylose and mannitol.

This strain was brought by H. Naganishi Faculty of Engineering, Hiroshima University, Hiroshima, Japan, in 1946, under the name of *Bacillus megaterium* de Bary.
[T. Sakane & I. Banno]

22. Bacillus circulans Jordan 1890
IFO 3967

Young cells: Gram-positive rods, 0.5–0.6 × 1.6–3.2 μm; motile by peritrichous flagella.
Spores: Ellipsoidal; terminal or subterminal; sporangia definitely swollen.
Catalase: Positive.
*Anaerobic growth: Negative.
V-P test: Negative.
pH in V-P broth: 5.2
*Egg-yolk reaction: Negative.
Growth at pH 5.7: Positive.
NaCl tolerance: Grow in 5 % NaCl broth, but not in 7 % NaCl broth.
Hydrolysis of starch: Positive.
Hydrolysis of hippurate: Positive
Hydrolysis of casein: Positive
Decomposition of tyrosine: Weakly positive.
Utilization of citrate: Negative.
Utilization of propionate: Negative.
Reduction of NO_3 to NO_2: Negative.
Production of indole: Negative.
*Formation of crystalline dextrins: Negative.
Production of dihydroxyacetone: Negative.
Deamination of phenylalanine: Negative.
Production of gas from glucose: Negative.
Temperature for growth: Maximum, 52 C; minimum, 20 C.

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*: See footnote on p. 97.

Decomposition of thiamine: Negative.
Acid from glucose, arabinose and mannitol, but not from xylose.

This strain was brought by K. Yamamoto, Research Laboratories, Takeda Chemical Industries Ltd., Osaka, Japan, in 1962, under the name of *Bacillus thiaminolyticus* Matsukawa & Misawa.

[T. Sakane & I. Banno]

23 to **25. Bacillus subtilis** (Ehrenberg) Cohn 1872
IFO 12112, 12113 and 12114

	IFO 12112	IFO 12113	IFO 12114
Cells: Width, μm	0.6–0.7	0.6–0.8	0.6–0.8
Length, μm	1.2–2.8	1.2–2.8	1.6–3.2
Gram-reaction	+	+	+
Spores: Shape	E	E	E
Dominant position	C	C	C
Distends sporangium distinctly	−	−	−
*Motility	−	−	−
Catalase	+	+	+
*Anaerobic growth	−	−	−
V-P reaction	+	+	+
pH in V-P broth	6.0	5.9	5.7
*Egg-yolk reaction	−	−	−
Growth at pH 5.7	+	+	+
Growth in 7% NaCl broth	+	+	+
*Tolerance for 0.02% azide	+	−	+
Acid production from			
Glucose	+	+	+
Arabinose	+	+	+
Xylose	w	w	−
Mannitol	+	+	+
Hydrolysis of			
Starch	+	+	+
Hippurate	−	w	w
Casein	+	+	+
Utilization of			
Citrate	+	+	+
Propionate	−	−	−
Decomposition of tyrosine	−	−	−
Reduction of NO_3 to NO_2	+	+	+
Temperature for growth:			
Maximum, C	54	54	54
Minimum, C	15	15	15

Abbreviations: E, ellipsoidal; C, central or paracentral; and w, weakly positive.
*: See footnote on p. 97.

These strains were obtained from Culture Collection of Entomogenus Bacteria, Institute of Entomology, Prague, CSSR, in 1965, under the name of *Bacillus pumilus* Gottheil.

[T. Sakane & I. Banno]

26 to **28. Flavobacterium lutescens** (Migula) Bergey, Harrison, Breed, Hammer & Huntoon 1923
IFO 3084, 3085 and 12997

	IFO 3084	IFO 3085	IFO 12997
Cells: Width, μm	0.3–0.4	0.3–0.4	0.3–0.4
Length, μm	0.8–2.4	1.0–1.6	1.0–2.0
Gram-reaction	−	−	−
Motility	−	−	−
Pigmentation	yellow	yellow	yellow
Catalase	+	+	+
Oxidase	w	w	+
Growth at 37C	+	+	+
Growth in 4 % NaCl broth	−	−	−
Require added NaCl	−	−	−
Hydrolysis of			
Gelatin	+	+	+
Casein	+	+	+
Starch	−	−	−
Agar	−	−	−
Cellulose	−	−	−
Tween 80	+	+	+
Decomposition of tyrosine	w	+	+
Methyl red test	−	−	−
V-P test	−	−	−
Utilization of citrate	−	w	−
Production of indole	−	−	−
Urease	−	−	−
Reduction of NO_3 to NO_2	+	+	+
Arginine dihydrolase	−	−	+
ONPG test	−	−	−
Oxidation of gluconate	−	−	−
Acid production from			
Glucose	−	−	−
Arabinose	−	−	−
Lactose	−	−	−
Sucrose	−	−	−
Maltose	−	−	−
Ethanol	−	−	−

