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No. 11

(Annual Report 1981-1982)

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INSTITUTE FOR FERMENTATION, OSAKA

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

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REPORT OF THE DIRECTOR

After the sudden decease of Mr. Chobei Takeda, the Chairman of the Board of Trustees of the Institute for Fermentation, Osaka, the position of Chairman remained vacant until March 1981.

At the 70th annual meeting in March 1981, Mr. Shinbei Konishi, chairman of the Board of Directors of Takeda Chemical Industries Ltd., was nominated as Chairman of the Board of Trustees. At the same time, Dr. Einosuke Ohmura was nominated as a new member of the Board of Trustees, and Dr. Masahiko Yoneda was nominated as Councilor. The Treasurer of the Institute, Mr. Tadashi Kato, retired from the Institute in October 1982 after four years service, and Mr. Tokusaburo Fujitani was appointed as Treasurer.

During 1981 to 1982, two new research sections were added to the Institute to handle the increasing number of microorganisms distributed and accessed, to maintain assurance of the authenticity of microorganisms distributed, and to keep pace with advances in taxonomy. A section for taxonomy of Actinomycetes was newly established, and Dr. Taiki Kusaka joined us as curator of Actinomycetes. He has also undertaken responsibility for part of the fungal collection, because of the large number of fungal cultures in the mycology section. In addition, the bacteriology section was seperated from yeast and bacteriology section, and Dr. Ko Imai was appointed as its curator. Thus the IFO has five research sections, mycology (Dr. Yokoyama), yeast (Dr. Banno), biochemistry and genetics (Dr. Sasajima), actinomycetes (Dr. Kusaka), and bacteriology (Dr. Imai), as well as an administrative office (Mr. Fujitani).

These five research sections have facilities for the efficient preservation of microorganisms, and for examination of chemical characteristics and fine structures of microorganisms, and these facilities are constantly being renovated or modernized. The research activities of these sections are reported in this issue of Research Communications.

In the past two years, the culture collection of the Institute has grown by about 700 strains, bringing the total number of strains maintained in the collection to about 11200 at the end of 1982. The total number of strains distributed in 1981 was 7600, and 10000 in 1982. These strains were distributed to research organizations, universities, hospitals and firms.

In accordance with the increase in cooperative activity between IFO and organizations in Asian countries, we received the following guest researchers from overseas; Miss Jin Kyung-Hee from Sookmyung Women's University in Seoul, Korea and Mr. Suparp Artjaryasripong from Bangkok MIRCEN, Dr. Yang Hsiu-Chu from the Plant Protection Center, Taiwan, and Mr. Yeh Kai Wun in National Taiwan University. They stayed in the Institute for between several weeks and six months carrying out research, and their cooperation contributed to research activities and maintenance of

the authenticity of cultures.

Lectures and seminars were given by the following guest speakers,

Dr. J.Z. Ying, Microbiology Institute, China Academy of Sciences

Prof. F.F. Busta, Food microbiology, University of Minesota

Prof. O.K. Miller Jr., Biology Department, Virginia Polytechnic Institute and State University

Dr. T.F. Myoda, A.I. du Pond Institute of the Nemours Foundation

Dr. I. Gandjar, Department of Biology, University of Indonesia

Dr. S.C. Jong, American Type Culture Collection

Dr. Z.Q. Li, Institute of Microbiology, China Academy of Science

Dr. K. Imai spent a year and a half as a postdoctoral scholar in Dr. B.G. Hall's Laboratory, Department of Microbiology, University of Connecticut. He returned to the Institute in June 1981 and resumed his work in the bacteriology section. Dr. T. Yokoyama has joined a research project on transformation of biomass planned by the Ministry of Agriculture, Forestry and Fishery.

