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REPORT OF THE DIRECTOR

In the last two years (1983 - 1984), the Institute for Fermentation, Osaka has realized a three-year project to modernize and renovate its facilities, and to recruit young talent so as to establish a solid basis for new steps to further development. Four members joined the institute and the total number of staff is now twenty three. Electrophoresis and high performance liquid chromatography equipment were installed to examine DNA homology, to analyse DNA sequences and to conduct chemical analyses of cell walls and lipopolysaccharide on the cell surface. A scanning electron microscope was installed to examine the fine structure of microorganisms. As a safeguard against earthquakes and other natural calamities one set of cultures was transferred to storage facilities in Hikari district, Yamaguchi Pref. At the annual meeting of the Board of Trustees in March 1984, a plan to establish a new section of cell lines was approved and Dr. Masao Takeuchi was appointed curator of cell lines. He joined us to collect, preserve and distribute cell lines and to do basic research on genetic and biochemical approaches. The cell line section started its work from May 1984. Preservation and provision of authentic and reference strains is one of the most important roles of culture collection. The basic strategy for preserving cell line is to have skilled staff and to be equipped with modern facilities and techniques for keeping cell lines viable and uncontaminated for a long period.

The Chairman, Mr. Shinbei Konishi, received new funds amounting to a hundred million yen from Takeda Chemical Industries Ltd., and added it to the foundation of the institute. The total amount of the foundation was thus raised to six hundred and fifty five million yen. On the establishment of the cell line section, Professor Yoshio Okada, Institute of Cell Technology, Osaka University, was nominated as a member of the Board of Trustees. Dr. Katsura Morita, Director of Central Research Division, Takeda Chemical Industries Ltd. was nominated as a councilor of the Board of Trustees.

The total number of cultures stored in the IFO culture collection reached 12,000 at the end of 1984. After the publication of the 6th

edition of the IFO List of Cultures in 1978, newly added strains have been listed in every IFO Research Communication, but publication of a new edition has been awaited. In 1984, the 7th edition of the IFO Catlogue of Cultures was published after a 6 year interval. The manuscript of the new edition was edited and arranged from the data base of the IFO culture collection and the final manuscripts for photo-set printing were prepared on a computer. It lists 4,848 fungi, 1,318 yeasts, 806 bacteria, 1,086 actinomycetes and 46 bacteriophages. It includes phytopathogenic strains and procedures for getting permission from the Phytoquarantine Office. The total number of strains distributed in 1983 was 7,800 and in 1984 8,000.

Given the increase in the number of accessions and in distribution of strains, new data processing facilities were urgently required for rapid and efficient administration of accession and distribution of strains. An IBM business computer system was introduced in July 1983, and all of the data in IBM OS-6 were transferred to the new system which went into use in November 1983. From January 1984, all documentation of acceptances and distributions was done using the new business system.

In November 1983 and June 1984, the Committee for Confirmation of ISP strains (International Streptomyces Projects) in Japan conducted the regular confirmatory tests of ISP strains stored at IFO. L-dried samples, which were prepared for distribution to customers, were tested for viablity, main characteristics and the authenticity of the strains. Almost all of the tested samples were confirmed to be satisfactory.

A three-year research project on transformation of biomass planned by the Ministry of Agriculture, Forestry and Fishery was finished in 1984. Dr. T. Yokoyama examined and isolated lignin-degrading fungi from the IFO culture collection and natural sources.

Cooperative work on examination of quinones and their correlation to taxonomy has been carried with Tokyo University of Agriculture and Technology. Prof. H. Kuraishi and Dr. T. Yokoyama are activly examining a number of fungus cultures in the IFO collection.

In Septmber 1983, the Third International Mycological Congress (IMC 3) was held in Tokyo, and a symposium on "Critical Problems of Culture Collections" was organzed by Dr. Batra of Northeastern Region Beltsville Agricultural Research, Agricultural Research Service, USDA. Speakers from culture collections throughout the world presented papers on problems of

preservation methods, patent procedures and management of culture collections. The record of the Symposium was published by IFO to commemorate the 40th anniversary of the establishment of IFO. These proceedings are available on request. Drs. T. Iijima, I. Banno and T. Yokoyama attended the congress and presented a paper. The Fifth International Congress of Culture Collections (ICCC V) was held in December 1984, in Bangkok. Dr. K. Imai presented a paper on preservation methods for chemolithotrophic bacteria, and Dr. T. Iijima reported on the activities of the Japan Federation for Culture Collections.

As a cooperative activity between IFO and organizations in Asia, a guest researcher, Mr. Fwu-Ling Lee from the Food Industry Research and Development Institute (FIRDI), Taiwan, spend three months (from March to May 1984) in this institute and worked on the taxonomy of yeasts. Dr. T. Iijima visited FIRDI in October 1983, and gave a lecture about present methods of preservation of microorganisms and management of culture collections. He spent one week in Taiwan, and visited universities and several organizations. Dr. I. Banno was invited to lecture on yeast taxonomy at the International Postgraduate University Course held annually in Osaka University. The students of this course work at various universities after the lecture to improve their research ability.

IFO received a number of guests in these two years. Lectures and Seminars were given by the following guest speakers.

Prof. R.R. Davenport (Long Ashton Research Station, University of Bristol): Environmental approaches for yeast studies.

Dr. C.P. Kurtzman (Agricultural Research Service, Northern Regional Research Unit): Molecular taxonomy of ascomycetous yeasts.

Dr. A.H.S. Onions (Commomwealth Mycological Institute): Preservation methods for fungus cultures.

Prof. M. Koĉur (Czechoslovak Collection of Microorganisms, Purkine University): Current views on the present classification of gram-positive catalase-positive cocci.

Prof. K. E. Sanderson (University of Calgary, Canada) : Genetic analysis of lipopolysaccharide synthesis in enteric bacteria.

Members of the institute have participated in mycological forays in Japan. Dr. T. Yokoyama joined the foray of IMC 3 in Nikko, and he also joined forays in Mt. Odai and in Hokkaido in 1984.

Distribution charges for the microorganisms in the IFO culture collection, were changed from April 1983 to ¥ 3,000 per culture for non-profit organizations and ¥ 6,000 per culture for commercial firms. There is a handling charge of ¥ 750 per package (10 strains / package on average). Postage is included in the above prices.

(T. IIJIMA)

IFO Res. Comm. 12,
5-18, 1985 (March)

ALTERATION OF THE MEMBRANE COMPOSITION OF BACILLUS SUBTILIS PLEIOTROPIC MUTANT LACKING TRANSKETOLASE

KEN-ICHI SASAJIMA, AKIRA YOKOTA and TOSHIO KUMADA*

Summary

The composition of cell wall and cytoplasmic membrane of Bacillus subtilis pleiotropic mutant lacking transketolase (tkt) (EC 2.2.1.1) was investigated. There was no difference in cell wall composition of the parental strain, IFO 12114, and the tkt mutant BG2607. However, SDS-polyacrylamide gel electrophoresis of membrane proteins revealed that the composition in the tkt mutant was altered. Lipid composition also differed in the tkt mutant; the amount of phosphatidylethanolamine in membrane was lower while that of phosphatidylglycerol and lysylphosphatidylglycerol was higher. These changes in membrane composition are good reflections of pleiotropic changes in the cell surface functions of the tkt mutant.

The transketolase (tkt) (EC 2.2.1.1) mutant of Bacillus subtilis is a pleiotropic mutant. Analysis of the pleiotropic changes revealed that the transport function of enzyme II of the D-Glucose:phosphoenolpyruvate phospho-

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transferase system was defective (40) and the regulation of synthesis of enzymes concerning D-mannitol and sorbitol catabolism under catabolite repression had changed (41). Functions relating to cell surface, such as morphogenesis, motility, flagellation, bacteriophage sensitivity and sporulation, were also altered (42,44). These pleiotropic changes were generated by a single mutation in the transketolase gene, which was elucidated by the isolation of true revertants (40). Strain BG2694, one of the true revertants isolated at the same frequency as the forward mutation, showed the same properties as the parental strain, IFO 12114 (40,41,43,44). These results imply that these pleiotropic changes were generated by transketolase deficiency through a cell surface change.

This paper deals with the difference in the membrane composition of the parental strain, IFO 12114, and the tkt mutant BG2607 that was detected by SDS-polyacrylamide gel electrophoresis of membrane proteins and thin layer chromatography of membrane lipids.

Materials and Methods

Bacterial strains. The bacterial strains employed were B. subtilis IFO 12114 and a tkt mutant BG2607 (45).

Media. Slightly modified Spizizen's medium (46) was used for cultivation of bacteria. Shikimic acid or a mixture of aromatic amino acids was added to the medium at the concentration of 0.5 mM to compensate for the growth requirements of the tkt mutant.

Preparation of cell walls, peptidoglycan and teichoic acid fractions. Preparation of the cell walls of bacteria was carried out according to the method of Kotani et al. (26) with a slight modification. The bacterial strains were grown in modified Spizizen's medium for 5 to 6 hr at 37 C with a rotary shaker. The cells were harvested by centrifugation, washed twice with 67 mM phosphate buffer (pH 7.8) and disrupted with a Kubota sonic oscillator. The crude cell walls were washed twice with distilled water and purified by differential centrifugation (300 x g, 20 min and 4000 x g, 60 min). The cell wall fractions were treated with 0.5% SDS for 5 min at 90 C to exclude lipids and proteins. The purified cell walls were washed twice with distilled water and dried over solid KOH.

Separation of teichoic acid from peptidoglycan was performed according to

the method described by Armstrong et al. (2). The dried cell walls were suspended in 10% trichloroacetic acid solution and mixed with a Voltex mixer. After standing overnight at 5 C in a refrigerator, the supernatant was separated by centrifugation (3000 x g, 10 min). The procedure was repeated several times. The supernatant fractions were combined, and 5 volumes of chilled ethanol was added. The precipitate was separated with a glass filter, washed with a small amount of acetone and ether, dried over solid KOH and used as the teichoic acid fraction. The residue of the 10% trichloroacetic acid extraction was washed twice with distilled water, dried over solid KOH and used as the peptidoglycan fraction.

Preparation of membrane proteins and lipids. The bacterial strains were grown in the same way as described for the preparation of cell walls and the cells were harvested by centrifugation, washed twice with 0.05 M phosphate buffer (pH 9.0) and suspended in the same buffer. The preparation of the membrane fraction was performed according to the method of Konings et al. (25).

The membrane fraction was suspended in 0.1 M phosphate buffer (pH 6.8). To the suspension was added the same volume of 0.05 M phosphate buffer (pH 7.1) containing 1% SDS and 2% 2-mercaptoethanol to resolve the membrane vesicles.

The lipids were extracted from the membrane fraction with methanol-chloroform according to the method of Bligh and Dyer (5).

Analysis of cell wall and membrane components.

(1) Peptidoglycan. Peptidoglycan was hydrolyzed with 4 N HCl at 100 C for 13 hr (37). The sample was concentrated by evaporation and dissolved in a small amount of distilled water. A small amount of the sample was spotted on Toyo filter paper No. 51 and developed descendingly with n-butanol-pyridine-water-acetic acid (100:40:30:3, by volume) as solvent. Sugars were detected with alkaline silver nitrate (48) and amino acids with ninhydrin (29).

(2) Teichoic acid. Teichoic acid was hydrolyzed with 2 N HCl in a sealed tube for 3 hr at 100 C. After evaporation, the samples were examined using paper chromatography. The solvent system was ethylacetate-acetic acid-water (3:3:1). The spray reagents used were alkaline silver nitrate for sugars (48), ninhydrin for amino acids (29), vanillin-perchloric acid for polyols (31) and ammonium molybdate for phosphoric acid esters (20).

Enzymatic assays of ribitol and glycerol were performed according to the methods of Fromm (16) and Noble and Sturgeon (33), respectively. The mixture of ribitol dehydrogenase and ribokinase was prepared according to the method

of Fossitt and Wood (15). The enzyme source was commercially obtained dried cells of Aerobacter aerogenes. Glycerokinase and α-glycerophosphate dehydrogenase preparations were also commercial products. The hydrolyzed samples described above were dephosphorylated with alkaline phosphatase (200 μg/ml, pH 9.0) at 37 C overnight, heated for 3 min at 80 C and added to the reaction mixture for determination of ribitol or glycerol.

(3) Membrane proteins. Slab SDS-polyacrylamide gel electrophoresis was carried out according to the method of Laemmli (28), using electrophoresis apparatus made by Atto Scientific Industries. Protein was determined according to the method of Lowry et al. (30).

(4) Lipids. Lipids were separated by thin layer chromatography on a silica gel plate (DC-Fertigplatten Kieselgel 60 F_{254}, Merk, Darmstadt) using chloroform-methanol-acetic acid (65:25:4, by volume) as solvent system. As spray reagents, 9 N H_2SO_4 was used for general (23), molybdate for phospholipids (12), ninhydrin for amino acids (29) and α-naphthol for glycolipids (24). Relative composition of lipids was estimated using a Gelman Chromatoscanner DCD-16.

Chemicals. Acrylamide, methylene bis-acrylamide, ammonium persulfate, N, N, N, N-tetramethylethylenediamine, vanillin, ninhydrin, α-naphthol, D-glucosamine, D-galactosamine, ribitol, glycerol, SDS, mercaptoethanol and sodium EDTA were produced by Wako Pure Chemicals. Deoxyribonuclease I, ribonuclease, lysozyme, α-glycerophosphate dehydrogenase, dried cells of Aerobacter aerogenes, phosphatidylglycerol, phosphatidylethanolamine, diaminopimeric acid, and muramic acid were produced by Sigma Chemical Co. Glycerokinase and alkaline phosphatase were produced by P-L Biochemcals Inc. Coomassie brilliant blue was purchased from Tokyo Kasei Co. Molecular weight standards were purchased from Pharmacia Fine Chemicals.

Results

Alteration of membrane protein composition

Our previous observation of pleiotropic changes in the tkt mutant of B. subtilis (40,41,43,44) implied that plural membrane proteins concerning D-glucose transport, flagellation, sporulation, etc., did not work well. So, to detect the difference in the composition of membrane proteins of the parental strain, IFO 12114, and the tkt mutant BG2607, SDS-polyacrylamide gel elec-

trophoresis of solubilized membrane proteins was performed. As shown in Fig. 1, seven bands of proteins were found to be different in comparing the parental and the tkt mutant strains. Some differed in quantity; the amount in the tkt mutant strain was lower. The molecular weight of one band appeared to be a little different. Newly recognized bands, which were not detected in the parental strain, were also found in the tkt mutant. These changes in membrane proteins seem to reflect pleiotropic changes in the tkt mutant; flagellation deficiency, D-glucose transport defect, morphological change, decreased frequen-

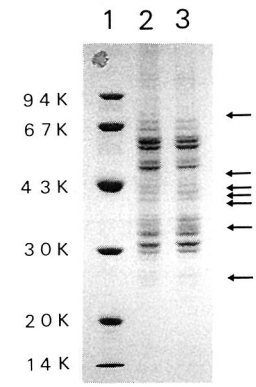
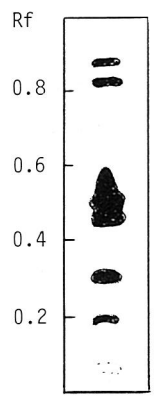


Fig. 1. SDS-Polyacrylamide gel electrophoresis of membrane proteins of B subtilis IFO 12114 and the tkt mutant BG2607.
Lane 1, standard proteins; lane 2, B. subtilis IFO 12114; lane 3, the tkt mutant BG2607.



Spot No.	Color reaction: Molybdate	Ninhydrin	α-Naphthol	Identification
1	-	-	-	Unknown
2	-	-	-	Unknown
3	+	-	-	Cardiolipin
4	-	-	+	Diglucosyldiglycerol
5	+	-	-	Phosphatidylglycerol
6	+	+	-	Phosphatidylethanolamine
7	-	-	+	Unknown
8	+	+	-	Lys-phosphatidylglycerol

Fig. 2. Thin layer chromatography of membrane lipids of B. subtilis IFO 12114.

cy of sporulation, etc.

Alteration of membrane lipid composition

As described above, the compositon of membrane proteins had altered in the tkt mutant. Next, composition of lipids, another major component of cytoplasmic membrane, was examined. Thin layer chromatography of membrane lipids of the parental strain, IFO 12114, is shown in Fig. 2. The spots were colored by spraying with H_2SO_4 and heating. Seven to eight spots of lipids were detected on the chromatogram, though spots No. 3, 4, and 5 were not separated clearly. Each lipid fraction was deduced to be the corresponding lipid by comparing their Rf values with those of standard samples, cardiolipin, phosphatidylglycerol and phosphatidylethanolamine and color reactions with molybdate, ninhydrin or α-naphthol reagent. Diglucosyldiglyceride and lysyl-phosphatidylglycerol were deduced from published data (4,8,35), because we could not obtain standard samples of these lipids. Three unknown lipid spots were also detected, one of which was a glycolipid (No. 7).

Densitometric estimation of the relative composition of the lipids is shown in Tables 1, 2 and 3. Cardiolipin and diglucosyldiglycerol were major lipid components in the parental strain IFO 12114. Though it had been thought that cardiolipin was a minor component of the membrane lipid of B. subtilis (4,8,35), it was recently found that cardiolipin is also one of the major components (14). This was confirmed in this study, as described above. Cardiolipin was extracted completely using membrane sample without cell wall components (14). Phospholipid composition and glycolipid composition are shown in Tables 2 and 3, respectively. Comparing the composition of lipids in the parental and the tkt mutant strains (Tables 1,2 and 3), phosphatidylethanolamine and unknown glycolipid decreased in the tkt mutant. Cardiolipin, phosphatidyl-

Table 1. Lipid composition of membranes of B. subtilis IFO 12114 and the tkt mutant BG2607

Lipid	Relative composition (%)	
	IFO 12114	BG2607
Cardiolipin	33.0	38.5
Diglucosyldiglyceride	32.8	31.1
Phosphatidylglycerol	4.9	9.5
Phosphatidylethanolamine	16.1	9.6
Unknown	9.8	5.6
Lysyl-phosphatidylglycerol	3.4	5.9

Table 2. Phospholipid composition of membranes of B. subtilis IFO 12114 and the tkt mutant BG2607

Lipid	Relative composition (%)	
	IFO 12114	BG2607
Cardiolipin	68.3	80.5
Phosphatidylglycerol	3.5	8.2
Phosphatidylethanolamine	27.8	9.0
Lysyl-phosphatidylglycerol	0.3	2.5

Table 3. Glycolipid composition of membranes of B. subtilis IFO 12114 and the tkt mutant BG2607

Glycolipid	Relative composition (%)	
	IFO 12114	BG2607
Diglucosyldiglycerol	85.8	91.7
Unknown	14.2	8.2

Table 4. Cell wall composition of B. subtilis IFO 12114 and the tkt mutant BG2607

Strain	Composition (%)	
	Teichoic acid	Peptidoglycan
IFO 12114	51.2	48.8
BG2607	40.0	56.4

Teichoic acid and peptidoglycan were prepared according to the methods described in "Materials and Methods". The weights of them were measured after being dried on solid KOH.

glycerol and lysyl-phosphatidylglycerol, instead, increased in the mutant strain.

Cell wall composition

Teichoic acid and peptidoglycan existed in the cell wall at a ratio of about 1:1 in the parental strain (Table 4). Peptidoglycan content was a little higher in the tkt mutant. As components of peptidoglycan, muramic acid, glucosamine, diaminopimeric acid, alanine and glutamic acid were found in both the parental and the tkt mutant strains using paper chromatography of hydrolyzed samples with HCl (Fig. 3), as is known in B. subtilis (18).

Similarly glucose, ribitol, glycerol, alanine and phosphoric acid were found using paper chromatography of hydrolyzed samples of teichoic acids in

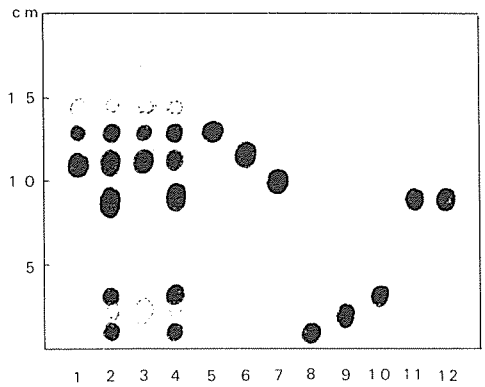


Fig. 3. Paper chromatography of components of peptidoglycans of B. subtilis IFO 12114 and the tkt mutant BG2607.
1, IFO 12114 (silver nitrate); 2, IFO 12114 (ninhydrin); 3, BG 2607 (silver nitrate); 4, BG2607 (ninhydrin); 5, muramic acid; 6, D-glucosamine; 7, D-galactosamine; 8, meso-diaminopimeric acid; 9, L-lysine; 10, L-glutamic acid; 11, D-alanine; 12, L-alanine.

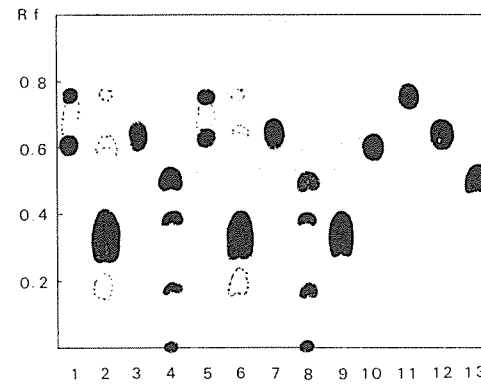


Fig. 4. Paper chromatography of components of teichoic acids of B. subtilis IFO 12114 and the tkt mutant BG2607.
1, IFO 12114 (vanillin); 2, IFO 12114 (silver nitrate); 3, IFO 12114 (ninhydrin); 4, IFO 12114 (molybdate); 5, BG2607 (vanillin); 6, BG2607 (silver nitrate); 7, BG2607 (ninhydrin); 8, BG2607 (molybdate); 9, D-glucose; 10, ribitol; 11, glycerol; 12, D-alanine; 13, L-alanine.

the both strains (Fig. 4). However, it was very difficult to estimate the amounts of ribitol and glycerol in the teichoic acid fraction by paper chromatography, so enzymatic assays of ribitol and glycerol using ribitol dehydrogenase and glycrokinase-α-glycerophosphate dehydrogenase mixture, respectively, were performed. As shown in Table 5, it was found that the major component polyalcohol was ribitol. Glycerol was a minor component in both strains and

Table 5. Ribitol and glycerol contents in teichoic acids from B. subtilis IFO 12114 and tkt mutant BG2607 determined by enzymic methods

Strain	Content (μg per mg of teichoic acid)	
	Ribitol	Glycerol
IFO 12114	29.8	4.31
BG2607	25.5	4.27

might come from linkage units between teichoic acid and peptidoglycan (10). So the teichoic acid of these strains was determined to be ribitol teichoic acid. It seemed that there was no difference in the composition of the cell walls of the parental and the tkt mutant strains.

Discussion

It has been shown in this paper that membrane composition is altered in the tkt mutant BG2607, i.e., several bands of membrane proteins in SDS-polyacrylamide gel elctrophoresis and lipid composition in thin layer chromatography differed in the parental and the tkt mutant strains.

Some membrane proteins seem to be synthesized in decreased quantities or to be defective in their integration into the membrane. And some proteins seem to have altered in molecular weight, probably due to defective processing or unknown mechanisms. More detailed studies are needed to clarify which protein band corresponds to which function, e.g., glucose transport, morphogenesis, motility, sporulation, bacteriophage sensitivity etc. The lower phosphatidylethanolamine content of the tkt mutant seems to be a cause of its decreased sporulation frequency in view of the reports of Lubochinsky and his coworkers concerning the phospholipid composition of asporogenous mutants of B. subtilis (7,39).

Compositional change of membrane proteins has been shown in various mutants, such as minicell-producing mutants of B. subtilis (9), protein excretion of Escherichia coli (50), polymyxin-resistant mutants of Pseudomonas aeruginosa (19), sporulation mutants of B. subtilis (6,32), a bacteriophage-resistant mutant of Caulobacter crescentus (13,17), an adenylate cyclase- or cyclic AMP receptor protein-deficient mutant of E. coli (1), alkaline phosphatase-negative mutants of E. coli (22) and membrane mutants of Salmonella typhimurium (36) and E. coli (51). Cyclic GMP derivatives also affected the membrane protein composition of C. crescentus (27). In these studies, the initial event or the entity which generates the pleiotropic changes in membrane protein composition has not been determined. In some cases, it is deduced that the pleiotropic change may be generated by deficiency in the correct insertion of membrane proteins (6,36) and in other some cases, it was assumed that the regulatory gene of membrane protein synthesis was defective (51).

Change in the lipid composition of bacterial membrane has also been shown in culture-conditional variation and various mutations in lipid synthesis and membrane biogenesis. However, the relationship between the lipid composition changes and membrane-functional changes has not been extensively studied in these reports, except the following.

Autolysis deficiency of Streptococcus faecium might be generated by change of lipid composition (47). Decreased phosphatidylethanolamine content caused sporulation deficiency in asporogenous mutants of B. subtilis (7,39), resistance to polymyxin in a P. aeruginosa mutant (11) and irregular cell size and shape in a B. subtilis mutant (3). Temperature-sensitive phosphatidylserine decarboxylase mutant (21), phosphatidylserine synthetase mutant (38), and the beta oxidation pathway mutant (49) are filamentous.

At this stage, it would be quite difficult to explain the relationship between the change in membrane composition and the pleiotropic, functional changes caused by the transketolase mutation in BG2607. The following possibilities exist; (i) mutant transketolase is hydrophobic and interacts with membrane to affect membrane construction, (ii) transketolase may be involved in regulation for the synthesis of various membrane-bound proteins and (iii) because transketolase is a key enzyme, both of the pentose phosphate pathway and of the aromatic biosynthesis pathway, metabolism disturbance caused by transketolase deficiency affects cytoplasmic environment resulting in the mal-construction of membrane.

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FORMATION OF 2,3-DIDEOXY-HEX-4-ULOSONIC ACID BY CARBOLIGASE ACTIVITY OF 2-KETOGLUTARATE DEHYDROGENASE IN MICROORGANISMS

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Summary

Cell-free extracts of Bacillus subtilis and Escherichia coli catalyzed decarboxylative condensation reactions between 2-ketoglutarate and such aldoses as glycolaldehyde, D-glyceraldehyde and L-glyceraldehyde. The product of 2-ketoglutarate and glycolaldehyde was purified, isolated as crystals, and identified as 2,3-dideoxy-hex-4-ulosonic acid.

Based on the mechanism of the carboligase reaction, the product of 2-ketoglutarate and D-glyceraldehyde (or L-glyceraldehyde) is postulated to be 2,3-dideoxy-hept-4-ulosonic acid.

A citK14 mutant of B. subtilis defective in 2-ketoglutarate dehydrogenase (EC 1.2.4.2) did not show 2-ketoglutarate-aldose carboligase activity, and spontaneous revertants (citK14$^+$) of the mutant restored carboligase activity. Deficiency of dihydrolipoamide succinyltransferase, pyruvate dehydrogenase or acetoin dehydrogenase in B. subtilis did not cause defective carboligase activity. Purified 2-ketoglutarate dehydrogenase comples (bovine heart) also catalyzed the carboligase reaction. From these results, it was concluded that 2-ketoglutarate dehydrogenase is responsible for the condensation reaction.

In previous papers (20, 21), we have reported that cell-free extracts of various microorganisms catalyzed an acyloin-type condensation reaction between aldose and pyruvate (or acetoin or methylacetoin) resulting in the formation of 1-deoxy-ketoses. These reactions could be ascribed to carboligase activities of pyruvate dehydrogenase (EC 1.2.4.1, PDH) of pyruvate dehydrogenase complex and acetoin dehydrogenase (AccDH) (Yokota and Sasajima, submitted for publication).

Further investigation of carboligase reaction by enzymes other than PDH and AccDH revealed that 2-ketoglutarate (KG)-aldose carboligase reaction is catalyzed by 2-ketoglutarate dehydrogenase (KG: lipoamide oxidoreductase (decarboxylating and acceptor-succinylating), KGDH), a component of 2-ketoglutarate dehydrogenase complex, and the reaction product of KG and glycolaldehyde was identified as 2,3-dideoxy-hex-4-ulosonic acid.

Schlossberg et al. (17, 18) reported that bovine heart KGDH was responsible for the condensation reaction between KG and glyoxylate (KG-glyoxylate carboligase activity). They identified the reaction product as 5-hydroxy-4-ketovarelic acid. Saito et al. (16) found that an enzyme preparation from Rhodopseudomonas spheroides catalyzes a carboligase reaction between KG and glyoxylate. Kubasik et al. (6) showed that Escherichia coli KGDH is associated with the KG-glyoxylate carboligase reaction. However, KG-glycolaldehyde or KG-glyceraldehyde carboligase reaction by KGDH has not hitherto been reported.

The present paper deals with the identification of the product of the KG-aldose carboligase reaction and also reports that KGDH of B. subtilis, E. coli and bovine heart is responsible for the condensation reaction between KG and such aldoses as glycolaldehyde, D-glyceraldehyde and L-glyceraldehyde.

Materials and Methods

Bacterial strains and culture conditions. The strains of B. subtilis and E. coli used in this study are listed in Table 1. Revertants R-1$\sim$R-5 (citK14$^+$) of the mutant JH414 (citK14) were selected by plating on minimal medium with sodium lactate (0.5%) as the carbon source according to the method described by Hoch and Coukoulis (5). B. subtilis wild-type and mutant strains were cultured by the method described by Rutberg and Hoch (15). The strain of E. coli was cultured in medium containing 1% peptone, 0.5% yeast

Table 1. Strains of Bacillus subtilis and Escherichia coli.

Strain	Genotype [a]	Source and reference
B. subtilis IFO 13719	Wild-type (Marburg strain)	IFO [b] Culture Collection
B. subtilis JH414	trpC2 citK14	J.A. Hoch (15)
B. subtilis JH414 R-1∼R-5	trpC2 citK14$^+$	Spontaneous revertants of JH414 (this work)
B. subtilis IA99	trpC2 citM1 ilvA3	Bacillus Genetic Stock Center (Ohio Univ.) (3, 15)
B. subtilis 61141	trpC2 metC7 aceA1	E. Freese (4)
B. subtilis 61411	pyrA3 fruB22 accB	E. Freese (7)
E. coli IFO 3301	Wild-type (K-12 strain)	IFO Culture Collection

[a] Enzymes deficiency: citK14, KGDH (EC 1.2.4.2) of 2-ketoglutarate dehydrogenase complex; citM1, dihydrolipoamide succinyltransferase (EC 2.3.2.61) of 2-ketoglutarate dehydrogenase complex; aceA, PDH (EC 1.2.4.1) of pyruvate dehydrogenase complex; and accB, acetoin dehydrogenase.

[b] Institute for Fermentation, Osaka (Japan).

extract and 0.5% NaCl.

Preparation of cell-free extracts. Cells for the preparation of extracts were grown to late log phase in Penassay broth (antibiotic medium No. 3, Difco Laboratory). The cells were collected by centrifugation and suspended in 0.1 M potassium phosphate buffer (pH 6.0) containing 0.1 mM EDTA, 0.1 mM $MgCl_2$, 1.43 mM 2-mercaptoethanol, and 2 μM thiamine pyrophosphate (TPP) (buffer A). Lysozyme (500 μg/ml) and deoxyribonuclease I (5 μg/ml) were added, and the cells were incubated at 37 C until lysis. The lysate was centrifuged at 13,000 x g for 20 min, and the clear supernatant obtained was used for enzyme assays.

Partial purification of KGDH. To the cell-free extract, solid ammonium sulfate was added to 30% saturation. After 15 min, the precipitate formed was separated by centrifugation and discarded. Solid ammonium sulfate was added to the supernatant to 60% saturation. The precipitate formed was collected by centrifugation and dissolved in buffer A. The solution was dialyzed against the same buffer, and then used as the partially purified KGDH preparation.

Preparation of apoenzyme of KGDH. Enzyme solution used for the examination of cofactor requirements was prepared as follows: The partially puri-

fied enzyme solution was submitted to ultracentrifugation at 140,000 x g for 180 min. The precipitate formed was dissolved in the same buffer and insoluble material was removed by centrifugation at 13,000 x g for 20 min. The supernatant containing 26 mg of protein was loaded onto a column of Sephadex G-25 (1.1 x 46 cm) preequillibrated with 0.02 M potassium phosphate buffer (pH 6.0) (buffer B) and eluted with the buffer. The active fractions were combined and used.

Enzyme assays. PDH was assayed using Reed and Willms' ferricyanide method (14). KGDH activity was determined by the substitution of pyruvate for KG in the PDH assay. Both assays were carried out at 30 C. Activities were calculated as nanomoles of substrate converted per min per mg of protein. Protein was determined using the method of Lowry et al. (9).

Detection of KG-aldose and pyruvate-aldose carboligase activities. Reaction mixtures (0.5 ml) containing 20 µmoles of Tris-HCl buffer (pH 8.0), 2.5 µmoles of $MgCl_2$, 0.25 µmoles of TPP, 1 µmole of EDTA, 50 µmoles of KG or pyruvate, 50 µmoles of aldose, and an enzyme preparation, were incubated at 37 C for 16 hr. The reaction was stopped by adding two volumes of ethanol. After centrifugation, the supernatants were subjected to paper chromatography with solvent B. The papers were dried and visualized with alkaline silver nitrate, alkaline triphenyltetrazolium chloride (TTC), vanillin-perchloric acid and 2,6-dichlorophenolindophenol reagent.

Isolation of compound 1. The reaction mixture (100 ml) containing 10 mmoles each of KG and glycolaldehyde and partially purified enzyme preparation (219 mg of protein) was incubated for 16 hr at 37 C and then used as a starting material for isolation of compound 1. Two volumes of ethanol was added to the solution and the precipitate formed was removed by centrifugation. The supernatant fluid was concentrated to about 10 ml in vacuo. Two volumes of methanol was added to the concentrate and the precipitate formed was removed by centrifugation. The supernatant fluid was concentrated in vacuo. The residue was extracted with methanol, and insoluble materials were removed by centrifugation. Methanol was removed by evaporation, and residual syrup was dissolved in a small volume of water and loaded onto a column (3.5 x 8 cm) of Dowex 1 (formate form). After washing the column with 50 ml of water, compound 1 was eluted with 100 ml of 1 M formate. The eluate was concentrated in vacuo, the residue extracted with acetone, and insoluble materials were removed by centrifugation. The acetone solution was evaporated to give a crystalline 1 (195 mg). Compound 1 was recrystallized

from cold acetone with addition of chloroform. It was colorless and soluble in water, methanol, ethanol and acetone, but was insoluble in chloroform.

Periodate oxidation of compound 1. Compound 1 (30 mg in 19 ml of water) was treated with 1 ml of 0.55 M periodate at room temperature for 15 min. Aliquots were then removed to determine the consumption of periodate, production of formaldehyde, formate and succinate. The remainder was treated as follows to examine succinate formed: an excess amount of ethylene glycol was added to the reaction mixture to decompose the periodate. The solution was passed through a column (1.2 x 8 cm) of IR 120 (H^+ form) and concentrated in vacuo. Succinate in the solution was examined by paper chromatography with solvent A. Consumption of periodate was measured by titrating with iodine solution (1). Production of formaldehyde was measured colorimetrically using Nash's method (12). Production of formate was measured colorimetrically with 2-thiobarbituric acid reagent (11). Total acids (formate plus succinate) formed were measured by titrating with 0.01 N $Ba(OH)_2$ in the presence of phenolphthalein.

Partial purification of compound 2. Reaction conditions were similar to those described for the isolation of 1 except that 2 mmoles of D-glyceraldehyde was used instead of glycolaldehyde in a total volume of 20 ml. The reaction mixture was treated with ethanol and methanol to remove protein and concentrated in vacuo. The concentrate was chromatographed on two sheets of Toyo Roshi No. 51 filter paper (40 x 40 cm). Compound 2 was extracted from the papers with hot water (100 ml) and the extract was concentrated in vacuo to a syrup.

Paper chromatography and thin-layer chromatography. Paper chromatography and thin-layer chromatography were performed by the method described previously (20) with the following solvent systems: (A), phenol-water-formate (75:25:1, by vol.); (B), n-butanol-ethanol-water (50:30:20, by vol.).

High performance liquid chromatography (HPLC). HPLC was performed on an Shimadzu Model LC-5A pumping system, equipped with a manual 20 µl loop injector. The column, a Shim-pack ISA-07/S2504 (4.0 x 250 mm), was heated to 65 C. The solvent system was 0.4 M borate buffer (pH 9.0) containing 0.04 M NaCl. The column effluent (flow rate 0.6 ml/min) and detection reagent, a mixture of 1% arginine and 3% borate (10) delivered by another Model LC-5A pump at a flow rate of 0.5 ml/min, were led to a Shimadzu Model CRB-3A chemical reaction bath and heated to 150 C. Fluorescence intensities of the effluent were measured with a Shimadzu Model RF-530 spectrofluorometer and a Chromatopac.