(Continued)

Litmus milk	alkaline	alkaline	alkaline
Colony migration on agar media	—	—	—

Abbreviation: w, weakly positive

Special characteristics: These three stains possess L-lysine-α-ketoglutarate aminotransferase*.

The strains IFO 3084 and IFO 3085 were brought by Tauchi, Tokai Regional Fisheries Research Laboratory, Japan, in 1950, under the names of *Achromobacter liquidum* (Frankland & Frankland) Bergey *et al.* and of *Flavobacterium flavescens* (Pohl) Bergey *et al.*, respectively.

The strain IFO 12997 was brought by K. Soda, Institute for Chemical Research, Kyoto University, Kyoto, Japan, in 1969, under the name of *Flavobacterium fuscum* (Zimmermann) Bergey *et al.*

[T. Sakane & I. Banno]

29. Flavobacterium okeanokoites ZoBell & Upham 1944
IFO 12536

Cells: Gram-negative rods, 0.6–0.7 × 1.2–2.0 μm, with rounded ends; motile by peritrichous flagella.
Colonies on sea water medium: Circular, entire, low-convex, smooth, orange; not migrated.
Catalase: Positive.
Oxidase: Weakly positive.
Urease: Negative.
Hydrolysis of casein: Positive.
Hydrolysis of gelatin: Positive.
Hydrolysis of starch: Negative.
Hydrolysis of agar: Negative.
Hydrolysis of cellulose: Negative.
Hydrolysis of tween 80: Negative.
Decomposition of tyrosine: Negative.
Methyl red test: Negative.
V-P test: Negative.
Reduction of NO_3 to NO_2: Negative.
Deamination of phenylalanine: Negative.
Arginine dihydrolase: Positive.
Production of indole: Negative.
Utilization of citrate: Negative.

*: Soda, K., H. Misono and T. Yamamoto. 1968. L-Lysine-α-ketoglutarate aminotransferase. Biochemistry **7**: 4102–4109

Require added NaCl: Positive.
Growth in 5% NaCl broth: Positive.
Growth at 37 C: Positive.
Litmus milk: Unchanged.
Oxidation of gluconate: Negative.
ONPG test: Negative.
No acid and no gas from glucose, arabinose, xylose, lactose, sucrose, maltose and ethanol.
Comment: These properties correspond to those originally described by ZoBell and Upham.* This strain originates from the culture studied by ZoBell who did not designate TYPE of the species. Therefore this strain is regarded as LECTOTYPE of the species.

This strain was brought to IFO in 1967 by K. Komagata, who obtained it from Czechoslovak Collection of Microorganisms, J.E. Prukyne University, Brno, CSSR.
[T. Sakane & I. Banno]

30. Pseudomonas caryophylli (Burkholder) Starr & Burkholder 1942
IFO 13694

Cells: Gram-negative rods, 0.5-0.6 × 1.2-1.6 μm; Motile by 1-3 polar flagella; Accumulate poly-β-hydroxybutyrate granules as intracellular carbon reserve especially in nitrogen-deficient media
Colonies on nutrient agar: Circular, entire, low-convex, smooth, gray; no diffusible pigments produced
Oxidase: Positive
Arginine dihydrolase: Weakly positive
Acylamidase: Negative
Denitrification: Positive
Production of indole: Negative
Production of acetoin: Negative
Hydrolysis of starch: Negative
Hydrolysis of casein: Negative
Hydrolysis of esculin: Weakly positive
Hydrolysis of tween 80: Negative
Hydrolysis of gelatin: Negative
Ketogenesis of glycerol: Negative
Oxidation of gluconate: Negative
Litmus milk: Alkaline
pH of growth: grow in pH=5.2, but not grow in pH=4.4

*: ZoBell, C.E. & H.C. Upham. 1944. A list of marine bacteria including descriptions of sixty new species. Bull. Scripps Inst. Oceanogr. Univ. Calif. (Tech. Ser.) **5**: 239–292.