Members of the Institute attended several international meetings: Dr. T. Iijima attended the VIth International Congress of Culture Collection in Brno, Czechoslovakia, July 1981, where he presented a paper. After the congress, he was able to visit the Deutsche Sammlung von Mikroorganismen in Göttingen and the Centraalbureau voor Schimmelcultures in Baarn and Delft and to see the facilities of the famous culture collections, and exchanged information on matters of mutual interests. Dr. I. Banno attended the 4th Korean-Japan Symposium on Industrial Fermentation and gave a lecture on "Preservation of bacterial and yeast cultures in IFO". He visited several organizations in Korea. He was also invited to lecture at the International Postgraduate University Course held annually in Osaka University. In November 1981, an ASEAN/UNESCO/UNEP/NIFtal training course was held in Bangkok, Thailand. Dr. T. Iijima attended as a lecturer and spoke on lecture and demonstrated a practical method of preserving microorganisms.

The present issue of IFO Research Communications, in addition to research papers, has a new section "Announcements", giving detailed information about the IFO culture collection.

(T. IIJIMA)

IFO Res. Comm. 11,
3-9, 1983 (March)

CELL SURFACE CHANGE OF *BACILLUS SUBTILIS* PLEIOTROPIC MUTANT LACKING TRANSKETOLASE: PROPERTIES OF VARIOUS REVERTANT STRAINS

Ken-ichi SASAJIMA and Toshio KUMADA

Summary

Cell morphology, motility, flagellation, bacteriophage sensitivity and sporulation of various revertant strains of a *tkt* mutant BG2607 of *Bacillus subtilis* IFO 12114 were examined. The true revertant BG2694 showed the same properties as the parental strain IFO 12114. Other suppressive revertant strains such as BG 2678, BG2680, BG2692 and BG2693, were altered in cell morphology, non-motile and non-flagellate. These suppressive revertant strains were also more sensitive to bacteriophages SP8, SP10 and SP01 than the parental strain. Sporulation frequency varied with the type of reversion. The suppressive revertant strains were similar in all respects to the *tkt* mutant BG2607. These results confirm that the functions relating to the cell surface have changed in the *tkt* mutant BG2607.

The *tkt* mutant BG2607 of *Bacillus subtilis* IFO 12114 showed pleiotropic changes in the functions related to the cell surface; (i) the transport function of the membrane-bound enzyme II^{glc} of the phosphoenolpyruvate-dependent phosphotransferase system (PTS) (Fig. 1) was defective (24), (ii) inducible synthesis of the activity of D-mannitol transport, which is also catalyzed by PTS (Fig. 1), and D-mannitol-1-phosphate dehydrogenase (Fig. 1) was hypersensitive to D-glucose repression (26), and synthesis of sorbitol permease and sorbitol dehydrogenase (Fig. 1) were no longer sensitive to D-glucose-repression (26). Further studies revealed that the *tkt* mutant BG 2607 formed chains during the exponential growth phase, was thicker than the parental strain, more sensitive to bacteriophages SP10 and SP01, underwent autolysis at a slower rate than the parental strain, and formed spores with remarkably reduced frequency (25). The *tkt* mutant BG2607 is also defective in flagellation (27). These pleiotropic changes of the *tkt* mutant BG2607 seem to be generated by a cell surface change caused by transketolase deficiency.

This study was undertaken to examine the properties of the various types of revertant strains derived from the *tkt* mutant BG2607 to confirm the cell surface change of the *tkt* mutant strain.

Materials and Methods

Bacterial strains and bacteriophages. The bacterial strains used were the parental strain *B. subtilis* IFO 12114, a *tkt* mutant BG2607 (23), a true revertant BG2694, suppressive revertants BG2678, BG2680, BG2692 and BG2693, and bacteriophage-

