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No. 10

(Annual Report 1979-1980)

1981

INSTITUTE FOR FERMENTATION, OSAKA

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

Chobei TAKEDA

1905–1980

Chobei TAKEDA, chairman of the Board of Trustees of the Institute for Fermentation, Osaka died suddenly of heart failure on September 1, 1980.

He was born in 1905 in Osaka, the eldest son of Mr. Chobei TAKEDA V, who worthly upheld the great traditions of the early pioneers of drug wholesale in Doshomachi, Osaka. After graduation from Keio University in 1927 he went to England and studied in Cambrige University for two years. During his stay in England, he acquired a modern and international appreciation of the need to develop the drug wholesale into a pharmaceutical industry.

In 1943 he became president of Takeda Chemical Industries Ltd. and served in that capacity till 1974, when he became chairman of the Board of directors. During these years he devoted his life to the development of the pharmaceutical industry. When the Institute for Fermentation, Osaka was founded in 1945, he became the chairman of its Board of Trustees. He was appointed Vice-President of the Osaka Chamber of Commerce and Industry in 1974 and served in these capacities till his death. He devoted his life unceasingly to service not only to the pharmaceutical industry but to many other fields of science and society.

With his death, we have lost a man of intellect and integrity. Those of us privileged to have known him grieve the loss of a great leader.

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THE REPORT OF THE DIRECTOR

The Institute for Fermentation, Osaka is a private, but publicly accessible organization which collects, preserves and provides authentic and important living cultures of microorganisms to universities and research organizations in Japan and other countries. The kinds of microorganisms accepted in the IFO collection are fungi, yeasts, bacteria and actinomycetes, all non-pathogenic to man. Preservation and provision of authentic and reference strains is one of the most important roles of a culture collection.

To accomplish these roles, the organization must have sufficient staff to examine the characteristics of strains in the collection in the broader scope of taxonomy. It must be equipped with modern facilities and techniques to ensure the deposited strains to remain viable and uncontaminated for a long period. And it must have the administrative ability to accession, store and release strains efficiently and precisely. A data center is essential for compilation of data and provision of up-to-date information about the strains. Beside of these basic roles, the organization has been involved in the accession of deposits of patented strains. In keeping with this increasing responsibility, the above capacities become indispensable.

In September 1979, an IBM OS-6 Information Processor was installed for processing records and documents concerning the accession and distribution of cultures. Records and data are stored in magnetic diskettes and can be promptly retrieved. In this issue of IFO research Communications, a new catalogue of cultures, "Catalogue of newly accepted strains", is published listing the cultures added to the collection since the publication of the most recent edition of the "IFO List of Cultures". The cultures appearing in this catalogue can be distributed under the same conditions as those strains listed in the "IFO List of Cultures". Details of these strians are available on request.

Besides raising the efficiency of management of the culture collection, we have made technological innovation in the preservation of cultures in recent years. Programs for authentication and long-term preservation of stored strains, are actively progressing, especially in the bacteria and yeast section. About 90% of bacterial cultures are stored by L-drying, and a modified method of L-drying is applied to yeasts. Since October 1980, some fungal cultures have been stored at ultra-low temperature. This method will be extended gradually to the remaining fungi and is expected to alleviate the laborious everyday workload. In the biochemical and genetic section, the mechanism of pleiotropy of a transketolase mutant of *Bacillus subtilis* has been investigated and shown to involve a change in the cell surface. The biosynthetic pathway of a new intermediate, 1-deoxy-D-altro-heptulose, is under investigation.

The Committee for Confirmation of ISP cultures (International Streptomyces Project) in Japan conducted the third confirmatory test of the ISP strains preserved in IFO. Almost all of the approximately 500 strains tested for their viability and

characteristics after preservation were found to be intact.

Mr. Akio Takeda, Vice-President of Takeda Chemical Industries Ltd., died in February 1980, and his bereaved family offered to donate their inheritance to the foundation of the institute. The Board of Trustees accepted the offer which has increased the foundation to ¥555,400,000. At the annual meeting of the Board of Trustees in June 1980, some changes of member were approved. Mr. Ryohei Kisaki, attorney-at-law, and Dr. Toshio Miwatani, Professor of Osaka University, were appointed Auditors, and Dr. Sueo Tatsuoka was appointed to the Board.

I am at a loss to express my heartful condolence to the bereaved familiy of Mr. Chobei Takeda, Chairman of the Board, who died suddenly on September 1, 1980, at the age of 75, after unceasing services not only to the pharmaceutical industry but to sciences and society in general. The foundation and development of the institute was due to his passionate support for this fundamental undertaking.

In the past two years, members of the institute have participated in mycological forays in Japan and other countries. Dr. Yokoyama attended the XIVth Pacific Science Congress in Khabarovsk, from August 18–30, 1979 and presented a paper on "Microfungal flora in Japan" and joined the Pre-Congress Tour around Magadan. The annual foray of the Mycological Society of Japan was held at Yamanaka, Ishikawa prefecture, in 1980, and staff of the institute joined the foray and collected samples. The total number of cultures stored in the IFO Collection reached to 10800 at the end of 1980.

Mr. Ko Imai received a doctorate from Hokkaido University in March 1979. He is now studying in Dr. Hall's laboratory, Department of Microbiology, University of Connecticut as a postdoctoral scholar. His research theme at the University of Connecticut is lactose utilization in *Klebsiella*. Director T. Iijima attended the symposium on the "Preservation of Microorganism and Management of Culture Collection" held in Seoul in September 1979, and was also able to visit some of the research organizations in Korea.

(T. IIJIMA)

Heartful condolences are offered to
Chairman Mr. Chobei Takeda who passed away on 1st September, 1980, and
Professor emeritus Yukinori Tsunematsu who passed away on 10th May, 1980.
They gave great contribution to the establishment and the development of the Institute for Fermentation, Osaka.

IFO Res. Comm. **10**,
3-9, 1981 (March)

CELL SURFACE CHANGE OF *BACILLUS SUBTILIS* PLEIOTROPIC MUTANT LACKING TRANSKETOLASE

Ken-ichi SASAJIMA and Toshio KUMADA

Summary

A *Bacillus subtilis* pleiotropic mutant lacking transketolase (*tkt*) exhibited the following properties relative to the parental strain: (i) cells of the *tkt* mutant formed chains during exponential growth and were thicker than those of the parental strain; (ii) the *tkt* mutant was more sensitive to bacteriophages SP10 and SP01 than the parental strain; (iii) walls of the *tkt* mutant underwent autolysis at a slower rate than did those of the parental strain; and (iv) sporulation frequency of the *tkt* mutant decreased remarkably. These data suggest some change(s) in the cell surface structure and function of the *tkt* mutant of *B. subtilis*.

The bacterial cell surface has a variety of complex and important functions; it is a barrier against toxic substances and invaders, and participates in nutrient transport, production of energy, excretion of extracellular enzymes and motility. How the cell surface exhibits these functions properly and timely whenever and wherever necessary is a mystery. And how is the cell surface constructed so elaborately and so orderly? Although much is known about the biogenesis of certain specific functional components of the cell surface, the total biogenesis of the orderly cell surface remains obscure.

In the course of studies on isolation and characterization of carbohydrate-metabolism mutants of *Bacillus subtilis*, we found that transketolase mutation generated pleiotropic changes; the transport function of the PEP-dependent D-glucose phosphotransferase system was defective (27), and the synthesis of sorbitol catabolic enzymes was insensitive to catabolite repression by D-glucose, while the synthesis of D-mannitol catabolic enzymes was hypersensitive to D-glucose repression and, moreover, sensitive to repression by such sugars as D-gluconate, L-arabinose and D-xylose which have the same configuration of hydroxyl groups at C-2 and C-3 as D-glucose. These pleiotropic properties seem to be related to a change in the cell surface of the *tkt* mutant which is caused by transketolase deficiency. The *tkt* mutant may be useful in studying bacterial cell surface biogenesis.

This study was undertaken to elucidate the changes in the cell surface of the *tkt* mutant of *B. subtilis*.

Materials and Methods

Bacterial strains and bacteriophages. The bacterial strains employed were *B.*

subtilis strains IFO 12114, ATCC 23059, ATCC 27370 and *tkt* mutant BG2607 derived from IFO 12114. Isolation of the *tkt* mutant BG2607 was described previously (25). Bacteriophages SP10 and SP01 were used to test bacteriophage sensitivity.

Media. Slightly modified Spizizen's synthetic medium (24) was mainly used for cultivation of bacteria. Shikimic acid was substituted for the mixture of aromatic amino acids to allow the growth of the *tkt* mutant. Complete medium (24) was used for bacteriophage experiments. Potato agar slants were used for sporulation (24).

Photography. Cells grown in the synthetic medium containing 1% sorbitol plus 1% D-glucose as carbon sources for 6 hr (exponential growth phase) were spread on thin agar layers on slide glasses and photographed with phase-contrast optics under a Nikon microscope.

Bacteriophage sensitivity. Bacteriophages SP10 and SP01 were grown on soft agar plates of complete medium in, respectively, *B. subtilis* strains ATCC 23059 and ATCC 27370 as propagating strains, then suspended in complete medium and stored in a refrigerator.

Zero point one milliliter of the overnight culture of bacteria in the complete medium and 0.2 ml of diluted bacteriophage suspension were spread with 5 ml of melted soft agar on complete medium plates. The number of plaque-forming units was counted after overnight incubation at 37 C.

Autolytic activity. Exponential phase cells grown in the synthetic medium containing 1% sorbitol as carbon source were harvested by centrifugation, washed with distilled water three times, resuspended in distilled water and disrupted with a Kubota sonic oscillator. Cell wall fraction was washed with distilled water and separated from unbroken cells by differential centrifugation. The cell wall fraction was suspended in glycine buffer (pH 9.5) at a final concentration of 0.04 M. Autolytic activity was determined by tracing the decrease of optical density at 650 nm.

Sporulation frequency. Cells grown on potato agar slants for 2 days at 37 C were maintained in a refrigerator for 2 more days to stimulate sporulation. The cells were suspended in 5 ml of sterilized distilled water, which was divided into two 2.5-ml portions. One portion was heated at 80 C for 10 min. Viable cells in the non-heated and heated cell suspensions were counted by plating on the complete medium and incubating at 37 C for 1 day. Sporulation frequency is expressed as the percentage of heat-resistant spores in the total of both spores and vegetative cells.

Results and Discussion

As shown in Fig. 1, the *tkt* mutant BG2607 has a changed morphology. Cells of strain BG2607 formed chains during exponential growth, while those of the parental strain *B. subtilis* IFO 12114 were rods. Moreover, cells of the *tkt* mutant were thicker than those of the parental strain. Morphological change of gram-positive bacteria has previously been described in *B. subtilis* mutants defective in teichoic acid syn-

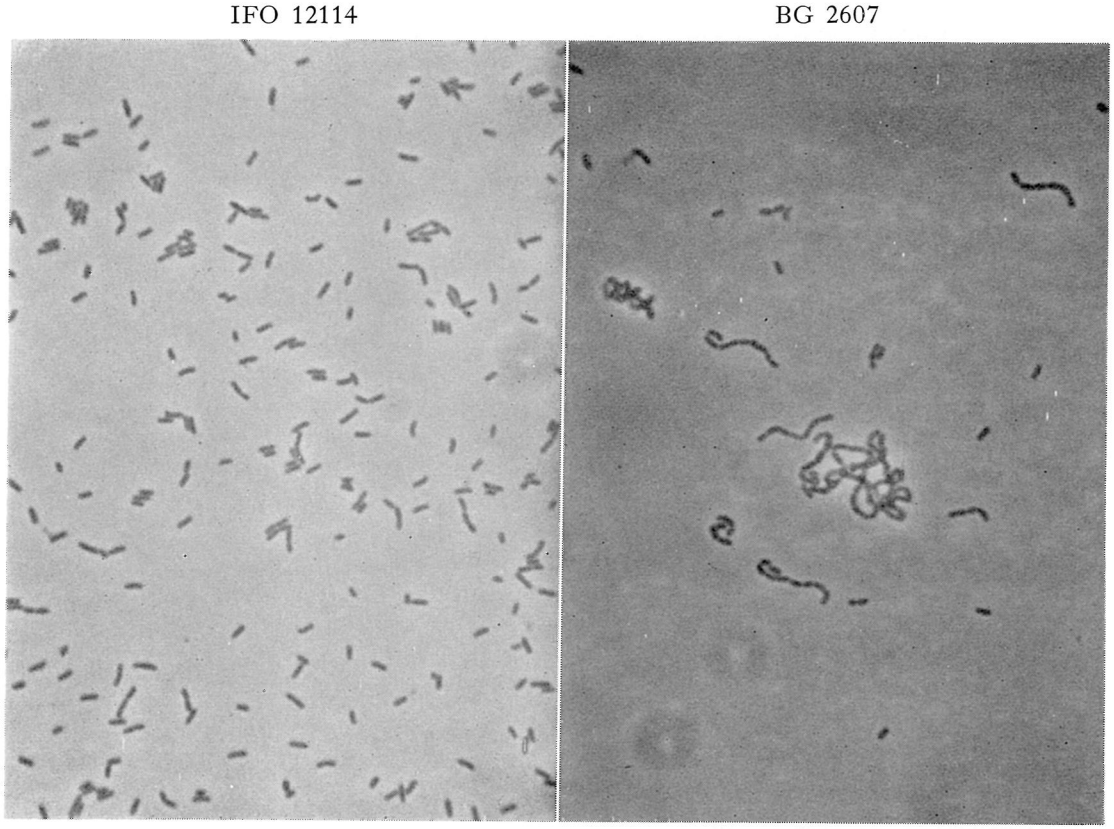

Fig. 1. Cell morphology of the parental strain IFO 12114 and the *tkt* mutant BG2607.

thesis (33), pneumococci whose teichoic acid is chemically modified by replacement of choline with ethanolamine (31, 32), bacteriophage-resistant mutants of *Staphylococcus aureus* (3, 7), autolytic-defective mutants of *B. subtilis* (2, 9, 10, 11, 12, 35), *B. licheniformis* (13) and *Streptococcus faecium* (30), a novobiocin-resistant mutant of *B. licheniformis* (20, 21) and NaCl-dependent mutants of *B. subtilis* and *B. licheniformis* (22, 23). Studies on the chemical composition of the cell surface of these mutants revealed that they are mostly defective in teichoic acid synthesis (3, 4, 28), teichuronic acid synthesis (13, 21) or lipoteichoic acid synthesis (30). The morphological change in the *tkt* mutant BG2607 suggests that there may be some change in its cell wall composition.

It has been reported that teichoic acid is required for adsorption of bacteriophage in *B. subtilis* (1, 15, 29, 36) and *S. aureus* (7). Since we suspected cell wall change in the *tkt* mutant, its sensitivity to bacteriophages was examined. The *tkt* mutant BG2607 was found to be more sensitive to bacteriophages SP10 and SP01 than the parental strain (Table 1), and thus the suspected change was confirmed.

As described above, autolytic enzymes seem to be involved in morphology. Autolytic enzymes have also been reported to have high affinity with teichoic acid (3, 5, 6, 16) or teichuronic acid (14, 21). The autolytic activity of the *tkt* mutant BG2607 was lower than that of the parental strain, though not markedly so (Fig. 2). This result also suggests a change in the composition of teichoic acid or teichuronic acid in the *tkt* mutant. Since transketolase is a key enzyme in the pentose phosphate path-

Table 1. Bacteriophage sensitivity of the parental strain IFO 12114 and the *tkt* mutant BG2607.

Strain	P.F.E.*	
	SP10	SP01
ATCC 23059	1	—
ATCC 27370	—	1
IFO 12114	0.27	0.24
BG 2607	4.1	5.1
	(15)	(21)

* P.F.E.: plaque forming efficiency. Figures indicate P.F.E. relative to that of the propagating strain. Figures in parentheses indicate P.F.E. relative to that of the parent strain.

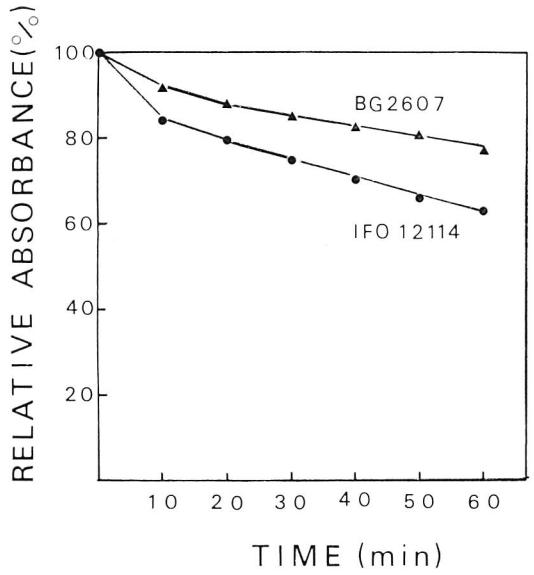

Fig. 2. Autolysis of cell walls of the parental strain IFO 12114 and the *tkt* mutant BG2607.

way, which is closely related to the synthesis of ribitol teichoic acid, this change is more likely to be in the teichoic acid composition.

Previous results, namely, the D-glucose transport deficiency (27) and the regulatory change in enzyme synthesis (26) in the *tkt* mutant, further suggest alteration of the cytoplasmic membrane. Rhaese *et al.* reported that adenosine 3'(2')-triphosphate-5'-triphosphate is involved in the initiation of sporulation and that this highly phosphorylated nucleotide is synthesized by membrane-bound enzymes (18, 19). The sporulation frequency of the *tkt* mutant BG2607 was decreased remarkably (Table 2). Regulation of sporulation seems to have changed in the *tkt* mutant. This result confirms the membrane change suggested by D-glucose transport deficiency (27).

As described the cell surface of the *tkt* mutant BG2607 of *B. subtilis* seems to be

Table 2. Sporulation frequencies of the parental strain IFO 12114 and the *tkt* mutant BG2607.

Strain	Frequency (%)
IFO 12114	92.5
BG 2607	4.8

altered. That the *tkt* mutant is also deficient in flagellation (unpublished data, K. Sasajima and T. Kumada) confirms the cell surface change. Similar pleiotropic staphylococcal mutants with altered utilization of sugars and composition of teichoic acid have been described by Korman (17) and Wolin *et al.* (34). A comparative investigation of the chemical composition of the cell wall and cytoplasmic membrane in the *tkt* mutant BG2607 and the parental strain *B. subtilis* IFO 12114 is now in progress.

The authors wish to express their thanks to Dr. T. Iijima, Director of Institute for Fermentation, 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. The authors also thank Dr. R. L. Gherna, American Type Culture Collection, for generously providing bacteriophages SP10 and SP01 with their propagating strains *B. subtilis* ATCC 23059 and ATCC 27370 and Takeda Chemical Industries, Ltd. for financial support of this research.

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ASCOMYCETOUS YEASTS ISOLATED FROM FOREST MATERIALS IN JAPAN

Isao BANNO and Kozaburo MIKATA

Summary

Yeasts were isolated from samples of soil, decayed fallen leaves, flowers, tree bark, mushrooms, dung, and fruits collected in forests in various parts of Japan, in order to survey naturally occurring yeasts. Since their population in these natural sources was minute, it was difficult to isolate yeasts by plating the samples directly on agar plate, and an enrichment method using 4 kinds of media was therefore adopted.

The isolated yeasts were identified from their morphology and physiological properties. This paper deals with the 633 strains regarded as ascomycetous yeasts, which were isolated from 332 of 519 samples. These included known species in the following genera: *Arthroascus* (1 species), *Debaryomyces* (3 species), *Hanseniaspora* (4 species) *Hansenula* (6 species), *Kluyveromyces* (4 species), *Metschnikowia* (2 species), *Pichia* (13 species), and *Saccharomyces* (15 species). In addition, 6 unknown species of *Pichia* and 2 unknown species of *Saccharomyces* were found.

The presence of yeasts has been investigated in Japan in slime-flux of trees (4, 5, 10), fruits (3, 11), *Drosophila* fly (13), sake brewery (6), and wine brewery (2, 9, 12). But yeast flora in forests, grass-land, and crop fields have not yet been surveyed. To expand our knowledge of the natural habitat of yeasts, we surveyed the yeasts present on miscellaneous forest materials. This paper deals with ascomycetous yeasts in seven forests across in Japan. The autoecology of the ascomycetous yeasts reported here is also discussed.

Materials

Samples. The localities and dates of sampling were as follows:

Mount Odaigahara	August 27, 1973
	August 4, 1975
	October 4, 1976
Mount Daisen	July 30, 1974
	August 7, 1975
Yakushima Island	October 2, 1975
Mount Ontakesan	August 31, 1976
Togakushi Heights	October 2, 1976
Myoko Heights	October 8, 1976
Towada	October 5, 1977

Fig. 1. Map of Japan showing forest localities where materials were collected.

1. Towada
2. Myoko Heights
3. Togakushi Heights
4. Mount Ontakesan
5. Mount Daisen
6. Mount Odaigahara
7. Yakushima island

These localities are shown on the map in Fig. 1.

Materials sampled were soil, half-decayed fallen leaves, flowers, tree bark, mushrooms, animal dung, and fruits.

Samples of 2–5 g were placed in sterilized polyethylene bags or small plastic tubes by means of spatula or forceps and transported to the laboratory within 3 days of collection.

Media. Four liquid media, orange-juice medium, malt medium, nitrate medium, and plum-juice medium prepared according to Banno and Mikata (1), were used for enrichement of yeasts.