Physical and chemical analyses. Melting point was uncorrected. Optical rotation was measured with a Japan Spectroscopic DIP-181 polarimeter. ^{1}H-NMR spectrum was measured with a Varian EM-390 (90 MHz) spectrometer with tetramethylsilane as a reference. ^{13}C-NMR spectrum was measured with a Varian XL 100-12 (25.2 MHz) spectrometer with dioxane as a reference. IR spectrum was measured with a Hitachi Model EPI-G3 spectrometer.

Chemicals. KG and glycolaldehyde were purchased from Wako Pure Chemical Industries. D- and L-glyceraldehyde, lysozyme, deoxyribonuclease I, and 2-ketoglutarate dehydrogenase complex (bovine heart) were purchased from Sigma Chemical Co.

Results and Discussion

Characterization and identification of compound 1

When KG was incubated with glycolaldehyde in the presence of a partially purified enzyme preparation of *B. subtilis* IFO 13719, a spot of a newly formed substance (Rf 0.20, solvent B) was detected by paper chromatography, as shown in Fig. 1. Cell-free extract of *E. coli* IFO 3301 also catalyzed the condensation reaction (data not shown). The product (compound 1) was purified and isolated as crystals from the reaction mixture as described in Materials and Methods.

Crystalline compound 1 showed as one spot in paper chromatography and thin-layer chromatography. It melted at 93-96 C and was found to have the chemical formula $C_6H_{10}O_5$ by elemental analysis: Anal. Found: C, 43.94; H, 6.24. Calcd. for $C_6H_{10}O_5$: C, 44.45; H, 6.22%. Compound 1 showed $[\alpha]_D$ +0.2° (c=2.0, H_2O) and gave positive results with alkaline TTC, alkaline silver nitrate and 2,6-dichlorophenolindophenol reagents, but gave negative results with vanillin-perchloric acid reagent. The ^{1}H-NMR spectrum of 1 in deuterium oxide (Fig. 2) showed an eight-line symmetrical pattern centered at δ3.0 (4H), which is consistent with R-CH_2-CH_2-R', and a doublet at δ4.12 (J=3Hz, 2H) for a O-C-$\underline{H}_2$- adjacent to another O-C-$\underline{H}$. The IR spectrum of 1 has the general features of strong acid (1720 cm^{-1}), ketone (1690 cm^{-1}) and alcohol (3390 cm^{-1}) absorptions.

In the ^{13}C-NMR spectrum shown in Table 1, the signals at 212.8 and 177.5 ppm were assigned to C=O and COOH, respectively, from the off-resonance decoupling spectrum. The signals at 78.3 and 63.6 ppm were assigned to $\begin{matrix} C \\ C \end{matrix}\!\!>\!\!\underline{C}\!<\!\!\begin{matrix} O \\ H \end{matrix}$ and

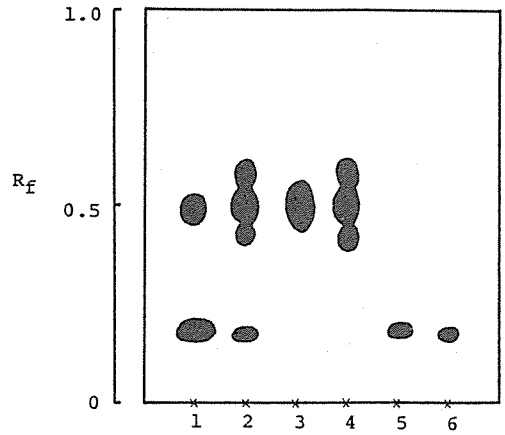


Fig. 1. Paper chromatography of reaction products formed from KG and glycolaldehyde or D-glyceraldehyde by wild-type and a citK14 mutant of Bacillus subtilis.
Compounds were visualized with alkaline TTC spray reagent. Strains used were: (1) and (2), IFO 13719 (wild-type); (3) and (4), JH414 (citK14). Substrates were: (1) and (3), KG and glycolaldehyde; (2) and (4), KG and D-glyceraldehyde. Standards: (5), 2,3-dideoxy-hex-4-ulosonic acid; (6), partially purified compound 2.

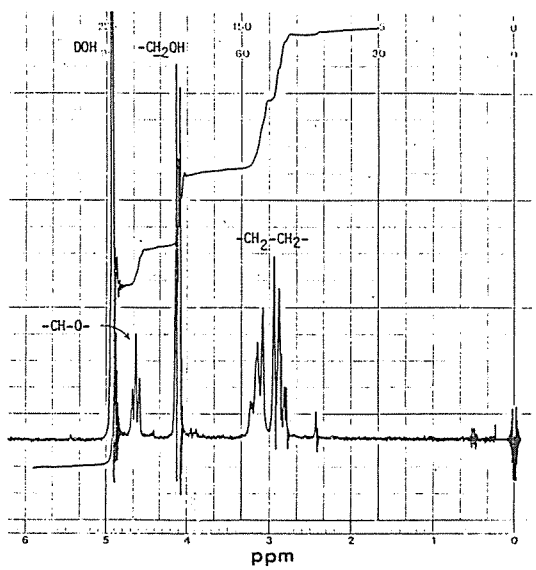
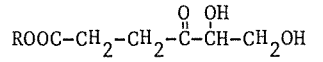


Fig. 2. ^{1}H-NMR spectrum of compound 1 in D_2O at 90 MHz.

C-$\underline{CH_2}$O, respectively, and signals at 34.4 and 28.4 ppm were assigned to C-$\underline{CH_2}$-groups. The comparison of the ^{13}C-NMR spectrum of $\underset{\sim}{1}$ with that of 2,3-dideoxy-L-glycero-hex-4-ulosonic acid methylester ($\underset{\sim}{3}$), which has been chemically synthesized by Dyong et al. (2), indicates reasonable correspondence of each carbon of $\underset{\sim}{1}$ and $\underset{\sim}{3}$, except for the signal of carbon of the methyl group (Table 2).

Table 2. Comparison of ^{13}C-NMR spectra of compound $\underset{\sim}{1}$ and 2,3-Dideoxy-L-glycero-hex-4-ulosonic acid methylester ($\underset{\sim}{3}$).



$\underset{\sim}{1}$: R=H

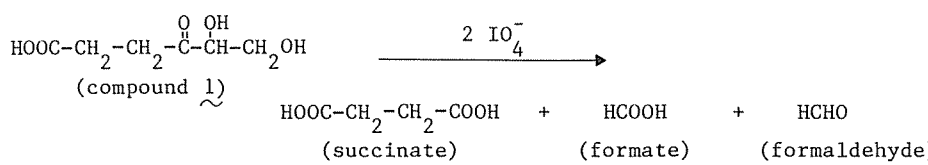
$\underset{\sim}{3}$: R=CH_3

Carbon	Chemical shift (ppm) $\underset{\sim}{1}$		$\underset{\sim}{3}$ [b]	
1	177.5	s [a]	173.3	s
2	34.4	t	33.0	t
3	28.4	t	27.3	t
4	212.8	s	209.4	s
5	78.3	d	77.8	d
6	63.6	t	63.5	t
(Methyl)	——		51.7	q

[a] Multiplicity on off-resonance decoupling: s, singlet; d, doublet; t, triplet; q, quartet.

[b] Assignments are based on the data of Dyong et al. (2).

The structure of compound $\underset{\sim}{1}$ was further examined by periodate oxidation. One mole of compound $\underset{\sim}{1}$ reacted with 2 moles of periodate, yielding 0.88 mole of formaldehyde, 0.92 mole of formate and 1.03 moles of succinate. Therefore, splitting by periodate in compound $\underset{\sim}{1}$ might proceed according to the following formula:



From these results, the product of the enzyme reaction between KG and glycolaldehyde, compound $\underset{\sim}{1}$, was determined to be 2,3-dideoxy-hex-4-ulosonic

acid.

Characterization of compound 2

When KG was incubated with D-glyceraldehyde using a similar method to that for the formation of compound 1, a spot of a newly formed substance (Rf 0.18, solvent B) was detected by paper chromatography, as shown in Fig. 1. It reduced alkaline silver nitrate and alkaline TTC, and was positive to 2,6-dichlorophenolindophenol reagent and vanillin-perchloric acid reagent (a brick red color).

When L-glyceraldehyde was used as a substrate instead of D-glyceraldehyde, similar results were obtained (data not shown).

When purification of product 2 was carried out by the same method as in compound 1, it changed into other compound(s) because of its instability at low pH. Therefore, we have not yet succeeded in isolating compound 2 as a pure substance. Purification and identification of the product are under investigation. However, based on the mechanism of carboligase reaction of KGDH as described below, and those of PDH and AccDH as described above, together with the results of characterization of 2 on paper chromatogram, we postulate the chemical structure of compound 2 to be 2,3-dideoxy-hept-4-ulosonic acid.

Compounds 1 and 2 have been chemically synthesized as their methyl esters from 2,3-dideoxy-ald-2-enonic acid esters (2). The syntheses involve several steps and further purification steps are required to isolate them. Therefore, the enzyme reaction described here can be used as a convenient method of preparing 2,3-dideoxy-4-ulosonic acids withgood yields.

Deficiency of KG-glycolaldehyde, KG-D-glyceraldehyde and KG-L-glyceraldehyde carboligase activities in KGDH-defective mutant of B. subtilis

It was presumed that the genuine enzyme which catalyzes the KG-aldose carboligase reaction is KGDH of 2-ketoglutarate dehydrogenase complex. To confirm this, we examined whether a KGDH-defective mutant (citK14) of B. subtilis has KG-aldose carboligase activity. As shown in Figs. 3 and 4, cell-free extract of the citK14 mutant JH414, which has no KGDH activity, did not show KG-aldose carboligase activity.

That deficiencies of both KGDH and KG-aldose carboligase activities were caused by a single mutation was shown by spontaneous revertants isolated with respect to sodium lactate utilization as a sole carbon source (4). All the citK14$^+$ revertants isolated showed the normal specific activity of both the enzymes. Wild-type strain, IFO 13719, also showed both the enzyme activ-

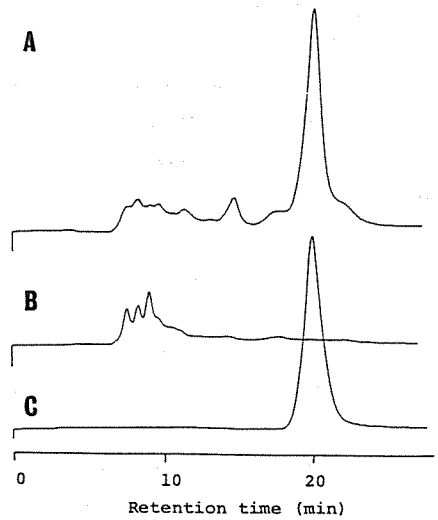


Fig. 3. HPLC analysis of reaction product of KG and glycolaldehyde by cell-free extracts of wild-type and mutant strains of Bacillus subtilis.
Enzyme preparation from: A, IFO 13719 (wild-type); B, JH414 (citK14). C, 2,3-dideoxy-hex-4-ulosonic acid as a standard.

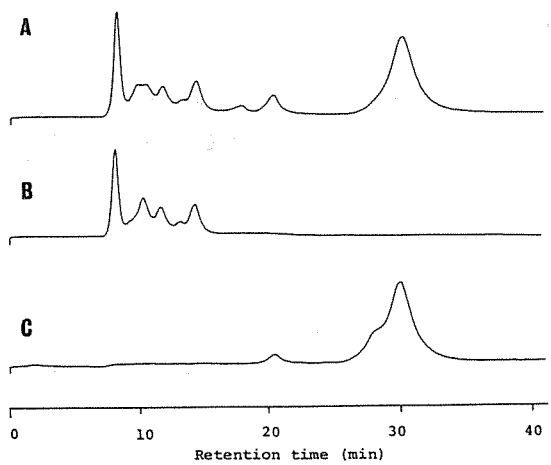


Fig. 4. HPLC analysis of reaction product between KG and D-glyceraldehyde by cell-free extracts of wild-type and citK14 mutant of Bacillus subtilis.
Enzyme preparation from: A, IFO 13719 (wild-type); B, JH414 (citK14). C, partially purified compound 2 as a standard.

Table 3. PDH, KGDH and carboligase activities in cell-free extracts of wild-type and mutant strains of Bacillus subtilis.

Strain	Enzyme activities (nmole/min per mg of protein)		Carboligase activities [b]			
	PDH	KGDH	Pyruvate + Glycol-aldehyde	Pyruvate + D-Glycer-aldehyde	KG + Glycol-aldehyde	KG + D-Glycer-aldehyde
IFO 13719	8.33	3.80	+	+	+	+
JH414	11.42	0	+	+	−	−
JH414 R-1	ND [a]	1.93	+	+	+	+
JH414 R-2	ND	1.18	+	+	+	+
JH414 R-3	ND	1.15	+	+	+	+
JH414 R-4	ND	0.55	+	+	+	+
JH414 R-5	ND	2.41	+	+	+	+
61141	0.20	3.23	−	−	+	+
61411	ND	1.09	+	+	+	+
IA99	ND	1.15	+	+	+	+

[a] ND, Not determined.
[b] The plus and minus signs indicate respectively the presence and absence of enzyme activities, tested by paper chromatography as described in the text.

ities (Table 3).

PDH-defective mutant 61141, AccDH-defective mutant 61411 and dihydrolipoamide succinyltransferase-defective mutant IA99 showed both KGDH and KG-aldose carboligase activities (Table 3). These results means that PDH, AccDH or dihydrolipoamide succinyltransferase (EC 2.3.2.61, a component of 2-ketoglutarate dehydrogenase complex) is not associated with the carboligase reaction.

2-Ketoglutarate dehydrogenase complex purified from bovine heart (19) also showed KG-aldose carboligase activity (data not shown).

From these results, it was concluded that KGDH is responsible for the formation of 2,3-dideoxy-hex-4-ulosonic acid (and maybe 2,3-dideoxy-hept-4-ulosonic acid) by the condensation reaction between KG and glycolaldehyde (or D- or L-glyceraldehyde).

Reaction mechanism of the enzyme

The results described above, together with the results reported by Schlossberg et al. (17, 18) and our previous reports on 1-deoxy-ketose formations (20, 21), show that the enzyme reactions synthesizing compounds 1 and

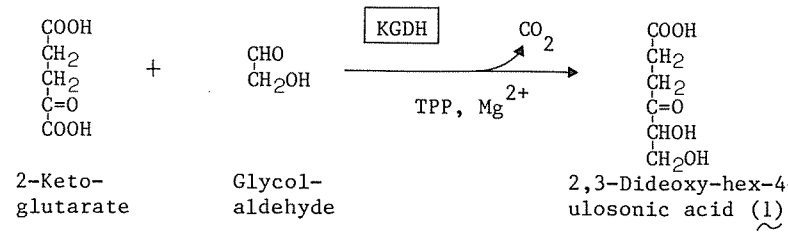


Fig. 5. Product of KG-glycolaldehyde carboligase reaction.

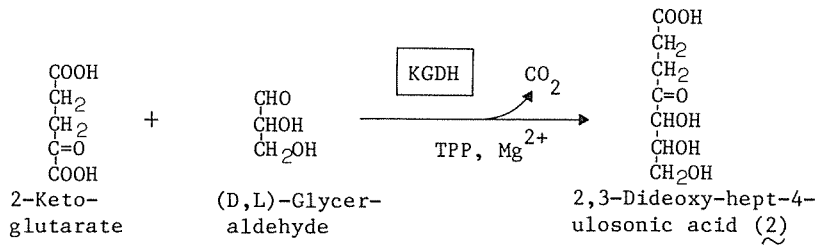


Fig. 6. Postulated product of KG-glyceraldehyde carboligase reaction.

2 by combinations of KG and glycolaldehyde, D- or L-glyceraldehyde might proceed according to the formulae shown in Figs. 5 and 6, respectively.

2-Ketoglutarate dehydrogenase complex is known to catalyze the oxidative decarboxylation of KG to form succinyl-CoA and carbon dioxide (13). It comprises multiple copies of three different enzymes: KGDH, dihydrolipoamide succinyltransferase and lipoamide dehydrogenase (EC 1.8.1.4). KGDH catalyzes the initial decarboxylation of KG and the reductative succinylation of lipoate. TPP is an essential cofactor for this process. The mechanism of the reaction involves the formation of an intermediate hydroxybutyryl-TPP which reacts further with lipoate covalently bound to dihydrolipoamide succinyltransferase and regenerate TPP (13).

The apoenzyme preparation, obtained by gel filtration as described in Materials and Methods, required Mg^{2+} and TPP for the carboligase reaction (Table 4). Therefore, hydroxybutyryl-TPP might participate as an intermediate in 2,3-dideoxy-4-ulosonic acid formation by KGDH. The mechanism of the carboligase reaction is presumed to be as shown in Fig. 7; TPP combines with KG and then produces carbon dioxide and hydroxybutyryl-TPP. The intermediate would then combine with aldoses to form 2,3-dideoxy-4-ulosonic acids.

Table 4. Cofactors required for KG-glycolaldehyde carboligase reaction by apoenzyme preparation of Bacillus subtilis.

Component ommited	2,3-Dideoxy-hex-4-ulosonic acid formed (mg/ml)
Complete [a]	2.45
minus TPP	0.70
minus $MgCl_2$	0.77
minus enzyme	0.00

[a] A complete reaction mixture contained 20 µmoles of Tris-HCl buffer (pH 8.0), 2.5 µmoles of $MgCl_2$, 0.25 µmoles of TPP, 1 µmoles of EDTA, 50 µmoles each of KG and glycolaldehyde, and 0.2 ml of apoenzyme solution (0.7 mg of protein) in a total volume of 0.5 ml, and was incubated at 37 C for 16 hr. The reaction was stopped by adding 2 volumes of ethanol. After centrifugation, the supernatant was subjected to HPLC. Method for HPLC analysis is described in Materials and Methods.

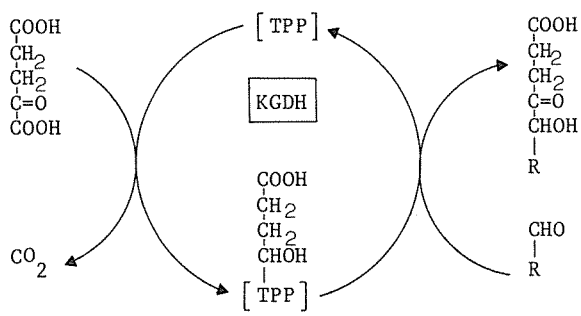


Fig 7. Possible mechanism for carboligase reaction by KGDH.

That a cell-free extract of E. coli and purified 2-ketoglutarate dehydrogenase complex of bovine heart catalyze the KG-aldose carboligase reaction indicates that KGDH of various organisms has catalytic activity. It is a future problem to study the physiological role of 2,3-dideoxy-4-ulosonic acids in organisms.

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FILAMENTOUS FUNGI COLLECTED IN THE FAR EASTERN USSR

Tadayoshi Ito and Tatsuo Yokoyama

Summary

A mycological survey was carried out in the Magadan and Upper Kolyma districts of the Far Eastern USSR (East Siberia). Attention was paid mainly to collect soil fungi associated with taiga and tundra vegetation. Some soil samples were also collected in Moscow and Leningrad in the European USSR.

Using four isolation methods, e.g., incubation at 42 C, treatment with 50% ethanol, treatment at 70 C and dilution plate, respectively, a total of 136 species with 11 varieties in 65 genera were isolated and identified.

The most predominant fungi in the East Siberian districts were Chrysosporium pannorum, Tolypocladium niveum and Pseudogymnoascus roseus. They are recognized as typical arctic fungi which can grow at 8 C. Mortierella alpina, M. isabellina, M. ramanniana, Oidiodendron griseum and Toluromyces lagena, which have been regarded as typical and dominant soil inhabitants in conifer forests in the temperate to cold temperate zones, are also dominant in these districts.

Seven noteworthy species are briefly described.

From August 11 through 29, 1979, Yokoyama attended the XIVth Pacific Science Congress which was held at Khabarovsk, USSR. Before the Congress special permission was given to him, together with a few scientists from the botanical and geological fields, to enter the Magadan and Upper Kolyma districts in the Far Eastern USSR (East Siberia). In fact he was invited to join a Pre-Congress Scientific Visit to these areas planned under the sponsorship of the USSR Academy of Sciences. Several staff from the botany and pedology laboratories of the Institute of Biological Problems of the North, Magadan, kindly arranged the tour and supported his mycological survey in the field. He collected soil, litter, peat and some mushroom samples both in the lowland forests and in the mountain areas in and around Magadan, Ola village, Snow Valley, Igandzha pass, Ust-Omchug, Ice River, the Aborigen Ecology Station and so on (Fig. 1). Most of these collection sites are located in the Upper Kolyma district (60-65°N, 148-152°E) and the northernmost is close to the arctic circle.

In addition, some soil samples were collected in Khabarovsk, Moscow and Leningrad. All of these, however, were either agricultural or city soil.

The microflora of soil fungi have been extensively investigated in many countries, including the developing countries. Although many works on the soil microfungal flora in Northern, Central and Far Eastern USSR have been published (6-8,13,18,19,22-25,34-37), a regular survey of soil fungal flora in the Far Eastern USSR was only started in 1965 (8). Therefore, much information is needed before we can evaluate the distribution and flora of the soil microfungi, especially of the tundra layer (permafrost zone) and the taiga (subarctic or cold temperate forest zone) in the Far Eastern USSR.

The purpose of this paper is to contribute information on the distribution and microflora of filamentous fungi, mainly in the Far Eastern USSR.

Materials and Methods

One hundred and sixty-seven samples were collected, mostly from undisturbed forest sites and mountain areas in the USSR (Table 1).

The samples were obtained from the subsurface after removing 1-2 cm of surface soil. In several collection sites, soil samples from around 10-40 cm were obtained. Each sample was stored in a sterile polyethylene bag. Fungi were isolated in our laboratory about one month later.

Fig. 1. Map of the Union of Soviet Socialist Republics showing where samples were collected.

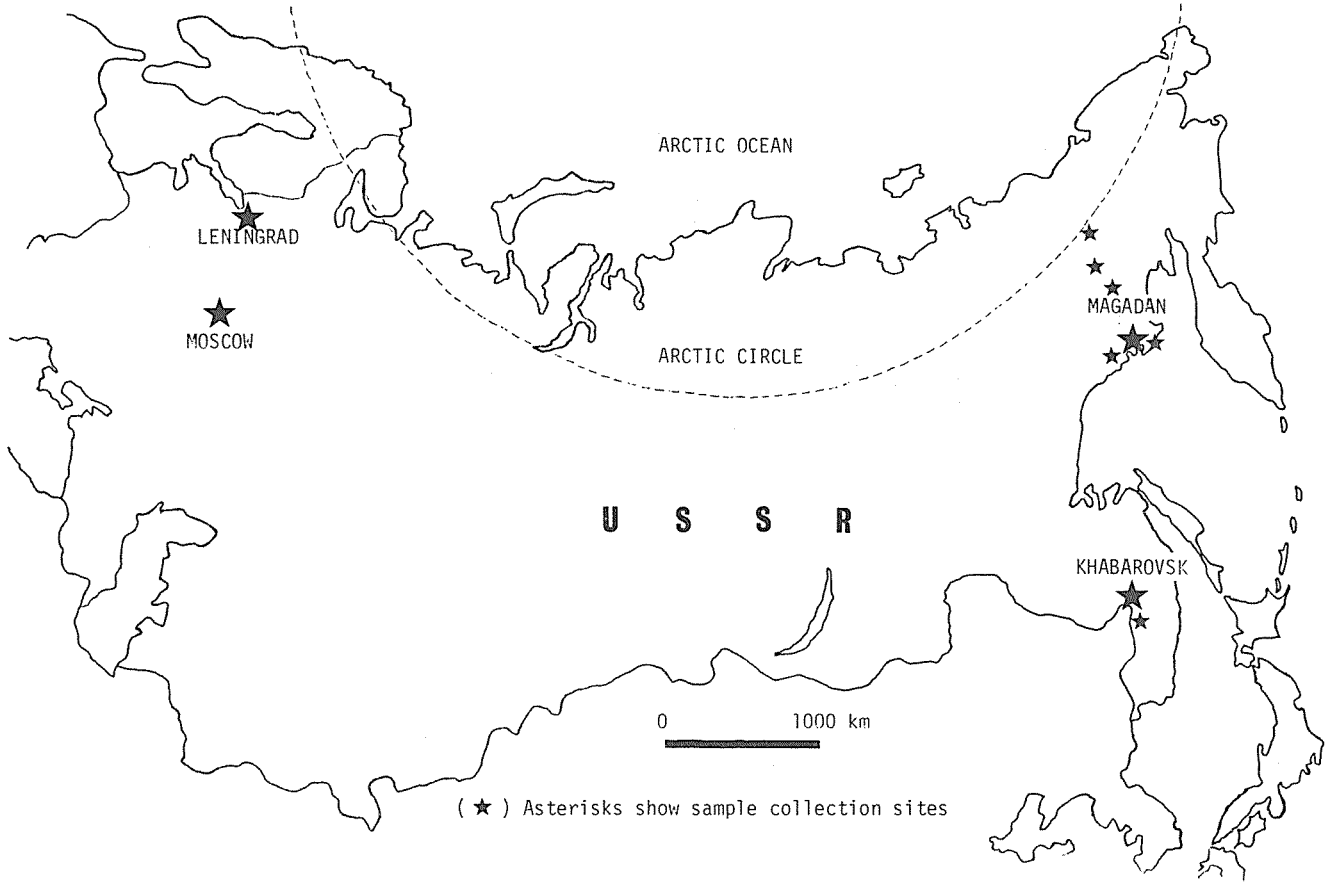

Table 1. Samples and isolates collected in the USSR.

Sample No.	Date sampled	Samples	Locality	Predominant vegetation	Remarks	Species numbers *
R 1453	Aug. 11, '79	Soil	Khabarovsk	Planted tree	road side, Lenin stadium	(7) 5,7,18,48,65, 89,125
R 1454	"	"	"	"	"	(10) 5,18,23,58,64, 76,93,100,102,119
R 1455	"	"	"	"	"	(12) 7,23,52,58,64,68, 72,79,80,94,95,126
R 1481	Aug. 12, '79	Fine sandy humus	Ola River, Ola village, west, Magadan		Riverbed	(9) 16,49,65,79,109, 116,123,124,130
R 1482	"	Sandy clay	"		"	(7) 12,33,49,65,79,97,128
R 1483	"	Fine soil	"		"	(7) 12,21,49,65,75,81,130
R 1484	"	Fine sandy clay	"		"	(6) 12,49,75,106,116,123
R 1485	"	"	"		"	(10) 3,12,49,75,79,81, 97,100,130,135
R 1486	"	Pebble	"		"	(4) 7,18,79,116
R 1487	"	Soil	"		"	(5) 16,39,123,124,130
R 1456	Aug. 13, '79	Soil	2 km north of Magadan	Larix dahurica		
R 1457	"	"	"	"		(1) 7
R 1458	"	"	"	"		(11) 7,9,14,76,97,108, 110,124,125,126,133
R 1459	"	"	"	Salix		(8) 6,7,18,113,117, 119,125,134
R 1460	"	"	"	"		(5) 7,49,109,126,133
R 1461	"	Moss box	"			(7) 7,9,13,18,39,97,133
R 1462	"	Soil	Igandzha pass	Pinus pumilus	ca 1,000 m elevation	(4) 7,49,75,97
R 1463	"	"	"	"	"	(8) 16,49,65,97,110, 112,116,125
R 1464	"	"	"	"	"	(4) 16,49,75,97
R 1465	"	"	"	Moss		(6) 7,9,12,49,75,97
R 1466	"	"	"	Lichen		(4) 12,49,97,133

Table 1. (continued)

Sample No.	Date sampled	Samples	Locality	Predominant vegetation	Remarks	Species numbers *
R 1467	Aug. 13, '79	Soil	Igandzha pass	Lichen		(4) 12,16,107,123
R 1468	"	"	"	Moss		(3) 12,75,97
R 1470	"	"	"			(5) 7,79,80,81,82
R 1575	"	"	"	*Sedum* sp.		(2) 16,18
R 1471	"	"	Ust-Omchug		Swamp near gold mine	(6) 16,79,100,108, 128,133
R 1472	"	"	"		"	(3) 6,80,133
R 1473	"	"	"		"	(6) 18,28,48,49,99,127
R 1474	"	"	"		Detrin River	(7) 18,31,38,72,79,88,116
R 1475	"	"	"		"	(6) 7,18,79,89,116,133
R 1476	"	"	"	Moss	"	(3) 7,18,79
R 1477	"	"	"	*Salix*	"	(2) 18,79
R 1478	"	Humus	"			(8) 7,9,14,39,46,113,128, 130
R 1479	"	Humus clay	"			(6) 9,66,79,108,124,130
R 1480	"	Soil	"	*Hordeum beckmania*		(9) 7,18,48,72,76,79,81, 116,130
R 1488	Aug. 14, '79	Peat	"			(12)8,13,16,18,28,48,79, 98,105,124,130,133
R 1489	"	"	"			(2) 13,125
R 1490	"	"	"			(6) 9,13,96,97,113,130
R 1491	"	Clay	"	Grass		(3) 48,79,130
R 1492	"	"	"	"		(8) 9,12,16,97,107,123, 124,130
R 1493	"	"	"			
R 1494	"	Sandy soil	"		Riverbed of Ice River	
R 1495	"	Humus sandy soil	"	*Salix* & *Astragalus*		(1) 130
R 1496	"	Humus soil	"			(8) 8,15,18,33,89,113, 123,124
R 1497	"	Sandy soil	"		30 cm depth	(2) 7,79
R 1498	"	Peat	"			(1) 79

Table 1. (continued)

Sample No.	Date sampled	Samples	Locality	Predominant vegetation	Remarks	Species numbers *
R 1499	Aug. 14, '79	Soil	Ust-Omchug	Cabbage	Crop field	(12)3,21,48,50,51,60,72, 79,101,104,134,136
R 1500	"	"	"	Turnip	"	(15)18,20,21,35,41,48,50, 60,63,72,78,79,83, 101,118
R 1501	"	"	"		Green house side	(11)35,45,48,60,63,72,79, 101,118,123,125
R 1502	"	Sandy soil	Ice River near Ust-Omchug		Riverbed	(2) 49,123
R 1503	"	Peaty soil	"	Salix	"	(2) 18,123
R 1504	"	Sandy soil	"	Moss	"	(3) 24,49,123
R 1505	"	Peaty soil	"	Larix dahurica	"	(4) 12,24,113,123
R 1506	"	Peat	"		"	(5) 35,48,72,92,118
R 1507	"	Sediments	"		Glacier	
R 1508	"	"	"		"	(1) 18
R 1509	"		"		"	(1) 18
R 1510	"	Semi-frozen soil	"	Larix dahurica	Permafrost	
R 1511	"	"	"	"	"	
R 1512	"	"	"	"	"	
R 1513	Aug. 15, '79	Soil	Aborigen	Lichen		(2) 18,130
R 1515	"	Litter	"	Larix dahurica		(5) 9,12,18,97,132
R 1516	"	"	"	Pinus pumilus		(3) 18,97,130
R 1517	"	"	"	"		(5) 9,12,18,100,112
R 1518	"	Humus	"			(2) 12,33
R 1519	"	Soil	"	Fern		(2) 11,97
R 1521	"	Humus soil	"			(1) 39
R 1522	"	Clay	"			(4) 7,77,80,119
R 1523	"	Frozen soil	"		Permafrost	(2) 16,49
R 1524	"	"	"		"	
R 1525	"	Humus	"			(2) 49,96
R 1526	"	Pinus seeds	"			(4) 9,12,18,133

Table 1. (continued)

Sample No.	Date sampled	Samples	Locality	Predominant vegetation	Remarks	Species numbers *
R 1527	Aug. 15, '79	Soil	Aborigen	Lichen		(6) 15,16,26,49,96,124
R 1528	"	Clay soil	"			
R 1529	"	"	"			(5) 7,10,15,33,124
R 1530	"	"	"			(6) 12,15,33,49,100,124
R 1531	"	"	"			(4) 15,18,33,123
R 1532	"	"	"	Pinus pumilus		
R 1533	"	"	"			(4) 14,16,28,123
R 1534	"	"	"			(3) 15,28,124
R 1535	"	"	"	Lichen		(3) 16,113,124
R 1536	"	Sandy clay	"		Swamp	(4) 16,96,123,130
R 1537	"	Clay soil	"		"	(2) 123,127
R 1538	"	Peaty humus	"		"	(7) 14,16,28,34,41,49,97
R 1539	"	Peat	"	Larix dahurica		(1) 46
R 1540	"	"	"			
R 1541	"	Dung	"		Snow hare	(2) 16,18
R 1542	"	"	"		Reindeer	(4) 9,13,28,80
R 1543	"	Humus soil	"			(4) 12,97,108,124
R 1544	"	"	"			(4) 12,14,16,130
R 1545	"	Clay	"			
R 1546	"	"	"			(1) 49
R 1547	"	Rocky soil	"			(3) 14,96,123
R 1548	"	Humus soil	"			(4) 13,14,15,25
R 1550	"	Soil	"	Moss & Grass		(1) 18
R 1572	"	"	"			(2) 13,16
R 1573	"	"	"			(1) 13
R 1574	"	Litter	"	Pinus pumilus		(5) 9,12,96,97,124
R 1551	Aug. 16, '79	Soil	"			(2) 12,64
R 1552	"	"	"			(3) 7,49,91
R 1553	"	Humus soil	"			(1) 49
R 1554	"	Clay soil	"	Moss		(1) 49
R 1555	"	Humus sandy soil	"	Lichen		(5) 13,14,18,49,113
R 1556	"	Humus	"			(3) 14,39,123

Table 1. (continued)

Sample No.	Date sampled	Samples	Locality	Predominant vegetation	Remarks	Species numbers *
R 1557	Aug. 16, '79	Humus soil	Aborigen			(4) 7,18,39,49
R 1558	"	Rocky soil	"			
R 1559	"	Soil	"	Carex sp.	above 16 cm depth	(3) 14,18,49
R 1560	"	"	"	"	under 16 cm depth	(1) 128
R 1561	"	"	"		10 cm depth	
R 1562	"	"	"		30 cm depth	
R 1563	"	"	"		40 cm depth	
R 1564	"	Peaty soil	"			(1) 9
R 1565	"	"	"	Lichen		
R 1566	"	Sandy soil	"			(2) 18,123
R 1567	"	Sandy clay	"		Swamp	
R 1568	"	Sandy soil	"	Lichen		(3) 13,49,123
R 1569	"	"	"			(4) 12,13,100,123
R 1570	"	"	near Aborigen		Polygon	
R 1571	"	"	"		"	(2) 16,18
R 1576	Aug. 18, '79	Litter	Magadan	Pinus pumilus	Snow valley	(4) 7,9,18,133
R 1577	"	Humus soil	"		"	(3) 9,79,97
R 1578	"	Rotten wood	"		"	(1) 9
R 1579	"	Humus	"		"	
R 1580	"	Soil	"		"	
R 1581	"	"	"		"	(2) 97,123
R 1582	"	"	"		"	(1) 97
R 1583	"	"	"		"	
R 1584	"	Humus soil	"		"	(4) 16,49,65,130
R 1585	"	"	"		"	(6) 2,9,18,113,124,130
R 1586	"	Swamp soil	"			(3) 48,79,116
R 1587	"	Rotten mushroom	"			(2) 9,130
R 1588	"	Humus	"			(3) 18,128,130
R 1589	"	"	"	Larix dahurica		(1) 130
R 1590	"	Soil & Larix rootlets	"	"		(4) 9,13,125,130
R 1591	"	Swamp soil	"			(6) 21,48,105,116,123,129

Table 1. (continued)

Sample No.	Date sampled	Samples	Locality	Predominant vegetation	Remarks	Species numbers *
R 1592	Aug. 18, '79	Humus	Magadan		Swamp	(4) 2,107,129,130
R 1593	"	Humus soil	"			(4) 9,97,113,130
R 1594	"	"	"			(4) 18,123,128,130
R 1595	"	"	"			(9) 9,12,18,39,46,62,97, 113,130
R 1596	"	Swamp soil	"			(2) 48,128
R 1597	"	Humus	"			(3) 9,130,133
R 1598	"	Litter	"	Pinus pumilus		(5) 9,13,18,79,113
R 1599	"	"	"	Larix dahurica		(5) 9,13,18,81,113
R 1601	Aug. 22, '79	Soil	Moscow			(12)4,7,18,43,44,57,68, 72,79,80,102,120
R 1602	"	"	"	Begonia		(23)15,18,22,30,32,40,42, 48,52,54,57,58,60,63, 72,79,80,87,90,112, 120,125,90
R 1603	"	"	"	Paeonia		(26)18,27,30,32,34,35,36, 37,43,47,48,51,52,53, 55,57,58,59,60,63,72, 79,81,83,86,130
R 1604	"	"	"			(12)1,4,7,15,18,49,79,81, 89,109,110,116
R 1605	Aug. 24, '79	"	Leningrad			(15)7,18,37,47,48,51,52, 54,58,60,72,80,91, 98,107
R 1606	"	"	"			(18)7,16,19,20,27,47,48, 51,54,63,72,79,83,86, 98,125,131,136
R 1607	"	"	"			(15)7,15,20,50,51,54,58, 63,70,72,79,80,81,84, 96
R 1608	"	"	"			(11)7,15,18,54,58,60,63, 72,79,80,81

Table 1. (continued)

Sample No.	Date sampled	Samples	Locality	Predominant vegetation	Remarks	Species numbers *
R 1609	Aug. 24, '79	Soil	Leningrad			(13) 18,31,32,47,48,51,56, 58,63,79,80,102,118
R 1610	Aug. 27, '79	"	Khabarovsk	Larix dahurica	Dendropark	(4) 7,15,97,124
R 1623	"	Litter	"	"	"	(10) 35,58,63,67,71,72,73, 80,81,114
R 1624	"	"	"	Picea	"	(3) 9,12,97
R 1611	Aug. 29, '79	"	near Khabarovsk	Prunus	Bolshe-Hechcirskij Forest	(9) 30,73,74,75,80,81,85, 111,129
R 1612	"	"	"	Betula	"	(6) 18,46,69,73,80,111
R 1613	"	Humus	"		"	(1) 123
R 1614	"	Soil	"		"	(1) 97
R 1615	"	Humus soil	"		"	(11) 4,12,15,16,24,28,33, 49,97,113,128
R 1616	"	Litter	"		"	(8) 9,29,46,61,72,80,111, 121
R 1617	"	Humus	"		"	(12) 1,9,21,59,72,81,114, 115,124,126,128,132
R 1618	"	Litter	"	Pinus	"	(9) 1,9,13,17,18,72,80, 122,128
R 1619	"	Humus soil	"		"	(6) 24,33,76,100,113,128
R 1620	"	Litter	"		"	(7) 1,7,12,18,33,79,132
R 1621	"	"	"	Pinus	"	(9) 1,7,46,52,80,81,113, 128,133
R 1622	"	Humus	"		"	(14) 1,4,7,9,15,46,49,64, 79,99,103,110,113,123

* These numbers refer to the isolated species number shown in table 2.
In parentheses are the total number of species.