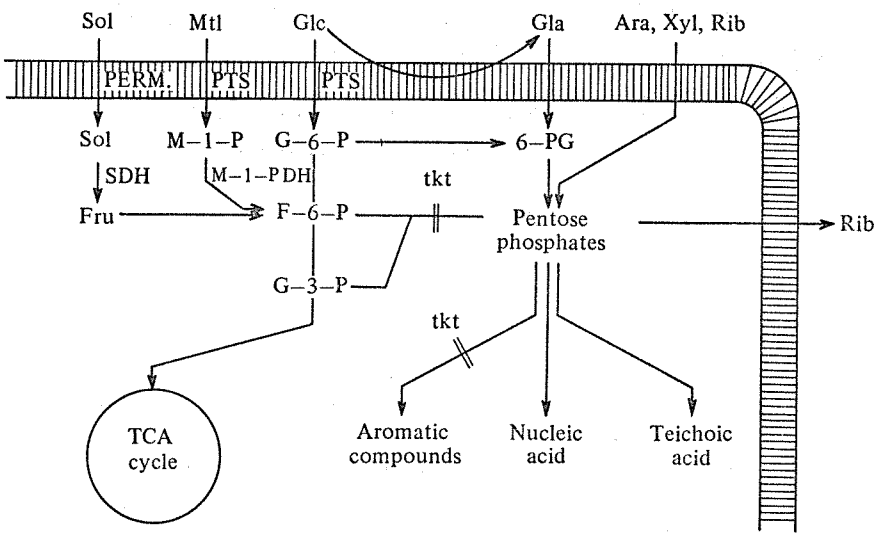

Fig. 1. Carbohydrate catabolic pathways in *Bacillus subtilis tkt* mutant BG2607. The *tkt* mutant BG2607 cannot utilize D-gluconate, L-arabinose, D-xylose and D-ribose as sole source of carbon (23), requires aromatic compounds for growth because of deficiency of aromatic biosynthesis (23), and produces a large amount of D-ribose in the culture medium (23).
Abbreviations: *tkt*, transketolase; PTS, phosphoenolpyruvate-dependent phosphotransferase system; PERM, permease; SDH, sorbitol dehydrogenase; M-1-PDH, D-mannitol-1-phosphate dehydrogenase; Glc, D-glucose; Mtl, D-mannitol; Sol, sorbitol; Fru, D-fructose; Gla, D-gluconate; Ara, L-arabinose; Xyl, D-xylose; Rib, D-ribose; M-1-P, D-mannitol-1-phosphate; G-6-P, D-glucose-6-phosphate; F-6-P, D-fructose-6-phosphate; 6-PG, 6-phospho-D-gluconate; G-3-P, D-glyceraldehyde-3-phosphate.

propagating strains *B. subtilis* ATCC 15563, ATCC 23059 and ATCC 27370. The suppressive revertant strains still lack transketolase (26) but have recovered some of the pleiotropic phenotypes of the *tkt* mutant BG2607 (24, 26). Strain BG2678 is no longer sensitive to D-glucose-repression of synthesis of both D-mannitol and sorbitol catabolic enzymes, but is still defective in D-glucose transport (26). Strain BG2680 has partially recovered D-glucose transport and is still sensitive to D-glucose-repression but not sensitive to D-gluconate-, D-xylose- and L-arabinose-repression of D-mannitol catabolic enzyme synthesis (26). Strain BG2692 has recovered D-glucose transport and at the same time become moderately resistant to D-glucose-repression of inducible synthesis of D-mannitol catabolic enzymes (24, 26). Strain BG2693 has recovered D-glucose transport (24). Strain BG2694 is a true revertant strain, with recovered transketolase activity and the same properties as the parental strain (24, 26). *B. subtilis* ATCC 15563, ATCC 23059 and ATCC 27370 were used respectively for propagation of bacteriophages SP8, SP10 and SP01. The bacteriophages were used to examine bacteriophage sensitivity of the parental, the *tkt* mutant and the revertant strains.

Media. Composition of media was described previously (25).

Morphology, motility and flagellation. Cells grown in a slightly modified Spizizen's synthetic medium containing 1% sorbitol plus 1% D-glucose as carbon sources

at 37 C for 6 hr (exponential growth phase) were spread on thin agar layers on slide glass and observed under phase-contrast optics with a Nikon microscope. Motility was examined by observing the culture with the microscope directly. Flagellation was examined similarly after flagella staining (17).

Bacteriophage sensitivity. Preparation of bacteriophage suspension and measurement of plaque-forming units were described in a previous paper (25).

Sporulation frequency. The method of estimation of sporulation frequency was also described previously (25).