Isolation of Yeasts. Yeasts could not be isolated by direct streaking on agar plates from most samples because of their minute populations and the predominance of filamentous fungi in collected materials. Since a differential medium for selecting yeast

while preventing fungal growth has not been devised, the enrichment method described in the previous paper (1) was used to isolate yeasts. About 0.5 g of sample was inoculated into the 4 enrichment media and incubated for 7–14 days at 25 C, then a loopful of enrichment culture was streaked on YM agar plates. After incubation for an appropriate period, the plates were inspected under a dissecting microscope and a visual estimate was made of the proportions of the various colony types. Yeasts were taken from all colonies differing in their micro- and macro-morphology, and isolates were purified by repeated streaking until plates containing only one type of colony were obtained. The pure cultures were stored on YM agar slopes.

Identification of yeasts. The isolates were identified by the procedures recommended in the 2nd edition of *The Yeasts, a taxonomic study* (1970) (7). When not identified with any species in this monograph, the isolates were compared with descriptions of more recently described species. In some cases, isolates were compared with cultures of type strains and authentic strains of species resembling them to complete the identification.

Results

A total of 519 samples was collected, consisting of 227 samples of soil, 89 of half-decayed leaves, 85 of flowers, 54 of tree bark, 50 of mushrooms, 12 of dung and 2 of fruits. Yeasts were isolated from 515 samples of all types, of which 332 contained ascomycetous and deuteromycetous yeasts and 183 only deuteromycetous yeasts. No yeast was recovered from the remaining 4 samples.

Table 1 shows the proportion of samples from which ascomycetous yeasts were obtained presented according to the type of material and locality of sampling. Ascomycetous yeasts were found in more than half (64%) of all samples. The frequency of isolation of ascomycetous yeast was highest (over 0.8) in half-decayed leaves in all forests. The frequencies of isolation from soil, bark, and mushrooms varied in the range of 0.5 to 0.6.

In total, 633 ascomycetous yeasts were isolated from 332 samples. Their genera and species are recorded in Table 2 together with the locality of sampling. *Debaryomyces hansenii, Hanseniaspora uvarum, Hanseniaspora valbyensis, Hansenula beijerinckii, Hansenula californica, Hansenula saturnus, Kluyveromyces thermotolerans, Metschnikowia pulcherrima, Pichia dispora, Pichia membranaefaciens, Saccharomyces cerevisiae,* and *Saccharomyces florentinus* were found in all localities and are considered to be distributed in forests across the Japanese islands. *Pichia pijperi, Pichia terricola,* and *Pichia toletana* were found only in Mount Odaigahara, while *Saccharomyces exiguus* was found only in Mount Daisen. The number of isolates of these species, however, was too small to conclude that they have particular relationship to their respective habitats. *Kluyveromyces phaffii*, on the other hand, was found only in Yakushima Island, in the relatively high frequency of 11 of the 18 samples, and may therefore be closely related

Table 1. Proportion of samples bearing ascomycetous yeasts.

Forest locality and year of sampling	Sample material							Total
	Soil	Decayed leaf	Flower	Tree bark	Mushroom	Dung	Fruit	
Odaigahara 1973	11/36	5/8	5/6	6/9	4/8	1/1	—	22/68
Odaigahara 1975	15/46	10/17	8/12	7/19	4/6	1/6	—	45/106
Odaigahara 1976	24/32	15/16	22/22	12/17	—	4/5	2/2	79/94
Daisen 1974	9/19	2/2	23/27	8/9	—	—	—	42/57
Daisen 1975	—	10/10	—	—	—	—	—	10/10
Yakushima 1975	8/10	10/11	—	—	—	—	—	18/21
Myoko 1976	7/14	3/9	—	—	—	—	—	10/23
Togakushi 1976	10/13	3/3	—	—	—	—	—	13/16
Ontakesan 1976	7/12	—	11/18	—	—	—	—	18/30
Towada 1977	34/45	13/13	—	—	18/36	—	—	65/94
Total	125/227	71/89	69/85	33/54	26/50	6/12	2/2	332/519

—: Sample not collected.

to the nature of Yakushima Island.

Mount Odaigahara was surveyed in summer and in autumn, but no significant difference in the yeast flora was found between the two seasons.

Table 3 shows the numbers of isolates classified by genera and species and sample source.

Arthroascus: *A. javanensis* was isolated from only one sample, a tree bark from Mt. Odaigahara. This species has also been isolated in Japan from slime-flux of *Quercus* by Phaff and others (10).

Debaryomyces: *D. cantarellii* was isolated from one sample each of soil, decayed leaf and bark. Seventeen strains of *D. hansenii* were obtained from soil, decayed leaf, and flowers. *D. marama* was isolated from two samples of decayed leaves collected at Mt. Odaigahara and Towada. This genus is comparatively rare in forest materials.

Hanseniaspora: *H. occidentalis* was isolated one sample each of soil and flower. Only

a few strains of this species are known, having been obtained from soil, *Drosophila* and orange juice. *H. osmophila, H. uvarum* and *H. valbyensis* were isolated from various samples, and seem to be distributed widely among forest materials. *H. uvarum*

Table 2. Numbers of ascomycetous yeasts isolated from the 7 forest localities.

Species \ Locality and year of sampling	Odaigahara 1973	Odaigahara 1975	Odaigahara 1976	Daisen 1974	Daisen 1975	Yakushima 1975	Myoko 1976	Togakushi 1976	Ontakesan 1976	Towada 1977	Total
Number of samples	68	106	94	57	10	21	23	16	30	94	519
Arthroascus javanensis			1								1
*Debaryomyces cantarellii**	1			1	1						3
hansenii	1	1	8		2	1				4	17
*marama**		1								1	2
Hanseniaspora occidentalis			1	1							2
osmophila	1	1	7						1	5	15
uvarum	2	8	7	2	1			2	4	8	34
valbyensis		3	6	2				1		3	15
Hansenula anomala	2	2					1		2		7
beijerinckii		1					1	2		2	6
californica	1	5	22		1	2		4		13	48
capsulata		2	6					2			10
*dimennae**								1		3	4
saturnus	1	6	1	3	1	6	1	3		15	37
Kluyveromyces fragilis									1		1
phaffii						11					11
thermotolerans	2	2	1	7	5	2			1	12	32
vanudenii				1							1
Metschnikowia pulcherrima	9		9	14	1	1			8	5	47
reukaufii		1	16	12					1		30
*Pichia bovis**								1			1
dispora	5	8	26	3	2	3	1			5	53
fluxuum		2	1		1						4
membranaefaciens	6		2	4	1	3	1		1	1	19
*pijperi**	2										2
pinus		3	2							3	8
pseudopolymorpha			1								1
*quercuum**					1						1
saitoi				1		1					2
*stipitis**				1							1
*terricola**	2										2
toletana			5								5
trehalophila							1				1
sp. 1		1									1
sp. 2			1							3	4
sp. 3			1								1
sp. 5		2	2							1	5
sp. 6										3	3
sp. 7								1			1

Table 2 (continued)

	Locality and year of sampling										Total
	Odaigahara 1973	Odaigahara 1975	Odaigahara 1976	Daisen 1974	Daisen 1975	Yakushima 1975	Myoko 1976	Togakushi 1976	Ontakesan 1976	Towada 1977	
Saccharomyces bailii						1					1
bayanus				1	3	1					5
cerevisiae		9	16	6	5		4	7		27	78
chevalieri			1								1
dairensis	1		2	2							5
exiguus				3							3
florentinus	10	4	17	4	1	2	1	1		8	48
globosus						2					2
kluyveri					2					10	12
microellipsodes								1			1
pretoriensis				1					2		3
rosei	1		2			1				2	6
rouxii	1		2			1					4
unisporus		2				2	4				8
uvarum		1		1	5			1		3	11
sp. 8		2									2
sp. 9					1					4	5
Total Genera	7	7	8	7	7	6	3	4	6	7	8
Total Species	18	22	27	20	17	16	9	13	9	23	56
Total Strains	52	67	166	70	34	40	15	27	21	141	633

* Not previously isolated in Japan.

showed an affinity for flowers above other materials.

Hansenula: This genus was represented by only 6 species. *H. anomala, H. beijerinckii, H. capsulata,* and *H. dimennae* were less common, while *H. californica* and *H. saturnus* were predominant, paticularly in soil samples. Although the number of species obtained in this genus was small, the number of isolates was relatively large.

Kluyveromyces: *K. fragilis* and *K. vanudenii* were each isolated once, from soil and bark respectively. *K. phaffii* was isolated from a few soil and decayed leaf samples collected only at Yakushima as mentioned earlier. *K. thermotolerans* was isolated from soil, decayed leaves, bark and mushrooms although not in great frequency. Some of these isolates differed from the type in being able to assimilate meliboise. Kodama (4) has also reported yeasts showing the same properties as the type strain of *K. thermotolerans* except for the ability to utilize melibiose. The GC content of a representative of these isolates, was found to be in the range of 44.6 to 44.9%, not significantly different from the 45.4% of the type culture. Consequently the melibiose assimilating yeast is regarded as a biotype of the species.

Metschnikowia: This genus was represented by two species, *M. pulcherrima* and *M. reukaufii*. Both were isolated in high frequency from flowers. This association of the two species with flowers is well known, and the present result shows that the two species are also common in flowers in the forests of Japan.

Table 3. Numbers of ascomycetous yeasts isolated from various forest materials.

Species	Sample material: Soil	Decayed leaf	Flower	Tree bark	Mushroom	Dung	Fruits	Total
Number of samples	227	89	85	54	50	12	2	519
Arthroascus javanensis				1				1
*Debaryomyces cantarellii**	1	1		1				3
hansenii	6	4	7					17
*marama**		2						2
Hanseniaspora occidentalis	1		1					2
osmophila	4	5	1	2	2	1		15
uvarum	8	4	15	1	5	1		34
valbyensis	4	6	4	1				15
Hansenula anomala	3	1	1	1		1		7
beijerinckii	6							6
californica	29	14	1	1	2	1		48
capsulata	2	4		4				10
*dimennae**	4							4
saturnus	31	6						37
Kluyveromyces fragilis	1							1
phaffii	5	6						11
thermotolerans	9	13	6	3	1			32
vanudenii				1				1
Metschnikowia pulcherrima	2	2	34	3	6			47
reukaufii	1		29					30
*Pichia bovis**		1						1
dispora	16	14	7	15		1		53
fluxuum		3		1				4
membranaefaciens	6	6	1	2	2	2		19
*pijperi**		1	1					2
pinus	3	3		2				8
pseudopolymorpha			1					1
*quercuum**		1						1
saitoi	1	1						2
*stipitis**	1							1
*terricola**		2						2
toletana	1			3			1	5
trehalophila	1							1
sp. 1				1				1
sp. 2	3	1						4
sp. 3				1				1
sp. 5	5							5
sp. 6					3			3
sp. 7	1							1
Saccharomyces bailii	1							1
bayanus	1	4						5
cerevisiae	30	32	2	5	5	4		78
chevalieri		1						1
dairensis	4			1				5
exiguus			3					3
florentinus	17	15	3	6	6		1	48
globosus	1	1						2
kluyveri	3	3			6			12
microellipsodes	1							1
pretoriensis	3							3
rosei	3	2				1		6

Table 3 (continued)

	Sample material							
	Soil	Decayed leaf	Flower	Tree bark	Mushroom	Dung	Fruits	Total
rouxii		1	3					4
unisporus	4	4						8
uvarum	3	7		1				11
sp. 8		1			1			2
sp. 9	1	4						5
Total Genera	7	7	7	8	6	4	2	8
Total Species	40	35	18	22	11	8	2	56
Total Strains	227	176	120	57	39	12	2	633

*Not previously isolated in Japan.

Pichia: Nineteen species of this genus were found. Only one strain each was obtained of *P. bovis, P. quercuum, P. stipitis, P. pseudopolymorpha,* and *P. trehalophila,* while isolates of *P. fluxuum, P. pijperi, P. saitoi, P. terricola,* and *P. toletana* were also few. *P. dispora* and *P. membranaefaciens* were frequently isolated from most kinds of materials. *P. dispora* has hitherto been obtained mainly from slime-flux of tree. *P. membranaefaciens* is known to be broadly distributed among natural substrates. The isolate of *P. pseudopolymorpha* from flower differed from the description of the species in sucrose fermentation and rhamnose assimilation, but had a GC content (35.8%) approximately equal to that of the type culture. Six yeasts designated *Pichia* spp. 1, 2, 3, 5, 6, and 7 could not be identified with any known species. Descriptions of these will be published elsewhere.

Saccharomyces: The 15 known species isolated in this survey have all been found frequently in many other sources. Of these, *S. bailii, S. bayanus, S. chevalieri, S. dairensis, S. exiguus, S. globosus, S. microellipsodes, S. pretriensis, S. rosei, S. rouxii, S. unisporus,* and *S. uvarum* were less frequently isolated from the forest materials. The commonest isolate, *S. cerevisiae,* was obtained predominantly from soil and decayed leaves and less commonly came from flowers, bark, and mushrooms. All isolates of *S. cerevisiae* abundantly produced asci on YM agar and corn meal agar. Five representative isolates were homothallic and cultures from a single spore formed asci spontaneously. *S. florentinus* was also frequently found in soil, decayed leaves, bark, flowers, mushrooms and fruits. Of this genus, *S. cerevisiae* and *S. florentinus* are probably distributed widely in the forest.

Two yeasts designated as *Saccharomyces* spp. 8 and 9 could not be identified with known species. Descriptions of the two yeasts will be published separately.

Eight species of yeasts, *Debaryomyces cantarellii, Debaryomyces marama, Hansenula dimennae, Pichia bovis, Pichia pijperi, Pichia quercuum, Pichia stipitis,* and *Pichia terricola* were found for the first time in Japan in this survey. The isolates of these species

are described in the descriptive catalogue of the IFO yeast collection (8).

Ascomycetous yeasts belonging to 47 species in 8 genera, accounting for about 60% of the total of 80 species in 16 genera so far described, were found in the forest materials in the present work.

Discussion

Our results indicate that although a minority population in the microbial world of the forest, ascomycetous yeasts are present in considerable variety. Forest materials thus present an important source in which a variety of yeasts with unique activities can be sought.

Phaff and others (10) and Kodama (4, 5) have investigated yeast flora in slime-flux of trees in Japan. This source yielded the following 17 species which were not found in this survey of forest materials: *Debaryomyces vanriji; Hansenula bimundalis, H. silvicola; Kluyveromyces dobzanskii, K. drosophilarum, K. waltii K. wickerhamii; Pichia farinosa, P. fermentans, P. naganishii, P. nakazawae, P. pastoris, P. strasburgensis, P. veronae, P. vini; Saccharomyces montanus;* and *Schizosaccharomyces japonicus.*
On the other hand the following 17 species were obtained from the forest materials but not from slime-flux: *Debaryomyces cantarellii, D. marama; Hanseniaspora occidentalis; Hansenula beijerinckii, H. dimennae; Kluyveromyces fragilis; Pichia bovis, P. pijperi, P. quercuum, P. stipitis, P. terricola; Saccharomyces bailii, S. bayanus, S. dairensis, S. globosus, S. microellipsodes,* and *S. unisporus.*

Twenty-eight species of yeasts have been found in both slime-flux and the forest materials: *Arthroascus javanensis; Debaryomyces hansenii; Hanseniaspora osmophila, H. uvarum, H. valbyensis; Hansenula anomala, H. calfornica, H. capsulata, H. saturnus; Kluyveromyces phaffii, K. thermotolerans, K. vanudenii*; *Metschnikowia pulcherrima, M. reukaufii; Pichia dispora, P. fluxuum, P. membranaefaciens, P. pinus, P. saitoi, P. toletana, P. trehalophila; Saccharomyces cerevisiae, S. chevalieri, S. florentinus, S. kluyveri, S. pretoriensis, S. rosei,* and *S. uvarum.*
These 28 species appear to be distributed widely in nature.

Hayashibe and others (3) studied the yeasts associated with plants in the sand-coast of the north-eastern provinces and obtained strains of *Saccharomyces uvarum, S. diastaticus, S. florentinus, Hansenula* sp. and *Schizosaccharomyces japonicus.* Of these, *S. diastaticus* and *Sch. japonicus* were not found in the forest materials.

Sasaki and Yoshida (11) surveyed the yeasts present on fresh fruit in Hokkaido. They isolated no ascomycetous yeast but only anasporogenous yeasts. If they had used enrichment method to isolate yeast, some ascomycetous yeasts could have been obtained.

The ecological niche of these yeasts in the forest could not be learned from the result of the present rough survey. Detailed research on their natural forest habitats

is required to clarify their role in the microecological system.

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THERMOPHILIC AND THERMOTOLERANT FUNGI IN PADDY FIELD SOILS

Tadayoshi ITO, Michiyo UEDA and Tatsuo YOKOYAMA

Summary

Thermophilic and thermotolerant fungi were repeatedly isolated by the heat incubation method at 42 C from the soils of four paddy fields in Osaka Prefecture in the period of 1976–1979.

Thermoascus aurantiacus, Aspergillus fumigatus and *Thermomyces lanuginosus* were the most common and predominant thermophilic and thermotolerant fungi found in these soils, although the last one was less predominant than the others.

Talaromyces emersonii, Thermoascus aurantiacus and *Aspergillus fumigatus* were the predominant thermophilic and thermotolerant fungi at Nose station and *A. fumigatus* was the most dominant at Habikino station. *Thermoascus aurantiacus* and *Aspergillus fumigatus* were predominant at Ikeda station and *Acremonium alabamense* and *Aspergillus fumigatus* were predominant at Ibaraki station.

Chaetomium thermophilum var. *dissitum, Thermoascus crustaceus, Malbranchea pulchella* var. *sulfurea, Sporotrichum thermophile, Absidia corymbifera, Rhizopus rhizopodiformis* were also detected but less frequently.

The isolated thermophilic and thermotolerant fungi were relatively abundant to a depth of 20 cm depth from the soil surface, below which their populations markedly decreased. No seasonal fluctuation in the predominant species was recognized.

The number of species detected was limited throughout the investigation, but was usually constant at each station, although many more species are known as thermophilic and thermotolerant fungi in the sense of Cooney & Emerson (1964).

Mesophilic species were also detected. Among these, *Neosartorya fischeri* var. *glabra* was dominant at Ibaraki, whereas *Thielavia terricola* and *Aspergillus terreus* were dominant at Ikeda and Habikino. *Neosartorya fischeri* var. *glabra* and *Thielavia terricola* were the most common mesophilic fungi detected in these soils.

Cooney & Emerson (6) have defined thermophilic fungi as those able to grow at a maximum temperature of at least 50 C and a minimum temperature of at least 20 C, and thermotolerant fungi as those which can grow at a maximum temperature of at least 50 C but can also grow at a minimum temperature of below 20 C. Thermophilic and thermotolerant fungi, therefore, may inhabit at the same niche and be isolable from the same samples.

Papers dealing with thermophilic and thermotolerant fungi include those by Apinis (1), Cooney & Emerson (6), Gochenaur (10), Huang & Schmitt (11) and Tansey & Jack (16). In Japan, several papers on these fungi have been published: Awao & Mitsugi (2), Awao & Otsuka (3), Minoura et al. (12, 13), Awao & Otsuka (4) and Tubaki et al.

(17). These reports, however, merely describe thermophilic and thermotolerant species which were isolated from various sources of substrata collected at various places.

Whether thermophilic and thermotolerant fungi inhabit the paddy fields of Japan and if so, how they are distributed, have not been reported, although a few papers dealing with fungal flora of paddy fields have been published in India (8), Italy (7) and USSR (5).

We have investigated the microflora of the soil fungi in paddy fields in Osaka Prefecture from 1976 to 1979. This paper describes part of a serial investigation and mainly deals with thermophilic and thermotolerant fungi isolated from four paddy fields by incubation at 42 C.