In order to obtain more detailed information on the distribution of filamentous fungi in these areas, four isolation methods were adopted; incubation at 42 C, treatment with 50% ethanol, heat treatment at 70 C and the ordinal dilution plate method.

To isolate thermophilic and thermotolerant fungi, heat incubation at 42 C for three days was used. A sample of approximately 2 g was suspended in 5 ml of sterilized water (original heavy suspension), then 0.2 ml of the suspension was spread with a sterilized L-shaped rod onto each of two plates containing Malt extract-Yeast extract-agar (MYA). For this isolation method only, MYA medium was adjusted to pH 4.5 with lactic acid instead of adding tetracycline.

The ethanol treatment was carried out by adding 2 ml of absolute ethanol to an aliquot of the original suspension. After 15 min, 0.2 ml of the ethanol-treated suspension was spread onto MYA plates.

The heat treatment was performed by heating the original suspension at 70 C for 15 min, then 0.2 ml samples were spread onto the agar plates mentioned above.

The dilution plate method consisted of diluting the original suspension to 1:25 and 1:250. Then 0.1 ml of each diluted suspension was spread onto the two MYA plates.

After inoculation, these plates, excepting those of the heat incubation procedure, were incubated at 24 C for three days and developed colonies were carefully taken off the plates under a dissecting microscope.

The Malt extract-Yeast extract-agar (MYA) described below was used for isolation. All isolates obtained using these procedures were transferred onto malt agar slant (MA). In addition to MYA and MA, various kinds of media used for identification are described below.

MYA medium: glucose, 10 g; peptone, 5 g; malt extract, 3 g; yeast extract, 3 g; agar, 20 g; tetracycline, 50 μg/ml; distilled water, 1000 ml. pH 5.6.

MA medium: malt extract, 10 g; glucose, 10 g; peptone, 1 g; agar, 20 g; distilled water, 1000 ml. pH 6.0.

CMA medium: corn meal agar (Nissui), 17 g; distilled water, 1000 ml. pH 6.0.

PCA medium: potato, 20 g; carrot, 20 g; agar, 20 g; distilled water, 1000 ml. pH 6.0.

PSA medium: potato, 200 g; sucrose, 30 g; agar, 20 g; distilled

water, 1000 ml. pH 5.6.

OA medium: oatmeal, 50 g; agar, 20 g; distilled water, 1000 ml. pH 7.0.

Results and Discussion

Table 1 shows the number of species isolated from each sample. Table 2 lists the species with a species number and a representative strain of each taxon isolated from the 167 samples collected in the USSR.

Judging from the number of colony forming units developed on the isolation plates, which might reflect the number of fungal propagules in each sample, the fungal population in soil, particularly of the natural stands in the Far Eastern USSR, seems very low. Accordingly, the number of fungal species isolated was also limited, and a total of 136 species with 11 varieties in 65 genera were identified and classified into 5 Zygomycotina, 22 Ascomycotina and 38 Deuteromycotina.

Some species of Mortierella which belong to Zygomycotina and are known as very common fungi in forest soil were often isolated. Mortierella alpina, M. isabellina and M. ramanniana var. angulispora were particularly predominant in these samples as has already been shown by Linnemann (20), Kobayasi et al. (14), Huang and Schmitt (10) and Yokoyama et al. (32). Other species of this genus were also isolated, but they were not so common.

Three species of Mucor were isolated; M. circinelloides, M. genevensis, M. hiemalis f. hiemalis and M. hiemalis f. silvaticus. The distribution of M. circinelloides and M. hiemalis in the Far East has also been reported by Zhukovskaya (34), Zhukovskaya and Egorova (36) and Egorova (7).

Twenty-two genera of Ascomycotina were encountered in this experiment. Many of them were obtained by heat incubation, ethanol- and heat-treatment isolation procedures. Six isolates of Diplogelasinospora grovesii, which was originally reported by Udagawa and Horie (31) from soil in Hokkaido, Japan, were isolated. This species is considered to be an inhabitant of cold areas, because we have isolated it frequently from forest soil in Alaska (unpublished). This is apparently the first record of this fungus in the USSR.

Two isolates of Hamigera striata were detected from soil samples from Ust-Omchug and the Aborigen Ecology Station. This species is often reported in soil in the USA, Sweden, the Philippines and Nepal, but is hitherto unpub-

Table 2. List of species and representative strains isolated from samples collected in the USSR.

ZYGOMYCOTINA			
1*	Absidia californica	R-1617-3	
2	A. coerulea	R-1585-3	
3	A. corymbifera	R42-1485-1	
4	A. cylindrospora	R70-1604-1	
5	A. spinosa	R-1453-2	
6	Basidiobolus ranarum	R-1459-8	
7	Mortierella alpina	R-1457-2	
8	M. humilis ?	R-1488-5	
9	M. isabellina	R70-1461-2	
10	M. longicollis	R-1529-4	
11	M. minutissima ?	R-1519-1	
12	M. ramanniana var. angulispora	R-1465-6	
13	M. ramanniana var. ramanniana	R-1461-6	
14	M. verticillata	R-1458-3	
15	M. vinacea	R-1527-3	
16	Mucor circinelloides	R70-1571-1	
17	M. genevensis	R-1618-3	
18	M. hiemalis f. hiemalis	R-1453-3	
19	M. hiemalis f. silvaticus	R-1606-2	
20	Rhizopus oryzae	R-1606-3	
ASCOMYCOTINA			
21	Byssochlamys fulva	RE-1499-2	
22	Chaetomium globosum	R-1602-16	(IFO 31387)
23	C. subspirale	R-1454-11	
24	C. torulosum	R-1505-13	
25	Coniochaeta tetraspora	R-1548-1	
26	Coniochaetidium savoryi	R-1527-1	
27	Dichotomomyces cejpii	RE-1603-7	
28	Diplogelasinospora grovesii	RE-1473-2	
29	Emericella nidulans var. acristata	R42-1616-4	
30	E. nidulans var. nidulans	R42-1502-5	
31	Emericellopsis glabra	R-1474-16	
32	Eupenicillium brefeldianum	RE-1602-5	
33	E. pinetorum	R70-1615-4	
34	E. shearii	R70-1538-2	
35	Eurotium amstelodami	RE-1500-3	(IFO 31389)
36	E. chevalieri	RE-1603-4	
37	E. repens	RE-1603-3	
38	E. rubrum	RE-1474-1	
39	Gelasinospora tetrasperma	RE-1461-1	
40	Hamigera avellanea	RE-1602-6	
41	H. striata	RE-1500-2	
42	Nectria inventa	R-1602-23	
43	Neosartorya fischeri var. fischeri	RE-1601-1	
44	N. fischeri var. glabra	R42-1601-1	
45	Petriella setifera	R-1501-8	
46	Podospora carbonaria	R70-1539-2	
47	Pseudeurotium ovalis	RE-1603-18	

Table 2. (continued)

48	Pseudeurotium zonatum	RE-1453-1	
49	Pseudogymnoascus roseus	R70-1484-2	
50	Talaromyces byssochlamydoides	R42-1499-5	
51	T. emersonii	R42-1499-4	
52	T. flavus var. flavus	R70-1602-1	
53	T. helicus var. helicus	RE-1603-14	
54	T. leycettanus	RE-1606-3	
55	T. luteus	RE-1603-13	
56	T. stipitatus	R70-1609-1	
57	T. trachyspermus	RE-1601-2	
58	T. ucrainicus	R70-1602-2	
59	T. wortmannii	RE-1603-12	
60	Thermoascus aurantiacus	R42-1499-3	
61	Thielavia arenaria	R42-1616-6	
62	Trichophaea abundans	R70-1595-1	
DEUTEROMYCOTINA			
63	Acremonium alabamense	R42-1500-4	
64	A. bacillisporum	R-1454-13	
65	A. butyri	R-1453-7	
66	A. curvulum	R-1479-8	
67	A. fusidioides	R70-1623-5	
68	Acrophialophora fusispora	R42-1455-2	(IFO 31383)
69	Alternaria alternata	R-1612-7	(IFO 31384)
70	Arthrinium phaeospermum	R-1607-10	
71	Aspergillus deflectus	R42-1623-4	
72	A. fumigatus	R42-1455-1	
73	A. niger	R42-1611-3	
74	A. restrictus	R70-1611-2	(IFO 31385)
75	Aureobasidium pullulans	R-1462-8	
76	Beauveria bassiana	R-1454-8	
77	Botrytis cinerea	R-1522-3	(IFO 31386)
78	Chrysosporium merdarium	RE-1500-4	
79	C. pannorum	RE-1500-6	
80	Cladosporium cladosporioides	R-1455-13	
81	C. herbarum	R-1470-9	(IFO 31388)
82	C. staurophorum	R70-1470-1	
83	Doratomyces microsporus	R-1500-9	
84	D. nanus	R-1607-21	
85	Fusarium tricinctum	R-1611-4	
86	Geotrichum candidum	R-16033	
87	Gilmaniella humicola	RE-1602-7	
88	Gliocladium deliquescens	R-1474-13	
89	G. roseum	R-1453-6	(IFO 31390)
90	G. virens	R-1602-2	
91	Gliomastix cerealis	R-1552-10	
92	Harposporium helicoides	R-1506-6	
93	Humicola grisea	R-1454-14	(IFO 31391)
94	Metarhizium anisopliae	R-1455-9	
95	Myrothecium roridum	R-1455-10	

Table 2. (continued)

96	Oidiodendron echinulatum	R-1525-7	
97	O. griseum	R70-1593-2	
98	O. truncatum	R-1488-19	
99	Paecilomyces carneus	R-1473-11	
100	P. farinosus	R-1454-9	
101	P. inflatus	R-1499-4	
102	P. lilacinus	R-1454-6	
103	P. marquandii	R-1622-18	
104	P. persicinus	R70-1499-1	
105	P. variotii	R70-1488-1	
106	Penicillium claviforme	R-1484-18	(IFO 31382)
107	P. frequentans	R70-1592-2	(IFO 31383)
108	P. funiculosum	R-1471-12	
109	P. janthinellum	R-1460-5	
110	P. nigricans	R-1622-14	
111	P. piceum	R42-1611-1	
112	P. purpurogenum var. rubri-sclerotium	R70-1517-1	
113	P. thomii	R-1619-7	(IFO 31384)
114	Periconia byssoides	RE-1623-1	(IFO 31385)
115	Phialocephala humicola	R-1617-11	
116	Phialophora alba	R1463-17	
117	P. fastigiata	R-1459-13	
118	Rhinocladiella mansonii ?	R-1609-15	
119	Scolecobasidium humicola	R-1454-15	
120	Sporotrichum thermophilum	R42-1601-2	
121	Thermomyces lanuginosus	R42-1616-2	
122	Thysanophora penicilloides	R-1618-14	
123	Tolypocladium niveum	R-1481-3	
124	Torulomyces lagena	R70-1585-5	
125	Trichoderma hamatum	R-1489-1	
126	T. harzianum	R-1458-1	
127	T. koningii	R-1473-5	(IFO 31386)
128	T. polysporum	R70-1588-3	
129	T. pseudokoningii	R42-1611-4	
130	T. viride	R-1478-1	(IFO 31386)
131	Trichophyton ajelloi	R-1606-11	
132	Verticillium catenulatum	R-1515-14	
133	V. psalliotae	R-1461-4	
134	Volutina concentrica	R-1459-19	
135	Wardomyces anomalus	R-1485-30	
136	W. inflatus	R-1499-9	

* Ordinal number shows the number of the species isolated in this work.

lished in Japan.

Only one isolate of Petriella setifera with Graphium anamorph was detected in soil from Ust-Omchug. This species is known from soil, horse dung and decayed oak trees in Germany, the USA and Japan.

Six isolates of Talaromyces leycettanus, which is a thermotolerant to thermophilic species, were found in soil samples from Moscow and Leningrad. This species is considered not so common in soil as has already shown by Ito and Yokoyama (12).

One isolate of Thielavia arenaria was also isolated from litter of a broad-leaf tree in Khavarovsk. As reported by Yokoyama and Ito (33), this species is rarely found in soil and is interesting in its geological distribution as compared with the original record from desert soil in Egypt.

Twenty-eight isolates of Pseudogymnoascus roseus were found in soil and humus samples mainly from the Ola River, Igandzha pass, Ust-Omchug and Aborigen. This fungus can grow well at 8 C and is considered one of the predominant fungi of the subarctic and arctic zones. It has been recorded in the soil of pear nursery in the Primorsky Region (36).

Many kinds of species and a number of isolates belonging to Deuteromycotina were obtained and identified in this investigation. Among these, Chrysosporium pannorum was frequently isolated from soil samples. The geological and ecological distribution of this species is well known (2,5,8,14-16,21,26,29,32). Barron (2) also showed that C. pannorum is very common in soils in Canada. Egorova (8) stressed that the fungi of the genus Chrysosporium with the dominant species C. pannorum are representative for tundra soils. This species seems to be the psychrophilic species which shows good growth at 8 C and is common in cold areas.

Only a single isolate of Cladosporium staurophorum was found in a soil sample from the Igandza pass. This may be the first described recording in the Far Eastern USSR. According to the Lists of Cultures, CBS and CMI, this species originated from soil in Colombia, the UK and the Netherlands, but seems not to occur commonly.

Cladosporium herbarum, which has been reported to be a common fungi in the Far Eastern USSR (7,34,36), but has not been reported in Japan, was also found in this survey. However, C. epiphyllum, which has been reported in the USSR (7,34,36), has not been isolated in this work so far.

Harposporium helicoides, known as one of the endozoic parasites of nematodes, was detected from peat from Ice River near Ust-Omchug. This

species has been obtained from soil in the USA, Canada and India, but has not yet been recorded in Japan.

Three species of Oidiodendron were also obtained. O. griseum was particularly dominant in soil from Magadan. This fungus was reported by Yokoyama et al. (32) in forest soil in Alaska. Barron (1) reported that Oidiodendron species have been isolated frequently from peat soil in southern Ontario, Canada, and Tokumasu (27) also reported that these fungi are widely distributed in forest soil in Japan. Egorova (8) reported that the fungi of the genera Oidiodendron and Botrytis are mainly associated with meadow soils and Acremonium and Monocillium with forest ones.

Many isolates of Tolypocladium niveum, which was originally described by Gams (9) from alpine soil in Germany as T. inflatum, were detected in soil from the Far Eastern USSR. This is the first record of this species in USSR. Recently, Bisset (3) noted that this species inhabits the soil of cold regions and the alpine soil. In addition, we confirmed that Beauveria sp., which was repeatedly reported by Kobayasi et al. (14-17) in soil from Alaska, Spitsbergen and Greenland, is conspecific with T. niveum.

Only a single isolate of Wardomyces anomalus was detected, in a clay sample from the Ola river. According to Ellis, this species is known from eggs, meat and soil in North America and Europe. However, it has hitherto not been reported from the Southeast Asian countries or Japan.

Species of Absidia, Neosartorya, Talaromyces, Pseudeurotium, Eurotium, Dichotomomyces, Aspergillus, Penicillium, Paecilomyces, Cladosporium, Trichoderma, Gliocladium, and some groups of Mortierella were frequently isolated from soil samples from Moscow and Leningrad throughout the present experiment. Bukhalo et al. (4) have reported similar results concerning the microflora from paddy field soils in the Ukraine.

A total of 127 species of Penicillium and 32 species of Aspergillus have been reported in soils in the Far East (8). Most of the species of Aspergillus predominated in meadow and cultural soils and A. flavus and A. versicolor were basically found in soil from the north mountain forests and tundra in the Far Eastern USSR (8). On the other hand, species of Penicillium were predominant in the forest soils; P. spinulosum, P. roseopurpureum, P. decumbens and P. implicatum in the hardwood and mixed forest soils in the south of the Far Eastern USSR, P. cyclopium, P. expansum and P. claviforme in coniferous forest soils, P. janthinellum, P. simplicissimum and P. jenseni in meadow soil, and P. lanosum, P. martensii and P. variabile

in tundra soils. Penicillium thomii, P. frequentans, P. nigricans, P. chrysogenum and P. brevi-compactum have been reported to occur more widely in the soils of the Far Eastern USSR (6,8).

Fusarium oxysporum, F. solani and Gliocladium roseum were also found predominantly in agricultural soils. These are associated with the root decay of cultivated plants in the south of the Soviet Far East (8,34,35). However, fungi belonging to these genera were not so common in soil samples from Magadan, Ust-Omchug and the Aborigen Ecology Station.

Though many species of Aspergillus, Fusarium and Penicillium have been described from agricultural and city soil samples in the Soviet Far East, as mentioned above, we found only some species of these fungi which were not so common.

Rhizoctonia solani and Thielaviopsis basicola have been reported to be predominant pathogenic soil fungi on cultivated plants in the Soviet Far East (34, 35). First evidence has also given for the wide distribution of Corynespora cassiicola as a severe soil-borne pathogen on soybean roots (35). These were, however, not found in soils from the forests and tundra of the Soviet Far East in this survey.

Species of Trichoderma have been found predominantly in soil samples from the Far Eastern USSR, as reported by Egorova (7, 8), Zhukovskaya (34), Zhukovskaya and Egorova (36) and Prjusnina et al. (24).

Species of Cladorrhinum, Cylindrocarpon, Cylindrocladium, Fusarium (except one isolate of F. tricinctum), Stachybotrys, and Trichocladium, which are very common in the temperate to tropical zones, were not isolated.

Myrothecium verrucaria and M. roridum are also known as common cosmopolitan fungi, but we did not find the former.

It is concluded that the predominant fungi characteristic of the Far Eastern USSR are two Deuteromycete fungi; Chrysosporium pannorum, and Tolypocladium niveum (T. inflatum) and one Ascomycete fungus Pseudogymnoascus roseus, all of which can grow well at 8 C. These fungi have also been reported from the subarctic and arctic areas such as Alaska, Spitsbergen, Greenland, etc., and seem to be psychlophylic or arctic fungi. On the other hand, Mortierella alpina, M. isabellina and M. ramanniana var. angulispora are also dominant and characteristic of the Far Eastern USSR. These fungi are those which have been recognized as characteristic of coniferous forest soils in the temperate and cold temperate zones.

Seven distributionally noteworthy species will be described below.

DESCRIPTION

Cladosporium staurophorum (Kendrick) M. B. Ellis (Fig. 2. A & B)
More dematiaceous Hyphomycetes. p. 333 (1976).
Syn. Hormodendrum staurophorum Kendrick, Can. J. Bot. 39: 833 (1961).

Colonies on oatmeal agar growing slowly and restrictedly, reaching a diameter of 1-1.5 cm in 2 weeks at 24 C, velvety, dark grey to dark brown; reverse dark green to almost black. Conidiophores straight or flexuous, branched, smooth, pale brown, thick wall, up to 150 µm long, 2-4 µm thick. Ramo-conidia often one septate, smooth, pale brown, 13-16 x 3-4 µm. Conidia fusiform or somewhat ellipsoid, 0-1 septate, solitary or catenate, branched, smooth, pale brown, 9-13 x 2-3.5 µm. Chlamydospores usually formed terminally or intercalarily, two to five-celled, dark brown, 12-15 x 9-12 µm.

Growth is nil in two weeks at 37 C but slow growth at 8 C.

Hab. Soil under Sedum sp., Igandzha pass (ca 1,000 m elevation), north of Magadan, Aug. 13, 1979, T. Yokoyama R70-1470-1.
Note. The fungus has been isolated from decaying fallen needles of Pinus sylvestris and from soil in the UK. It is also known from Colombia and the Netherlands. The fungus is characterized by its cylindrical, smooth conidia. This record seems to be the first in the Far Eastern USSR.

Diplogelasinospora grovesii Udagawa & Horie (Fig. 2. C, D & E)
Journ. Jap. Bot. 47: 298 (1972).

Colonies on potato carrot agar growing rapidly, reaching a diameter of 9 cm in 2 weeks at 24 C; vegetative mycelium floccose to partly immersed, hyaline to olivaceous brown; reverse pale brown at the center and hyaline to pale grey at the margin. Cleistothecia discrete, spherical, non-ostiolate, black, 250-350 µm in diameter, covered with long, brown, septate, 3-4 µm wide hyphal hairs. Peridium thick-walled composed of 5-8 layers of flattened, 5-10 µm broad cells, light brown in the outer layer, hyaline in the inner part; ascogonial initials branched, of coiled hyphae, hyaline. Asci cylindrical, evanescent, hyaline, 140-160 x 14-16 µm. Ascospores ellipsoid to broadly fusiform, pitted, two-celled; one cell dark brown, with an apical germ pore; the other cell hyaline; 22-26 x 12-14 µm. Two types of

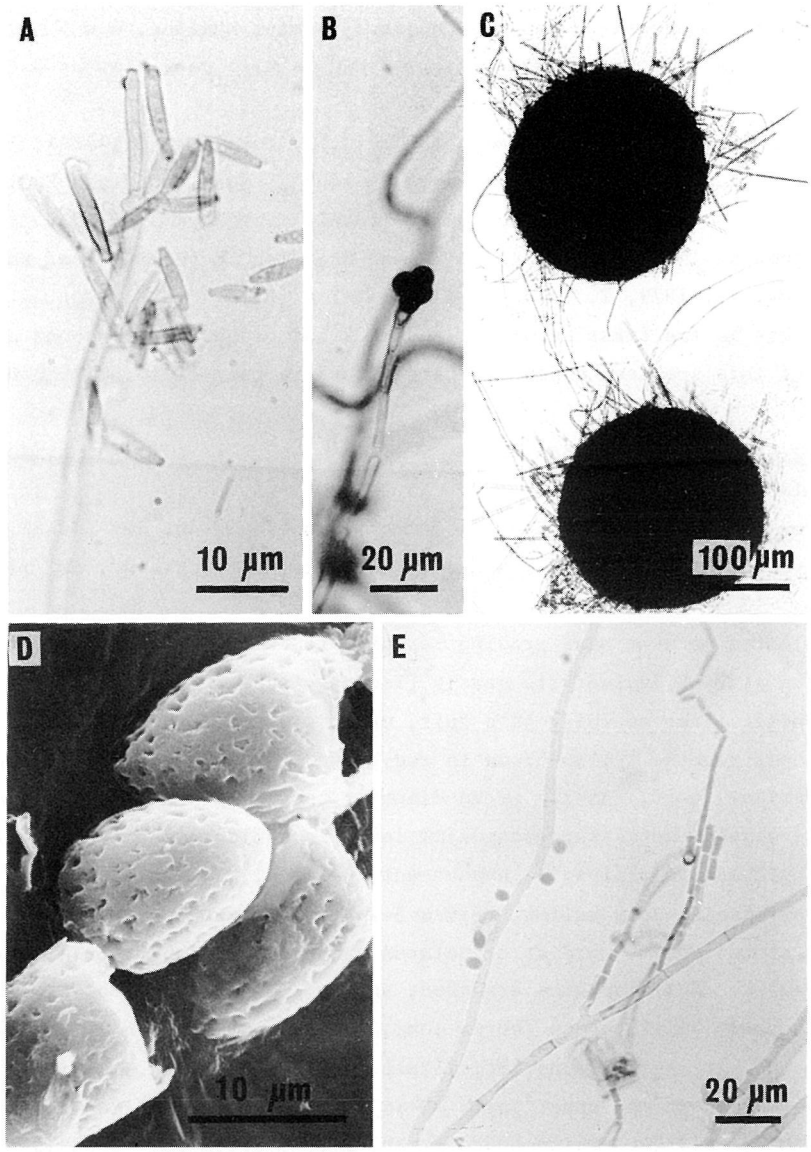

Fig. 2. A & B. Cladosporium staurophorum. A. Conidial structure. B. Chlamidospore. C, D & E. Diplogelasinospora grovesii. C. Cleistothecia. D. Ascospores. E. Anamorphs.

conidia appeared; arthroconidia formed from vegetative hyphae as an intercalary cell, cylindrical, hyaline, 4-15 x 2-4 μm; conidia of "aleuriospore"-type directly produced on hyphae, solitary, ovate, hyaline, 6 x 5 μm.

Growth is slow in two weeks at 37 C and is also very slow at 8 C.

Hab. Swamp soil, Ust-Omchug, Aug. 13, 1979, T. Yokoyama RE-1473-2; Peat, Ust-Omchug, Aug. 14, 1979, T. Yokoyama RE-1488-2; Soil, Aborigen Ecology Station, Aug. 15, 1979, T. Yokoyama RE-1533-2; T. Yokoyama RE-1534-1; T. Yokoyama RE-1538-3; Humus soil, Bolshe-Hechcirskij forest, near Khabarovsk, Aug. 29, 1979, T. Yokoyama R70-1615-2.

Note. This is the first record in the USSR and probably the second described record of this species, which was first found in beet-field soil in Hokkaido.

Hamigera striata Stolk & Samson (Fig. 3. A, B & C)

Persoonia 6: 347 (1971).

Syn. Penicillium striatum Raper & Fennell, Mycologia 40: 521 (1948), Talaromyces striatus (Raper & Fennell) Benjamin, Mycologia 47: 682 (1955).

Colonies on malt agar growing rapidly, reaching a diameter of 8 cm in two weeks at 24 C, velvety to partly floccose, submerged at the margin, at first white, later becoming pale buff, often with sectors; exudate in hyaline drops; reverse yellow-brown to red-brown. Ascocarps produced abundantly, spherical, buff, 135-175 μm in diameter, covering hyphae plenty, consisting of irregular networks; ascogonial initials coiled, septate, hyaline, Asci subglobose to ellipsoid, evanescent, hyaline, 20-25 x 12-15 μm. Ascospores ellipsoid, pale yellow, 8-10 x 5-7 μm, ornamented with 8 to 12 longitudinal striations. Conidial structures produced abundantly on M40Y (Harrold's Agar). Conidiophores branched, smooth, thick-walled, pale yellow green, usually short, up to 100 μm long, 2.5-3 μm wide. Penicilli often complicated, irregular, monoverticillate to biverticillate. Rami present. Metulae swollen at the upper part, 10-14 x 2.5-5 μm. Phialides cylindrical, with a thick-walled conidium bearing tip, 9-11 x 2.5-3 μm. Conidia ovoid to ellipsoid, smooth, hyaline, 4-7 x 2.5-4 μm.

Growth is slow in two weeks at 37 C and very slow at 8 C.

Hab. Turnip field soil, Ust-Omchug, Aug. 14, 1979, T. Yokoyama RE-1500-2; Peaty humus, Aborigen, Aug. 15, 1979, T. Yokoyama RE-1538-1.

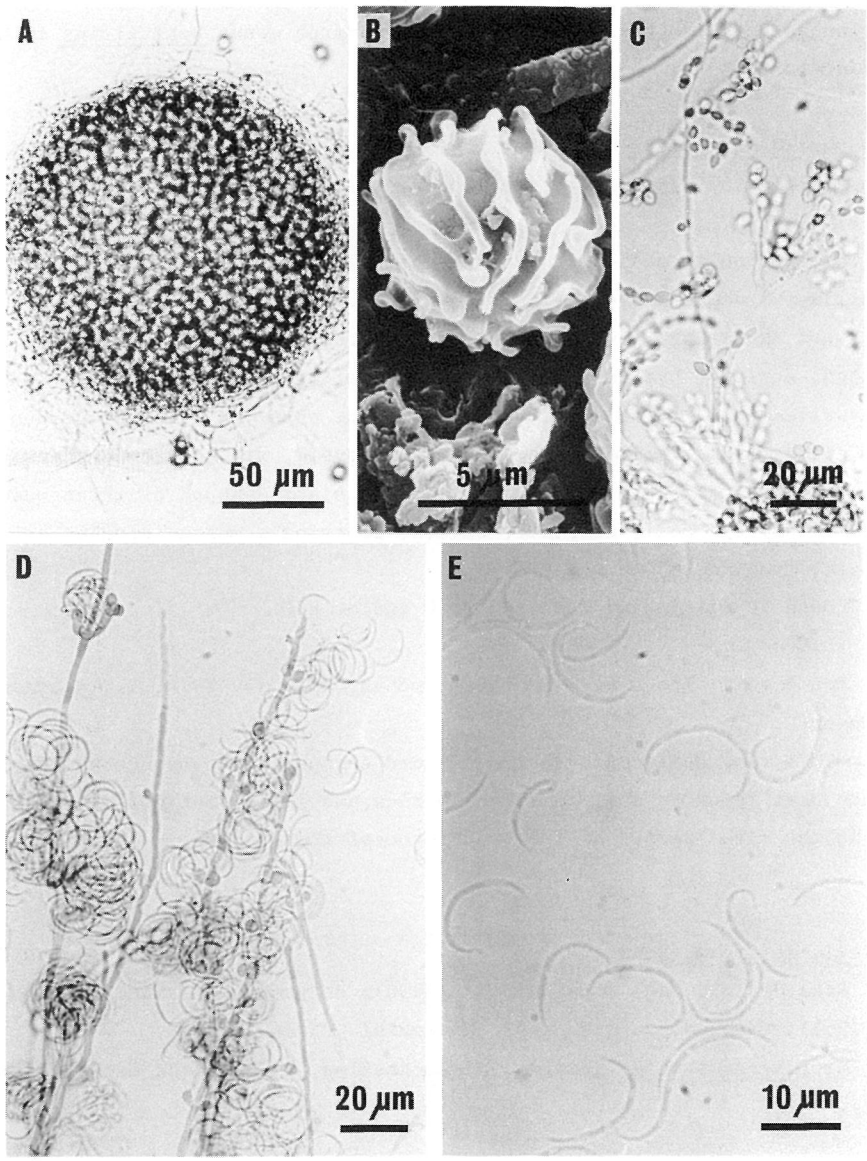

Fig. 3. A, B & C. Hamigera striata. A. Cleistothecium. B. Ascospore. C. Anamorph. D & E. Harposporium helicoides. D. Conidial structures. E. Conidia.

Note. This fungus seems to be a soil inhabitant since it has been found from soil in the USA, Sweden, the Phillipines and Nepal. No record in Japan. This fungus has also been reported under anamorph genus Penicillium in the Far Eastern USSR (6, 36).

Harposporium helicoides Drechsler (Fig. 3. D & E)
Phytopathology 31: 794 (1941); Patil & Pendse, Kavaka 11: 25 (1983).

Colonies on oatmeal agar growing very slow, reaching a diameter of 2.5 cm in two weeks at 24 C, immersed or velvety, partly floccose, pure white; not exudating; reverse grey to pale brown. Vegetative mycelium branched, septate, hyaline, 2-3 µm wide, sometimes produced swollen and rough-walled cell. Distinct conidiophores not observed. Conidiogenous cells solitary and lateral, subglobose to obovoid, with a slender neck, hyaline, 3-10 x 2.5-5 µm. Conidia filiform, sickle-shaped or three quarters of a helical coil, aseptate, smooth, cohering a muscus droplet at the base, hyaline, 25-40 x 1-1.5 µm.

Growth is nil in two weeks at 37 C and at 8 C.

Hab. Peaty soil, Ice River, near Ust-Omchug, Aug. 14, 1979, T. Yokoyama R-1506-6.
Note. This fungus is characterized by its endoparasitic nature to nematodes and has been found in soil in North America and India, but not in Japan. This is the first record of the occurrence of this fungus in the Far Eastern USSR.

Petriella setifera (Schmidt) Curzi (Fig. 4. A, B & C)
Boll. Staz. Patol. veg. Roma 10: 411 (1930); Barron et al. Can. J. Bot. 39: 842 (1961); Udagawa, J. gen. appl. Microbiol. 9: 142 (1963).
Syn. Microascus setifer Schmidt, Diss. Breslau, W. C. Korn, Germany, p. 81 (1912).

Colonies on potato carrot agar moderate growth, reaching a diameter 5 cm in two weeks at 24 C, velvety or partly floccose at the center, hyaline to pale grey; no exudation; reverse hyaline to pale grey. Teleo- and anamorphic state well produced on the surface of agar medium. Perithecia usually pushed out ascospores from a neck, spherical, solitary, superficial or

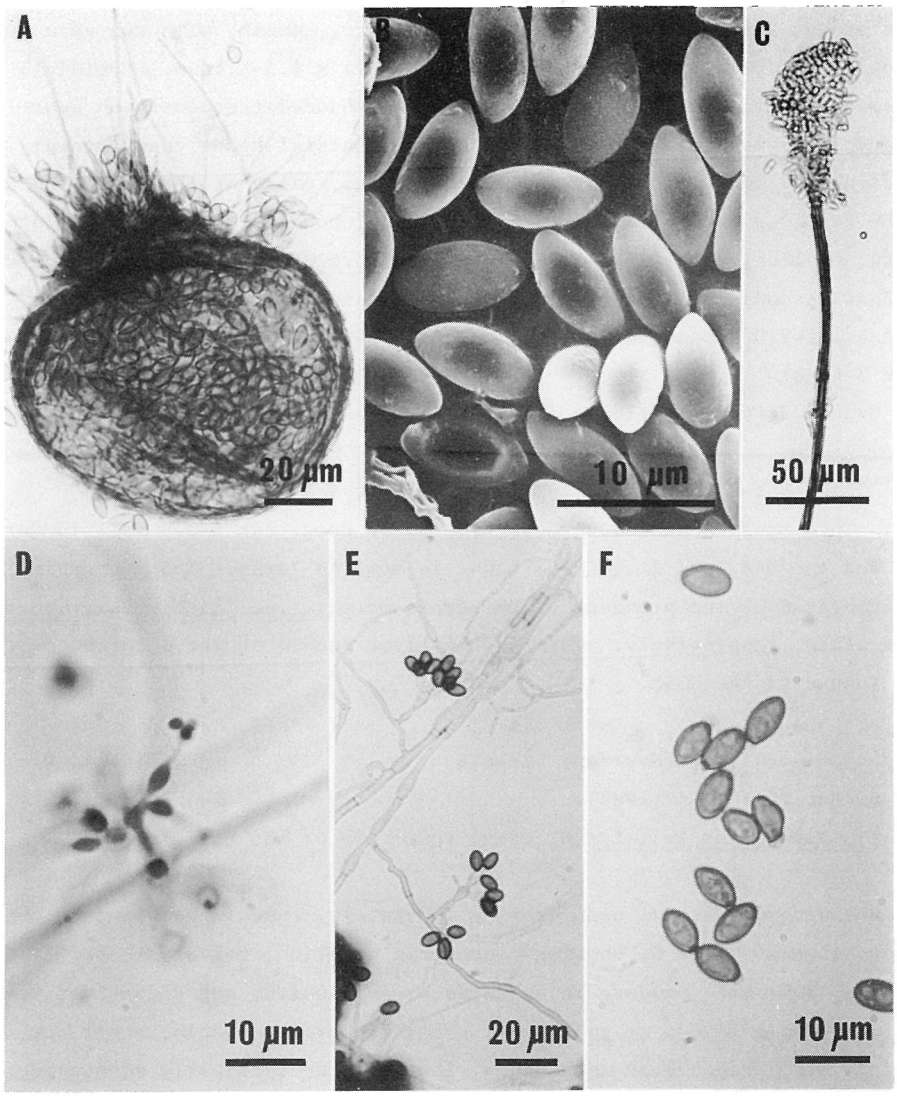

Fig. 4. A, B & C. Petriella setifera. A. Perithecium. B. Ascospores. C. Anamorph. D. Tolypocladium niveum, conidial structure. E & F. Wardomyces anomalus. E. Conidial structures. F. Conidia.

immersed, with a short neck, dark brown, 120-160 μm; Peridium 5-7 layers, 7.5 μm thick, membranaceous, covered with long, straight, olive brown setae of up to 130 μm long and 1.5-2 μm wide. Ascogonial initials coiled, septate, olive brown, 2.5-3 μm thick. Ascospores oblate, smooth, with two germ pores at both ends, pale brown to reddish brown, 9-11 x 4.5-5.5 μm. Conidial structure of two types. The Graphium state produced frequently on agar surface; coremia erect, stalky, with muscus conidial heads, dark brown; conidiophores long, slender, somewhat divergent, paler at the tip, truncate at the base, up to 300 μm long, 2-2.5 μm wide; conidia oblong to pyriform, smooth, hyaline, 6-10 (-14) x 3-4 μm. The Sporothrix state also produced abundantly; conidiophores simple, smooth, straight to flexuous, hyaline, 15-25 x 1.5-2.0 μm; conidia elliptical to pyriform, smooth, hyaline, 5-9 x 3-4 μm.

