NUMBER 9

INSTITUTE FOR FERMENTATION
OSAKA

RESEARCH COMMUNICATIONS

(ANNUAL REPORT 1977-1978)

1979

財団法人 発酵研究所

RESEARCH COMMUNICATIONS

No. 9

(*Annual Report 1977-1978*)

1979

INSTITUTE FOR FERMENTATION, OSAKA

17–85, JUSO-HONMACHI 2-CHOME
YODOGAWA-KU, OSAKA 532, JAPAN

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THE REPORT OF THE DIRECTOR

The Institute for Fermentation, Osaka is engaged in the preservation and distribution of reference strains of microorganisms of importance to science and technology. In the IFO culture collection about seven thousands strains are available for distribution to researchers in scientific organizations and factories upon request. In the past two years (1977–1978) the institute has added about six hundreds strains to its culture collection. In accordance with the increase in the number of strains stored in the collection, and the accumulation of results of authentication of strains in the culture collection during these years, publication of a revised edition of the List of Cultures (5th Edition 1972) has been a matter of urgent necessity. In September 1978, the 6th edition of the List of Cultures was published after one year's preparation; it lists 4050 fungi, 1125 yeasts, 695 bacteria, 743 actinomycetes and 35 bacteriophages. The manuscripts of this edition were transcribed onto computer tape and the final manuscripts for photo-set were prepared automatically. This allows easy updating of the list and editing manuscripts for the next issue.

In 1977, Professor Osamu Hayaishi, Faculty of Medicine, Kyoto University and Professor Hideaki Yamada, Faculty of Agriculture, Kyoto University were nominated as new members of the Board of Trustees. In November 1978, Dr. Masao Isono was nominated as a Councilor of the Board of Trustees. All of the members of the Board are represented in this report. The Treasurer of the institute, Mr. Ihei Tanida retired after one year's service and Mr. Kazuo Matsumoto succeeded to the position in August 1977. In July 1978, he moved to other organization and Mr. Tadashi Kato was appointed as Treasurer.

In the three research sections of the institute, the bacteriology and yeast science section, the mycology section and the biochemistry and genetics section, steady progress has been made. In the bacteriology and yeasts science section under Dr. Banno authentication of the maintaining strains has been proceeded. Reexamination of aerobic bacteria and yeasts in the culture collection was completed at the end of 1977, and authenticated strains have been preserved as L-dried specimens to prevent further change during preservation. A reexamination of anaerobic bacteria in the collection is under way. Recently, reports have been published stating that in the process of freeze-drying, there breaks occure in DNA strands and these result in mutations in the treated cells. The possibility that mutations occur in L-dried specimens represents an important problem as almost all of the strains are intended to be preserved as L-dried specimens. At the Meeting of the Society of Cryobiology held in Tokyo in August 1978, Dr. Banno presented a paper concerning this problem. In it he stated that possible mutation could be prevented by preserving specimens at a low temperature. The mycology section under Dr. Yokoyama, carried out a survey of microflora in the soil of the rice paddy fields, by a project which lasted three years (1976–1978). In 1976 a preliminary

survey was carried out at four experimental rice paddy fields in Osaka Prefecture, and from 1977 to 1978 a full-scale survey was performed and data on seasonal and regional changes in number and species of fungi were compiled. Dr. Yokoyama attended the 2nd International Mycology Congress held at the University of South Florida, Tampa, Florida in 1977 and presented a paper about microflora in leaf-litter: Fungi imperfecti of Japan and South Pacific islands. After the congress he joined a foray in the area of the Apparachian Mountains, and visited several organizations engaged in the preservation of fungi, such as ATCC, Virginia Institute of Polytechnics and State University and the Squibb Institute for Medical Research. The annual foray of the Japanese Mycological Society was held at the Lake Towada, in October 1977 and at Miyazaki in October 1978. Staff of this institute joined the foray and collected samples, including soil, leaves, mashrooms and decayed woods and have worked on the isolation of bacteria, yeasts and fungi from these samples using various methods including enrichment method. Some of them were found to be the first recorded in Japan. In the biochemistry and genetics section under Dr. Sasajima, the mechanism of pleiotropy of the transketolase mutant of *Bacillus subtilis* have been investigated and some of the results are presented in this report. A new substance was accumulated in the medium by cultivating a transketolase mutant of *Bacillus pumilus*, and the substance was identified as 1-deoxy-D-*altro*-heptulose, a novel monosaccharide.

During these two years we have had lectures or seminar by the following guests speakers;

Dr. C. W. Hesseltine (NRRL) "Activities in NRRL and ARS Culture Collection"

Prof. T. Yanagishima (Nagoya University) "Sexual conjugation in *Saccharomyces*"

Prof. T. Takemaru (Okayama University) "Sexuality in Basidiomycetes"

Dr. R. F. Gomez (Massachusetts Institute of Technology) "DNA damage and repair in *Escherichia coli*."

Dr. V. D. Kuznetsov (Institute of Microbiolgy Academy of Science U.S.S.R.) "Homological series of hereditary variation in Streptomyces"

In September 1978, the European Patent Office and the IFO signed an Agreement concerning the deposit of microorganisms for the patent procedures, the same agreement will be concluded between the Swedish Patent Office and the IFO in the near future.

(T. IIJIMA)

IFO Res. Comm. 9,
3–16, 1979 (March)

ENRICHMENT AND ISOLATION OF BACTERIA CAPABLE OF UTILIZING TRICARBALLYLIC ACID

Ko IMAI

Summary

Bacteria capable of utilizing tricarballylic acid (tri$^+$) were isolated from soil samples by enrichment culture, and their taxonomic properties were examined. Before the enrichment cultivation, tri$^+$ bacteria accounted for 6 to 8% of the bacterial population. After incubation for 3 days, the percentage of tri$^+$ bacteria to the bacterial population increased to 60 to 70%, and the majority of tri$^+$ bacteria isolated were *Pseudomonas putida*. After incubation for 7 days, microorganisms belonging to *Pseudomonas acidovorans* group became dominant for *P. putida*, though the percentage of tri$^+$ bacteria to the bacterial population decreased to 10% or less. Among the 474 tri$^+$ isolates obtained in the present study, the gram-negative species predominated, and no gram-positive species were found, the sole exception being *Corynebacterium* sp., which was gram-variable. The tri$^+$ gram-negative species were distributed not only among aerobs, such as *Pseudomonas, Alcaligenes* and *Acinetobacter*, but also among facultative anaerobs, such as *Citrobacter* and *Klebsiella* which were recovered less frequently.

Tricarballyilc acid, a kind of tricarboxylic acid, is known to occur as a minor constituent in plant materials (15, 16, 19, 23). There are a few reports (1, 4, 5, 6, 18) on bacteria capable of utilizing tricarballylic acid (tri$^+$). The author and the colleagues have studied transport systems for tricarboxylic acids in some species belonging to Enterobacteriaceae (8–14), and shown that *Salmonella typhimurium* utilizes tricarballylate and possesses a system that is induced by tricarballylate and transports tricarballylic, citric, and *cis*-aconitic acids (8, 14). In order to reveal the distribution of tri$^+$ bacterial species and the properties of their transport systems for tricarballylic acid, an attempt was made to isolate tri$^+$ bacteria. In this report, the isolation of tri$^+$ bacteria by enrichment culture and the taxonomic properties of the isolates are described.

Materials and Methods

Constituents of media used. Enrichment of tri$^+$ bacteria was achieved in medium A, consisting of 5 g of sodium tricarballylate, 1 g of $(NH_4)_2SO_4$, 1 g of NaCl, 0.7 g of K_2HPO_4, 0.3 g of KH_2PO_4, 0.1 g of $MgSO_4 \cdot 7H_2O$, 100 μg of *p*-aminobenzoic acid, 1 μg of biotin, 500 μg of choline, 200 μg of calcium pantothenate, 1 μg of folic acid, 200 μg of niacin, 100 μg of riboflavin, 200 μg of thiamine and 1,000 ml of distilled water. In order to isolate tri$^+$ bacteria specifically, agar medium A containing the constituents of medium A and 15 g of agar was employed. The bacterial population in the enrichment cultures were determined by the pour plate method (17) using medium B consisting of

10 g of Polypepton (Daigo Eiyo Co., Osaka), 2 g of yeast extract, 2 g of NaCl, 15 g of agar and 1,000 ml of distilled water. Soft agar media A and B containing 0.8 % agar were employed in the pour plate method. Ability to utilize tricarballylic acid was examined on the slants of medium C which consisted of 3 g of tricarballylate, 0.2 g of glucose, 0.5 g of yeast extract, 1 g of KH_2PO_4, 5 g of NaCl, 12 mg of phenol red, 15 g of agar and 1,000 ml of distilled water. The media used in the taxonomic examination were prepared according to relevant manuals (3, 20, 21),

Sources of inoculum. Soil samples were collected at rice fields in the cities of Habikino (sample H) and Ikeda (sample I), Osaka prefecture, in January 1977, and in Minoo city (sample M), Osaka prefecture, in June 1977.

Enrichment and isolation of tri$^+$ bacteria. Approximately 1 g of the soil samples were inoculated into 200 ml of Erlenmeyer flasks containing 100 ml of medium A and incubated on a rotary shaker at 28 C. Immediately after inoculation and again after incubation for 3 days and 7 days, 0.5 ml of the cultures were withdrawn and diluted with medium A without tricarballylate. The diluents (0.1 ml) with appropriate inoculum sizes were spread onto the plates of medium B with 5 ml of soft agar medium B, premelated and maintained at 50 C. The plates were incubated at 28 C for 7 days, and the bacterial population was estimated from the colony count on these plates. After recording the colony count, 144 colonies were transferred from the medium B plates to the slants of medium C at random and incubated at 28 C for 7 days. The percentage of tri$^+$ bacteria to the bacterial population was estimated from the number of isolates among the 144 on the slants of medium C giving alkaline reaction (method I).

In order to isolate tri$^+$ bacteria specifically, the diluents of the cultures were plated onto agar medium A with premelted soft agar medium A and incubated at 28 C for 7 days. All visible colonies were transferred to the slants of medium C and incubated at 28 C for 7 days. The number of tri$^+$ bacteria in the cultures and the percentage of tri$^+$ bacteria to the bacterial population were estimated on the basis of the number of the slants showing positive reaction (method II).

The tri$^+$ bacteria were transferred from the medium C slants to the medium B plates and purified repeatedly.

Characterization and identification of tri$^+$ isolates. The tri$^+$ isolates purified were divided into several groups by preliminary examination, as follows: Culture characteristics, cell morphology, gram-reaction, motility, oxidase reaction, acid and gas production from glucose, fluorescent pigments, gelatin liquefaction, hydrolysis of starch and growth on a medium containing glucose and $(NH_4)_2SO_4$ as a sole source of carbon and nitrogen. A representative strain of each group was chosen and examined as to its taxonomic properties in more detail according to appropriate manuals (3, 20, 21). Identification of the tri$^+$ bacteria were performed according to *Bergey's Manual* (2).

Results and Discussion

Enrichment of tri$^+$ bacteria

Table 1 shows the changes of the bacterial population, number of tri$^+$ bacteria,

Table 1. Changes in bacterial population, number of tri+ bacteria and percentage of tri+ bacteria to bacterial population during the enrichment cultivation.

	Sample	Incubation period (day) 0	3	7
Number of bacteria grown on medium B/1 ml of culture (bacterial population)	I	5.6×10^4 (——)	1.5×10^9 (——)	1.7×10^7 (——)
	H	1.3×10^5 (7.6%)	1.4×10^9 (68.1%)	9.6×10^7 (10.4%)
(Percentage of tri+ bacteria to bacterial population determined by method I)	M	2.0×10^5 (5.6%)	4.9×10^7 (11.8%)	2.5×10^4 (1.4%)
Number of tri+ bacteria on agar medium A	I	3.8×10^3 (6.8%)	9.0×10^8 (60.0%)	1.1×10^5 (0.7%)
	H	7.5×10^3 (5.8%)	5.7×10^8 (40.7%)	5.3×10^6 (5.5%)
(Percentage of tri+ bacteria to bacterial population determined by method II)	M	1.3×10^3 (0.7%)	1.4×10^6 (2.9%)	—— (——)

tri+, able to utilize tricarballylate.

and the percentage of tri+ bacteria to the bacterial population during enrichment cultivation. Immediately after inoculation with soil samples (incubation period 0), the bacterial population in 1 ml of the medium was about 10^4 to 10^5, and the percentage of tri+ bacteria was 6 to 8 % of the bacterial population. After incubation for 3 days, the bacterial population reached 10^9, and the percentage of tri+ bacteria to the bacterial population increased to 60 to 70 %. A longer incubation period (7 days) resulted in a decrease of the bacterial population and of the percentage of tri+ bacteria to the bacterial population. In the case of sample M, which was collected from an irrigated rice field and had a high moisture content, the culture incubated for 3 days is considered probably to have entered the decline phase of grwoth, considering the low bacterial population (4.9×10^7) and low percentage of tri+ bacteria to the bacterial population (11.8 %) during this incubation period.

The percentages of tri+ bacteria determined by method II using agar medium A were lower than those by the method I using medium B. However, all tri+ species obtained by using medium B were recovered by using agar medium A (This will be described later).

Characteristics and identification of tri+ isolates

In the present study, 474 tri+ isolates were obtained and divided into 9 groups by preliminary examination. The representative strain of each group was examined as to its taxonomic properties in more detail and identified. The results are given in the descriptions below.

Group 1 (*Corynebacterium* sp.): The 17 tri+ isolates, which were gram-variable, pleomorphic (Pl. 1, A) and nonmotile rods, are similar to *Corynebacterium sepedonicum*,

a phytopathogenic coryneform bacterium, but different from this species in that they grow rapidly on nutrient agar at 37 C. The pathogenicity to animals or plants of these 17 strains is unknown, and so their taxonomic position remains undetermined. Table 2 shows the properties of the strain HA0-5 as a representative of the 17 isolates.

Group 2 (the same species as the unidentified strain IA0-141): The 7 isolates, which were obtained by using agar medium A before enrichment cultivation, are gram-negative motile rods with a polar flagellum (Pl. 1, B) and formed reddish-brown colonies on nutrient agar. These 7 isolates are similar to photoheterotrophic orgnaisms, such as *Rhodopseudomonas* and *Rhodospirillum*, in morphological characteristics, but do not

Table 2. Taxonomic properties of *Corynebacterium* sp. HA0-5.

Gram-reaction	V
Motility	−
Catalase	+
Oxidase	−
OF test	−
Acid and gas from glucose	−
Indole	−
MR	−
VP	−
Pigments	−
Urease	−
Arginine dihydrolase	−
Lysine decarboxylase	−
Ornithine decarboxylase	−
Hydrolysis of gelatin	+
starch	+
esculin	+
Tween 80	+
Litmus milk	unchanged
Utilization of glucose	+
fructose	+
galactose	+
mannose	+
arabinose	+
xylose	+
lactose	−
maltose	+
sucrose	+
dulcitol	−
mannitol	+
glycerol	+
Growth at 37 C	+

V, variable; +, positive; and −, negative.

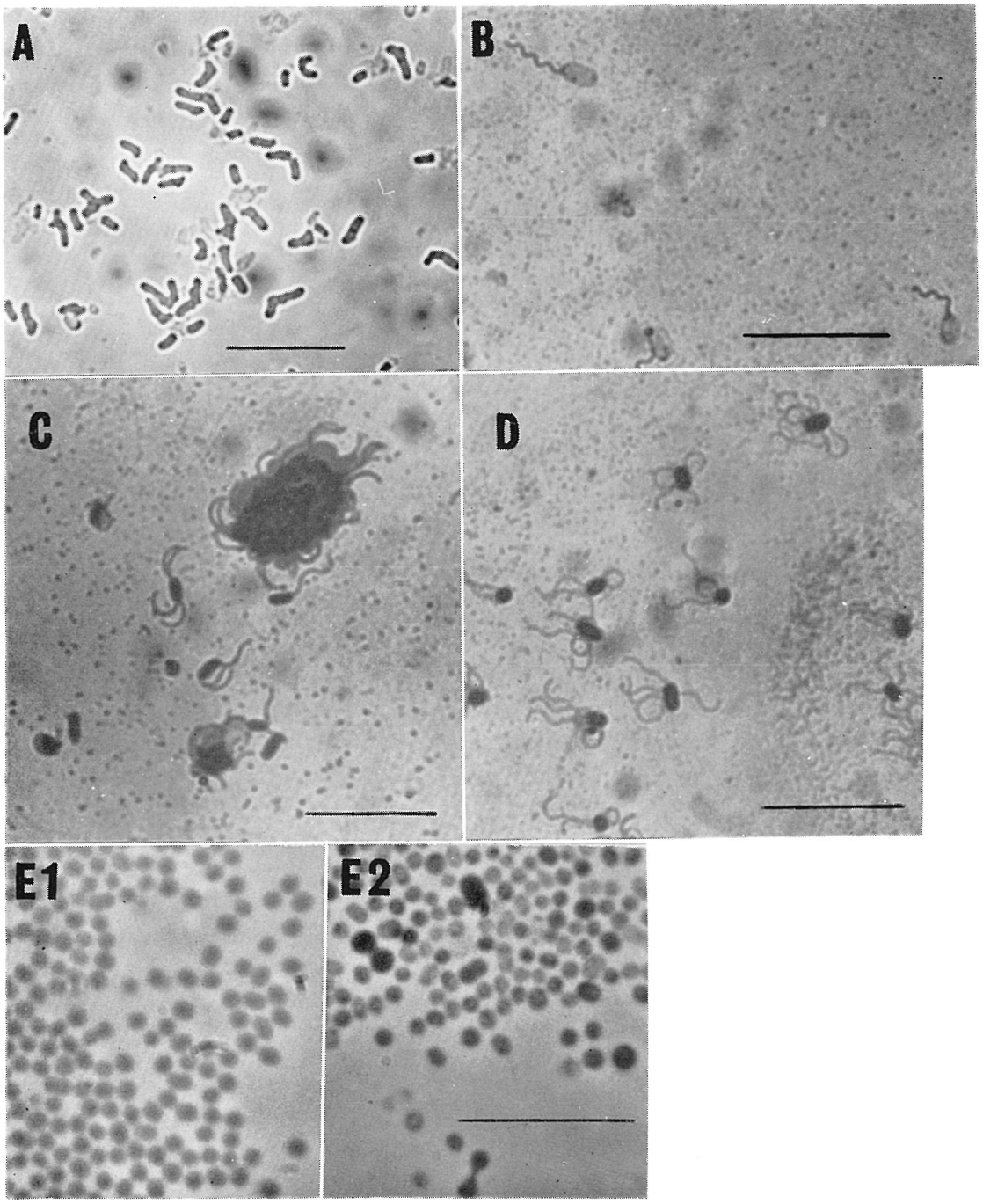
grow in a photoorganotrophic medium (20) under anaerobic conditions in light. The 15 species of the genus *Pseudomonas*, which produce pink, red or brown pigments associated with colonies, are described in *Bergey's Manual* (2), but the taxonomic position of these 7 isolates is not yet clarified. Table 3 shows the properties of the strain IA0-141 as a representative of the 7 isolates.

Group 3 (*Pseudomonas acidovorans* group): The 142 tri$^+$ isolates, which are gram-negative, oxidase positive and motile rods with polar flagella (Pl. 1, C) and do not uti-

Table 3. Taxonomic properties of unidentified strain IA0-141.

Gram-reaction	−
Motility	+
Flagellation	polar
Photosynthesis	−
Catalase	+
Oxidase	−
OF test	O
MR	−
VP	−
Indole	−
Arginine dihydrolase	−
Lysine decarboxylase	−
Ornithine decarboxylase	−
Phenylalanine deaminase	−
Urease	+
Nitrate reduction	−
Malonate	−
Hydrolysis of gelatin	−
cellulose	−
starch	+
esculin	−
Tween 80	−
Utilization of glucose	+
fructose	−
galactose	+
mannose	−
arabinose	+
xylose	+
lactose	−
maltose	+
sucrose	−
dulcitol	−
mannitol	−
glycerol	+

O, oxidative; +, positive; and −, negative.



Pl. 1. A. Cells of *Corynebacterium* sp. HA0-5 grown on nutrient agar for 20 hr.
B. Polar monotrichous flagellum of the unidentified strain IA0-141 stained by Leifson's method (3).
C. Bipolar multitrichous flagella of *Comamonas terrigena* IA7-364.
D. Peritrichous flagella of *Alcaligenes faecalis* HA7-52.
E. Cells of *Acinetobacter calcoaceticus* HA3-53 grown on nutrient agar for 20 hr (E1) and for 7 days (E2).
The bars represent 10 μm.

lize glucose as carbon and energy source, consisted of microorganisms belonging to *Pseudomonas acidovorans* group (22). Of these 142 isolates, 135 isolates which did not hydrolyze Tween 80, are considered to be *Comamonas terrigena*, and the remaining 7 strains which hydrolyzed Tween 80, are considered to be *Pseudomonas acidovorans* or *Pseudomans testosteroni*. Since there is a report (7) that these 3 species possess compositions similar to cellular fatty acids, these 142 isolates are listed as microorganisms belonging to *P. acidovorans* group in Table 10. Table 4 shows the taxonomic properties of *Comamonas terrigena* IA7-364.

Table 4. Taxonomic properties of *Comamonas terrigena* IA7-364.

Gram-reaction	−
Motility	+
Flagellation	bipolar
Oxidase	+
OF test	−
Fluorescent pigments	−
Arginine dihydrolase	−
Lysine decarboxylase	−
Ornithine decarboxylase	−
Nitrate reduction	+
Hydrolysis of gelatin	−
starch	−
esculin	−
Tween 80	−
Indole	−
MR	−
VP	−
Growth at 37 C	+
Utilization of glucose	−
fructose	−
galactose	−
mannose	−
arabinose	−
xylose	−
lactose	−
maltose	−
sucrose	−
dulcitol	−
mannitol	−
glycerol	−
succinate	+
sebacate	+
p-hydroxybenzoate	+

+, positive and −, negative.

Groups 4 and 5 (*Pseudomonas putida* and *Pseudomonas fluorescens*): Of 293 tri$^+$ isolates, which were gram-negative, oxidase-positive motile rods with polar flagella and produced fluorescent pigments on King B medium, the 265 gelatinase-negative strains were identified as *Pseudomonas putida*, and the remaining 28 strains, which liquefied gelatin, were identified as *Pseudomonas fluorescens*. Table 5 shows the taxonomic properties of strains HA0-2 and HB0-3 as representatives of *P. putida* and *P. fluorescens*, respectively.

Table 5. Taxonomic properties of *Pseudomonas putida* HA0-2 and *Pseudomonas fluorescens* HB0-3.

	HA0-2	HB0-3
Gram-reaction	−	−
Motility	+	+
Flagellation	polar	polar
OF test	O	O
Oxidase	+	+
Catalase	+	+
Fluorescent pigments	+	+
Pyocyanine	−	−
Carotenoids	−	−
Arginine dihydrolase	+	+
Lysine decarboxylase	−	−
Ornithine decarboxylase	−	−
Denitrification	−	−
Indole	−	−
Hydrolysis of gelatin	−	+
Utilization of trehalose	−	+
inositol	−	−
glucose	+	+
L-valine	+	+
β-alanine	+	+
DL-arginine	+	+

O, oxidative; +, positive; and −, negative.

Group 6 (*Alcaligenes faecalis*): The 9 tri$^+$ isolates, which were gram-negative, oxidase-positive coccal rods, motile with peritrichous flagella (Pl. 1, D), were identified as *Alcaligenes faecalis*. Table 6 shows the taxonomic properties of the strain HA7-52 as a representative of these 9 isolates.

Group 7 (*Acinetobacter calcoaceticus*): The strain HA3-53, which was gram-negative, oxidase-negative, nonmotile, short and plump rods (Pl. 1, E), was identified as *Acinetobacter calcoaceticus*. The taxonomic properties of this strain are shown in Table 7.

Group 8 (*Citrobacter intermedius* biotype a): The 3 tri$^+$ isolates, which were

gram-negative, oxidase-negative motile rods and produced acid and gas from glucose, were identified as *Citrobacter intermedius* biotype a. The properties of the representative strain IA7-343 are shown in Table 8.

Group 9 (*Klebsiella pneumoniae* biotype oxytoca): The 2 tri+ isolates, which were gram-negative nonmotile rods and produced acid and gas from glucose, were identified as *Klebsiella pneumoniae* biotype oxytoca. The taxonomic properties of the strain

Table 6. Taxonomic properties of *Alcaligenes faecalis* HA7-52.

Gram-reaction	−
Motility	+
Catalase	+
Oxidase	+
OF test	−
MR	−
VP	−
Arginine dihydrolase	−
Lysine decarboxylase	−
Ornithine decarboxylase	−
Phenylalnine deaminase	−
Urease	−
Indole	−
Malonate	−
Nitrate reduction	+
Hydrolysis of gelatin	−
starch	−
esculin	−
Tween 80	−
Litmus milk	alkaline
Production of 3-ketolactose	−
Utilization of glucose	−
fructose	−
galactose	−
mannose	−
arabinose	−
xylose	−
trehalose	−
sucrose	−
maltose	−
lactose	−
mannitol	−
glycerol	−
citrate	+
isocitrate	+

+, positive and −, negative.