Materials and Methods

Stations. The paddy fields investigated are all located in Osaka Prefecture, Japan, and have been planted with rice for more than a century. As Figure 1 shows, Nose station is located in the northern part of Osaka Prefecture, in Kuragaki, Nose-cho, Toyono-gun. The village is surrounded by hills and is one of the coldest places in Osaka. The paddy field belongs to the Nose branch of the Osaka Agricultural Re-

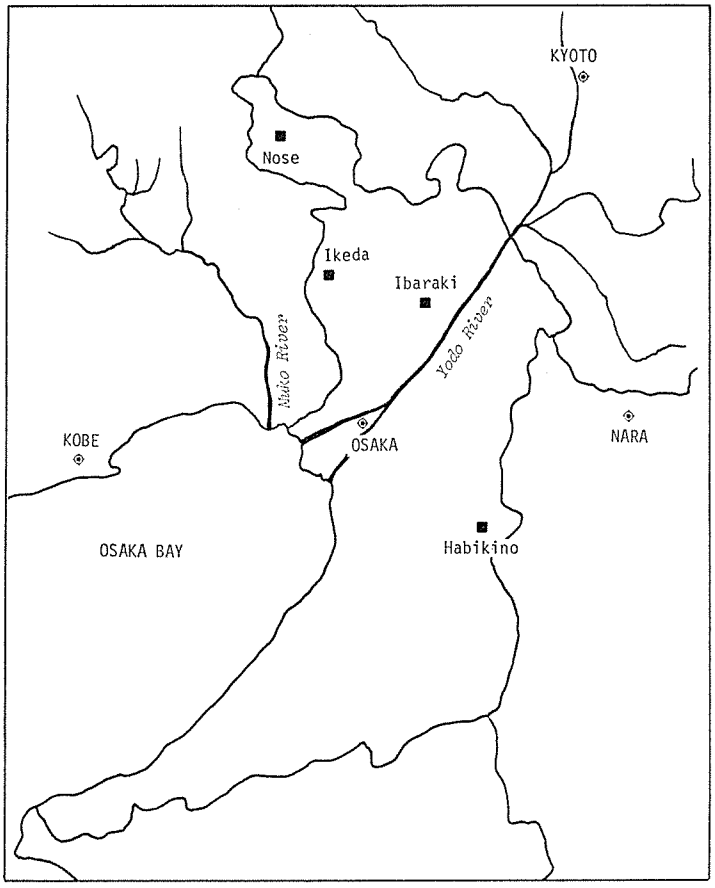

Fig. 1. Map of Osaka Prefecture indicating the four stations investigated.

search Center, and rice has been cropped once a year. This field is considered typical of paddy fields in Japan. Habikino station is located at Shakudo, Habikino, in the Osaka plain in the center of Osaka Prefecture, and is a suburb of Habikino City. This station is controlled by the Osaka Agricultural Research Center and has been planted once a year only for rice production. Ikeda station is located at Hachioji, Ikeda, close to the residential zone of this city. This station belongs to the Osaka Prefectural Horticultural High School and has been used in the training of the students in a rotation system of rice and vegetables. Ibaraki station belongs to an old farmer and is located at Nakatsu-cho, Ibaraki. It is surrounded by residential streets and is very close to the town center. This paddy field has been planted only with rice for more than three hundred years.

Sampling method. Soil samples were collected every three months from each station from July 1976 through March 1979, except at the Ikeda station, where they were collected only five times from August 1976 to August 1977. Soils were drawn vertically from five sites in each field with sterilized stainless-steel soil samplers fitted with a narrow side-window (600 mm long and 20 mm in inside diam). Soil samples were divided into three fractions representing depths of 0–10 cm, 10–20 cm and 20–30 cm from the surface, and 1-g, 2-g and 3-g samples were taken from the 0–10 cm, 10–20 cm and 20–30 cm fractions, respectively, in the laboratory.

Isolation and identification. Final soil samples were suspended in 5 ml of sterilized water, and 0.2 ml of the suspension was spread onto MYA medium (see below), adjusted to pH 4.5 with lactic acid, in two 9-cm disposable plastic Petri dishes. These plates were incubated at 42 C for three days and colonies were picked up from the plates under a dissecting microscope. These isolates were transferred to malt agar (MA) slants and incubated at 37 C.

The media used for isolating and identifying the fungi were as follows. MYA medium: glucose, 10 g; peptone, 5 g; malt extract, 3 g; yeast extract, 3 g; agar, 20 g; distilled water, 1000 ml. MA medium: malt extract, 10 g; glucose, 10 g; peptone, 1 g; agar, 20 g; distilled water, 1000 ml. CMA medium: corn meal agar (Nissan), 17 g; distilled water, 1000 ml. PCA medium: potato, 20 g; carrot, 20 g; agar, 20 g; distilled water, 1000 ml. Y_pS_s medium: soluble starch, 15 g; yeast extract, 4 g; K_2HPO_4, 1 g; $M_gSO_4 \cdot 7H_2O$, 0.5 g; agar, 20 g; distilled water, 1000 ml. PSA medium: potato, 200 g; sucrose, 30 g; agar, 20 g; distilled water, 1000 ml. OA medium: oatmeal, 50 g; agar, 20 g; distilled water, 1000 ml.

The isolates were identified mainly from the CMI Description of Pathogenic Fungi and Bacteria, Nos. 521 & 522, Rifai (14), Evans (9) and Samson et al. (15). IFO strains were also used for comparison.

Results

Difference in fungal species between stations

Table 1 lists the fungal species detected at the four stations and the total number

Table 1. Fungi isolated from paddy field soils by heat incubation method at 42 C.

SPECIES	Station*			
ZYGOMYCOTINA				
*Absidia corymbifera****			Y	
*Rhizopus rhizopodiformis****	W			
Rhizopus sp.***	W	X		
ASCOMYCOTINA				
Anixiella reticulata	W	X		Z
Chaetomium thermophilum var. *dissitum***			Y	
Emericella nidulans var. *nidulans*			Y	
E. nidulans var. *echinulata*			Y	
Hamigera avellanea				Z
Monascus ruber			Y	
Neosartorya fischeri var. *fischeri*		X		Z
N. fischeri var. *glabra*	W	X	Y	Z
N. fischeri var. *spinosa*	W	X	Y	Z
N. quadricincta	W			Z
Talaromyces flavus var. *flavus*			Y	
*T. emersonii***	W	X	Y	
T. trachyspermus				Z
*Thermoascus aurantiacus***	W	X	Y	Z
*T. crustaceus***			Y	
Thielavia terricola	W	X	Y	Z
DEUTEROMYCOTINA				
*Acremonium alabamense***		X	Y	Z
Acrophialophora levis		X	Y	
Aspergillus carneus		X		Z
A. flavus			Y	
*A. fumigatus****	W	X	Y	Z
A. niger		X	Y	Z
A. terreus	W	X	Y	Z
Malbranchea pulchella var. *sulfurea***	W	X	Y	
Paecilomyces variotii		X		Z
Penicillium funiculosum	W	X		Z
P. piceum	W		Y	
P. restrictum			Y	
Penicillium sp. 4		X		
Penicillium sp. 5				Z
Phoma sp.				Z
*Sporotrichum thermophile***	W	X	Y	
*Thermomyces lanuginosus***	W	X	Y	Z
Trichoderma pseudokoningii				Z
Total number of species	16	20	23	20

* W=Nose, X=Habikino, Y=Ikeda, Z=Ibaraki
** Thermophilic species *sensu* Cooney & Emerson (1964).
*** Thermotolerant species *sensu* Cooney & Emerson (1964).

Table 2. Serial isolation of fungi from paddy field soils at Nose Station.

SPECIES	1976						1977												1978												1979			TOTAL		
	SEP.			DEC.			MAR.			JUN.			SEP.			DEC.			MAR.			JUN.			SEP.			DEC.			MAR.					
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
ZYGOMYCOTINA																																				
*Rhizopus rhizopodiformis**																															1			1		
Rhizopus sp.*				1						1						1			1														3	1	
ASCOMYCOTINA																																				
Anixiella reticulata					1	1																												1	1	
Neosartorya fischeri var. *glabra*					2	1		2		1	1		1			1	1		1	2					2	1		1			2	1	4	13	3
N. fischeri var. *spinosa*							1				2																							1	2	
N. quadricincta							1	1														1									1			2	2	
*Talaromyces emersonii**	2	3	1	3	4	3	2	4	3	3	3		3	5		4	3	2	2	3	3	3	4	1	3	3	3	4	4	4	3	3	3	31	39	23
*Thermoascus aurantiacus**	4	3	4	3	5	5	4	5	4	5	5	2	5	4	5	5	5	4	4	5	5	5	5	4	5	5	5	5	5	4	5	5	4	50	52	46
Thielavia terricola																												1						1		
DEUTEROMYCOTINA																																				
*Aspergillus fumigatus**	4	1	1	4	1	1	2	1	1	3	3	1	2	1	1	4	2	1	4	3	1	4	1	1	4	3	1	4	3	1	3	2	1	38	21	11
A. terreus		1			1																														2	
Malbranchea pulchella var. *sulfurea**	2	1		2						1												2	1		2	2		3	3		1	1		13	8	
Penicillium funiculosum								1												1											1	1		1	3	
P. piceum																															1			1		
*Sporotrichum thermophile**	1			1				1		1												1			1	2		2			1			8	3	
*Thermomyces lanuginosus**	2									1						1				1								1						5	1	

NOTE:

A=0–10 cm, B=10–20 cm, C=20–30 cm

Figures indicate number of soil samples from which a fungus was isolated/5 soil samples tested.

* Thermophilic and thermotolerant fungi *sensu* Cooney & Emerson (1964).

Table 3. Serial isolation of fungi from paddy field soils at Habikino Station.

SPECIES	1976						1977												1978												1979			TOTAL		
	AUG.			NOV.			FEB.			MAY			AUG.			NOV.			FEB.			MAY			AUG.			NOV.			FEB.					
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
ZYGOMYCOTINA																																				
Rhizopus sp.*										1																								1		
ASCOMYCOTINA																																				
Anixiella reticulata		1																																1		
Neosartorya fischeri var. *fischeri*								2																										2		
N. fischeri var. *glabra*			1											1	1		2		1	1		1				1					1		2	6	2
N. fischeri var. *spinosa*													1		1																		1		1
*Talaromyces emersonii**																								1												1
*Thermoascus aurantiacus**		2						1	1			2								1	1		1		3	1				1	1			4	6	5
Thielavia terricola	4	3	1	2	3	1	4	1	1	2	1			1		1	3	1	4	2	1	4	2		3	3		2	2	1	4	2	2	30	23	8
DEUTEROMYCOTINA																																				
*Acremonium alabamense**	1									1	1	1	2	1			1			1					3	1	1				1			8	5	2
Acrophialophora levis				1																					1									2		
Aspergillus carneus		1		2																														2	1	
*A. fumigatus**	2	1		1			2		1	2	2					2	3		2						2	1		1		1	1	1	1	15	8	3
A. niger	1			1						1		1					2					1												4	2	1
A. terreus	5	2		4	2		5	3	1	4	2	1	2	2		3	3	1	5	3	1	5	3	1	3	2		2	3	1	4	1		42	26	6
Malbranchea pulchella var. *sulfurea**	1																					2			2									5		
Paecilomyces variotii																															1			1		
Penicillium funiculosum																		1													1			1		1
Penicillium sp. 4																			1			2												3		
*Sporotrichum thermophile**	1																																	1		
*Thermomyces lanuginosus**							1													1	1		1											2		2

NOTE:
A=0–10 cm, B=10–20 cm, C=20–30 cm
Figures indicate number of soil samples from which a fungus was isolated/5 soil samples tested.
* Thermophilic and thermotolerant fungi *sensu* Cooney & Emerson (1964).

Table 4. Serial isolation of fungi from paddy field soils at Ibaraki Station.

SPECIES	1976						1977												1978												1979			TOTAL		
	JUL.			OCT.			JAN.			APR.			JUL.			OCT.			JAN.			APR.			JUL.			OCT.			JAN.					
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
ASCOMYCOTINA																																				
Anixiella reticulata		1		1				1		1	1	1																						2	3	1
Hamigera avellanea																															1				1	
Neosartorya fischeri var. *fischeri*																									1										1	
N. fischeri var. *glabra*	2	1		2	1		2	2		3	1		2	2	1	2	3	1	2	3		3	2		1	3			1			1	2	1	20	21	3
N. fischeri var. *spinosa*																				1						1									2	
N. quadricincta										1							1						1								1			2	2	
Talaromyces trachyspermus				2																														2		
*Thermoascus aurantiacus**				1						1						1										1			1					3	2	
Thielavia terricola			1	1	1			1		3		1	1				1		2	1			1			3	1		1			1			8	9	3
DEUTEROMYCOTINA																																				
*Acremonium alabamense**	1			1	2		2	1		1			1		1				1	1	1	1	1		1	1	1				1	1	1	10	7	4
Aspergillus carneus				1																											1			2		
*A. fumigatus**				1	1		1			2	2		2			2	1		2	1		2	1								2	1		14	7	
A. niger				3	1			1		1						2													1					6	2	1
A. terreus				1	2		2												2			1			1		1	2			1	1		10	3	1
Paecilomyces variotii					1																														1	
Penicillium funiculosum				4	3	3																												4	3	3
Penicillium sp. 5					1																														1	
Phoma sp.					1																														1	
*Thermomyces lanuginosus**																												1	1					1	1	
Trichoderma pseudokoningii										1																					1			2		

NOTE:
A=0–10 cm, B=10–20 cm, C=20–30 cm
Figures indicate number of soil samples from which a fungus was isolated/5 soil samples tested.
* Thermophilic and thermotolerant fungi *sensu* Cooney & Emerson (1964).

of the species found there in three years' investigation. In total only 37 species classified in 21 genera were obtained, comprising 3 species of Zygomycotina, 16 of Ascomycotina and 18 of Deuteromycotina. Basidiomycotina were not isolated. The species common to the four stations were *Neosartorya fischeri* var. *glabra, N. fischeri* var. *spinosa, Thermoascus aurantiacus, Thielavia terricola* (four fungi belonging to Ascomycotina), *Aspergillus fumigatus, A. terreus, Thermomyces lanuginosus* (three belonging to Deuteromycotina). The total number of fungal species isolated was highest at Ikeda station,

Table 5. Serial isolation of fungi from paddy field soils at Ikeda Station.

SPECIES	1976						1977									TOTAL		
	AUG.			NOV.			FEB.			MAY			AUG.					
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
ZYGOMYCOTINA																		
*Absidia corymbifera**							1									1		
ASCOMYCOTINA																		
Chaetomium thermophilum var. *dissitum**					1	1											1	1
Emericella nidulans var. *nidulans*	1			2				1								3	1	
E. nidulans var. *echinulata*	1												1			2		
Monascus ruber								1									1	
Neosartorya fischeri var. *glabra*							2			2	3		1	3		5	6	
N. fischeri var. *spinosa*										1						1		
Talaromyces flavus var. *flavus*						1												1
*T. emersonii**	1					1	1	2		2			1	2	1	5	4	2
*Thermoascus aurantiacus**	1		1				3	1		5	3	1	3	4	1	12	8	3
*T. crustaceus**							1						2			3		
Thielavia terricola	1	1	1	1	2		5	3	1	4	3	1	5	2		16	11	3
DEUTEROMYCOTINA																		
*Acremonium alabamense**	1		1	1	3	1				2			2	1		6	4	2
Acrophialophora levis				1												1		
Aspergillus flavus				1												1		
*A. fumigatus**	2	2		5	1	1	3			3			4	1		17	4	1
A. niger				3												3		
A. terreus	4	2	1	4	5	1	3	1		2	1		4	1		17	10	2
Malbranchea pulchella var. *sulfurea**		2	1	4	2	1										4	4	2
Penicillium piceum			1	3		1										3		2
P. restrictum					1												1	
*Sporotrichum thermophile**				2				1					1			3	1	
*Thermomyces lanuginosus**						1							1			1		1

NOTE:
A=0-10 cm, B=10-20 cm, C=20-30 cm
Figures indicate number of soil samples from which a fungus was isolated/5 soil samples tested.
* Thermophilic and thermotolerant fungi *sensu* Cooney & Emerson (1964).

although soil was sampled there only five times, and lowest at Nose station.

As Tables 2–5 show, *Talaromyces emersonii, Thermoascus aurantiacus* and *Aspergillus fumigatus* were detected more frequently at Nose station than at the other three stations. *Thielavia terricola, Aspergillus terreus* and *A. fumigatus* were predominant at Habikino station, *Thermoascus aurantiacus, Thielavia terricola, Aspergillus fumigatus* and *A. terreus* were predominant at Ikeda station, and *Neosartorya fischeri* var. *glabra, Acremonium alabamense* and *Aspergillus fumigatus* were predominant at Ibaraki station.

Figure 2 shows histograms for several predominant species, which reveal their distributional difference between stations.

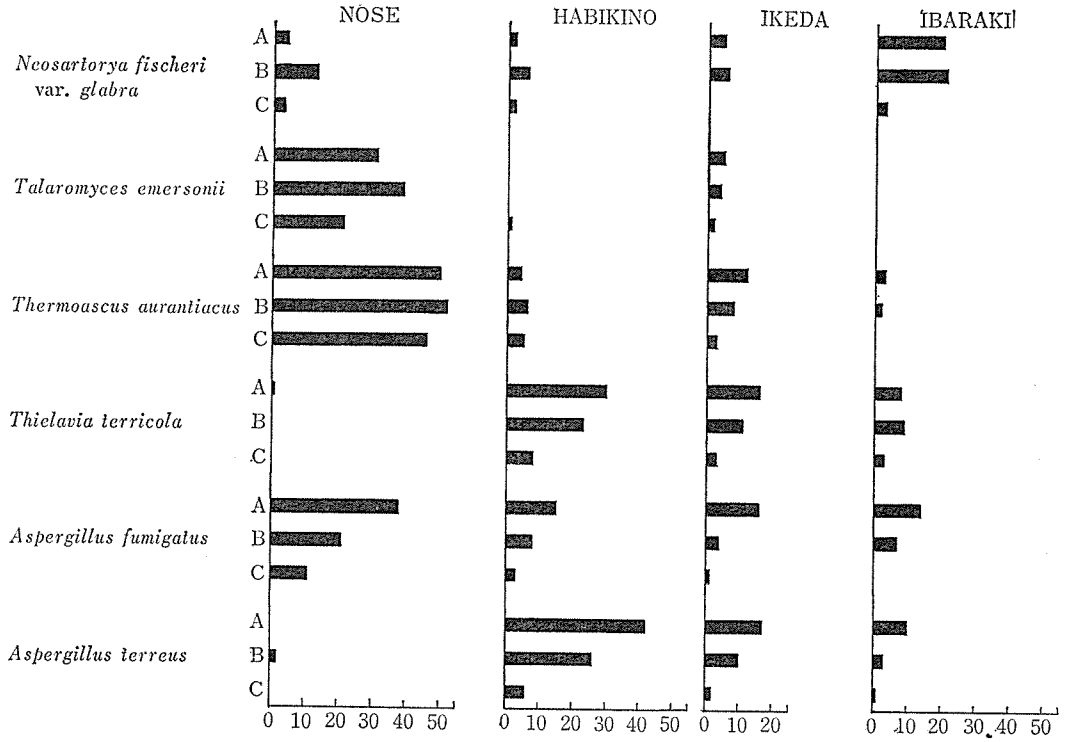

Fig. 2. Distribution of dominant fungi between stations.
Bars represent the number of soil samples from which a fungus was isolated from a total of 55 soil samples tested (or 25 samples at Ikeda station).
A=0–10 cm depth; B=10–20 cm depth; C=20–30 cm depth.

Differences in fungal species at different depths of soil

As Tables 2–5 and Figure 2 indicate, the predominant species, i.e., *Neosartorya fischeri* var. *glabra, Talaromyces emersonii, Thermoascus aurantiacus, Thielavia terricola, Aspergillus fumigatus* and *A. terreus* were usually isolated in abundance from the 0–10 cm and 10–20 cm layers at the four stations, but were remarkably fewer in the 20–30 cm layer. The less predominant species showed a similar tendency.

Seasonal fluctuations in the dominant species

As Tables 2–5 and Figure 3 show, *Talaromyces emersonii*, *Thermoascus aurantiacus* and *Aspergillus fumigatus* were repeatedly isolated at Nose station from September 1976 through March 1979; *Thielavia terricola, Aspergillus fumigatus* and *A. terreus* were predominant at Habikino station from August 1976 to February 1979; *Thielavia terricola, Aspergillus fumigatus* and *A. terreus* were predominant at Ikeda station where soil was sampled only five times, from August 1976 through August 1977; and *Neosartorya fischeri* var. *glabra, Thielavia terricola, Acremonium alabamense, Aspergillus fumigatus* were constantly isolated at Ibaraki station from July 1976 to January 1979. Virtually no seasonal fluctuation in the predominant species was recognized in this serial experiment. The less predominant species are omitted from the discussion because they seem to be of sporadic occurrence.

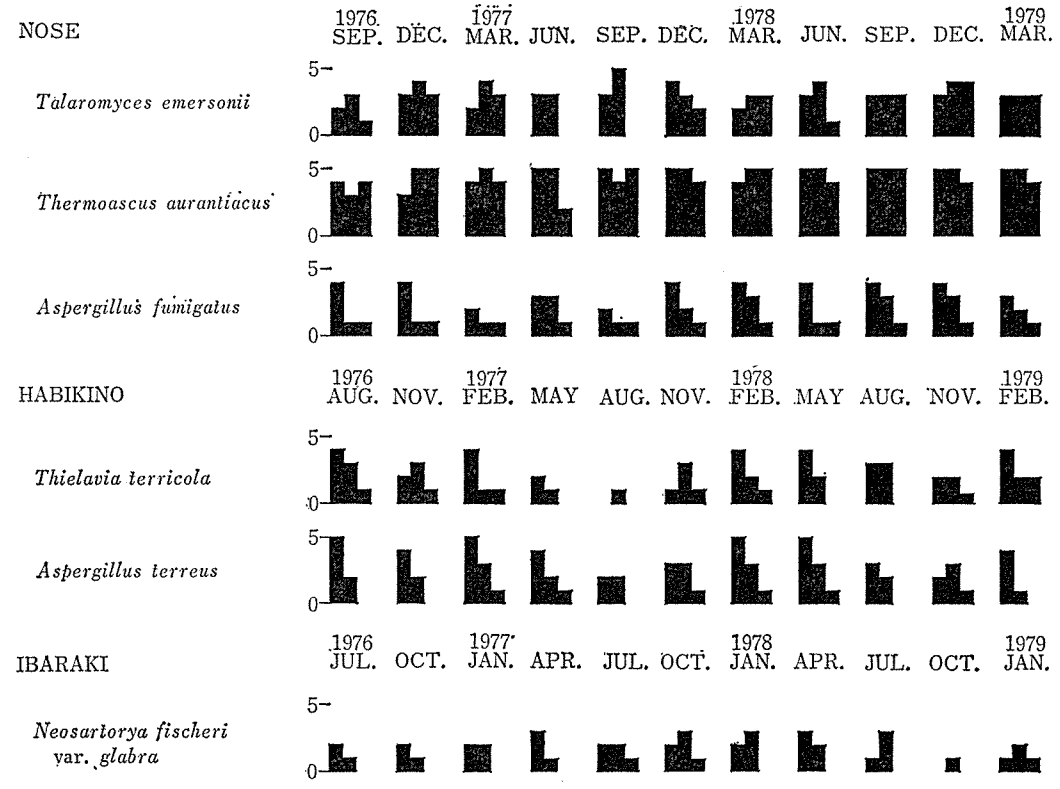

Fig. 3. Serial detection of dominant fungi.
Bars represent the number of soil samples from which a fungus was isolated from a total of 5 soil samples tested.
Left=0–10 cm depth; Middle=10–20 cm depth; Right=20–30 cm depth.