Growth is very little in two weeks at 37 C but is nil at 8 C.

Hab. Soil, Ust-Omchug, Aug. 14, 1979, T. Yokoyama R-1501-8.
Note. This species has been known from horse dung, oak tree, soil in Germany and the USA, and it is also found in soil in Japan. The fungus is characterized by the presence of the synanamorph; i.e. Graphium and Sporothrix state, respectively. This is the first record of the occurrence of this fungus in the USSR.

Tolypocladium niveum (Rostrup) Bissett (Fig. 4. D)
Can. J. Bot. 61: 1311 (1983).
Syn. Tolypocladium inflatum W. Gams, Persoonia 6: 185 (1971).

Colonies on oatmeal agar growing moderately, reaching a diameter of 5 cm in two weeks at 24 C, showing concentric zonation, velvety to partly floccose, sometimes powdery in appearance, pure white; not exudating; reverse hyaline to pale yellow or pale pink. Conidiophores variable, short, straight, verticillate, branched or unbranched, laterally or terminally on hyphae, 3-5 x 2-3 μm. Phialides oblong to cylindrical or subglobose, single to clustered, constricted at the base, hyaline, 3-5 x 2.5-3 μm, with a filamentous conidium bearing tube 3-4 μm long. Conidia subglobose to ovoid, smooth, hyaline, grouped in mass, 2-2.5 x 1.5-2 μm.

Growth is nil in two weeks at 37 C but slight at 8 C.

Hab. Sandy soil, Ola river, Ola village, west of Magadan, Aug. 12, 1979, T. Yokoyama R-1481-3; T. Yokoyama R-1484-4; T. Yokoyama R-1487-5; Soil under lichen, Igandzha pass (ca 1,000 m elavation), north of Magadan, Aug. 13, 1979, T. Yokoyama R-1467-11; Humus soil, Ust-Omchug, Aug. 14, 1979, T. Yokoyama R-1492-7; T. Yokoyama R-1496-11, T. Yokoyama R-1501-3; T. Yokoyama R-1502-3; T. Yokoyama R-1503-6; Sandy soil under Salix sp., Ice River near Ust-Omchug, Aug. 14, 1979, T. Yokoyama R-1504-4 and peaty soil under moss, T. Yokoyama R-1505-10; Clay soil, Aborigen, Aug. 15, 1979, T. Yokoyama R-1531-6; T. Yokoyama 1533-2; T. Yokoyama R-1536-5; T. Yokoyama R-1537-2; T. Yokoyama R-1547-4; Aug. 16, 1979, T. Yokoyama R-1556-3; T. Yokoyama R-1566-4; T. Yokoyama R-1568-4; T. Yokoyama R-1569-1; Soil, Snow Valley, Magadan, Aug. 18, 1979, T. Yokoyama R-1581-1; Swamp soil, Magadan, Aug. 18, 1979, T. Yokoyama R-1591-2; T. Yokoyama R-1594-7; Humus under moss, Bolshe-Hecheirskij Forest, Khabarovsk, Aug. 29, 1979, T. Yokoyama R-1613-1; T. Yokoyama R-1622-5.

Note. The fungus was originally found from alpine soil in Germany. Isolation data in this work clearly indicate this is one of the commonest soil inhabitants in the Far Eastern USSR. This is the first record in the USSR.

Wardomyces anomalus Brooks & Hansford (Fig. 4. E & F)

Trans. Br. mycol. Soc. 8: 135 (1923); Dickinson, C. H., Trans. Br. mycol. Soc. 47: 321 (1964).

Colonies on oatmeal agar growing slowly, reaching a diameter of 3.5 cm in two weeks at 24 C, velvety to floccose, pale grey to dark grey, hyaline at the margin; exudating hyaline droplets on the colony surface; reverse grey to dark brown. Conidiophores short, straight, branched, septate, hyaline to pale brown, 12-15 x 3-5 μm. Conidiogenous cells clavate to doliiform, smooth, hyaline, 5-8 x 2-4 μm. Conidia ovoid to navicular, smooth, truncate at the base, brown, 6-7.5 x 3.5-4 μm.

Growth is nil in two weeks at 37 C but slight at 8 C.

Hab. Sandy clay, Ola river, Ola village, west of Magadan, Aug. 12, 1979, T. Yokoyama R-1485-30.

Note. This fungus has been reported from eggs, meat and soil in North America and Europe, but hitherto has not been found in Southeast Asia or Japan.

The junior author wishes to thank Dr. Vitautas Kontrimavichus and Dr. Ivan Bogdanov, Director and Scientific Secretary of the Institute of Biological Problems of the North, Magadan, for their kind invitation to undertake the mycological survey in their area and also invaluable support in collecting the fungal materials.

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IFO Res. Comm. 12
63-69, 1985 (March)

SCANNING ELECTRON MICROSCOPY OF ASCOSPORES FROM VARIOUS STRAINS OF DEBARYOMYCES HANSENII (ZOPF) LODDER ET KREGER-VAN RIJ

ISAO BANNO and KOZABURO MIKATA

Summary

Mature ascospores of 26 various strains of Debaryomyces hansenii were examined by SEM. Ornamentation of the surface structure of 24 strains was pleomorphic and varied from irregular warts to short ridges, or intertwining of thread-like projections. Two strains showed a unique structure consisting of several irregularly curved, wale-like ranges. D. hansenii could be distinguished by these irregular shaped protuberances from D. marama which have a spiral ridge system, and from all other species which have separated, single warts on the ascospore surface.

Ascospore morphology of Endomycetous yeast has been considered highly useful as a criterion and diagnostic key to distinguish genera and species (7). The species of genus Debaryomyces all have a warty surface when viewed with a light microscope. The ultrastructure of ascospores of the genus was examined with a transmission electron microscope by Kawakami (2), and Kreger-van Rij and Veenhuis (3,4), and has been found to have a wavy pattern.

Kurtzman et al. (5) examined ascospores from species of Debaryomyces

with a scanning electron microscope (SEM). Wart like protuberances were found on the surface of ascospores of D. cantarellii, D. castellii, D. coudertii, D. formicarius, D. phaffii, D. vanriji, and D. yarrowii. A spiral ridge system was detected on the ascospores of D. marama. Ornamentation on those of D. hansenii varied from short to long interconnected ridges or broad based, elongated conical protuberences. They said that strain separation of D. hansenii based on ascospore surface structure was not possible.

In order to ascertain whether such variation of surface fine structures of the ascospores of D. hansenii is found in mature ascospores, and whether these spore morphologies correlate to any known physiologocal property, we reexamined the fine surface structures of spores completely matured by prolonged incubation on a sporulation medium.

Materials and Methods

Organisms. The twenty four strains of Debaryomyces hansenii examined are listed in Table 1. They consist of 11 authentic strains preserved in the Institute for Fermentation, Osaka and 13 strains newly isolated from various sources. The two strains of Torulopsis famata (Harrison) Lodder et Kreger-van Rij, which had been believed to be anamorph of D. hansenii, IFO 0623 (Type culture of T. famata) and IFO 0728 were also examined, as ascospore formation was found in these two strains.

Sporulation. A mass of cells harvested from a colony on YM agar slant incubated for 3 days at 24 C was transferred on to corn-meal agar and incubated for a further 2 to 4 weeks at 17 C in order to obtain completely mature asci and ascospores.

Preparation of ascospores for SEM. After confirmation of sufficient production of ascospores using a light microscope, the ascogenous cells were treated with an enzyme mixture of 2 mg of Zymolyase 5000 and 4 mg of crude enzymes of Trametes sanguinea (1) per 1 ml for 2 hr at 28 C to digest ascus-wall, and washed twice with 0.1 M phosphate buffer (pH 7.2) by centrifugation. The free ascospores were then fixed with 2% glutaraldehyde in the phosphate buffer for 2 hr at 4 C and with 1% osmic acid in the phosphate buffer for 16 hr at 4 C. After washing several times with the phosphate buffer, the fixed samples were dehydrated by passing

Table 1. List of strains examined.

Strain	Original name	Source
IFO 0045 (CBS 787)	Debaryomyces matruchoti	
IFO 0046 (CBS 790)	Debaryomyces grustzii	diseased nail
IFO 0063	Debaryomyces sp. Fm	
IFO 0085		
IFO 0086		
IFO 0087		
IFO 0097 (CBS 117)	Debaryomyces novazeelandicus	rennet
IFO 0564	Debaryomyces tyrocola	cheese
IFO 0623 (CBS 1795)	Torulopsis famata	wound of hand
IFO 0728	Torulopsis famata	black figs
IFO 1428	Debaryomyces nepalensis	soil
IFO 1751		flower
IFO 1752		soil
AM 74		beetle
Di-208u8		litter leaf
Di-209u8		litter leaf
F-Y-1		raw meat
F-Y-2		raw meat
Mm-122a2		soil
My-16a1		fallen leaf
O-25a3		soil
Od-4m3		flower
Shake		salted salmon
Tw-103k1		soil
Xy 12		soil
Xy 43		soil

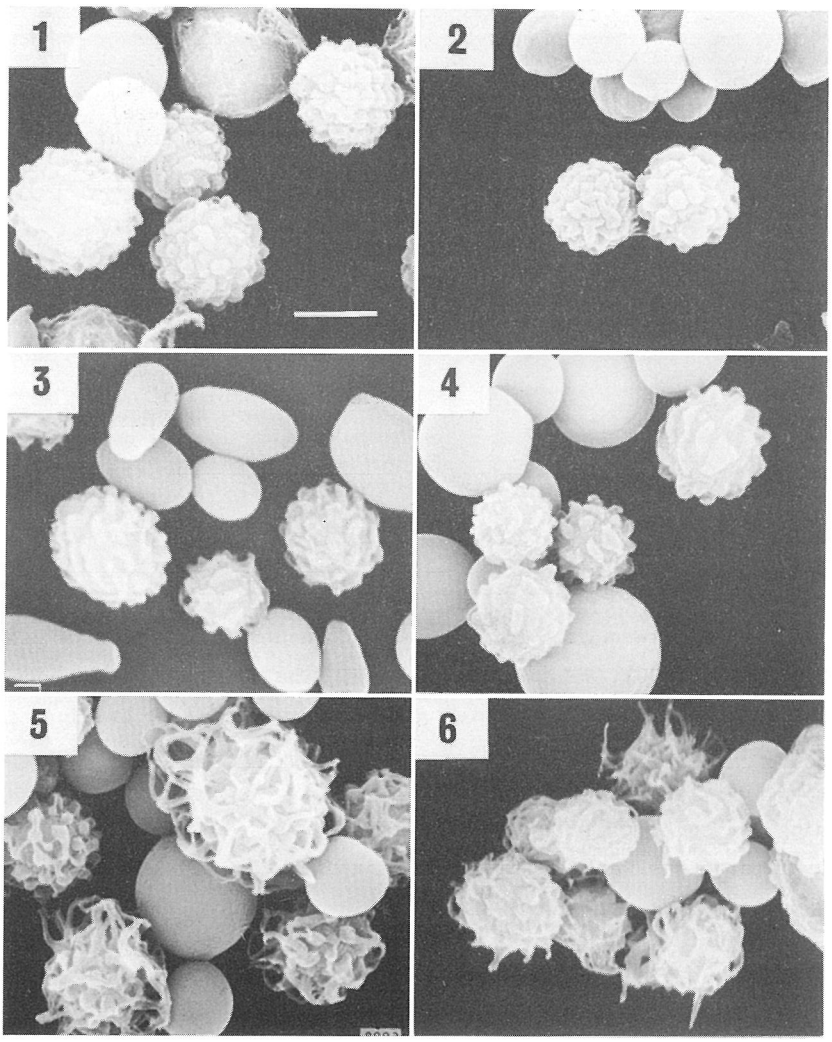
at 15 min intervals through a graded aceton concentration series of 30%, 50%, 60%, 70%, 80%, 90%, 95%, and 100% twice. The spore preparations were mounted on a small disk of cover glass and subjected to critical point drying in a JCDP-3 (JEOL Ltd.). The glass disk with the dried spore sample on it was coated with gold at 1.2 kv, 5 mA for 5 min *in vacuo* using ion sputtering aparatus JFC-1100 (JEOL Ltd.).

The preparations were examined in a scanning electric microscope JSM T-20 (JEOL Ltd.) at a voltage of 20 Kv.

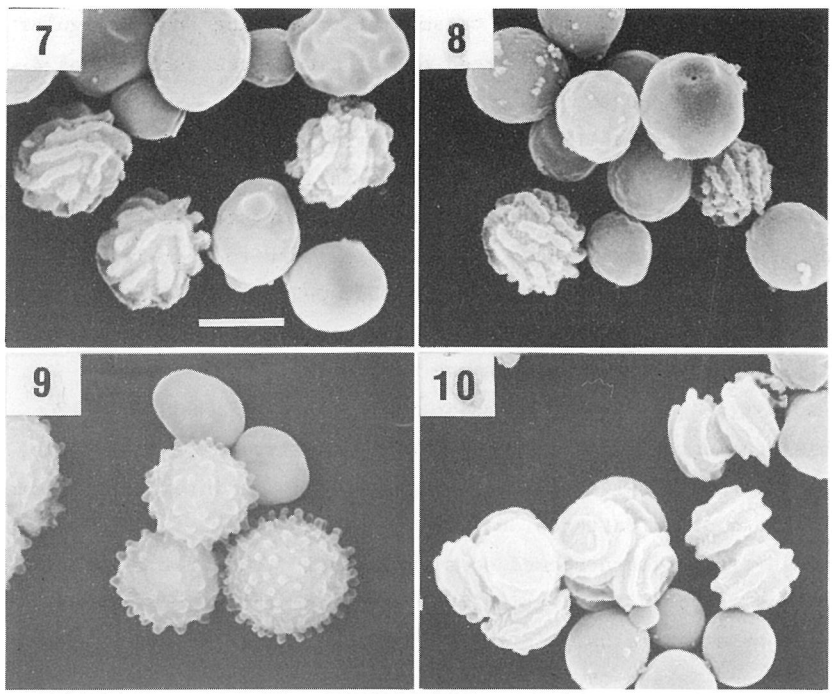
Results and Discussion

Typical examples of four patterns of surface ornamentation of ascospore found among the strains examined are presented in Figs. 1 to 8. The first pattern is represented by ascospores shown in Figs. 1 and 2. The

ascospores were covered with two or three connecting wart-like protuberances. Some protuberances were broad based and conical. This pattern of ornamentation is referred to as irregular wart-type below. For the second pattern, Figs. 3 and 4 show variable short ridges on the surface of ascospores. This will be called short ridge-type. The third pattern



Figs. 1 - 6. Scanning electron microscopies of ascospores of Debaryomyces hansenii.
1, IFO 1428; 2, IFO 0046; 3, strain Mm-122a2;
4, strain Shake; 5, strain F-Y-2; 6, strain Di-208u8.
White bar represents 2 μm.



Figs. 7 & 8. Scanning electron microscopies of ascospores of Debaryomyces hansenii. 7, IFO 0085; 8, IFO 1751.
Fig. 9. Scanning electron microscopy of ascospores of Debaryomyces polymorpha IFO 1352.
Fig. 10. Scanning electron microscopy of ascospores of Debaryomyces marama IFO 1878.
White bar represents 2 μm.

occurred on ascospores shown in Figs. 5 and 6. An intertwining of tapered projections was found on the surface. It seems like a loose hair ball and will be referred to as hair-like-type. Figs. 7 and 8 present the fourth pattern, in which a small number of irregularly curved, large, wale-like ranges were found on the spherical surface. The ascospores with this pattern were shaped like the kernel of a walnut. This fourth type of ornamentation will be called wale-type pattern.

Irregular wart-type, short ridge-type, and hair-like-type patterns have already been described by Kurtzman et al.(5). The wale-type pattern was first observed in the present investigation.

Irregular wart-type ornamentation was observed in ascospores from 6 strains: IFO 0086, IFO 0564, IFO 0728 (formerly T. famata), IFO 1428, Di-209u8, and F-Y-1. The short ridge-type pattern was found in 3 strains;

IFO 0063, IFO 0097, and Mm-122a2. Ornamentation varying from irregular wart-type to short ridge-type occurred on the ascospores surfaces of 4 strains; IFO 0045, IFO 0046, IFO 1752, and Shake.

Ascospores of strains IFO 0623 (formerly T. famata), Di-208u8, and Od-4m3 showed hair-like-type patterns. A variation from wart-type pattern to hair-like-type pattern was found in ascospores of strains IFO 0087 and F-Y-2, and a variation from short ridge-type pattern to hair-like-type pattern in those of 5 strains, AM 74, My-16a1, O-25a3, Tw-103k1, and Xy 12. Mixed patterns of wart-type, short ridge-type, hair-like-type, and intermediate types were observed in one strain, Xy 43.

The wale-type pattern was found in two strains, IFO 0085 and IFO 1751. This ornamentation was quite unique among strains of the species and was exclusively observed in these two strains. An intermediate form, between this and other patterns, was not found.

The wale-type pattern seemed so significantly to differ from the other patterns as to be a criterion for separating these strains from strains with other patterns. In the DNA-DNA reassociation test carried out by Suzuki and Nakase (6, personal communication from Nakase), however, strains of D. hansenii producing the ascospores of the four different patterns gave levels of DNA homology higher than 35% with each other. Consequently, all these strains of variable ascospore shapes should be embraced in a single taxon at the specific level and the strain with wale-type pattern is obviously regarded as a member of the species. The pleomorphism found in mature ascospores of these strains is evidently characteristic of D. hansenii.

These results demonstrate that the range of diversity of spore surface of the species is wider than that indicated by Kurtzman, and a wale-type pattern is included in the diversity of spore shapes.

No correlation was found between variation of the surface ornamentation and particular physiological properties or the sources from which the strains were isolated.

It is noteworthy that protuberances of ascospores of other species of Debaryomyces, except D. marama, are regularly isolated, single wart-shape, as seen in spores of D. polymorpha of Fig. 9 for example (5), and ornamentation of D. marama is a spiral ridge system (5, and Fig. 10), whereas those of D. hansenii are pleomorphic and varied from irregular warts, short ridges to wale-like range, or hair-like intertwining. In this

respect, D. hansenii obviously is distinct from other species of the genus. The finestructure of the spore surface is a useful criterion for to identifying Debaryomyces hansenii, memberes of which are hardly distinguished from the other species by physiological properties because of their diversity in physiological characteristics.

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IFO Res. Comm. 12,
70-77, 1985 (March)

SCANNING ELECTRON MICROSCOPY OF ASCOSPORES OF PICHIA MEMBRANAEFACIENS HANSEN

KOZABURO MIKATA and ISAO BANNO

Summary

Three light microscopical forms of completely matured ascospores produced by strains of Pichia membranaefaciens Hansen were intensively examined with a scanning electron microscope (SEM). Finestructures of the ascospores were divided into two patterns: a semisphere with a remarkable brim at the edge, and an oblate sphere with a fine ledge.

Morphology of ascospores of Pichia membranaefaciens Hansen observed under a light microscope has been found to be diverse. Kreger-van Rij (8) described the spores of the species as round or hemispherical, without a ledge or with a ledge of variable length and width, and Kurtzman (9) also stated that the spores may be spheroidal or hemispheroidal, with or without a ledge, and spores with ledges appear nearly hat-shaped or nearly saturn-shaped.

Kawakami (4,5) examined the spores of the species through a transmission electron microscope (TEM) and found two patterns of spore forms: one possessing a large ledge with a swollen band and the other round or hemispherical without a clear ledge. He regarded the former as helmet shaped perhaps corresponing to Phaff's "helmet" (11) developed using a light

microscope. Kurtzman (9) occasionally found a single strain to produce the whole range of spore shapes. Such a wide range of variation of spore morphology seems to us to be too diverse to include these in one species. In order to ascertain whether strains of the species are further separated according to the fine morphology of the spore, we conducted a reexamination of ascospores with an electron microscope on the strains included in Pichia membranaefaciens Hansen sensu Kurtzman (9). We used a scanning electron microscope (SEM) for this purpose, since an SEM could give full information about the actual, three dimensional finestructure of the spores which could not be perceived from examination with a transmission electron microscope.

Materials and Methods

Organisms. Seventy-five strains of P. membranaefaciens were examined; 29 authentic strains, including 7 former varieties of the species which have been preserved in the Institute for Fermentaion, Osaka; 5 strains obtained from the Faculty of Engineering, Hiroshima University; 28 strains newly isolated from galleries of ambrosia beetles in Japan; 12 strains newly isolated from forest materials in Japan; and one strain isolated from tea-fungus (a local beverage).

Sporulation. A mass of cells harvested from a colony on YM agar incubated for 3 days at 24 C was transferred onto Dalmau plate of corn meal agar and 10% malt-extract agar slant, and incubated for more than one week at 24 C to obtain completely matured ascospores.

Preparation of ascospore for SEM. After confirmation of sufficient production of ascospores under a light microscope, sporogenous cultures were suspended in 0.1 M phosphate buffer (pH 7.2) and washed twice with the same buffer by centrifugation. When the ascospores were not released from the ascus, the suspension was treated with an enzyme mixture of 2 mg of Zymolyase 5000 and 4 mg of crude enzyme of Trametes sanguinea (2) per 1 ml for 2 hr at 28 C to digest ascus-wall and washed twice with 0.1 M phosphate buffer (pH 7.2) by centrifugation. Fixation, dehydration, and Au-coating of spore samples were carried out according to the procedure described in a previous paper (1). The prepared ascospores were examined using Scanning electron microscope JSM T-20(JEOL Ltd.) at a voltage of 20 kv.

Results and Discussion

Light microscopic examination

Before examination with an SEM, we surveyed ascospores produced by 75 strains under a light microscope. No difference in shape of ascospore was found for the two sporulation media, although sporulation was better on malt-extract agar than corn meal agar. The light microscopic shapes of ascospores produced by the strains could be divided into three forms; Form-1 was hemispherical (Fig. 1); Form-2 was hat shaped (Fig. 2); and Form-3 was spherical to angular spherical (Figs. 3 and 4).

Two strains, IFO 0182 and IFO 0189, produced spores of Form-1. There appeared an ultrafine ledge at the margin of the hemisphere in this form, although this was not clear under the light microscope.

Spores of Form-2 were produced by 33 strains. Form-1 and Form-2 probably correspond to Kodama's a-type concept (7). Differentiation between Form-1 and Form-2 would not be possible with a light microscope with low resolving power. Spores of Form-3 were produced by 25 strains. All the Form-3 spores are considered to be actually angular spherical, as an angular sphere appears to be round if looked at from a different angle. Thsis was made clear by SEM.

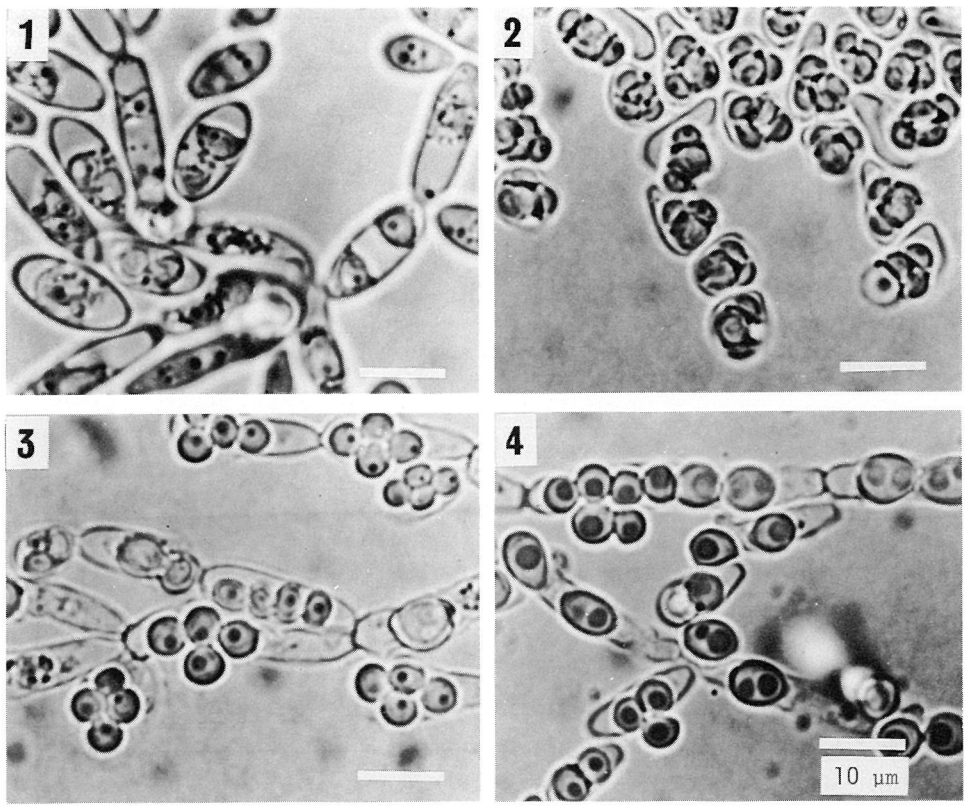
Only one strain, IFO 0188, produced irregular formed ascospores. Sporulation of this strain was very poor in both sporulation media, so that spore isolation and examination with SEM were impossible. The spores are probably abortive.

Lodder (10), and Kodama et al. (6,7) reported that some strains had variable shaped ascospores, from spherical to semispherical. But we found no strain with spores of two or three shapes. The difference between their reports and the present result might depend on spore maturation.

Examination with SEM

One representative strain was chosen from Form-1, one from Form-2, and ten from Form-3, respectively, for SEM examination. Their strain numbers, original names, origins, and light microscopical forms of spores are listed in Table 1. Spores produced on malt-extract agar were subjected to SEM examination at a magnification of 10,000.

Finestructure of the spores of IFO 0182 (Form-1) was hemispherical with a thin brim at the edge (Fig. 5). The surface was wrinkled or looked



Light micrographs of ascospores.
Fig. 1 Hemispherical-shape, IFO 0182;
Fig. 2 Hat-shape, IFO 1788;
Figs. 3 and 4 Spherical- to angular spherical-shapes,
3 = IFO 0461, 4 = IFO 0989.

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as though it were covered with a thin, wrinkled membrane. The large wrinkle near the brim looked like another brim.

The spores of IFO 1788 (Form-2) showed the same fine structure pattern as IFO 0182, being a wrinkled hemisphere with a thin brim (Fig. 6).

Spores of six of the ten strains chosen from Form-3 are shown in Figs. 7 to 12. These spores were hemispherical with narrow and somewhat thick brims, helmet-shaped. The surface was rough with wrinkles. The

Table 1. The strains of Pichia membranaefaciens examined

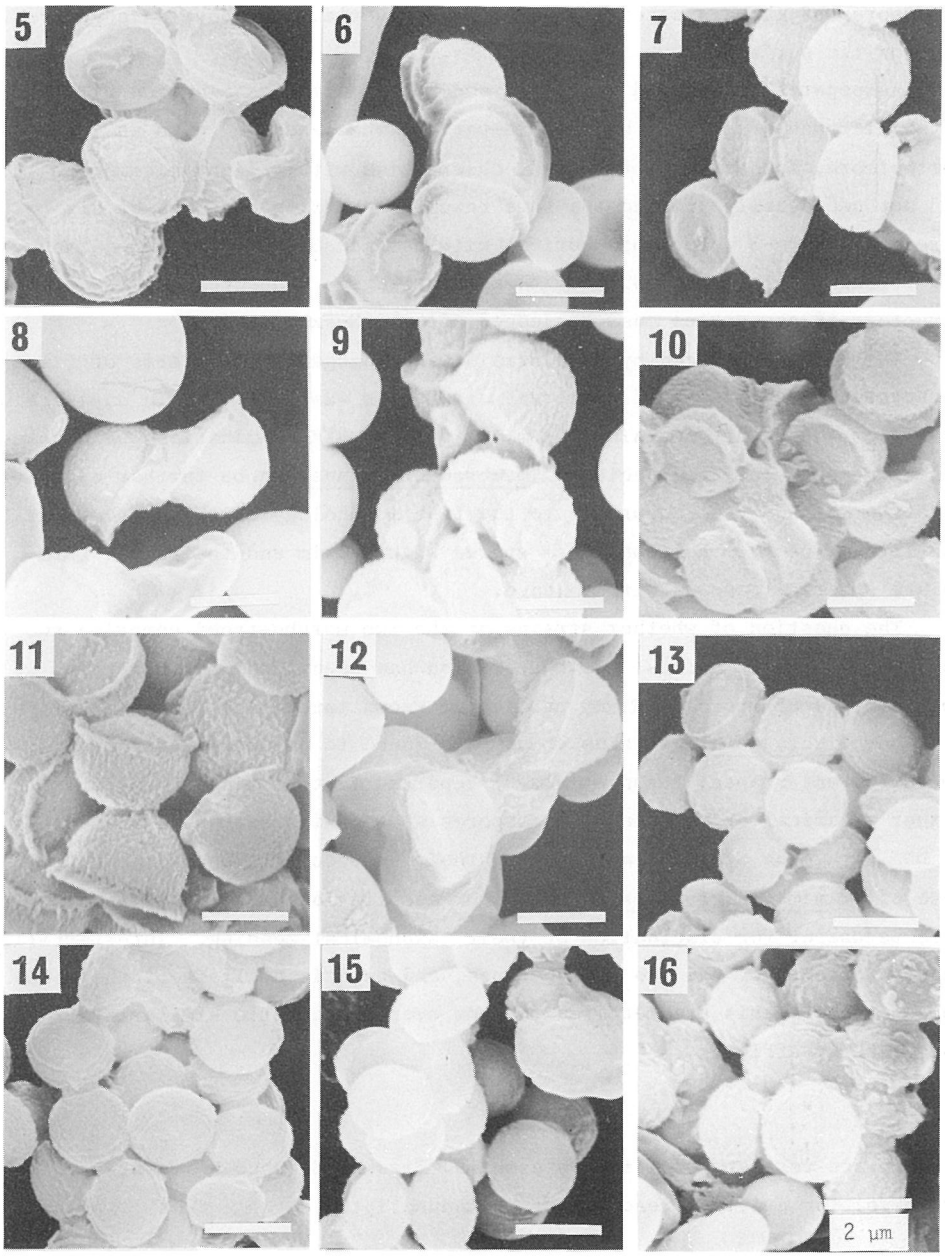
Number of strain	Original name	Origin	Ascospore form under light microscope
IFO 0182		RIB	Form-1
IFO 0183	P. miyazi	FAG	Form-3
IFO 0185	P. hyalospora	RIB	Form-3
IFO 0461	Z. guilliermondii	HUT	Form-3
IFO 0563 (NI 7373)	Z. chevalieri		Form-3
IFO 0864 (RIFY 7714)		RIFY	Form-3
IFO 0989 (Kodama No.34)	P. membranaefaciens var. mandshurica	Kodama	Form-3
IFO 1004 (Kodama No.14)	P. membranaefaciens var. mandshurica	Kodama	Form-3
IFO 1788			Form-2
HUT 7295 (Kodama Y-124)	P. membranaefaciens var. mandshurica	Kodama	Form-3
HUT 7302 (Kodama Y-463)	P. membranaefaciens var. mandshurica	Kodama	Form-3
HUT 7303 (Kodama Y-460)	P. membranaefaciens var. mandshurica	Kodama	Form-3

FAG; Faculty of Agriculture, Gifu University. RIB; National Research Institute of Brewing, Tax Administration Agency, Tokyo. HUT; Faculty of Engineering, Hiroshima University. RIFY; Research Institute of Fermentation, Yamanashi University. Kodama; Laboratory of Kodama Brewing Co., Ltd. Akita.

ascospore-finestructure patterns of IFO 0182 (Form-1), IFO 1788 (Form-2), and these 6 strains are substantially the same with respect to their semi-spherical shape with a brim, although the size of the brim varies. This pattern, semisphere with a brim, corresponds to the spore surrounded by a large ledge with a swallen band reported by Kawakami using TEM (5).

The other four strains of Form-3, IFO 0461, HUT 7295, HUT 7302, and HUT 7303 have a different ascospore finestructure pattern. The spores of all the four strains were oblate spherical with a fine ledge along a parallel, but not equatol and had irregular wrinkles all over the surface (Figs. 13 to 16). The three HUT strains were reported by Kawakami to produce spheroidal or hemispheroidal ascospores without a clear ledge (4). But we have never found spores without any ledge. Careful inspection of the TEM photographs

Explanation of figures on next page.
Scanning electron micrographs of ascospores.
Fig. 5 IFO 0182; Fig. 6 IFO 1788; Fig. 7 IFO 0183;
Fig. 8 IFO 0185; Fig. 9 IFO 0563; Fig. 10 IFO 0864;
Fig. 11 IFO 0989; Fig. 12 IFO 1004; Fig. 13 IFO 0461;
Fig. 14 HUT 7295; Fig. 15 HUT 7302; Fig. 16 HUT 7303.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2 µm

which Kawakami presented as examples of ascospores without a clear ledge (Figs. 6 and 7 of reference 5) shows the cross section of a fine ledge. The spores of these species are considered invariably to have a ledge or brim on the surface.

Ascospores classified as Form-1 and Form-2 after examination with a light microscope showed a hemisphere-pattern when examined with an SEM. Furthermore, for strains of Form-3, observation with a light microscope did not necessarily produce the same results as SEM: ascospores of six strains of Form-3 were hemispherical with a thick brim and those of the other four strains were oblate with a fine ledge. Consequently, the actual structure of ascospores must be judged after SEM-examination.

The spores produced by each strain were homogeneous in terms of fine-structure. Coexistance of different structures was not found in any strain.

From the results obtained from the present SEM-examination, we conclude that the yeasts of the species P. membranaefacience can be further classified into two morpho-types according to the fine morphology of their ascospores: the first type form hemispherical spores with a brim and the second form oblate spherical spores with a ledge.

The question of whether strains of the two morpho-types comprise two independent taxons arises. No correlation has been found between the morpho-types of spores and any particular known taxonomical chracteristics.

For Pichia ohmeri strains which were found to produce ascospores of two different shapes, Fuson et al.(3) reported that strains which form either spherical or hat shaped ascospores showed high sequence relatedness of DNA with each other, demonstrating their conspecific kinship. In the case of P. membranaefaciens, a DNA-DNA reassociation experiment might also be useful for elucidation of their taxonomical meaning. Interfertility between strains of the two morpho-types, and genetic analysis of the ascospore morphogenesis are required to know over all genetic similarity or phylogenetic affinity.