Temperature of growth: Optimum 28-30 C; grow at 41 C; Not grow at 4 C and 46 C
Acid production from glucose, xylose, galactose, lactose, mannitol and ethanol, weakly produced from maltose and sucrose, not produced from starch.
The following compounds utilized as sole carbon sources, glucose, galactose, xylose, rhamnose, sucrose, erythritol, citrate, acetate, lactate, succinate, arginine, asparagine, alanine, glycollate, ethanol, betain and *p*-hydroxybenzoate. Starch and *m*-hydroxybenzoate not utilized as sole carbon sources.

This strain was originally isolated by Fewkes *et al.*[*1] as an organisms which assimilated L-glucose and found by Sasajima and Sinskey[*2] to have a novel aldose dehydrogenase in cells, oxidizing L-xylose, D-arabinose, L-fucose and L-glucose in the presence of NAD^+.

[T. Sakane & I. Banno]

*1: Fewkes, R. C., A. J. Sinskey & D. I. C. Wang, Abstracts of the 4th International Fermentation Symposium, March 1972, Kyoto, p. 226.
*2: Sasajima, K. & A. J. Sinskey, Abstracts of the annual meeting of the Agricultural Chemical Society of Japan, April 1974, p. 12.

LIST OF STRAINS EXCLUDED FROM THE IFO LIST OF CULTURES, 5TH EDITION (1972) AND ITS SUPPLEMENT (1975).

The IFO Culture Collection has not been able to distribute the following strains because of their doubtful taxonomic positions or extinction.

Moulds

Scientific name and authors	IFO No.
Aleurodiscus amorphus (Pers.) Rabenh.	6247
Arachniotus purpureus Müller & Pacha-Aue	9638
Aspergillus oryzae (Ahlburg) Cohn	4192
Chaetomium angustispirale Sergejeva	7013
Chaetomium thermophilum La Touche	9736
Coccomyces hiemalis Higgins	7718
Didymobotryum verrucosum Hino & Katumoto	6836
Gloeophyllum trabeum (Pers. ex Fr.) Murill	6268
Gonatorrhodiella parasitica Thaxter	8524
Helminthosporium sigmoideum Cavara var. *irregulare* Cralley & Tullis	5904
Lindra thalassiae Orpurt, Meyers, Boral & Sims	8818
Mucor inaequisporus Dade	8623
Myxotrichum chartarum (Nees) Kunze	7012
Neurospora crassa Shear & Dodge	6659
Ovularia schwarziana Magnus	7428
Rosellinia necatrix (Hart.) Berlese	6323
Saccobolus kerverni Boud. var. *platensis* Speg.	7804
Syzygites megalocarpus Ehrenberg ex Fr.	6991
Trichometasphaeria turcica Luttrell	7352
Trichometasphaeria turcica Luttrell	7366
Trichophyton megnini Blanchard	5931

Yeast

Candida albicans (Robin) Berkhout	1387
Candida melinii Diddens & Lodder	1430
Candida melinii Diddens & Lodder	1431
Candida mesenterica (Geiger) Diddens & Lodder	0009
Candida mesenterica (Geiger) Diddens & Lodder	0748
Candida mesenterica (Geiger) Diddens & Lodder	1294
Candida mesenterica (Geiger) Diddens & Lodder	1301
Candida tropicalis (Cast.) Berkhout	1499

Hanseniaspora valbyensis Klöcker 0670
Metschnikowia reukaufii Pitt & Miller 0681
Pichia polymorpha Klöcker 1153
Saccharomyces bisporus (Naganishi) Lodder & van Rij var. *mellis* (Fab. & Quin.) van der Walt 0687
Saccharomyces cerevisiae Hansen 0255
Saccharomyces delbrueckii Lindner 0724
Saccharomyces delbrueckii Lindner 1139
Saccharomyces delbrueckii Lindner 1140
Saccharomyces microellipsodes Osterwalder 1016
Saccharomyces montanus Phaff et al 0044
Saccharomyces rosei (Guilliermond) Lodder & van Rij 0252
Saccharomyces rouxii Boutroux 0562
Torulopsis apicola Hajsig 1093
Torulopsis bovina (van Uden & do Carmo-Sousa) van Uden & Vidal-Leiria 1312
Torulopsis molischiana (Zikes) Lodder 0646