Ecology and distribution of the thermophilic and thermotolerant fungi

Although approximately 70 species of thermophilic and thermotolerant fungi *sensu* Cooney & Emerson (6) are known in the literature, only 8 species of thermophilic fungi were isolated in this experiment: *Chaetomium thermophilum* var. *dissitum, Talaromyces*

emersonii, Thermoascus aurantiacus and *T. crustaceus,* which belong to Ascomycotina, and *Acremonium alabamense, Malbranchea pulchella* var. *sulfurea, Sporotrichum thermophile* and *Thermomyces lanuginosus,* which belong to Deuteromycotina. These species are shown by ** in Table 1.

Absidia corymbifera, Rhizopus rhizopodiformis and *Rhizopus* sp., which belong to Zygomycotina, and *Aspergillus fumigatus* which belongs to Deuteromycotina, were also detected. These are thermotolerant fungi in the sense of Cooney & Emerson (6) and are indicated by *** in Table 1.

Among the thermophilic and thermotolerant fungi detected in this investigation, *Thermoascus aurantiacus, Aspergillus fumigatus* and *Thermomyces lanuginosus* were common to the four stations, but most frequently at Nose station. *Talaromyces emersonii, Malbranchea pulchella* var. *sulfurea* and *Sporotrichum thermophile* were detected at the three stations other than Ibaraki station, whereas *Acremonium alabamense* was found at the three stations other than Nose station and predominant at Ibaraki station. *Absidia corymbifera, Chaetomium thermophilum* var. *dissitum* and *Thermoascus crustaceus* were detected only at Ikeda station.

As mentioned above, the predominant thermophilic and thermotolerant fungi at Nose station were *Talaromyces emersonii, Thermoascus aurantiacus* and *Aspergillus fumigatus* throughout the investigation. However, the predominant thermophilic and thermotolerant fungi at Habikino and Ibaraki stations were *Acremonium alabamense* and *Aspergillus fumigatus*; those at Ikeda station were *Thermoascus aurantiacus* and *Aspergillus fumigatus.* These species are considered to be the main element of thermophilic and thermotolerant fungi in the paddy field soils in Osaka.

Generally thermophilic and thermotolerant fungi grow faster than mesophilic fungi and mostly inhabit plant debris, manure, stored crops, mushroom beds, peat and tips, which are all self-heated. Together with mesophilic fungi, they also inhabit soils, hay, herviborous dung, water sediments, birds' nests and animals' dens. Thermophilic and thermotolerant fungi, therefore, are distributed widely on a variety of substrates. This investigation has confirmed that these fungi also naturally inhabit paddy field soils.

Discussion

In our three years of investigation on the filamentous fungi in the soils of four paddy fields, the kinds of thermophilic and thermotolerant fungi, including mesophilic species, were recognized to depend on the soil types in which they live, as suggested by Apinis (1) and Evans (9). Moreover, it was confirmed that these fungi could be constantly isolated at the given sites throughout the investigation.

At below 20 cm depth, the types and total numbers of fungal species isolated at each station decreased remarkably. Similar results were obtained for isolation by ethanol treatment, heat treatment (70 C), and the dilution plate method (unpublished data). This agreed well with the findings of Tansey & Jack (16), who studied the thermophilic and thermotolerant fungi of the grassland in Indiana, U.S.A. at different

depths of soil.

Thermoascus aurantiacus was detected in high frequencey throughout the investigation, but *Thermoascus crustaceus* was isolated from only three soil samples at Ikeda station. Tubaki & Tsuruta (18) investigated the thermophilic and thermotolerant fungi in imported maize and milo and found that *Thermoascus crustaceus* was predominant on these grains but *T. aurantiacus* was much less abundant. Therefore it is supposed that *T. aurantiacus* is predominant in soils, while *T. crustaceus* is predominant on grains. Thus both species may occupy suitable substrates independently and inhabit their own niches.

Although *Absidia corymbifera, Rhizopus rhizopodiformis* and unidentified species of *Rhizopus* were sometimes detected in the paddy field soils, such thermophilic species of *Mucor* as *M. pusillus* were not isolated.

The flora of thermophilic and thermotolerant fungi isolated in this investigation indicate that the fungal population in the ecosystem of the paddy field is remarkably stable. Why this is so is not clear, but it is generally considered that the removal of the excess substrates, often together with the toxic substances to rice plants, by irrigating and covering the paddy fields with water for a given interval may stabilize the nutritional condition of the field so that the rice plants can grow stably and continuously for a long year without a significant loss of the yield and injury. Such a favourable environmental condition may also allow the soil fungi to survive in a very stable condition.

In addition, the survival potency of the fungi in the paddy fields may be constant almost all the year round, because the temperature and water content of the soils below 5 cm depth change little down to 30 cm depth either in the hot and wet season or the cold and dry season. This is supported by the population of mesophilic fungi obtained by three other methods for isolation, including the dilution plate method (unpublished data).

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PREDICTION OF PROSPECTIVE VIABILITY OF L-DRIED CULTURES OF BACTERIA AFTER LONG-TERM PRESERVATION

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Summary

A study was undertaken to predict the viability of L-dried specimens of bacteria after long-term preservation. Cultures of 53 bacterial strains were dried from liquid state *in vacuo* (L-drying). One set of dried specimens was preserved at 37 C in an accelerated storage test and another set at 5 C in a long-term preservation. Their viability were determined after 2 weeks and 80 to 92 months, respectively.

Viable cells were recovered from the dried specimens of all of the 53 strains, though individual recovery rate varied from 0.2 to 100 percent of the viable count immediately after drying. For all strains, the survival value in the accelerated test was comparable to that after 80 to 92 months of preservation at 5 C. The results indicate that the viability of a dried specimen stored at 5 C can be predicted by the accelerated storage test in a short time, and that the method is valid for various bacteria.

Freeze-drying or L-drying is generally used for long-term preservation of microbial cultures. We have improved the method of L-drying and succeeded in preparing dried specimens of almost all bacterial stock cultures maintained in the collection of the Institute for Fermentation, Osaka (1, 3).

It is desirable to be able to predict the period over which L-dried cultures will survive. The fact that dried viral suspensions are inactivated following Arrhenius' equation (2) led us to think that the viability of a dried culture after long-term preservation might be predictable from data obtained in an accelerated test at a high temperature. The present experiment was conducted to examine the general validity of this idea in long-term preservation of various bacteria by L-drying.

Materials and Methods

Fifty-three bacterial strains were selected at random from the collection. Cells were harvested from early stationary-phase cultures on suitable media and suspended in suitable fluids (1, 5). Samples of 0.1 ml of these suspensions were dried under vacuum without freezing in small ampoules by the method of Iijima and Sakane (3, 4). Dried specimens were prepared in triplicate. The first series was used to estimate viable count immediately after drying, the second was incubated at 37 C for 2 weeks

in an accelerated storage test, and the third was stored at 5 C for 80 to 92 months. The number of viable cells in the dried specimens was counted by the procedure described previously (1), whereby a dried specimen was rehydrated with Bacto nutrient broth and a known volume of suspension was plated onto an agar medium suitable for growth by the soft-agar double layer method. Viable count is expressed in colony forming unit (CFU). The experimental deviation of log CFU in determination of viability was within ± 0.3. The recovery rate from dried specimens preserved at 37 and 5 C is expressed by the viable count as a percentage of that estimated immediately after drying.

Results and Discussion

Fig. 1 shows the survival versus time data obtained with L-dried cells of *Escherichia coli* F682 stored at 5 C, 25 C, and 37 C. Semilog plots show that the viable count decreases with storage period, and that the rate of decrease is higher at the higher temperature. At each temperature the decrease was linear up to 12 months, thereafter becoming gradually slower and eventually stopping. The following equation holds for

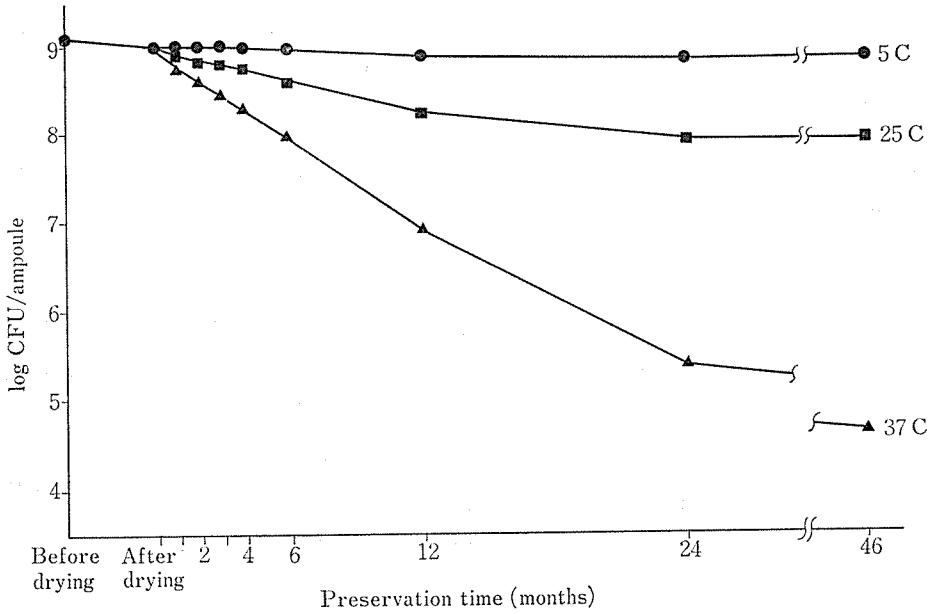

Fig. 1. Change in survival value of L-dried cells of *Escherichia coli* strain F 682 during preservation at 5 C, 25 C, and 37 C.

between 0 and 12 months after drying:

$$\log Y = \log Y_0 - K_t M$$

where Y is the viable counts after preservation for M months at t C, Y_0 is the viable count immediately after drying, and K_t is the specific rate of degradation of the dried

cell at t C. From data in Fig. 1, the values of K at 5 C, 25 C, and 37 C are calculated to be 0.0125, 0.065 and 0.179, respectively.

The semilog plot of these K values versus the reciprocal of temperature (1/T) in Fig. 2 shows a linear relationship between K and 1/T. This indicates that the degradation follows Arrhenius' kinetics.

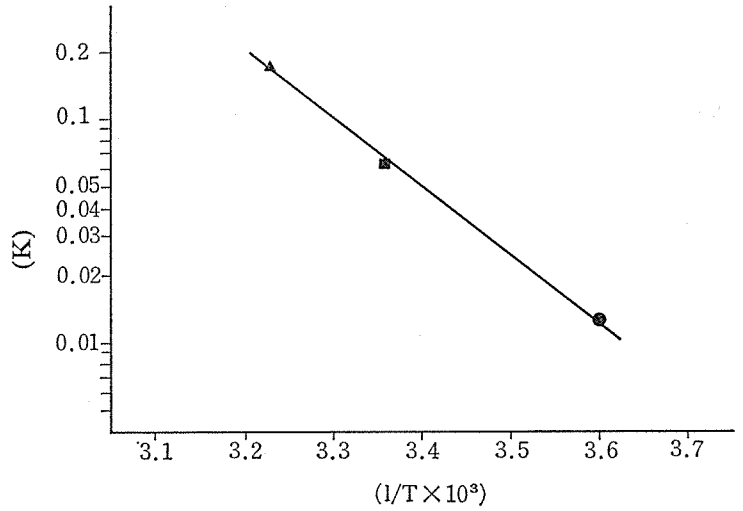

Fig. 2. Thermal degradation plots for L-dried cells of *E. coli* strain F 682.
The curve is the plots of rate of thermal degradation (K) against the reciprocals of absolute temperature (1/T).

If the rate of degradation remained constant during preservation after 12 months, this L-dried culture would die out after 4 years at 37 C or after 50 years at 5 C. Fig. 1 shows, however, that the degradation gradually get slower, and eventually ceases. In other words, the viability of the dried culture preserved at low temperature ceases to decline after 24 months, suggesting that dried cultures of this bacterium will survive semi-premanently if stored at low temperature. It should be noted in Fig. 1 that the survival value when degradation has ceased of the L-dried culture stored at 5 C is equal to that of the culture stored for 2 weeks at 37 C. To ascertain whether this observation is true in general, 53 bacterial strains were selected at random from the collection, and the survival values of their dried cultures were compared after preservation for 2 weeks at 37 C and 80–92 months at 5 C.

The data are presented in Table 1. Recovery rate after 80–92 months at 5 C varied between 0.4 and 118%. The percent survival immediately after drying, which indicate sensitivity to drying, do not correlate with recovery after preservation. In general high recovery after preservation was obtained in gram-positive bacteria. In *Aeromonas hydrophila* IFO 3820, *Acetobacter xylinus* IFO 3288 and *Pseudomonas fluorescens* IFO 3903, the recovery from the L-dried cultures was less than 2% after 80 months at 5 C. Further investigation into conditions of drying is required to increase the recovery rate in these bacteria.

Table 1. Survival value of L-dried cells of various bacteria after drying and preservation.

Strains	IFO No.	log CFU per ampoule			% Recovery*	
		After drying	Preservation for 2 weeks at 37 C	Preservation for 80 to 92 months at 5 C	Preservation at 37 C	Preservation at 5 C
Acetobacter aurantius	13331	8.68 (34**)	7.92	8.04	18	23
Acetobacter xylinus	3288	6.97 (95)	5.51	5.28	3	2
Achromobacter xerosis	12668	8.46 (18)	7.98	8.11	33	45
Aeromonas hydrophila	3820	7.48 (0.9)	4.44	5.11	0.2	0.4
Agrobacterium radiobacter	13256	9.28 (79)	8.79	8.80	32	33
Alcaligenes faecalis	13111	9.77 (72)	8.91	9.28	14	32
Arthrobacter tumescens	12960	8.76 (81)	8.69	8.32	84	36
Azotobacter chroococcum	12994	7.48 (5)	6.04	6.25	4	6
Azotobacter vinelandii	12018	7.58 (2)	6.70	7.52	13	87
Bacillus cereus	3132	8.04 (100)	8.15	8.11	127	118
Bacillus subtilis	13169	7.49 (10)	7.08	7.36	39	74
Beijerinckia indica	3744	7.71 (10)	7.42	7.00	51	20
Brevibacterium ammoniagenes	12071	9.08 (100)	9.04	8.97	92	78
Cellulomonas flavigena	3748	9.20 (70)	9.11	9.15	81	88
Chromobacterium violaceum	12614	7.25 (2)	6.49	6.54	17	19
Comamonas terrigena	12685	6.95 (0.8)	6.32	6.34	23	24
Corynebacterium nephridii	12159	9.23 (89)	9.18	9.30	88	118
Enterobacter cloacae	12935	9.53 (92)	9.38	9.56	71	106
Erwinia carotovora	3380	9.04 (44)	8.45	8.26	25	17
Erwinia carotovora	3830	8.74 (39)	7.88	8.38	14	44
Erwinia carotovora	12380	7.90 (40)	7.23	7.58	22	48
Erwinia herbicola	12686	9.61 (100)	9.40	9.53	61	83
Escherichia coli	13168	9.42 (93)	8.38	8.08	9	5
Flavobacterium suaveolens	3752	9.62 (81)	9.52	9.56	79	86
Gluconobacter melanogenes	3293	7.98 (35)	7.11	6.72	15	6
Klebsiella pneumoniae	3318	8.53 (8)	8.32	8.53	62	100
Klebsiella pneumoniae	3512	8.61 (80)	8.59	8.58	95	93
Lactobacillus casei	3533	7.73 (68)	7.32	7.67	39	87
Microbacterium thermosphactum	12167	7.88 (54)	7.40	7.61	33	54
Micrococcus luteus	12708	9.28 (100)	9.30	9.26	105	95
Micrococcus roseus	3764	9.00 (100)	9.00	9.04	100	110
Mycobacterium smegmatis	3083	9.04 (16)	9.00	9.04	91	100
Paracoccus denitrificans	13301	8.46 (32)	7.70	7.60	17	14
Proteus inconstans	12930	8.84 (75)	8.65	8.61	65	59
Proteus mirabilis	3849	9.11 (43)	8.69	8.97	38	72
Proteus morganii	3168	8.23 (39)	7.56	8.11	21	76
Proteus morganii	3848	8.55 (53)	8.40	8.12	71	37
Proteus vulgaris	3167	8.28 (41)	7.28	8.11	10	68
Proteus vulgaris	3851	7.71 (16)	6.52	6.98	6	19

Table 1. (continued)

Strains	IFO No.	log CFU per ampoule			% Recovery*	
		After drying	Preservation for 2 weeks at 37 C	Preservation for 80 to 92 months at 5 C	Preservation at 37 C	Preservation at 5 C
Pseudomonas aeruginosa	13520	9.18 (68)	8.65	8.25	30	12
Pseudomonas auricularis	13334	8.00 (9)	6.68	7.23	5	17
Pseudomonas denitrificans	13302	9.18 (72)	8.23	8.28	11	13
Pseudomonas fluorescens	3903	7.32 (5)	6.08	5.66	6	2
Rhizobium japonicum	13338	5.73 (2)	4.73	5.00	10	19
Rhodopseudomonas spheroides	13521	8.18 (25)	7.18	7.25	10	12
Salmonella typhimurium	13245	9.30 (69)	8.85	8.76	36	29
Serratia liquefaciens	12979	9.28 (78)	8.99	9.11	54	78
Serratia marcescens	3759	9.42 (100)	9.18	9.15	58	54
Spirillum lunatum	3985	8.36 (12)	7.62	7.11	18	6
Staphylococcus epidermidis	12993	8.82 (100)	8.54	8.70	53	76
Streptococcus faecalis	12968	8.66 (77)	8.43	8.51	59	70
Xanthomonas campestris	13303	8.72 (78)	8.53	8.52	65	63
Xanthomonas oryzae	3995	7.32 (4)	6.94	6.64	41	21

* Recovery rate; percentage of CFU (viable count) after preservation to that immediately after drying.
** Percentage of CFU immediately after drying to that in suspension before drying.

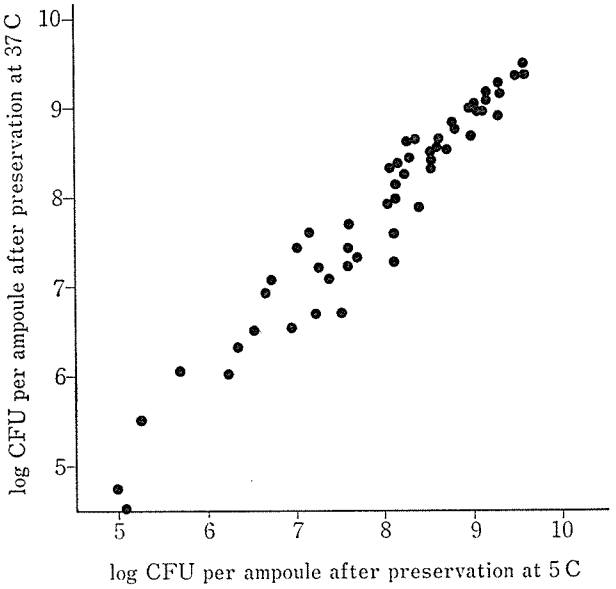

Fig. 3. Correlation between the survival values of L-dried cells of various bacceria after preservation at 37 C for 2 weeks and 5 C for 80 to 92 months.

The recoveries of L-dried cultures in the accelerated storage test varied between 0.2 and 127%. With the limits of experimental error, the survival values of individual strains after 2 weeks at 37 C were equal to those after preservation of 80–92 months at 5 C. The linear correlation between the viable counts after 2 weeks at 37 C and after 80–90 months at 5 C is shown in Fig. 3. The correlation coefficient wsa calculated to be 0.96.

These result demonstrate that for a variety of bacteria, the survival value obtained by the accelerated storage test is indicative of the number of cells which will survive prolonged preservation at such a low temperature as 5 C. The accelerated storage test is effective for predicting the viability of L-dried cultures in long-term preservation and is applicable to various microorganisms.

We are grateful to Dr. T. Miwatani, Professor of the Research Institute for Microbial Disease, Osaka University, for his critical reading of the manuscript.

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PRESERVATION OF YEAST CULTURES ON ANHYDROUS SILICA GEL

Isao BANNO, Kozaburo MIKATA and Sakae YAMAUCHI

Summary

A variety of yeasts was preserved on anhydrous silica gel. Two hundred yeast strains tested were all alive after 2 years. After 10 years, 181 strains were still viable.