IA3-218 are shown in Table 9.

Change of tri$^+$ species during enrichment cultivation

On the basis of the results mentioned above, changes in the tri$^+$ bacterial species during the enrichment cultivation are summarized in Table 10.

Before enrichment cultivation, the 11 tri$^+$ strains were isolated from the plates of medium B inoculated with sample H. Of these 11 strains, 6 strains were *Corynebac-*

Table 7. Taxonomic properties of *Acinetobacter calcoaceticus* HA3-53.

Gram-reaction	−
Motility	−
Oxidase	−
OF test	−
Indole	−
MR	−
VP	−
KCN	−
Arginine dihydrolase	−
Lysine decarboxylase	−
Ornithine decarboxylase	−
Phenylalanine deaminase	−
Nitrate reduction	−
Hydrolysis of gelatin	−
starch	−
esculin	−
Tween 80	+
Malonate	+
Litmus milk	alkaline
Utilization of glucose	−
fructose	−
galactose	−
mannose	−
arabinose	−
xylose	−
lactose	−
maltose	−
sucrose	−
dulcitol	−
mannitol	−
glycerol	−
citrate	+
isocitrate	+
Resistance to 5 units of penicillin	+

+, positive and −, negative.

terium sp., 3 strains were *P. putida*, and 2 strains were *P. fluorescens*. After incubation for 3 days, the 98 tri$^+$ strains were isolated from the culture of sample H by using medium B, and *P. putida* accounted for 78 % (76 strains) of these 98 isolates. Of the remaining 22 strains, 14 strains were microorganisms belonging to *P. acidovorans* group, 7 strains were *P. fluorescens*, and 1 strain was *Corynebacterium* sp. After incubation for 7 days, the microorganisms belonging to the *P. acidovorans* group predominated, accounting for 93 % (13 isolates) of the 14 tri$^+$ isolates: The remaining one was identified as *Alcaligenes faecalis*.

When agar medium A was employed to isolate tri$^+$ bacteira from the culture of sample H, all species obtained by using medium B were recovered. Furthermore, the frequency of isolation of each species did not vary geratly between medium B and

Table 8. Taxonomic properties of *Citrobacter intermedius* IA7-343.

Gram-reaction	−
Motility	+
Catalse	+
Oxidase	−
OF test	F
MR	+
VP	−
Phenylalnine deaminase	−
Nitrate reduction	+
Urease	+
H_2S on TSI	−
Malonate	−
KCN	+
Citrate (Koser)	+
Arginine dihydrolase	+
Lysine decarboxylase	−
Ornithine decarboxylase	+
Gas from glucose	+
Acid from adonitol	−
arabinose	+
dulcitol	−
inositol	−
lactose	+
maltose	+
mannitol	+
rhamnose	+
sorbitol	+
trehalose	+
xylose	+
Hydrolysis of gelatin	−

F, fermentative; +, positive; and −, negative.

agar medium A.

In the isolation of tri^+ bacteria from samples I and M, only agar medium A was employed. The dominant species among the tri^+ bacteria isolated from the culture of sample I were *P. putida* after incubation for 3 days and microorganisms belong to *P. acidovorans* group after incubation for 7 days. This result is the same as in the case of sample H. The microorganisms belonging to *P. acidovorans* group, which were isola-

Table 9. Taxonomic properties of *Klebsiella pneumoniae* biotype oxytoca IA3-218.

Gram-reaction	−
Motility	−
Catalase	+
OF test	F
MR	−
VP	+
KCN	+
Indole	+
Oxidase	±
Brown pigment (water soluble)	+
Arginine dihydrolase	−
Lysine decarboxylase	+
Ornithine decarboxylase	−
Hydrolysis of gelatin	+
Malonate	+
Gas from glucose	+
glycerol	+
Acid from arabinose	+
adonitol	−
lactose	+
maltose	+
mannitol	+
rhamnose	+
raffinose	+
salicin	+
sorbitol	+
trehalose	+
sucrose	+
adonitol	+
inositol	+
xylose	+
Growth at 37 C	+
Ammonium ion and glucose as sole source of nitrogen and carbon	+

F, fermentative; +, positive; ±, doubtfully positive; and −, negative.

Table 10. Change in tri$^+$ bacterial species during incubation.

Sample	H						I			M	
Medium used for isolation	B			A			A			A	
Incubation period (day)	0	3	7	0	3	7	0	3	7	0	3
Corynebacterium sp.	6	1		7			2			1	
Unidentified strain IA0-141				4			1			2	
Pseudomonas putida	3	76		4	80		1	93		2	6
Pseudomonas fluorescens	2	7		1	4			14			
Pseudomonas acidovorans group		14	13	4	10	23		48	15		15
Alcaligenes faecalis			1	1		3				1	3
Acinetobacter calcoaceticus					1						
Citrobacter intermedius									3		
Klebsiella pneumoniae biotype oxytoca								1	1		
Number of isolates	11	98	14	21	95	26	4	156	19	6	24

ted from the cultures of samples H and I when incubated for 7 days, were predominatly isolated from the culture of sample M when incubated for 3 days. This result shows that the culture of sample M had entered the decline phase of growth after incubation for 3 days.

Isolation of *A. faecalis, A. calcoaceticus, C. intermedius* and *K. pneumoniae* varied in the samples and was less frequent than that of *P. putida* and *P. acidovorans* group microorganisms. *Corynebacterium* sp. was isolated frequently from the samples before the enrichment cultivation. The same species as the undentified strain IAO-141 was isolated from the samples before the enrichment cultivation when using agar medium A but not when using medium B.

We can conclude that, under the conditions employed in this study to isolate tri$^+$ bacteria, *P. putida* predominates at the maximal growth phase of enrichment cultures, and longer incubation periods result in elimination of *P. putida* and in the dominancy of microorganisms belonging to *P. acidovorans* group.

Among the 474 tri$^+$ isolates obtained in the present study, gram-negative species predominated, and no gram-positive species were found, the sole exception being *Corynebacterium* sp., which was gram-variable. Tri$^+$ gram-negative species were distributed not only among facultative anaerobs but also among aerobs.

I am grateful to Dr. H. Iizuka, Dr. T. Iijima, and Dr. I. Banno for critical reading of the manuscript.

References

1) Altekar, W. W., and M. R. R. Rao. 1963. Microbiological dissimilation of tricarballylate and *trans*-aconitate. J. Bacteriol. **85**: 604–613.
2) Buchanan, R. E., and N. E. Gibbons (ed.). 1974. Bergey's manual of determinative bacteriology, 8th ed. The Williams & Wilkins Co., Baltimore.

3) Cowan, S. T., and K. J. Steel (ed.). 1965. Manual for the identification of medical bacteria. Cambridge University Press, London.
4) Gray, C. T., and M. S. Brooke. 1954. The relation of tricarballylic and itaconic acids of the citric acid cycle. Bacteriol. Pro. p. 115–116.
5) Gray, C. T., and M. Raulett. 1962. Tricarballylic dehydrogenase, a succinic dehydrogenase-like bacterial enzyme. Federation Proc. **21**: 45.
6) Gutnick, D., J. M. Calvo, T. Klopotowski, and B. N. Ames. 1969. Compounds which serve as the sole source of carbon or nitrogen for *Salmonella typhimurium* LT-2. J. Bacteriol. **100**: 215–219.
7) Ikemoto, S., H. Kuraishi, K. Komagata, R. Azuma, T. Suto, and H. Murooka. 1978. Cellular fatty acid composition in *Pseudomonas* species. J. Gen. Appl. Microbiol. **24**: 199–213.
8) Imai, K. 1975. Isolation of tricarboxylic acid transport-negative mutants of *Salmonella typhimurium*. J. Gen. Appl. Microbiol. **21**: 127–134.
9) Imai, K. 1976. Transport of tricarboxylic acids in *Serratia marcescens* (I). Nippon Nogei-kagaku Kaishi **50**: 217–220. in Japanese.
10) Imai, K. 1976. Transport of tricarboxylic acids in *Serratia marcescens* (II). Nippon Nogei-kagaku Kaishi **50**: 221–224. in Japanese.
11) Imai, K. 1977. Transport system for citric acid in *Proteus vulgaris*. Agric. Biol. Chem. (Tokyo) **41**: 733–735.
12) Imai, K. 1978. Tricarboxylic acid transport systems in *Proteus mirabilis*. J. Gen. Appl. Microbiol. **24**: 279–285.
13) Imai, K., T. Iijima, and I. Banno. 1977. Location of *tct* (tricarboxylic acid transport) genes on the chromosome of *Salmonella typhimurium*. IFO Res. Comm. **8**: 63–68.
14) Imai, K., T. Iijima, and T. Hasegawa. 1973. Transport of tricarboxylic acids in *Salmonella typhimurium*. J. Bacteriol. **114**: 961–965.
15) Miller, B. S., and T. Swain. 1960. Chromatographic analysis of amino acids, organic acids and sugars in wheat plant extracts. J. Sci. Food Agric. **11**: 344–348.
16) Nelson, E. K., and H. H. Mottern. 1931. Some organic acids in barley, maize, oats and rye plants. J. Am. Chem. Soc. **53**: 3046–3048.
17) Postgate, J. R. 1969. Viable counts and viability. *In* J. R. Norris and D. W. Ribbons (ed.), Methods in microbiology, vol. 1, p. 611–628. Academic Press Inc., New York.
18) Rao, M. R. R., and W. W. Altekar. 1962. Tricarballylate dehydrogenase. Biochem. Biophys. Res. Commun. **7**: 62–66.
19) Roberts, E. J., and L. F. Martin. 1954. Identification and determination of non-nitrogenous organic acids of sugar cane by partition chromatography. Anal. Chem. **26**: 815–818.
20) Skerman, V. B. D. (ed.). 1967. A guide to the identification of the genera of bacteria. 2nd ed. The Williams & Wilkins Company, Baltimore.
21) Society of American Bacteriologists (ed.). 1957. Manual of microbiological methods. McGraw-Hill Book Company, Inc., New York.
22) Stanier, R. Y., N. J. Palleroni, and M. Doudoroff. 1966. The aerobic Pseudomonads: a taxonomic study. J. Gen. Microbiol. **43**: 159–271.
23) Vavruch, I. 1954. Chromatographic studies on sugar beet. II. Nitrogen-free organic acids. Chem. Listy **48**: 442–445.

IFO Res. Comm. 9,
17–26, 1979 (March)

DEFICIENCY OF D-GLUCOSE TRANSPORT IN TRANSKETOLASE MUTANT OF *BACILLUS SUBTILIS*

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Summary

The mechanism of malutilization of D-glucose, one of the pleiotropic properties of the transketolase mutant of *Bacillus subtilis*, was investigated. The cells of the mutant grown even in the presence of D-glucose could not consume oxygen in the presence of D-glucose nor take up D-glucose. However, the phosphorylating activity for methyl-α-D-glucoside by the phosphoenolpyruvate-dependent phosphotransferase system could be detected in cell-free extract of the mutant as well as in the parent. These results indicate that the transport function of the phosphoenolpyruvate: D-glucose phosphotransferase system of the mutant may be defective even though the phosphorylating activity is present. Revertants, which regained the ability to utilize D-glucose by suppressive mutation but were still lacking transketolase, were isolated. This implies that transketolase mutation is not directly related to the defect in the transport function of the phosphotransferase system. It seems that transketolase mutation may affect formation of some substance(s) which is essential to the construction of an orderly and functional transport system for D-glucose.

The transketolase mutant of *Bacillus subtilis* is a pleiotropic mutant as described in a previous paper (30). Among the pleiotropic properties, deficiency of utilization of pentoses and requirement of aromatic amino acids and vitamins are readily explained by a consideration of the role of transketolase in carbohydrate metabolism (30). However, malutilization of D-glucose, D-mannose, maltose, sucrose, trehalose and pyruvate and inhibition of utilization of D-mannitol, D-fructose and glycerol by D-glucose, D-gluconate, D-xylose or L-arabinose can not be explained directly by transketolase deficiency. This research was undertaken to elucidate the mechanism of the malutilization of these sugars.

Materials and Methods

Bacterial strains. *B. subtilis* IFO 12114, the transketolase mutant BG2607, the threonine-less derivative of strain BG2607, BG2623, and the revertants BG2694, BG2692 and BG2693 derived from strain BG2623 were used. Isolation of the transketolase mutant BG2607 was described previously (30). Revertants were isolated according to the method described in a previous paper, as follows (28). The threonine-less mutant BG2623 was derived from strain BG2607 in order to distinguish the revertants from contaminating wild-type strains. The spore suspension of strain BG2623 was irradiated with ultraviolet rays and spread on a D-glucose medium, which was incubated at 37 C for 7 days. The larger colonies were picked up as D-glucose utilizers.

Strain BG2623 formed very small colonies on the medium because the transketolase mutant could utilize D-glucose only to a small degree (30). Isolated revertants showed very slow growth on various carbon sources because of threonine requirement. So threonine markers were reverted to *thr*$^+$. Strains BG2692 and BG2693 were thus isolated as D-glucose utilizers but still required shikimic acid for their growth, showing that the reversion was the result of a second suppressive mutation. The true revertant BG2694 was isolated as a strain which no longer required shikimic acid for its growth. Its *thr* marker was also reverted.

Medium. Slightly modified Spizizen's synthetic medium (28) was used.

Oxygen consumption. Cells grown at 37 C overnight in a medium containing 1 % of sorbitol and 1 % of D-glucose as carbon sources were harvested by centrifugation and washed twice with a basal medium containing only phosphate salts and ammonium sulfate and suspended in the basal medium so that the absorbance at the wavelength of 600 nm was 25. One milliliter of the cell suspension was poured into a glass vessel with a Clark-type electrode and was agitated with a magnetic stirrer for 5 to 10 min, after which 4 ml of the basal medium was added. After an additional 10 min, 10 μl of 0.5 M sugar solution was added to start the reaction. Oxygen consumption was determined by a Beckman oxygen analyzer 777 and expressed in percent saturation in the vessel open to the air.

Uptake of sugars. Two point five milliliters of cell suspension prepared as described above (A_{650}: 2) was mixed with 2.5 ml of a basal medium containing 1.0 mM labeled sugar: D-(U-^{14}C)glucose (specific activity, 0.1 μCi/μmole) or (U-^{14}C)sorbitol (specific activity, 0.1 μCi/μmole) which was preincubated at 30 C. After 1, 3, 5, and 7 min of reaction, 1 ml of the assay mixture was filtered through a millipore filter (0.45μ pore size). The cells were then rapidly washed twice with 5 ml portions of the basal medium. The filter was dried and then transferred to a scintillation vial containing 6 ml of scintillation fluid (4.0 g of 2, 5-diphenyl oxazole and 0.1 g of 1, 4-bis-2-(4-methyl-5-phenyl-oxazol-2-yl)-benzene in 1,000 ml of toluene and 500 ml of Triton X-100). Radioactivity was counted with an Aloka liquid scintillation spectrometer model LSC 671.

Preparation of cell-free extracts. Cells grown at 37 C on a rotary shaker were harvested at exponential growth by centrifugation, washed with 0.01 M Tris-HCl buffer (pH 7.5) containing 0.01 M mercaptoethanol, 0.01 M $MgCl_2 \cdot 6H_2O$ and 0.01 mM EDTA and suspended in the same buffer (A_{650}: 100). The cells were disrupted with a Kubota sonic oscillator at 160 W for 10 min. The supernatant solution obtained by centrifugation at 3,000$\times g$ for 10 min was used as the cell-free extract.

Protein was determined according to the method of Lowry *et al.* (17).

Assay for phosphoenolpyruvate-dependent phosphotransferase activity. Determination of methyl-α-D-glucoside-6-phosphate formed from ^{14}C-labeled methyl-α-D-glucoside was carried out according to the method of Marquet *et al.* (19). The reaction mixture (0.5 ml) containing 1 mM methyl-α-D-(U-^{14}C)glucoside (specific activity, 0.1 μCi/μmole),

10 m*M* $MgCl_2$, 1 mM dithiothreitol, 5 mM phosphoenolpyruvate (PEP), 20mM NaF, 40 mM Tris-HCl buffer (pH 7.5) and the cell-free extract was incubated at 30 C. The reaction was started by addition of the enzyme solution. After 60 min of incubation the reaction mixture was applied to a BIO RAD AG-1×2 column (0.5×4.0 cm) in the formate form. Free methyl-α-D-(U-^{14}C)glucoside was washed out with 3.5 ml of distilled water and methyl-α-D-(U-^{14}C)glucoside-6-phosphate was subsequently eluted with 3.5 ml of 0.5 M ammonium formate in 0.2 M formic acid. One milliliter of the eluate was dissolved in 12 ml of toluene-based scintillation liquid as described above. Radioactivity was counted with an Aloka scintillation spectrometer model LSC 671.

Growth test. Growth test was carried out according to the method described previously (29).

Chemicals. PEP and ATP were purchased from Sigma Chemical Co. D-(U-^{14}C) glucose, (U-^{14}C)sorbitol and methyl-α-D-(U-^{14}C)glucoside were products of Radiochemical Center, Amersham. 1,4-Bis-2-(4-methyl-5-phenyl-oxazol-2-yl)-benzene and 2,5-diphenyloxazole were purchased from Wako Pure Chemical Ind. Ltd. All other reagents were the usual commercial products.

Results

Deficiency of utilization of D-*glucose and maltose*

As described in a previous paper (30), the transketolase mutant of *B. subtilis* could scarcely grow on D-glucose, D-mannose, maltose, sucrose, trehalose and pyruvate. The transketolase mutant could utilize only D-mannitol, sorbitol, D-fructose, glycerol and L-glutamate as sole source of carbon. Moreover, D-glucose inhibited the utilization of D-mannitol, D-fructose and glycerol by the mutant also described previously (30). Utilization of L-glutamate was also inhibited by D-glucose (unpublished data). This is another pleiotropic property of the mutant. So only sorbitol could be used as a source of carbon to obtain the cells grown in the presence of D-glucose. As shown in Fig. 1, the cells of the transketolase mutant BG2607 thus obtained could not consume oxygen in the presence of D-glucose. Apparently, ability to utilize D-glucose was not induced in the cells in spite of the presence of D-glucose in the medium. Maltose could not be utilized either by cells grown on sorbitol in the presence of maltose; ability of maltose utilization was not induced in the cells. Thus, it was shown that induction of ability to utilize these sugars was defective in the transketolase mutant.

Deficiency of D-*glucose transport*

As the mutant could still utilize sorbitol, D-mannitol, D-fructose, glycerol and L-glutamate, it appeared that the defect might not be in the central pathway of carbohydrate metabolism, EMP pathway, and rather in a branch pathway reaching to the central pathway. In the case of D-glucose metabolism, a defect in the transport system was thought to be the point. As shown in Fig. 2, the cells of the mutant BG2607 grown

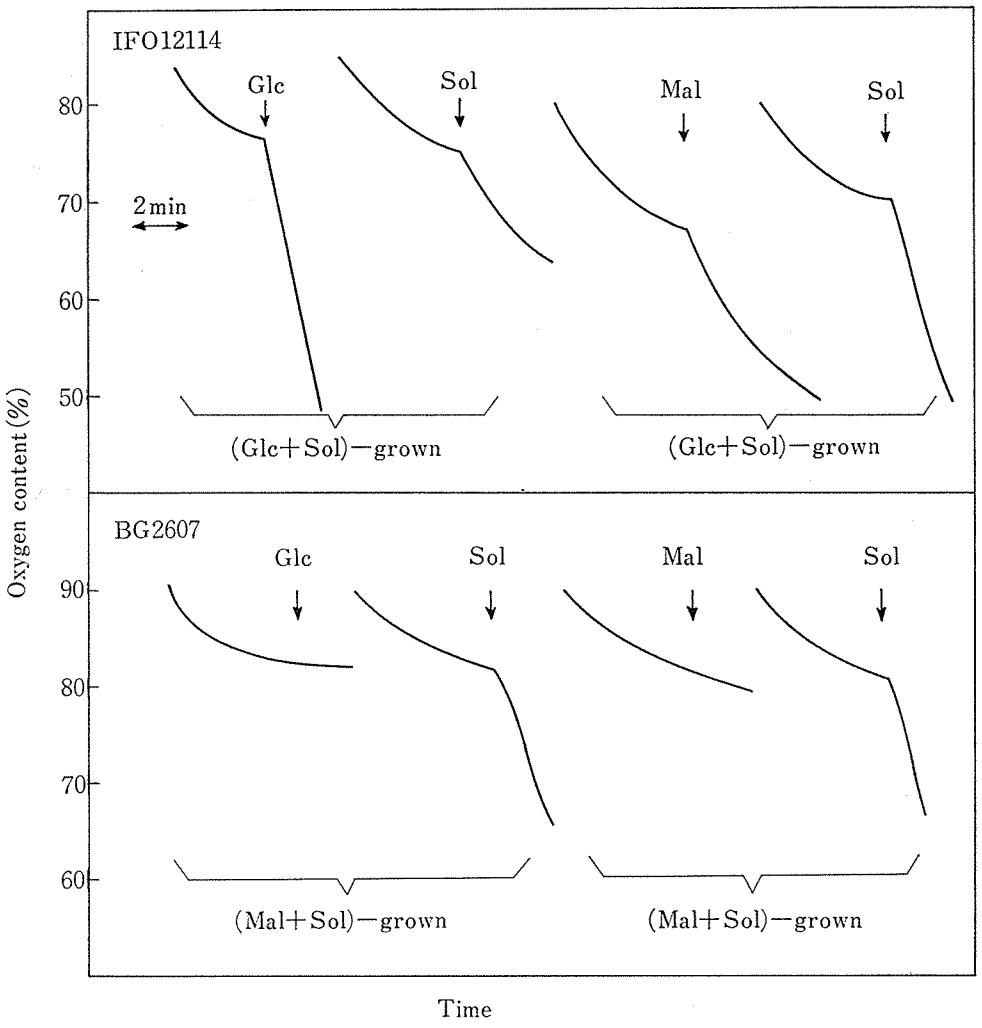

Fig. 1. Oxygen consumption in the presence of D-glucose or sorbitol by *B. subtilis* IFO 12114 grown on sorbitol plus D-glucose and in the presence of maltose or sorbitol by the transketolase mutant BG2607 grown on sorbitol plus maltose. Each substrate was added at the arrow. Abbreviations: Glc, D-glucose; Sol, sorbitol; Mal, maltose.

even in the presence of D-glucose could not take up D-glucose. Meanwhile, the parent strain IFO 12114 could take up D-glucose. In the parent, sorbitol as the sole source of carbon also induced D-glucose transport activity. The ability of sorbitol to induce D-glucose transport activity was also lost in the mutant BG2607. Thus, the transport system for D-glucose appeared to be defective in the transketolase mutant.

It was also interesting that sorbitol transport activity was induced in the mutant BG2607 even in the presence of D-glucose though it was repressed in the parent strain IFO 12114. Catabolite repression of sorbitol metabolism appeared to have changed in the mutant so as to be insensitive to D-glucose repression, probably because of a defect in D-glucose transport.

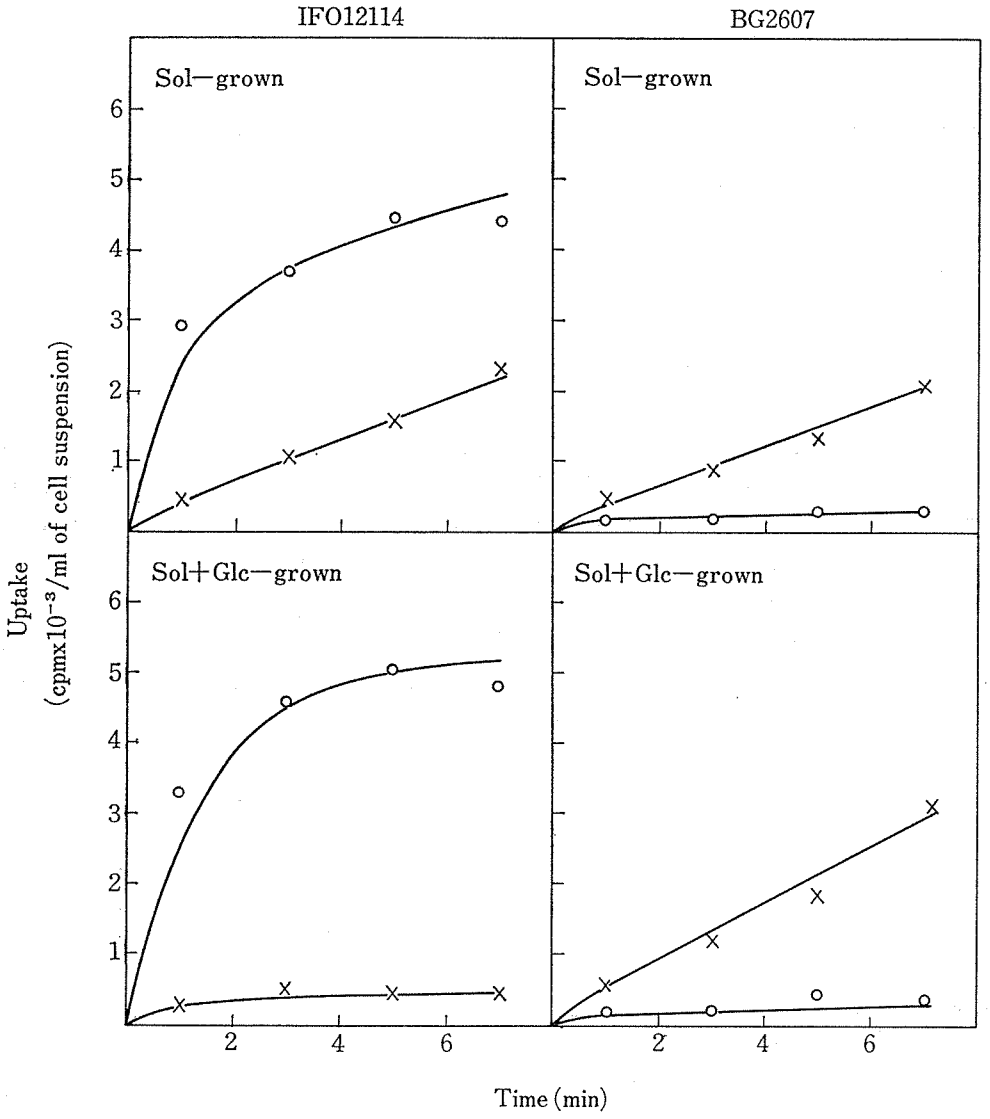

Fig. 2. Uptake of ^{14}C-sugars by *B. subtilis* IFO 12114 and the transketolase mutant BG2607 grown on sorbitol or sorbitol plus D-glucose. D-(U-^{14}C)glucose, —○—; (U-^{14}C)sorbitol, —×—. Abbreviations: Sol, sorbitol; Glc, D-glucose.