We are very grateful to Professor H. Iizuka, Science University of Tokyo, for critical reading of the manuscript.

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MAINTENANCE OF RECOMBINANT PLASMIDS IN *SACCHAROMYCES CEREVISIAE* AFTER L-DRYING

YOSHINOBU KANEKO, KOZABURO MIKATA and ISAO BANNO

Summary

Four *Saccharomyces cerevisiae* strains carrying recombinant plasmid were preserved by L-drying. The viability of these strains and their maintenance of recombinant plasmids were determined after drying and after accelerated storage at 37 C. The results suggest that L-drying is a useful method for preserving yeast strains carrying recombinant plasmid for a long period.

The advent of recombinant DNA technology enables us to manipulate or engineer microorganisms' genetic material and to create microorganisms which produce desired characteristics. Microorganisms have already been engineered to produce human insulin, interferon, growth hormone, urokinase, somatostatin, etc., industrially. It is very important to preserve such genetically engineered microorganisms. L-drying has successfully been used for long-term preservation of bacterial (1,3,4) and yeast strains (2,6). In this study, L-drying was used to preserve *S. cerevisiae* strains harboring recombinant plasmid and accelerated storage tests were performed.

Materials and Methods

Strains and plasmids. S. cerevisiae strains used in this study were strain NA75-2A/pAL2 (MATa pho8-2 leu2 trp1 his4 can1 [pAL2]), strain NA79-10C/pAL51 (MATα pho8-2 leu2 trp1 his3 can1 [pAL51]), strain NA79-10C/pAL109 (MATα pho8-2 leu2 trp1 his3 can1 [pAL109]) and strain NA87-11A/pAT90 (MATα pho3-1 pho5-1 leu2 trp1 his3 can1 [pAT90]). All strains were obtained from Y. Oshima (Osaka University) and A. Toh-e (Hiroshima University). The recombinant plasmids which these yeast strains carried are shown in Fig.1. These plasmids are 2-μm circle-based chimeric plasmids.

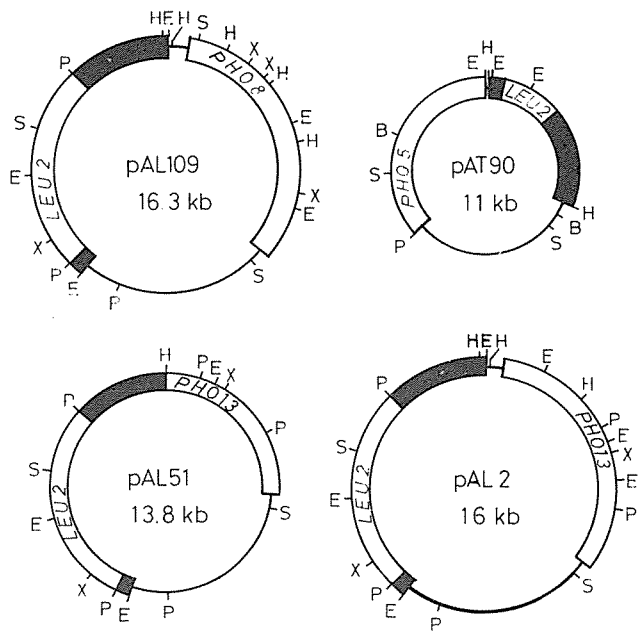

Fig. 1. Structure of plasmids used. A single thin line on the circle indicates the DNA fragment derived from pBR322. A thick line indicates a 2μ DNA sequence. Double thin lines indicate a yeast chromosomal DNA sequence. Restriction enzyme sites are abbreviated as follows : B, BamHI; E, EcoRI; H, HindIII; P, PstI; S, SalI; X, XhoI.

Media. Nutrient medium (YPD) contained 1% yeast extract, 2% polypeptone and 2% glucose. Modified Burkholder's synthetic medium (8)

was used as basal minimal medium. Leucine (Leu) omission medium was prepared by adding adenine, uracil and amino acids except for leucine to basal minimal medium according to the formula described in the Cold Spring Harbor yeast manual (7). Solid media were prepared by adding 2% agar.

Preparation of L-dried specimens. Cells grown on Leu omission plate at 28 C for 2 to 3 days were suspended in suspending medium C (2) at about 10^8 cells per ml. The cell suspension was dried according to the method described by Banno et al. (2).

Determination of viability and maintenance of recombinant plasmid in the L-dried cultures. Rehydration was carried out as described previously (2), except that basal minimal medium was used instead of YM broth. After appropriate dilution, the cells were spread out, using the soft-agar double layer method, on YPD plate for determination of viability, and on Leu omission plate for determination of maintenance of recombinant plasmid, and incubated at 28 C for 2 to 5 days. Maintenance of recombinant plasmid was expressed as the ratio of Leu prototroph (Leu^+) count to viable count in the cell suspension.

Results and Discussion

Survival values of strains carrying recombinant plasmid after drying and after accelerated storage at 37 C are shown in Fig. 2A. The survival percentages were 33 to 43% immediately after drying and 16 to 31% after accelerated storage. Ordinary strains of S. cerevisiae showed survival rates of 6.2 to 76% immediately after drying and of 3.3 to 56% after preservation at 37 C for 60 days (6). Therefore, it is unlikely that strains carrying recombinant plasmid are more sensitive to L-drying than ordinary strains.

To test the maintenance of recombinant plasmid in the above strains, except NA75-2A/pAL2, the cell suspension was spread on a Leu omission plate after appropriate dilution. The cells carrying the recombinant plasmid must show Leu^+ phenotype, because the recombinant plasmid contains the yeast LEU2 gene (Fig. 1). Therefore, the maintenance of recombinant plasmid is expressed as the ratio of Leu^+ cells to surviving cells. In the case of NA75-2A/pAL2, the maintenance of recombinant plasmid was

estimated by counting Leu^+ colonies in 100 survival colonies that appeared on the YPD. As shown in Fig. 2B, the percentage of cells carrying the recombinant plasmid in the 4 strains was slightly reduced immediately after drying, but not further reduced after accelerated storage. The reason why the percentage of cells carrying recombinant plasmid was reduced after drying is not known. Differences in maintenance of recombinant plasmids between the 4 strains were found. NA79-10C/pAL51 and NA79-10C/pAL109 were constructed by transforming the same host strain (NA79-10C) with pAL51 and pAL109 respectively. Futcher and Cox (5) reported that the stability of artificial 2-μm circle-based plasmids varies greatly from plasmid to plasmid. It seems that differences in maintenance of recombinant plasmids is caused by variations in the stability of recombinant plasmids.

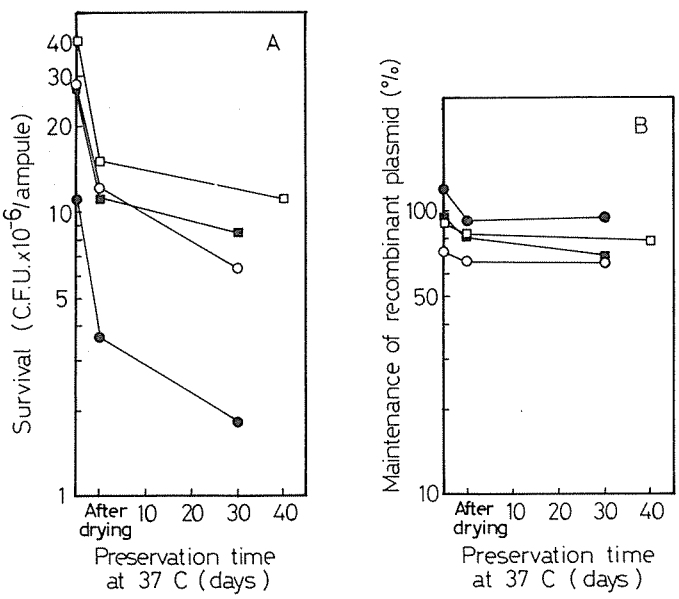

Fig. 2. Survival (A) and maintenance of recombinant plasmid (B) of L-dried cultures. Symbols : ○, NA79-10C/pAL109; ●, NA87-11A/pAT90; □, NA75-2A/pAL2; ■, NA79-10C/pAL51.

For the strains other than NA75-2A/pAL2, eight clones were chosen from the Leu^+ colonies that appeared on the Leu omission plate after accelerated storage and subjected to the curing test. All of these showed instability of Leu^+ phenotype after growing on non-selective

medium (YPD). This result suggests that the recombinant plasmid is not integrated into a host chromosome but exists as a plasmid.

We can predict the viabilities of these dried cultures stored at 5 C from the data on the accelerated storage test using the equation of Mikata et al. (5). Calculations using our data indicate that these L-dried cultures carrying recombinant plasmid will keep survival values of 10 to 30% after presevation at 5 C for 5 years. Therefore, we conclude that L-drying is a useful method for long-term storage of S. cerevisiae strains carrying recombinant plasmid.

We thank Y. Oshima and A. Toh-e for providing the yeast strains carrying recombinant plasmid.

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FURTHER QUALIFICATION OF ISP STRAINS DEPOSITED IN IFO BY THE SOCIETY FOR ACTINOMYCETES, JAPAN (SAJ)

YOSHIRO OKAMI* and TAIKI KUSAKA

Summary

A frame-work for reaffirmation of deposited ISP strains at IFO was organized in cooperation with the check-committee of SAJ. The Committee, consisting of experts nominated by SAJ, examined the characteristics of the strains every four years. By comparison with the description of the ISP strain in IJSB, the appropriateness of the deposited strain for preservation and distribution was assessed by the Committee who forwarded their advice to IFO.

The urgent need for an authentic reference collection, accompanied by standardized characterization for species of actinomycetes, has been pointed out (1). An international cooperative effort to collect and re-examine the type cultures of streptomycetes was organized as the International Streptomyces Project (ISP) in 1964 by the Sub-Committee on Taxonomy of Actinomycetes of the International Committee on Bacteriologi-

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cal Nomenclature (1). Type and neotype strains of the genus Streptomyces were collected, examined, and redescribed by experts throughout the world, using a standardized method (7). Description of and comments on the strains were published (8-11), and a set of them was deposited in each of four nominated culture collections in order to ensure free availability of the strains for requestors.

Thus, subcultures of the strains were deposited in the American Type Culture Collection, Rockville (ATCC), the USSR Research Institute for Antibiotics, Moscow (RIA), the Centraalbureau voor Schimmelcultures, Baarn (CBS) and the Institute for Fermentation, Osaka (IFO). IFO received the strains through SAJ and undertook responsibility for their preservation and distribution. These strains are referred to as ISP strains in this paper.

SAJ has been conscious of the fact that the ISP strains will be of significant value in use as references for identification and other scientific or industrial applications. In 1970, SAJ organized a permanent Committee consisting of 12 experts to confirm the characteristics of the ISP strains maintained at IFO every 4 years (2).

Qualification results till 1979 have been published (6). This paper deals with the confirmation results in 1984 and with former results.

Materials and Methods

Strains used. IFO received 286 strains in 1969 (group 1) and 154 strains in 1973 (group 2) through SAJ. All the strains were preserved by periodically transferring subcultures.

Confirmation. For group 1, four examinations (1971, 1975, 1979, and 1984), and for group 2, three examination (1974, 1979, and 1984) were conducted by the Committee.

Checking-criteria. The Committee adopted the criteria, growth, color of aerial hyphae, reverse color of colony, formation of soluble pigment, and microscopic morphology in accordance with the ISP method (2).

Results

In 1971, IFO supplies lyophilized ISP strains to the Committee for the first confirmation. This produced no questions concerning the original descriptions.

The second confirmation was conducted in 1975, using periodically transferred subcultures, and found no inappropriate strains. From the subcultures, lyophiles were prepared and supplied for the third confirmation in 1979. As seen in Table 1, IFO 12840 (ISP 5252) and IFO 13011

Table 1. Problems and judgements produced by the Committee testing group 1 strains

Strain	Year	Am	Sp	Rv	Mo	Judge
S. arabicus	1971	OK	OK	OK	OK	OK
IFO 12840(ISP 5252)	1975	OK	OK	OK	OK	OK
	1980	None	OK	OK	?	Replace(IFO 14035)
S. albidus	1971	OK	OK	OK	OK	OK
IFO 13011(ISP 5320)	1975	OK	OK	OK	OK	OK
	1980	?	OK	OK	OK	Replace(IFO 14052)
S. psammoticus	1971	OK	OK	OK	OK	OK
IFO 13076(ISP 5341)	1975	OK	OK	OK	OK	OK
	1980		Dead			Replace (IFO 13971)

OK: Same as the ISP description in IJSB.
? or remarks: Difference from ISP description in IJSB.
AM: Aerial mycelium. Sp: Soluble pigment. Rv: Reverse color of colony.
Mo: Microscopic morphology.

(ISP 5320) offered some problems and IFO 13076 (ISP 5341) was found to be a dead strain. Accordingly, the Committee advised the IFO to suspend the distribution of these strains and their unavailability was announced in a timely issue of SAJ's "Actinomycetologist". The Comittee collected samples of these strains from other depositories, including the ISP Center (Dr. E.B. Shirling, Ohio), among which they found appropriate ones, and announced the replacement of the cultures in question together with their new IFO numbers.

IFO 14035, 14052 and 13971 replaced the three inappropriate strains mentioned above (Table 1), and were reconfirmed at the fourth confirmation

Table 2. Problems and judgement produced by the Committee testing group 2 strains

Strain	Year	Am	Sp	Rv	Mo	Judge
S. xantholiticus	1974	No growth				Suspend
IFO 13354 (ISP 5244)	1979	OK	OK	OK	OK	OK
	1984	OK	OK	OK	OK	OK
S. horton	1974	None				Suspend
IFO 13355 (ISP 5266)	1979	OK	OK	OK	OK	OK
	1984	OK	OK	OK	OK	OK
S. sclerotialus	1974	None				Suspend
IFO 13356 (ISP 5269)	1979	OK (poor)	OK	OK	OK	Replace(IFO13904)
S. flavisclerotícus	1974	None				Suspend
IFO 13357 (ISP 5270)	1979	Blue	Brown		Spiral	Replace(IFO14019)
S. purpurogeni-scleroticus	1974	None				Suspend
IFO 13358 (ISP 5271)	1979	OK (poor)	OK(weak)	OK	OK	Replace(IFO13903)
Chainia nigra	1974	None				Suspend
IFO 13362 (ISP 5302)	1979	None	OK	OK	?	Replace(IFO13902)
S. roseisclerotícus	1974	None				Suspend
IFO 13363 (ISP 5303)	1979	OK	OK	OK	OK	OK
	1984	OK	OK	OK	OK	OK
Streptoverticillium kashimirense	1974	No growth				Suspend
IFO 13364 (ISP 5336)	1979	OK	OK	OK	No whirl	Replace(IFO13906)
S. novaecaesareae	1974	None				Suspend
IFO 13368 (ISP 5358)	1979	OK	OK	OK	OK	OK
	1984	OK	OK	OK	OK	OK
S. thermophilus	1974	None				Suspend
IFO 13370 (ISP 5365)	1979	OK	OK	OK	OK	OK
	1984	OK	OK	OK	OK	OK
S. flavofungini	1974	None				Suspend
IFO 13371 (ISP 5366)	1979	OK	OK	OK	OK	OK
	1984	None	OK	OK	OK	OK
S. thermoviolaceus	1974	Not examined				Suspend
subsp. thermo-violaceus IFO 13387 (ISP 5443)	1979	None	None	OK	?	Replace(IFO13905)
Streptoverticillium parvisporogenes	1974	White-gray	OK	OK	No whirl	Suspend
IFO 13394 (ISP 5473)	1979	White-gray	OK	OK	No whirl	Replace(IFO13907)

Remarks are same as those in Table 1.

in 1984. L-dried ampoules made in 1983 were supplied for this confirmation study. All the strains were substantially appropriate, though some of the L-dried ampoules had low revived colonial numbers. These strains were rechecked using newly prepared L-dried ampoules.

IFO accepted 154 strains (group 2) in 1973 and these were lyophilized.

However, 30 of these strains could not be lyophilized at that moment because of their extremely poor growth. They were transferred to various media to find ones that would promote good growth or aerial sporulation, and this produced 11 barely lyophilizable strains. These were supplied to the Confirmation Committee in 1974, together with other lyophilized strains, but the nineteen strains which could not be lyophilized were not. None of the test organisms proved to have serious problems. However, the Committee were obliged to recommend that the IFO suspend distribution of 12 strains, owing to their extremely poor growth. After many attempts to obtain good growth of the poor growth strains, IFO was successful in lyophilizing the unlyophilized 19 strains discussed above before the second confirmation in 1979. As seen in Table 2, the Committee found that 7 strains; IFO 13356 (ISP 5269), IFO 13357 (ISP 5270), IFO 13358 (ISP 5271), IFO 13362 (ISP 5302), IFO 13364 (ISP 5336), IFO 13387 (ISP5443), and IFO 13394 (ISP 5473) were inappropriate.

The Committee then collected these strains from other depositories and examined them in the same manner as those in group 1. Consequently, the 7 defective cultures were replaced by newcomers which were accepted under the numbers IFO 13904, 14019, 13903, 13902, 13906, 13905, and 13907, respectively. L-dried ampoules of all the strains in group 2 were reprepared in 1983 and supplied, with the 7 replacement strains for the third confirmation in 1984, at which some colonies of IFO 13346 and IFO 13418 were found to have lost soluble pigment formation, spore of IFO 13410 were found to be sparse and other strains offered no problems. To these inappropriate strains, IFO successfully conducted monospore isolation.

Discussion

Lyophilized preparations of the group 1 strains were prepared for the first, third and fourth confirmation studies. These preparations, plus subcultures on agar slants for the second confirmation, were used. It was confirmed that successive subculturing on agar slants, at intervals of 3 or 6 months, did not affect their characteristics over a period of more than 10 years. On the other hand, the lyophilized strains were also preserved well in a lyophilized condition for at least 5 years, as the results of confirmation studies of group 2 strains showed, though

some strains could not be lyophilized which inconvenienced requestors. So all the strains in both groups have been lyophilized and preserved in the Culture Collection of IFO.

ISP strains at IFO, which have been thus guaranteed by the Committee, could be used as references for identification of streptomycetes. Whenever IFO distributes ISP strain(s) to requestors, they are accompanied by an announcement that the strain is authorized by the Committee of SAJ and is appropriate for taxonomical studies.

Since 1982, IFO has distributed L-dried ampoules of actinomycetes because the L-drying method in more convenient in its technique than lyophilization, it having been demonstrated that this method saves processing labour and is good for long-term preservation of microorganisms (3). Therefore, IFO has applied the method to the preservation of ISP strains, and supplied such samples for the confirmation studies in 1984 by the Committee of SAJ.

The number and variety of actinomycetes cultures at IFO have been increasing year by year (5), and it is almost impossible for the limited staff to manage all the cultures, to check their taxonomical character, physiological activity, or production abilities. One possible countermeasure to improve the culture collection would be cooperative work with outside experts (4). The confirmation system of ISP strains, which we have described, is a good example of such a countermeasure which has not been adopted yet worldwide.

We propose that the exchange of information about ISP strains and, if possible, the exchange of these strains with culture collections that preserve other sets of ISP strains, will afford a more effective guarantee for the ISP strains through extramural checking of their taxonomical features.

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PRESERVATION OF ANTIMICROBIAL ACTIVITY IN L-DRIED AND AGAR-TRANSPLANTED ACTINOMYCETES

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Summary

Preservation of antimicrobial activity was studied using both an L-dried actinomycetes strain preserved for 14 years and one repeatedly transplanted for each of 222 strains selected at random from the ISP strains. Activity against five indicator microorganisms was detected by the cross streak method. Thirty-eight strains did not show any antimicrobial activity. Of the L-dried strains 76.7% showed activity, 92.4% if the 38 strains mentioned above are excluded. For the repeatedly transplanted strains, the corresponding figures were 69.5% and 83.8%, respectively.

Liquid drying in vacuo (L-dried method) (3,5) requires less labour than either transplanting or lyophilization and has fewer problems in terms of viability (8) and preservation of taxonomical characteristics, but the production ability of useful substances and the preservation of sensitivity to chemicals by L-dried strains has not been well studied.

This paper deals with the antimicrobial activities of L-dried strains compared with that of repeatedly transplanted strains.

Materials and methods

Tested actinomycetes. From the ISP (International Streptomyces Project) strains (2,6), 222 were chosen at random. Strains which have been stored at 8 C for 14 years after drying by the L-drying method in 1969 (refer to L strain) and those which have been maintained for the same period by transplanting from agar medium to agar medium at intervals of 3 or 6 months (refer to M strain) were used.

Indicator organisms for antimicrobial spectrum. The following micro-organisms were used:

a) Bacillus subtilis PCI219 IFO 3513-------(B)
b) Staphylococcus aureus FDA209P IFO 12732------(St)
c) Escherichia coli JC-2 IFO 12734------(Co)
d) Candida albicans IFO 0583-------(Ca)
e) Penicillium chrysogenum Q176 IFO 4626-------(Pen)

Cells including spores of the slant which were transplanted 2 or 7 days before the test and incubated at 28 C were suspended in sterilized water.

Media. The media for the actinomycetes in Table 1-5 were as follows (also listed in the IFO List of Cultures (1984) (2)). Fifteen ml of agar medium was poured into each Petri dish. No. 227 medium(ISP-2); yeast extract 4 g, glucose 4 g, malt extract 10 g, dist. water 1 liter, agar 20 g, pH 7.3. No. 228 medium(Bennett's agar); yeast extract 1 g, beef extract 1 g, N.Z. amine (A type) 2 g, glucose 10 g, dist. water 1 liter, agar 20 g, pH 7.3. No. 231 medium; same as No. 228 medium, but with maltose instead of glucose as carbon source. No. 1 medium; potato (decoction) 200 g, sucrose 20 g, dist. water 1 liter, agar 20 g, pH 5.6.

Antimicrobial test. A strain of actinomycetes was streaked on the agar plate and incubated for 4 or 5 days. A suspension of each indicator organism was streaked at right angles to the growth of actinomycetes and the plate was incubated 28 C for 3 or 4 days.

Evaluation. The inhibition zone of indicator organism around the growth of aristeromycin was measured in mm and antimicrobial activity was expressed using following index:

index 0: inhibition zone; 0-- 5 mm.
index 1: inhibition zone; 6--15 mm.
index 2: inhibition zone; > 16 mm.

Results

According to the difference of the indices in the spectrum between L and M strain, the strains were classified into following five groups:

Group 1, exactly the same spectrum: The spectra of both strains are exactly the same. The thirty-five strains belonging to this group are listed in Table 1.

Table 1. Antimicrobial spectra of actinomycetes culture stored by repeated transfer and L-dry. Group 1; exactly the same spectra

IFO	Med. No.	Tem.	Antimicrobial spectra M strain B	St	Co	Ca	Pen	L strain B	St	Co	Ca	Pen	Name of strain
12738	227	28	1	1	0	0	0	1	1	0	0	0	S. alboniger
12741	228	28	2	2	0	0	0	2	2	0	0	0	S. atroolivaceus
12742	231	28	2	2	0	0	0	2	2	0	0	0	S. aureoverticillatus
12755	231	28	2	2	0	0	0	2	2	0	0	0	S. chrysomallus subsp. chrysomallus
12772	227	28	2	2	0	0	0	2	2	0	0	0	S. flavoviridis
12788	231	28	2	2	0	0	0	2	2	0	0	0	S. lateritius
12789	227	28	2	2	2	1	2	2	2	2	1	2	S. lavendulae subsp. lavendulae
12801	227	28	2	2	0	0	0	2	2	0	0	0	S. narbonensis
12812	228	28	2	2	1	0	1	2	2	1	0	1	S. ramulosus
12824	227	28	2	2	0	2	2	2	2	0	2	2	S. toyocaensis
12837	231	28	1	2	0	1	1	1	2	0	1	1	S. aminophilus
12844	227	28	2	2	0	0	0	2	2	0	0	0	S. bellus
12859	227	28	2	2	0	2	2	2	2	0	2	2	S. endus
12886	231	28	2	2	0	0	0	2	2	0	0	0	S. longisporoflavus
12887	228	28	1	1	1	1	1	1	1	1	1	1	Stv. luteoverticillatum
12903	227	28	2	2	2	1	2	2	2	2	1	2	A. pseudolavendulae
12907	228	28	2	2	2	2	2	2	2	2	2	2	S. rimosus subsp. rimosus
12910	228	28	1	0	0	0	0	1	0	0	0	0	S. roseosporus
12914	231	28	1	0	0	0	0	0	0	0	0	0	S. scabies
12917	231	28	2	2	0	0	0	2	2	0	0	0	S. spheroides
12918	231	28	2	2	2	0	0	2	2	2	0	0	A. streptomycini
12919	228	28	2	2	0	0	0	2	2	0	0	0	S. tanashiensis
13061	227	28	2	2	0	2	2	2	2	0	2	2	S. melanosporofaciens
13064	227	28	2	2	0	0	0	2	2	0	0	0	S. nashvillensis
13074	231	28	2	2	0	0	0	2	2	0	0	0	S. pristinaespiralis
13088	227	37	2	2	0	0	0	2	2	0	0	0	S. thermotolerans
13100	231	28	1	1	0	0	0	1	1	0	0	0	S. violaceochromogenes
13107	231	28	0	2	0	1	1	0	2	0	1	1	A. vulgaris
13198	228	28	2	2	1	0	0	2	2	1	0	0	S. bikiniensis
13357	227	28	0	1	0	0	0	0	1	0	0	0	C. flava
13382	227	28	2	2	0	0	0	2	2	0	0	0	S. helvaticus

(to be continued)

Table 1. (continued)

IFO	Med. No.	Tem.	Antimicrobial spectra M strain B	St	Co	Ca	Pen	L strain B	St	Co	Ca	Pen	Name of strain
13385	227	28	1	2	0	0	0	1	2	0	0	0	S. lividans
13396	227	28	2	2	0	0	0	2	2	0	0	0	S. tenebrarius
13401	227	28	1	2	0	0	0	1	2	0	0	0	S. iakyrus
13474	231	37	0	0	0	2	0	0	0	0	2	0	S. tetanusemus

S; Streptomyces, Stv; Streptoverticillium, A; Actinomyces, C; Chainia.
Tem.; cultural temperature.
B, St, Co, Ca, Pen, and indices of 0,1,2; refer to Materials and methods.

Group 2, almost the same spectrum: Indices for four indicator organisms of the five for one strain are the same as those for the other strain and the difference in the index which is not the same is less than one. Table 3 lists the 106 strains, with their spectra, belonging to this group.

Table 2. Antimicrobial spectra of actinomycetes culture stored by repeated transfer and L-dry. Group 2; almost the same spectra

IFO	Med. No.	Tem.	Antimicrobial spectra M strain B	St	Co	Ca	Pen	L strain B	St	Co	Ca	Pen	Name of strain
12737	228	28	2	2	0	2	2	2	2	1	2	2	Stv. albireticuli
12740	231	28	0	1	0	0	0	0	0	0	0	0	S. althioticus
12746	231	28	1	0	0	0	0	0	0	0	0	0	S. bicolor
12747	231	28	2	2	1	2	2	2	1	0	1	2	Stv. blastmyceticum
12748	227	28	1	2	0	0	0	1	1	0	0	0	S. cacaoi subsp. cacaoi
12753	227	28	0	0	0	0	0	1	1	0	0	1	S. chartreusis
12760	227	28	2	1	0	2	2	2	2	0	2	1	S. cremeus
12761	227	28	1	1	1	0	1	2	2	0	0	0	S. curacoi
12766	227	28	0	0	1	1	0	0	0	0	0	0	S. felleus
12771	228	28	0	1	0	0	0	0	0	0	0	0	S. flavovirens
12773	231	28	2	2	2	2	1	2	2	1	2	2	S. fradiae
12774	227	28	1	2	0	0	0	1	1	0	0	0	S. glaucescens
12775	227	28	1	0	0	0	0	0	1	0	0	0	S. griseobrunneus
12780	1	28	0	0	0	0	1	1	1	0	0	0	S. griseorubens
12782	227	28	2	0	0	2	2	2	1	1	2	2	Stv. hachijoense
12784	227	28	2	2	0	0	0	2	2	0	0	1	S. hawaiiensis
12785	227	28	2	2	0	1	1	1	1	0	1	1	Stv. hiroshimense
12796	227	28	1	0	0	0	0	1	1	1	0	0	S. massasporeus
12798	227	28	0	0	0	0	0	1	0	0	0	0	S. minoensis
12803	227	28	2	1	1	0	0	2	2	0	0	0	S. nitrosporeus

(to be continued)

Table 2. (continued)

IFO	Med. No.	Tem.	Antimicrobial spectra M strain B	St	Co	Ca	Pen	L strain B	St	Co	Ca	Pen	Name of strain
12807	227	28	1	0	0	0	0	0	0	0	0	0	S. pilosus
12814	227	28	0	1	0	0	0	1	1	0	0	0	S. resistomycificus
12817	227	28	1	1	2	2	2	1	1	1	1	2	Stv. roseoverticillatum
12823	227	28	2	0	0	0	1	2	0	0	0	0	S. toxytricini
12824	227	28	2	2	0	2	2	1	2	0	2	2	S. toyocaensis
12827	227	28	1	1	1	1	0	1	0	1	0	0	S. virginiae
12828	227	28	2	2	0	0	1	2	1	0	0	0	S. xanthochromogenes
12830	231	28	1	1	0	1	1	2	1	0	0	0	S. aburaviensis
12838	227	28	0	0	0	0	0	0	1	0	0	0	S. antibioticus
12841	227	28	1	0	0	0	0	2	1	0	0	0	S. argenteolus
12845	231	28	0	1	0	0	0	0	0	0	0	0	Stv. biverticillatum
12846	231	28	0	1	0	2	2	1	1	0	1	1	A. candidus
12847	228	28	2	2	2	0	0	2	2	1	0	0	S. capreolus
12849	231	28	1	2	0	0	1	2	2	0	0	1	S. cellostaticus
12852	227	28	2	1	2	2	2	2	1	1	2	2	Stv. cinnamoneum subsp. cinnamoneum
12853	227	28	2	0	0	0	0	2	0	0	0	1	S. citreofluorescens
12855	227	28	1	1	0	0	0	1	0	0	0	0	S. coeruleorubidus
12858	228	28	1	1	0	1	0	1	2	0	1	0	Stv. ehimense
12861	227	28	1	0	0	0	0	2	1	0	1	1	S. fluorescens
12867	227	28	2	2	0	0	0	1	1	0	0	0	S. globisporus subsp. globisporus
12869	227	28	2	1	0	0	0	2	2	0	0	0	S. griseinus
12870	227	28	2	2	1	1	2	2	2	1	2	2	S. griseofuscus
12873	227	28	1	0	0	0	1	0	0	0	0	0	S. griseoruber
12879	228	28	0	0	0	0	0	1	1	0	0	0	S. janthinus
12880	231	28	2	2	2	1	2	2	1	2	1	2	Stv. kentuckense
12881	228	28	2	2	2	1	1	2	2	1	1	0	S. lavendulocolor
12882	227	28	1	2	0	0	0	0	2	0	0	0	S. lavendofoliae
12891	227	28	2	1	0	0	1	1	1	0	0	1	S. misakiensis
12893	227	28	2	2	0	1	2	2	1	1	2	2	Stv. netropsis
12894	228	28	1	1	0	2	2	1	0	0	2	2	S. nigrescens
12895	231	28	0	0	0	2	0	0	0	0	1	0	S. nodosus
12897	231	28	2	2	0	0	0	2	1	0	0	0	S. olivoviridis
12900	227	28	0	0	0	0	0	1	1	0	0	0	S. phaeoviridis
12905	227	28	1	2	0	0	1	0	0	0	0	0	S. purpeofuscus
12906	231	28	2	2	2	1	2	2	2	2	2	2	S. racemochromogenes
12909	231	28	2	1	0	1	2	2	2	0	2	2	S. roseoluteus
12911	231	28	0	1	1	0	0	0	2	1	0	0	S. roseoviridis
12915	231	28	1	2	0	0	0	2	2	0	0	0	S. sindenensis
13009	228	28	1	2	1	0	0	2	2	1	0	0	S. actuosus
13012	227	28	0	2	0	0	0	1	2	0	0	0	A. albohelvatus
13021	227	28	1	2	1	0	0	1	1	0	0	0	Stv. aureoversile
13023	227	28	0	1	0	0	0	0	2	0	0	0	S. bottropensis
13026	231	28	0	0	0	0	0	0	1	0	0	0	S. cavourensis
13029	231	28	0	2	1	1	0	0	1	0	1	0	S. citreus
13030	228	28	1	1	1	0	0	1	1	0	0	0	A. coeliatus

(to be continued)

Table 2. (continued)

IFO	Med. No.	Tem.	Antimicrobial spectra M strain B	St	Co	Ca	Pen	L strain B	St	Co	Ca	Pen	Name of strain
13035	227	28	0	0	0	0	0	0	1	0	0	0	A. cyanogenus
13036	227	28	1	2	0	0	0	2	2	0	0	0	A. cyanoglomerus subsp. cellulose
13038	227	28	1	2	0	0	0	2	2	0	0	0	A. flavescens
13039	231	28	1	2	0	0	0	0	1	0	0	0	S. flavidovirens
13041	227	28	1	0	0	0	0	0	0	0	0	0	S. flocculus
13042	227	28	1	1	0	0	0	0	0	0	0	0	S. fumanus
13045	227	28	2	2	2	2	2	2	2	1	1	2	S. griseolavendus
13048	231	28	1	0	0	0	0	0	0	0	0	0	S. heimi
13050	231	28	0	0	0	0	0	1	0	0	0	0	S. ipomoeae
13051	231	28	2	2	0	1	1	2	2	0	1	2	S. karnatakensis
13052	228	28	2	2	2	2	2	2	2	1	1	2	Stv. kishiwadense
13053	227	28	0	0	0	1	0	0	0	0	0	0	S. krainskii
13055	227	28	2	2	0	2	2	2	2	0	1	1	A. longissimus
13063	227	28	1	2	0	1	1	1	1	0	2	2	S. misionensis
13070	228	28	2	2	2	2	0	2	2	1	2	1	S. paucisporogenes
13072	231	28	1	2	2	2	2	2	2	2	2	2	S. polychromogenes
13073	227	28	0	1	0	0	0	0	0	0	0	0	S. praecox
13077	227	28	1	2	0	0	0	1	1	0	0	0	S. purpurascens
13079	228	28	2	2	2	0	0	1	2	2	0	0	Stv. rectiverticillatum
13080	227	28	1	1	0	0	0	1	2	0	0	0	S. roseochromogenes
13084	231	28	1	2	0	0	1	2	2	0	0	2	S. saraceticus
13086	228	28	2	2	1	0	0	2	2	2	0	0	S. sparsogenes
13090	227	28	2	1	0	0	0	2	1	0	1	1	S. tubercidicus
13092	231	28	1	1	0	1	0	2	2	0	1	0	S. umbrosus
13098	228	28	2	2	0	1	1	2	2	0	1	2	S. vinaceus
13099	227	28	2	2	2	1	1	1	2	1	1	0	S. vinaceusdrappus
13101	231	28	0	0	0	0	0	1	1	0	0	0	S. violaceolatus
13102	231	28	2	2	0	0	0	2	2	0	1	1	S. violaceorectus
13104	227	28	1	0	0	0	0	0	0	0	0	0	S. violarus
13105	227	28	1	2	0	0	0	1	1	0	0	0	A. violochromogenes
13106	227	28	0	1	0	0	0	0	2	0	0	0	S. viridodiastaticus
13108	231	28	0	1	0	0	1	1	2	0	0	0	S. yokosukanensis
13128	228	28	2	2	1	0	0	2	2	0	0	0	S. caespitosus
13197	231	28	2	1	2	1	1	2	2	2	1	1	S. baarnensis
13388	228	28	2	1	1	0	0	2	2	2	0	0	S. subrutilus
13394	228	28	2	2	1	0	2	2	2	0	0	2	Stv. parvisporogenes
13407	228	28	0	0	0	0	0	1	0	0	0	0	S. rishiriensis
13418	231	28	2	0	0	0	0	1	0	0	0	0	S. tuirus
13453	231	28	0	0	1	1	0	0	0	2	2	0	S. alni
13454	231	28	2	1	2	1	1	2	2	1	1	2	S. colombiensis
13473	227	37	2	2	1	0	0	2	2	0	0	0	S. thermonitrificans

S; Streptomyces, Stv; Streptoverticillium, A; Actinomyces.
Tem.; cultural temperature.
B, St, Co, Ca, Pen, and indices of 0,1,2; refer to Materials and methods.

Group 3, different spectra; L strain preserved activities, but M strain did not: Although some indices in the spectrum of L strain are 2, the corresponding indices of M strain are 0. These 30 strains are as shown in Table 3.