Results

Cell morphology, motility and flagellation are shown in Table 1. The true revertant BG2694 showed the same morphology as the parental strain IFO 12114. The suppressive revertant strains showed morphology of filaments or chains as similar to the *tkt* mutant BG2607. The true revertant BG2694 was motile and had flagella,

Table 1. Morphology, motility and flagellation of the parental strain *B. subtilis* IFO 12114, the *tkt* mutant BG2607 and revertant strains BG2678, BG2680, BG2692, BG2693 and BG2694.

Strain	Morphology	Motility	Flagellation
IFO 12114	rods	motile	+
BG2607	chains, thick	non-motile	—
BG2678	chains, thick	non-motile	—
BG2680	chains, thick	non-motile	—
BG2692	filaments, thin	non-motile	—
BG2693	chains, thick	non-motile	—
BG2694	rods	motile	+

Table 2. Bacteriophage sensitivity of the parental strain *B. subtilis* IFO 12114, the *tkt* mutant BG2607 and revertant strains BG2678, BG2680, BG2692, BG2693 and BG2694.

Strain	Plaque-forming efficiency*		
	SP8	SP10	SP01
IFO 12114	1	1	1
BG2607	12	21	21
BG2678	14	16	33
BG2680	16	21	32
BG2692	22	18	37
BG2693	12	21	27
BG2694	0.5	1	0.9
ATCC 15563	0.02	—	—
ATCC 23059	—	5	—
ATCC 27370	—	—	4

* Figures are relative to plaque-forming units of the parental strain *B. subtilis* IFO 12114.

like the parental strain IFO 12114. In contrast, all the suppressive revertant strains were non-motile and non-flagellate, like the *tkt* mutant BG2607.

Sensitivity to bacteriophage infection of the revertant strains is shown in Table 2. The true revertant BG2694 showed almost the same sensitivity as the parental strain IFO 12114. However, the suppressive revertant strains were more sensitive to the three bacteriophages SP8, SP10 and SP01 than the parental strain, in which they resembled to the *tkt* mutant BG2607.

Sporulation frequency is shown in Table 3. The values varied with the type of reversion. The true revertant BG2694 sporulated with the same frequency as the parental strain IFO 12114. No sporulation was observed in strain BG2692. Sporulation frequency of strain BG2693 was lower than that of the *tkt* mutant BG2607. Strain BG2678 sporulated almost as well as the parental strain. Strain BG2680 sporulated moderately well, but not as well as the parental strain.

Table 3. Sporulation frequency of the parental strain *B. subtilis* IFO 12114, the *tkt* mutant BG2607 and revertant strains BG2678, BG2680, BG2692, BG2693 and BG2694.

Strain	Sporulation frequency (%)
IFO 12114	92.5
BG2607	4.8
BG2678	78.7
BG2680	58.3
BG2692	<0.1
BG2693	0.5
BG2694	86.6

Discussion

This study showed that the true revertant BG2694 has recovered the normal cell surface functions related to morphology, flagellation, bacteriophage infection and sporulation, but that the suppressive revertant strains have not recovered normal cell morphology and flagellation. Sensitivity to bacteriophage infection of the suppressive revertant strains was similar to that of the *tkt* mutant BG2607. The sporulation frequencies of the suppressive revertant strains varied with the type of reversion. These various properties of the suppressive revertant strains that are expressed in functions relating to the cell surface seem to be a reflection of a partial recovery of pleiotropic properties of the *tkt* mutant BG2607 by a secondary mutation in genes other than transketolase gene: recovery of D-glucose transport and regulation of enzyme synthesis in strain BG2692 (24, 26), recovery of D-glucose transport in strain BG2693 (24) and recovery of regulation of enzyme synthesis in strains BG2678 and BG2680 (26).

The above results with the various revertant strains confirm the presence of a cell surface change in the *tkt* mutant BG2607 which affects cell morphology (25), flag-

ellation (27), sporulation (25), bacteriophage infection (25), sugar transport (24) and regulatory mechanism of inducible enzyme synthesis (26).