PEP-dependent phosphotransferase activity in the mutant

It is known that D-glucose is transported through the cytoplasmic membrane with concomitant phosphorylation in *B. subtilis* by the PEP-dependent phosphotransferase system (3, 7, 8, 18, 19, 24). The phosphotransferase system was first described by Kundig *et al.* (14) and involves the following two reactions (21, 25, 26, 27):

$$\text{PEP}+\text{HPr} \xrightarrow{\text{enzyme I}} \text{P}-\text{HPr}+\text{Pyruvate}$$

$$\text{P}-\text{HPr}+\text{Sugar} \xrightarrow[\text{complex}]{\text{enzyme II}} \text{Sugar}-\text{P}+\text{HPr}$$

Table 1. PEP-dependent phosphotransferase activities for methyl-α-D-glucoside in cell-free extracts of *B. subtilis* IFO 12114 and the transketolase mutant BG2607.

Reaction mixture	Phosphotransferase activity (nmoles/mg/60 min)			
	IFO 12114		BG2607	
	Sol-grown	Sol+Glc-grown	Sol-grown	Sol+Glc-grown
Complete	7.0	22.5	5.0	14.4
No PEP	1.9	2.4	<1	<1
ATP§	<1	1.2	<1	<1

§ ATP was substituted for PEP (at the same concentration) in the complete system.

Enzyme I and HPr (histidine-containing phosphocarrier protein) are general, soluble components, while enzyme II complex is a sugar-specific, membrane-bound component. Another soluble, sugar-specific component factor III which cooperates with enzyme II is also required in some cases (31). The phosphotransferase system for D-glucose in *B. subtilis* has been reported to be induced by D-glucose (19).

Because glucokinase activity was very high in the extract, PEP-dependent phosphorylation of D-glucose could not be determined. However, when D-glucose analogue methyl-α-D-glucoside was used, the effect of glucokinase was excluded. As shown in Table 1, phosphorylation of methyl-α-D-glucoside in the presence of PEP was detected in the cell-free extract of the transketolase mutant BG2607 as well as the parent IFO 12114. Weak activity was induced in sorbitol-grown cells of both strains. ATP scarcely facilitated the phosphorylation of methyl-α-D-glucoside. Thus, no difference could be detected between the parent and mutant strains, as to the phosphorylating activity of the phosphotransferase system. It appeared that the transport function of the system was defective in the transketolase mutant.

It was interesting that this defect in D-glucose transport did not affect the induction of the phosphotransferase system. It seems that the phosphotransferase system is induced by D-glucose outside the cell surface. Similar phenomena have been described by several investigators (9, 20, 27, 32).

Isolation of revertants

Revertants which could utilize D-glucose were isolated and the growth patterns on 15 carbon sources are shown in Table 2. Strain BG2694, one of the five true revertants, showed the same properties as the parent strain in its utilization of carbon sources. Strains BG2692 and BG2693, the supressive mutants, were still defective in transketolase and so could not utilize pentoses and D-gluconate, although the malutilization of D-glucose, D-mannose, maltose, sucrose, trehalose and pyruvate was reverted except that in strain BG2693 pyruvate was barely utilized still. All the revertants restored D-glucose transport function as shown in Fig. 3. In strains BG2692 and BG2693, it appeared that the second mutation suppressed the defective transport function of the phosphotransferase system for D-glucose. It was thus shown that transketolase mu-

Table 2. Utilization of various carbon sources by *B. subtilis* IFO 12114, the transketolase mutant BG2607 and the revertants BG2694, BG2692 and BG2693.

Carbon source	Utilization of carbon sources§				
	IFO 12114	BG2607	BG2694	BG2692	BG2693
Maltose	+	∓	+	+	+
Sucrose	+	∓	+	+	+
Trehalose	+	∓	+	+	+
D-Glucose	+	∓	+	+	+
D-Mannose	+	∓	+	+	+
D-Fructose	+	+	+	+	+
D-Mannitol	+	+	+	+	+
Sorbitol	+	+	+	+	+
D-Gluconate	+	−	+	−	−
D-Xylose	+	−	+	−	−
L-Arabinose	+	−	+	−	−
D-Ribose	+	−	+	−	−
Glycerol	+	+	+	+	+
Pyruvate	+	∓	+	+	∓
L-Glutamate	+	+	+	+	+

§ −, no growth; ∓, malutilization probably because of simple diffusion; +, good growth.

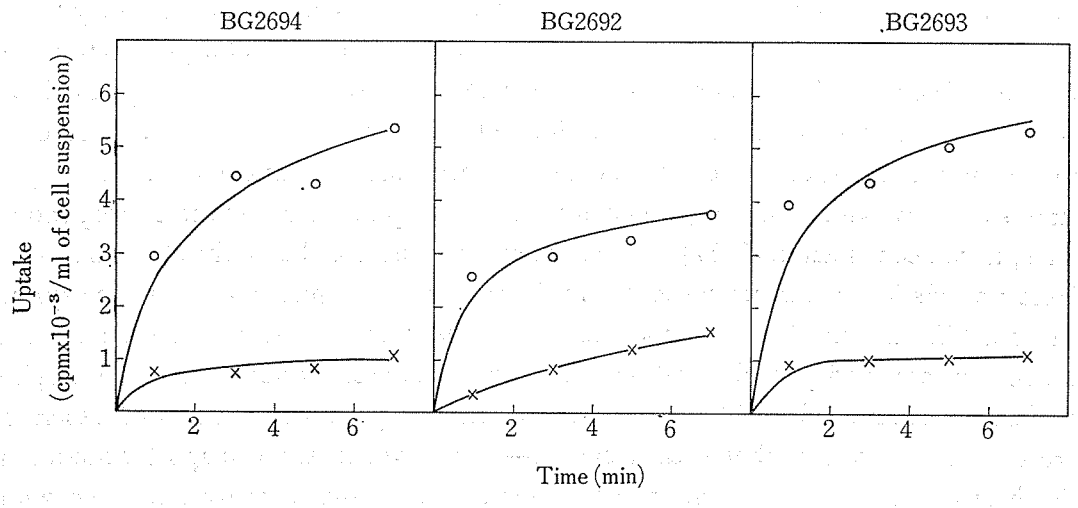

Fig. 3. Uptake of ^{14}C-sugars by the true and supressive revertants BG2694, BG2692 and BG2693. D-(U-^{14}C)glucose, —○—; (U-^{14}C)sorbitol, —×—.

tation was not directly related to D-glucose transport function. The true and suppressive revertants were isolated at frequencies of 9.5×10^{-4} and 7.7×10^{-5}, respectively. These results indicate that the initial transketolase mutation was a point mutation. The properties of the second mutation are unknown but it appears to be related to membrane function or structure.

All the revertants were again sensitive to repression of the synthesis of the sorbitol permease system, probably because D-glucose was taken up and metabolized to catabolite(s) which caused catabolite repression.

Discussion

The mechanism of the malutilization of D-glucose in the transketolase mutant of *B. subtilis* was shown to be caused by deficiency of D-glucose transport function of the PEP-dependent phosphotransferase system for D-glucose. In the parent strain, both transport function and phosphorylating activity could be induced by D-glucose and sorbitol though sorbitol was a weaker inducer. In contrast to this, neither D-glucose nor sorbitol could induce the transport function of the phosphotransferase system in the transketolase mutant though they could induce phosphorylating activity. Non-function of phosphotransferase system due to PEP shortage has been described by Kornberg and Smith (13) and Roehl and Vinopol (23) in the phosphofructokinase mutants of *Escherichia coli* which could not form PEP through the EMP pathway. However, PEP shortage might not be the cause in the transketolase mutant of *B. subtilis* described in this paper because D-manntiol and D-fructose, which were described to be phosphorylated and transported by the PEP-dependent phosphotransferase system in *B. subtilis* (4, 5, 11, 12, 18), were utilized by the mutant, indicating that the general soluble components of the system, enzyme I, HPr and PEP are not the cause of the transport deficiency. So the membrane-bound enzyme II complex may be defective in the mutant. Enzyme II complex catalizes both the phosphorylation and transport of sugars. The components of enzyme II complex have been separated and role in the phosphorlyating activity examined (15). However, the role of these components in the transport function has not yet been studied extensively. Transport mutants of *E. coli* and *B. subtilis*, which are still able to phosphorylate the corresponding sugars, have been isolated (1, 12). It has been also reported that sorbitol is a substrate of phosphorylation but is not taken up by PEP: D-manntiol phosphotransferase system in *E. coli* (32) and in *Staphylococcus aureus* (9), respectively. Gachelin reported that transport process and phosphorylation process were different functions of the phosphotransferase system in *E. coli* (10). In view of these reports and the results described in this paper, it appears that such a change—one in which the transport function is defective though the phosphorylating activity is functional—has occurred in the enzyme II complex of the transketolase mutant of *B. subtilis*.

How the transketolase mutation renders the transport function of the phosphotransferase system for D-glucose inactive is unknown. That supressive mutants, which could take up D-glucose though they were still lacking tansketolase, were isolated implies that transketolase is indirectly related to membrane function or structure through unknown mechanism. As described previously (30), the transketolase mutant of *B. subtilis* is a pleiotropic mutant. Evidence for membrane change in the transketolase mutant has been obtained in our studies; the mutant is non-motile and has no flagella

(unpublished data). Recently, a pleiotropic membrane mutant of *Salmonella typhimurium* was described by Postma *et al* (22). Induction or insertion of membrane proteins may be defective in the mutant.

Little is known about the mechanism of catabolite repression in gram-positive bacteria, compared with the considerable progress made in the field of gram-negative bacteria. However, it has been reported that the accumulation of anionic substances such as sugar phosphates may be the initial event in the catabolite repression of enzyme synthesis in *B. subtilis* (2, 6, 16). In the transketolase mutant, the synthesis of transport system or permease for sorbitol was induced even in the presence of D-glucose. The derepressed synthesis might be due to a failure to accumulate sugar phosphates in the mutant cell as a result of a defect in D-glucose transport. This fact is accords well with the results described by other investigators heretofore.

The authors wish to express their thanks to Dr. T. Iijima, Director of this Institute, for his encouragement and valuable discussions. Grateful acknowledgement is also made to Dr. G. Tamura, Professor of the University of Tokyo, for his criticism of the manuscript.

References

1) Bourd, G., R. S. Erlagaeva, T. N. Bolshakova, and V. N. Gershanovitch. 1977. Glucose catabolite repression in *Escherichia coli* K-12 mutants defective in methyl-α-D-glucoside transport. Eur. J. Biochem. **53**: 419–427.
2) Coote, J. G. 1974. Comparative studies on induction of sporulation and synthesis of inducible enzyme in *Bacillus subtilis*. J. Bacteriol. **120**: 1102–1108.
3) Delobbe, A., R. Haguenauer, and G. Rapoport. 1971. Studies on the transport of α-methyl-D-glucoside in *Bacillus subtilis* 168. Biochimie **53**: 1015–1021.
4) Delobbe, A., H. Chalumeau, and P. Gay. 1975. Existance of two alternative pathways for fructose and sorbitol metabolism in *Bacillus subtilis* Marburg. Eur. J. Biochem. **51**: 503–510.
5) Delobbe, A., H. Chalumeau, J.-M. Claverie, and P. Gay. 1976. Phosphorylation of intracellular fructose in *Bacillus subtilis* mediated by phosphoenolpyruvate-1-fructose phosphotransferase. Eur. J. Biochem. **66**: 485–491.
6) Dowds, B., L. Baxter, and M. McKillen. 1978. Catabolite repression in *Bacillus subtilis*. Biochim. Biophys. Acta **541**: 18–34.
7) Falcone, G., and A. H. Romano. 1971. Sugar uptake by germinating *Bacillus subtilis* spores. F.E.B.S. Lett. **16**: 57–59.
8) Freese, E., W. Klofat, and E. Galliers. 1970. Commitment to sporulation and induction of glucose-phosphoenolpyruvate transferase. Biochim. Biophys. Acta **222**: 265–289.
9) Friedman, S. A., and J. B. Hays. 1977. Initial characterization of hexose and hexitol phosphoenolpyruvate-dependent phosphotransferases of *Staphylococcus aureus*. J. Bacteriol. **130**: 991–999.
10) Gachelin, G. 1970. Studies in the α-methylglucoside permease of *Escherichia coli*: A two-step mechanism for the accumulation of α-methylglucoside 6-phosphate. Eur. J. Biochem. **16**: 342–357.
11) Gay, P., P. Cordier, M. Marquet, and A. Delobbe. 1973. Carbohydrate metabolism and transport in *Bacillus subtilis*: A study of *ctr* mutations. Molec. gen. Genet. **121**: 355–368.
12) Gay, P., and A. Delobbe. 1977. Fructose transport in *Bacillus subtilis*. Eur. J. Biochem. **79**: 363–373.
13) Kornberg, H. L., and J. Smith. 1970. Role of phosphofructokinase in the utilization of

glucose by *Escherichia coli*. Nature (London) **227**: 44–46.
14) Kundig, W., S. Ghosh, and S. Roseman. 1964. Phosphate bound to histidine in a protein as an intermediate in a novel phospho-transferase system. Proc. Natl. Acad. Sci. USA. **52**: 1067–1074.
15) Kundig, W., and S. Roseman. 1971. Sugar transport. II. Characterization of constitutive membrane-bound enzymes II of the *Escherichia coli* phosphotransferase system. J. Biol. Chem. **246**: 1407–1418.
16) Lopez, J. M., and B. Thoms. 1977. Role of sugar uptake and metabolic intermediates on catabolite repression in *Bacillus subtilis*. J. Bacteriol. **129**: 217–224.
17) Lowry, O. H., J. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**: 265–275.
18) Marquet, M., M.-C. Wagner, A. Delobbe, P. Gay, and G. Rapoport. 1970. Mise en évidence de systémes de phosphotransférases dans le transport du glucose, du fructose et du saccharose chez *Bacillus subtilis*. C. R. Acad. Sci. **271**: 449–452.
19) Marquet, M., M.-C. Wagner, and R. Dedonder. 1971. Separation of components of the phosphoenolpyruvate-glucose phosphotransferase system from *Bacillus subtilis* Marburg. Biochimie **53**: 1131–1134.
20) Phibbs, P. V., Jr., S. M. McCowen, T. W. Ferry, and W. T. Blevins. 1978. Mannitol and Fructose Catabolic Pathways of *Pseudomonas aeruginosa* Carbohydrate-Negative Mutants and Pleiotropic Effects of Certain Enzyme Deficiencies. J. Bacteriol. **133**: 717–728.
21) Postma, P. W., and S. Roseman. 1976. The bacterial phosphoenolpyruvate: sugar phosphotransferase system. Biochim. Biophys. Acta **457**: 213–257.
22) Postma, P. W., C. Cordaro, and S. Roseman. 1977. Sugar transport: A pleiotropic membrane mutant of *Salmonella typhimurium*. J. Biol. Chem. **252**: 7862–7876.
23) Roehl, R. A., and R. T. Vinopal. 1976. Lack of glucose phosphotransferase function in phosphofructokinase mutants of *Escherichia coli*. J. Bacteriol. **126**: 852–860.
24) Romano, A. H., S. J. Eberhard, S. L. Dingle, and J. D. McDowell. 1970. Distribution of the phosphoenolpyruvate: glucose phosphotransferase system in bacteria. J. Bacteriol. **104**: 808–813.
25) Roseman, S. 1969. The transport of carbohydrates by a bacterial phosphotransferase system. J. gen. Physiol. **54**: 138s–184s.
26) Roseman, S. 1972. Carbohydrate transport in bacterial cells. *In* L. E. Hokin (ed), Metabolic transport, vol. 6, p. 41–89. Academic Press Inc., New York.
27) Saier, M. H., Jr. 1977. Bacterial phosphoenolpyruvate: sugar phosphotransferase systems: structural, functional and evolutionary interrelationships. Bacteriol. Rev. **41**: 856–871.
28) Sasajima, K., I. Nogami, and M. Yoneda. 1970. Carbohydrate metabolism mutants of a *Bacillus* species. Part I. Isolation of mutants and their inosine formation. Agric. Biol. Chem. **34**: 381–389.
29) Sasajima, K., and M. Yoneda. 1974. D-Sedoheptulose-7-phosphate: D-glyceraldehyde-3-phosphate glycolaldehydetransferase and D-ribulose-5-phosphate 3-epimerase mutants of a *Bacillus* species. Agric. Biol. Chem. **38**: 1305–1310.
30) Sasajima, K., T. Kumada, and A. Yokota. 1977. A pleiotropy in carbohydrate metabolism of *Bacillus subtilis* mutant lacking transketolase. IFO Res. Comm. **8**: 69–77.
31) Simoni, R., M. Smith, and S. Roseman. 1968. Resolution of a staphylococcal phosphotransferase system into four protein components and its relationship to sugar transport. Biochem. Biophys. Res. Commun. **31**: 804–811.
32) Solomon, E., and E. C. C. Lin. 1972. Mutations affecting the dissimilation of mannitol by *Escherichia coli* K-12. J. Bacteriol. **111**: 566–574.

IFO Res. Comm. 9,
27–34, 1979 (March)

VIABILITY OF VARIOUS YEASTS AFTER L-DRYING

Isao BANNO, Kozaburo MIKATA and Takeshi SAKANE

Summary

The technique of drying of cell suspension *in vacuo* without freezing was applied in the preservation of yeasts. Dried specimens of 149 yeast strains prepared using suspending medium containing 5% monosodium glutamate, 5% lactose and 6% polyvinylpyrrolidone in phosphate buffer (0.1M, pH 7.0) were subjected to an accelerated storage test at 37 C. Survival value after storage at 37 C for 60 days indicated that almost all yeasts dried by this method will survive long-term preservation at 5 C.

Many laboratories employ freeze-drying as the best method for long-term preservation of yeast strains (5, 6). On the other hand Iijima and Sakane (3) have improved the L-drying method described by Annear (1, 2). L-drying has advantages over freeze-drying on three points: drying without freezing, use of a cotton wool plug preventing contamination, and the need for a shorter time to dry the cells. If this elicite and efficient method is applied to the preservation of yeasts, it would be helpful for culture collections maintaining a large number of strains. In a previous experiment (7) factors affecting the survival of cells after L-drying were investigated. It was clear that cells harvested at stationary growth phase survived better after drying, and sodium glutamate, sugars and polyvinylpyrrolidone were effective as protectants for drying. It was also reported that compared with a freeze-dried specimen, an L-dried specimen prepared using the same suspending medium was superior in survival after preservation at 37 C for 45 days. Subsequently various yeasts were dried by this method and examined for survival value. The present paper deals with the result obtained immediately after drying and after preservation at a elevated temperature for an accelerated storage test.

Materials and Methods

Organisms. Yeast strains maintained in the IFO culture collection were subjected to L-drying.

Growth medium. YM medium was used for pre-drying culture and counting of viable cells. YM medium contains 1 % glucose, 0.5 % peptone, 0.3 % yeast extract dehydrated, and 0.3 % malt extract dehydrated (pH 6.0). Agar was added for solid medium.

Suspending medium. The following suspending media were used:

A. Solution containing 5 % skim milk dehydrated and 5 % monosodium glutamate.
B. Solution containing 5 % skim milk dehydrated and 10 % sucrose.

C. Phosphate buffer (0.1 M, pH 7.0) containing 5 % monosodium glutamate, 5 % lactose and 6 % polyvinylpyrrolidone (PVP).

Preparation of cell suspension. Yeast strains were incubated on YM agar slant at optimal temperatures until their growth reached the stationary phase. The cell were harvested from the slant and suspended in suspending medium so that the density of cells would be more than 10^7 per ml.

Preparation of dried specimen. (1) Round-bottomed and straight-necked tubes (8 mm×110 mm) were plugged with cotton wool and sterilized in an oven.

(2) One tenth ml aliquots of cell suspension were introduced into the sterile tubes.

(3) Cotton wool plugs were trimmed at the top of the tubes and pushed half way into the tube using a sterilezed glass rod.

(4) The tubes were constricted in a gas flame about 1.5 cm above the plug.

(5) The tubes were attached to the manifold connected to a vacuum pump assembly.

(6) When the valve to the manifold was opened, the water of the cell suspension was evaporated *in vacuo*. The temperature of the suspension decreased to below 10 C, but, because of the impediment of the cotton wool in the tube, it did not fall below freezing point.

(7) The suspension superficially appeared to be dried after about 1 hr but drying was continued for a further 1 hr after this stage had been reached.

(8) The tubes were sealed at the constricted portion by a gas flame *in vacuo*.

(9) The resulting ampoules were tested for maintainance of vacuum by a High Frequency Tester (Edward High Vacuum Ltd. model T2).

Some of the ampoules were stored at 37 C and checked for viability. The other ampoules were stored at 5 C for long-term preservation.

Rehydration. After opening an ampoule, 0.5 ml of YM broth was added to the ampoule and the content was carefully mixed to make a homogeneous suspension. After appropriate dilution the viable cells were estimated by plating on YM agar plates using the soft-agar double layer method. Survival value was expressed as percentage of viable count after drying to initial viable count in the cell suspension.

Results and Discussion

Twelve yeast strains selected at random were dried using 3 suspending media A, B and C. The survival values of the dried specimens were determined after drying and after preservation at 37 C. The results are given in table 1. Immediately after drying, no significant difference was found in survival among the 3 suspending media. But after preservation for 15 days and 45 days, the survival varied according to the suspending media. The difference appears to be remarkable in strains sensitive to drying (*Saccharomycodes ludwigii, Schwanniomyces occidentalis*). The highest survival value was obtained in specimens dried using suspending medium C. In this suspending medium all the strains except *Saccharomyces exiguus* showed survival values of more than 1 % after preservation at 37 C for 45 days. Judging by this result, long-term

Table 1. Effect of suspending media on viability of 12 yeasts after drying and preservation at 37 C.

Species	Suspending medium	Survival value (%) after preservation of dried specimens at 37 C for		
		0[a]	15 days	45 days
Saccharomyces cerevisiae IFO 0234	A	59	11	6
	B	51	4	0.3
	C	55	36	39
Saccharomyces exiguus IFO 0271	A	10	0.2	0.09
	B	3	0.01	0
	C	9	0.6	0.2
Saccharomycodes ludwigii IFO 1266	A	25	2	1.4
	B	44	0.1	0.01
	C	73	61	62
Schizosaccharomyces pombe IFO 0350	A	61	23	22
	B	73	26	11
	C	85	60	50
Schwanniomyces occidentalis IFO 0371	A	10	0.3	0.2
	B	16	0.07	0.002
	C	8	5	4
Wickerhamia fluorescens IFO 1116	A	71	6	3
	B	68	18	1
	C	79	50	46
Brettanomyces anomalus IFO 0796	A	51	1.9	1.7
	B	67	27	28
	C	70	34	45
Candida tenuis IFO 0716	A			
	B	40	3	0.1
	C	51	32	25
Cryptococcus laurentii IFO 1312	A			
	B	28	1	0.4
	C	47	32	20
Kloeckera javanica IFO 1157	A			
	B	73	15	3
	C	39	21	14
Torulopsis bovina IFO 0873	A	37	0.2	0.2
	B	30	0.2	0.001
	C	21	4	1
Trigonopsis variabilis IFO 1051	A			
	B	74	14	6
	C	66	29	26

[a] Immediately after drying.

preservation by L-drying seems to be possible for almost all yeasts, if the dried specimen is prepared using suspending medium C and stored at 5 C or colder. Consequently, yeast cultures maintained in the IFO collection have been scheduled to be subjected to this L-drying method.