Bacteria

Agrobacterium tumefaciens (Smith & Townsend) Conn 13262
Alcaligenes bookeri (Ford) Bergey et al 12948
Bacillus aneurinolyticus Kimura & Aoyama 3968
Bacillus brevis Migula 3864
Bacillus brevis Migula 12364
Bacillus subtilis (Ehrenberg) Cohn 3021
Brevibacterium saperdae Lysenko 12129
Brucella bronchiseptica (Ferry) Topley & Wilson 3159
Brucella bronchiseptica (Ferry) Topley & Wilson 3515
Corynebacterium michiganense (Smith) Jensen 12526
Corynebacterium sepedonicum (Spieckermann & Kotthoff) Skaptason & Burkholder 3306
Desulfotomaculum nigrificans (Werkman & Weaver) Campbell & Postgate 12545
Escherichia coli (Migula) Castellani & Chalmers 3043
Flavobacterium aquatile (Frankland & Frankland) Bergey et al 3772
Gluconobacter albidus (Kondo & Ameyama) Asai 3252
Halobacterium halobium (Petter) Elazari-Volcani 12986
Micrococcus morrhuae Klebahn 12987
Pseudomonas atlantica Humm 12984
Pseudomonas desmolytica Gray & Thornton 12570
Pseudomonas indoloxidans Gray 3783
Pseudomonas ovalis Chester 12051
Pseudomonas riboflavina Foster 3140
Serratia marcescens Bizio 3047

Serratia marcescens Bizio 3048
Serratia marcescens Bizio 3049
Serratia marcescens Bizio 3050
Serratia marcescens Bizio 3051
Serratia marcescens Bizio 3054
Serratia marcescens Bizio 3057
Sphaerotilus natans Kützing 12078
Sphaerotilus natans Kützing 12079
Sphaerotilus natans Kützing 12080
Sphaerotilus natans Kützing 12081
Spirillum polymorphum Williams & Rittenberg 3962
Xanthomonas citri (Hasse) Dowson 3311

Actinomycetes

Agromyces ramosus Gledhill & Casida 13152
Streptomyces hygroscopicus (Jensen) Waksman & Henrici var. *limoneus* Iwasa 13255
Streptomyces olivochromogenes (Bergey et al.) Waksman & Henrici 3562
Streptomyces phaeoviridis Shinobu 3843

ABSTRACTS 1975-1976

Aquatic sediment as a habit of thermophilic fungi

Keisuke TUBAKI, Tadayoshi ITO and Yoshio MATSUDA*

Ann. Micr. **24**: 199–207 (1974)

An experiment was carried out to find the thermophilic fungi from aquatic sediment because no mention has been made to the aquatic occurrence of them. Seven thermophilic and six thermotolerant species have been found from the sediment of the lake and river. The cardinal temperatures for the growth of them were determined.

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Isolation of tricarboxylic acid transport-negative mutants of *Salmonella typhimurium*

Ko IMAI

J. Gen. Appl. Microbiol. **21**: 127–134 (1975)

Tricarboxylic acid transport-negative mutants of *Salmonella typhimurium* were isolated and partially characterized. It was found that *S. typhimurium* possesses another transport system in addition to the three systems described previously. The fourth system was induced by citrate and carried citric acid.

Phenol formation from alkylparabens by bacteria

Ritsuo NAKAMORI*, Fumihiko TANAKA*, Tsutomu NOJIRI*, Hiroshi YOSHINO*, Minoru OKUMURA* and Ko IMAI

J. Pharm. Sci. **64**: 1071–1073 (1975)

Three microorganisms were isolated from the pharmaceutical manufacturing processes using gelatin and were able to grow in a gelatin paste containing alkylparabens. They were identified as *Klebsiella aerogenes* (strain 3) and *Pseduomonas aeruginosa* (strains 4 and 5). It was found that the strains 4 and 5 have esterolytic activity on alkylparabens, and the strain 3 contains a decarboxylating system for *p*-hydroxybenzoic acid. It was also suggested that the rather rapid hydrolysis of alkylparabens by esterolytic activity of either strains 4 or 5 favors the survival of the strain 3, which plays the leading role in the phenol formation from alkylparabens *via p*-hydroxybenzoic acid.