Perkins (1962) (5) reported a simple preservation method using anhydrous silica gel. He preserved cultures of *Neurospora crassa* by pipetting a suspension of conidia or mycelia in skim milk into tubes containing anhydrous silica gel particles. This method was applied to 700 cultures of *N. crassa*. The cultures recovered from the dried state over several years yielded identical inocula free from mutation. Subsequently the method was tested with other fungi. *Aspergillus nidulans, Asp. parasiticus* (3), *Claviceps paspali, Ustilago maydis, Endothia parasitica, Fusarium oxysporum* and *Verticillium* spp. (6) were reported to be preserved successfully in a few years. Several species of yeast, *Saccharomyces cerevisiae* (2), *Candida* spp. (4) and *Rhodotorula* spp. (7) were reported to be viable for at least 3 years on the silica gel. This technique has been also used for maintaining some green algae, cellular slime mould and several species of bacteria.

Trollope (8) tested a modification of Perkins' method with 33 bacterial and 22 fungal cultures including 2 yeasts and reported that when stored at 4 C, all 22 of the fungi tested and 73% of the bacteria survived over 1 year, and 36% of fungi and 60% of bacteria were still viable after 3 to 4 years. Storage at 4 C increased the survival period compared with that at 37 C.

In the many cases reported, the storage period has been within 4 years. For *N. crassa* and *Asp. parasiticus*, however 5 to 7 years have been reported (3, 5). In the present work, we assess the usefulness of a simple modification of Perkins' method for long-term preservation of a variety of yeast cultures.

Materials and Methods

Strains. Strains liable to die within a short time when preserved on agar slants were selected from the collection of the Institute for Fermentation, Osaka.

Drying on silica gel. Screw-capped vials (10×60 mm) were half-filled with silica gel (30:1 mixture of white and blue small granular: Wako chemicals Ltd.) and sterilized at 180 C with dry heat for 3 hr. Each strain was grown on a slope of YM agar medium

at appropriate temperature until good confluent growth was obtained. Cells were harvested by means of a fine spatula and deposited on the inner wall of the vial. Then the vial was tightly closed and stored in a tightly sealed box in a cold room at 4 C.

Viability assessment. The viability of cultures stored on silica gel was determined by removing a fine piece of dried cell mass from the vials with a hook and spreading the cells on YM agar plates. After incubation at optimal temperature, colonies growing on the plate were examined macroscopically and microscopically to determine wether the original characteristics had been retained.

Results and Discussion

Cultures of the two hundred yeasts listed in Table 1 were dried on the silica gel

Table 1. Viability of yeasts preserved on silica gel at 4 C over 10 years.

Species	Number of strains dried	Number of strains viable after 10 years
Brettanomyces		
B. anomalus	2	2
B. bruxellensis	3	3
B. claussenii	1	1
B. lambicus	4	4
Bullera		
B. alba	4	4
Candida		
C. albicans	2	2
C. bovina	6	3
C. curiosa	1	0
C. diddensii	1	1
C. diversa	2	2
C. glaebosa	1	1
C. humicola	1	1
C. melinii	2	2
C. mesenterica	8	6
C. mogii	1	1
C. pseudotropicalis	2	1
C. rugosa	1	1
C. sake	1	1
C. salmonicola	1	1
C. slooffii	1	1
C. stellatoidea	2	2
C. tropicalis	1	1
C. utilis	2	2
Cryptococcus		
C. albidus	4	4

Table 1. (continued)

Species	Number of strains dried	Number of strains viable after 10 years
C. flavus	4	4
C. laurentii var. *flavescens*	6	6
C. laurentii var. *laurentii*	1	1
C. laurentii var. *magnus*	2	2
C. macerans	1	1
C. neoformans	1	1
Debaryomyces		
D. coudertii	1	1
D. hansenii	1	1
D. tamarii	1	1
Eremascus		
E. fertilis	1	1
Hansenula		
H. anomala	2	2
H. jadinii	1	1
Kluyveromyces		
K. marxianus	1	1
Leucosporidium		
L. scottii	4	3
Lipomyces		
L. starkeyi	2	0
L. lipofer	1	0
Metschnikowia		
M. pulcherrima	3	3
M. reukaufii	1	1
Nadsonia		
N. elongata	1	1
N. fulvescens	1	1
Nematospora		
N. coryli	2	2
Pichia		
P. mogii	1	1
Pityrosporum		
P. ovale	1	1
P. pachydermatis	1	1
Rhodosporidium		
R. infirmo-miniatum	3	3
R. toruloides	4	4
Rhodotorula		
R. aurantiaca	3	3
R. glutinis	7	6

Table 1. (continued)

Species	Number of strains dried	Number of strains viable after 10 years
R. graminis	1	1
R. lactosa	3	3
R. marina	3	3
R. minuta	5	5
R. psychrophila	1	1
R. rubra	7	6
Saccharomyces		
S. bailii	1	1
S. cerevisiae	2	2
S. chevalieri	1	1
S. italicus	1	1
S. kloeckerianus	1	1
S. rouxii	3	2
S. telluris	4	4
S. uvarum	2	2
Saccharomycopsis		
S. fibuligera	1	1
Schizosaccharomyces		
S. octosporus	3	3
S. pombe	2	2
Sporobolomyces		
S. gracilis	2	1
S. holsaticus	5	5
S. odorus	3	3
S. pararoseus	5	5
S. roseus	7	7
S. salmonicolor	4	4
Sterigmatomyces		
S. halophilus	1	1
Torulopsis		
T. candida	1	1
T. cantarellii	1	1
T. etchellsii	1	1
T. globosa	1	1
T. holmii	1	1
T. lactis-condensi	5	3
T. magnoliae	1	1
T. pintolopesii	1	1
T. pinus	1	1
T. stellata	4	4
T. versatilis	3	1
Trichosporon		
T. cutaneum	2	2
T. margaritiferum	1	1

and stored. The listed cultures were all alive after 2 years of preservation. Viability was next assessed after 10 years. The results are shown in Table 1.

Ten strains of *Brettanomyces* and 4 strains of *Bullera* tested were still alive. Twenty-nine of 36 strains of *Candida* survived, but viable colonies were not recovered for 3 of 6 strains of *C. bovina*, 1 strain of *C. curiosa*, 2 of 8 strains of *C. mesenterica*, and 1 of 2 strains of *C. pseudotropicalis*. The recovery in strains of *C. bovina* was especially low, and this species is also known to give a low survival value in L-dried culture. Fifteen strains of *Cryptococcus*, 3 strains of *Debaryomyces*, 1 strain of *Eremoascus*, 3 strains of *Hansenula*, and 1 strain of *Kluyveromyces* tested were all still alive. In *Leucosporidium*, 3 of 4 strains of *L. scotii* were still alive. In the genus *Lipomyces*, the 2 strains of *L. starkeyi* and 1 strain of *L. lipofer* tested were all inactivated. This genus is characterized by the presence of a large fat globule in the cell, which may be the reason why these yeasts are unstable in drying.

Four strains of *Metschnikowia*, 2 strains of *Nadsonia*, 2 strains of *Nematospora*, 1 strain of *Pichia*, 2 strains of *Pityrosporum*, and 7 strains of *Rhodosporidium* all remained viable, though the number of strains tested was small. Of 36 strains of *Rhodotorula*, 34 were viable but 1 each of *R. glutinis* and *R. rubra* was not. Of 15 strains of *Saccharomyces*, 14 survived but 1 strain of *S. rouxii* did not. All of 1 strain of *Saccharomycopsis fibuligera*, 5 strains of *Schizosaccharomyces*, 1 strain of *Sterigmatomyces*, and 3 strains of *Trichosporon* were viable. Of 26 strains of *Sporobolomyces*, 25 remained viable. Of 20 strains of *Torulopsis*, 2 of 5 strains of *T. lactis-condensi* and 2 of 3 strains of *T. versatilis* did not survive, but the others remained viable.

Of a total of 200 strains of various yeasts, 181 (90.5%) survived over 10 years of preservation. Compared with the results of Trollope (8) and others, this result indicates that yeast cultures tend to survive on silica gel better than most fungi and bacteria. The method has proved reliable for storing various yeasts over several years, and will allow laboratories without such facilities as lyophilization equipment to maintain many cultures, because it is simple and inexpensive compared with other preservation methods.

We are indebted to Dr. T. Miwatani, Professor of the Research Institute for Microbial Disease, Osaka University, for his critical reading of the manuscript.

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45-48, 1981 (March)

DESCRIPTIVE CATALOGUE OF IFO FUNGUS COLLECTION VII.

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 published records of their occurrence in Japan, many fungi have been found to be taxa either new to Japan or obscurely or insufficiently described. In some cases, the first record of a fungus in Japan gives only the name of a taxon, without an adequate description of the species concerned. The object of this series of publications is to provide descriptions of the fungi preserved or newly deposited in the IFO culture collection and/or in the IFO herbarium 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. Authors of the description of these fungal taxa are shown in brackets.

74. Acremonium fusidioides (Nicot) W. Gams (Pl. 1, A) Hyphomycetes *Cephalosporium*-artige Schimmelpilze (Hyphomycetes), P. 70 (1971).
Syn. *Paecilomyces fusidioides* Nicot, Cah. Maboké **6**: 17 (1968).

Colonies on oatmeal agar growing somewhat restrictedly at 24 C (16–18 mm in diam after 10 days), more or less zonate, thin to lanose, partly floccose, white through rosy buff to reddish grey, white at the margin; reverse uncolored or pale brown. Conidiophores erect, simple but rarely branched, tapering towards the apex, septate, smooth-walled, 20–28×2.5—3.0 μm, 0.8–1.0 μm wide at the apex. Conidia of two types; one catenate in a long chain, fusiform, 1-celled, pale green, smooth, 4.8—6.4×1.5—2.5 μm, the other irregularly catenate in a short chain of 1–7 conidia, globose, 1-celled, hyaline, thick-walled, scarcely roughened, 4–5 μm in diam. Teleomorph not observed.

Growth is nil at 37 C.

Hab. From soil: Yakeyama, Kamikita-gun, Aomori Pref., Oct. 4, 1977, T. Ito S52E-51-3 (IFO 31139); Tsuta Spa, Kamikita-gun, Aomori Pref., Oct. 5, 1977, T. Ito S52E-63-4 (IFO 31140) and Shakudo, Habikino, Osaka Pref., Feb. 20, 1979, T. Yokoyama XXI-5-5-27 (IFO 31141).
Additional strain examined: IFO 6813.

This species differs distinctly from other related species in having two types of conidia, fusiform and globose, on the same hypha. These two types of conidia were observed in all of three strains isolated, but only in old age. These isolates fit very well with Gams' description as well as with the strain IFO 6813, which was once erroneously identified by Tubaki (1954) as *Fusidium coccineum* Fuckel sensu Tubaki and treated as identical to this species by Gams (1971).

[T. Ito & T. Yokoyama]

75. Chaetomium convolutum Chivers (Pl. 1, B-C) Sphaeriales
Proc. Amer. Acad. Arts Sci. **48**: 83 (1912); Chivers, Mem. Torrey Bot. Club, **14**: 155 (1915); Skolko & Groves, Can. J. Bot. **31**: 779 (1953); Seth, Beih. Nova Hedwigia, **37**: 55 (1970).

Colonies on oatmeal agar growing rapidly at 24 C, white to grey, then becoming dark grey to black in age. Vegetative mycelium submerged, thin, floccose at the margin; reverse uncolored or pale yellow. Perithecia abundant, scattered, dark brown to black at maturity, subglobose to ovate, 275—330×175—225 μm; terminal hairs dark brown, straight near the base, spirally coiled at the attenuated tip, 5–6 μm wide at the base, unbranched, septate, rough-walled; lateral hairs straight with a tapering tip, scarcely roughened; peridium dark brown, membranaceous. Asci 8-spored, clavate, hyaline, 30—36×12—15 μm, shortly stipitate, evanescent. Ascospores biseriate, ellipsoid to ovoid with apiculate ends, 1-celled, pale brown to dark brown, 8—9×5—6 μm. Anamorph not observed.

Growth is nil at 37 C.

Hab. Isolated as an air contaminant in a culture of *Choanephora cucurbitarum* received from Tokyo. Aug. 1, 1979 (IFO 30917).
Additional strains examined: IFO 9107 and IFO 9910.

This species closely resembles *Chaetomium bostorycodes* in its morphological characteristics but differs clearly in having pale brown and lemon shaped ascospores at maturity.

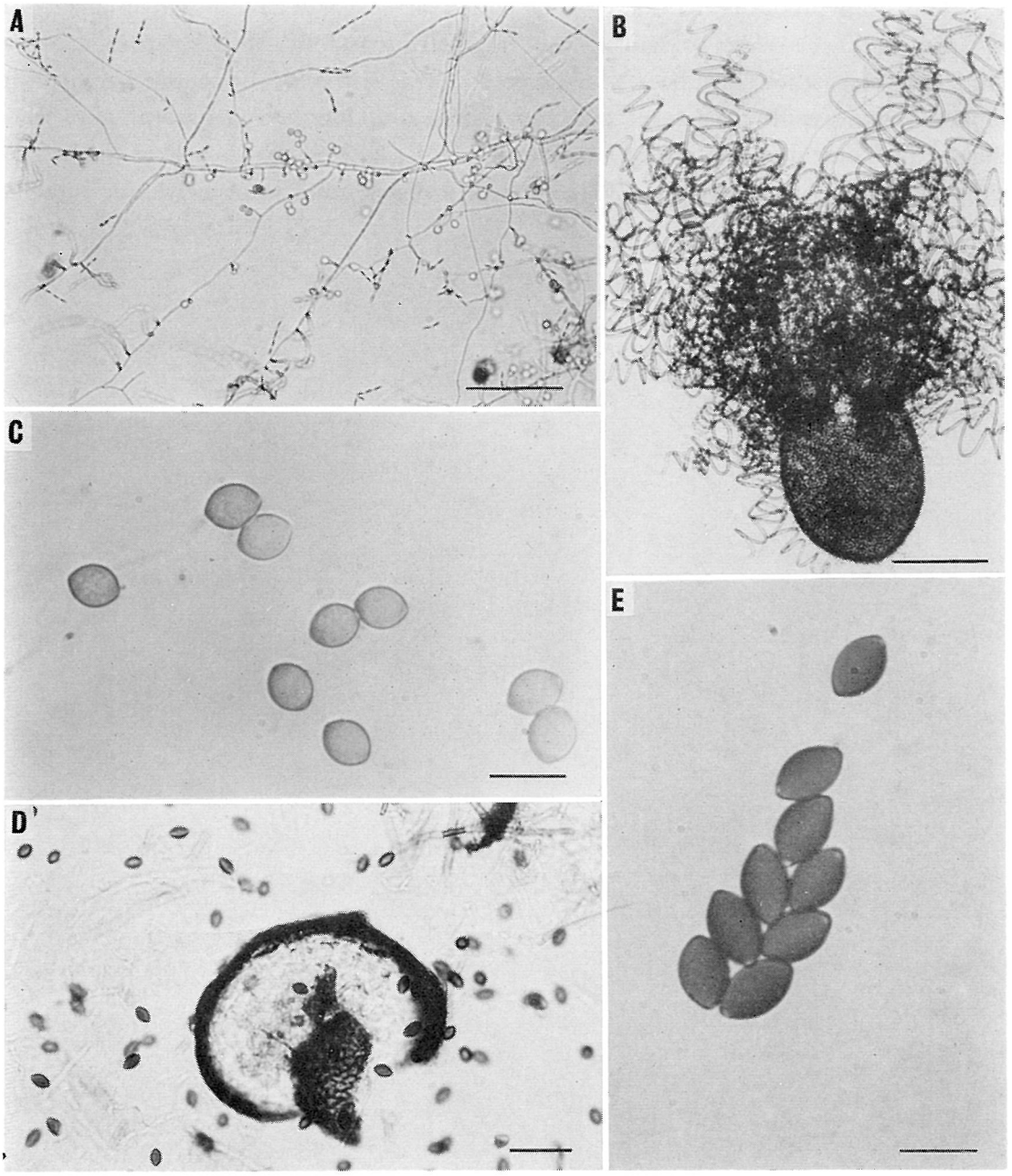
[T. Yokoyama & T. Ito]

76. Thielavia arenaria Mouchacca (Pl. 1, D-E) Eurotiales
Bull. Trim. Soc. Mycol. Fr. **89**: 295 (1973); von Arx, Studies in Mycology **8**: 1 (1975).

Colonies on potato carrot agar growing rapidly at 37 C (8.0 cm in diam after 7 days), grey brown to brown, lanose to floccose; aerial mycelium creeping on agar medium, pale brown at first, later dark brown, smooth, rigid, septate, 3–4 μm wide. Ascocarps globose to subglobose, non-ostiolate, dark brown to almost black, covered with dark brown hyphae, 100—250×90—230 μm; peridium consist of the tissue type *textura epidermoidea,* thin, 1–3 layered, 5–8 μm wide. Asci 8-spored, ovoid to subglobose, hyaline, evanescent, 20—25×18—23 μm. Ascospores ovoid to ellipsoid, at first hyaline, later becoming dark brown, with a subapical germ pore, 10—12.5× 6—7.5 μm. Conidia pyriform to clavate, sometimes irregular in shape, 1-celled, pale brown, thin walled, smooth, 3—6×2.5—5 μm, of aleuriospore-type.

Growth is observed even at 45 C.

Hab. From soil: Sukayu Spa, Aomori, Aomori Pref., Oct. 5, 1977, T. Ito S5242-68-3 (IFO 31142).



Pl. 1. A. *Acremonium fusidioides* (IFO 31141). B & C. *Chaetomium convolutum* (IFO 30917). B. Perithecium. C. Ascospores. D & E. *Thielavia arenaria* (IFO 31142). D. Section of a cleistothecium. E. Ascospores. Bars A=50 μm; B=125 μm; C & E=10 μm; D=20 μm

Additional strains examined: CBS 507.74 (IFO 31060) and CBS 508.74 (IFO 31061).

This strain produces ascocarps well on potato carrot agar at 28 C, but mycelial growth is better at 37 C. The present species originally found by Mouchacca in a desert soil in Egypt has not since been rediscovered. This is the second isolate from soil in Japan. Very recently, we succeeded in isolating four more strains from soils; three isolates in Korea and one in the far eastern USSR, both of which belong to the cool temperate zone (unpublished). This geological distribution is of particular interest.

[T. Yokoyama & T. Ito]

IFO Res. Comm. **10**,
49–53, 1981 (March)

DESCRIPTIVE CATALOGUE OF IFO YEAST COLLECTION III.

In the routine work of updating data on strains in the collection, interesting strains worthy of record have been found and a few strains have been reidentified as different species. The object of this catalogue is to provide descriptions of taxonomical characteristics of these strains. The following descriptions are arranged in alphabetical order of the scientific names of strains. The authors of descriptions are indicated in brackets.

18. Candida guilliermondii (Castellani) Langeron & Guerra var. **guilliermondii** Castellani, A. 1912. Brit. Med. J. **2**: 1208; van Uden, N. & H. Buckley. 1970. *In* The Yeasts, A Taxonomic Study, ed. by J. Lodder, North-Holland Publ. Co., Amsterdam, p. 967.

IFO 0437

Morphological and cultural characteristics of the strain are almost the same as those of *Candida guilliermondii* var. *guilliermondii* described in *The Yeasts* (1970).

Physiological characteristics are presented in Table 1.

This strain was obtained from M. Mogi, Noda Institute for Scientific Research, Noda, Japan, in 1949, under the name *Torulopsis miso* α var. 1 Mogi and has been entered in the IFO List of Cultures 6th ed. under the name *Candida mogii*.

[K. Mikata & I. Banno]

19. Cryptococcus albidus (Saito) Skinner var. **albidus**

Saito, K. 1922. Japan J. Bot. **1**: 1; Phaff, H. J. & J.W. Fell. 1970. *In* The Yeasts, A Taxonomic Study, ed. by J. Lodder, North-Holland Publ. Co., Amsterdam, p. 1093.

IFO 1420

Morphological and cultural characteristics of the strain are almost the same as those of *Cryptococcus albidus* var. *albidus* described in *The Yeasts* (1970).

Physiological characteristics are presented in Table 1.

This strain was obtained from Herbarium of the Department of Botany, Facullty of Science, University of Tokyo, in 1967, under the name *Cryptococcus neoformans*. (Sugiyama, J. & S. Goto. 1967. J. Jpn. Bot. 42: 75)

[K. Mikata & I. Banno]

20. Cryptococcus laurentii (Kufferath) Skinner var. **flavescens** (Saito) Lodder & Kreger-van Rij

Saito, K. 1922. Japan J. Bot. **1**: 1; Phaff, H.J. & J.W. Fell. 1970. *In* The Yeasts, A Taxonomic Study, ed. by J. Lodder, North-Holland Publ. Co., Amsterdam, p. 1120.

IFO 1376

Morphological and cultural characteristics of the strain are almost the same as those of *Cryptococcus laurentii* var. *flavescens* described in *The Yeasts* (1970).

Physiological characteristics are presented in Table 1.

This strain was given by D.G. Ahearn, University of Miami, in 1966, under the name *Cryptococcus laurentii*.