Viability of L-dried specimens of 149 yeast strains after drying and after preservation at 37 C are presented in Table 2. The yeasts which gave survival values less than 1 % after 60 days storage are *Ambrosiozyma cicatricosa* IFO 1846, *A. philentoma* IFO 1847, *Debaryomyces coudertii* IFO 1817, *Hansenula dimennae* IFO 1771, *Leucosporidium frigidum* 1851, *L. nivale* IFO 1852, *Pichia stipitis* IFO 1720, and *Schwanniomyces occidentalis* IFO 1841. The proportion of these 8 strains to a total of 149 strains is 5.3 %. The reason why these 8 strains decreased remerkably in survival after drying is unknown, and further investigation is necessary to search for factors which may bring about better survival.

Table 2. Viability of various yeast strains after L-drying and after preservation of dried specimen at 37 C.

Species	IFO No.	Survival value (%) after preservation at 37 C for		
		0[a]	30 days	60 days
Aessosporon salmonicolor	1845[b]	12	6.4	2.8
Ambrosiozyma cicatricosa	1846[b]	2.3	0.08	0.05
A. philentoma	1847	1.2	0.1	0.05
Arthroascus javanensis	1848	17.3	6.9	3.7
Candida brumptii	1695	—[c]	42	—
C. brumptii	1697	—	49	—
C. guilliermondii	0455	92	34	20
C. parakurusei	1068	100	73	69
C. pseudointermedia	1693	—	19	—
C. ravautii	1698	—	41	—
C. solani	0762	68	18	12
C. tropicalis	1401	100	62	49
C. utilis	1086	67	13	3
C. zeylanoides	0738[d]	77	39[e]	45[f]
Cryptococcus laurentii	0698[d]	86	57[e]	50[f]
Debaryomyces cantarellii	1716	44	31	28
D. cantarellii	1717	37	18	19
D. coudertii	1817	46	2.5	0.01
D. hansenii	1751	65	7.4	2.1
D. hansenii	1752	52	8.5	7.9
D. yarrowii	1818	61	34	18
Hanseniaspora occidentalis	1718	38	18	25
H'spora occidentalis	1819	46	16	8.6
H'spora osmophila	1753	45	40	31
H'spora osmophila	1754	43	35	35
H'spora uvarum	1755	80	48	63
H'spora uvarum	1756	60	54	58

Table 2. (continued)

Species	IFO No.	Survival value (%) after preservation at 37 C for		
		0[a]	30 days	60 days
Hanseniaspora uvarum	1757	70	42	37
H'spora valbyensis	1758	34	35	23
H'spora valbyensis	1759	50	35	42
Hansenula anomala	0146[d]	79	7[e]	10[f]
H. anomala	1760	50	35	33
H. anomala	1761	21	12	14
H. beijerinckii	1762	38	16	22
H. beijerinckii	1763	27	21	13
H. californica	1764	46	20	30
H. californica	1765	39	25	25
H. californica	1766	47	32	44
H. californica	1767	34	19	18
H. capsulata	1768	54	12	20
H. capsulata	1769	52	16	19
H. capsulata	1770	59	22	18
H. dimennae	1771	28	8.4	1
H. dryadoides	1820	17	1.9	1.4
H. holstii	1076	82	54	18
H. muscicola	1704	—	52	—
H. philodendra	1821	65	33	11
H. saturnus	1772	41	23	16
H. saturnus	1773	41	27	33
H. saturnus	1774	40	30	30
H. saturnus	1775	44	27	25
H. saturnus	1776	32	21	23
H. sydowiorum	1705	—	43	—
H. wickerhamii	1706	—	33	—
Kloeckera africana	0633[d]	100	66[e]	55[f]
K. javanica	1157[d]	73	15[e]	3[f]
Kluyveromyces fragilis	1777	35	16	15
K'myces thermotolerans	1778	70	33	30
K'myces thermotolerans	1779	75	46	36
K'myces thermotolerans	1780	48	22	34
Leucosporidium frigidum	1851	4.4	0.8	0.7
L. nivale	1852	4.8	1.0	0
Nadsonia elongata	0665[d]	34	13[e]	11[f]
Pichia abadieae	1822	47	55	32
P. besseyi	1707	—	12	—
P. castillae	1823	58	54	21
P. dispora	1781	56	35	26
P. dispora	1782	48	26	23
P. farinosa	0716[d]	100	100[e]	43[f]
P. fluxuum	1784	42	29	21
P. fluxuum	1785	32	22	16
P. fluxuum	1786	51	15	10
P. fluxuum	1787	55	24	24

Table 2. (continued)

Species	IFO No.	Survival value (%) after preservation at 37 C for		
		0[a]	30 days	60 days
Pichia media	1824	43	48	42
P. mucosa	1825	39	16	21
P. membranaefaciens	1788	46	35	30
P. membranaefaciens	1789	64	16	22
P. membranaefaciens	1790	55	40	35
P. naganishii	1670	56	39	51
P. norvegensis	1694	—	40	—
P. pijperi	1791	65	55	52
P. pijperi	1792	63	32	34
P. pinus	1793	52	26	20
P. pinus	1794	80	30	30
P. pinus	1795	59	16	15
P. quercuum	1719	45	30	34
P. quercuum	0949[d]	74	15[e]	20[f]
P. saitoi	1796	55	43	31
P. saitoi	1797	56	34	26
P. sargentensis	1826	18	14	10
P. spartinae	1827	20	3.9	7.0
P. stipitis	1720	2.6	1.1	0.7
P. terricola	1798	68	66	52
P. toletana	1799	42	16	9
P. toletana	1800	74	30	30
Rhodosporidium diobovatum	1828	67	39	45
R'sporidium diobovatum	1829	51	24	14
R'sporidium diobovatum	1830	69	34	23
Rhodotorula glutinis	0871[d]	98	91[e]	76[f]
R. flava	0710[d]	65	15[e]	15[f]
Saccharomyces bailii	1801	45	33	27
S. bayanus	1802	59	53	52
S. bayanus	1803	22	15	17
S. cerevisiae	1699	—	65	—
S. cerevisiae	1700	—	71	—
S. cerevisiae	1701	—	54	—
S. cerevisiae	1702	—	71	—
S. cerevisiae	1703	—	58	—
S. cerevisiae	1804	67	57	53
S. cerevisiae	1805	76	68	54
S. cordubensis	1832	34	21	30
S. coreanus	1833	37	36	33
S. florentinus	1806	76	68	53
S. florentinus	1807	78	51	2.1
S. florentinus	1808	80	44	12
S. florentinus	1809	82	44	33
S. florentinus	1810	77	60	43
S. gaditensis	1834	60	46	58
S. kluyveri	1811	57	37	31

Table 2. (continued)

Species	IFO No.	Survival value (%) after preservation at 37 C for		
		0[a]	30 days	60 days
Saccharomyces mrakii	1835	31	25	19
S. norbensis	1836	31	21	25
S. prostoserdovii	1837	35	20	22
S. rouxii	1812	75	35	4.6
S. rouxii	1813	10	6	4
S. rouxii	1814	53	22	20
S. servazzii	1838	72	55	39
S. uvarum	1815	30	22	23
S. uvarum	1816	34	27	28
Saccharomycopsis crataegensis	1708	—	46	—
S'copsis crataegensis	1709	—	43	—
S'copsis malanga	1710	—	57	—
S'copsis fibuligera	1711	—	46	—
Schwanniomyces alluvius	1839	22	13	8.8
Schwanniomyces castellii	1840	18	15	7
Schwanniomyces occidentalis	1841	1.6	0.4	0.1
Schwanniomyces persoonii	1842	15	9	10
Selenozyma peltata	1853	31	15	14
Sporidiobolus ruinenii	1689	5.2	2.3	2.5
Sporidiobolus ruinenii	1689[b]	10	5.2	4.6
Sporobolomyces odorus	1597[d]	75	64[e]	26[f]
Sporobolomyces roseus	1037[d]	79	24[e]	11[f]
Stephanoascus ciferrii	1854	60	54	55
Stephanoascus ciferrii	1855[b]	71	49	39
Sterigmatomyces elviae	1843	39	36	30
Sterigmatomyces indicus	1844	26	25	19
Syringospora albicans	1856	47	44	38
Torulopsis candida	0863	80	53	18
T. stellata	0703[d]	100	4[e]	1[f]
T. stellata	0858[d]	86	9[e]	1[f]
Wickerhamiella domercqii	1857	73	51	24

[a] Immediately after drying.
[b] Cells grown in YM broth on rotary shaker were dried.
[c] Not determined.
[d] Dried using suspending medium B.
[e] Determination after 15 days preservation.
[f] Determination after 45 days preservation.

Survival value has no relation to the taxonomic position of the strain dried. The value varied with strains in a species. But in general, yeasts having round to oval cell such as *Saccharomyces, Pichia* and *Rhodotorula* appear to give high survival after preservation.

Investigation of L-drying of bacteria (4, 8) predicted that the L-dried specimen giving a survival value of more than 1 % after preservation at 37 C would probably sur-

vive after preservation at 5 C for period longer than 5 years. The L-dried specimens of most yeasts presented in Table 2 are considered to be likely to remain viable during fairly long-term preservation at 5 C.

The authors wish to express deepest appreciation to Dr. T. Miwatani, Professor of the Research Institute for Microbial Disease, Osaka University, for his kindness in reviewing the manuscript.

References

1) Annear, D. I. 1962. Recoveries of bacteria after drying on cellulose fibers (A method for the routine preservation of bacteria). Aust. J. Exp. Biol. Med. Sci. **40**: 1–8.
2) Annear, D. I. 1970. Preservation of microorganisms by drying from the liquid state. *In* H. Iizuka and T. Hasegawa (ed.) Proceedings of the first international conference on culture collections. p. 273–279. Univ. of Tokyo Press, Tokyo.
3) Iijima, T., and T. Sakane. 1973. A method for preservation of bacteria and bacteriophages by drying *in vacuo*. Cryobiology **16**: 379–385.
4) Iijima, T., and T. Sakane. 1973. Method for preservation of bacteria and bacteriophages by drying *in vacuo*. IFO Res. Comm. **6**: 4–17.
5) Kirsop, B. 1974. The stability of biochemical, morphological and brewing properties of yeast cultures maintained by subculturing and freeze-drying. J. Inst. Brew. **80**: 565–570.
6) Rodrigues de Miranda, L., and D. Yarrow. 1972. The maintenance of stock cultures of yeasts. *In* A. Kockova-Kratochvilova and E. Minarik (ed.) Proceedings of the 1st specialized symposium on yeasts. p. 59–67.
7) Sakane, T., K. Mikata, and I. Banno. 1975. L-drying of yeasts. J. Japan. Soc. Research Freez. and Dry. **21**: 29–35, in Japanese.
8) Sakane, T., and I. Banno. 1976. Preservation of bacterial strains by L-drying. J. Japan. Soc. Research Freez. and Dry. **22**: 101–108, in Japanese.

IFO Res. Comm. 9,
35–45, 1979 (March)

VIABILITY OF VARIOUS BACTERIA AFTER L-DRYING

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Summary

Various bacteria maintained in the IFO culture collection were subjected to L-drying. When cells grown in a ordinary medium were suspended in a suspending medium (phosphate buffer 0.1M, pH 7.0 containing 3% glutamate) and dried *in vacuo*, most bacteria gave good dried specimens showing a high survival value. But a few bacteria such as *Myxococcus*, *Sphaerotilus*, *Azotobacter*, halophilic bacteria and *Thiobacillus* required modification of pre-drying medium and/or suspending medium for drying so as to yield desirable viability after drying.

The L-drying method described by Annear (1) was improved and a practical method of L-drying was established by Iijima and Sakane (2, 3). This method has advantages over freeze-drying on three points: the use of a cotton plug to prevent contamination, drying without freezing and taking a shorter time to dry the cells. Survival after L-drying of the bacteria tested has been found not to be less than that after freeze-drying. The method is so elicite and efficient as to be considered a practical method of preserving a great number of strains, and the method has been applied to drying-preservation of various bacteria in the IFO culture collection. This paper describes pre-drying media and suspending media suitable for drying of various bacteria as well as survival values after L-drying.

Materials and Methods

Bacterial strains. Strains of 178 species which had been preserved by subculturing were subjected to drying.

Pre-drying media. The media mentioned below were used to obtain cells for drying:

Medium No. 202

Potato*	200	g
Press yeast	30	g
Liver, infusion from*	25	g
Meat extract	5	g
Thioglycollate medium dehydrated	10	g
Glucose	5	g
Glycerol	15	g
$CaCO_3$	15	g
Distilled water	make up to 1	liter
Agar	15	g
	pH 7.0	

*Gently boil sliced potatoes in 500 ml of the water (or sliced liver in 150 ml of the water) for 30 min and remove solids by filtration through cloth.

Medium No. 203

Peptone		10	g
Yeast extract		5	g
Liver, infusion from*		25	g
Glucose		3	g
Glycerol		15	g
NaCl		3	g
Distilled water	make up to	1	liter
Agar		15	g
		pH 7.0	

Medium No. 204

Peptone		4	g
Yeast extract		2	g
Liver, infusion from*		25	g
$FeSO_4 \cdot 7H_2O$		10	mg
Vitamin mixture**		5	ml
Sea water		250	ml
Distilled water	make up to	1	liter
Agar		20	g
		pH 7.2	

*See medium 202

**Biotin 0.2μg, calcium pantothenate 40μg, folic acid 0.2μg, inositol 200μg, niacin 40μg, *p*-aminobenzoic acid 20μg, pyridoxine·HCl 40μg, riboflavin 40μg, thiamine·HCl 20μg, cholin 100μg, cyanocobalamin 0.002μg per ml.

Medium No. 205

Skim milk dehydrated		100	g
Press yeast		30	g
Liver, infusion from*		130	g
Tomato juice		100	ml
Malt extract		5	g
Thioglycollate medium dehydrated		5	g
$CaCO_3$		15	g
Distilled water	make up to	1	liter
Agar		15	g
		pH 7.0	

*Gently boil sliced liver in 700 ml of the water for 30 min and remove solids by filtration through cloth.

Medium No. 206

Potato*		200	g
Thioglycollate medium dehydrated		10	g
Glucose		5	g
$CaCO_3$		15	g
Distilled water	make up to	1	liter
Agar		15	g

*Boil peeled and sliced potatoes in 500 ml of the water for 30 min and mash solids to make thick soup.

Medium No. 207

Mannitol		15	g
K_2HPO_4		0.2	g
$MgSO_4 \cdot 7H_2O$		0.2	g
NaCl		0.2	g
$CaSO_4 \cdot 2H_2O$		0.1	g
$CaCO_3$		5	g
Distilled water		1	liter
Agar		15	g
		pH 7.2	

Medium No. 216

Bacto-Heart Infusion Agar dehydrated (Difco)		40	g
Distilled water		1	liter

Medium No. 219

Casitone (Difco)		20	g
$MgSO_4 \cdot 7H_2O$		1	g
Potassium phosphate buffer (0.01M, pH 7.2)		1	liter
Agar		15	g

Medium No. 221

Uric acid		2	g
Yeast extract		1	g
K_2HPO_4		1	g
$MgSO_4 \cdot 7H_2O$		35	mg
$FeSO_4 \cdot 7H_2O$		35	mg
$CaCl_2 \cdot 2H_2O$		4.2	mg
Mercaptoacetic acid (80% solution)		2	ml
Distilled water		1	liter
Agar		15	g
		pH 7.0	

Medium No. 222

Sodium lactate	3.5	g
Yeast extract	1	g
NH_4Cl	0.5	g
K_2HPO_4	1	g
$MgSO_4 \cdot 7H_2O$	2	g
$CaSO_4 \cdot 2H_2O$	1	g
$Fe_2(SO_4)_3 \cdot (NH_4)_2SO_4 \cdot 24\ H_2O$	0.5	g
Distilled water	1	liter
	pH 7.2～7.4	

Medium No. 223

Peptone	5	g
Beef extract	3	g
Yeast extract	0.2	g
Glucose	5	g
$MgSO_4 \cdot 7H_2O$	1.5	g
Na_2SO_4	1.5	g
$Fe_2(SO_4)_3 \cdot (NH_4)_2SO_4 \cdot 24H_2O$	0.1	g
Distilled water	1	liter
	pH 7.0～7.2	

Medium No. 224

$(NH_4)_2SO_4$	2	g
KNO_3	3	g
KH_2PO_4	3	g
$MgCl_2 \cdot 6H_2O$	0.5	g
$CaCl_2 \cdot 2H_2O$	0.25	g
$FeSO_4 \cdot 7H_2O$	0.01	g
$Na_2S_2O_3 \cdot 5H_2O$	5	g
$Na_2MoO_4 \cdot 2H_2O$	0.3	mg
Yeast extract	0.1	g
Sulfur, powdered	1	g
Bromophenol blue	10	mg
Distilled water	1	liter
	pH 4.0～4.6	

Medium No. 226

Yeast extract	5	g
Glucose	20	g
Distilled water	1	liter
	pH 6.8～7.0	

Medium LA

Trypticase (BBL)	10	g
Phytone (BBL)	5	g
Yeast extract	5	g
Glucose	5	g
NaCl	5	g
K_2HPO_4	1.5	g
Distilled water	1	liter
Agar	15	g

Medium YG

Yeast extract	1	g
Glucose	5	g
Distilled water	1	liter
Agar	15	g
	pH 7.0	

Suspending media. The harvested cells were suspended in the suspending media mentioned below so that the density of cells might be 10^9 per ml or more.

Suspending media:

No. 1

Monosodium glutamate	3	g
Potassium phosphate buffer, 0.1M, pH 7.0	100	ml

No. 2

Monosodium glutamate	5	g
Sea water	100	ml
	pH 7.0	

No. 3

Skim milk dehydrated	5	g
Actocol*	0.1	ml
Distilled water	100	ml

No. 4

Monosodium glutamate	5	g
Skim milk dehydrated	5	g
Actocol*	0.1	ml
Distilled water	100	ml

No. 5

Sucrose	12	g
Peptone	0.5	g
Meat extract	0.3	g
Sea water	100	ml

pH 7.0

No. 6

Sucrose	12	g
Peptone	0.5	g
Meat extract	0.3	g
Distilled water	100	ml

pH 7.0

* Purchased from Takeda Chemical Industries, Ltd, Osaka. A polymer of propylene oxide, mol. wt. about 3,000. An agent added to the fluid to prevent bubbling in evaporation *in vacuo*.

Preparation of L-dried specimen. One tenth ml aliquots of the cell suspension were introduced into small tubes and dried under vacuum according to the method described by Iijima and Sakane (3).

Results and Discussion

Table 1 shows survival values of various bacteria after L-drying, when cells grown on a suitable pre-drying medium and suspended in a suitable suspending medium were dried. It is clear from the results given in Table 1 that survival value has no relation to the taxonomic position of the strain dried. The value varied with strains in a species. Most bacterial strains showed high survival values after drying, when ordinary growth media were used as pre-drying medium and suspending medium No. 1 was used for drying. However with a few bacteria mentioned below, special treatment was required to obtain a dried specimen with a desirable survival value.

In the case of *Myxococcus xanthus*, a dried specimen yielding a high viability was obtained by drying a cell-suspension containing many microcysts, which were produced in abundance by transfering a culture grown once on pre-drying medium No. 219 onto a cyst-forming medium composed of 1 g casitone Difco, 4.6g glycerol, 0.17g $MgSO_4 \cdot 7H_2O$ and 1.5 g agar in 100 ml of potassium phosphate buffer 0.01 M, pH 7.2.

Sphaerotilus natans showed an appreciable survival value when a culture consisting of single cells without trichomes was dried. This bacterium produced mainly single cells on pre-drying medium No. 203.

Halophilic bacteria such as *Vibrio* and *Aeromonas* gave a good result when cells were dried using sea water containing 5 % sodium glutamate as a suspending medium.

Cells of strains of genus *Azotobacter* died as soon as suspended in suspending medium No. 1, but survived well in suspending medium No. 3. When dried using this suspending medium, they showed survival of 1 % or so.

Cells of *Thiobacillus thiooxidans*, a obligate chemolithotroph, were harvested at

Table 1. Viability of various bacteria immediately after drying.

Species	Number of strains dried	Pre-drying medium	Suspending medium	Survival* (%)	Remarks
Acetobacter aceti	3	202	1	31–66	
Acetobacter aceti subsp. *orleanensis*	2	202	1	43, 68	
Acetobacter acetigenus	4	202	1	12–85	
Acetobacter acetosus	3	202	1	28–98	
Acetobacter ascendens	2	202	1	52, 56	
Acetobacter aurantius	10	202	1	13–66	
Acetobacter kuetzingianus	1	202	1	53	
Acetobacter pasteurianus	1	202	1	52	
Acetobacter pasteurianus subsp. *estunensis*	1	202	1	65	
Acetobacter pasteurianus subsp. *lovaniensis*	1	202	1	53	
Acetobacter peroxidans	1	202	1	11	
Acetobacter rancens	3	202	1	39–41	
Acetobacter turbidans	1	202	1	73	
Acetobacter xylinus	4	202	1	9–95	
Achromobacter liquefaciens	1	203	1	32	
Achromobacter parvulus	2	203	1	45, 70	
Achromobacter xerosis	1	203	1	18	
Acinetobacter calcoaceticus	3	203	1	38–88	
Actinobacillus sp.	1	203	1	77	
Aerococcus viridans	2	LA	1	80, 84	
Aeromonas hirudinus	1	203	1	0.2	
Aeromonas hydrophila	3	203	1	2–5	
Aeromonas hydrophila	3	204	2	4–31	halophilic strains
Aeromonas hydrophila subsp. *anaerogenes*	1	203	1	42	
Aeromonas hydrophila subsp. *proteolytica*	1	204	2	8	
Aeromonas ichthyosmia	1	203	1	42	
Aeromonas punctata	2	204	2	18, 30	
Aeromonas punctata subsp. *caviae*	1	204	2	33	
Aeromonas salmonicida	2	204	2	5, 6	
Aeromonas salmonicida subsp. *masoucida*	1	203	1	0.5	
Agrobacterium radiobacter	8	203	1	31–94	
Agrobacterium rhizogenes	1	203	1	11	
Agrobacterium rubi	2	203	1	11, 19	
Agrobacterium tumefaciens	5	203	1	22–60	
Alcaligenes faecalis	2	203	1	42, 72	
Arthrobacter citreus	2	203	1	41, 78	
Arthrobacter duodecadis	1	203	1	13	
Arthrobacter globiformis	10	203	1	9–100	
Arthrobacter simplex	2	203	1	55, 90	
Arthrobacter tumescens	1	203	1	81	
Arthrobacter viscosus	1	203	1	14	
Azotobacter chroococcum	2	207	3	1, 2	(a)
Azotobacter vinelandii	3	207	3	0.06–1	(a)
Bacillus alvei	1	203	1	100	(b)

Table 1. (continued)

Species	Number of strains dried	Pre-drying medium	Suspending medium	Survival* (%)	Remarks
Bacillus brevis	6	203	1	29–97	(b)
Bacillus cereus	12	203	1	18–100	(b)
Bacillus cereus subsp. *fluorescens*	1	203	1	68	(b)
Bacillus cereus subsp. *mycoides*	4	203	1	14–100	(b)
Bacillus circulans	22	203	1	3–77	(b)
Bacillus coagulans	5	203	1	0.4–24	(b)
Bacillus firmus	1	203	1	33	(b)
Bacillus licheniformis	9	203	1	80–100	(b)
Bacillus macerans	1	203	1	33	(b)
Bacillus megaterium	4	203	1	0.9–80	(b)
Bacillus polymyxa	1	203	1	3	
Bacillus pumilus	15	203	1	32–100	(b)
Bacillus sphaericus	9	203	1	22–100	(b)
Bacillus stearothermophilus	2	203	1	82, 100	(b)
Bacillus subtilis	34	203	1	10–100	(b)
Bacillus subtilis subsp. *aterrimus*	1	203	1	66	(b)
Bacillus subtilis subsp. *niger*	2	203	1	68, 89	(b)
Bacillus thiaminolyticus	1	203	1	100	(b)
Bacillus thuringiensis	3	203	1	34–79	(b)
Bacterium gracile	1	LA	1	100	
Beijerinckia indica	2	207	3	5, 9	(a)
Bordetella bronchiseptica	2	203	1	62, 90	
Brevibacterium acetylicum	1	203	1	53	
Brevibacterium ammoniagenes	3	203	1	84–100	
Brevibacterium citreum	1	203	1	55	
Brevibacterium fuscum	1	203	1	52	
Brevibacterium helvolum	1	203	1	24	
Brevibacterium imperiale	1	203	1	58	
Brevibacterium incertum	1	203	1	68	
Brevibacterium linens	4	203	1	48–75	
Brevibacterium lipolyticum	1	203	1	76	
Brevibacterium luteum	1	203	1	28	
Brevibacterium protophormiae	1	203	1	46	
Brevibacterium pusillum	1	203	1	77	
Brevibacterium stationis	1	203	1	100	
Brevibacterium sulfureum	1	203	1	57	
Brevibacterium testaceum	1	203	1	63	
Brevibacterium vitarumen	1	203	1	68	
Cellulomonas flavigena	7	203	1	44–83	
Chromobacterium chocolatum	1	203	1	79	
Chromobacterium iodinum	1	203	1	100	
Chromobacterium lividum	2	203	1	2, 27	
Chromobacterium violaceum	1	203	1	1	
Citrobacter freundii	1	203	1	64	