Table 3. Antimicrobial spectra of actinomycetes culture stored by repeated transfer and L-dry.
Group 3; different spectrum (L strains maintain activities)

IFO	Med. No.	Tem.	Antimicrobial spectra M strain B	St	Co	Ca	Pen	L strain B	St	Co	Ca	Pen	Name of strain
12744	227	28	0	0	0	0	0	2	2	0	0	0	S. azureus
12758	227	28	2	0	0	0	0	2	2	0	0	0	S. coerulescens
12762	227	28	2	0	0	0	0	2	2	0	0	0	S. daghestanicus
12765	227	28	1	0	0	1	2	2	2	0	1	2	S. fasciculatus
12792	227	28	2	0	0	0	0	2	2	0	0	0	S. litmocidini
12797	227	28	2	0	0	0	0	2	2	0	0	0	S. michiganensis
12799	227	28	2	0	0	1	2	2	2	0	2	2	S. murinus
12804	227	28	2	0	0	0	0	2	2	0	0	0	S. niveus
12806	227	28	1	0	0	0	0	2	2	0	0	1	Nocardia orientalis
12811	227	28	2	0	0	0	0	2	2	0	1	1	S. puniceus
12815	227	28	1	2	1	0	0	0	0	0	0	2	S. roseolilacinus
12818	227	28	1	0	0	0	0	2	2	0	0	0	S. roseus
12820	227	28	2	0	0	0	1	2	2	0	0	2	S. sioyaensis
12821	227	28	2	0	0	1	0	2	2	0	1	0	S. spiroverticillatus
12822	227	28	1	0	0	0	0	2	2	0	0	0	S. tendae
12831	227	28	1	0	0	0	0	2	2	0	0	0	S. afghaniensis
12839	227	28	2	0	0	2	2	2	2	0	2	2	S. antimycoticus
12843	227	28	0	2	0	0	0	2	2	0	0	0	S. aureofaciens
12860	227	28	0	0	0	0	0	0	0	0	1	2	S. filipinensis
12868	231	28	1	2	0	1	1	2	1	2	1	2	S. goshikiensis
12871	227	28	2	0	0	0	0	2	2	0	0	0	S. griseoincarnatus
12883	228	28	1	0	0	0	0	0	0	0	2	0	A. levoris
12884	231	28	0	2	0	0	0	2	2	1	1	1	Stv. lilacinum
12885	227	28	0	0	0	0	0	1	2	0	0	0	S. longisporus
12899	231	28	0	0	0	0	0	2	1	0	0	0	S. phaeopurpureus
12901	227	28	1	0	0	0	0	2	2	1	1	1	S. platensis
13022	231	28	2	2	0	0	0	2	2	2	0	0	A. aurigineus
13054	227	28	0	0	0	0	0	1	2	0	0	0	S. lincolnensis
13056	227	28	0	0	0	0	1	1	2	0	2	2	S. lucensis
13062	227	28	0	0	0	0	0	2	0	0	0	0	S. microflavus

S; Streptomyces, Stv; Streptoverticillium, A; Actinomyces.
Tem.; cultural temperature.
B, St, Co, Ca, Pen, and indices of 0,1,2; refer to Materials and methods.

Group 4, different spectra; M strain preserved activities, but L strain did not: Although some indices in the spectrum of M strain are 2, the corresponding indices of L strain are 0. These 14 strains are shown in Table 4.

Table 4. Antimicrobial spectra of actinomycetes culture stored by repeated transfer and L-dry.
Group 4; different spectrum (M strains maintain activities)

IFO	Med. No.	Tem.	Antimicrobial spectra M strain B	St	Co	Ca	Pen	L strain B	St	Co	Ca	Pen	Name of strain
12757	227	28	2	0	0	0	1	0	1	0	0	1	S. coeruleofuscus
12768	227	28	2	0	0	1	0	0	0	0	0	0	S. flaveolus
12790	227	28	0	2	0	0	0	0	0	0	0	0	S. limosus
12815	227	28	1	2	1	0	0	0	0	0	0	2	S. roseolilacinus
12908	231	28	2	2	2	1	1	1	0	0	0	0	S. rochei
13037	227	28	0	2	0	0	0	0	0	0	0	0	S. fimicarius
13069	228	28	2	2	2	2	1	2	2	2	0	1	S. ornatus
13075	227	28	2	2	0	0	0	0	0	0	0	0	S. prunicolor
13083	227	28	2	1	0	0	1	0	0	0	0	0	S. sampsonii
13190	227	28	2	2	1	0	0	0	0	0	0	1	S. cyaneofuscatus
13191	231	28	0	2	0	0	0	0	0	0	0	0	S. exfoliatus
13347	227	28	0	2	0	0	0	0	0	0	0	0	S. viridochromogenes
13383	231	28	2	2	2	0	0	1	1	0	0	0	S. indigocolor
14035	227	28	2	2	0	0	0	0	2	0	0	0	S. arabicus

S; Streptomyces.
Tem.; cultural temperature.
B, St, Co, Ca, Pen, and indices of 0,1,2; refer to Materials and methods.

Group 5, no activity: Both L and M strains showed no antimicrobial activities. The 38 strains in this group are listed in Table 5. The majority of them are considered to produce no antimicrobial substance before preservation.

The strain IFO 12815 is included not only in Group 4, because of its index for Staphylococcus aureus, but also in Group 3, because of its index for Penicillium chrysogenum. As summarized in Table 6, Groups 1 and 2 were eventually considered to be same. The percentages of the strains in Groups 1 and 2, 3, 4 and 5 were 63.2%, 13.5%, 6.3% and 17.0%, respectively. The percentage of the strains which preserved activities for 14

Table 5. Antimicrobial spectra of actinomycetes culture stored by repeated transfer and L-dry. Group 5; no antimicrobial activities

IFO	Med. No.	Tem.	Antimicrobial spectra M strain B	St	Co	Ca	Pen	L strain B	St	Co	Ca	Pen	Name of strain
12735	231	28	0	0	0	0	0	0	0	0	0	0	S. achromogenes subsp. achromogenes
12739	227	28	0	0	0	0	0	0	0	0	0	0	S. albovinaceus
12777	227	28	0	0	0	0	0	0	0	0	0	0	S. griseolus
12783	227	28	0	0	0	0	0	0	0	0	0	0	S. halstedii
12786	227	28	0	0	0	0	0	0	0	0	0	0	S. hirsutus
12791	227	28	0	0	0	0	0	0	0	0	0	0	S. lipmanii
12795	227	28	0	0	0	0	0	0	0	0	0	0	S. malachiticus
12802	227	28	0	0	0	0	0	0	0	0	0	0	S. nigrifaciens
12805	227	28	0	0	0	0	0	0	0	0	0	0	S. olivaceus
12809	227	28	0	0	0	0	0	0	0	0	0	0	S. prasinopilosus
12816	227	28	0	0	0	0	0	0	0	0	0	0	S. roseolus
12832	231	28	0	0	0	0	0	0	0	0	0	0	A. albocyaneus
12835	228	28	0	0	0	0	0	0	0	0	0	0	S. amakusaensis
12850	227	28	0	0	0	0	0	0	0	0	0	0	S. chibaensis
12857	227	28	0	0	0	0	0	0	0	0	0	0	S. cyanoalbus
12862	227	28	0	0	0	0	0	0	0	0	0	0	S. fragilis
12863	231	28	0	0	0	0	0	0	0	0	0	0	A. fulvoviridis
12878	231	28	0	0	0	0	0	0	0	0	0	0	S. indigoferus
12898	227	28	0	0	0	0	0	0	0	0	0	0	S. phaeochromogenes
12913	227	28	0	0	0	0	0	0	0	0	0	0	S. rubiginosus
13011	227	28	0	0	0	0	0	0	0	0	0	0	S. albidus
13013	228	28	0	0	0	0	0	0	0	0	0	0	S. alboviridis
13014	231	28	0	0	0	0	0	0	0	0	0	0	S. albus subsp. albus
13015	228	28	0	0	0	0	0	0	0	0	0	0	S. almquistii
13028	231	28	0	0	0	0	0	0	0	0	0	0	S. cinnabarinus
13034	227	28	0	0	0	0	0	0	0	0	0	0	A. cyanocolor
13059	228	28	0	0	0	0	0	0	0	0	0	0	A. malachitofuscus
13060	228	28	0	0	0	0	0	0	0	0	0	0	A. malachitorectus
13078	228	28	0	0	0	0	0	0	0	0	0	0	S. rangoon
13082	227	28	0	0	0	0	0	0	0	0	0	0	Stv. waksmanii
13085	227	28	0	0	0	0	0	0	0	0	0	0	S. setonii
13087	228	28	0	0	0	0	0	0	0	0	0	0	S. termitum
13089	231	37	0	0	0	0	0	0	0	0	0	0	S. thermovulgaris
13091	227	28	0	0	0	0	0	0	0	0	0	0	S. umbrinus
13201	227	28	0	0	0	0	0	0	0	0	0	0	S. finlayi
13425	231	28	0	0	0	0	0	0	0	0	0	0	S. vinaceus
13509	231	28	0	0	0	0	0	0	0	0	0	0	N. globerula
14052	227	28	0	0	0	0	0	0	0	0	0	0	S. albidus

S; Streptomyces, A; Actinomyces, N; Nocardia, Stv; Streptoverticillium.
Tem.; cultural temperature.
B, St, Co, Ca, Pen, and indices of 0,1,2; refer to Materials and methods.

years in the L-dried condition was 76.7%, excluding those in Groups 4 and 5.

The corresponding percentage for repeatedly transplanted was 69.5%. If we assume that all the strains in Group 5 had no antimicrobial activity before preservation, the number of strains tested would be 185. In this case, the percentages of the strains preserving activities will be 92.4% and 83.8%, for L-drying and repeated transplanting, respectively.

Table 6. Numbers and percentages of stocked actinomycetes grouped by antimicrobial activities.

G R O U P								
	Antimicrobial spectra	I N D E X						
No.	by M & L strain	M strain		L strain	Number	%	Number	%
1)	exactly the same	=			35		35	
2)	almost the same	0		1	106	63.2	106	76.2
		1		0				
		1		2				
		2		1				
3)	different	0		2	30	13.5	30	16.2
4)	different	2		0	14	6.3	14	7.6
5)	no activity	0		0	38	17.0	--	--
				Total	223	100.0	185	100.0

Discussion

A grisin producing strain, Streptomyces griseus is reported to have been preserved for two years by lyophilization or the paraffin seal method (7). Novobiocin, streptomycin and chloramphenicol-producing strains have been preserved for 25 years at 20 C below zero using paraffin seal or lyophilization methods, and, except the chloramphenicol-producing one, showed almost the same antimicrobial activities after preservation as they had 25 years before, using the same checking method. Although the chloramphenicol-producing strain showed lower activity, strains which recovered their activity were easily obtained from fifty monospore cultures (1). Similarly, it is reported that enzyme inhibitory activities were well maintained for a long period in the lyophilized or agar slant frozen condition, in spite of a decrease in the revived colonial number (4).

The results of this report reveal that from 76.7 to 92.4% of randomly selected actinomycetes strains preserved their antimicrobial activities. However, 7.6-23.3% of the strains preserved using the L-drying method did not maintain their biological activities in this experiment. Other preservation methods should be applied to such strains.

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PRESERVATION OF THE ANTIBIOTIC PRODUCING ABILITY OF A STREPTOMYCETE BY LYOPHILIZING FOR 13 YEARS

TAIKI KUSAKA

Summary

The cultured broth of the L strain of aristeromycin producer, Streptomyces citricolor IFO 13005, which was revived from a lyophilized ampoule preserved for 13 years at 8 C, showed the same antimicrobial spectrum as that of M strain of the same producer which was maintained for the same period by transplanting from agar medium to agar medium at intervals of 3 or 6 months. From the broth, antibiotic having the same physicochemical properties and NMR spectrum of aristeromycin was isolated. The antibiotic producing ability of this streptomycete was preserved for a long period in the lyophilized state, while decreased by transplantation on agar medium.

The lyophilization method has been widely adopted to the long term preservation of stock cultures in culture collection (8), and guarantees a high survival ratio and good preservation of the morphological characteristics of the cultures (6,10). Although antimicrobial activities were reported to be experimentally preserved well for a long period (1,5,7), there are very few reports which reveal the preservation of producing

abilities of the same antibiotics. Different kinds of antibiotics may produce the same antimicrobial spectrum if indicator organisms are limited. Therefore, antibiotic producing abilities can not be judged from the antimicrobial spectrum of the cultivated broth, but from the actual isolation of the antibiotic from cultured broth.

This paper deals with the preservation of the antibiotic producing ability of a lyophilized strain in comparison with that of a repeatedly transplanting strain.

Materials and Methods

Strains. A strain (refer to L strain) of Streptomyces citricolor IFO 13005 (=B16757) (3) revived in May of 1983 from an ampoule kept for 13 years at 8 C, and a strain (refer to M strain) transplanted at intervals of 3 or 6 months for 13 years were used. Indicator organisms for the antimicrobial spectra were as follows;

a)	Bacillus subtilis PCI219	IFO 3513
b)	Staphylococcus aureus FDA209P	IFO 12732
c)	Escherichia coli JC-2	IFO 12734
d)	Candida albicans	IFO 0583
e)	Penicillium chrysogenum Q176	IFO 4626
f)	Xanthomonas oryzae N5801	IFO 3995

Bacterial and fungal cells including spores, which were transplanted to slant agar medium 2 or 7 days before the test and incubated at 28 C, were suspended in sterilized water. In the case of X. oryzae, a cell suspension of the culture incubated for 24 hrs at 28 C in glucose bouillon medium was obtained.

Media. Malt extract yeast extract medium (ISP-2) (9) was used for the growth of actinomycete. Glucose bouillon agar medium (4) (glucose 10 g, meat extract 5 g, peptone 5 g, NaCl 5 g, tap water 1 liter, pH 7.0) was used for the growth of indicator organisms employed for testing antimicrobial spectrum. The components of S medium (4), which was employed for fermentation of antibiotic production, was as follows (in g/liter): glucose 20 g, soluble starch 30 g, soybean flour 10 g, corn steep liquor 10 g, peptone 5 g, NaCl 3 g. Later pH adjustment to 7.0 with 2 N NaOH, $CaCO_3$ 5 g was added.

Test of antimicrobial spectrum. L or M strain of S. citricolor IFO 13005 was streaked on an agar plate of ISP-2 in a Petri dish and incubated for 4 or 5 days until sufficient growth was obtained. A suspension of each indicator organism was streaked at right angles to the growth of the streptomycete strain and the plate was incubated at 28 C for a further 5 days. The inhibition zone of each indicator organim, apart from the growth of streptomycetes, was measured in mm and thus, an antimicrobial spectrum was obtained. In the case of the test against X. oryzae, 5 ml of the glucose bouillon agar containing 1% cell suspension of X. oryzae was poured onto an agar plate of the same medium in a Petri dish. A paper disk containing filtered broth or antibiotic solution was put on the agar plate and incubated for 2 days at 28 C. The diameter of the inhibition zone appearing around disk was measured.

Test for antibiotic production; (1) Fermentation. The same fermentation method (3,4) used for the antibiotic producer before lyophilization of the streptomycete was adopted for the test. Forty ml of S medium in a 200 ml Erlenmeyer's flask was sterilized by autoclaving at 1.5 kg/cm^2 for 20 min. A small agar block of the cultured strain was inoculated and incubated at 28 C for 4 or 6 days on a rotary shaker (220 rpm/min) until antibiotic activity against X. oryzae appeared. The fermented broth, without sterilization, was filtered and used for the isolation of antibiotic. The filtered broth boiled for 3 min was used for the test of antimicrobial spectrum.

(2) Isolation. The same procedure to isolate antibiotic from the strain (3,4) as had been used before lyophilization of the strain was adopted in principle. The filtered broth was absorbed in active carbon (2% w/v) at pH 8.0. The carbon was washed with water and eluted with 80% aqueous acetone. After concentration of the eluate under reduced pressure the concentrate was subjected to an ion exchange resin Dowex 50(H^+). The column was then washed with a solution of water, pyridine, and 1 N NH_4OH (7:2:1, v/v/v). The biologically active fractions were collected and concentrated under reduced pressure. The concentrate was subjected to HP-20 resin column chromatography. The column was then washed and eluted with 10% aqueous ethanol. The eluate was then decolorized with active carbon and concentrated until red brown powder was obtained. Colorless prismatic crystals were obtained from a water solution of the powder.

Results

M strain of S. citricolor IFO 13005 showed weak inhibition of the growth of X. oryzae in the broth of 4 days culture. On the other hand, strong inhibition by L-strain was observed in both 4 and 6 days cultured broth (Table 1). Both strains kept their antimicrobial substance produc-

Table 1. Comparison of antimicrobial activities against Xanthomonas oryzae of the cultured broth of lyophilized (L strain) and repeated transplanting strain (M strain)

Strain	Cultured broth (4 days) pH	Inhibition zone (mm)		Cultured broth (6 days) pH	Inhibition zone (mm)	
L strain	4.8	32	27	5.6	25	24
M strain	6.2	14	16	6.8	0	0
aristeromycin(1000 μg/ml)		23	24		22	26

ing ability, though L strain produced it more constantly than M strain. The antimicrobial spectra of both strains were the same and showed strong antimicrobial activity selectively to X. oryzae (Table 2). This charac-

Table 2. Comparison of antimicrobial spectrum by lyophilized (L strain) and repeated transplanting strain (M strain)

Indicator organism		Inhibition zone (mm) L strain	M strain	Aristeromycin (1000 μg/ml)
Bacillus subtilis PCI219	IFO 3513	0	0	0
Staphylococcus aureus FDA209P	IFO 12732	0	0	0
Escherichia coli JC-2	IFO 12734	0	0	0
Candida albicans	IFO 0583	0	0	0
Penicillium chrysogenum Q176	IFO 4626	0	0	0
Xanthomonas oryzae N5801	IFO 3995	25	18	24

teristic antimicrobial spectrum indicated a long period of preservation of the same antibiotic production by lyophilization. Furthermore, antimicrobial substance, which inhibits the growth of X. oryzae, was isolated from the broth of L strain, and identified with aristeromycin, because the physicochemical properties as well as the NMR spectrum of this com-

pound coincided well with those of aristeromycin (Table 3). The lyophi-

Table 3. Comparison of physico-chemical properties of aristeromycin and that obtained from the cultured broth of lyophilized strain.

Properties	Antibiotic from lyophilized strain		Aristeromycin	
characteristic	colorless prism		colorless prism	
melting point	216 - 218 C		213 - 215 C	
molecular weight	269.78		265	
chemical analysis	Found	Calcd. for	Found	Calcd. for
C	48.97	48.97	49.88	49.80
H	5.78	5.79	5.65	5.70
N	25.83	25.96	26.46	26.40
molecular formula	$C_{11}H_{15}N_5O_3 \cdot 1/4H_2O$		$C_{11}H_{15}N_5O_3$	

lized strain of S. citricolor IFO 13005 maintains the aristeromycin producing ability of the strain before lyophilization.

Discussion

Useful substance producer must preserve production ability for a long period.

When some special strains, such as novobiocin or streptomycin producing strains, had been preserved for 25 years at 20 C below zero using paraffin sealing or lyophilization, most showed almost the same antimicrobial activities as those shown 25 years earlier using the same method used (1). Of 200 lyophilized Streptomyces strains selected at random from ISP strains, about 90% showed the same, or almost the same, antimicrobial spectra as those of the corresponding strains repeatedly transplanted from agar medium to agar medium for the same period (5). However, this conclusion is due only to the recognition of antimicrobial spectra as phenomena, but not to the confirmation of the substance produced. During preservation for more than ten years, it is possible for antibiotic producing strains to change their pathway of antibiotic production. If a variant produces another substance, which has the same antimicrobial spectrum and a different chemical structure, it can not be concluded that the strain produces the same antibiotic in its cultured broth judging

only by its antimicrobial spectra. S. citricolor IFO 13005 produces the antibiotic aristeromycin in its filtered broth whose chemical structure is related to that of adenosine. Among antibiotics isolated from the cultured broth of actinomycetes, formycin (2) is reported to have the same characteristic antimicrobial spectrum as that of aristeromycin and their adenosine related structures resemble each other. Our experimental result indicated that the L strain of this species showed a characteristic antimicrobial spectrum and the production of aristeromycin in its broth. We conclude that this species holds its antibiotic ability in a lyophilized condition.

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DESCRIPTIVE CATALOGUE OF IFO FUNGUS
COLLECTION IX.

In routine identification work on fungi newly isolated in Japan and in checking the list of the 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 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 published in other papers. The authors of the descriptions of these fungal taxa are shown in parentheses.

81. Acrophialophora levis Samson & Tariq Mahmood

(Fig. 1, A & B) Hyphomycetes Acta Bot. Neerl. 19: 807 (1970); Morinaga et al., Journ. Jap. Bot. 54: 1 (1979); Ito et al., IFO Res. Comm. 10: 20 (1981).

Colonies on malt agar floccose, at first white, later becoming pale yellow to light brown, sometimes pale grey, radially furrowed, rarely with sectors; reverse fuscous to dark brown, or almost black, sometimes orange-brown at the inoculated site. Conidiophores rarely formed, erect, stout, coursely rough-walled, brown, 600-1300 x 2.5-4.0 μm. Phialides mainly formed on aerial mycelium, rarely on undifferentiated conidiophores, flask-shaped to ampulliform, hyaline, smooth, 8-12 x 3-4 μm. Conidia ellipsoidal to fusiform, hyaline, smooth, 4-7.5 x 2.5-3.5 μm.

Growth is good at 37 C.

Hab. paddy field soil; Hachioji-cho, Ikeda, Osaka Pref., 8 November, 1976, T. Yokoyama YII42-1-5-6 (IFO 31155); Shakudo, Habikino, Osaka Pref., 22 November, 1976, T. Yokoyama XII42-4-5-6 (IFO 31156) and 21 August, 1978, T. Yokoyama XIX42-3-5-4 (IFO 31157).
Additional strains examined: IFO 30991 (CBS 102.61), IFO 30992 (CBS 942.69), IFO 30993 (CBS 484.70, type = ATCC 22557), IFO 30994 (CBS 732.70).

This fungus has been isolated from soil and compost and the most remarkable feature by which the fungus can be separated from the other two species is its hyaline smooth conidia.

In the course of our three year investigation on the fungus flora of the paddy field soils in Osaka prefecture, we obtained three isolates by incubating soil plates at 42 C. We temporally identified them as A. fusispora at that time. After reexamining the fungus, we concluded that these three isolates are conspecific to A. levis (Ito et al., 1981). Morphologically all these isolates agree well with the description given by Samson and Mahmood (1970), except that pigmented conidiophores are formed in very small numbers as known in the strain CBS 102.61.

Morinaga et al.(1979) described the species as a rare fungus from soil in Singapore.

(T. Ito & T. Yokoyama)

82. Gamsia dimera (Gams) Morelet (Fig. 1, C & D) Hyphomycetes
Ann. Soc. Sci. nat. Archeol. Toulon 21: 105 (1969); Ellis, More Dematiaceous Hyphomycetes, p. 349 (1976); Morinaga et al., Journ. Jap. Bot. 54: 3 (1979).
Syn. Wardomyces dimerus Gams, Trans. Brit. Mycol. Soc. 51: 800 (1968).

Colonies on modified Leonian's agar growing rapidly, restrict, velvety, dark grey to black, partially floccose; reverse almost black. Conidiogenous cells of two types; one monoblastic, scattered, straight or flexuous, simple or branched, hyaline, smooth, thin-walled, percurrent, cylindrical at the base, slightly attenuated towards the apex, indistinctly annellate, 15-50 x 2.5-3.5 µm; the other polyblastic, determinate, cylindrical or clavate, hyaline, smooth, thin-walled, 10-30 x 2-3 µm. Conidia of two kinds; one catenate, two-celled, ellipsoidal to oval, with a broad basal scar, hyaline, smooth, 7-9 x 3.5-5 µm; the other single, one-celled, pear-shaped or navi-

cular, truncate at the base, brown to dark brown, smooth, mostly with a longitudinal germ slit, 7-11 x 4-6 μm.

Growth is nil at 37 C.

Hab. paddy field soil, Nakatsu-cho Ibaraki, Osaka Pref., 16 January, 1978, T. Yokoyama ZVII-1-10-30 (IFO 30713).

This fungus was originally described by Gams as Wardomyces dimerus based on three isolates from wheat field soils in the Netherlands, together with additional isolate made by Hennebert from air contaminant in Belgium.

Morelet (1969) transferred this species to Gamsia mainly because of the dimorphic characteristic of the conidial structures. Morinaga et al. (1979) also reported the fungus from soil in Hongkong as a noteworthy species.

(T. Ito & T. Yokoyama)

83. Stachybotrys bisbyi (Srinivasan) Barron (Fig. 1, E & F) Hyphomycetes Mycologia 56: 315 (1964); Watanabe, Trans. Mycol. Soc. Japan 16: 176 (1975); Jong and Davis, Mycotaxon 3: 409 (1976).

Syn. Hyalostachybotrys bisbyi Srinivasan, J. Ind. Bot. Soc. 37: 341 (1958).
Hyalostachybotrys sacchari Srinivasan, J. Ind. Bot. Soc. 37: 341 (1958).
Stachybotrys aurantia Barron, Can. J. Bot. 40: 258 (1962).

Colonies on malt agar growing rapidly, plane or partly floccose, salmon to peach color; reverse at first uncolored, later becoming pale buff; slimy heads formed. Conidiophores arising from submerged and aerial mycelium, terminating in a cluster of 4-6 phialides, thick-walled, slightly roughed, simple or branched one to three times near the base, 4-6 septate, gradually tapering towards the apex, 80-120 μm long, 3.5-4.5 μm wide near the base, 3-3.5 μm wide at the apex. Phialides hyaline, smooth or slightly roughed, closely arranged, short clavate to cylindrical, unseptate, 12-16 x 3.5-4 μm. Conidia borne on the tip of phialides and aggregated in a slimy ball on the phialides, variable in size and shape, elliptical, oval to ovate, continuous, hyaline to slightly pale peach, smooth, 6-9 x 3-5.5 μm.

Growth is nil at 37 C.

Hab. paddy field soil, Shakudo, Habikino, Osaka Pref., 22 November, 1976, T. Yokoyama XII-3-10-1 (IFO 30399).

This fungus was originally described by Srinivasan as the type species of his new genus, Hyalostachybotrys, only because of the hyaline or sub-hyaline nature of conidiophores and conidia (Srinivasan, 1958). However, Barron (1964) reduced Hyalostachybotrys to synonymy with Stachybotrys because he argued that a genus based on the color of the conidium would have no taxonomic validity. At the same time, he recognized that Stachybotrys aurantia Barron (1962) is conspecific to S. bisbyi (1958). Later, Jong and Davis (1976) concluded that S. sacchari is a synonym of S. bisbyi because there is no clear difference between their conidial morphology.

(T. Ito & T. Yokoyama)

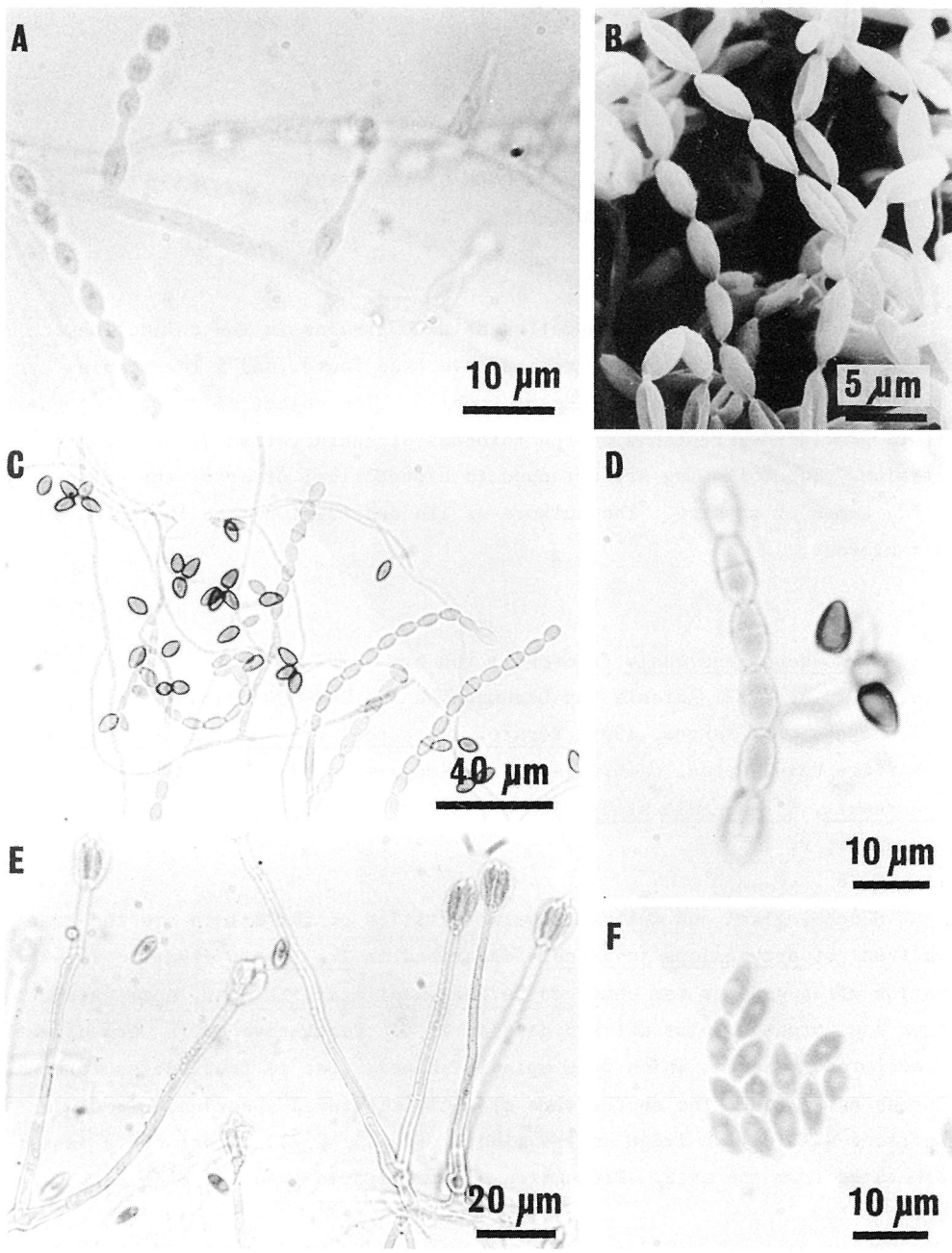

Fig. 1. A & B. Acrophialophora levis (IFO 31156). A. Conidial structures. B. Conidia. C & D. Gamsia dimera (IFO 30713). C. Conidial structures. D. Two types of conidia. E & F. Stachybotrys bisbyi (IFO 30399). E. Conidial structures. F. Conidia.

IFO Res. Comm. **12**,
112-119, 1985 (March)

DESCRIPTIVE CATALOGUE OF IFO YEAST COLLECTION V.

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 the taxonomical characteristics of these strains. The following are arranged in alphabetical order of the scientific names of strains. The authors of the descriptions are indicated in parentheses.

30. Arthroascus javanensis (Kloecker) von Arx

von Arx, J.A. 1972. Antonie van Leeuwenhoek 38: 289-309; Barnett, A.J., R.W. Payne, & D. Yarrow. 1983. Yeasts: characteristics and identification. Cambridge Univ. Press, Cambridge p. 66; Kreger-van Rij, N.J.W. 1984. In The Yeasts, a taxonomic study, 3rd ed. N.J.W. Kreger-van Rij. Elsevier, Amsterdam, P. 114-116.

IFO 10138

Morphological and cultural characteristics of the strain are the same as those of Arthroascus javanensis described in The Yeasts (1984). Formation of ascospores was observed on corn meal agar, YM agar, malt extract agar and Gorodkowa agar after 5 days at 24 C. Vegetative cells were directly transformed to asci, which were spindle-shaped. One to four spores were formed per ascus. The spores were slightly flattened spherical and smooth surfaced with a fine ledge in the middle (Figs. 1 & 2). Spores were easily liberated from the asci. Production of many spores gave the culture a brown color.

Physiological characteristics presented in Table 1 differ from those of the standard description of the species in The Yeasts (1984) with respect to ability to ferment glucose, presence of sucrose assimilation and absence

of succinate assimilation. But it has been found by Barnett et al. that assimilation of sucrose and succinate are variable with strains in the species. Difference only in the ability of fermentation is not sufficient to distinguish this strain as a separate species from Arthroascus javanensis.

IFO 10138 was isolated from the flower of Tricyrtis flava collected in a forest in Miyazaki Prefecture on Oct. 13, 1978 (isolation No. My-3n9).

(K. Mikata)

31. and 32. Candida diversa Ohara, Nonomura et Yunome ex van Uden et Buckley

Ohara, Y., H. Nonomura, & H. Yunome. 1960. Bull. Res. Inst. Ferment. Yamanashi Univ. 7: 3-5; Meyer, S.A., D.G. Ahearn, & D. Yarrow. 1984. In The Yeasts, a taxonomic study, 3rd ed. N.J.W. Kreger-van Rij, Elsevier, Amsterdam. p. 664-665.

IFO 0861 and 1085.

The two strains entered under the name Torulopsis glabrata in IFO List of Cultures 6th ed. 1978, were reidentified as Candida diversa on the basis of a reexamination of their properties.

Morphological and cultural characteristics of both strains are almost the same as those described in The Yeasts (1984).

Physiological characteristics are presented in Table 1.

(K. Mikata)

33 - 35. Issatchenkia scutulata (Phaff, Miller et Miranda) Kurtzman, Smiley et Johnson var. exigua (Phaff, Miller et Miranda) Kurtzman, Smiley et Johnson

Kurtzman, C.P., M.J. Smiley, & C.J. Johnson. 1980. Int. J. Syst. Bacteriol. 30: 503-513.

IFO 10049, 10050, and 10051

Morphological and cultural characteristics of the three strains are almost the same as those of Issatchenkia scutulata var. exigua, described by Kurtzman et al. (1980). Formation of ascospores was observed on corn meal agar and YM agar after 1 week at 24 C. Single vegetative cells were directly transformed into asci. Two to four ascospores were produced per ascus. The spores were spherical with a warty surface (Fig. 3). The presence of many spores made the colony brown. The three strains were heterothallic. Haploid cultures from single spores of IFO 10049 each conjugated

with opposite mating type cultures of type strains (IFO 1896, NRRL Y-10920) of this variety, and produced viable ascospores.

Physiological characteristics are presented in Table 1. The three strains differ from the type strain in absence of fermentation and weak growth at high osmotic pressure. On the basis of their interfertility with the type strain and absence of assimilation of glycerol, however, the three are regarded as members of the variety I. scutulata var. exigua.

The three strains were isolated from mushrooms collected in forest around Lake Towada, Aomori Prefecture on Oct. 5, 1977 (isolation Nos. Tw-31a3, Tw-32a2, and Tw-35a2). This is the first record of the variety in Japan. Two mating types of IFO 10049 have been deposited as IFO 10079 and IFO 10080.

(K. Mikata and I. Banno)

36 to 39. Issatchenkia scutulata (Phaff, Miller et Miranda) Kurtzman, Smiley et Johnson var. scutulata
Kurtzman, C.P., M.J. Smiley, & C.J. Johnson. 1980. Int. J. Syst. Bacteriol. 30: 503-513.
IFO 10068, 10083, 10084, and 10085

Morphological and cultural characteristics of the two strains are almost the same as those described by Kurtzman et al. (1980). Formation of ascospores was observed on corn meal agar and YM agar after one week at 24 C. Single vegetative cells were directly transformed to asci which contained mainly four ascospores. The spores were spherical with a warty surface (Fig. 4). The strains were heterothallic. Haploid cultures from single spores of strains IFO 10068 and 10085 were each conjugated with opposite mating type cultures of both type strain of I. scutulata var. exigua (IFO 1896) and I. scutulata var. scutulata (IFO 1895, NRRL Y-7663), and produced asci containinig two to four warty ascospores in them.

Physiological characteristics are presented in Table 1.

On the basis of their rapid fermentation of glucose, assimilation of glycerol and ability to grow at a moderately high osmotic pressure, the four strains are regarded as members of the variety I. scutulata var. scutulata.