[K. Mikata & I. Banno]

Table 1. Physiological

	C. guilliermondii var. *guilliermondii* IFO 0437	*Cry. albidus* var. *albidus* IFO 1420	*Cry. laurentii* var. *flavescens* IFO 1376
Fermentation			
Glucose	+	−	−
Galactose	+	−	−
Sucrose	+	−	−
Maltose	−	−	−
Trehalose	−	−	−
Lactose	−	−	−
Raffinose	−	−	−
Inulin	−	−	−
Soluble starch	−	−	−
α-Methyl-D-glucoside	−	−	−
Assimilation of carbon compounds			
Glucose	+	+	+
Galactose	+	+	+
L-Sorbose	+ s	+ w s	+ w
Sucrose	+	+	+
Maltose	+	+	+
Cellobiose	+ s	+	+
Trehalose	+	+	+
Lactose	−	+ s	+ s
Melibiose	−	−	+
Raffinose	+	+ w	+ s
Melezitose	+	+	+
Inulin	+ s	−	−
Soluble starch	−	+ s	+ s
D-Xylose	+	+	+
L-Arabinose	+	+	+
D-Arabinose	+	+ w	+ s
D-Ribose	+	+ w	+
L-Rhamnose	+	+	+ s
Ethanol	+	+	−
Glycerol	+	+ w	+ w s
Erythritol	−	−	−
Ribitol	+	+ w	+ w s
Galactitol	+	−	+
D-Mannitol	+	+ w	+
D-Glucitol	+	+	+
α-Methyl-D-glucoside	+	+	+ s
Salicin	+ s	+	+ w
DL-Lactic acid	+ w s	−	+ w
Succinic acid	+	−	−
Citric acid	+	+	−
Inositol	−	+	+ w
Splitting of arbutin	+ s	+	+
Assimilation of potassium nitrate	−	+	−
Growth in vitamin-free medium	−	+	−
Growth at 37 C	−	−	−
Hydrolysis of urea	−	+	+

+ =positive, − =negative, w=weak, s=slow, NT=not tested.

21. Hansenula beijerinckii van der Walt

van der Walt, J.P. 1957. Antonie van Leeuwenhoek **23**: 23; Wickerham, L.J. 1970. *In* The Yeasts, A Taxonomic Study, ed. by J. Lodder, North-Holland Publ. Co., Amsterdam, p. 254.

characteristics

H. beijerinckii IFO 0810	*P. membranaefaciens* IFO 0839 IFO 0840	*Sp. pararoseus* IFO 0471	*Sp. pararoseus* IFO 1105	*Saccharomycopsis lipolytica* IFO 1542
+	+	−	−	−
−	−	−	−	−
+	−	−	−	−
−	−	−	−	−
−	−	−	−	−
−	−	−	−	−
+	−	−	−	−
+ w	−	−	−	−
−	−	−	−	−
−	−	−	−	−
+	+	+	+	+
−	−	+ w s	+ s	−
−	+	+ w s	+ s	+ w
+	−	+	+	−
+	−	+	+	−
+	−	+	+	−
+	−	+	+	−
−	−	−	−	−
−	−	−	−	−
+	−	+	+	−
+	−	+	+	−
+ s	−	−	−	−
−	−	+ w	+	−
+	+ w s	−	+ w	−
−	−	−	−	−
−	−	+ w	+ w	−
−	−	−	−	+ s
−	−	−	−	−
+	+	+	+ s	+
+	+	+ w	+ s	+
−	−	−	−	+
−	−	+	−	+ s
−	−	−	−	−
+	−	+	+	+
+	−	+	+	+
+	−	−	+	−
+	−	+ w	+	−
+	+	−	+	+
+	−	+	+ w	+
−	−	+ w	−	+
−	−	−	−	−
+	−	+	+	−
+	−	−	−	−
+	+	+	+	−
−	+	−	−	−
−	−	NT	NT	+

IFO 0810

Morphological and cultural characteristics of the strain are almost the same as those of *Hansenula beijerinckii* described in *The Yeasts* (1970).

Physiological characteristics are presented in Table 1.

This strain was obtained from Laboratory of Kodama Brewing Co., Akita, Japan, in 1956, under the name of *Hansenula saturnus* (LKB 157) and has been entered in the IFO List of Cultures 6th ed. under this name.

[K. Mikata & I. Banno]

22. and **23. Pichia membranaefaciens** Hansen

Hansen, E. Chr. 1888. Medd. Carlsberg Lab. **2**; 220; Hansen, E. Chr. 1904. Zentr. Bakteriol. Parasitenk., Abt. II, **12**: 529; Kreger-van Rij, N.J.W. 1970. *In* The Yeasts, A Taxonomic Study, ed. by J. Lodder, North-Holland Publ. Co., Amsterdam, p. 500.

IFO 0839 and 0840

Morphological and cultural characteristics of these strains are almost the same as those of *Pichia membranaefaciens* described in *The Yeasts* (1970). Formation of ascospores is observed on corn meal agar. Vegetative cells are directly transformed into asci. One to four spherical spores mostly containing lipid globule are formed per ascus.

Physiological characteristics are presented in Table 1.

These strains were obtained from Y. Ohara, Research Institute of Fermentation, Yamanashi University, Kofu, Japan, in 1957, under the name *Candida krusei* (RIFY 7711 and RIFY 7712).

[K. Mikata & I. Banno]

24. and **25. Sporobolomyces pararoseus** Olson & Hammer

Olson, H.C. & B.W. Hammer. 1937. Iowa State Coll. J. Sci. **11**: 207; Phaff, H.J. 1970. *In* The Yeasts, A Taxonomic Study, ed. by J. Lodder, North-Holland Publ. Co., Amsterdam, p. 846.

IFO 0471 and IFO 1105

Morphological and cultural characteristics of these strains are almost the same as those of *Sporobolomyces pararoseus* described in *The Yeasts* (1970). Mirror images of the inoculation line on the agar medium of inverted dish are found on the bottom dish. Reniform ballistospores are formed on sterigmata of cells.

Physiological characteristics are presented in Table 1.

Strain IFO 0471 was obtained from H. Naganishi, Faculty of Engineering, Hiroshima University, Hiroshima, Japan, in 1946, under the name *Torula sanguinea.*

Strain IFO 1105 was obtained from the Nagao Institute, Tokyo, Japan, in 1961, under the name *Sporobolomyces salmoneus* (NI 7485).

IFO 0471 and 1105 have been entered in the IFO List of Cultures 6th ed. under the names *Rhodotorula rubra* and *Sporobolomyces roseus* respectively.

[K. Mikata & I. Banno]

26. Saccharomycopsis lipolytica (Wickerham *et al.*) Yarrow

Yarrow, D. 1972. Antonie van Leeuwenhoek **38**: 357.

Nomenclatural synonym. *Endomycopsis lipolytica* Wickerham, I.J.,C.P. Kurtzman, & A.I. Herman. 1970. *in* Recent trends in Yeast Research, ed. by D.G. Ahearn, School of Arts and Sciences, Georgia State Univ., Atlanta, p. 81.

IFO 1542

Morphological and cultural characteristics of the strain are almost the same as those of *Endomycopsis lipolytica* described by Wickerham *et al.* (1970).

Physiological characteristics are presented in Table 1.

This strain was obtained from Institute of Applied Microbiology, University of Tokyo, in 1970, under the name *Candida rugosa.*

[K. Mikata & I. Banno]

IFO Res. Comm. **10**,
54-66, 1981 (March)

DESCRIPTIVE CATALOGUE OF IFO BACTERIAL COLLECTION V.

In the routine work of updating data on strains in the collection, a few strains have been reidentified as different species, and some strains that have frequently been used in research but whose characteristics have been insufficiently described have been identified as known species. In the identification of newly isolated strains, interesting strains worth adding to the collection have been found. The object of this catalogue is to provide descriptions of taxonomical characteristics of these strains. Below, the descrioptions are arranged in alphabetical order of the scientific name of the strains. The authors of descriptions are shown in brackets.

48. Aeromonas hydrophila subsp. **hydrophila** (Chester) Stanier 1943, biotype 1. IFO 13283

Young cells: Gram-negative rods, 0.6—0.8×2—3 μm, motile by single polar flagellum.
Colonies: Circular, convex, entire, translucent, pale-brown.
Pigment (water-soluble) on trypticase soy agar: Negative.
Oxidase: Positive.
Catalase: Positive.
Hugh-Leifson's OF test: Fermentative.
Voges-Proskauer test: Positive.
Methyl red test: Negative.
Hydrolysis of
- gelatin: Positive.
- casein: Positive.
- starch: Positive.

Oxidation of
- gluconate: Positive.
- malonate: Negative.

Production of
- indole: Positive.
- H_2S: Positive.

Litmus milk: Slowly peptonized, acidified.
Denitrification: Negative.
Reduction of NO_3 to NO_2: Positive.
Decarboxylation of
- lysine: Negative.
- arginine: Positive.
- ornithine: Negative.

Deamination of phenylalanine: Positive.

Utilization of citrate: Positive.
Urease: Positive.
Utilization as sole sources of carbon
glucose: Positive.
trehalose: Positive.
valine: Negative.
alanine: Negative.
arginine: Positive.
Utilization as sole sources of nitrogen
ammonium sulfate: Positive.
potassium nitrate: Positive.
Acid and gas from galactose, glucose, mannitol, maltose, salicin, sucrose and glycerol.
No acid and no gas from lactose, adonitol, L-arabinose, dulcitol, inositol, and sorbitol.
DNase: Positive.
Growth at 37 C: Positive.
Growth in 7.5 % NaCl broth: Negative.

This strain was obtained from ARS Culture Collection, Northern Regional Research Center, U.S. Department of Agriculture, Peoria, in 1971, under the name *Aeromonas hirudinus*.

[M. Takeuchi & I. Banno]

49. Bacillus polymyxa Nacé 1889
IFO 13003

Young cells: Gram-positive rods, 0.7—0.9×4—7 μm: motile by peritrichous flagella.
Spores: Elliptical; subterminal to terminal; sporangia distinctly swollen.
Colonies: Irregular, low convex, undulate, opaque, smooth, pale-brown.
Oxidase: Negative.
Catalase: Positive.
Anaerobic growth: Positive.
Voges-Proskauer test: Positive.
Methyl red test: Positive.
Hydrolysis of
gelatin: Negative.
casein: Positive.
starch: Positive.
Oxidation of
gluconate: Negative.
malonate: Positive.
Production of
indole: Negative.
H_2S: Positive.
Litmus milk: Coagulated, reduced, not peptonized.

Denitrification: Negative.
Reduction of NO_3 to NO_2: Positive.
Decarboxylation of
lysine: Negative.
arginine: Negative.
ornithine: Negative.
Deamination of phenylalanine: Negative.
Utilization of citrate: Negative.
Urease: Negative.
Novobiocin sensitivity, 0.6 μg/ml: Sensitive.
Utilization as sole sources of nitrogen
ammonium sulfate: Positive.
potassium nitrate: Weakly positive
Acid and gas from glucose, cellobiose, maltose, mannitol, lactose, salicin, sucrose, D-xylose, and glycerol. Acid but no gas from inositol and sorbitol. No acid and no gas from adonitol, L-arabinose and dulcitol.
Growth in 5 % NaCl broth: Negative.
Temperature for growth: Grows at 37 C, does not grow at 10 C or 45 C.

This strain was obtained from Y. Kimura, Faculty of Pharmaceutical Sciences, Mukogawa Women's University, Japan, in 1969, under the name *Bacillus polymyxa* T-14.

[M. Takeuchi & I. Banno]

50. Bacillus pumilus Meyer and Gottheil 1901
IFO 13004

Young cells: Gram-positive rods, 0.7—0.8×2—3 μm; motile by peritrichous flagella.
Spores: Elliptical; central; sporangia not swollen.
Oxidase: Positive.
Catalase: Positive.
Anaerobic growth: Negative.
Voges-Proskauer test: Positive.
Methyl red test: Positive.
Hydrolysis of
gelatin: Positive.
casein: Positive.
starch: Negative.
Oxidation of
gluconate: Negative.
malonate: Negative.
Production of
indole: Negative.
H_2S: Positive.

Litmus milk: Unchanged.
Denitrification: Negative.
Reduction of NO_3 to NO_2: Negative.
Decarboxylation of
lysine: Negative.
arginine: Negative.
ornithine: Negative.
Deamination of phenylalanine: Negative.
Utilization of citrate: Positive.
Urease: Negative.
Novobiocin sensitivity, 0.6 μg/ml: Sensitive.
DNase: Negative.
Utilization as sole sources of nitrogen
ammonium sulfate: Positive.
potassium nitrate: Negative.
Acid but no gas from glucose, cellobiose, mannitol, salicin, sucrose and glycerol. No acid and no gas from adonitol, L-arabinose, dulcitol, inositol, lactose, sorbitol, and D-xylose.
Temperature for growth: Grows at 10 C to 45 C, dose not grow at 55 C.

This strain was obtained from Y. Kimura, Faculty of Pharmaceutical Sciences, Mukogawa Women's University, Japan, in 1969, under the name *Bacillus polymyxa* T-39.

[M. Takeuchi & I. Banno]

51. Enterobacter aerogenes Hormaeche and Edwards 1960
IFO 13285

Young cells: Gram-negative rods, 0.6—0.7×2—3 μm; motile by peritrichous flagella.
Colonies: Circular, low convex, entire, opaque, pale-brown.
Oxidase: Negative.
Catalase: Positive.
Hugh-Leifson's OF test: Fermentative.
Voges-Proskauer test: Positive.
Methyl red test: Negative.
Hydrolysis of
gelatin: Positive.
casein: Positive.
starch: Negative.
Oxidation of
gluconate: Positive.
malonate: Negative.
Production of indole: Negative.

Production of H_2S: Negative.
Litmus milk: Coagulated, reduced, peptonized.
Denitrification: Negative.
Reduction of NO_3 to NO_2: Positive.
Decarboxylation of
lysine: Positive.
arginine: Negative.
ornithine: Positive.
Deamination of phenylalanine: Negative.
Urease: Negative.
Utilization as sole sources of carbon
glucose: Positive.
acetate: Positive.
citrate: Positive.
gluconate: Positive.
malonate: Positive.
Utilization as sole sources of nitrogen
ammonium sulfate: Positive.
potassium nitrate: Positive.
Acid and gas from glucose at 45 C: Positive.
Acid and gas from L-arabinose, galactose, mannitol, sorbitol, D-xylose, maltose, sucrose, salicin and glycerol. Acid but no gas from lactose and inositol. No acid and no gas from adonitol and dulcitol.

This strain was obtained from ARS Culture Collection, Northern Regional Research Center, U.S. Department of Agriculture, Peoria, under the name *Aeromonas ichthyosmia*, in 1971.

[M. Takeuchi & I. Banno]

52. **Erwinia carotovora** (Jones) Bergey *et al.* subsp. **carotovora** Dye 1969
IFO 13921

Cells: Gram-negative rods; motile by peritrichous flagella.
Colonies on nutrient agar: Circular, entire, low-convex, smooth, pale-brown; no diffusible pigments produced.
Catalase: Positive.
Oxidase reaction: Negative.
Methyl red test: Weakly positive.

Voges-Proskauer test: Positive.
Hydrolysis of
gelatin: Positive.
casein: Negative.
starch: Negative.
Tween 80: Negative.
esculin: Positive.
Reduction of NO_3 to NO_2: Positive.
Production of indole: Negative.
Dihydrolation of arginine: Negative.
Decarboxylation of
lysine: Negative
ornithine: Negative.
Deamination of phenylalanine: Negative.
Oxidation of
malonate: Negative.
gluconate: Positive.
Mucoidal growth in sucrose medium: Positive.
Growth in 5 % NaCl broth: Positive.
Growth at 37 C: Positive.
Production of H_2S from cysteine: Positive.
ONPG test: Positive.
Growth factors not required.
Acid and gas produced from glucose, fructose, L-arabinose, xylose, sucrose, maltose, lactose, trehalose, melibiose, raffinose, sorbitol, mannitol and dulcitol. No acid and no gas produced from melezitose, inositol, glycerol and α-methylgulcoside.
The following compounds utilized as sole sources of carbon: Glucose, L-arabinose, trehalose, sucrose, cellobiose, citrate, lactate and propylene glycol. Inositol, malonate, formate, tartrate and arginine not utilized as sole sources of carbon.

This strain which produces two exopectinases, exopectate lyase (EC.4.2.2.9) and oligo-D-galactosiduronate lyase (EC.4.2.2.6) was isolated from sewage and designated W-2 by C. Hatanaka, Faculty of Applied Biological Science, Hiroshima University, Japan, in 1970.*

[T. Sakane & I. Banno]

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* Hatanaka, C. & J. Ozawa. 1972. Agric. Biol. Chem. **36**: 2307; 1973. *ibid.* **37**: 593.

53 to 57. **Lactobacillus casei** (Orla-Jensen) Hansen and Lessel 1971
IFO 3532, 3533, 3831, 3863 and 3953

	IFO 3532	IFO 3533	IFO 3831	IFO 3863	IFO 3953
Cells: Form	R	R	R	R	R
Gram-reaction	+	+	+	+	+
Motility	−	−	−	−	−
Endospore formation	−	−	−	−	−
Oxygen requirement for growth	F	F	F	F	F
Catalase	−	−	−	−	−
Oxidase reaction	−	−	−	−	−
Type of lactic acid fermentation	Homo	Homo	Homo	Homo	Homo
Optical activity of lactic acid produced	L(+)	L(+)	L(+)	L(+)	L(+)
Gas production from: Glucose	−	−	−	−	−
Gluconate	+	+	+	+	+
Litmus milk: Change of pH	Ac	Ac	Ac	Ac	Ac
Reduction of litmus	−	+	+	+	−
Coagulation	−	+	+	+	−
Peptonization	−	−	−	−	−
Production of NH_3 from arginine	−	−	−	−	−
Growth at: 10 C	+	+	+	+	+
20 C	+	+	+	+	+
45 C	−	−	−	+	−
pH 4.0	+	+	+	+	+
pH 5.0	+	+	+	+	+
pH 7.4	+	+	+	+	+
Tolerance for: 4% NaCl	+	+	+	+	+
6% NaCl	+	+	+	+	+
10% Ethanol	+	+	+	+	+
15% Ethanol	+	w	+	−	+
Requirement for: Thiamine	−	−	−	−	−
Riboflavine	+	+	+	+	−
Pyridoxal	+	+	+	+	−
Niacin	+	+	+	+	+
Folid acid	−	−	+	−	−
Acid production from: Glucose	+	+	+	+	+
L-Arabinose	−	−	−	−	−
Cellobiose	w	w	w	+	+
Galactose	+	+	+	+	+
Lactose	+	+	+	+	−
Maltose	+	+	+	+	+
Mannitol	+	+	+	+	+
Mannose	+	+	+	+	+
Melezitose	+	+	+	+	+

(continued)

	IFO 3532	IFO 3533	IFO 3831	IFO 3863	IFO 3953
Melibiose	−	−	−	−	−
Pyruvate	+	−	−	+	+
Raffinose	−	−	−	−	−
L-Rhamnose	+	−	−	+	−
Ribose	+	+	+	+	+
Salicin	+	+	+	+	+
Sorbitol	+	w	w	w	w
Sucrose	w	+	w	w	w
Trehalose	+	+	+	+	+
D-Xylose	−	−	−	−	−
Esculin	+	+	+	+	+

Abbreviations: R, rods; F, facultative anaerobes; Homo, homofermentative; L(+), dextrorotatory; Ac, acidic; and w, weakly positive.

Strains IFO 3532, 3533 and 3863 were obtained from Institute of Applied Microbiology, University of Tokyo, Japan, in 1953, 1957, and 1960, under the names *Lactobacillus acidophilus* (Moro) Holland, *Lactobacillus bulgaricus* (Luerssen and Kühn) Holland, and *Lactobacillus thermophilus* Ayers and Johnson, respectively. Strains IFO 3831 and 3953 were isolated by R. Kodama, Institute for Fermentation, Osaka, from moromi in sake brewing, and were entered in the IFO List of Cultures, 6th edition, as *Lactobacillus acidophilus* (Moro) Hansen and Mocquot. These have been reidentified as *Lactobacillus casei* on the basis of classification system described by M. Rogosa in Bergey's Manual of Determinative Bacteriology, 8th edition (1974).

[T. Sakane & I. Banno]

58 and 59. **Pediococcus acidilactici** Lindner 1887
IFO 12218 and 12231

	IFO 12218	IFO 12231
Cells morphology	Cocci occurring singly, in pairs, in tetrads, and in irregular clusters	
Gram-reaction	+	+
Catalase	−	w
Oxygen requirement for growth	F	F
Type of lactic acid fermentation	Homo	Homo
Type of lactic acid produced	DL	DL
Reduction of NO_3 to NO_2	−	−

(continued)

	IFO 12218	IFO 12231
Hydrolysis of:		
Gelatin	−	−
Tween 80	−	−
Esculin	+	+
Growth in 6% NaCl broth	−	−
Growth at:		
37 C	+	+
45 C	+	+
50 C	+	+ (slow)
pH 5.0	+	+
pH 7.0	+	+
pH 9.0	−	−
Gas production from glucose	−	−
Final pH in glucose broth	4.1	4.3
Acid production from:		
Glucose	+	+
Fructose	+	+
Galactose	+	+
Mannose	+	+
L-Arabinose	+	+
D-Xylose	+	+
Maltose	−	−
Lactose	−	−
Sucrose	−	+
Sorbitol	−	−
Mannitol	−	−
Starch	−	−

Abbreviations: w, weakly positive; F, facultative anaerobes; Homo, homofermetative; and DL, DL-lactic acid.