* Biochemistry and Bacteriology Section, Quality Control Department, Osaka Plant, Takeda Chemical Industries Ltd.

Hypomyces and the conidial states in Japan

Keisuke TUBAKI

Rept. Tottori Mycol. Inst. **12**: 161–169 (1975)

Nine species of the fungicolous *Hypomyces* from Japan were shown. They are *H. aurantius, H. camphorati, H. cervigenus, H. chrysospermus, H. hyalinus, H. lateritius, H. rosellus, H. trichothecoides* and *H. tulasneanus*. In addition, the ontogeny of the conidial development was discussed.

Notes of the Japanese Hyphomycetes VI. *Candelabrum* and *Beverwykella* gen. nov.

Keisuke TUBAKI

Trans. mycol. Soc. Japan **16**: 132–140 (1975)

Two new Hyphomycetes are described: *Candelabrum brocchiatum* Tubaki and *Beverwykella pulmonaria* (v. Bev.) Tubaki. The former is the third species of the genus and the latter is a new comb. transferred from *Papulaspora*. Both fungi are aero-aquatic species.

Notes on the Japanese Hyphomycetes VII. *Cancellidium*, a new Hyphomycetes genus.

Keisuke TUBAKI

Trans. mycol. Soc. Japan **16**: 357–360 (1975)

A new aero-aquatic hyphomycete genus *Cancellidium* was made and a new species *C. applanatum* Tubaki was proposed. The fungus is characteristic in the large, black, multicellular and flat conidia.

Pestalotia distincta Guba refound in Japan

Tatsuo YOKOYAMA

Mycologia **67**: 1032–1035 (1975)

Pestalotia distincta Guba, a Coelomycetes, was redescribed based on three newly isolated cultures and the holotype specimen deposited in the Herbarium of the New York Botanical Garden. *P. distincta* was originally described by Guba (1961) as the

fungus parasitic on *Castanopsis cuspidata* Schottky in Japan and has been thought as unusual species of *Pestalotia* because of the absence of conspicuous hyaline apical cells. Microscopic examination of the conidia formed in cultures revealed that the hyaline, conical, conspicuous apical cells were usually present. Although this is the second described record of the occurrence of *P. distincta*, this fungus seems to be distributed fairly commonly along the coastal areas of southwestern Japan in the evergreen oak forests in which species of *Castanopsis* are dominant. In addition, new combination, *Pestalotiopsis distincta* (Guba) Yokoyama, was proposed.

A new species of *Monochaetia* with arthroconidia

Tatsuo YOKOYAMA

Trans. Br. mycol. Soc. **65**: 499–503 (1975)

A new species of Coelomycetes, *Monochaetia dimorphospora* Yokoyama was described. This fungus was found on the fallen leaves of *Castanea pubinervis* Schneid. in Ibaraki Pref., Japan and is unique in the formation of arthroconidia together with typical *Monochaetia*-type conidia in culture. This is the first published record of arthroconidia associated with the *Monochaetia-Pestalotia-Seimatosporium* complex of fungi. After publishing the paper, the second record of distribution of this species was informed with culture by the personal communication from Mr. G.A. Kuter, Department of Botany, University of Wisconsin-Madison, U.S.A., which was said to represent only one out of the many different fungi that he has isolated from decomposing sugar maple leaf litter.

Yeast species and varieties hitherto described in Japan

Shoji GOTO*, Takashi NAKASE**, Isao BANNO and Takeshi TSUCHIYA***

J. Gen. Appl. Microbiol., **21**: 263–271 (1975) & **22**: 177–182 (1976)

A survey was conducted to verify the original descriptions of the species and varieties reported in Japan until 1971. As a result, the list of the 236 species and 76 varieties is presented, including names (accession numbers) of type strains in culture collections, references and synonyms.

* Research Institute of Fermentation, Yamanashi University, Kofu.
** Central Research Laboratories, Ajinomoto Co., Inc., Kawasaki.
*** School of Medicine, Juntendo University, Tokyo.