Morphological changes of gram-positive bacteria have been described in *B. subtilis* grown in the presence of tunicamycin (32), a *B. subtilis* mutant defective in teichoic acid synthesis (35), *Diplococcus pneumoniae*, whose teichoic acid is chemically modified by replacement of choline with ethanolamine (33, 34), a bacteriophage-resistant mutant of *Staphylococcus aureus* (5, 7), autolysis-defective mutants of *B. subtilis* (4, 8, 9, 10, 11, 37), *B. licheniformis* (12) and *Streptococcus faecium* (31), a novobiocin-resistant mutant of *B. licheniformis* (19, 20), and NaCl-dependent mutants of *B. subtilis* and *B. licheniformis* (21, 22). Further studies revealed that the morphological changes seem to be related to a defect of teichoic acid synthesis (5, 6, 29), teichulonic acid synthesis (12, 20) or lipoteichoic acid synthesis (31). It has been reported that bacteriophage infection requires teichoic acid (1, 7, 15, 30, 38) and/or phospholipid (2, 14). The *tkt* mutant BG2607 may be defective in teichoic acid or phospholipid synthesis.

As sporulation is accompanied by morphological development, it must require elaborate membrane structure. A small change in the membrane will thus influence sporulation. Pleiotropic asporogenous mutants of *B. subtilis* have been described as being defective in the membrane (3).

Pleiotropic membrane mutants of *Salmonella typhimurium* (18) and *Escherichia coli* (36) and pleiotropic morphological mutants of *Caulobacter crescentus* (13, 16, 28) have been described. In the *S. typhimurium* mutant and the *E. coli* mutant, various biochemical functions have been altered pleiotropically by a single mutation. The *C. crescentus* mutant has altered sensitivity to bacteriophage infection, flagellation and pilli formation.

Pleiotropic changes in functions relating to cell surface of the *tkt* mutant BG2607 seem to be generated by a change in cell wall or plasma membrane caused by transketolase mutation. Analysis of cell wall and plasma membrane of the *tkt* mutant and the various revertant strains is now in progress.

The authors wish to express their thanks to Dr. G. Tamura, Professor of the University of Tokyo, for his critisism of the manuscript. Grateful acknowledgement is also made to Dr. R.L. Gherna, American Type Culture Collection, for generously providing bacteriophages SP8, SP10 and SP01 with their propagating strains *B. subtilis* ATCC 15563, ATCC 23059 and ATCC 27370, and to Takeda Chemical Industries, Ltd. for financial support of this research.

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A *PHOTOBACTERIUM PHOSPHOREUM* STRAIN EXHIBITS CHEMOTAXIS FOR SODIUM CHLORIDE

Ko IMAI

Summary

The chemotactic response of *Photobacterium phosphoreum* IFO 13896, whose motility was hardly detected in a hanging drop preparation, was examined by the capillary tube method. The bacterium was found to exhibit chemotaxis toward sodium chloride and glucose.

A luminescent bacterium IFO 13896, which was isolated from a squid and identified as *Photobacterium phosphoreum*, exhibited a chemotactic response to a concentration gradient of sodium chloride in a medium, though its motility was hardly detected in hanging drop preparations (4). Most of the studies reported on bacterial chemotaxis deal with attraction to sugars and amino acids (2, 3), but little information is available on the chemotactic response to salts. The purpose of this investigation was to confirm that *P. phosphoreum* IFO 13896 is really motile and exhibits chemotaxis for sodium chloride.

Materials and Methods

Strain used. *Photobacterium phosphoreum* IFO 13896 was deposited by Dr. K. Matsui; its taxonomic properties have been described previously (4).

Constituents of media used. Peptone-yeast extract medium (PY) contained 10 g of polypeptone, 2 g of yeast extract, and 1,000 ml of distilled water and was adjusted to pH 7.2 with NaOH. Basal medium (BM) consisted of 3 g of KH_2PO_4, 7 g of K_2HPO_4, 1 g of $(NH_4)_2SO_4$, 0.1 g of $MgSO_4 \cdot 7H_2O$, and 1,000 ml of distilled water. To determine colony forming units, nutrient agar supplemented with 2% sodium chloride and 0.5% glycerol (NA) was employed.