Table 1. (continued)

Species	Number of strains dried	Pre-drying medium	Suspending medium	Survival* (%)	Remarks
Citrobacter intermedius	5	203	1	20–73	
Clostridium acetobutylicum	3	206	1	69–100	(c)
Clostridium botulinum	1	206	1	100	(c)
Clostridium butylicum	3	206	1	60–100	(c)
Clostridium cylindrosporum	1	221	1	100	(c)
Clostridium kainantoi	1	206	1	100	(c)
Clostridium sporogenes	2	206	1	66, 100	(c)
Comamonas terrigena	2	203	1	0.8, 39	
Corynebacterium aquaticum	1	203	1	65	
Corynebacterium erythrogenes	1	204	2	67	
Corynebacterium fascians	2	203	1	40, 100	
Corynebacterium flaccumfaciens	1	203	1	62	
Corynebacterium michiganense	2	203	1	34, 63	
Corynebacterium paurometabolum	1	203	1	20	
Corynebacterium rathayi	1	203	1	63	
Corynebacterium sepedonicum	2	203	1	38, 91	
Corynebacterium tritici	1	203	1	49	
Corynebacterium xerosis	1	216	1	8	
Cytophaga sp.	2	204	2	1, 8	
Desulfotomaculum nigrificans	1	222	1	1	(d)
Desulfovibrio desulfuricans	1	223	1	1	(d)
Enterobacter aerogenes	2	203	1	70, 84	
Enterobacter cloacae	5	203	1	8–100	
Erwinia amylovora	2	203	1	52, 55	
Erwinia carotovora	3	203	1	39–44	
Erwinia herbicola	1	203	1	100	
Escherichia coli	20	203	1	14–93	
Flavobacterium arborescens	1	203	1	89	
Flavobacterium capsulatum	1	203	1	40	
Flavobacterium esteroaromaticum	1	203	1	100	
Flavobacterium gasogenes	1	203	1	66	
Flavobacterium heparinum	1	203	1	45	
Flavobacterium lutescens	3	203	1	28–38	
Flavobacterium meningosepticum	1	203	1	61	
Flavobacterium okeanokoites	1	203	1	11	
Flavobacterium suaveolens	1	203	1	81	
Gaffkya homari	1	205	1	41	(e)
Gluconobacter albidus	4	202	1	23–77	
Gluconobacter capsulatus	1	202	1	76	
Gluconobacter cerinus	9	202	1	64–100	
Gluconobacter dioxyacetonicus	4	202	1	52–100	
Gluconobacter gluconicus	3	202	1	79–83	
Gluconobacter industrius	2	202	1	28, 76	
Gluconobacter liquefaciens	1	202	1	17	

Table 1. (continued)

Species	Number of strains dried	Pre-drying medium	Suspending medium	Survival* (%)	Remarks
Gluconobacter melanogenus	4	202	1	35–54	
Gluconobacter nonoxygluconicus	2	202	1	57, 100	
Gluconobacter oxydans	2	202	1	53, 76	
Gluconobacter oxydans subsp. *sphaericus*	1	202	1	55	
Gluconobacter roseus	1	202	1	100	
Gluconobacter rubiginosus	1	202	1	45	
Gluconobacter suboxydans	6	202	1	47–96	
Gluconobacter suboxydans var α	5	202	1	59–75	
Hafnia alvei	1	203	1	85	
Klebsiella pneumoniae	11	203	1	8–91	
Kurthia zopffi	2	203	1	25, 41	
Lactobacillus bulgaricus	1	205	1	58	(e)
Lactobacillus casei	1	205	1	57	(e)
Lactobacillus lactis	1	205	1	1	(e)
Leuconostock dextranicum	1	LA	1	78	
Leuconostock mesenteroides	3	LA	1	67–80	
Microbacterium thermosphactum	1	203	1	54	
Micrococcus aurantiacus	1	203	1	8	
Micrococcus luteus	9	203	1	74–100	
Micrococcus roseus	2	203	1	69, 100	
Micrococcus varians	1	203	1	96	(b)
Mycobacterium fortuitum	1	203	1	100	(b)
Mycobacterium phlei	2	203	1	100, 100	(b)
Mycobacterium rhodochrous	6	203	1	75–100	(b)
Mycobacterium smegmatis	3	203	1	16–100	(b)
Mycoplana bullata	1	203	1	75	
Mycoplana dimorpha	1	203	1	17	
Myxococcus xanthus	1	219	1	76	(f)
Nocardia erythropolis	5	203	1	71–100	
Paracoccus denitrificans	2	203	1	32, 68	
Pediococcus acidilactici	3	LA	1	66–100	
Pediococcus homari	2	LA	1	67, 100	
Pediococcus parvulus	4	LA	1	62–85	
Pediococcus pentosaceus	6	LA	1	62–91	
Pediococcus soyae	1	LA	1	53	
Pediococcus urinae-equi	1	LA	1	36	
Protaminobacter alboflavus	1	203	1	56	
Protaminobacter ruber	1	203	1	41	
Proteus inconstans	2	203	1	70, 75	
Proteus mirabilis	2	203	1	43, 92	
Proteus morganii	3	203	1	39–87	
Proteus rettgeri	1	203	1	20	
Proteus vulgaris	4	203	1	16–43	
Pseudomonas acidovorans	1	203	1	25	

Table 1. (continued)

Species	Number of strains dried	Pre-drying medium	Suspending medium	Survival* (%)	Remarks
Pseudomonas aeruginosa	42	203	1	0.5–71	
Pseudomonas aureofaciens	5	203	1	18–48	
Pseudomonas auricularis	2	203	1	9, 26	
Pseudomonas azotoformans	1	203	1	24	
Pseudomonas carrageenovora	1	204	2	9	
Pseudomonas caryophylli	6	203	1	12–100	
Pseudomonas cepacia	1	203	1	5	
Pseudomonas chlororaphis	2	203	1	8, 19	
Pseudomonas cruciviae	1	203	1	27	
Pseudomonas dacunhae	1	203	1	23	
Pseudomonas denitrificans	1	203	1	72	
Pseudomonas diminuta	1	203	1	57	
Pseudomonas fluorescens	6	203	1	5–51	
Pseudomonas fragi	2	203	1	21, 34	
Pseudomonas maltophilia	3	203	1	21–76	
Pseudomonas marginata	1	203	1	14	
Pseudomonas nitroreducens	1	203	1	33	
Pseudomonas oleovorans	1	203	1	5	
Pseudomonas oxalaticus	1	203	1	36	
Pseudomonas pavonaceae	1	203	1	14	
Pseudomonas putida	4	203	1	21–74	
Pseudomonas putrefaciens	3	203	1	0.01–0.1	
Pseudomonas reptilivora	1	203	1	72	
Pseudomonas riboflavina	1	203	1	54	
Pseudomonas striafaciens	1	203	1	6	
Pseudomonas stutzeri	4	203	1	2–30	
Pseudomonas syncyanea	1	203	1	1	
Pseudomonas synxantha	3	203	1	10–29	
Pseudomonas syringae	4	203	1	0.9–14	
Pseudomonas taetrolens	2	203	1	9, 40	
Pseudomonas trifolii	1	203	1	84	
Pseudomonas vesicularis	1	203	1	14	
Rhizobium japonicum	1	YG	1	2	
Rhizobium meliloti	1	YG	1	10	
Rhizobium trifolii	1	YG	1	55	
Rhodopseudomonas spheroides	2	203	1	6, 25	
Rhodospirillum rubrum	1	203	1	3	
Salmonella enteritidis	1	203	1	65	
Salmonella gallinarum	1	203	1	58	
Salmonella typhimurium	2	203	1	69, 72	
Serratia liquefaciens	2	203	1	75, 78	
Serratia marcescens	6	203	1	55–100	
Serratia marinorubra	1	203	1	67	
Sphaerotilus natans	1	203	4	6	

Table 1. (continued)

Species	Number of strains dried	Pre-drying medium	Suspending medium	Survival* (%)	Remarks
Spirillum hiroshimense	1	204	5	0.01	(g)
Spirillum itersonii subsp. *nipponicum*	1	203	6	0.1	(g)
Spirillum lunatum	1	203	1	12	
Spirillum metamorphum	1	203	1	0.5	
Spirillum pelagicum	1	204	5	0.01	(g)
Spirillum psychrophilum	1	203	6	0.01	(g)
Spirillum pusillum	1	204	5	0.01	(g)
Sporolactobacillus inulinus	2	205	1	39, 40	
Sporosarcina ureae	2	203	1	56, 64	
Staphylococcus aureus	8	203	1	63–91	
Staphylococcus epidermidis	2	203	1	92, 100	
Streptococcus bovis	3	LA	1	0.8–15	
Streptococcus cremoris	1	LA	1	94	
Streptococcus equinus	1	LA	1	18	
Streptococcus faecalis	3	LA	1	59–100	
Streptococcus faecalis subsp. *liquefaciens*	7	LA	1	77–100	
Streptococcus faecalis subsp. *zymogenes*	1	LA	1	98	
Streptococcus faecium	5	LA	1	83–100	
Streptococcus faecium subsp. *mobilis*	4	LA	1	91–100	
Streptococcus lactis	2	LA	1	69, 96	
Thiobacillus novellus	1	203	1	12	
Thiobacillus thiooxidans	2	224	1	1, 1	
Vibrio alginolyticus	1	204	2	0.4	
Vibrio anguillarum	2	204	2	0.2, 2	
Vibrio parahaemolyticus	1	204	2	3	
Xanthomonas campestris	2	203	1	36, 78	
Xanthomonas citri	1	203	1	23	
Xanthomonas cucurbitae	1	203	1	24	
Xanthomonas oryzae	1	203	1	4	
Xanthomonas phaseoli	2	203	1	7, 27	
Xanthomonas physalidicola	1	203	1	28	
Xanthomonas pisi	1	203	1	52	
Xanthomonas pruni	2	203	1	5, 32	
Xanthomonas translucens	2	203	1	40, 61	
Zymomonas mobilis subsp. *mobilis*	1	226	1	12	
Zymomonas mobilis subsp. *pomaceae*	1	226	1	8	

Remarks:

a) Rehydration with distilled water.
b) Cells grown for 5 to 7 days were harvested.
c) Growth under anaerobic conditions (Alkali-Pyrogallol method).
d) The cells were washed with fresh medium before drying.
e) Growth under anaerobic conditions (Air was replaced with N_2 gas).
f) Microcysts were dried.
g) Recovery of viable cells was better in liquid medium than on agar medium.

*: Survival value is expressed as percentage of viable count after drying to initial viable count in the cell-suspension.

early stationary growth phase and submitted to drying after separation from sulfur particles and washing with the fresh medium. The dried specimen exhibited a survival value of about 1 %. It was necessary to rehydrate the dried specimen with a large amount of the medium for recovery of the cells, in order to minimize the growth-inhibitory effect of the organic compound used as a protective agent in the suspending medium (5).

After examination of changes in survival value of *Escherichia coli* K12 during preservation of the dried specimen up to 46 months at 5 C, 25 C and 37 C, Sakane and Banno (4) predicted that a dried specimen which yielded survival values higher than 1 % immediately after drying could survive long-term preservation at 5 C. It seems probable from this prediction that the L-dried specimens of most of the bacteria presented in Table 1 will be able to survive fairly long term at 5 C. However a dried specimen yielding a lower survival value than 1 % should be checked for viability and renewed after a short time.

The authors are grateful to Dr. T. Miwatani, Professor of the Research Institute for Microbial Disease, Osaka University, for his criticisms of the manuscript.

References

1) Annear, D. I. 1970. Preservation of microorganisms by drying from the liquid state. *In* H. Iizuka and T. Hasegawa (ed.) Proceedings of the first international conference on culture collections. p. 273–279. Univ. of Tokyo Press, Tokyo.
2) Iijima, T., and T. Sakane. 1973. A method for preservation of bacteria and bacteriophages by drying *in Vacuo*. Cryobiology **10**: 379–385.
3) Iijima, T., and T. Sakane. 1973. Method for preservation of bacteria and bacteriophages by drying *in Vacuo*. IFO Res. Comm. **6**: 4–17.
4) Sakane, T., and I. Banno. 1976. Preservation of bacteria by L-drying. J. Japan. Soc. Research Freez. and Dry. **22**: 101–108, in Japanese.
5) Sakane, T., and I. Banno. 1977. Preservation of *Thiobacillus* by L-drying. J. Japan. Soc. Research Freez. and Dry. **23**: 18–22, in Japanese.

IFO Res. Comm. 9,
46–61, 1979 (March)

NOTES ON THE FILAMENTOUS FUNGI ISOLATED FROM FOREST SOILS IN ALASKA

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Summary

Twenty three species of the filamentous fungi which were isolated from the soil samples of several subarctic forests in Alaska were described briefly. Of the 23 species identified 3 belong to the Zygomycotina, 3 to the Ascomycotina, and 17 to the Deuteromycotina. The most prevalent species was *Mortierella ramanniana* var. *angulispora* followed by *Penicillium terlikowskii* and *Mortierella isabellina*.

During field trips to survey the wild mushrooms in Alaska, U.S.A., in the midsummer of 1976, 19 soil samples were collected by Dr. Kazumasa Yokoyama, Shiga University. All of the soil samples including one peat sample were collected in the forests near Anchorage and Fairbanks as shown in Fig. 1 and Table 1.

A total of 19 samples was taken aseptically for mycoflora investigation and put into a sterile polyethylene bag to bring back to the laboratory.

Isolation of the fungi was carried out about one month after the soil samples were collected, although these samples were kept in a refrigerator or at a controlled temperature kept as low as possible. To isolate the filamentous fungi from the soils, two isolation methods were carried out; the dilution plate method described by Waksman in 1922 (1) and the ethanol treatment method described by Warcup & Baker in 1963 (2).

The dilution plate method was carried out by suspending a soil sample weighing approximately 2 g into a 5 ml of sterilized water. A 0.1 ml of the suspension thus prepared was then spread by a sterilized L-shaped rod all over the petri dish containing malt extract-yeast extract-agar medium (MYA).

The composition of MYA medium is detailed below:

> Malt extract, 0.3 g; yeast extract, 0.3 g; peptone, 0.5 g; glucose, 1 g; agar, 1.5 g; distilled water, 100 ml. (containing tetracycline, 50 μg/ml).

The second method was carried out by adding a 5 ml of absolute ethanol for 15 min onto 5 ml of soil suspension containing 2 g of soil in sterilized water as above. A 0.2 ml of the ethanol-treated soil suspension was then spread over the petri dish as described above.

Two replicates for each sample were made and the petri dish cultures were incubated at 24 C for 3 days. All the colonies which developed on the medium were carefully picked up under a dissecting microscope and transferred to make pure slant cultures at 24 C.

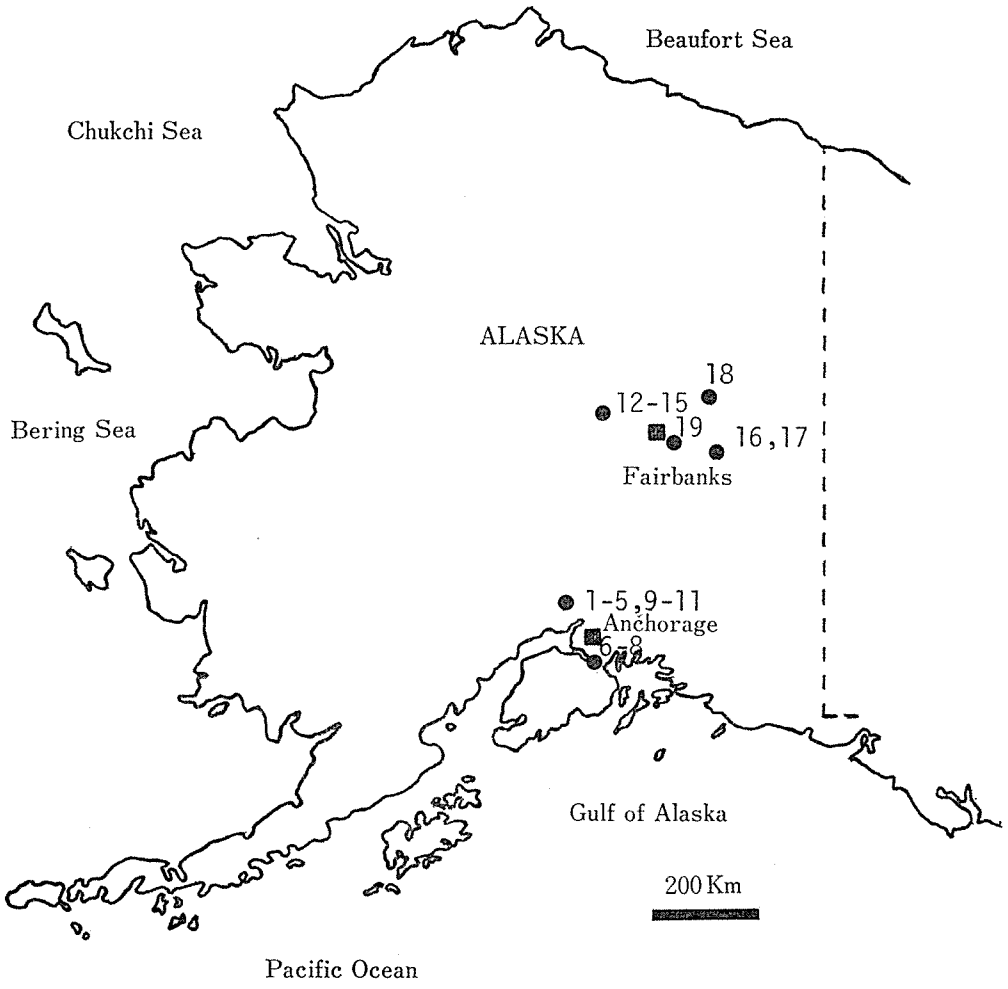


Fig. 1. Map of Alaska

Table 2 represents a list of the fungi isolated and identified. Of the 164 strains isolated, 52 strains were identified; 14 strains belong to the Zygomycotina, 4 strains to the Ascomycotina, and 34 strains to the Deuteromycotina. However, 112 strains have not yet been identified mainly because of absence of sporulation.

A total of 23 species in 13 genera were identified, of which the Zygomycotina was represented by 3 species in 2 genera, the Ascomycotina by 3 species in 2 genera, and the Deuteromycotina by 17 species in 9 genera. The most prevalent fungus in this collection was *Mortierella ramanniana* var. *angulispora,* which was found in 8 different soil samples, followed by *Penicillium terlikowskii* (in 6 different samples) and *Mortierella isabellina* (in 5 different samples).

In this report, we briefly describe 23 species hitherto identified.

We wish to thank Dr. Kazumasa Yokoyama, Associate Professor, the Biological Institute, Shiga University, Otsu, for providing us with soil samples and laboratory facilities. We are grateful to Dr. Teiji Iijima, Director of the Institute for Fermentation,

Table 1. Soil samples collected in Alaska.

Number of soil	Date of collection	Location	Vegetation	Remarks
1	11 Aug., 1976	Campus of Methodist University of Anchorage	*Abies-Betula* forest	
2	〃	〃	*Abies* forest	upper layer
3	〃	〃	〃	lower layer
4	〃	Earthquake Park, Anchorage	*Betula* forest	
5	〃	〃	Peat	
6	12 Aug., 1976	Turnagain Pass, ca 40 Km SE of Anchorage	*Tsuga-Picea* forest	upper layer
7	〃	〃	〃	lower layer
8	〃	Winner Creek, ca 60 Km SE of Anchorage	〃	
9	13 Aug., 1976	Chugach State Park, ca 20 Km E of Anchorage	*Tsuga* forest	Subalpine tundra
10	〃	〃	Formerly *Tsuga-Populus* forest	Burned soil
11	〃	〃	*Tsuga* forest	
12	16 Aug., 1976	White Mountain Trail, ca 100 Km NW of Fairbanks	*Picea-Betula* stand	Subalpine tundra
13	〃	〃	〃	〃
14	〃	〃	〃	〃
15	〃	〃	〃	〃
16	17 Aug., 1976	Harding Lake, ca 70 Km SE of Fairbanks	*Picea-Populus-Betula* forest	
17	〃	〃	〃	
18	19 Aug., 1976	Experimental Forest of University of Alaska, ca 50 Km NE of Fairbanks	*Populus-Betula* forest	
19	20 Aug., 1976	Arboretum of University of Alaska, Fairbanks	*Picea* forest	

Collection of soil samples was made by Dr. Kazumasa Yokoyama.

Osaka, for his valuable suggestions and general encouragements. The technical assistance of Mrs. Michiyo Ueda for this work is gratefully acknowledged.

Table 2. List of fungi isolated from soil samples collected in Alaska.

Fungus	soil sample 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	Number of strains isolated
[Zygomycotina]																				
Mortierella isabellina	○		○								○					○		○		5
M. ramanniana var. *angulispora*	○					○	○	○	○					○		○			○	8
Mucor sp.																			○	1
[Ascomycotina]																				
Eupenicillium lapidosum																	○			1
E. pinetorum			○										○							2
Pseudogymnoascus roseus										○										1
[Deuteromycotina]																				
Acremonium butyri									○											1
Beauveria bassiana			○																	1
Chrysosporium pannorum									◎							◎				4
C. merdarium var. *merdarium*									○											1
Oidiodendron griseum															○	○		○		3
Penicillium adametzi					○										○					2
P. janthinellum										○										1
P. lanosum								○	○											2
P. nigricans			○								○								○	3
P. odoratum												○								1
P. raistrickii		○							○						○				○	4
P. terlikowskii								○			○			○	○		○	○		6
P. velutinum																○				1
Trichoderma polysporum																○				1
Troposporella fumosa			○																	1
Verticillium fungicola																		○		1
Truncatella truncata				○																1
unidentified strain	5	8	6	6	6	4	2	4	4	9	6	8	6	8	4	5	10	3	8	112
Total number of strain identified	2	1	5	1	1	1	1	3	7	2	3	1	1	2	4	7	2	4	4	52
Total number of strain isolated	7	9	11	7	7	5	3	7	11	11	9	9	7	10	8	12	12	7	12	164

○ represent single strain; ◎ represent two strain.

Descriptions

[Zygomycotina]

Mortierella isabellina Oudemans & Koning (Pl. 1, A)

Arch. Néerl. Sci. (Sér. 2) **7**: 276 (1902); Linnemann, G., Die Mucorineen Gattung Mortierella Coemans, p. 18 (1941); Linnemann, G., in Zycha, H., & Siepmann, R., Mucorales, p. 159 (1969); Cooke, W.B., & Fournelle, H.T., J. Arctic Inst. N. Amer., Arctic **3**: 266 (1960); Kobayasi, Y., *et al.*, Ann. Rept. Inst. Ferm. Osaka **3**: 17 (1967).

Colonies on malt agar growing rapidly, grey to dark grey, velvety with white margin, often with concentric zones of 1–3 mm intervals; reverse uncolored or pale grey. Sporangiophores simple or irregularly branched, 120–300 μm; Collar broad, distinct. Sporangia globose, dark grey at maturity, 10–20 μm in diam. Sporangiospores numerous, subglobose, oval to slightly angular, 2–3 μm. Chlamydospores produced in abundance, globose.

Strains examined; Ala-1-2 (1)*, Ala-3-1 (3), Ala-11-1 (11), Ala-16-4 (16), Ala-18-2 (18).

This species has already been reported by Cooke & Fournelle (1960) and Kobayasi *et al.* (1967) from Alaskan soils.

Mortierella ramanniana (Möller) Linnemann var. **angulispora** (Naumov) Linnemann (Pl. 1, B)

Linnemann, G., in Zycha, H., & Siepmann, R., Mucorales, p. 162 (1969); Naumov, N.A., Mucorales, p. 140 (1935); Kominami, K., *et al.*, Nagaoa **1**: 14 (1952); Cooke, W.B., & Fournelle, H.T., J. Arctic Inst. N. Amer., Arctic **3**: 266 (1960); Indoh, H., Trans. Mycol. Soc. Japan **3**: 24 (1962); Kobayasi, Y., *et al.*, Ann. Rept. Inst. Ferm. Osaka **3**: 18 (1967).

Colonies on malt agar growing rapidly, velvety, russet-vinaceous; reverse uncolored. Sporangiophores branched near the base, 100–400 μm long, with distinct septum close to the base of the sporangia. Sporangia globose to subglobose, pale red, 15–20 μm in diam; Collar distinct; Collumella distinct, subglobose, 3–10 μm. Sporangiospores 1-celled, angular or cuneiform, 2–3.5 μm.

Strains examined: Ala-1-1 (1), Ala-6-1 (6), Ala-7-1 (7), Ala-8-1 (8), Ala-9-3 (9), Ala-14-1 (14), Ala-16-3 (16), Ala-19-2 (19).