Preservation of bacteriophages by freezing and drying

Teiji IIJIMA and Takeshi SAKANE

Jap. Soc. Research Freez. Dry. **22**: 9–12 (1976)

Practical method for preserving bacteriophages, i.e., storing phage lysate at a low temperature, storing lysate in liquid nitrogen, storing phage particles in a lyophilized state and storing particles by L-drying are reviewed from a practical point of view. The results of preserving L-dried samples for 5 years showed that L-drying procedure for bacteria and yeasts was also efficient for preserving phages over long term without loss of titer. [In Japanese]

Transport of tricarboxylic acids in *Serratia marcescens* (I)

Ko IMAI

J. Agr. Chem. Soc. Japan **50**: 217–220 (1976)

Serratia marcescens utilized citric, isocitric, *cis*-aconitic and *trans*-aconitic acids as a sole carbon source and possessed at least three inducible transport systems for these acids. The first system was induced by citrate, isocitrate, or *cis*-aconitate, and transported citric and isocitric acids. The second system was also induced by the same acids as in the first system and carried *cis*-aconitic acid. This system required Mg^{2+} ions and was stable at pH 8.6 but unstable at pH 7.0. The third system, induced by *trans*-aconitate or *cis*-aconitate, transported *trans*-aconitic, *cis*-aconitic, citric, and isocitric acids, and was stable at pH 7.0 but unstable at pH 8.6.

[in Japanese]

Transport of tricarboxylic acids in *Serratia marcescens* (II)

Ko IMAI

J. Agr. Chem. Soc. Japan **50**: 221–224 (1976)

Serratia marcescens utilized citric, isocitric, *cis*-aconitic and *trans*-aconitic acids and possessed at least three inducible transport systems for these acids. In this paper tricarboxylic acid transport-negative mutants of *S. marcescens* were isolated and partially characterized. It was confirmed that *S. marcescens* possesses another transport system in addition to the three systems. The fourth system was induced by citrate and carried citric acid. [in Japanese]

Pink root rot of onion caused by *Pyrenochaeta terrestris* (Hansen) Gorenz, Walker & Larson

Fujio KODAMA*, Yukio SUGAWARA** and Tatsuo YOKOYAMA

Ann. Phytopath. Soc. Japan **42**: 320–321 (1976)

Occurrence of onion pink root rot disease was reported for the first time in Japan. This disease has been known as a hitherto unknown disease which seems to be caused by some physiological factors since several years ago in Hokkaido. The fungus can be detected dominantly around the areas where the onions were continuously cropped every year. On the other hand, the fungal population at the newly cropped fields is very low. In addition to the similarity of the symptom on the onion roots, morphological and cultural characteristics of the fungus concerned agreed well with those reported from several foreign countries. It was concluded that the causal pathogen of onion pink root rot disease in Japan should be best treated as *Pyrenochaeta terrestris* (Hansen) Gorenz, Walker & Larson. [in Japanese]

* Hokkaido Kamikawa Agricultural Experiment Station, Asahikawa, Hokkaido
** Furano Agricultural Co-operation, Furano, Hokkaido

Preservation of bacterial strains by L-drying method

Takeshi SAKANE and Isao BANNO

Jap. Soc. Research Freez. and Drying, **22**: 101–108 (1976)

Concerning 40 genera preserved in the IFO culture collection, 380 strains were examined for viability after L-drying and rehydration. When the cells grown in ordinary media for bacteria were dried *in vacuo* from suspending solution of 1/10 M phosphate buffer containing 3 % sodium glutamate, high survival-recovery of the dried cells was found in almost all the strains with exception of the following bacteria. In the strains of *Myxococcus, Sphaerotilus, Azotobacter,* and halophilic bacteria, the cells were recovered at negligible rates from the dried condition. The recovery rates of these strains were greatly increased by selecting media for preculture in the cases of *Myxococcus* and *Sphaerotilus* or by modifying components of the solution to suspend cells of *Sphaerotilus, Azotobacter,* and the holophiles. After all, the dried specimens capable of surviving after long-term preservation were obtained in all the tested bacteria. [in Japanese]

A New variety of *Fusarium merismoides*

Keisuke TUBAKI, C. BOOTH* and T. HARADA**

Trans. Br. mycol. Soc. **66**: 355–356 (1976)

A new variety, *Fusaium merismoides* Cda. var. *acetilereum* Tubaki, Booth & Harada was described. This fungus is characteristic in utilizing 2-butyne-1,4 diol, an acetylene compound, as a sole source of carbon. Two strains of this variety have been so far isolated from those soils collected in Osaka, Japan and Perth, Australia.