Chemotaxis assay. Chemotactic response was assayed based on the capillary tube method described by Adler (1). A capillary tube, sealed at one end in a flame, was filled with suspending medium containing the attractant, and the open end was inserted into a 10-ml centrifuge tube containing 1 ml of bacterial suspension (ca. 2×10^7 cells). After incubation at 20 C for 1 hr, the capillary was removed from the centrifuge tube. The sealed end of the capillary was broken off, and the contents were squirted into a tube containing 10 ml of BM with 3% NaCl. Suitable dilutions were made in BM with 3% NaCl, then 0.1 ml portions of diluents were mixed on NA plates with 5 ml of nutrient soft agar premelted and kept at 50 C. After incubation at 20 C, colonies were counted. Assay points were duplicated for all experiments.

Results and Discussion

Effect on survival of sodium chloride in suspending medium

Cells grown on NA were suspended in 10 ml of PY containing 2% sodium chloride, and 0.1 ml of this suspension was added into 10 ml of one of the test media. Immediately after the addition and again after incubation for 30 and 60 min at 20 C, colony forming units in the media were determined by the pour-plate method with NA. The results obtained are given in Fig. 1. When the cells were added into BM

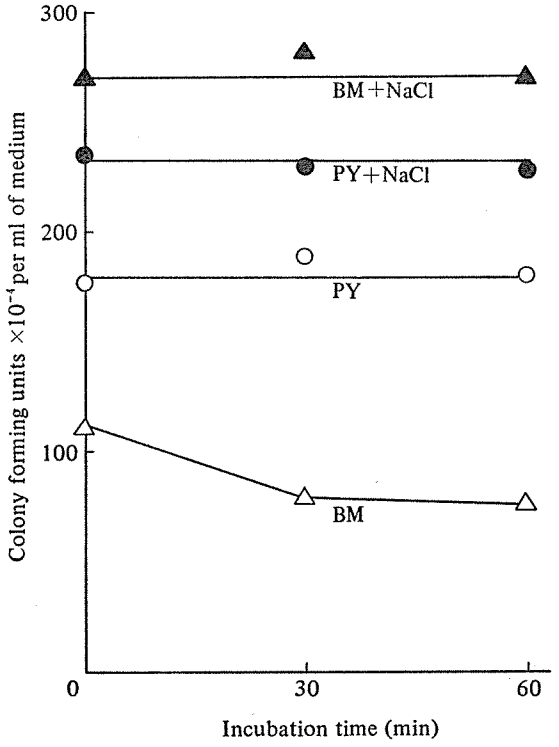

Fig. 1. Effect of suspending media on survival. Symbols indicate survival in PY (○), PY plus 3% NaCl (●), BM (△), and BM plus 3% NaCl (▲).

without sodium chloride, survival decreased during the incubation, whereas in BM with 3% sodium chloride, survival remained relatively high level. Therefore, BM with 3% NaCl was used for the dilution of bacteria accumulated in the capillary. In PY, the survival was unchanged during incubation, irrespective of the presence of sodium chloride. This finding indicates that PY is a more suitable suspending medium than BM for assay of the chemotactic response to sodium chloride.

Concentration response for sodium chloride

Cells grown on NA were suspended in PY at a density of ca. 2.0×10^7 cells per ml and assayed for chemotaxis for sodium chloride. Figure 2 demonstrated the 1-hr

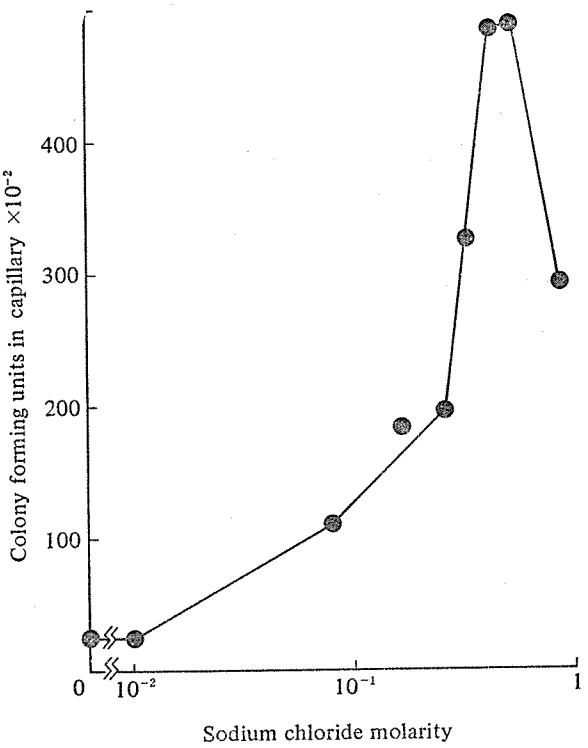

Fig. 2. Concentration response to sodium chloride.