This species has already been reported by Cooke & Fournelle (1960) and Kobayasi *et al.* (1967) from Alaska.

Mucor sp.

Colonies on malt agar growing rapidly, pale-yellow to buff; reverse yellow. Spor-

* Number of soil sample listed in Table 1.

angiophores long, straight, simple or branched. Sporangia brown at maturity, with deliquescent wall. Collumella at first globose, then ellipsoid, truncate at the base, up to 30 μm high and 20 μm broad. Sporangiospores oblong, ellipsoid, smooth, hyaline, variable in size, 5–8×3–5 μm.
Strain examined: Ala-19-3 (19).

Morphologically this isolate is identical to *M. hiemalis* f. *hiemalis,* but was not able to form zygospores with either IFO 9404 (+) or IFO 9405 (—) strains of *M. hiemalis* f. *hiemalis.*

[Ascomycotina]
Eupenicillium lapidosum Scott & Stolk (Pl. 2, A & B)
Ant. Leeuwenh. **33**: 298 (1976); Scott, D.B., CSIR Res. Rept., Pretoria, S. Africa, **272**: 100 (1968); Udagawa, S., & Horie, Y., Trans. Mycol. Soc. Japan **14**: 383 (1973).
St. conid. *Penicillium lapidosum* Raper & Fennell, Mycologia **40**: 524 (1948); Raper, K.B., & Thom, C., A Manual of the Penicillia, p. 163 (1949).

Colonies on malt agar growing rapidly and spreading broadly, 6 cm in diam in 1 week at 25 C, greyish green especially around the center; reverse pale yellowish brown. Cleistothecia spherical to ovate, 70–250 μm in diam, yellowish brown to pale orange, often covered by the conidial structures. Ascospores not seen. Conidiophores borne on the aerial hyphae, 25–100 μm high or more, 2–3 μm thick, with smooth to definitely roughened walls; penicilli variable in pattern, mostly monoverticillate to biverticillate. Conidia elliptical, 2.2–3.5×2–2.5 μm, smooth-walled or nearly so.
Strain examined: Ala E-17-1 (17).
Other strain examined: IFO 6100 (E.J. Cameron, isolated from canned blue berries, an authentic culture derived from the type; =NRRL 718=ATCC 10462=CBS 343.48= IMI 39743=QM 1928).

The present strain has not produced matured asci and ascospores so far examined, but closely fits the original description given for the present species in its cultural and microscopical characteristics.

Eupenicillium pinetorum Stolk (Pl. 2, C & D)
Ant. Leeuwenh. **34**: 37 (1968); Scott, D.B., CSIR Res. Rept., Pretoria, S. Africa, **272**: 123 (1968); Udagawa, S., Trans. Mycol. Soc. Japan **10**: 104 (1970).
St. conid. *Penicillium pinetorum* Christensen & Backus, Mycologia **53**: 457 (1961).
Syn. *Penicillium silvaticum* Suprum (1956) non Oudemans (1902), nec (Wehmer) Biourge (1923), nec (Wehmer) Gäumann (1926); *Penicillium macedonense* Verona & Mickovski (1962).

Colonies on malt agar growing rather rapidly, 2 cm in diam in 1 week at 25 C;

margin slightly lobate; conidial area olive grey; reverse brownish. Cleistothecia abundant, subglobose to ellipsoid, 50–190 μm in diam, yellowish brown. Ascospores lenticular with two closely appressed, 0.5 μm wide, equatorial ridges, 3–4.5×2.5–3 μm; convex surface smooth. Conidiophores borne on the creeping hyphae as short perpendicular branches, smooth walled, 15–50×2–2.5 μm; penicilli strictly monoverticillate. Conidia mostly globose, echinulate, 3–3.5 μm.
Strains examined: Ala–3–7 (3), Ala–13–5 (13).
Other strain examined: IFO 7743 (M. Christensen WSF–15–C, isolated from soil of *Pinus* and *Betula* forest, an authentic culture derived from the type ;=ATCC 14770=CBS 295.62=IMI 94209).

Pseudogymnoascus roseus Raillo (Pl. 1, C)
Zentbl. Bakt. ParasitKde (Abt. 2) **78**: 520 (1929); Samson, R.A., Acta Bot. Neerl. **21**: 518 (1972).

Colonies on oatmeal agar growing slowly, restricted, floccose, white at first, soon becoming pinkish brown; reverse yellow to yellowish brown. Ascomata globose to subglobose, yellow brown to red brown, 100–200 μm in diam. Peridial hyphae thick, up to 2–3.5 μm, solid, H-shaped, with hyaline to pale yellow, short, slightly rough-walled appendages. Asci globose to oval, with a short stalk, 8-spored, 6–9×5–7 μm. Ascospores spindle-shaped to fusiform, pale yellow, smooth, 3–4×2–2.5 μm. Conidia of aleurioconidium-type, oval to pyriform, hyaline, 2.5–3×2–2.5 μm.
Strain examined: Ala–10–5 (10).

[Deuteromycotina]
Acremonium butyri (van Beyma) W. Gams (Pl. 1, D)
Cephalosporium-artige Schimmelpilze (Hyphomycetes), p. 126 (1971).
Syn. *Tilachlidium butyri* van Beyma (1938); *Gliomastix lavitskiae* Zhdanova (1966); *Cephalosporium khandalense* Thirumalachar & Sukapure (1966).

Colonies on malt agar growing rapidly, tinged with some shade of yellowish green to olivaceous brown, white at maturity, floccose to funiculose; reverse dark olive to almost black. Conidiophores borne on the aerial mycelium, erect, simple or branched, septate, slightly rough-walled, tapering towards the apex, yellowish, 20–50×2–3.5 μm. Conidia variable in shape and size, oval, ellipsoid, sometimes cylindric and slightly curved, smooth, yellowish, 4–8×2–3 μm.
Strain examined: Ala–9–8 (9).

According to Gams (1971), this species has been treated as an imperfect state of *Nectria viridescens* Booth (Mycol. Pap. **73**: 89, 1959).

Beauveria bassiana (Balsamo) Vuillemin (Pl. 1, E)
Bull. Soc. Bot. France **29**: 34 (1912); Hoog, G.S. de, Studies in Mycology **1**: 4 (1972).

Colonies on oatmeal agar growing rapidly, velvety or powdery, partially floccose to funiculose, at first white, then creamish or yellowish; reverse yellowish to pale brown. Conidiophores borne directly on the aerial mycelium or short branches, clustered, each with one to five conidiogenous cells. Conidiogenous cells ovate to flask-shaped at the base, 3–6×2.5–3.5 μm, proliferating into narrow, geniculate and denticulate terminal parts of up to 20 μm long. Conidia globose to broadly ellipsoid, hyaline to pale yellow in mass, 1-celled, smooth, 2–3×2–2.5 μm.
Strain examined: Ala–3–8 (3).

Chyrsosporium merdarium (Link) Carmichael var. **merdarium** (Pl. 1, F)
Can. J. Bot. **40**: 1160 (1962); Matsushima, T., Icon. Microfung. a Matsushima Lect., p. 30 (1975); Sigler, L., & Carmichael, J.W., Mycotaxon **4**: 374 (1976).

Colonies on malt agar growing moderately, at first white, then pale yellow to olivaceous, floccose; reverse yellow to yellowish brown. Mycelium submerged or partially floccose, hyaline to pale yellow, 2–4 μm thick. Conidiophores terminally on the aerial mycelium or intercalary, branched at a right angle or at an obtuse angles. Aleurioconidia subglobose, doliiform to pyriform, sometimes with a scar, hyaline, smooth or rarely rough-walled, 5–6 (8)×4–5 μm.
Strain examined: Ala–9–9 (9).

Chrysosporium pannorum (Link) Hughes (Pl. 1, G)
Can. J. Bot. **36**: 749 (1958); Carmichael, J.W., Can. J. Bot. **40**: 1162 (1962); Kobayasi, Y., *et al.*, Ann. Rept. Inst. Ferm. Osaka **3**: 108 (1967); Matsushima, T., Icon. Microfung. a Matsushima Lect., p. 30 (1975).

Colonies on malt agar growing slowly, restricted, finely powdery to velvety, with some shade of grey, brown, olive, yellow; reverse pale yellow to brown. Conidiophores branched at an acute angle or verticillate, hyaline to pale yellow, bearing aleurioconidia at the tip. Conidia of aleurioconidia type, cuneiform or pyriform, smooth or slightly rough-walled, hyaline to pale yellow, 3–4×2–3 μm.
Strains examined: Ala–9–10 (9), Ala–16–10 (16).

This species has already been reported in Alaska by Kobayasi *et al.* (1967) and Matsushima (1975).

Oidiodendron griseum Robak (Pl. 1, H)
Can. J. Bot. **40**: 606 (1962); Tokumasu, S., Trans. Mycol. Soc. Japan **14**: 248 (1973).

Colonies on malt agar growing slowly, restricted, greyish at first, later becoming olivaceous grey to brown; reverse grey to almost black. Conidiophores erect, verticillately branched at the upper, brown, septate, smooth, up to 100 μm high, sometimes

reaching to 200 μm high, 2–3.5 μm at the base. Conidia globose, subglobose or oval, rarely short cylindric, pale brown, 2–3×1.5–2 μm.
Strains examined: Ala–15–8 (15), Ala–16–11 (16), Ala–18–5 (18).

Penicillium adametzi Zaleski (Pl. 2, E & F)
Bull. Acad. Polonaise Sci. Math. Nat. Ser. B. p. 507 (1927); Thom, C., The Penicillia, p. 194 (1930); Raper, K.B., & Thom, C., A Manual of the Penicillia, p. 228 (1949).

Colonies on potato sucrose agar growing rapidly, 3–3.5 cm in diam in 1 week at 25 C, plane, composed of velvety aerial mycelium, dull green or greyish green, becoming dark green in age; reverse pale yellowish brown. Conidiophores short, 30–50×2–2.5 μm; Penicilli strictly monoverticillate. Conidia globose to subglobose, with delicately granulated wall, 2.5–3.5 μm.
Strains examined: Ala–5–1 (5), Ala–15–5 (15).

Penicillium janthinellum Biourge (Pl. 2, G & H)
Monogr., La Cellule **33**: 258 (1923); Thom, C., The Penicillia, p. 238 (1930); Raper, K.B., & Thom, C., A Manual of the Penicillia, p. 299 (1949).

Colonies on potato sucrose agar growing rapidly, 3.2–3.5 cm in diam in 1 week at 25 C, composed of delicately floccose aerial mycelium, greyish green or greenish grey, with a white margin, 2–2.5 mm wide; reverse pale rose. Conidiophores smooth or finely roughened; penicilli asymmetric, strongly divaricate, variable in pattern. Conidia globose to subglobose, often with apiculate ends, rough-walled, 2.5–3 μm.
Strain examined: Ala–10–2 (10).

This is the most abundant and worldwide fungus among the species of *Penicillium* in soils.

Penicillium lanosum Westling (Pl. 2, I & J)
Arkiv Botanik **11**: 55 & 97 (1911); Thom, C., The Penicillia, p. 317 (1930); Raper, K.B., & Thom, C., A Manual of the Penicillia, p. 431 (1949); Kobayasi, Y., *et al.*, Ann. Rept. Inst. Ferm. Osaka **3**: 110 (1967).

Colonies on potato sucrose agar growing rather restrictedly, 1.5–2 cm in diam in 1 week at 25 C, floccose, slightly wrinkled at the central or subcentral areas, white at margin; margin 1 mm wide; conidial area olive grey or greyish yellow-green; reverse uncolored or pale yellowish brown. Conidiophores borne on the aerial hyphae or arising from the substrate; penicilli asymmetric, irregularly branched, often becoming divergent. Conidia globose to subglobose, rough-walled, 2.5–3 μm.
Strains examined: Ala–8–5 (8), Ala–9–6 (9).
Other strain examined: IFO 6099 (R. Westling, an authentic culture derived from the type; =NRRL 2009=ATCC 10458=CBS 106.11=IMI 40224=QM 7591).

This species has already been recorded in the soil of Glacier Bay by Cooke & Lawrence (1959) and on decayed mushroom collected at Umiat (Kobayasi *et al.*, 1967) in Alaska.

Penicillium nigricans (Bainier) Thom (Pl. 3, A & B)
The Penicillia, p. 351 (1930); Raper, K.B., & Thom, C., A Manual of the Penicillia, p. 325 (1949).
Syn. *Penicillium echinatum* Dale (1923).

Colonies on potato sucrose agar growing restrictedly, 1.5 cm in diam in 1 week at 25 C, composed of comparatively deep basal felt, floccose, radially furrowed, light greenish grey to greenish grey, becoming olive grey in age; conidial structures crowded; margin white, broad; reverse yellow orange to dark yellow orange. Conidiophores arising from the substrate, occasionally borne on the aerial mycelium, variable in length; penicilli asymmetric, divaricate, variable in pattern. Conidia globose, echinulate, 2.5–3 μm.
Strains examined: Ala–3–6 (3), Ala–11–4 (11), Ala–19–8 (19).
Other strain examined: IFO 6103 (=ATCC 10115=CBS 354.48=IMI 39767=NRRL 915=QM 1933=C. Thom 4640–448).

Penicillium odoratum Christensen & Backus (Pl. 3, E & F)
Mycologia **53**: 459 (1961).

Colonies on potato sucrose agar growing rapidly, 3.5 cm in diam in 1 week at 25 C, velvety, plane, dark blue green; margin white, 1 mm wide; odor strong, aromatic; reverse uncolored or pale olive. Conidiophores arising from the substrate or basal felt, with delicately granulated wall; penicilli monoverticillate, occasionally branched. Conidia globose to subglobose, slightly echinulate, 2.5–3.5 μm.
Strain examined: Ala–12–6 (12).
Other strain examined: IFO 7741 (M. Christensen, isolated from peaty soil, an authentic culture derived from the type; =ATCC 14769=CBS 294.62).

Penicillium raistrickii Smith (Pl. 3, C & D)
Trans. Brit. Mycol. Soc. **18**: 90 (1933); Raper, K.B., & Thom, C., A Manual of the Penicillia, p. 275 (1949).

Colonies on malt agar growing rapidly, 3 cm in diam in 1 week at 25 C, plane, showing a marked tendency to sector, sometimes with blue green sporulating areas; sclerotia appear dominantly and sterile hyphae increase both on adjacent sectors, light brownish; reverse dull brown. Conidiophores rough-walled, 250×3.5 μm; penicilli asymmetric, divaricate. Conidia globose or subglobose, with delicately roughened wall, 2.3–2.8 μm.
Strains examined: Ala–2–6 (2), Ala–9–7 (9), Ala–15–7 (15), Ala–19–10 (19).

Other strain examined: IFO 6104 (G. Smith, isolated from cotton yarn, an authentic culture derived from the type; =NRRL 2039=ATCC 10490=CBS 261.33=IMI 40231).

Penicillium terlikowskii Zaleski (Pl. 3, G & H)
Bull. Acad. Polonaise Sci. Math. Nat. Ser. B., p. 501 (1927); Thom, C., The Penicillia, p. 203 (1930); Raper, K.B., & Thom, C., A Manual of the Penicillia, p. 231 (1949).

Colonies on potato sucrose agar growing rapidly, 3.2–3.5 cm in diam in 1 week at 25 C, velvety, plane, greyish olive or olive grey; reverse uncolored. Conidiophores short, mostly less than 100 μm high, but occasionally longer, 2–2.5 μm thick; penicilli strictly monoverticillate. Conidia globose to subglobose, with delicately roughened wall, 2–2.5 μm.
Strains examined: Ala–8–4 (8), Ala–11–3 (11), Ala–14–10 (14), Ala–15–4 (15), Ala–17–6 (17), Ala–18–4 (18).
Other strain examined: IFO 6108 (P.W. Brian No. 241;=NRRL 2067=ATCC 10504=CBS 379.48=IMI 40572).

Penicillium velutinum van Beyma (Pl. 3, I & J)
Zentbl. Bakt., II **91**: 352 (1935); Raper, K.B., & Thom, C., A Manual of the Penicillia, p. 250 (1949).

Colonies on potato sucrose agar growing rapidly, 2.5 cm in diam in 1 week at 25 C, composed of floccose aerial mycelium, olive grey or slate olive, becoming dark olive grey in age, raised and irregularly wrinkled at the central area; margin white, broad; reverse uncolored. Conidiophores arising from the substrate or borne on aerial hyphae; penicilli sometimes monoverticillate, usually ramigenously branched. Conidia globose or subglobose, echinulate, 2–3 μm.
Strain examined: Ala–16–7 (16).

This species was originally isolated from sputum.

Trichoderma polysporum (Link ex Persoon) Rifai, aggr.
Mycol. Pap. **116**: 1 (1969).

Colonies on malt agar growing slowly, floccose, white, partially dispersed as pure white conidial areas; reverse pale brown. Conidiophores numerous on the compact tufts; sterile main axis elongate, straight or flexuous, branched laterally to verticillately; phialides flask-shaped, tapering towards the apex. Conidia subglobose to ovoidal, hyaline, smooth, 3.5×2 μm, formed in slime drops on the phialides.
Strain examined: Ala–16–9 (16).

This fungus is quite common in soil and said to be worldwide in soil and on plant

debris. Many isolates from England, Germany, Netherlands, U.S.A. and Australia have been studies by Rifai (1969) and many other pioneer workers.

Troposporella fumosa Karsten (Pl. 1, I)
Hedwigia **31**: 299 (1892); Ellis, M.B., Dematiaceous Hyphomycetes, p. 85 (1971).

Colonies on malt agar growing slowly restricted, fawn to brown, velvety to lanose; reverse dark brown, almost black. Conidiophores simple or irregularly branched, aggregated, pale brown, multiseptate, smooth, up to 75 μm long, 3–5 μm thick. Conidiogenous cells monoblastic, short, cylindric. Conidia solitary, acrogenous, helicoid, hyaline at first, then becoming pale brown, smooth, coiled 1 1/2 to 2 times at maturity, 12–18 μm in diam in coiled conidia; filaments 3–5 μm thick, 7–15 septate, constricted at the septum.
Strain examined: Ala–3–12 (3).

Truncatella truncata (Léveillé) Steyaert
Bull. Jard. Bot. Bruxelles **19**: 295 (1949); Linder, D.H., Bull. Nat. Mus. Canada No. 97 (1947); Guba, E.F., Monograph of Monochaetia and Pestalotia, p. 65 (1961); Kobayasi, Y., *et al.*, Ann. Rept. Inst. Ferm. Osaka **3**: 99 (1967).
Syn. *Pestalotia truncata* Léveillé

Colonies on cornmeal agar growing slowly, white to pale brown, floccose. Acervuli developed partially on agar surface, scattered singly or aggregated in groups, covered by white mycelial tufts at first, then naked to form slimy conidial mass. Conidia in mass greyish to olivaceous brown at first, then almost black at maturity. Conidia obclavate, 3-septate, 15–25×5–6 μm; two intermediate colored cells concolor, dark brown, thick-walled; apical cell hyaline to very pale brown, with 1 to 3 simple or 2– to 5-branched, hyaline appendage of 10–20 μm long and 1 μm thick; basal cell hyaline to very pale brown, truncate at the base.
Strain examined: Ala–4–4 (4).
Other strain examined: IFO 8584 (K. Tubaki B–4–4).

This fungus is known as one of the common fungi on various kind of the vascular plants and has already been recorded in the tundra of L. Peters and soil of C. Thompson, Alaska (Kobayasi *et al.*, 1967). Linder (1947) has also reported this fungus in S. Baffin, Canadian E. Arctic.

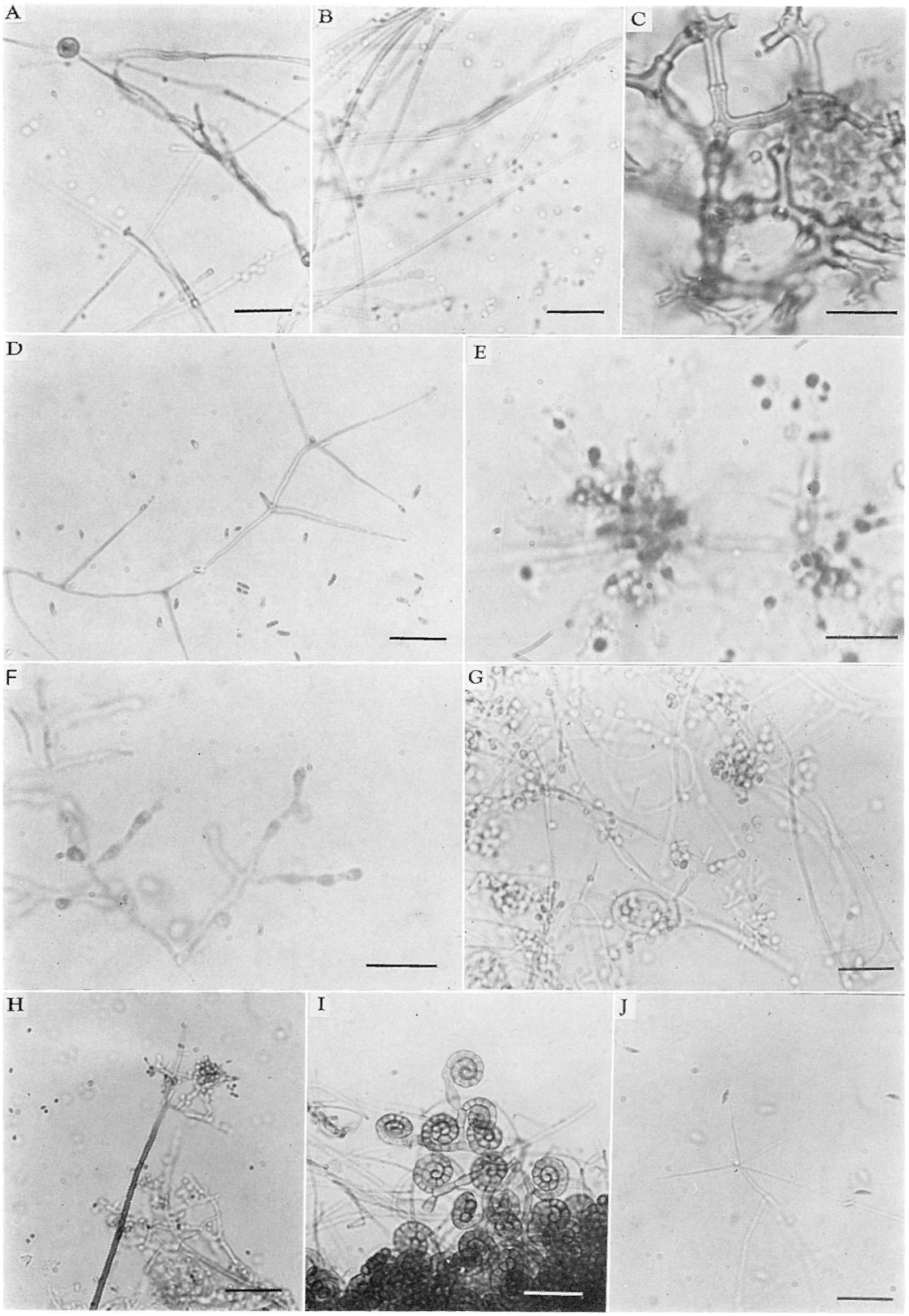
Verticillium fungicola (Preuss) Hassebrauk (Pl. 1, J)
Phytopath. Z. **9**: 514 (1936); Kobayasi, Y., *et al.*, Ann. Rept. Inst. Ferm. Osaka **3**: 111 (1967); Gams, W., Cephalosporium-artige Schimmelpilze (Hyphomycetes), p. 169 (1971).
Syn. *Verticillium malthousei* Ware (1933).

Colonies on malt agar growing slowly, delicate, matted, pure white; reverse uncolored. Conidiophores erect, septate, verticillately branched with 2 to 3 phialides, sometimes with single phialide. Phialides narrowed towards the apex, hyaline, 18–24 (30)×1–1.5 (2.5) μm. Conidia variable in shape, cylindric, plano-convex or elliptical, rarely curved, 1-celled, hyaline, 4–10×1.5–2.5 μm.
Strain examined: Ala–18–6 (18).
Other strain examined: IFO 8578 (ATCC 18163; Tubaki A–1–5).