* Commonwealth Mycological Institute, Kew, Surrey.
** Institute of Scientific and Industrial Research, Osaka University, Suita, Osaka.

Identification of *Endomycopsis fibuligera* isolated from *Ragi* in Indonesia and properties of its crystalline glucoamylase

Kanji KATO*, Kapti KUSWANTO*, Isao BANNO and Tokuya HARADA*

J. Ferment. Technol., **54**: 831–837 (1976)

A strain of yeast R1, isolated as an amylase producer from *Ragi* in Indonesia, was identified as a strain of *Endomycopsis fibuligera*. Its amylase was purified to a crystalline form and found to be a glucoamylase (α-D-1→4 glucan glucohydrolase, EC 3.2.1.3). It released the β-form of glucose by hydrolysis and had high specific activities towards maltodextrins with over four degrees of polymerization, amylose, amylopectin and glycogen but little or no activity towards α-methyl- or *p*-nitrophenyl-α-glucoside. Its enzymatic properties were similar to those of enzymes from other strain of *E. fibuligera*.

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

PRESENTATION OF PAPERS AT SCIENTIFIC MEETINGS 1975-1976

Author(s)	Title	Scientific Meeting
I. BANNO, K. SAKANE, K. MIKATA & T. IIJIMA	Preservation of microbial strains by liquid-drying.	Japanese Society for Bacteriology. Kanazawa (April, 1975)
T. ENDO*1, O. WAKAE*1, T. FUJIMORI*1 & T. YOKOYAMA	Studies on control effect of sheeth blight of rice plant by validamycin. XIV. Effect on microflora of rice plant (2).	Phytopathological Society of Japan. Fukuoka (April, 1975)
K. IMAI & I. BANNO	A taxonomic study on *Klebsiella* and *Enterobacter*.	Japanese Society for Bacteriology. Kanazawa (April, 1975)
F. KODAMA*2, Y. SUGAWARA*3 & T. YOKOYAMA	Pink root rot of onion caused by *Pyrenochaeta terrestris* (Hansen) Gorenz, Walker & Larson.	Phytopathological Society of Japan. Fukuoka (April, 1975)
T. YOKOYAMA	Angular leaf-spot disease of *Alisma plantago-aquatica* var. *orientale* caused by *Cylindrocarpon* sp.	Phytopathological Society of Japan. Fukuoka (April, 1975)
K. IMAI & I. BANNO	Transport systems for tricarboxylic acids in the species belonging to Enterobacteriaceae (III).	Agricultural Chemical Society of Japan. Sapporo (July, 1975)
K. TUBAKI	Production of arsenic gasses by the annellation forming fungi.	5th Congress of International Society for Human and Animal Mycology. Tokyo (July, 1975)
Y. SAKAMOTO, T. IIJIMA, S. IYOBE*4 & S. MITSUHASHI*4	Typing of *Pseudomonas aeruginosa* by bacteriophages (II).	Symposium on bacterial resistance to anti-biotics. Ikaho (September, 1975)
T. YOKOYAMA	On some Coelomycetes in Japan.	Mycological Society of Japan. Fukuoka (October, 1975)
T. IIJIMA & T. SAKANE	Preservation of bacteriophages by Freezing and Drying.	Japanese Society of Research of Freezing and Drying. Tokyo (November, 1975)
T. YOKOYAMA	Preservation of the fungal cultures.	Japanese Society of Research of Freezing and Drying. Tokyo (November, 1975)

*1 Research Laboratories, Agricultural Chemical Division, Takeda Chemical Industries, Ltd.
*2 Hokkaido Kamikawa Agricultural Experiment Station.
*3 Furano Agricultural Co-operation.
*4 Department of Microbiology, School of Medicine, Gunma University.