These strains were obtained from the Noda Institute for Scientific Research, Noda, Japan in 1965, under the names; IFO 12218 as *Pediococcus homari* Deibel and Niven, and IFO 12231 as *Pediococcus cerevisiae* Balcke.

These have been reidentified as *Pediococcus acidilactici* according to the description of species given by E. I. Garvie in 1974.*

[T. Sakane & I. Banno]

* Garvie, E. I. 1974. Int. J. Syst. Bacteriol. **24**: 301.

60 to 62. **Pediococcus pentosaceus** Mees 1934

IFO 12229, 12230 and 12232

	IFO 12229	IFO 12230	IFO 12232
Cells morphology	Cocci occurring singly, in pairs, in tetrads, and in irregular clusters		
Gram-reaction	+	+	+
Catalase	+	−	w
Oxygen requirement for growth	F	F	F
Type of lactic acid fermentation	Homo	Homo	Homo
Type of lactic acid produced	DL	DL	DL
Reduction of NO_3 to NO_2	−	−	−
Hydrolysis of:			
Gelatin	−	−	−
Tween 80	−	−	−
Esculin	+	+	+
Growth in:			
6% NaCl broth	+	−	−
10% NaCl broth	−	−	−
Growth at:			
37 C	+	+	+
45 C	w	w	w
50 C	−	−	−
pH 5.0	+	+	+
pH 7.0	+	+	+
pH 9.0	−	−	−
Gas production from glucose	−	−	−
Acid production from:			
Glucose	+	+	+
Fructose	+	+	+
Galactose	+	+	+
Mannose	+	+	+
L-Arabinose	+	+	−
D-Xylose	−	−	−
Maltose	+	+	+
Lactose	+	+	+
Sucrose	−	−	−
Sorbitol	−	−	−
Mannitol	−	−	−
Starch	−	−	−
Final pH in glucose broth	4.0	4.1	4.2

Abbreviations: w, weakly positive; F, facultative anaerobes; Homo, homofermentative; and DL, DL-lactic acid.

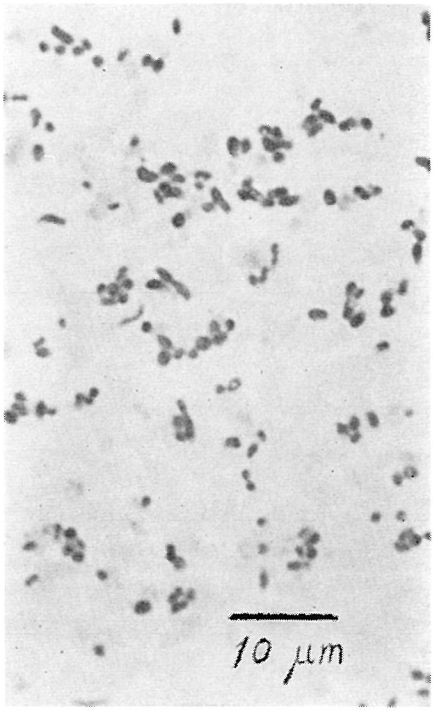
These strains were obtained from the Noda Institute for Scientific Research, Noda, Japan, in 1965, under the name *Pediococcus cerevisiae* Balcke.

These have been reidentified as *Pediococcus pentosaseus* according the description of species given by E. I. Garvie in 1974.*

[T. Sakane & I. Banno]

63. **Photobacterium phosphoreum** (Cohn) Ford 1927
IFO 13896

Shape: Rods to coccal rods. Pleomorphic forms occur (Plate 1). Asporogenous.
Gram-reaction: Negative.
Motility: Motile. But not detected microscopically in a hanging drop preparation.
Oxidase reaction: Negative.
Acid and gas from D-glucose, D-mannose, D-galactose and maltose, but no acid and no gas from L-arabinose, D-xylose and lactose.
Hugh-Leifoson's OF test: Fermentative.
Production of indole: Negative.
Methyl red test: Positive.
Voges-Proskauer test: Positive.
Hydrolysis of
starch: Negative.
gelatin: Negative.
Tween 80: Negative.
Utilization of citrate (Christensen): Negative.
Bioluminescence: Positive.
Growth on nutrient agar without NaCl: Negative.
Growth at 5 C: Positive.
Growth at 37 C: Negative.



Pl. 1.

[K. Imai]

64. **Pseudomonas fluorescens** Migula 1895
IFO 13922

Cells: Gram-negative rods; motile by 2 to 5 polar flagella.
Colonies on nutrient agar: Circular, entire, low-convex, gray to pale-brown.
Production of pigments: Produces diffusible yellow fluorescent pigments.
Catalase: Positive.

* Garvie, E. I. 1974. Int. J. Syst. Bacteriol. **24**: 301.

Oxidase reaction: Positive.
Accumulation of poly-β-hydroxybutyrate: Negative.
Arginine dihydrolase: Positive.
Reduction of NO_3 to NO_2: Positive.
Denitrification: Negative.
Mucoidal growth in sucrose medium: Positive.
Hydrolysis of
gelatin: Positive.
casein: Positive.
starch: Negative.
Tween 80: Negative.
Hugh-Leifson's OF test: Oxidative.
Gas production from glucose: Negative.
Growth at 41 C: Negative.
Growth factors not required.
The following compounds utilized as sole sources of carbon: Glucose, trehalose, L-arabinose, inositol, arginine, histidine, betain, malonate, citrate, lactate, propylene glycol and DL-β-hydroxybutyrate. Sucrose, cellobiose, formate, tartrate and ethanol not utilized as sole sources of carbon.

This strain was isolated from soft-rotted radish as organism which produces an exopectinase, D-galacturonandigalacturono hydrolase (EC. 3.2.1.82) and designated P-1 by C. Hatanaka, Faculty of Applied Biological Science, Hiroshima University, Japan, in 1970.*

[T. Sakane & I Banno]

65. Pseudomonas maltophilia Hugh and Ryschenkow 1960
IFO 13923

Cells: Gram-negative rods; motile by 2 to 5 polar flagella.
Colonies on nutrient agar: Circular, entire, low-convex, smooth, pale-yellow.
Production of pigments: No pigments produced.
Catalase: Positive.
Oxidase reaction: Weakly positive.
Arginine dihydrolase: Negative.
Reduction of NO_3 to NO_2: Positive.
Denitrification: Negative.
Mucoidal growth in sucrose medium: Negative.
Hydrolysis of
gelatin: Positive.

* Hatanaka, C. & T. Imamura, 1974. Agric. Biol. Chem. **38**: 2267.

casein: Positive.
starch: Negative.
Tween 80: Positive.
Decarboxylation of
lysine: Positive.
ornithine: Negative.
Hugh-Leifson's OF test: Oxidative.
Gas production from glucose: Negative.
Growth at 41 C: Negative.
Methionine required as the only organic growth factor.
The following compounds utilized as sole sources of carbon: Glucose, trehalose, sucrose, cellobiose, malonate, citrate and lactate. L-Arabinose, inositol, arginine, histidine, betain, formate, tartrate, propylene glycol, DL-β-hydroxybutyrate and ethanol not utilized as sole sources of carbon.

This strain was isolated from soil as organism which produces an exopectinase, oligo-D-galactosiduronate lyase (EC. 4.2.2.6) and designated S-2 by C. Hatanaka, Faculty of Applied Biological Science, Hiroshima University, Japan, in 1969.*

[T. Sakane & I. Banno]

* Hatanaka, C. & J. Ozawa. 1971. Agric. Biol. Chem. **35**: 1617.

CATALOGUE ON NEWLY ACCEPTED STRAINS

(ALPHABETICAL)

The cultures involved in this catalogue can be distributed under the same condition as other strains listed in "IFO List of Cultures" 6th edition 1978. Detailed data about these strains are available on request.

YEASTS

IFO No.	NAME	MATING TYPE
1912	Candida acutus	
1910	Candida cariosilignicola	
1913	Candida mamillae	
1914	Candida placentae	
1911	Candida succiphila	
1940	Candida tsukubaensis	
1862	Cryptococcus ater	
1863	Cryptococcus dimennae	
1866	Cryptococcus kuetzingii	
1867	Cryptococcus lactativorus	
1898	Cryptococcus laurentii var. laurentii	alpha
1870	Cryptococcus macerans	
1871	Cryptococcus melibiosum	
1872	Cryptococcus skinneri	
1878	Debaryomyces marama	
1879	Debaryomyces marama	
1900	Debaryomyces melissophilus	
1901	Debaryomyces melissophilus	
1915	Filobasidium floriforme	A
1916	Filobasidium floriforme	a
1880	Hansenula dimennae	
1881	Hansenula dimennae	
1882	Hansenula dimennae	
1963	Kluyveromyces fragilis	
1902	Kluyveromyces lactis	
1903	Kluyveromyces lactis	
1883	Kluyveromyces phaffii	
1884	Kluyveromyces phaffii	
1885	Kluyveromyces phaffii	
1917	Leucosporidium antarcticum	
1918	Leucosporidium antarcticum	alpha
1919	Leucosporidium antarcticum	a
1920	Leucosporidium frigidum	
1921	Leucosporidium gelidum	
1922	Leucosporidium nivalis	
1923	Leucosporidium scottii	A2B2
1924	Leucosporidium scottii	A1B1
1925	Leucosporidium scottii	
1926	Leucosporidium stokesii	
1886	Pichia bovis	
1909	Pichia cellobiosa	
1904	Pichia kudriavzevii	

1887	Pichia pijperi	
1896	Pichia scutulata var. exigua	
1895	Pichia scutulata var. scutulata	
1905	Pichia spartinae	
1888	Pichia terricola	
1907	Pichia terricola	
1927	Rhodosporidium bisporidiis	A1B1
1928	Rhodosporidium bisporidiis	A2B2
1929	Rhodosporidium capitatum	
1930	Rhodosporidium dacryoidum	A1B1
1931	Rhodosporidium dacryoidum	A2B2
1933	Rhodosporidium infirmo-miniatum	A2
1934	Rhodosporidium infirmo-miniatum	A3
1935	Rhodosporidium malvinellum	a
1936	Rhodosporidium malvinellum	alpha
1937	Rhodosporidium sphaerocarpum	a
1938	Rhodosporidium sphaerocarpum	alpha
1939	Rhodosporidium sphaerocarpum	
1943	Saccharomyces bayanus	
1944	Saccharomyces bisporus var. bisporus	
1945	Saccharomyces bisporus var. mellis	
1946	Saccharomyces bisporus var. mellis	
1947	Saccharomyces cerevisiae	
1948	Saccharomyces cerevisiae	
1949	Saccharomyces cerevisiae	
1950	Saccharomyces cerevisiae	
1951	Saccharomyces cerevisiae	
1952	Saccharomyces cerevisiae	
1953	Saccharomyces cerevisiae	
1954	Saccharomyces cerevisiae	
1955	Saccharomyces chevalieri	
1956	Saccharomyces delbrueckii	
1957	Saccharomyces delbrueckii	
1958	Saccharomyces diastaticus	
1959	Saccharomyces fermentati	
1889	Saccharomyces globosus	
1890	Saccharomyces globosus	
1891	Saccharomyces globosus	
1892	Saccharomyces kluyveri	
1893	Saccharomyces kluyveri	a
1894	Saccharomyces kluyveri	alpha
1876	Saccharomyces rouxii	a
1877	Saccharomyces rouxii	alpha
1960	Saccharomyces rouxii	
1897	Saccharomyces telluris	
1961	Saccharomyces uvarum	
1962	Saccharomyces uvarum	
1906	Torulopsis halonitratophila	
1941	Torulopsis halophilus	
1908	Torulopsis mannitofaciens	
1942	Torulopsis nodaensis	

BACTERIA & ACTINOMYCETES

13772 Acetobacter xylinus
13773 Acetobacter xylinus
13908 Actinomadura dassonvillei
13909 Actinomadura madurae
13910 Actinomadura pelletieri
13967 Actinoplanes aurantiacus
13992 Actinoplanes auranticolor
13993 Actinoplanes azureus
13938 Actinoplanes brasiliensis
13939 Actinoplanes caeruleus
13994 Actinoplanes deccanensis
13995 Actinoplanes garbadiensis
13996 Actinoplanes ianthinogenes
13911 Actinoplanes italicus
13997 Actinoplanes liguriae
14063 Actinoplanes nipponensis
13968 Actinoplanes pallido-aurantiacus
13878 Actinoplanes philippinensis
13940 Actinoplanes philippinensis
14020 Actinoplanes purpeobrunneus
14030 Actinoplanes pyriformis
13941 Actinoplanes rectilineatus
13969 Actinoplanes roseosporangius
13970 Actinoplanes rutilosporangius
13998 Actinoplanes sarveparensis
13943 Actinoplanes sp.
13944 Actinoplanes sp.
13999 Actinoplanes teichomyceticus
14021 Actinoplanes tuftoflagellus
13942 Actinoplanes utahensis
14064 Actinosynnema mirum
13784 Aeromonas salmonicida subsp. masoucida
13899 Agromyces ramosus
13912 Amorphosporangium globisporus
14065 Ampullariella regularis subsp. intermedia
14066 Ampullariella violaceochromogenes
13865 Bacillus thuringiensis
13866 Bacillus thuringiensis
13902 Chainia nigra
13948 Clostridium acetobutylicum
13949 Clostridium butylicum
13950 Clostridium sporogenes
13913 Dermatophilus congolensis
13921 Erwinia carotovora subsp. carotovora
14082 Erwinia carotovora subsp. carotovora
13898 Escherichia coli
13965 Escherichia coli
13966 Escherichia coli
14087 Flavobacterium keratolyticus
13914 Jensenia canicruria
13890 Kineosporia aurantiaca
14067 Kineosporia aurantiaca

13924 Kitasatoa diplosporus
13809 Kitasatoa griseophaeus
13925 Kitasatoa kauaiensis
13926 Kitasatoa nagasakiensis
13927 Kitasatoa purpurea
13951 Lactobacillus acidophilus
13952 Lactobacillus acidophilus
13953 Lactobacillus bulgaricus
13954 Lactobacillus fructivorans
14039 Microbispora amethystogenes
14040 Microbispora amethystogenes subsp. nonreducans
14041 Microbispora diastatica
14042 Microbispora echinospora
14043 Microbispora parva
14044 Microbispora rosea
14045 Microbispora rosea subsp. nonnitritogenes
14046 Microbispora thermodiastatica
14047 Microbispora thermorosea
13867 Micrococcus luteus
14068 Micromonospora aurantiaca
14069 Micromonospora brunnea
14024 Micromonospora brunnescens
14025 Micromonospora citrea
14022 Micromonospora echinoaurantiaca
14023 Micromonospora fulvoviolacea
14026 Micromonospora fulvoviridis
14070 Micromonospora rubra
13915 Microtetraspora fusca
13916 Microtetraspora glauca
14027 Microtetraspora incanescens
13917 Nocardioides albus
14031 Nocardioides flavus
14032 Nocardioides flavus
14033 Nocardioides fulvus
14034 Nocardioides fulvus
13896 Photobacterium phosphoreum
13879 Planobispora longispora
13918 Planobispora longispora
13880 Planomonospora parontospora subsp. parontospora
14074 Pseudomonas cepacia
13922 Pseudomonas fluorescens
13923 Pseudomonas maltophilia
13934 Pseudomonas paucimobilis
13935 Pseudomonas paucimobilis
13936 Pseudomonas paucimobilis
13937 Pseudomonas paucimobilis
14053 Pseudomonas syringae
14054 Pseudomonas syringae
14055 Pseudomonas syringae
14075 Pseudomonas syringae
14076 Pseudomonas syringae
14077 Pseudomonas syringae
14078 Pseudomonas syringae
14079 Pseudomonas syringae
14080 Pseudomonas syringae
14081 Pseudomonas syringae
14083 Pseudomonas syringae

14084 Pseudomonas syringae
14085 Pseudomonas syringae
14086 Pseudomonas syringae
13919 Saccharopolyspora hirsuta
13959 Spirillum giesbergeri
13958 Spirillum lunatum
13960 Spirillum metamorphum
13961 Spirillum polymorphum
13962 Spirillum putridiconchylium
13889 Staphylococcus epidermidis
14056 Streptoalloteichus hindustanus
13955 Streptococcus mutans
13956 Streptococcus salivarius
13957 Streptococcus thermophilus
13781 Streptomyces aburaviensis subsp. tuftformis
14000 Streptomyces achromogenes subsp. rubradiris
14001 Streptomyces achromogenes subsp. streptozoticus
13820 Streptomyces achromogenes subsp. tomaymyceticus
13811 Streptomyces adephospholyticus
14052 Streptomyces albidus
13846 Streptomyces albospinus
13840 Streptomyces albus subsp. coleimyceticus
13812 Streptomyces albus subsp. pathocidicus
13831 Streptomyces amagasakensis
14035 Streptomyces arabicus
13847 Streptomyces argenteolus subsp. toyonakensis
14002 Streptomyces aureofaciens
14003 Streptomyces aureofaciens
14004 Streptomyces aureofaciens
13824 Streptomyces brunneogriseus
13813 Streptomyces cacaoi subsp. asoensis
13803 Streptomyces candidus subsp. azaticus
13821 Streptomyces candidus subsp. enterostaticus
14057 Streptomyces cattleya
13780 Streptomyces celluloflavus
13822 Streptomyces cinerochromogenes
13814 Streptomyces diastatochromogenes subsp. luteus
14005 Streptomyces endus
14006 Streptomyces endus
14058 Streptomyces fimbriatus
14019 Streptomyces flavisclerotícus
13848 Streptomyces fungicidicus
13974 Streptomyces gardneri
13775 Streptomyces globifer
13776 Streptomyces griseochromogenes subsp. suitaenesis
13804 Streptomyces griseoflavus subsp. pyrindicus
14059 Streptomyces griseus
13849 Streptomyces griseus subsp. rhodochrous
13785 Streptomyces hikiziensis
13810 Streptomyces hofunensis
13825 Streptomyces humidus subsp. antitumoris
13976 Streptomyces humidus subsp. antitumoris
13850 Streptomyces hyalinus
14011 Streptomyces hygroscopicus
14012 Streptomyces hygroscopicus
14013 Streptomyces hygroscopicus
14014 Streptomyces hygroscopicus

14015 Streptomyces hygroscopicus
14016 Streptomyces hygroscopicus
13815 Streptomyces hygroscopicus subsp. aabomyceticus
14017 Streptomyces hygroscopicus subsp. angustmyceticus
13981 Streptomyces hygroscopicus subsp. ascomyceticus
13868 Streptomyces hygroscopicus subsp. azalomyceticus
13977 Streptomyces hygroscopicus subsp. decoyicus
13978 Streptomyces hygroscopicus subsp. enhygrus
13786 Streptomyces hygroscopicus subsp. glebosus
13982 Streptomyces hygroscopicus subsp. glebosus
13946 Streptomyces hygroscopicus subsp. hialomyceticus
13983 Streptomyces hygroscopicus subsp. ossamyceticus
13869 Streptomyces jumonjinensis
14060 Streptomyces jumonjinensis
13805 Streptomyces kagawaensis
13816 Streptomyces kagoshimanus
13790 Streptomyces kaniharaensis
13851 Streptomyces kasugaensis
13852 Streptomyces kasugaspinus
13783 Streptomyces kobenensis
14028 Streptomyces lavendulae subsp. fuscus
13870 Streptomyces lividoclavatus
13787 Streptomyces lividus
13826 Streptomyces luteocolor
13853 Streptomyces macromomyceticus
13897 Streptomyces macrosporeus
13871 Streptomyces malachiticus subsp. griseospinosus
13854 Streptomyces mauvecolor
13791 Streptomyces miharaensis
13855 Streptomyces misawanensis
13973 Streptomyces mucoflavus
13777 Streptomyces multispiralis
13792 Streptomyces mycarofaciens
13793 Streptomyces myxogenes
13806 Streptomyces nodosus subsp. asukaensis
13794 Streptomyces nojiriensis
13817 Streptomyces ogaensis
13873 Streptomyces olivochromogenes
13874 Streptomyces olivochromogenes
13875 Streptomyces olivochromogenes
13876 Streptomyces olivochromogenes
13795 Streptomyces olivogriseus
13832 Streptomyces owasiensis
13841 Streptomyces phaeoverticillatus subsp. takatsukiensis
14029 Streptomyces piloviolofuscus
13839 Streptomyces piomogenus
14007 Streptomyces platensis
14008 Streptomyces platensis
13827 Streptomyces platensis subsp. malvinus
13979 Streptomyces platensis subsp. malvinus
13818 Streptomyces platensis subsp. robigocidicus
13872 Streptomyces polychromogenes subsp. arenicolus
13842 Streptomyces propurpuratus
13877 Streptomyces psammoticus
13971 Streptomyces psammoticus
13833 Streptomyces pseudogriseolus subsp. glucofermentans
13778 Streptomyces purpureofuscus subsp. acoagulans