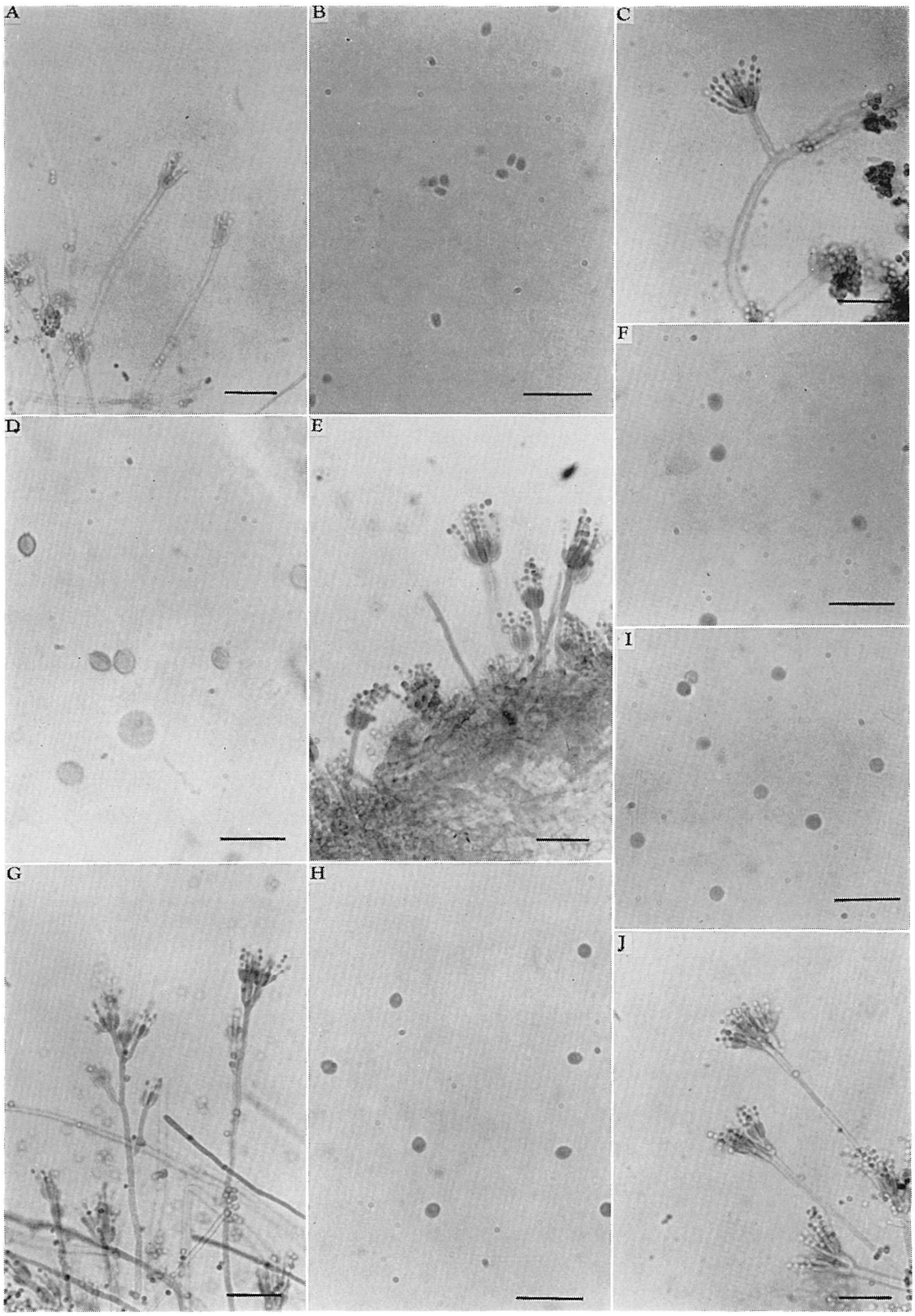
In this strain, conidia are more or less longer than those given by Gams (1971). According to Tubaki, this fungus has already been recorded in the tundra soil of Point Barrow, Alaska (Kobayasi *et al.*, 1967).

References

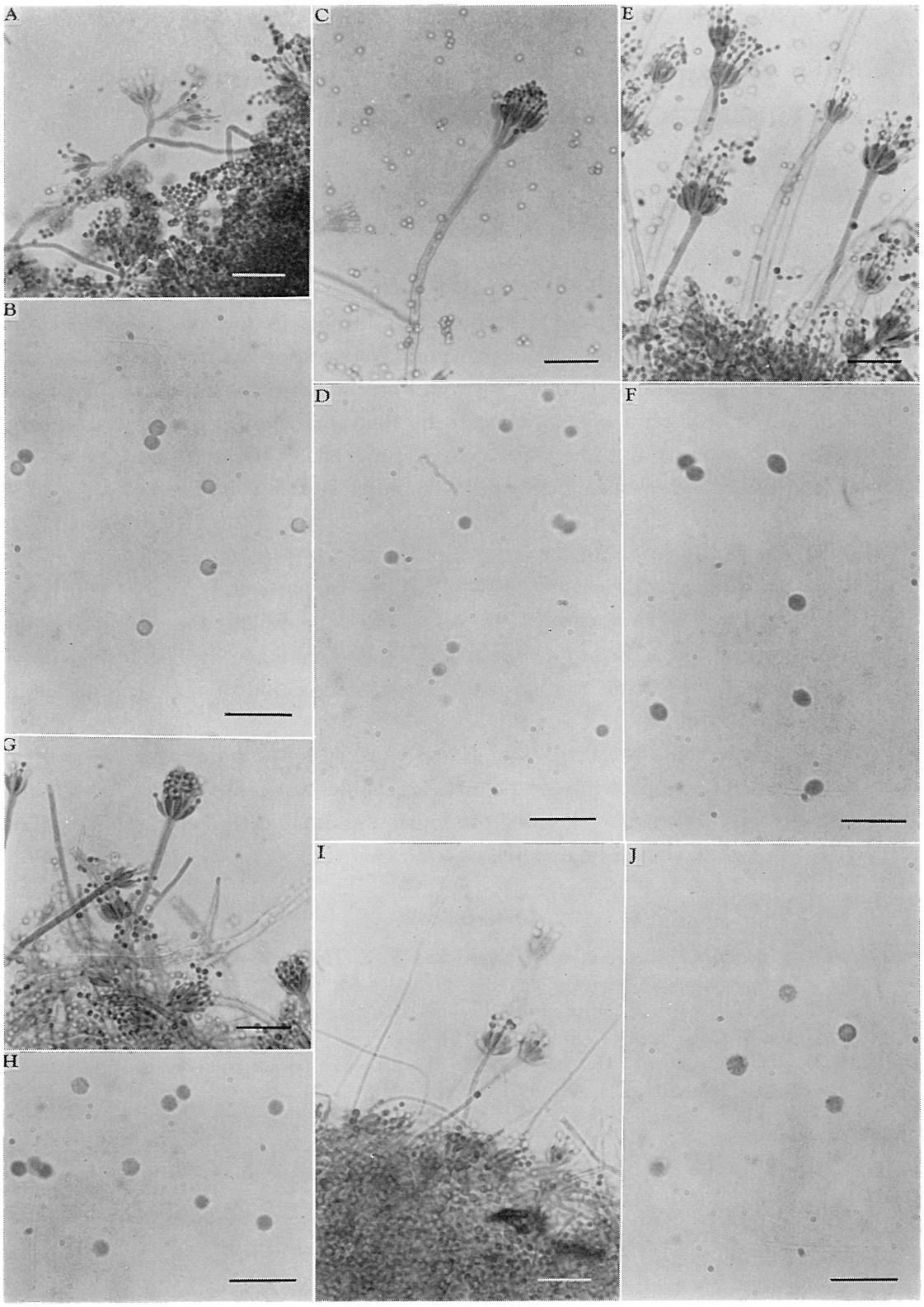
1) Waksman, S. A. 1922. A method of counting the number of fungi in the soil. J. Bacteriol. **7**: 339–341.
2) Warcup, J. H., and K. F. Baker. 1963. Occurrence of dormant ascospores in soil. Nature **197**: 1317–1318.



Pl. 1. A. *Mortierella isabellina* (Ala-1-2). B. *Mortierella ramanniana* var. *angulispora* (Ala-6-1). C. *Pseudogymnoascus roseus* (Ala-10-5). D. *Acremonium butyri* (Ala-9-8). E. *Beauveria bassiana* (Ala-3-8). F. *Chrysosporium merdarium* var. *merdarium* (Ala-9-9). G. *Chrysosporium pannorum* (Ala-9-10). H. *Oidiodendron griseum* (Ala-16-11). I. *Troposporella fumosa* (Ala-3-12). J. *Verticillium fungicola* (Ala-18-6). Bars A, B, D, G, H, I & J=20 μm; C, E & F=10 μm



Pl. 2. A & B. *Eupenicillium lapidosum* (Ala E-17-1), A. Conidial structures. B. Conidia. C & D. *Eupenicillium pinetorum* (Ala-3-7), C. Conidial structure. D. Ascospores and an immature ascus. E & F. *Penicillium adametzi* (Ala-15-5), E. Conidial structures. F. Conidia. G & H. *Penicillium janthinellum* (Ala-10-2), G. Conidial structures. H. Conidia. I & J. *Penicillium lanosum* (Ala-9-6), I. Conidia. J. Conidial structures. Bars A, C, E, G & J=20 μm; B, D, F, H & I=10 μm



Pl. 3. A & B. *Penicillium nigricans* (Ala-3-6), A. Conidial structures. B. Conidia. C & D. *Penicillium raistrickii* (Ala-9-7), C. Conidial structure. D. Conidia. E & F. *Penicillium odoratum* (Ala-12-6), E. Conidial structures. F. Conidia. G & H. *Penicillium terlikowskii* (Ala-11-13), G. Conidial structures. H. Conidia. I & J. *Penicillium velutinum* (Ala-16-7), I. Conidial structures. J. Conidia. Bars A, C, E, G & I=20 μm; B, D, F, H & J=10 μm

IFO Res. Comm. **9**,
62, 1979 (March)

EMENDATION OF DENOTATION OF *tct* (TRICARBOXYLIC ACID TRANSPORT) GENES

Ko IMAI, Teiji IIJIMA, and Isao BANNO

In previous papers (2, 3) from this laboratory, the authors revealed that *Salmonella typhimurium* possesses four inducible transport systems for the tricarboxylic acids. The first system, induced by citrate, isocitrate, or *cis*-aconitate, transports citric and isocitric acids. The second system, induced by the same acids as in the first system, transports *cis*-aconitic acid. The third system, induced by tricarbalyllate, transports tricarballylic, citric, and *cis*-aconitic acids. The fourth system is induced by citrate and transports citric acid.

In the article "Location of *tct* (tricarboxylic acid transport) genes on the chromosome of *Salmonella typhimurium*" by K. Imai, T. Iijima, and I. Banno, which appeared in the March 1977 issue of IFO Res. Comm. **8**: 63–68, the symbols of *tct* genes were not denoted according to the recommendations made by Demerec *et al* (1) for genetic nomenclature, and the authors request emendation of their denotation of *tct* genes as follows:

The genes governing the first, second, and third systems, which were denoted as *tctI*, *tctII*, and *tctIII*, respectively, in the article, should be denoted as *tctA*, *tctB*, and *tctC*, respectively. The gene governing the fourth system, which is induced by citrate and carries citric acid, should be denoted as *tctD*.

References

1) Demerec, M., E. A. Adelberg, A. J. Clark, and P. E. Hartman. 1966. A proposal for a uniform nomenclature in bacterial genetics. Genetics **54**: 61–76.
2) Imai, K. 1975. Isolation of tricarboxylic acid transport-negative mutants of *Salmonella typhimurium*. J. Gen. Appl. Microbiol. **21**: 127–134.
3) Imai, K., T. Iijima, and T. Hasegawa. 1973. Transport of tricarboxylic acids in *Salmonella typhimurium*. J. Bacteriol. **114**: 961–965.

IFO Res. Comm. **9**,
63–64, 1979 (March)

DESCRIPTIVE CATALOGUE OF IFO FUNGUS COLLECTION VI.

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 taxa either new to Japan or obscurely or insufficiently described. Sometimes, the first records of the occurrence of particular fungi in Japan have been made solely by referring to the name of a taxon, without an adequate description of the species concerned. The object of this series of papers is to provide description of fungi preserved or newly deposited in the IFO culture collection and/or 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 descriptions of taxa are shown in brackets.

73. Wardomyces inflatus (Marchal) Hennebert (Pl. 1, A–C) Hyphomycetes
Trans. Brit. Mycol. Soc. **51**: 755 (1968); Ellis, Dematiaceous Hyphomycetes, p. 77 (1971).
Syn. *Trichosporium inflatum* Marchal, Bull. Soc. Bot. Belg. **34**: 142 (1895), *Wardomyces hughesii* Hennebert, Can. J. Bot. **40**: 1027 (1962).

Colonies on oatmeal agar growing rapidly, velvety to lanose, dark grey to black, white at margin; droplets present; reverse black. Conidiophores erect, simple or irregularly branched, straight or flexuous, narrow at the base, septate, smooth-walled, hyaline, 15–20×2–4 μm; conidiogenous cells polyblastic with one to four conidia, subspherical, ampulliform or clavate, hyaline, 3–6×2–4 μm. Conidia ellipsoidal or oblong, rounded at the apex, truncate at the base, with a longitudinal germ slit, 1-celled, dark brown to black, 5–7×3–4 μm.

Growth and sporulation not very good on cornmeal agar, potato carrot agar, malt agar and potato sucrose agar.

Hab. From soil, Mt. Hakkoda, Aomori City, Aomori Prefecture, 5 October, 1977, T. Ito S5270–69–1 (IFO 30558).

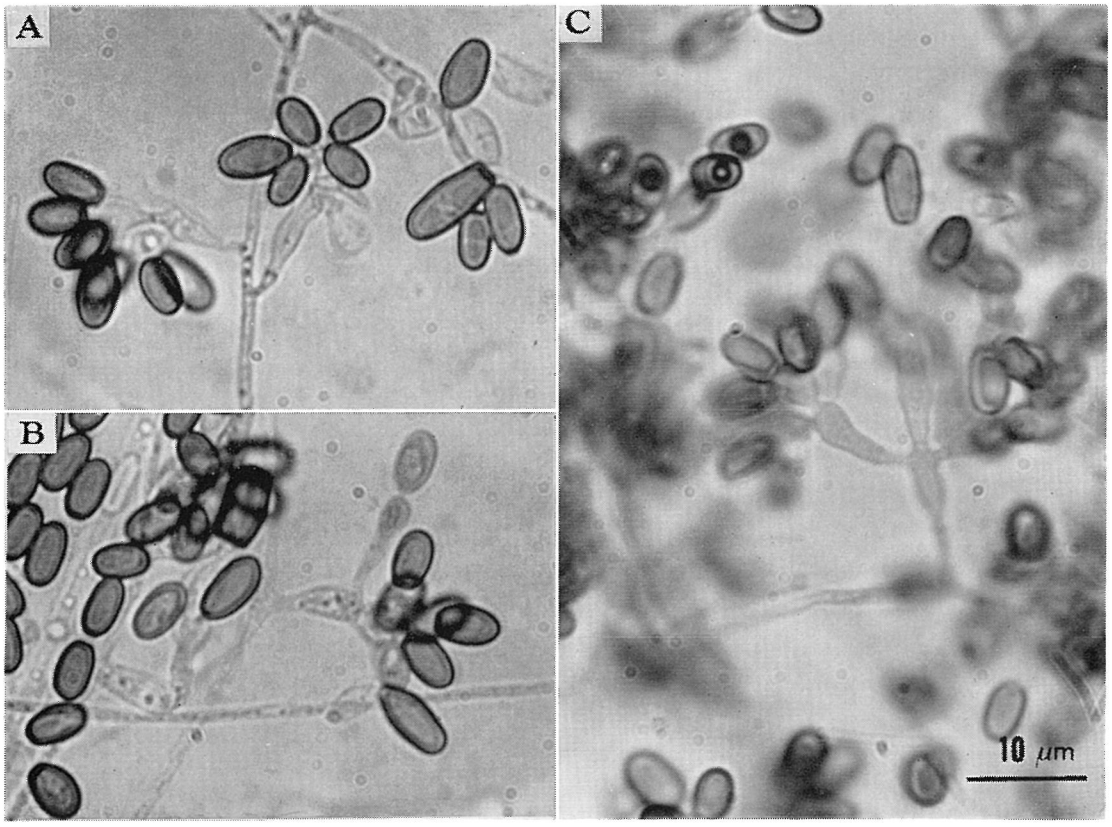
Trichosporium inflatum was originally described from pig dung in Brussels, Belgium. However, one of the later isolates, GLH 669 from green house humic soil in Heverlee, Belgium, has been proposed as the neotype of the species, because of absence of type material and slide in Marchal's collection.

Wardomyces hughesii was recorded by Hennebert (1962) on *Acer* and *Pteridium* from North America and treated as a different taxon with smaller conidia. Later, in

1968, he reexamined the type specimen and six cultural strains of additional collections of *Trichosporium inflatum* and concluded that the species should be treated in the genus *Wardomyces* as *W. inflatus*. Furthermore, he found that the range of conidia of this species was 4.5–7.5×3–4 μm (av. 6×3.5 μm) and therefore *W. hughesii* was identical with this fungus.

The present strain agrees well with those given by Hennebert in its morphological characteristics.

[T. Ito & T. Yokoyama]



Pl. 1. A-C conidiophores and conidia of *Wardomyces inflatus*.

IFO Res. Comm. 9,
65–70, 1979 (March)

DESCRIPTIVE CATALOGUE OF IFO YEAST COLLECTION 2

In the routine work of identifying strains newly isolated 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 yet been reported or have only insufficiently been described in Japan. New taxa will be described in original papers. The following descriptions are arranged in alphabetical order of the scientific name. The authors of the descriptions are indicated in brackets.

6 and **7. Debaryomyces marama** di Menna

di Menna, M. E. 1954. J. Gen. Microbiol. **10**: 65; Kreger-van Rij, N. J. W. 1970. *In* The Yeasts, A Taxonomic Study, ed. by J. Lodder, North-Holland Publ. Co., Amsterdam, p. 146.

IFO 1878 and 1879

Growth in glucose-yeast extract-peptone water: After 3 days at 25 C the cells are short-oval to oval, 2.4–3.8×2.8–4.4 μm; single, in pairs or in small cluster. A sediment and a very thin, dull, smooth pellicle are formed.

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

Growth on YM agar: After 3 days at 25 C the cell are spherical to short-oval, 2.3–5.1×2.4–5.5 μm; single, in pairs or in small cluster.

After one month at 20 C the streak culture is cream-colored, soft, shiny, low-convex and smooth. The margin is entire to undulate.

Dalmau plate cultures on corn meal agar: Pseudomycelium is not formed. Short chains of cells are present.

Formation of ascospores on YM agar and corn meal agar: Conjugation between mother cell and bud precedes ascus formation. The spores are spherical to oval, and mostly contain lipid globules; one to four are formed per ascus. Under the light microscope the spore wall is smooth. The presence of many spores give the cultures a brown color.

Physiological characteristics are presented in Table 1.

The two strains differ from each other in assimilation of lactose and melibiose. The variation in the assimilation of the two sugars has been allowed for in *Debaryomyces marama*.

IFO 1878 was isolated from a partially decayed leaf of *Fagus crenata* collected on Mt. Odaigahara, Nara Pref., on Aug. 4, 1975. (strain No. Od-2a1)

IFO 1879 was isolated from a mushroom collected in Towada, Aomori Pref., on Oct. 5, 1977. (strain No. Tw-52k5)

These are the first record of the species in Japan.

[K. Mikata & I. Banno]

Table 1. Physiological characteristics.

	D. marama		*H. dimennae*	*P. bovis*	*P. pijperi*	*P. terricola*
	IFO 1878	IFO 1879	IFO 1771 IFO 1880 IFO 1881 IFO 1882	IFO 1886	IFO 1791 IFO 1792 IFO 1887	IFO 1798 IFO 1888
Fermentation						
Glucose	−	−	+	+	+	+
Galactose	−	−	−	−	−	−
Sucrose	−	−	−	−	−	−
Maltose	−	−	−	−	−	−
Trehalose	−	−	−	−	−	−
Lactose	−	−	−	−	−	−
Raffinose	−	−	−	−	−	−
Inulin	−	−	−	−	−	−
Soluble starch	−	−	−	−	−	−
α-Methyl-D-glucoside	−	−	−	−	−	−
Assimilation of carbon compounds						
Glucose	+	+	+	+	+	+
Galactose	+	+	−	−	−	−
L-Sorbose	+	+	+s	−	+s	−
Sucrose	+	+	−	+	−	−
Maltose	+	+	−	+	−	−
Cellobiose	+	+	+	+	+	−
Trehalose	+	+	−	+	−	−
Lactose	−	+s	−	−	−	−
Melibiose	+s	−	−	−	−	−
Raffinose	+	+	−	−	−	−
Melezitose	+	+	−	+	−	−
Inulin	w	w	−	−	−	−
Soluble starch	+	w	−	w	−	−
D-Xylose	+	+	+	+	+	−

Table 1. (continued)

L-Arabinose	+	+s	−	−	−	−
D-Arabinose	−	−	−	−	−	−
D-Ribose	+s	+	−	−	−	−
L-Rhamnose	−	−	−	+	−	−
Ethanol	+	+	+	+	+	+
Glycerol	+	+	+	+	+	+
Erythritol	+	+	−	−	−	−
Ribitol	+	+	−	−	−	−
Galactitol	−	−	−	−	−	−
D-Mannitol	+	+	+s	+	+s	−
D-Glucitol	+	+	+s	+	+s	−
α-Methyl-D-glucoside	+	+	−	+	−	−
Salicin	+	+	+	+	+	−
DL-Lactic acid	+	+	+	+	+	−
Succinic acid	ws	ws	ws	+s	w	ws
Citric acid	+	+	−	+s	−	−
Inositol	−	−	−	−	−	−
Splitting of arbutin	positive	positive	positive	positive	positive	negative
Assimilation of potassium nitrate	negative	negative	positive	negative	negative	negative
Growth in vitamin-free medium	negative	negative	negative	negative	negative	negative
Growth at 37C	negative	negative	negative	negative	negative	negative
Growth on 50% (w/w) glucose-yeast extract agar	positive	positive	negative	negative	negative	negative

w=weak, s=slow.

8 to 11. Hansenula dimennae Wickerham

Wickerham, L. J. 1969. Mycopath. Mycol. Appl. **37**: 28; and 1970. *In* The Yeasts, A Taxonomic Study, ed. by J. Lodder, North-Holland Publ. Co., Amsterdam, p. 270.

IFO 1771, 1880, 1881 and 1882

Growth in glucose-yeast extract-peptone water: After 3 days at 25 C the cells are oval to long-oval, 3.2–3.7×4.1–7.5 μm; single, in pairs or in short chains. A sediment and a thin, dull, creeping pellicle are formed.

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

Growth on YM agar: After 3 days at 25 C the cells are oval, long-oval and cylindrical, 2.4–5.5×3.4–8.2 μm; single, in pairs or short chains.

After one month at 20 C the streak culture is cream-colored to pale-brown, soft, dull, flat and smooth. The margin is undulate.

Dalmau plate cultures on corn meal agar: Pseudomycelium is not formed.

Formation of ascospores on YM agar and V-8 agar: Conjugation occurs between mother cell and bud and between independent cells. The spores are Saturn-shaped and mostly contain lipid globules; one to four are formed per ascus. They are liberated soon after maturation.

Physiological characteristics are presented in Table 1.

IFO 1771 was isolated from soil collected on Mt. Togakushi, Nagano Pref., on Oct. 2, 1976. (strain No. To-13k5)

IFO 1880, 1881 and 1882 were isolated from soil collected in Towada, Aomori Pref., on Oct. 5, 1977. (strain No. Tw-106a6, Tw-108a3, and Tw-111n4)

These are the first record of the species in Japan.

[K. Mikata & I. Banno]

12. Pichia bovis van Uden & do Carmo-Sousa

van Uden, N. & do Carmo-Sousa, L. 1957. J. Gen. Microbiol. **16**: 385; Kreger-van Rij, N. J. W. 1970. *In* The Yeasts, A Taxonomic Study, ed. by J. Lodder, North-Holland Publ. Co., Amsterdam, p. 470.

IFO 1886

Growth in glucose-yeast extract-peptone water: After 3 days at 25 C the cells are oval to long-oval, 2.7–4.4×3.9–6.6 μm; single or in pairs. A sediment and a thin, dull, creeping pellicle are formed.

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

Growth on YM agar: After 3 days at 25 C the cells are oval to long-oval, 2.1–4.2× 3.2–6.6 μm; single or in pairs.

After one month at 20 C the streak culture is creamish-brown, dull, smooth, low-convex and butyrous. The margin is undulate.

Dalmau plate cultures on corn meal agar: A primitive pseudomycelium is formed.

Formation of ascospores on YM agar and corn meal agar: Vegetative cells are directly transformed into asci. The spores are hat-shaped; one to four are formed per ascus. They are liberated soon after maturation.

Physiological characteristics are presented in Table 1.

The strain shows resemblance in its assimilation pattern of carbon compounds to *Pichia rhodaensis* and *P. toletana* as well as to *P. bovis*. However the authors consider the strain a member of *P. bovis* because of the fact that the pseudomycelium produced is very primitive and no conjugation between vegetative cells precedes ascus formation.

IFO 1886 was isolated from a partially decayed leaf collected on Mt. Togakushi, Nagano Pref., on Oct. 2, 1976. (strain No. To-16a2)

This is the first record of the species in Japan.

[K. Mikata & I. Banno]

13 to **15. Pichia pijperi** van der Walt & Tscheuschner

van der Walt, J. P. & I. T. Tscheuschner. 1957. Antonie van Leeuwenhoek **28**: 186; Kreger-van Rij, N. J. W. 1970. *In* The Yeasts, A Taxonomic Study, ed. by J. Lodder, North-Holland Publ. Co., Amsterdam, p. 513.