Author(s)	Title	Scientific Meeting
K. IMAI & I. BANNO	Transport systems for tricarboxylic acids in the species belonging to Enterobacteriaceae (IV).	Agricultural Chemical Society of Japan. Kyoto (April, 1976)
K. KATO[*1], K. KUSWANTO[*1], I. BANNO & T. HARADA[*1]	Glucoamylase produced by yeast isolated from *Ragi*.	Agricultural Chemical Society of Japan. Kyoto (April, 1976)
S. OUCHI[*2], H. OKU[*2], T. SHIRAISHI[*2], T. YOKOYAMA, M. HATAMOTO[*3], M. TATEISHI[*3] & S. FUJII[*3]	Brown spot of grape.	Phytopathological Society of Japan. Tokyo (April, 1976)
K. SAKANE & I. BANNO	Preservation of bacterial strains by L-drying	Japanese Society for Research of Freezing and Drying. Tokyo (April, 1976)
Y. SAKAMOTO[*4], K. YAMAMOTO[*4], T. IIJIMA, S. IYOBE[*5] & S. MITSUHASHI[*5]	A phage typing of Pseudomonas.	3rd International Symposium on Antibiotics. Castle of smolenice, Czechoslovakia (June 1976)
T. YOKOYAMA	Conidial state of *Shiraia bambusicola* P. Henn.	Mycological Society of Japan. Tokyo (July, 1976)
I. BANNO & K. MIKATA	Isolation of yeasts from natural materials by enrichment method.	The Society of Fermentation Technology, Japan. Osaka (October, 1976)

[*1] Institute of Scientific and Industrial Research, Osaka University, Suita, Osaka.
[*2] Department of Plant Pathology, Faculty of Agriculture, Okayama University.
[*3] Okayama Agricultural Experiment Station.
[*4] Central Research Division, Takeda Chemical Industries Ltd.
[*5] Department of Microbiology, School of Medicine, Gunma University.

MISCELLANEOUS SCIENTIFIC PAPERS

Yutaka SAKAMOTO, Teiji IIJIMA, Shizuko IYOBE, and Susumu MITSUHASHI, 1975. Typing of *Pseudomonas aeruginosa* by phage resistance and lysogeny. *In* Microbial Drug Resistance p. 307–320. University of Tokyo Press.

Tatsuo YOKOYAMA. 1975. Preservation of the fungal cultures. Rinsho-Kensa 19: 311–318. (in Japanese)

Ken-ichi SASAJIMA. 1976. D-Ribose. *In* K. Ogata, S. Kinoshita, T. Tsunoda and K. Aida (ed.) Microbial Production of Nucleic acid-related Substances, p. 199–204. Kodansha Ltd., Tokyo.

Kiichi TAKANO, Yasumoto KIKUCHI, Teiji IIJIMA, and Kiyoshi TSUCHIKAWA 1975. Genetic Toxicology Testing. *In* Fetotoxicity and Genetic Toxicology. Nankodo Press Tokyo. (in Japanese)

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CORRECTIONS

In the issue of IFO Research Communication No. 7, the following corrections should be made.

Page	Line	Type	Should read
38	4	strations	stations
45	31	*Verticllium*	*Verticillium*
46	15	*microsporus*	*microspora*
47	33	siolated	isolated
51	12	extremly	extremely
61	6	sustem (s)	system (s)
61	14	enzyme	enzymes
	25	L-amino acids	L-Amino acids
63	Table 2	(STR 69)	(STF 69)
	Fig. 1	STF 90	STF 69
66	23	*flavotritici**	*flavotricini**
	37	*hawaiensis*	*hawaiiensis*
	44	21883	12883
67	5	13799	12799
	11	*S. perviensis*	*S. peruviensis*
	13	12092	12902
	17	*S. roseofluvus*	*S. roseofulvus*
68	1	Streptomycetes	streptomycetes
76	12	sotck	stock
77	8	wsa	was
79	4	preceeding	preceding
80	23	preceeding	preceding
97	9	adsorttion adsorption	adsorption
107	8	rhumatoid	rheumatoid
108	36	cencentration	concentration
109	6	cencentration	concentration
	25	Labzoffski	Labzoffsky
	32	Labzoffski	Labzoffsky
	39	both	bath
	40	Labzoffski	Labzoffsky
	41	rhumatoid	rheumatoid
110	8	Labzoffski	Labzoffsky
113	20	Canad.	Can.
114	14	soc.	Soc.
117	19	Canad.	Can.
143	13	12012	12010
149	18	*Stachybotris*	*Stachybotrys*

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