13903 Streptomyces purpurogeniscleroticus
13796 Streptomyces ribosidificus
13807 Streptomyces rosa subsp. notoensis
13828 Streptomyces roseochromogenes subsp. albocyclini
13829 Streptomyces roseocinereus
13834 Streptomyces ryensis
13928 Streptomyces sahachiroi
13823 Streptomyces sapporonensis
13904 Streptomyces sclerotialus
13843 Streptomyces senoensis
13797 Streptomyces setonensis
13835 Streptomyces shiodaensis
13856 Streptomyces spinichromogenes
13857 Streptomyces spinicoumarensis
13980 Streptomyces sviceus
13929 Streptomyces tanashiensis subsp. cephalomyceticus
13858 Streptomyces testaceus
13905 Streptomyces thermoviolaceus subsp. thermoviolaceus
13798 Streptomyces tosaensis
13799 Streptomyces triangulatus
13836 Streptomyces triostinicus
13782 Streptomyces tsusimaensis
13800 Streptomyces varius
14061 Streptomyces viridochromogenes
13859 Streptomyces viridochromogenes subsp. komabensis
13830 Streptomyces viridochromogenes subsp. sulfomycini
14062 Streptomyces wedmorensis
13845 Streptomyces xylophagus
13901 Streptosporangium albidum
13900 Streptosporangium album
13986 Streptosporangium amethystogenes
13972 Streptosporangium corrugatum
13964 Streptosporangium indianensis
13990 Streptosporangium nondiastaticum
13991 Streptosporangium pseudovulgare
14048 Streptosporangium roseum
13947 Streptosporangium rubrum
13975 Streptosporangium rubrum
13988 Streptosporangium viridialbum subsp. reducens
13987 Streptosporangium viridialbum subsp. viridialbum
13989 Streptosporangium viridogriseum subsp. kofuense
13985 Streptosporangium vulgare
13860 Streptoverticillium abikoense
13861 Streptoverticillium alboverticillatum
13838 Streptoverticillium eurocidicum subsp. asterocidicum
13862 Streptoverticillium fervens subsp. phenomyceticus
13906 Streptoverticillium kashmirense
13930 Streptoverticillium kitasatoense
13819 Streptoverticillium mobaraense
13801 Streptoverticillium novoverticillium
13907 Streptoverticillium parvisporogenes
13931 Streptoverticillium phaeoverticillatum subsp. phaeoverticillatum
13932 Streptoverticillium reticulum subsp. protomycicum
13933 Streptoverticillium reticulum subsp. shimofusaense
13802 Streptoverticillium rimofaciens
13808 Streptoverticillium taitoensis
13863 Streptoverticillium verticillum subsp. quintum

13864 Streptoverticillium verticillum subsp. vericillum
14050 Thermoactinomyces monosporus
13920 Thermoactinomyces sacchari
14051 Thermoactinomyces vulgaris
14049 Thermomonospora fusca
14071 Thermomonospora fusca

FUNGI

30793 Achaetomiella megaspora
30998 Achaetomiella megaspora
30999 Achaetomiella megaspora
31000 Achaetomiella megaspora
30535 Acremonium luzulae
30538 Acremonium terricola
30988 Acrophialophora fusispora
30989 Acrophialophora fusispora
30990 Acrophialophora fusispora
30991 Acrophialophora levis
30992 Acrophialophora levis
30993 Acrophialophora levis
30994 Acrophialophora levis
30995 Acrophialophora nainiana
30996 Acrophialophora nainiana
30997 Acrophialophora nainiana
30774 Agaricus bisporus
30782 Agaricus bisporus
30874 Agaricus bisporus
30914 Albertiniella polyporicola
30835 Anixiella endodonta
30577 Anixiella reticulata
30712 Ardhachandra selenoides
30523 Armillariella mellea
30743 Armillariella mellea
30500 Arthrinium japonicum
30798 Arthrinium japonicum
30897 Aspergillus carneus
30898 Aspergillus carneus
30899 Aspergillus carneus
30870 Aspergillus fumigatus
31012 Aspergillus niger
30615 Aspergillus penicilloides
30537 Aspergillus terreus
30536 Aspergillus terreus var. aureus
30557 Aureobasidium pullulans
30562 Aureobasidium pullulans
30778 Auricularia polytricha
30711 Boothiella tetraspora
30915 Botryotinia fuckeliana
30591 Botryotrichum piluliferum
30569 Byssochlamys nivea
30918 Calonectria rigidiuscula
30920 Calonectria rigidiuscula
30921 Calonectria rigidiuscula

30922 Calonectria rigidiuscula
30923 Calonectria rigidiuscula
30924 Calonectria rigidiuscula
30925 Calonectria rigidiuscula
31008 Calonectria rigidiuscula
30530 Calvatia craniiformis
30664 Camposporium japonicum
30802 Camposporium japonicum
30803 Camposporium pellucidum
30596 Cephaloascus albidus
30597 Cephaloascus fragrans
30693 Cephaloascus fragrans
30694 Cephaloascus fragrans
30794 Cephaloascus fragrans
30501 Ceratocystis fimbriata
30956 Ceratocystis fimbriata
30466 Cercosporella aesculi
30804 Chaetendophragmia triseptata
30916 Chaetomium aureum
30576 Chaetomium cochliodes
30917 Chaetomium convolutum
31030 Chaetospermopsis boninensis
30566 Chorioactis geaster
30658 Chorioactis geaster
31006 Cladosporium cladosporioides
30567 Clitocybe acromelalga
30524 Clitocybe clavipes
30884 Cochliobolus intermedius
30883 Cochliobolus lunatus
30676 Cochliobolus sativus
30677 Cochliobolus sativus
30678 Cochliobolus sativus
30665 Codinaea simplex
30747 Collybia butyracea
30770 Collybia peronata
30592 Coniella castaneicola
30584 Coniochaeta nepalica
30670 Conioscypha lignicola
30671 Conioscypha varia
30672 Conioscypha varia
30673 Conioscypha varia
30674 Conioscypha varia
30675 Conioscypha varia
30760 Conocybe tenera
30971 Coprinus angulatus
30626 Coprinus atramentarius
30627 Coprinus cinereus
30628 Coprinus cinereus
30480 Coprinus comatus
30972 Coprinus disseminatus
30629 Coprinus echinosporus
30630 Coprinus echinosporus
30631 Coprinus echinosporus
31058 Coprinus filamentifer
30476 Coprinus neolagopus
30477 Coprinus phlyctidosporus
30478 Coprinus phlyctidosporus

30479 Coprinus stercorarius
30769 Coriolus consors
30657 Corticium rolfsii
30502 Corynespora cassiicola
30503 Corynespora cassiicola
30504 Corynespora cassiicola
30505 Corynespora cassiicola
30506 Corynespora cassiicola
30507 Corynespora cassiicola
30508 Corynespora cassiicola
30509 Corynespora cassiicola
30510 Corynespora cassiicola
30986 Corynespora cassiicola
30987 Corynespora cassiicola
30561 Cylindrocarpon tonkinense
30660 Cymatoderma elegans
30766 Daedalea dickinsii
30895 Diaporthe medusae
30961 Diaporthe nomurai
30962 Diaporthe nomurai
30580 Diplogelasinospora inaequalis
30862 Echinopodospora effusa
30863 Echinopodospora vermicularis
30531 Eleutherascus lectardii
30532 Eleutherascus lectardii
30533 Eleutherascus lectardii
30534 Eleutherascus lectardii
30799 Eleutherascus lectardii
30666 Ellisiopsis gallesiae
30836 Emericella arizonica
30837 Emericella aurantiobrunnea
30838 Emericella bicolor
30839 Emericella cleistominuta
30840 Emericella desertorum
30559 Emericella foveolata
30560 Emericella foveolata
30841 Emericella fruticulosa
30842 Emericella heterothallica
30843 Emericella heterothallica
30844 Emericella nidulans var. acristata
30907 Emericella nidulans var. dentata
30908 Emericella nidulans var. dentata
30909 Emericella nidulans var. dentata
30910 Emericella nidulans var. dentata
30845 Emericella nidulans var. dentata
30846 Emericella nidulans var. dentata
30896 Emericella nidulans var. echinulata
30847 Emericella nidulans var. lata
30872 Emericella nidulans var. nidulans
30848 Emericella parvathecia
30849 Emericella purpurea
30911 Emericella quadrilineata
30912 Emericella quadrilineata
30850 Emericella quadrilineata
30851 Emericella quadrilineata
30913 Emericella rugulosa
30852 Emericella rugulosa

30853 Emericella rugulosa
30854 Emericella spectabilis
30855 Emericella stellata
30856 Emericella striata
30906 Emericella sublata
30857 Emericella unguis
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30570 Eurotium chevalieri
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30815 Exobasidium gracile
30756 Exobasidium japonicum
31001 Fibulobasidium inconspicuum
31002 Fibulobasidium inconspicuum
31003 Fibulobasidium inconspicuum
31004 Fibulobasidium inconspicuum
30904 Flammulina velutipes
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30598 Flammulina velutipes
30599 Flammulina velutipes
30600 Flammulina velutipes
30601 Flammulina velutipes
30602 Flammulina velutipes
30603 Flammulina velutipes
30875 Flammulina velutipes
30653 Flosculomyces floridaensis
30654 Flosculomyces floridaensis
30655 Flosculomyces floridaensis
30656 Flosculomyces floridaensis
30777 Fomes fomentarius
30700 Fusarium oxysporum
30701 Fusarium oxysporum
30702 Fusarium oxysporum
30703 Fusarium oxysporum
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30707 Fusarium oxysporum
30708 Fusarium oxysporum
30709 Fusarium oxysporum
30710 Fusarium oxysporum
30467 Fusarium oxysporum f. sp. spinaciae
30468 Fusarium oxysporum f. sp. spinaciae
30469 Fusarium oxysporum f. sp. spinaciae
30966 Fusarium roseum cultivar. "Equiseti"
30926 Fusarium semitectum
30713 Gamsia dimera
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31044 Gliomastix murorum

30967 Gonatophragmium mori
30968 Gonatophragmium mori
30522 Grifola frondosa
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30582 Gymnoascus reessii
30761 Gymnopilus aeruginosus
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30973 Hebeloma crustuliniforme f. microspermum
30481 Hebeloma radicosum
30974 Hebeloma radicosum
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30632 Hebeloma vinosophyllum
30667 Helicoma dennisii
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30608 Hypocrea albocornea
30609 Hypocrea albofulva
30610 Hypocrea cerebriformis
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30919 Hypomyces rosellus
30964 Hypomyces solani f. sp. mori
30748 Kuehneromyces mutabilis
30483 Laccaria proxima
30745 Laetiporus sulphureus
30719 Lentinus edodes
30720 Lentinus edodes
30721 Lentinus edodes
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30723 Lentinus edodes
30724 Lentinus edodes
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31052 Lepista irina
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30495 Lepista sordida
31013 Lepista sordida
31045 Leucoagaricus excoriatus
31046 Leucoagaricus naucinus
31047 Leucoagaricus naucinus
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31057 Leucocoprinus birnbaumii
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30765 Leucocoprinus luteus
30864 Lophotrichus plumbescens
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30888 Melanconium fuligineum
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30572 Neosartorya fischeri var. glabra
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30690 Penicillium loliense
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30900 Penicillium piceum
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30594 Sclerotium cepivorum
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30652 Zopfiella lundqvistii

ABSTRACTS 1979-1980

Mutational problem in preservation of bacteria by L-drying

T. SAKANE, I. BANNO and T. IIJIMA

J. Japan. Soc. Res. Freez. Dry. **25**: 59–64 (1979)

Various auxotrophic mutants of strain B/r of *Escherichia coli*, strain Holloway of *Pseudomonas aeruginosa* and strain Marburg of *Bacillus subtilis* were examined for induction of reverse mutation by preservation of their L-dried cells.

When the dried cells were rehydrated immediately after drying, no increase in mutation frequency was found in any mutant tested. After preservation at lower temperature than 3 C, no induction of mutation was found in B/r of *E. coli* but at higher temperatures the frequency of mutation increased exponentially with the temperature. The frequency at 37 C was about 10 times the spontaneous level.

In the mutants of *P. aeruginosa* and *B. subtilis*, the mutation frequency during preservation at 37 C was almost equal to that before drying. The result indicates that preservation of L-dried cells does not generally induce mutation in bacterial strains.

[in Japanese]

Reactivation of inactivated D-glucose dehydrogenase of a *Bacillus* species by pyridine and adenine nucleotides

A. YOKOTA, K. SASAJIMA and M. YONEDA*

Agric. Biol. Chem. **43**: 271～278 (1979)

D-Glucose dehydrogenase [β-D-glucose: NAD(P) oxidoreductase (EC1.1.1.47)] was synthesized derepressively in a mutant of a *Bacillus* species which was isolated as an improved strain for D-ribose production. The enzyme was very unstable and inactivated during storage or column chromatography. The inactivation was prevented in the presence of NAD^+, $NADP^+$ or certain salts. The inactive enzyme was reactivated by the addition of NAD^+, NADH, $NADP^+$, NADPH, AMP, ADP, ATP or certain salts. The molecular weights of the inactive and active form of the enzyme were estimated to be about 45,000 and 80,000, respectively, by Sephadex G-150 gel filtration. Thus, it seems that the enzyme activity is regulated by monomer-dimer interconversion of the enzyme molecule.

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Distribution of *Descolea flavoannulata* (Vasilieva) Horak in Far Eastern Asia

T. YOKOYAMA, Y.-H. PARK*, Y.-S. KIM*, B.-K. KIM** and T. HONGO***

Trans. Mycol. Soc. Japan **20**: 63–72 (1979)

Descolea flavoannulata, one of the two hitherto described species of the genus *Descolea* in the Far Eastern Asia, is fully redescribed with some macroscopic and microscopic figures and monochromatic photographs. The distribution of this fungus has been stretched from Siberia and Japan to Korea, as two new localities were recently found in the southern part of Korea. Geographical distribution and mycorrhizal association of the northern hemispheric species of *Descolea* are also discussed.

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** Department of Microbial Chemistry, College of Pharmacy, Seoul National University, Seoul, Korea.
*** Biological Institute, Faculty of Education, Shiga University, Otsu.

Arthrinium japonicum Pollack & Benjamin from its natural habitat in Japan

T. YOKOYAMA and Y. HARADA*

Trans. Mycol. Soc. Japan **20**: 149–157 (1979)

Arthrinium japonicum, a basauxic Hyphomycete originally reported from the United States as an introduced fungus on straw packing from Japan, was found on the dead leaves and culms of *Carex dispalata* in Hirosaki, Aomori Prefecture, Japan. This is the first record of this fungus from its natural habitat. Some cultural characteristics are also described based on the Japanese isolate IFO 30500 and two additional collections in Aomori Prefecture are reported.

* Faculty of Agriculture, Hirosaki University, Hirosaki, Aomori.

Oxidation of L-glucose by a pseudomonad

K. SASAJIMA and A.J. SINSKEY*

Biochim. Biophys. Acta **571**: 120–126 (1979)

A new enzyme, D-*threo*-aldose dehydrogenase(2*S*, 3*R*-aldose dehydrogenase), found in *Pseudomonas caryophylli*, was capable of oxidizing L-glucose, L-xylose, D-arabinose, and L-fucose in the presence of NAD^+. The enzyme was synthesized constitutively and purified about 120-fold from D-glucose-grown cells. The K_m values for L-glucose,

L-xylose, D-arabinose, and L-fucose were $1.5 \cdot 10^{-2}$, $4.5 \cdot 10^{-3}$, $2.8 \cdot 10^{-3}$, and $2.1 \cdot 10^{-3}$, respectively. D-Glucose and other aldoses inhibited the enzyme reaction; this inhibition was competitive with L-glucose as substrate and D-glucose as inhibitor. The optimum pH for the enzyme reaction was 10; the molecular weight of the enzyme was determined by gel filtration to be $7 \cdot 10^4$.

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Estimate of viability of L-dried specimen after long-term preservation

T. SAKANE and I. BANNO

Japan. J. Freez. Dry. **26**: 96–100 (1980)

A study was undertaken to predict the viability of L-dried specimens of bacteria after long-term preservation. Cultures of 53 bacterial strains were L-dried, and one set of dried specimens was preserved at 37 C in an accelerated storage test and another set at 5 C for long-term preservation. Viable cells were recovered from the dried specimens of all of the 53 strains after preservation for 2 weeks at 37 C and also after 92 months at 5 C. The recovery of viable cell varied from strain to strain from 0.2 to 100 % of the viable count immediately after drying. For all strains, the survival value in the accelerated storage test was comparable to that after 92 months at 5 C. The result demonstrated that the viability of L-dried specimens stored at 5 C can be predicted by the accelerated storage test.

[in Japanese]

A mating pheromone produced by *Saccharomyces kluyveri*

A. SAKURAI*, Y. SATO*, K.H. PARK*, N. TAKAHASHI*,
N. YANAGISHIMA** and I. BANNO

Agric. Biol. Chem. **44**: 1451–1453 (1980)

A haploid strain of *S. kluyveri* produced a mating pheromone which induced sexual agglutinability in cells of mating type *a* of *S. cerevisiae*. The active compound (α-K substance) was collected from culture filtrate of the mating type α strain of *S. kluyveri*, IFO 1894, and purified by chromatography on gels. The purified compound induced agglutinability at a concentration of 6–12 ng/ml. Amino acid analysis indicated the compound was a peptide of Ser 2, Glu 1, Pro 1, Gly 1, Met 1, Leu 1, Tyr 1, Phe 1, Lys 1, His 1, Trp 1.

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PRESENTATION OF PAPERS AT SCIENTIFIC MEETINGS 1979-1980

Author(s)	Title	Scientific Meeting
I. BANNO, T. SAKANE & T. IIJIMA	Prevention of mutation during preservation of L-dried specimen of bacteria.	Agricultural Chemical Society of Japan. Tokyo (April, 1979)
T. IIJIMA, T. SAKANE & I. BANNO	Induction of mutation in bacterial samples prepared by L-drying.	Agricultural Chemical Society of Japan. Tokyo (April, 1979)
K. IMAI & I. BANNO	Utilization of tricarboxylic acid by *Klebsiella pneumoniae*.	Agricultural Chemical Society of Japan. Tokyo (April, 1979)
K. SASAJIMA & T. KUMADA	Deficiency of D-glucose transport in *Bacillus subtilis* transketolase mutant.	Agricultural Chemical Society of Japan. Tokyo (April, 1979)
I. BANNO	Long-term preservation of yeast stock cultures.	Japanese Society for Bacteriology. Isebara city (April, 1979)
T. SAKANE, I. BANNO & T. IIJIMA	Mutational problem in preservation of bacteria by L-drying.	Japanese Society for Research of Freezing and Drying. Tokyo (April, 1979)
T. ITO & T. YOKOYAMA	Distribution of *Limnoperdon incarnatum* Escobar in rice paddy field soils.	Mycological Society of Japan. Otsu (May, 1979)
T. YOKOYAMA & T. ITO	*Zopfiella lundqvistii* Shearer & Crane and *Eleutherascus lectardii* (Nicot) von Arx from rice paddy field soils.	Mycological Society of Japan. Otsu (May, 1979)
T. YOKOYAMA	Microfungal flora of rice paddy field soils and oak forest litters in Japan.	XIV Pacific Science Congress. Khabarovsk, USSR (August, 1979)
T. IIJIMA	The history of IFO and the roles of culture collection.	Korean-Japan Joint Symposium. Seoul (September, 1979)
A. YOKOTA & K. SASAJIMA	Derepressed synthesis of D-glucose dehydrogenase in a mutant of a *Bacillus* species.	Japanese Biochemical Society. Tokyo (October, 1979)
K. IMAI	Taxonomic studies on a bacterium involving with decomposition of poly(vinyl alcohol).	Agricultural Chemical Society of Japan. Kyoto (October, 1979)

Author(s)	Title	Scientific Meeting
K. SASAJIMA & T. KUMADA	Cell surface change of *Bacillus subtilis* mutant lacking transketolase.	3rd Symposium on Studies of *Bacillus subtilis*. Tokyo (October, 1979)
K. IMAI	A new species of *Flavobacterium* capable of decomposing Validamycin which is used to control sheath blight in rice plants.	Society of Fermentation Technology, Japan. Osaka (November, 1979)
K. SASAJIMA & T. KUMADA	Cell surface change in the transketolase mutant of *Bacillus subtilis*.	Agricultural Chemical Society of Japan. Fukuoka (April, 1980)
T. SAKANE & I. BANNO	Estimate of viability of L-dried specimen after long-term preservation.	Japanese Society for Research of Freezing and Drying. Tokyo (April, 1980)
T. ITO & T. YOKOYAMA	Thermophilic and thermotolerant fungi in rice paddy field soils.	Mycological Society of Japan. Hiroshima (May, 1980)
K. MIKATA, I. BANNO & K. KODAMA*	Ascomycetous yeasts isolated from tunnel of ambrosia beetle in Japan.	Mycological Society of Japan. Hiroshima (May, 1980)
T. YOKOYAMA	Ascigerous state of *Shiraia bambusicola* P. Hennings.	Mycological Society of Japan. Hiroshima (May, 1980)
T. IIJIMA	On the WIPO Budapest Treaty.	JFCC Annual Meeting. Osaka (June, 1980)
T. IIJIMA	Coping for the new rule.	Symposium on the Taxonomy and Nomenclatural Code of Bacteria. Tokyo (July, 1980)
A. YOKOTA & K. SASAJIMA	Derepressed syntheses of sporulation marker enzymes in a *Bacillus* species mutant.	6th Spore Seminar. Kyoto (October, 1980)
T. IIJIMA	On the Budapest Treaty on the international recognition of the deposit of microorganism for the purposes of patent procedures.	Society of Fermentation Technology, Japan. Osaka (November, 1980)

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CORRECTIONS

In the issue of IFO Researeh Communications No. 9, the following corrections should be made.

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20	Fig. 1.	(Glc+Sol)-grown (right)	(Mal+Sol)-grown
〃	〃	(Mal+Sol)-grown (left)	(Glc+Sol)-grown
24	27	D-manntiol	D-mannitol
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