IFO 10068 and IFO 10084 were isolated from partially decayed leaves and soil collected in a forest near Lake Towada, Aomori Prefecture on Oct. 5, 1977 respectively (isolation Nos. Tw-52m6 and Tw-118a2); IFO 10083 from

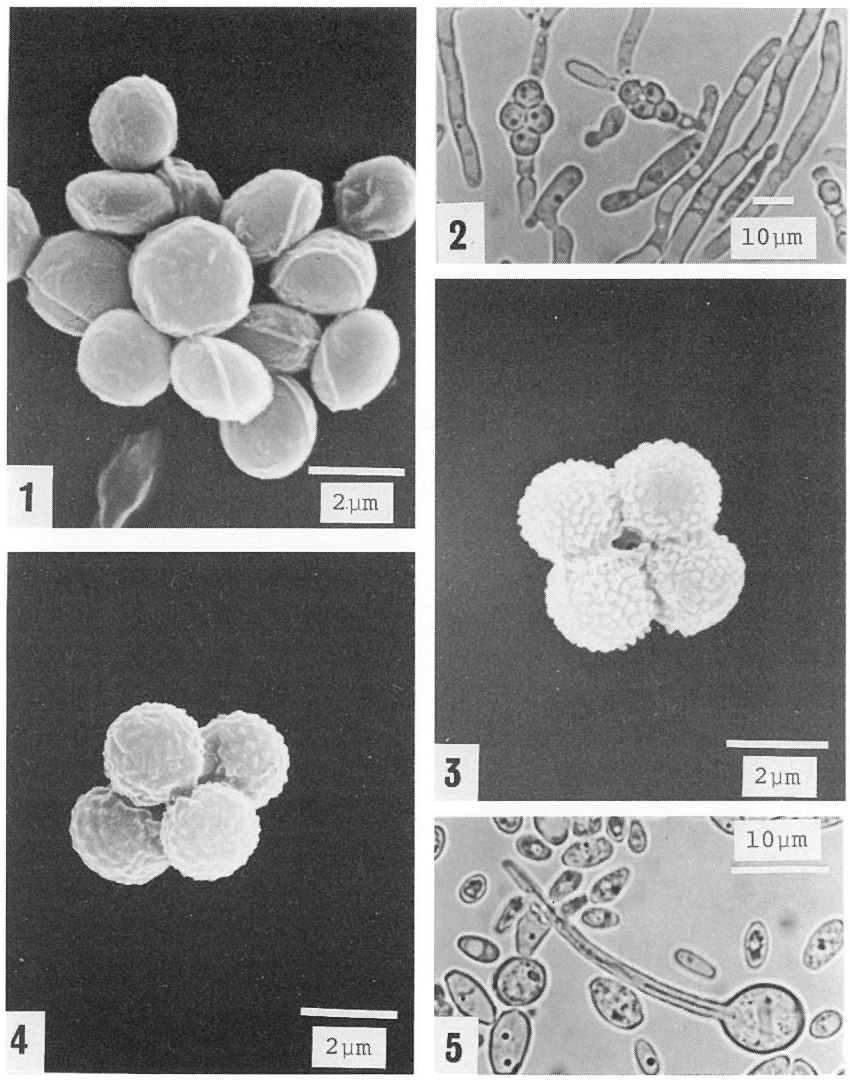

Fig. 1. Scanning electron microscopy of ascospores from *Arthroascus javanensis* IFO 10138
Fig. 2. Light microscopy of ascospores from *Arthroascus javanensis* IFO 10138
Fig. 3. Scanning electron microscopy of ascospores from *Issatchenkia scutulata* var. *exigua* IFO 10050
Fig. 4. Scanning electron microscopy of ascospores from *Issatchenkia scutulata* var. *scutulata* IFO 10068
Fig. 5. Light microscopy of a ascus containing needle-shaped ascospore from *Metschnikowia pulcherrima* IFO 0863

Table 1. Physiological characteristics

	A. javanensis IFO 10138	*C. diversa* IFO 0861 IFO 1085	*I. scutulata* var. *exigua* IFO 10049 IFO 10050 IFO 10051
Fermentation			
Glucose	+w	+	-
Galactose	-	-	-
Sucrose	-	-	-
Maltose	-	-	-
Trehalose	-	-	-
Lactose	-	-	-
Raffinose	-	-	-
Inulin	-	-	-
Soluble starch	-	-	-
α-Methyl-D-glucoside	-	-	-
Assimilation of carbon compounds			
Glucose	+	+	+
Galactose	-	-	-
L-Sorbose	-	-	-
Sucrose	+s	-	-
Maltose	-	-	-
Cellobiose	-	-	-
Trehalose	+w	-	-
Lactose	-	-	-
Melibiose	-	-	-
Raffinose	-	-	-
Melezitose	-	-	-
Inulin	-	-	-
Soluble starch	-	-	-
D-Xylose	-	-	-
L-Arabinose	-	-	-
D-Arabinose	-	-	-
D-Ribose	-	-	-
L-Rhamnose	-	-	-
Ethanol	+	+s	+
Glycerol	+w	-	-
Erythritol	-	-	-
Ribitol	-	+s	-
Galactitol	-	-	-
D-Mannitol	-	+	-
D-Glucitol	-	+	-
α-Methyl-D-glucoside	-	-	-
Salicin	-	-	-
DL-Lactic acid	-	-	+w
Succinic acid	-	-	-
Citric acid	-	-	-
Inositol	-	-	-
D-Glucosamine hydrochloride	NT	NT	+w
Splitting of arbutin	-	-	-
Assimilation of			
potassium nitrate	-	-	-
ethylamine hydrochloride	-	+	+
Growth in vitamin-free medium	-	-	+
Growth at 37 C	-	-	-
Growth in 10% sodium chloride plus 5% glucose in yeast nitrogen base	NT	NT	+w
Ubiquinone type	Q8	Q7	Q7

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

I. scutulata var. *scutulata* IFO 10068 IFO 10083-10085	*K. marxianus* var. *bulgaricus* IFO 0617	*M. pulcherrima* IFO 0863	*P. ohmeri* IFO 0158	*T. delbrueckii* IFO 0381 IFO 1083
+	+	+	+	+
-	+	-	+s	-
-	+	-	+	+
-	-	-	-	-/+s
-	-	-	-	-/+s
-	+	-	-	-
-	-	-	+	-/+
-	-	-	-	-/+s
-	-	-	-	-
-	-	-	-	-/+s
+	+	+	+	+
-	+	+	+	-
-	-	+	+	+s
-	+	+	+	+
-	-	+	+	+
-	+	+	+	-
-	-	+	+	+s
-	+	-	-	-
-	-	-	-	-
-	+	-	+	+
-	-	+	-	-
-	-	-	-	+
-	-	-	-	-
-	+	+	-	-
-	+s	-	-	-
-	-	-	-	-
-	-	+	-	-
-	-	-	-	-
+	+s	+	+	+
+w	+s	+	+	+
-	-	-	-	-
-	-	+	+	-
-	-	-	-	-
-	-	+	+	+
-	+s	+	+	+
-	-	+	+	-/+
-	+	+	-	-
+	+	+ws	+w	+
-	+s	+	-	-
-	-	-	+	-
-	-	-	-	-
-	NT	NT	NT	NT
-	+	+	+s	-
-	-	-	-	-
+	+	+	+	-
+	-	-	-	+
-	+	-	+	-
+w	NT	NT	NT	NT
Q7	Q6	NT	Q9	Q6

partially decayed leaves collected at Mt. Daisen, Tottori Prefecture, on Aug. 7, 1975 (isolation No. Di-207u2); IFO 10085 from partially decayed leaves collected in a forest in Miyazaki Prefecture on Oct. 13, 1978 (isolation No. Mm-22a6). This is the first record of the variety in Japan. Two mating types of IFO 10068 have been deposited as IFO 10069 and IFO 10070.

(K. Mikata and I. Banno)

40. Kluyveromyces marxianus (Hansen) van der Walt var. bulgaricus (Santa Maria) Johannsen et van der Walt

Johannsen, E., & J.P. van der Walt. 1980. Arch. Microbiol. 118: 45-48; Kreger-van Rij, N.J.W., & E. Johannsen. 1984. In The Yeasts, a taxonomic study. 3rd ed. N.J.W. Kreger-van Rij. Elsevier, Amsterdam p. 236-237.

IFO 0617

This strain, which has been deposited under the name Candida pseudotropicalis, was reidentified, since production of ascospores was found.

Morphological and cultural chracteristics of the strain are almost the same as those of Kluyveromyces marxianus var. bulgaricus described in The Yeasts (1984). Formation of ascospores was observed on corn meal agar after 5 days at 24 C. Vegetative cells were directly transformed into evanescent asci. Two to four spheroidal ascospores were formed per ascus, liberated easily from ascus. The spores were agglutinated.

Physiological characteristics are presented in Table 1.

(K. Mikata)

41. Metschnikowia pulcherrima Pitt et Miller

Pitt, J.I., & M.W. Miller. 1968. Mycologia 60: 663-685; Miller, M.W. & H.J. Phaff. 1984. in The Yeasts, a taxonomic study. 3rd ed. N.J.W. Kreger-van Rij, Elsevier, Amsterdam. p. 273-275.

IFO 0863

This strain, which was deposited under the name Torulopsis famata, was reidentified, since ascospore formation was found.

Morphological and cultural characteristics of the strain are almost the same as those of Metschnikowia pulcherrima described by Pitt et al.

Formation of ascospores was observed on corn meal agar culture after one month at 12 C. Asci arose from spheroidal chlamydospores which generally contained a single, prominent lipid globule. Asci were club shaped,

containing one to two, needle-shaped ascospores(Fig. 5).

Physiological characteristics are presented in Table 1.

(K. Mikata)

42. Pichia ohmeri (Etchells et Bell) Kreger-van Rij

Kurtzman, C.P. 1984. In The Yeasts, a taxonomic study, 3rd ed. N.J.W. Kreger-van Rij. Elsevier, Amsterdam. p. 348-350.

IFO 0158

This strain was obtained from the Government Research Institute of Formosa in 1941 under the name Mycotorula sp. N.

Morphological and cultural characteristics are almost the same as those of Pichia ohmeri described in The Yeasts (1984). The strain produced ascospores when mated with a mating type strain IFO 1483 (NRRL Y-2079). Asci including four ascospores were formed on 10% malt extract agar slant after one week at 24 C. The spores were hat-shaped.

Physiological characteristics are presented in Table 1.

(K. Mikata & I. Banno)

43 and 44. Torulaspora delbrueckii (Lindner) Lindner

Yarrow, D. 1984. In The Yeasts, a taxonomic study. 3d ed. N.J.W. Kreger-van Rij. Elsevier, Amsterdam. p. 435-437.

IFO 0381 and 1083

The two strains, which were deposited under the name Torulopsis colliculosa, were taxonomically reexamined, since ascospore formation was found.

Morphological and cultural characteristics are almost the same as those of Torulaspora delbrueckii described in The Yeasts (1984). Formation of ascospores was observed on corn meal agar after one week at 17 C. Conjugation between a cell and its bud preceded the formation of asci containing one to two, globose and rough ascospores. Cells with protuberances which resemble a copulation tube were also observed.

Physiological characteristics are presented in Table 1.

(K. Mikata & I. Banno)

IFO Res. Comm. 12,
120-121, 1985 (March)

DESCRIPTIVE CATALOGUE OF IFO BACTERIAL COLLECTION VII.

The purpose of this catalogue is to describe the taxonomic properties of strains which had been misclassified but have since been reclassified in routine reidentification work on the IFO bacterial collection.

71. Bacillus alvei Cheshire & Cheyne

IFO 14175

This strain was obtained from M. Kitamikado, Faculty of Agriculture, Kyushu University, Fukuoka, Japan, as a microorganism which produces endo-β-N-acetylglucosamidase.* It was identified as Bacillus alvei by the following properties.

Cells: Gram-negative rods, 0.5-0.6 x 1.5-4.0 μm; motile by peritrichous flagella.

Spores: Elliptical shape; dominant position is central; sporangia distinctly swollen.

Colonies on nutrient agar: Irregular, thin, translucent, whitish gray; give rise to small motile colonies.

Catalase is produced.

Voges-Proskauel test is positive.

Indole and dihydroxyacetone are produced.

Nitrate is not reduced to nitrite.

Growth in anaerobic agar** is positive.

Growth in 0.001% lysozyme broth is positive.

Casein is hydrolyzed.

Temperature relations: Grows between 20 and 38 C, but not at 45 C.

Acid but no gas from glucose, ribose and trehalose. No acid no gas from arabinose, lactose and xylose.

The G+C content of the DNA is 47.8 mol% (Tm).

* Morinaga, T., M. Kitamikado, H. Iwase, S. Li, and Y. Li. 1983. Biochem. Biophys. Acta. 749: 211-213.
** Gordon, R.E., W.C. Haynes, and C.H-N. Pang. 1973. The genus Bacillus. U.S.Dept. Agr. Handbook No. 427, Washington, D.C.

(T. Sakane)

72. Cytophaga keratolytica (Kitamikado and Ito) Imai
IFO 14087

This strain was deposited under the name of Flavobacterium keratolyticus Kitamikado and Ito but reclassified as a species of the genus Cytophaga by the following properties: Cells were gram-negative and no flagellation. Obvious gliding movement was not found. Hydrolysis of gelatin and starch was positive. Cellulose, agar and chitin were not hydrolyzed. Menaquinone was the sole respiratory quinone, and the menaquinone isoprenologue was unsaturated MK-7. The G+C content of DNA was 40 mol%.

(K. Imai)

ANNOUNCEMENTS

CULTURE COLLECTION OF ANIMAL CELL LINES AT IFO

The Institute for Fermentation, Osaka (IFO) started to collect animal cell lines in May, 1984. The IFO accepts for deposit new cell lines which have properties of special interest. Individuals wishing to deposit a cell line should write to the curator giving relevant details, including characteristics and conditions of distribution. When the cell line is considered to be acceptable on the basis of this information, the IFO will send the depositor a data sheet for the accession of a cell line (IFO DATA SHEET FOR ACCESSION). Before acceptance of a cell line the Accession Form must be completed by the depositor. It should be sent to the IFO with several bottles of the cultured cell line, if possible, and copies of papers citing the cell line. Frozen cell lines are also acceptable. All cell cultures are maintained in the frozen state using liquid nitrogen (-190°C) and are stored for many years.

The IFO stands on the principle that all of the microorganisms and cell lines preserved are open to the public. If the depositor desires to impose some restrictions on distribution, the IFO accepts cultured cell lines for safe deposit with all proprietary rights to the cell line retained by the depositor.

One of the most important functions of the IFO is to distribute mycoplasma (and other microorganisms)-free cell lines. The cell lines have been tested for mycoplasmal contamination using modified Chanock's medium and staining with fluorescent stain. The IFO has also carried out analyses of some cellbiological properties of these cell lines, such as examinations of species verification and karyotype analysis. In the section "Catalogue of newly accepted strains" of this annual report, we show animal cell lines which are not contaminated with mycoplasmas. Furthermore, we will list the collected cell lines in the eighth edition of the IFO catalogue, after characterization of the cell lines have been finished.

(Masao Takeuchi)

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財団法人 発酵研究所

IFO DATA SHEET

(Cell lines)

(IFO Form AC1)

The depositor is requested
to supply data on this form.
Please print clearly.

Do not write in this box.

IFO No.
Name of Bank
Stock No.
Reception No.
Reception Date
Accession Date

1. Name of this cell line:
   1.1. Scientific name, name of cell _____
   1.2. Common name or synonym _____
   1.3. Type of cell _____
   1.4. Name of original cell line _____
   1.5. Reference citing this cell line _____
2. Depositor : 2.1. Name _____
   2.2. Address _____
   _____ 2.3. Tel. _____
3. Establisher: 3.1. Name _____
   3.2. Address _____
   3.3. Name of established cell line _____
   3.4. Reference citing the established cell line _____
   _____
4. History of this cell line since the establishment: IFO ← Depositor ← _____
   _____
   _____
   _____
5. Source of the established cell line: 5.1. Genus _____
   5.2. Species _____ 5.3. Race _____
   5.4. Organ _____ 5.5. Tissue _____
6. Characteristic of the source: 6.1. Sex _____
   6.2. Developing stage _____
   6.3. Clinical phenotype _____
   6.4. Genetical background _____
7. Parent cell of hybridoma: 7.1. Name of the parent cell _____
   7.2. Reference _____
   7.3. Accession No. of IFO or other organization _____
8. Characteristic of this deposited cell line: _____
   8.1. Morphology _____
   8.2. Growth: 8.2.1. Number of subcultivation since the establishment _____
   8.2.2. Cloning _____

(Continued on reverse side)

IFO

8.2.3. Colony formation

8.2.4. Plating efficiency 8.2.5. Doubling time

8.3. Pathogenicity

8.4. Tumorigenicity: 8.4.1. Type of tumor

8.4.2. Host used 8.4.3. *In vivo* growth

8.5. Virus: 8.5.1. Viral particle

8.5.2. Viral gene

8.5.3. Virus susceptibility

8.6. Hereditary and biochemical characteristics: 8.6.1. Karyology

8.6.2. Genetic marker

8.6.3. Isoenzyme marker

8.6.4. Enzyme marker

8.6.5. Surface marker

8.6.6. Factor produced

8.7. Others

9. Cultivation: 9.1. Medium

9.2. Serum 9.3. Additive

9.4. Procedure of subcultivation

9.5. Inoculum size 9.6. Subcultivation interval

9.7. Saturation density

9.8. Temperature 9.9. Viability

10. Freezing and thawing: 10.1. Freezing medium

10.2. Procedure for thawing

10.3. Viability after thawing

10.4. Stability in frozen state

11. Sterility

12. Distribution (free or restricted)

13. Patent No.

14. Reason for deposition: 14.1. Request (IFO or depositor)

14.2. Importance or application of this cell line

15. Remarks

16. Please write in Japanese (Domestic use only):

16.1. Name of depositor

16.2. Address

_____, _____

Date ***Signature of depositor***

CATALOGUE OF NEWLY ACCEPTED STRAINS

Sep. 1982 - Sep. 1984

(NUMERICAL)

The culture involved in this catlogue can be distributed under the same condition as strains listed in IFO LIST OF CULTURES 7TH EDITION 1984.

10075 Rhodosporidium toruloides
HISTORY: IFO (K. Mikata; R-3-75; air).
TEMP: 24 MEDIUM: 101

10076 Rhodosporidium toruloides
HISTORY: IFO (K. Mikata; R-5-33; air).
TEMP: 24 MEDIUM: 101

10077 Issatchenkia scutulata var. exigua
HISTORY: IFO (K. Mikata; 1896(1A); single spore isolate of IFO 1896).
TEMP: 24 MEDIUM: 101

10078 Issatchenkia scutulata var. exigua
HISTORY: IFO (K. Mikata; 1896(1B); single spore isolate of IFO 1896).
TEMP: 24 MEDIUM: 101

10079 Issatchenkia scutulata var. exigua
HISTORY: IFO (K. Mikata; 10049(2A); single spore isolate of IFO 10049).
TEMP: 24 MEDIUM: 101

10080 Issatchenkia scutulata var. exigua
HISTORY: IFO (K. Mikata; 10049(2B); single spore isolate of IFO 10049).
TEMP: 24 MEDIUM: 101

10081 Issatchenkia scutulata var. scutulata
HISTORY: IFO (K. Mikata; 1895(3A); single spore isolate of IFO 1895).
TEMP: 24 MEDIUM: 101

10082 Issatchenkia scutulata var. scutulata
HISTORY: IFO (K. Mikata; 1895(3B); single spore isolate of IFO 1895).
TEMP: 24 MEDIUM: 101

10083 Issatchenkia scutulata var. scutulata
HISTORY: IFO (K. Mikata; Di-207u2; partially decayed leaf).
TEMP: 24 MEDIUM: 101

10084 Issatchenkia scutulata var. scutulata
HISTORY: IFO (K. Mikata; Tw-118a2; soil).
TEMP: 24 MEDIUM: 101

10085 Issatchenkia scutulata var. scutulata
HISTORY: IFO (K. Mikata; Mm-22a6; partially decayed leaf).
TEMP: 24 MEDIUM: 101

10086 Issatchenkia scutulata var. scutulata
HISTORY: IFO (K. Mikata; Mm-22a6(1A); single spore isolate of Mm-22a6).
TEMP: 24 MEDIUM: 101

10087 Issatchenkia scutulata var. scutulata
HISTORY: IFO (K. Mikata; Mm-22a6(1C); single spore isolate of Mm-22a6).
TEMP: 24 MEDIUM: 101

10088 Issatchenkia sp.
HISTORY: IFO (K. Mikata; Tw-32m7; mushroom).
TEMP: 24 MEDIUM: 101

10090 Pichia kodamae
HISTORY: K. Kodama -- CBS 7081 -- J.P. van der Walt.
TEMP: 24 MEDIUM: 101

10092 Saccharomyces cerevisiae
HISTORY: B. Ono, IS419-23A, recombinant, genotype: alpha ade1 cdc19 gal1 his7 leu2-1o lys2 met8-1a pet9 tyr1o.
TEMP: 24 MEDIUM: 109

10093 Saccharomyces cerevisiae
HISTORY: B. Ono, IS448-4C, recombinant, genotype: alpha ade8 aro1B cdc9 his4-1176o ilv1-2o leu2-1o lys1-1o met8-1a trp4.
TEMP: 24 MEDIUM: 109

10094 Saccharomyces cerevisiae
HISTORY: B. Ono, IS447-19B, recombinant, genotype: alpha can1 cdc4 his2 hom3 leu2-1o lys9o met1 pet8 rad4 trp2 ura3.
TEMP: 24 MEDIUM: 109

10095 Saccharomyces cerevisiae
HISTORY: B. Ono, IS488-21A, recombinant, genotype: alpha arg4 cdc11 his6 ilv3 leu2-1o lys11o trp3 ura2.
TEMP: 24 MEDIUM: 109

10096 Saccharomyces cerevisiae
HISTORY: B. Ono, IS409-6D, recombinant, genotype: alpha ade3 asp5 gal2 leu2-1o rna1.
TEMP: 24 MEDIUM: 109

10097 Saccharomyces cerevisiae
HISTORY: B. Ono, IS483-3D, recombinant, genotype: alpha ade2 arg1 aro7-1a gal4-69 his3 leu2-1o lys4 met4 pet17 prt1 rad1 trp4 ura2 ade2-linked gal.
TEMP: 24 MEDIUM: 109

10098 Saccharomyces cerevisiae
HISTORY: B. Ono, IS446-12C, recombinant, genotype: alpha ade5 cyh2 his5-2o leu2-1o lys5 met2 pet14 pha2 rna3 trp5.
TEMP: 24 MEDIUM: 109

10099 Saccharomyces cerevisiae
HISTORY: B. Ono, IS475-18B recombinant, genotype: alpha ade6 arg8 leu2-1o lys7 pet trp1-1a ura4-1o.
TEMP: 24 MEDIUM: 109

10100 Saccharomyces cerevisiae
HISTORY: B. Ono, IS481-4B, recombinant, genotype: alpha asp5 cdc4 leu2-1o pet17.
TEMP: 24 MEDIUM: 109

10101 Saccharomyces cerevisiae
HISTORY: B. Ono, IS491-13C, recombinant, genotype: alpha arg4 aro7-1a his4-1176o leu2-1o lys1-1o met14 trp1-1a.
TEMP: 24 MEDIUM: 109

10102 Saccharomyces cerevisiae
HISTORY: B. Ono, IS502-7C, recombinant, genotype: alpha ade1 arg4 his6 ilv3 leu2-1o lys7 trp5 ura3.
TEMP: 24 MEDIUM: 109

10103 Candida agrestis
HISTORY: RIFY 4611 (S. Goto; No. 611; Vitis coignetiae).
TEMP: 24 MEDIUM: 101

10104 Candida montana
HISTORY: RIFY 4865 (S. Goto; No. 865; Vitis coignetiae).
TEMP: 24 MEDIUM: 101

10105 Rhodotorula fujisanensis
HISTORY: RIFY 4870 (S. Goto; Vitis coignetiae).

TEMP: 24 MEDIUM: 101

10106 Pichia guilliermondii
HISTORY: T. Shinoda, M 5004 -- CBS 2030 -- NRRL Y-2075 (L.J. Wickerham; frass on Ulms americana).
TEMP: 24 MEDIUM: 101

10107 Pichia guilliermondii
HISTORY: T. Shinoda, M 5003 -- CBS 2031 -- NRRL Y-2076 (L.J. Wickerham; frass on Ulms americana).
TEMP: 24 MEDIUM: 101

10108 Candida albicans
HISTORY: T. Shinoda, M 1445 -- Division of Mycotic Diseases, CDC (L. Kaufman) -- NIH, B-792.
TEMP: 24 MEDIUM: 101

10109 Hansenula arabitolgenes
HISTORY: AS 2.887 (Chang S.-C.; 275; jam hongguojiang).
TEMP: 24 MEDIUM: 101

10110 Rhodosporidium infirmo-miniatum
HISTORY: AS 2.1391 (Lee M.-H.; L-82; frozen diseased pear).
TEMP: 24 MEDIUM: 101

10111 Saccharomycodes sinensis
HISTORY: AS 2.1395 (Yue, J.-Z.; HS 506; soil in forest).
TEMP: 28 MEDIUM: 101

10112 Bullera armeniaca
HISTORY: CBS 7091 -- R.W.M. Buhagiar, cabbages.
TEMP: 17 MEDIUM: 101

10113 Bullera crocea
HISTORY: CBS 6714 -- R.W.M. Buhagiar, strawberries.
TEMP: 17 MEDIUM: 101

10114 Bullera dendrophila
HISTORY: CBS 6074 -- J.P. van der Walt, frass of Buprestid larvae in Dichrostachys cinerea
TEMP: 24 MEDIUM: 101

10115 Bullera grandispora
HISTORY: CBS 6982 -- R.J. Bandoni, decayed wood.
TEMP: 17 MEDIUM: 101

10116 Bullera piricola
HISTORY: CBS 6754 -- F. Stadelmann, 141L, pear leaf.
TEMP: 17 MEDIUM: 101

10117 Sporopachydermia quercuum
HISTORY: CBS 8070 -- M.-A. Lachance, UWO(PS) 80-118, exudate from Quercus rubra.
TEMP: 24 MEDIUM: 101

10118 Sterigmatomyces nectairii
HISTORY: CBS 6405 -- E.G. Dale, cheese.
TEMP: 24 MEDIUM: 101

10119 Sterigmatomyces penicillatus
HISTORY: CBS 5492 -- G. Kraepelin, contaminant on agar plate.
TEMP: 24 MEDIUM: 101

10120 Sterigmatomyces polyborus
HISTORY: CBS 6072 -- J.P. van der Walt, tunnel of Xyleborus torquatus in Cussonia umbellifera.
TEMP: 24 MEDIUM: 101

10121 Sterigmatosporidium polymorphum
HISTORY: CBS 8088 -- G. Kraepelin, Ra 18, pitwood planks.
TEMP: 24 MEDIUM: 101

10122 Sterigmatosporidium polymorphum
HISTORY: CBS 8089 -- G. Kraepelin, Ra 18-a, single spore isolate from Ra 18.
TEMP: 24 MEDIUM: 101

10123 Sterigmatosporidium polymorphum
HISTORY: CBS 8090 -- G. Kraepelin, Ra 18-alpha, single spore isolate from Ra 18.
TEMP: 24 MEDIUM: 101

10124 Aciculoconidium aculeatum
HISTORY: ATCC 28680 -- CBS 5578 -- L. do Carmo-Sousa -- H.J. Phaff, UCD 51-227, Drosophila occidentalis.
TEMP: 24 MEDIUM: 101

10125 Aciculoconidium aculeatum
HISTORY: ATCC 15540 -- D. Dupont -- NRRL YB-4297.
TEMP: 24 MEDIUM: 101

10126 Cryptococcus asgardensis
HISTORY: ATCC 46399 -- H.S. Vishniac, MYSW 302Y310, soil.
TEMP: 17 MEDIUM: 101

10127 Cryptococcus lupi
HISTORY: ATCC 44529 -- H.S. Vishniac, MYSW 202Y252, soil.
TEMP: 17 MEDIUM: 101

10128 Cryptococcus vishniacii var. asocialis
HISTORY: ATCC 46402 -- H.S. Vishniac, MYSW 302Y312, soil.
TEMP: 17 MEDIUM: 101

10129 Phaffia rhodozyma
HISTORY: ATCC 24202 -- H.J. Phaff, UCD 67-210, exudate from Fagus crenata.
TEMP: 24 MEDIUM: 101

10130 Phaffia rhodozyma
HISTORY: ATCC 24228 -- H.J. Phaff, UCD 68-653C, exudate from Betula papyrifera.
TEMP: 24 MEDIUM: 101

10131 Sarcinosporon inkin
HISTORY: ATCC 18020 -- L.R. Hedrick -- IGC 3727.
TEMP: 24 MEDIUM: 101

10132 Sympodiomyces parvus
HISTORY: ATCC 22869 -- J.W. Fell, 23-188, antarctic water.
TEMP: 17 MEDIUM: 101

14290 Streptomyces rubicolor
HISTORY: Yunnan Inst. Microbiol. (D. Jiang; JH-2710).
TEMP: 28 MEDIUM: 227

14291 Bacteroides vulgatus
HISTORY: Daigo Nutritive Chemicals (H. Yoshino) -- ATCC 8482.
TEMP: 37 MEDIUM: 236

14292 Clostridium sporogenes
HISTORY: Daigo Nutritive Chemicals (H. Yoshino) -- ATCC 7955.
TEMP: 37 MEDIUM: 201

14293 Clostridium sporogenes
HISTORY: Daigo Nutritive Chemicals (H. Yoshino) -- ATCC 11437.
TEMP: 37 MEDIUM: 201

14294 Actinosporangium vitaminophilum
HISTORY: Cent. Res. Lab., Meiji Seika (T. Shomura; SF-2080; soil).

TEMP: 28 MEDIUM: 227

14295 Promicromonospora enterophila
HISTORY: Eötvös L. Univ. (K. Márialigeti; DFA-19; Chromatoiulus projectus millipede).
TEMP: 28 MEDIUM: 230

14296 Promicromonospora enterophila
HISTORY: Eötvös L. Univ. (K. Márialigeti; DFA-17; Chromatoiulus projectus millipede).
TEMP: 28 MEDIUM: 230

14297 Nitrobacter agilis
HISTORY: T. Yamanaka (Tokyo Inst. of Tech.) -- ATCC 14123.
TEMP: 28 MEDIUM: 239

14298 Nitrosomonas europaea
HISTORY: T. Yamanaka (Tokyo Inst. of Tech.) -- J.D. Nicholas (Adelaide, Australia).
TEMP: 28 MEDIUM: 240

14301 Streptomyces sclerogranulatus
HISTORY: IAM (A. Shimazu; 7672-MC_4; soil).
TEMP: 28 MEDIUM: 228

14302 Streptomyces sclerogranulatus
HISTORY: IAM (A, Shimazu; 8898-CC_1; soil).
TEMP: 28 MEDIUM: 228

14303 Streptomyces sclerogranulatus
HISTORY: IAM (A. Shimazu; 9143-S_1; soil).
TEMP: 28 MEDIUM: 228

14304 Micromonospora olivasterospora
HISTORY: Tokyo Res. Lab., Kyowa Hakko Co. (I. Kawamoto; MK70; soil).
TEMP: 28 MEDIUM: 227

14309 Sebekia benihana
HISTORY: Upjohn Co. (A. Dietz & G.P. Li; UC 5762; soil).
TEMP: 28 MEDIUM: 231

14310 Nocardiopsis mutabilis
HISTORY: ATCC 31520 -- Smith Kline & French Labs. (M.C. Shearer; SK & F-AAA025; soil).
TEMP: 28 MEDIUM: 227

14311 Streptosporangium fragile
HISTORY: ATCC 31519 -- Smith Kline & French Labs. (M.C. Shearer; SK & F-BC2496; soil).
TEMP: 28 MEDIUM: 227

14325 Pseudonocardia compacta
HISTORY: Fachbereich Biologie Universität (A. Henssen; MB H-147; soil).
TEMP: 28 MEDIUM: 231

14326 Nocardia sp.
HISTORY: ATCC 31319 -- Chugai Pharm. Co., Ltd. (H. Oishi; 2-200; soil).
TEMP: 28 MEDIUM: 231

14333 Nocardia thermoflava
HISTORY: IMAS (Y.Y. Lu; T526; soil).
TEMP: 45 MEDIUM: 227

14334 Actinopolyspora thermovinacea
HISTORY: IMAS (Y.Y. Lu; T522; soil).
TEMP: 45 MEDIUM: 231

14335 Nocardioides thermolilacinus
HISTORY: IMAS (Y.Y. Lu; T505; soil).
TEMP: 45 MEDIUM: 227

14336 Nocardioides thermolilacinus

HISTORY: IMAS (Y.Y. Lu; T511; soil).
TEMP: 45 MEDIUM: 227

14337 Micropolyspora roseoalba
HISTORY: IMAS (Y.Y. Lu; T496; soil).
TEMP: 45 MEDIUM: 231

14338 Micropolyspora cinereoflava
HISTORY: IMAS (Y.Y. Lu; T493; soil).
TEMP: 45 MEDIUM: 227

14339 Microtetraspora cyaneoviridis
HISTORY: IMAS (Y.X. Deng; 79t-13; soil).
TEMP: 30 MEDIUM: 231

14340 Nocardia fusca
HISTORY: IMAS (Z.H. Liu; 1.128a; soil).
TEMP: 28 MEDIUM: 227

14341 Nocardia flavorosea
HISTORY: IMAS (Z.H. Liu; 10.268-1; soil).
TEMP: 28 MEDIUM: 227

14342 Nocardia flavorosea subsp. fusca
HISTORY: IMAS (Z.H. Liu; 22.29-P; soil).
TEMP: 28 MEDIUM: 231

14343 Pseudonocardia compacta
HISTORY: Fachbereich Biologie Universität (A. Henssen; MB H-146; soil).
TEMP: 28 MEDIUM: 227

14344 Aureobacterium sp.
HISTORY: Osaka City Univ. (A. Misaki) -- Osaka Univ. (S. Kotani; M-2; soil).
TEMP: 30 MEDIUM: 201

14345 Actinomadura polychroma
HISTORY: INA 2755 (T.P. Preobrazhenskaya; soil).
TEMP: 37 MEDIUM: 8

14346 Actinomadura umbrina
HISTORY: INA 2309 (T.P. Preobrazhenskaya; soil).
TEMP: 28 MEDIUM: 228

14347 Actinomadura fulvescens
HISTORY: INA 3321 (T.P. Preobrazhenskaya; soil).
TEMP: 28 MEDIUM: 228

14348 Actinomadura turkmeniaca
HISTORY: INA 3344 (T.P. Preobrazhenskaya; soil).
TEMP: 28 MEDIUM: 227

14349 Thermopolyspora flexuosa
HISTORY: KCC A-0056 -- Moscow State Univ., 435 (N.A. Krassilnikov; soil).
TEMP: 45 MEDIUM: 243

14352 Dactylosporangium roseum
HISTORY: Meiji Seika Kaisha, Ltd. (T. Shomura; SF-2186; soil).
TEMP: 28 MEDIUM: 227

14353 Streptomyces tsukiyonensis
HISTORY: Pharm. Div., Josei Univ. (K. Tsuchiya; AS631; soil).
TEMP: 28 MEDIUM: 227

14354 Micromonospora thermoauberginospora
HISTORY: IMAS (Y.Y. Lu; T524; cow dungs).
TEMP: 28 MEDIUM: 228

14357 Bacillus subtilis
HISTORY: NIH (E. Freese; 61141).
TEMP: 37 MEDIUM: 201

14358 Bacillus pumilus
HISTORY: Univ. Maryland (P.S. Lovett; ATCC 12140).
TEMP: 37 MEDIUM: 201

14359 Escherichia coli
HISTORY: IFO (Y. Kaneko; JA221) -- OUT (T. Oshima).
TEMP: 37 MEDIUM: 201

14360 Escherichia coli
HISTORY: IFO (Y. Kaneko; KBC 12).
TEMP: 37 MEDIUM: 201

14361 Nocardia thermoflava
HISTORY: IMAS (Y.Y. Lu; T521; soil).
TEMP: 45 MEDIUM: 227

14362 Nocardiopsis streptosporus
HISTORY: IMAS (Z.H. Liu; 4-4C; soil).
TEMP: 28 MEDIUM: 227

14363 Rhodococcus marinonascens
HISTORY: Inst. Meeresforschung Bremerhaven (H. Weyland; 3438W; marine sediment).
TEMP: 20 MEDIUM: 244

14367 Bacillus pumilus
HISTORY: National Inst. Hygienic Sciences (M. Kurusu) -- ATCC 27142.
TEMP: 30 MEDIUM: 201

14369 Microtetraspora caesia
HISTORY: Bristol-Myers Co. -- Bristol-Banyu Res. Inst. (K. Tomita; G432-4; soil).
TEMP: 28 MEDIUM: 227