IFO 1791, 1792 and 1887

Growth in glucose-yeast extract-peptone water: After 3 days at 25 C the cells are oval, long-oval, and cylindrical, 2.4–5.1×3.8–8.8 μm; single, in pairs or in short chains. A sediment and a thin, dull, creeping pellicle are formed.

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

Growth on YM agar: After 3 days at 25 C the cells are oval to ellipsoidal, cylindrical and apiculate, 2.3–4.0×4.0–12.5 μm; single, in pairs or in chains.

After one month at 20 C the streak culture is cream-colored to yellowish, soft, dull, flat and partly wrinkled in the middle. The margin is entire to undulate.

Dalmau plate cultures on corn meal agar: Pseudomycelium is well developed. It consists of chain of curved and elongate cells arranged in trees.

Formation of ascospores on corn meal agar and V-8 agar: Vegetative cells are directly transformed into asci. The spores are hat-shaped; two to four are formed per ascus. They are liberated soon after maturation.

Physiological characteristics are presented in Table 1.

IFO 1791 was isolated from a flower collected on Mt. Odaigahara, Nara Pref., on Aug. 27, 1973. (strain No. O-107m7)

IFO 1792 was isolated from moss collected on Mt. Odaigahara, Nara Pref., on Aug. 27, 1973. (strain No. O-110m5)

IFO 1887 was isolated from a partially decayed leaf collected on Mt. Odaigahara, Nara Pref., on Aug. 27, 1973. (strain No. O-127m6)

These are the first record of the species in Japan.

[K. Mikata & I. Banno]

16 and **17. Pichia terricola** van der Walt

van der Walt, J. P. 1957. Antonie van Leeuwenhoek **23**: 25; Kreger-van Rij, N. J. W. 1970. *In* The Yeasts, A Taxonomic Study, ed. by J. Lodder, North-Holland Publ. Co., Amsterdam, p. 537.

IFO 1798 and 1888

Growth in glucose-yeast extract-peptone water: After 3 days at 25 C the cells are oval to long-oval, 3.8–6.4×4.5–7.1 μm; single or in pairs. A sediment and a thin, dull, creeping pellicle are formed.

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

Growth on YM agar: After 3 days at 25 C the cells are oval to long-oval and cylindrical, 2.6–5.1×4.3–7.4 μm; single or in pairs.

After one month at 20 C the streak culture is yellowish-brown, semi-dull, flat and butyrous. The margin is undulate.

Dalmau plate cultures on corn meal agar: Pseudomycelium is not formed.

Formation of ascospores on YM agar, corn meal agar and V-8 agar: Vegetative cells are directly transformed into asci. The spores are spherical and have a slightly warty appearance; one or two are formed per ascus. The presence of many spores give the culture a brown color.

Physilogical characteristics are presented in Table 1.

IFO 1798 and 1888 were isolated from partially decayed leaves collected on Mt. Odaigahara, Nara Pref., on Aug. 27, 1973. (strains No. O-126a8, O-127a12)

These are the first record of the species in Japan.

[K. Mikata & I. Banno]

IFO Res. Comm. 9,
71–77, 1979 (March)

DESCRIPTIVE CATALOGUE OF IFO BACTERIAL COLLECTION 4

Some strains used in various researches have been identified as known species. However the descriptions of their characteristics in published papers are incomplete. In the routine work of updating data of strains in the collection, a few of the strains obtained from other organization were reidentified as different species. The object of this catalogue is to provide descriptions of these strains. The following descriptions are arranged in alphabetical order of the scientific name. The authors of the descriptions are indicated in brackets.

31. **Azotobacter vinelandii** Lipman 1903

IFO 3741

Cells: Large ovoid rods, 1.4–1.6×2.0–3.6 μm; as cultures age, the cells become coccoid; motile by peritrichous flagella; gram-negative; thick-walled cysts present.

Colonies on Ashby's medium: Circular, entire, convex, translucent, mucoidal, gray; water-soluble yellow-green pigment produced.

Catalase: Positive.
Growth in nitrogen-free medium: Positive.
Growth in peptone water: Negative.
Hydrolysis of casein: Negative.
Hydrolysis of starch: Positive.
Hydrolysis of gelatin: Negative.
Litmus milk: Alkaline.
Temperature for growth: Optimum, 28–30 C; grows at 17 C and 37 C.
The following compounds utilized as sole sources of carbon: glucose, galactose, mannitol, rhamnose, starch, glycerol, acetate, succinate and lactate. Xylose, arabinose, lactose and citrate not utilized as sole sources of carbon.

This strain was obtained from the Institute of Applied Microbiology, University of Tokyo, Japan, in 1958, under the name of *Azotobacter agilis* Beijerinck.

[T. Sakane & I. Banno]

32 to 35. **Cellulomonas flavigena** (Kellerman & McBeth) Bergey *et al.* 1923

IFO 3747, 3748, 3753 and 12680

	IFO 3747	IFO 3748	IFO 3753	IFO 12680
Cells: Width, μm	0.4	0.4	0.4–0.5	0.4–0.5
Length, μm	0.8–1.8	0.5–1.0	0.8–1.6	0.8–1.2

(Continued)

Gram-reaction	+	−	+	+
Pigmentation	white	gray	yellow	yellow
Catalase	+	+	+	+
Reduction of NO_3 to NO_2	+	+	+	+
Decomposition of cellulose	+	+	+	+
Hydrolysis of				
Casein	w	+	+	w
Starch	+	−	+	+
Esculin	+	+	+	+
Gelatin	w(late)	w(late)	+	+
Acid production from				
Glucose	+	+	+	+
Arabinose	+	+	+	+
Xylose	+	+	+	+
Trehalose	+	+	−	w
Mannitol	−	−	+	−
Utilization as sole carbon sources				
Glucose	+	+	+	+
Xylose	+	+	w	+
Arabinose	+	+	w	+
Citrate	−	−	−	−
Acetate	w	−	−	−
Succinate	−	−	−	−
Arginine	−	−	−	−
Asparagine	−	−	−	−
Ethanol	−	−	−	−
p-Hydroxybenzoate	−	−	−	−
Growth at 37 C	+	+	+	+
Growth in vitamin-deficient medium	−	−	−	−

Abbreviation: w, weakly positive.

The strains IFO 3747, 3748 and 3753 were obtained from the American Type Culture Collection, in 1958, under the name of *Cellulomonas uda* (Kellerman *et al.*) Bergey *et al.*, *Cellulomonas gelida* (Kellerman *et al.*) Bergey *et al.*, and *Cellulomonas cellasea* (Kellerman *et al.*) Bergey *et al.*, respectively. The strain IFO 12680 was obtained from the Central Research Laboratories, Ajinomoto Co., Inc., Kawasaki, Japan, in 1958, under the name of *Cellulomonas biazotea* (Kellerman *et al.*) Bergey *et al.*

[T. Sakane & I. Banno]

36 and **37. Chromobacterium lividum** (Eisenberg) Bergey *et al.* 1923
IFO 3740 and 12613

	IFO 3740	IFO 12613
Cells: Width, μm	0.5–0.6	0.4–0.5
Length, μm	1.2–4.0	1.0–2.4
Gram-reaction	−	−

(Continued)

Flagellation	polar	polar
Pigmentation	violet	violet
Catalase	+	+
Oxidase	+	+
Methyl red test	−	−
V-P test	−	−
Reduction of NO_3 to NO_2	−	w
Arginine dihydrolase	−	−
Hydrolysis of		
Casein	+	−
Esculin	+	+
Gelatin	+	w
Starch	−	−
Sensitivity to		
Benzylpenicillin, 50 μg/ml	R	R
Tetracycline, 10 μg/ml	S	S
Growth at 4 C	+	+
Growth at 37 C	−	−
Gas production from glucose	−	−
Acid production from		
Glucose	−	−
Arabinose	−	−
Xylose	−	−
Trehalose	−	−

Abbreviation: w, weakly positive; R, resistant; and S, sensitive.

The strain IFO 3740 was obtained from the Institute of Biology, Czechoslovak Academy of Sciences, Prague, CSSR, in 1958, under the name of *Chromobacterium violaceum* Bergonzini. The strain IFO 12613 was obtained from the Department of Microbiology, Shizuoka University, Japan, in 1968, under the name of *Chromobacterium amethystinum* (Chester) Holland.

[T. Sakane & I. Banno]

38. **Clostridium kluyveri** Barker & Taha 1942

Barker, H. A., & S. M. Taha, 1942. J. Bacteriol. **43**: 347

IFO 12016

Young cells: Gram-negative rods, 0.8–0.9×2.3–10.0 μm; motile by peritrichous flagella.

Spores: Ovoid; subterminal; sporangia not definitely swollen.

Colonies on yeast extract-ethanol agar medium under anaerobic condition: Circular, entire, low-convex, smooth, white; growth is slow and scant.

No growth in the usual laboratory media.

Condition for growth: Strictly anaerobic; requires 1 % or more concentration of yeast extract.

Temperature for growth: Grows at 25 C to 37 C.

The strain was obtained from the American Type Culture Collection as number 8527, in 1963, and examined for characteristics after purification in 1976. The properties described above agree with those originally reported by H. A. Barker and S. M. Taha.

[T. Sakane & I. Banno]

39. **Haffnia alvei** Møller 1954

IFO 3731

Cells: Gram-negative rods, 0.5–0.6×0.6–1.2 μm; motile by lateral flagella.
Colonies on nutrient agar: Circular, entire, low-convex, smooth, gray.
Catalase: Positive.
Oxidase: Negative.
Urease: Negative.
Reduction of NO_3 to NO_2: Positive.
Methyl red test: Negative.
V-P test: Positive.
Production of indole: Negative.
Hydrolysis of gelatin: Negative.
Hydrolysis of arginine: Negative.
Decarboxylation of lysine: Positive.
Decarboxylation of ornithine: Positive.
Deamination of phenylalanine: Negative.
Oxidation of malonate: Positive.
Oxidation of gluconate: Positive.
Utilization of citrate: Positive.
Temperature for growth: Grows at 10 C to 37 C, not grow at 47 C.
Acid and gas from glucose, xylose, maltose, sucrose, mannitol, glycerol and starch. No acid and no gas from arabinose, lactose, salicine, esculin, sorbitol, dulcitol and inositol.

This strain was obtained from the Faculty of Agriculture, Tohoku University, Japan, in 1958, under the name of *Bacterium cadaveris* Gale & Epps.

[T. Sakane & I. Banno]

40 to 42. **Mycobacterium smegmatis** (Trevisan) Lehmann & Neumann 1899

IFO 3082, 3153 and 3154

	IFO 3082	IFO 3153	IFO 3154
Cells: Width, μm	0.4–0.5	0.4–0.5	0.4–0.5
Length, μm	1.6–4.0	2.0–6.0	2.0–4.0
Gram-reaction	+	+	+

(Continued)

Acid-alcohol fast	+	+	+
Pigmentation	creamy	creamy	creamy
Catalase	+	+	+
Urease	+	+	+
Reduction of NO_3 to NO_2	+	+	+
Deamination of phenylalanine	−	−	−
Acetamidase	+	+	+
Hydrolysis of			
Casein	−	−	−
Starch	−	−	−
Tween 80	+	+	+
Gelatin	−	−	−
Growth at			
37 C	+	+	+
46 C	+	+	+
55 C	−	−	−
Tolerance for			
Malachite green, 0.01%	−	−	−
Methyl violet, 0.01%	−	−	−
Pyronin G, 0.01%	+	+	+
Heat tolerance, 60 C for 4 hr	−	−	−
Acid production from			
Glucose	w	+	+
Xylose	w	w	w
Galactose	−	−	−
Rhamnose	w	w	w
Glycerol	w	+	w
Mannitol	w	+	w
Inositol	w	w	w
Utilization as sole sources of carbon			
Glucose	+	+	+
Mannitol	+	+	+
Citrate	w	w	w
Acetate	+	+	+
Arginine	w	w	w
Glutamate	+	+	+
p-Hydroxybenzoate	−	−	−

Abbreviation: w, weakly positive.

These strains were obtained under the name of *Mycobacterium avium* Chester; IFO 3082 from the Research Institute for Microbial Diseases, Osaka University, Japan, in 1952, IFO 3153 from the Medical School, Osaka University, Japan, in 1952, and IFO 3154 from the Faculty of Agriculture, University of Tokyo, Japan, in 1952.

[T. Sakane & I. Banno]

43 to **46. Pseudomonas caryophylli** (Burkholder) Starr & Burkholder 1942
IFO 12950, 12951, 12952 and 12953

	IFO 12950	IFO 12951	IFO 12952	IFO 12953
Gram-reaction	−	−	−	−
Oxidase	+	+	+	+
Numbers of flagella	1	>1	>1	>1
Production of diffusible pigments	−	−	−	−
Accumulation of poly-β-hydroxybutyrate	+	+	+	+
Arginine dihydrolase	w	w	w	w
Denitrification	+	+	+	+
Hydrolysis of				
Starch	−	−	−	−
Casein	−	−	−	−
Gelatin	−	−	−	−
Esculin	−	w	−	−
Tween 80	−	−	−	−
Growth factors required	−	−	−	−
Utilization as sole sources of carbon				
Glucose	+	+	+	+
Xylose	+	+	+	+
Sucrose	+	+	+	+
D-Arabinose	+	+	+	+
L-Arabinose	+	+	+	+
Citrate	+	+	+	+
Lactate	+	+	+	+
Glycollate	NT	+	w	+
Malonate	−	−	−	−
Arginine	+	+	+	+
Alanine	NT	+	+	+
Betain	+	+	+	+
Ethanol	+	+	+	+
p-Hydroxybenzoate	−	+	−	−
m-Hydroxybenzoate	−	−	−	−
β-Hydroxybutyrate	+	+	w	w
Growth at 40 C	+	+	+	+

Abbreviation: w, weakly positive; and NT, not tested.

These strains were isolated by Y. Nakasuji, Institute for Fermentation, Osaka, from diseased silkworm, and were entered in the IFO List of Cultures, 5th edition, as *Pseudomonas ovalis* Chester.

[T. Sakane & I. Banno]

47. **Pseudomonas cepacia** Burkholder 1950

IFO 3739

Cells: Gram-negative rods, 0.4–0.6×0.8–1.2 μm; motile by 1 to 4 polar flagella. Colonies on nutrient agar: Circular, entire, low-convex, smooth, dark yellow; no diffusible pigment produced.

Catalase: Positive.

Oxidase: Positive.

Accumulation of poly-β-hydroxybutyrate: Positive.
Arginine dihydrolase: Negative.
Denitrification: Negative.
Hydrolysis of starch: Negative.
Hydrolysis of gelatin: Positive.
Hydrolysis of casein: Positive.
Hydrolysis of esculin: Positive.
Reduction of NO_3 to NO_2: Weakly positive.
Litmus milk: Alkaline; peptonized.
Growth at 40 C: Positive.
Growth in a simple mineral medium with ammonium salts and glucose: Positive.
Sensitivity to benzylpenicillin, 50 μg/ml: Resistant.
Sensitivity to tetracycline, 50 μg/ml: Resistant.
Acid from glucose, fructose, arabinose, trehalose and mannitol.
The following compounds utilized as sole sources of carbon: glucose, galactose, arabinose, mannitol, cellobiose, glycerol, citrate, acetate, succinate, lactate, arginine, asparagine and betain. Xylose, rhamnose, starch, ethanol and *p*-hydroxybenzoate not utilized as sole sources of carbon.

This strain was obtained from the Institute of Biology, Czechoslovak Academy of Sciences, Prague, CSSR, in 1958, under the name of *Chromobacterium ianthinum* Gilman.

[T. Sakane & I. Banno]

ABSTRACTS 1977-1978

Transport system for citric acid in *Proteus vulgaris*

K. IMAI

Agric. Biol. Chem. **41**: 733–735 (1977)

Strains of *Proteus vulgaris* utilized only citric acid among the tricarboxylic acids. Citric acid uptake in *P. vulgaris* IFO 3045 was mediated by a single transport system induced by citrate, isocitrate or *cis*-aconitate and possessed a weak affinity for isocitric acid. This citric acid trasnport system corresponds to the system of *Salmonella typhimurium* or *Serratia marcescens*, which is induced by citrate, isocitrate or *cis*-aconitate and transports citric and isocitric acids.

Preservation of *Thiobacillus* by L-drying

T. SAKANE and I. BANNO

J. Japan. Soc. Research Freez. Dry. **23**: 18–22 (1977)

L-drying was applied to the preservation of two species of *Thiobacillus*. The cells of *Thiobacillus thiooxidans*, an obligate chemolithotroph, harvested at early stationary growth phase were submitted to L-drying after separation from sulfur particles and washing with the fresh medium. The dried specimen exhibited a survival of about 1 % of the cells immediately after drying. It was neccssary to rehydrate the dried specimen with a large amount of the medium for recovery of the cells, to minimize growth-inhibitory effect of the organic compound used as a protective agent against drying. While the cells of *T. novellus*, a heterochemolithotroph, grown on nutrient agar medium were dried according to the same simple procedure as *Escherichia coli* and 10 % of the cells survived.

After preservation of the dried specimen for 6 months at 5 C, no appreciable decrease in survival was found in the two species. Long term preservation of *Thiobacillus* by L-drying is probably successful.

[in Japanese]

List of microfungi recorded at myclogical forays held at Mt. Daisen, Tottori Prefecture 1974-1975

T. YOKOYAMA and T. ITO

Trans. Mycol. Soc. Japan **18**: 234–241 (1977)

Twenty four species of the Deuteromycotina and one species of the Ascomycotina

collected at Mt. Daisen, Tottori Prefecture, were described briefly. Among them, *Articulospora tetracladia, Tetracladium setigerum* and *Varicosporium elodeae* which have been reported as the most common worldwide aquatic Hyphomycetes were found on the fallen leaves in deciduous oak forests. *Chaetopsina ramifera* was reported as the first record in Japan, although this has often been found on the fallen leaves of *Castanopsis* spp. in the southwestern area of Japan. *Pseudodictyosporium wauense, Sporidesmium flexum* and *Arxiella terrestris* were also reported as the second record in Japan. *Kamatia indica* was reduced to a synonym of *P. wauense.*

Tricarboxylic acid transport systems in *Proteus mirabilis*

K. IMAI

J. Gen. Appl. Microbiol. **24**: 279–285 (1978)

Tricarboxylic acid transport systems in *Proteus mirabilis* IFO 3849, which utilized citric, isocitric, and *cis*-aconitic acids, were characterized. The results show that *P. mirabilis* possesses three inducible transport systems for the tricarboxylic acids. The first system is induced by citrate, isocitrate, or *cis*-aconitate and transports citric and isocitric acids. The affinity of isocitric acid for this system is lower than that of citric acid. The second system, induced by the same acids as in the first system, transports *cis*-aconitic acid, requires Mg^{2+} ions, and is stable at pH 8.6 but unstable at pH 7. The third system is induced by citrate and carries citric acid.

Accumulation of a new monosaccharide, 1-deoxy-D-*altro*-heptulose (1-deoxy-sedoheptulose) by transketolase mutants of *Bacillus pumilus*

A. YOKOTA, K. SASAJIMA and S. HORII*

Agric. Biol. Chem. **42**: 2245–2252 (1978)

Transketolase mutants derived from *Bacillus pumilus* IFO 12089 produced two unknown compounds. One of them was isolated from the culture broth and was determined to be a new monosaccharide, 1-deoxy-D-*altro*-heptulose (1-deoxy-sedoheptulose) (**1**). Compound **1** was easily converted into its non-reducing anhydride in acidic solution at room temperature, which was identified as 2,7-anhydro-1-deoxy-β-D-*altro*-heptulopyranose (**2**). Compounds **1** and **2** were also chemically synthesized from 2,7-anhydro-β-D-*altro*-heptulopyranose (**3**) to confirm the chemical structures.

* Medicinal Research Laboratories, Central Research Division, Takeda Chemical Industries, Ltd.

PRESENTATION OF PAPERS AT SCIENTIFIC MEETINGS 1977-1978

Author(s)	Title	Scientific Meeting
K. IMAI & I. BANNO	Transport systems for tricarboxylic acids in the species belonging to Enterobacteriaceae (V).	Agricultural Chemical Society of Japan. Yokohama (April, 1977)
K. SASAJIMA, T. KUMADA & A. YOKOTA	Pleiotropic properties of *Bacillus subtilis* mutant lacking transketolase.	Agricultural Chemical Society of Japan. Yokohama (April, 1977)
K. IMAI, I. BANNO & T. IIJIMA	Mapping of *tct* (tricarboxylic acid transport) genes in *Salmonella typhimurium*.	Japanese Society for Bacteriology. Osaka (April, 1977)
T. SAKANE & I. BANNO	Preservation of *Thiobacillus* by L-drying.	Japanese Society for Research of Freezing and Drying. Tokyo (April, 1977)
T. YOKOYAMA	On a species of *Ciliochorella* on *Magnolia ovobata*.	Mycological Society of Japan. Okayama (May, 1977)
T. YOKOYAMA	Fungi on the fallen leaves. Symposium on the role of fungi in ecosystems.	Japan Academy of Sciences. Tokyo (June, 1977)
T. YOKOYAMA & K. TUBAKI*1	Fungi Imperfecti of Japan and South Pacific Islands.	2nd International Mycological Congress. Tampa, Florida (August, 1977)
K. IMAI	Properties of tricarboxylic acid transport systems in bacteria belonging to Enterobacteriaceae and its relation to the taxonomy.	Society of Fermentation Technology, Japan. Osaka (November, 1977)
H. MORI*2 & I. BANNO	Study on ploidy of strains of *Saccharomyces rouxii* preserved in IFO.	Society of Fermentation Technology, Japan. Osaka (November, 1977)
K. IMAI	Isolation of bacteria capable of utilizing tricarballylate by enrichment culture.	Agricultural Chemical Society of Japan. Nagoya (April, 1978)
A. YOKOTA, K. SASAJIMA & S. HORII*3	Accumulation of a new monosaccharide, 1-deoxy-D-*altro*-heptulose by the transketolase mutants of *Bacillus pumilus*.	Agricultural Chemical Society of Japan. Nagoya (April, 1978)

*1 Institute of Biological Sciences, The University of Tsukuba.
*2 Noda Institute for Scientific Research.
*3 Medicinal Research Laboratories, Central Research Division, Takeda Chemical Industries, Ltd.

Author(s)	Title	Scientific Meeting
K. MIKATA & I. BANNO	Flora of ascomycetous yeasts in forest.	Mycological Society of Japan. Tsukuba (April, 1978)
I. BANNO, T. SAKANE & T. IIJIMA	Mutational problem in preservation of bacteria (*Escherichia coli*) by L-drying.	Society for Cryobiology. Tokyo (August, 1978)
K. SASAJIMA & T. KUMADA	Change of catabolite repression in *Bacillus subtilis* mutant lacking transketolase.	Japanese Biochemical Society. Kyoto (November, 1978)

MISCELLANEOUS SCIENTIFIC PAPERS

T. IIJIMA, 1977. Preservation of bacteriophages. *In* T. Nei (ed.) Preservation of microorganisms. p. 107–114. University of Tokyo Press. (in Japanese)

T. YOKOYAMA, 1977. Preservation of filamentous fungi (II). *In* T. Nei (ed.) Preservation of microorganisms. p. 317–353. University of Tokyo Press. (in Japanese)

T. YOKOYAMA, 1977. Tobacco mosaic virus. *In* A. Kawamura, Jr. (ed.) Fluorescent antibody techniques and their application (2nd Edition). p. 217–220. University of Tokyo Press. (in Japanese)

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No. 8, 1977

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CORRECTIONS

In the issue of IFO Research Communications No. 8, the following corrections should be made.

Page	Line	Type	Should read
2	12	Insernational	International
	13–14	for this for this	for this
9	Running head	on	of
10	20	isolated	isolates
12	Table 3, column 2		
	21	*Hansnula californica*	*Hansenula californica*
40	Table 5, right column		
	14	*citrium*	*citrinum*
60	21	samll	small
64	Table 1, footnote a	thord	third
66	Table 2, column 1		
	5	SU418 × M189(P22)$^+$	SU418 × M272(P22)$^+$
73	Fig. 4.	pyruvate, ▽—△	pyruvate, ▽—▽
76	4	L-arabonose	L-arabinose
79	21	sphaerical	spherical
	28	sphaerical	spherical
81	11	latin	Latin
82	2	sphaerical	spherical
	38	latin	Latin
83	39	sphaerical	spherical
84	31	sphaerical to subsphaerical	spherical to subspherical
86	Plate 1, legend		
	4	A = 500 μm	A = 250 μm
86–89	Plates 1–4, legends	All of magnifications in parentheses should be deleted.	
91	7	sci entific	scientific
112	5	2-butyne-1,4 diol	2-butyne-1,4-diol

発酵研究所研究報告　第9号

昭和54年2月25日　印刷
昭和54年3月1日　発行

定価 800 円

編　集　坂　野　勲
発行人　飯　島　貞　二
発行所　財団法人 発 酵 研 究 所
大阪市淀川区十三本町2丁目17番85号
印刷所　日 本 印 刷 出 版 株 式 会 社
大阪市福島区吉野1丁目2番7号