14370 Microtetraspora caesia
HISTORY: Bristol-Myers Co. -- Bristol-Banyu Res. Inst. (K. Tomita; G434-6; soil).
TEMP: 28 MEDIUM: 227

14371 Kitasatosporia griseola
HISTORY: Kitasato Inst., AM-9660 (Y. Takahashi; soil).
TEMP: 28 MEDIUM: 228

14372 Kitasatosporia phosalacinea
HISTORY: Kitasato Inst., KA-338 (Y. Takahashi; soil).
TEMP: 28 MEDIUM: 227

14381 Dactylosporangium fulvum
HISTORY: Pharm. Res. Lab., Meiji Seika Kaisha, Ltd. (T. Shomura; SF-2113; soil).
TEMP: 28 MEDIUM: 227

14382 Microbispora viridis
HISTORY: Cent. Res. Lab., Meiji Seika Kaisha, Ltd. (S. Miyadoh; SF-2240; soil).
TEMP: 28 MEDIUM: 245

20059 Bacillus pumilus phage PBP-1
HISTORY: Univ. Maryland (P.S. Lovett; PBP-1).

20060 Bacillus pumilus phage NP-5
HISTORY: IFO (K. Imai; NP-5).

31383 Acrophialophora fusispora
HISTORY: IFO (T. Yokoyama; R42-1455-2; soil).
TEMP: 28 MEDIUM: 5 54-K-1640

31384 Alternaria alternata

HISTORY: IFO (T. Yokoyama; R1612-7; litter).
TEMP: 24 MEDIUM: 1 54-K-1640

31385 Aspergillus restrictus
HISTORY: IFO (T. Yokoyama; R70-1611-2; Prunus litter).
TEMP: 24 MEDIUM: 3 54-K-1640

31386 Botrytis cinerea
HISTORY: IFO (T. Yokoyama; R1522-3; humus soil).
TEMP: 24 MEDIUM: 1 54-K-1640

31387 Chaetomium globosum
HISTORY: IFO (T. Yokoyama; R1602-16; soil).
TEMP: 24 MEDIUM: 8 54-K-1640

31388 Cladosporium herbarum
HISTORY: IFO (T. Yokoyama; R1470-9; soil).
TEMP: 24 MEDIUM: 1 54-K-1640

31389 Eurotium amsterodami
HISTORY: IFO (T. Yokoyama; RE1500-3; turnip field soil).
TEMP: 28 MEDIUM: 9 54-K-1640

31390 Gliocladium roseum
HISTORY: IFO (T. Yokoyama; R1453-6; soil).
TEMP: 24 MEDIUM: 1 54-K-1640

31391 Humicola grisea
HISTORY: IFO (T. Yokoyama; R1454-14; soil).
TEMP: 24 MEDIUM: 1 54-K-1640

31392 Penicillium claviforme
HISTORY: IFO (T. Yokoyama; R1484-18; clay soil).
TEMP: 24 MEDIUM: 1 54-K-1640

31393 Penicillium frequentans
HISTORY: IFO (T. Yokoyama; R70-1592-2; humus).
TEMP: 24 MEDIUM: 1 54-K-1640

31394 Penicillium thomii
HISTORY: IFO (T. Yokoyama; R1619-7; humus soil).
TEMP: 24 MEDIUM: 1 54-K-1640

31395 Periconia byssoides
HISTORY: IFO (T. Yokoyama; RE1623-1; Larix litter).
TEMP: 24 MEDIUM: 1 54-K-1640

31396 Trichoderma koningii
HISTORY: IFO (T. Yokoyama; R1473-5; soil).
TEMP: 24 MEDIUM: 1 54-K-1640

31397 Trichoderma viride
HISTORY: IFO (T. Yokoyama; R1478-1; humus).
TEMP: 24 MEDIUM: 1 54-K-1640

31398 Mucor circinelloides f. circinelloides
HISTORY: IFO (T. Yokoyama; R70-1571-1; gold mine soil).
TEMP: 24 MEDIUM: 1 54-K-1640

31399 Coprinus atramentarius
HISTORY: IFO (T. Yokoyama; R1626).
TEMP: 24 MEDIUM: 1 54-K-1640

31605 Peloronectriella sasae
HISTORY: IFO (T. Yokoyama; T. Yokoyama 5806-3-1; Sasa kurilensis).
TEMP: 24 MEDIUM: 8

31606 Peloronectriella sasae
HISTORY: Fac. Agr., Hirosaki Univ. (Y. Harada; 326; Sasa kurilensis).
TEMP: 24 MEDIUM: 8

31607 Peloronectriella sasae
HISTORY: Fac. Agr., Hirosaki Univ. (Y. Harada; 328; Sasa kurilensis).
TEMP: 24 MEDIUM: 8

31608 Ascosphaera apis
HISTORY: Hatano Res. Inst., Food & Drug Safety Cent. (K. Takatori; M#702-3; honey bee)
TEMP: 24 MEDIUM: 8

31609 Sabouraudites canis
HISTORY: Hatano Res. Inst., Food & Drug Safety Cent. (K. Takatori; M#428; Bengal tiger).
TEMP: 28 MEDIUM: 6

31610 Trichophyton equinum
HISTORY: Hatano Res. Inst., Food & Drug safety Cent. (K. Takatori; Me 1; horse).
TEMP: 28 MEDIUM: 6

31611 Arthorobotryum hyalospora
HISTORY: TKBC 1367 (G. Okada; OFC 1211; decaying branch of broad-leaved tree).
TEMP: 24 MEDIUM: 5

31612 Graphilbum macrospora
HISTORY: TKBC 1368 (G. Okada; OFC 1312; rotting wood).
TEMP: 24 MEDIUM: 5

31613 Graphilbum pleomorphum
HISTORY: TKBC 1369 (G. Okada; OFC 1371; decaying branch).
TEMP: 24 MEDIUM: 1

31614 Graphilbum pleomorphum
HISTORY: TKBC 1370 (G. Okada, OFC 1373; decaying branch).
TEMP: 24 MEDIUM: 1

31615 Penicillium eberhardtii
HISTORY: IFO (T. Yokoyama; T. Yokoyama 5506-30-1).
TEMP: 24 MEDIUM: 1

31616 Armillariella mellea
HISTORY: Fac. Agr., Kagoshima Univ. (T. Terashita; 204; tuber of Gastrodia elata; oni-no-yagara).
TEMP: 24 MEDIUM: 5

31617 Armillariella mellea
HISTORY: Fac. Agr., Kagoshima Univ. (T. Terashita; 207; tuber of Gastrodia elata; oni-no-yagara).
TEMP: 24 MEDIUM: 5

31618 Armillariella mellea
HISTORY: Fac. Agr., Kagoshima Univ. (T. Terashita; 208; root of Galeola septentrionalis; tutiakebi).
TEMP: 24 MEDIUM: 5

31619 Armillariella mellea
HISTORY: Fac. Agr., Kagoshima Univ. (T. Terashita; 1051; Quercus acutissima; kunugi)
TEMP: 24 MEDIUM: 5

31620 Armillariella mellea
HISTORY: Fac. Agr., Kagoshima Univ. (T. Terashita; 1052; root of Galeola septentrionalis; tutiakebi).
TEMP: 24 MEDIUM: 5

31621 Armillariella mellea
HISTORY: Fac. Agr., Kagoshima Univ. (T. Terashita; 1053; root of Galeola

septentrionalis; tutiakebi).
TEMP: 24 MEDIUM: 5

31622 Phytophthora cryptogea
HISTORY: Hokkaido Nat. Agr. Exp. St. (N. Matsumoto; S-16; soil).
TEMP: 24 MEDIUM: 1

31623 Phytophthora megasperma
HISTORY: Hokkaido Nat. Agr. Exp. St. (N. Matsumoto; 57; soil).
TEMP: 24 MEDIUM: 1

31624 Phytophthora megasperma
HISTORY: Hokkaido Nat. Agr. Exp. St. (N. Matsumoto; K-1-1; soil).
TEMP: 24 MEDIUM: 1

31625 Morchella esculenta
HISTORY: IFO (T. Yokoyama; T. Yokoyama 5905-1-1).
TEMP: 24 MEDIUM: 1

31626 Penicilliopsis clavariaeformis
HISTORY: IFO (T. Yokoyama; T. Yokoyama 5810-10-1; seed of Diospyros kaki).
TEMP: 24 MEDIUM: 5

31627 Urnula craterium
HISTORY: IFO (T. Yokoyama; T. Yokoyama 5805-1-1; rotten trunk of Quercus sp.).
TEMP: 24 MEDIUM: 1

31628 Aspergillus niger
HISTORY: Fac. Agr., Saga Univ. (K. Tanaka; A-1; bulb of Allium cepa).
TEMP: 24 MEDIUM: 1

31629 Fusarium oxysporum f. sp. adzukicola
HISTORY: Hokkaido Nat. Agr. Exp. St. (K. Kitazawa & K. Yanagita; FPA 1; Phaseolus angularis).
TEMP: 24 MEDIUM: 1

31630 Fusarium oxysporum f. sp. adzukicola
HISTORY: Hokkaido Nat. Agr. Exp. St. (K. Kitazawa & K. Yanagita; FPA 2; Phaseolus angularis).
TEMP: 24 MEDIUM: 1

31631 Fusarium oxysporum
HISTORY: IFO (T. Yokoyama) -- AKU (T. Tochikura; SA 252; soil under Musa Basjoo).
TEMP: 24 MEDIUM: 1

31632 Myrothecium advena
HISTORY: IFO (T. Yokoyama; Co 1-5; stem of Coffea arabica).
TEMP: 24 MEDIUM: 1

31633 Myrothecium advena
HISTORY: IFO (T. Yokoyama; Co 1-14; Coffea arabica).
TEMP: 24 MEDIUM: 1

31634 Coniella diplodiella
HISTORY: IFO (T. Yokoyama; T. Yokoyama 5811-4-1; Vitis vinifera cultivar. "Kyoho").
TEMP: 24 MEDIUM: 1

31635 Neurospora sitophila
HISTORY: IFO (T. Ito; culture contaminant).
TEMP: 24 MEDIUM: 1

31636 Talaromyces flavus var. macrosporus
HISTORY: IFO (T. Ito; DIC 1; thick paper).
TEMP: 24 MEDIUM: 5

31637 Chaetomium hispanicum
HISTORY: Biol. Dept., Fac. Med., Univ. Barcelona (J. Guarro; FMR 313; soil).
TEMP: 24 MEDIUM: 8

ANIMAL CELL LINES

These 23 cell lines shown in this list are available for distribution. If you would like to know the condition of the distribution of these cell lines, please ask the curator (Dr. Masao Takeuchi). The detail description of these cell lines will be shown in the supplement of seventh edition of IFO List of Cultures.

50003 CKT-1
HISTORY: RTCI -- Tokushima Univ. (Kimura).
TEMP: 37 MEDIUM: 501

50004 WISH
HISTORY: IFO -- RTCI -- Flow Laboratories Inc. -- ATCC CCL 25.
TEMP: 37 MEDIUM: 501

50005 J-111
HISTORY: IFO -- RTCI -- Flow Laboratories Inc. --
TEMP: 37 MEDIUM: 501

50007 Ca SKi
HISTORY: IFO -- RTCI -- Flow Laboratories Inc. -- ATCC CRL 1550
TEMP: 37 MEDIUM: 551

50009 G-361
HISTORY: IFO -- RTCI -- Flow Laboratories Inc. -- ATCC CRL 1424.
TEMP: 37 MEDIUM: 501

50010 C 6/36
HISTORY: RTCI -- RIMD -- ATCC CRL 1660.
TEMP: 30 MEDIUM: 501

50011 HeLa S3
HISTORY: IFO -- RTCI -- Flow Laboratories Inc. -- ATCC CCL 2.2.
TEMP: 37 MEDIUM: 501

50012 Pt K2
HISTORY: IFO -- RTCI -- Flow Laboratories Inc. -- ATCC CCL 56.
TEMP: 37 MEDIUM: 503

50013 RPMI 8226
HISTORY: IFO -- RTCI -- Flow Laboratories Inc. -- ATCC CCL 155.
TEMP: 37 MEDIUM: 551

50014 MDBK
HISTORY: IFO -- RTCI -- Flow Laboratories Inc. -- ATCC CCL 22.
TEMP: 37 MEDIUM: 501

50015 F2408
HISTORY: RIMD -- Sork Inst. (W. Eckhalt).
TEMP: 37 MEDIUM: 531

50016 Chang Liver
HISTORY: IFO -- RTCI -- Flow Laboratories Inc. -- ATCC CCL 13.
TEMP: 37 MEDIUM: 501

50017 XC
HISTORY: RIMD -- ATCC CCL 165.
TEMP: 37 MEDIUM: 501

50018 NIL
HISTORY: RIMD -- Washington Univ. (S. Hakomori).
TEMP: 37 MEDIUM: 501

50020 SIRC
HISTORY: RIMD -- ATCC CCL 60.
TEMP: 37 MEDIUM: 501

50021 A31-714 C4
HISTORY: RIMD -- Washington Univ. (P. Vogt) -- ATCC CCL 163
TEMP: 37 MEDIUM: 501

50022 HL-60
HISTORY: IFO -- RTCI -- Osaka Univ. -- ATCC CCL 240.
TEMP: 37 MEDIUM: 554

50023 J774A.1
HISTORY: RTCI -- ATCC TIB 67.
TEMP: 37 MEDIUM: 551

50025 IM-9
HISTORY: IFO -- RTCI -- Flow Laboratories Inc. -- ATCC CCL 159.
TEMP: 37 MEDIUM: 551

50026 CCRF-SB
HISTORY: IFO -- RTCI -- Flow Laboratories Inc. -- ATCC CCL 120.
TEMP: 37 MEDIUM: 501.

50027 EL4
HISTORY: IFO -- RTCI -- ATCC TIB 39.
TEMP: 37 MEDIUM: 551

50028 EB-3
HISTORY: IFO -- RTCI -- Flow Laboratories Inc. -- ATCC CCL 85.
TEMP: 37 MEDIUM: 551

50029 P815
HISTORY: IFO -- RTCI -- ATCC TIB 64.
TEMP: 37 MEDIUM: 551

ABSTRACTS 1983-1984

Leaf spot disease of Stevia caused by Septoria steviae

C. Ishiba*, T. Yokoyama and T. Tani*

Ann. Phytopath. Soc. Japan 48: 34-43 (1982)

A new disease of Stevia rebaudiana Bertoni was found in Kagawa Prefecture in the early July of 1978 and named as the leaf spot disease of stevia. In the beginning, small brown spot occurred on the leaves and gradually developed to round to rectangle or irregularily expanded, large, brown to dark-brown spots. Eventually these leaves were defoliated. Many pycnidia of a species of the genus Septoria were formed on relatively old spots. Original isolate (S-O=IFO 31181) was obtained from a pin-size spot on the leaves. The growth of this isolate on PSA medium was very slow. Sectors of the black (S-B) and the white (S-W) colonies which grew considerably well on various agar media were formed in abundance during culture. Inoculation tests indicated that all of these three isolates (S-O, S-B and and S-W) were pathogenic on stevia leaves, but not on leaves of Erigeron annuus and Chrysanthemum morifolium. The maximum mycelial growth of the S-B isolate on PSA and stevia leaf decoction agar was found at 25 C and in a range of pH 5.5 to 7.0. Since the leaf spot symptoms caused by S. erigerontis and S. solidaginicola were found on the leaves of two Compositae plants, E. annuus and Solidago altissima, respectively, which were growing near stevia fields, the morphological and pathogenic characteristics of these fungi were compared with the stevia fungus. S. solidaginicola was different from the stevia fungus in the size of the pycnospores on host plants. S. erigerontis was different from the stevia fungus in its cultural characteristics and did not show any pathogenicity on stevia leaves. On the basis of the pathogenicity on stevia and its morphological characteristics, the present fungus was considered as a new species causing the leaf spot disease of stevia and a new name for the fungus, Septoria steviae sp. nov., was proposed. [in Japanese]

* Faculty of Agriculture, Kagawa University.

Black spot disease of Stevia caused by *Alternaria steviae*

C. Ishiba*, T. Yokoyama and T. Tani*

Ann. Phytopath. Soc. Japan 48: 44-51 (1982)

A new disease of *Stevia rebaudiana* Bertoni caused by a hitherto undescribed species of the genus *Alternaria* was found in Kagawa Prefecture in the late August of 1978 and was named as the black spot disease of stevia. Symptom on leaves appeared as small spots at an early stage of the infection, similar to that of the leaf spot caused by *Septoria steviae*. The spots expanded to irregular shape in black color and were surrounded with a chlorotic zone. The spots also occurred on stems, petiols and involucral scales. Among several *Alternaria* species isolated from the diseased stevia leaves, the fungus showed pathogenicity on stevia leaves by an artificial inoculation. The fungus was also pathogenic on the injured leaves of taxonomically related plants: *Eupatorium chinense* and *E. fortunei*, but not on the leaves of *Chrysanthemum frutescens*, *C. morifolium*, *Erigeron annuus* and *Tagetes patula*. Maximum mycelial growth of the causal fungus *Alternaria* sp. (Kagawa-a=IFO 31182) on PSA medium was at 25 C and in a range of pH 6.0 to 6.5. Based on the morphological characteristics and specific pathogenicity on stevia plants, the present fungus was considered as a new species of the genus *Alternaria* and *Alternaria steviae*, sp. nov. was proposed. The occurrence of black spot disease in fields was diversed depending on stevia strains and this was highly correlated with the susceptibility of leaves to the fungus as tested by the artificial inoculation. The culture filtrate of the pathogen growing in Richard's liquid medium induced a necrotic lesion on the leaves of susceptible strains at a concentration of 1:8. On the leaves of resistant strains, however, necrosis appeared only when undiluted culture filtrate was applied. The use of culture filtrate was suggested as effective for selection of the stevia strains resistant to the black spot of stevia caused by *Alternaria steviae*. [in Japanese]

* Faculty of Agriculture, Kagawa University.

Deficiency of flagellation in a *Bacillus subtilis* pleiotropic mutant lacking transketolase

K. Sasajima and T. Kumada*

Agric. Biol. Chem. 47: 1375-1376 (1983)

A *Bacillus subtilis* mutant lacking transketolase has pleiotropic defects in the cell surface functions: defective transport function of $EnzII^{glc}$ of the PEP-dependent phosphotransferase system, regulatory change in enzyme synthesis under catabolite repression, morphological change, change of bacteriophage sensitivity, and decrease in sporulation frequency. In this study, deficiency of flagellation was elucidated. This deficiency, together with the phenomena described previously indicates that there is some structural or compositional change in the cell surface of the *tkt* mutant.

* Central Research Division, Takeda Chemical Industries, Ltd.

Enzymatic formation of a new monosaccharide, 1-deoxy-D-*altro*-heptulose phosphate, from DL-acetoin and D-ribose 5-phosphate by a transketolase mutant of *Bacillus pumilus*

A. Yokota and K. Sasajima

Agric. Biol. Chem. 47: 1545-1553 (1983)

1-Deoxy-D-*altro*-heptulose phosphate (DAHP) synthase activity was found in the cell-free extract of a transketolase mutant, BG2532, of *Bacillus pumilus* IFO 12089. The enzyme was partially purified by protamine sulfate treatment, ammonium sulfate fractionation, DEAE-cellulose and hydroxylapatite column chromatography. When DL-acetoin and D-ribose 5-phosphate were incubated with the partially purified enzyme preparation, DAHP and acetaldehyde were detected as reaction products. Thiamine pyrophosphate (TPP) and Mg^{2+} were required as cofactors. The results of the stoichiometric measurements indicate that DAHP synthesis proceeds according to the following formula:

$$\text{DL-Acetoin} + \text{D-Ribose 5-phosphate} \xrightarrow{TPP, Mg^{2+}} \text{Acetaldehyde} + \text{DAHP}$$

Intracellular DAHP formed by this reaction may be excreted and accumulated in culture broth as 1-deoxy-D-altro-heptulose after dephosphorylation.

Ascomycetous yeasts isolated from galleries of the ambrosia beetles in Japan

I. Banno, K. Mikata and K. Kodama*

Trans. mycol. Soc. Japan 24: 441-450 (1983)

An introductory study of the yeasts recovered from tunnels bored in wood by ambrosia beetles has been carried out. Materials in the form of cut wood or timber were collected from various localities in Japan from Hokkaido to Yaku Island. The samples represented 35 species of broad-leaved trees and 4 species of coniferous trees. The beetles encountered comprised 16 species of 7 genera belonging to Scolytidae and two species of two genera belonging to Platypodidae. A total of 385 strains was isolated from 162 samples. Among these, 188 strains were found to form ascospores and were identified as representative of Debaryomyces hansenii, Hansenula anomala, H. bimundalis, H. californica, H. capsulata, H. saturnus, H. wingei, Hormoascus platypodis, Kluyveromyces thermotolerans, Pichia acaciae, P. membranaefaciens, P. nakazawae var. akitaensis, P. pinus, P. rhodanensis, and P. veronae. Three yeasts (AM13, AM72, and AM105), representative of three undescribed Pichia species were also isolated. Although no specific and exclusive relationships were found between kinds of yeasts and beetles or trees, tendencies were nevertheless noted: Hansenula anomala was isolated most frequently from wood samples infected by the genus Xyleborus; Hormoascus platypodis was prominently associated with invasions of Crossotarsus nipponicus in the tree Fagus crenata; The Pichia species coded AM72 was associated mainly with infestations of Scolytoplatypus shogun in the tree F. crenata; and the predominant yeast recovered from the tree Machilus thunbergii was Pichia acaciae. Most of the isolates of Hormoascus platypodis were found in the area north of Yamagata, in contrast to the majority of Pichia acaciae being found in the area south of Gifu.

* Laboratory of Kodama Brewing Co., Ltd.

Preservation of filamentous fungal cultures by freezing

T. Yokoyama and T. Ito
Japan. J. Freez. Dry. 29: 28-29 (1983)

Among 940 strains of basidiomycete cultures in the IFO culture collection, 879 (93.5%) survived after storage by freezing at -80 C for one year. Some strains of Lycoperdales, Hymenogastrales, and Auriculariales were found to be very sensitive to cryogenic preservation. Most of the Zygomycete, Ascomycete, and Deuteromycete strains survived well after storage for 3 to 6 months by this procedure. Glycerol at a concentration of 10% (v/v) was found effective as a protective agent. [in Japanese]

Practical methods for preserving microorganisms

I. Banno
Japan. J. Freez. Dry. 30: 60-64 (1984)

The development of methods for preservation and their application to various microorganisms since 1980 are reviewed. Although no new method was elaborated, simplification of existing methods and an increase in the kinds of microorganisms which were successfully preserved by each method were found. In conclusion, L-drying, freeze-drying, and deep freezing at temperatures under -70 C were considered to be the most reliable for long-term preservation. [in Japanese]

Long-term preservation of fungal cultures

T. Yokoyama and T. Ito
Japan. J. Freez. Dry. 30: 65-67 (1984)

The viabilities of fungal strains after storage by freezing in 10% glycerol at -80 C for 6 months were 97.5% for Zygomycetes, 94.6% for Asco-

mycetes, and 99.4% for Deuteromycetes. After storage for 12 months the viabilities did not show any remarkable decline. However, Mastigomycete strains showed only 22.1% viability after 6 months by the same procedure; this fact indicates that most of them are very sensitive to the freezing process. It is concluded that the cryogenic preservation of fungal cultures at -80 C is an effective long-term preservation method except for Mastigomycete strains. [in Japanese]

Compounds protecting L-dried cultures from mutation. II. Prevention of DNA strand breakage in L-dried Escherichia coli by thiourea, adonitol, and cysteine

T. Sakane, K. Imai and I. Banno

Japan. J. Freez. Dry. 30: 17-22 (1984)

DNA injury in L-dried Escherichia coli cells was investigated using the alkaline sucrose gradient sedimentation technique. When the cells were dried in 0.1 M potassium phosphate buffer containing 3% monosodium glutamate, no DNA single-strand breaks were detected immediately after drying, but temperature-dependent DNA breakage was induced during the preservation process of dried cells. When 30 mM thiourea or 100 mM adonitol was added to the suspending fluid, no significant DNA breakage resulted either immediately after drying or after preservation for 6 months at 5 C. After preservation for 8 weeks at 37 C, there were fewer DNA breakages in cells dried with thiourea or adonitol than in those dried without the protectants. Furthermore, thiourea and adonitol protected vegetative cells from damage by radicals derived from hydrogen peroxide. On the basis of these results, thiourea and adonitol are thought to prevent DNA strand breakage by acting as radical-scavengers. No evidence for a preventive effect of cysteine on DNA strand breakage has been obtained, and role of cysteine in increasing the survival of dried cells is not clear. [in Japanese]

Formation of 1-deoxy-D-threo-pentulose and 1-deoxy-L-threo-pentulose by cell-free extracts of microorganisms

A. Yokota and K. Sasajima
Agric. Biol. Chem. 48: 149-158 (1984)

A new enzymatic acyloin-type condensation between pyruvate (or acetoin or methylacetoin) and D-glyceraldehyde was found to be catalyzed by cell-free extracts of a transketolase mutant of Bacillus pumilus IFO 12089. The reaction product (1) was isolated and determined to be 1-deoxy-D-threo-pentulose (D-DTP), which is considered to be a precursor of the five-carbon unit of the thiazole ring of thiamine. 1-Deoxy-L-threo-pentulose (L-DTP, 2) was synthesized similarly when L-glyceraldehyde was used instead of D-glyceraldehyde. The configurations of 1 and 2 were confirmed by reduction to the corresponding 1-deoxy-pentitols.

Similar enzyme activities were also detected in cell-free extracts of all the wild-type strains tested of bacteria, actinomycetes, yeasts, and molds. These results suggest that the D-DTP synthesizing enzyme plays an important role in the biosynthesis of the thiazole ring of thiamine in vivo.

Formation of 1-deoxy-D-fructose, 1-deoxy-D-sorbose, 1-deoxy-D-tagatose and 1-deoxy-erythrulose by cell-free extracts of bacteria and actinomycetes

A. Yokota and K. Sasajima
Agric. Biol. Chem. 48: 1643-1645 (1984)

New enzymatic acyloin-type condensations between pyruvate (or acetoin or methylacetoin) and aldehydes were found to be catalyzed by cell-free extracts of a transketolase mutant of Bacillus pumilus IFO 12089. Each reaction product was isolated from the enzyme reaction mixture and its chemical structure was determined. When D-erythrose was used as the aldehyede, 1-deoxy-D-fructose was formed. When D-threose was used, 1-deoxy-D-sorbose and a small amount of 1-deoxy-D-tagatose were formed. 1-Deoxy-erythrulose (3,4-dihydroxy-2-butanone) was formed when glycolaldehyde was used as substrate.

Similar enzyme activities were also found in cell-free extracts of many strains of bacteria and actinomycetes.

Composition of the 6-deoxy-L-talose-containing cell wall of Flavobacterium arborescens IFO 3750

M. Takeuchi and K. Imai

Nippon Nogeikagaku Kaishi 58: 385-386 (1984)

The cell wall of Flavobacterium arborescens IFO 3750 contains 6-deoxy-L-talose. In the wall the peptide moiety of the peptidoglycan possesses alanine, glycine, lysine, glutamic acid (plus 3-hydroxyglutamic acid), and homoserine (ratio; 1:3:1:1:1). The polysaccharide in the wall is composed of galactose, mannose, 6-deoxy-L-talose, and glucosamine (ratio; 2:1:1:1). [in Japanese]

Mating-type differentiation in ascosporogenous yeasts on the basis of mating-type-specific substances responsible for sexual cell-cell recognition

M. Yamaguchi*, K. Yoshida*, I. Banno and N. Yanagishima*

Mol. Gen. Genet. 194: 24-30 (1984)

Sexual agglutination occurred only between cells of opposite mating types of the same species in all the Saccharomyces, Hansenula, Saccharomycodes, and Pichia yeasts tested. We succeeded in solubilizing the sex-specific glycoprotein, cell wall agglutination substance responsible for sexual agglutinability by briefly autoclaving these yeasts. The agglutination substances of all the above yeasts were univalent and sensitive to the enzyme pronase. The formation of complementary complexes was observed only between agglutination substances of opposite mating types of the same species. In general, the agglutination substance of one mating type was more resistant to heat treatment at 100 C in 3% acetic acid and more sensitive to 5% 2-mercaptoethanol treatment than the agglutination substance of

the other mating type in these yeasts. On the basis of these results together with the pheromone response and production, we expect that almost all ascosporogenous yeasts can be classified into the two mating types corresponding to a and mating types in Saccharomyces cerevisiae, respectively.

* Biological Institute, Faculty of Science, Nagoya University.

PRESENTATION OF PAPERS AT SCIENTIFIC MEETINGS 1983-1984

The 60th Symposium of Research Committee on Sterilization of Medical Products (March, 1983, Tokyo)

K. Imai

Properties of Bacillus subtilis strains which are used as the biological indicator for sterilization.

Agricultural Chemical Society of Japan (March-April, 1983, Sendai)

I. Banno and K. Mikata

Ethanol production from D-xylose by yeasts.

M. Takeuchi and K. Imai

Isolation and identification of 6-deoxy-L-talose from the cell wall of Flavobacterium arborescens IFO 3750.

A. Yokota and K. Sasajima

Enzymatic synthesis of 1-deoxy-D-threo-pentulose, a precursor in the biosynthesis of the thiazole ring of thiamine, by cell-free extracts of microorganisms.

Mycological Society of Japan (May, 1983, Tokyo)

M. Shibata[*1], Y. Fujimura[*1], H. Kuraishi[*1], J. Sugiyama[*2] and T. Yokoyama

Ubiquinone system in the classification of higher filamentous fungi.

The 6th Japanese Carbohydrate Symposium (August, 1983, Sendai)

A. Yokota and K. Sasajima

Biosynthesis of 1-deoxy-ketoses by microorganisms.

[*1] Faculty of Agriculture, Tokyo University of Agriculture and Technology.

[*2] Institute of Applied Microbiology, University of Tokyo.

The 3rd International Mycological Congress (August-September, 1983, Tokyo)

I. Banno, K. Mikata and K. Kodama[*1]

The occurrence of Ascomycetous yeasts in nature in Japan.

H. Kuraishi[*2], J. Sugiyama[*3] and T. Yokoyama

Coenzyme Q system in filamentous fungi.

T. Yokoyama

Pleoanamorphism of acervulous Coelomycetes.

The 1st International Symposium for Chemobiodynamics (September, 1983, Chiba)

T. Yokoyama

Recent trend of taxonomy of Coelomycetes.

Japanese Society for Research of Freeze and Drying (November, 1983, Nishinomiya)

I. Banno

Practical methods for preserving microorganisms.

T. Yokoyama and T. Ito

Long-term preservation of fungal cultures.

Society for Fermentation Technology, Japan (November, 1983, Osaka)

M. Aoki[*2], M. Shibata[*2], Y. Fujimura[*2], H. Kuraishi[*2], J. Sugiyama[*3] and T. Yokoyama

Ubiquinone branches in the classification of Paecilomyces, Aspergillus and Penicillium.

[*1] Laboratory of Kodama Brewing Co., Ltd.

[*2] Faculty of Agriculture, Tokyo University of Agriculture and Technology.

[*3] Institute of Applied Microbiology, University of Tokyo.

Kansai Mycological Club (March, 1984, Kyoto)

T. Ito and T. Yokoyama

Thermophilic and thermotolerant fungi in paddy field soils.

Agricultural Chemical Society of Japan (April, 1984, Tokyo)

M. Aoki[*1], Y. Fujimura[*1], H. Kuraishi[*1], J. Sugiyama[*2] and T. Yokoyama

Ubiquinone branches in the calssification of Discomycetes.

K. Imai and M. Takeuchi

Immunological relationship between Microbacterium arborescens and other coryneform bacteria.

M. Takeuchi and K. Imai

Taxonomic studies on the genus Microbacterium. II. Composition of cell wall.

A. Yamaguchi[*3], K. Imai and A. Misaki[*3]

α-D-Arabinofuranosidase produced by a bacterium M-2: Purification of the enzyme and taxonomic properties of M-2.

A. Yokota and K. Sasajima

Formation of 1-deoxy-ketoses by pyruvate dehydrogenase.

Japanese Society for Research of Freezing and Drying (April, 1984, Tokyo)

T. Sakane, K. Imai and I. Banno

Compounds protecting L-dried cultures from mutation. II. Prevention of DNA strand breakage in L-dried Escherichia coli by thiourea, adonitol and cysteine.

[*1] Faculty of Agriculture, Tokyo University of Agriculture and Technology.

[*2] Institute of Applied Microbiology, University of Tokyo.

[*3] Osaka City University, Faculty of the Science of Living.

Phytopathological Society of Japan (April, 1984, Tokyo)

H. Nasu[*1], T. Yokoyama and H. Okamoto[*1]

Rice sheath rot disease in Okayama and its causal fungus Sarocladium attenuatum.

The Japanese Society for Tuberculosis (April, 1984, Tokyo)

J. Mizuno[*2], M. Takeuchi, K. Imai and I. Yano[*3]

Mycolic acids of a novel, acid-fast bacterium which exhibits motility.

Symposium on the Utilization of Computors in Culture Collections. Japan Federation for Culture Collections (June, 1984, Tokyo)

T. Iijima

Critical problems of culture collections; from the symposium in The 3rd International Mycological Congress.

T. Kusaka

Application of computor systems to the information services of culture collections.

Yeast Genetics and Molecular Biology Society of Japan (July, 1984, Nagoya)

Y. Kaneko, T. Toh-e[*4] and Y. Oshima[*5]

Genetic mapping and DNA sequencing of the Saccharomyces cerevisiae gene for specific alkaline p-nitrophenylphosphatase.

Mycological Society of Japan (September, 1984, Sapporo)

T. Ito and T. Yokoyama

Studies on the filamentous fungi collected in the Far Eastern USSR.

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*1 Okayama Prefectural Agricultural Experiment Station.
*2 Osaka City University, Medical School.
*3 Niigata University, School of Medicine.
*4 Department of Fermentation Technology, Hiroshima University.
*5 Department of Fermentation Technology, Osaka University.

K. Mikata and I. Banno
Scanning electron microscopy of ascospores of Debaryomyces and Pichia.

Y. Yamada[*1], Y. Fujimura[*1], H. Kuraishi[*1], J. Sugiyama[*2] and T. Yokoyama
The system of ubiquinone in Basidiomycotina.

The 20th Symposium on Soil-born Diseases. Phytopathological Society of Japan (October, 1984, Kyoto)

T. Yokoyama
Preservation of Pythium and Phytophthora.

The 5th International Congress of Culture Collections (November, 1984, Bangkok)

T. Iijima
History and activities of JFCC.

K. Imai, T. Sakane, I. Banno and T. Iijima
Application of L-drying method to the preservation of Thiobacillus ferrooxidans.

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*1 Faculty of Agriculture, Tokyo University of Agriculture and Technology.

*2 Institute of Applied Microbiology, University of Tokyo.

MISCELLANEOUS SCIENTIFIC PAPERS

T. Iijima, 1983. The Japanese Patent Law. In K. Aoshima, K. Tubaki and K. Miura (ed.) Kinrui Kenkyuho. p. 372-374. Kyoritsu Shuppan Co., Ltd., Tokyo. [in Japanese]

K. Imai, 1983. Reviews of Bacillus subtilis strains which are used as biological indicator for sterilization. The Japanese Journal of Medical Instrumentation 53: 41-44. [in Japanese]

T. Iijima, 1984. Critical problems in culture collections related to bioengineering. In L.R. Bartra and T. Iijima (ed.) Critical problems of culture collections. p. 59-64. Institute for Fermentation, Osaka, Osaka.

T. Kusaka, 1983-1984. Activities of actinomycetes collection in IFO. The Actinomycetes 18: 100-103.

T. Kusaka, 1984. Isolation methods and application of rare microorganisms and their application. In case of Actinomycetes. In Screening, isolation, and breeding of microorganisms, p. 26-41. CMC Co., Tokyo. [in Japanese]

K. Sasajima and M. Yoneda,[*1] 1984. Production of pentoses by microorganisms. In G.E. Russell (ed.) Biotechnology and genetic engineering reviews. Vol. 2, p. 175-213. Intercept, Newcastle upon Tyne.

[*1] Personnel Training Center, Takeda Chemical Industries, Ltd.

CORRECTIONS

In the issue of IFO Research Communications No. 11, the following corrections should be made.

Page	Line	Type	Should read
7	12	teichulonic	teichuronic
36	19	Leucosporidium artarcticum	Leucosporidium antarcticum
40	17	C. succophila	C. succiphila
44	6	T. cutaneum var. autarcticum	T. cutaneum var. antarcticum
68	26	Volvariella speciosa	Volvariella speciosa var. gloiocephala