responses to different concentrations of sodium chloride. When the capillary contained PY without sodium chloride, a relatively small number of bacteria (ca. 2.5×10^3) entered the capillary. This background accumulation is presumably caused by random movement (1). The capillaries containing 2 to 3% sodium chloride (0.34 to 0.51 M), the optimal concentration for the growth of *Photobacterium*, accumulated ca. 5×10^4 bacteria, i.e., 20 times the background accumulation.

Concentration response for glucose

It is well known that glucose is an effective attractant for *Escherichia coli* (2, 3). To avoid the influence of NaCl on the survival during the chemotaxis assay, cells of IFO 13896 were suspended in BM with 3% NaCl and assayed for the chemotaxis toward glucose. The 1-hr responses to different concentrations of glucose are shown in Fig. 3. When the capillaries contained more than 5 mM glucose, motile bacteria were attracted.

From the results shown in figures 2 and 3, it can be concluded that *P. phosphoreum* IFO 13896 is really motile and exhibits chemotaxis for sodium chloride and glucose.

Bergey's Manual of Determinative Bacteriology, 8th edition, says that strains of *Photobacterium* are "motile by one or more polar flagella, or occasionary non-motile." Some *Photobacterium* strains may not exhibit motility in hanging drop preparations, though really motile. The motility of such strains can be detected by the capillary

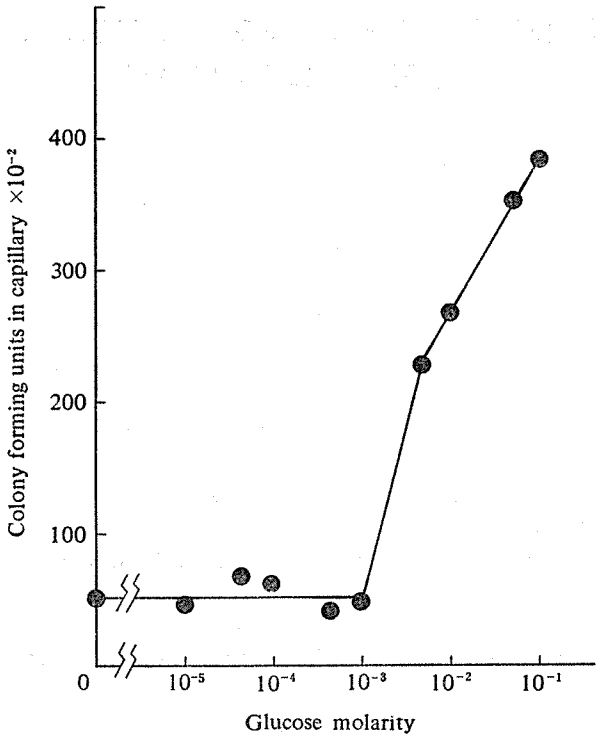

Fig. 3. Concentration response to glucose.

tube method. The growth and chemotactic responses to various salts will be examined using chemically defined media.

I am grateful to Dr. K. Matsui and Dr. S. Takao, for critical reading of the manuscript.

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COMPOUNDS PROTECTING L-DRIED CULTURES FROM MUTATION

Takeshi SAKANE, Isao BANNO and Teiji IIJIMA

Summary

Sixty-nine compounds were screened as additives to suspending medium that increase the survival value of L-dried cells of *Escherichia coli* B/r *recA*$^-$, which was sensitive to L-drying for lack of a system of post-replication repair of damaged DNA.

Adonitol, AICA, arabitol, cysteine, dithioerythritol, dithiothreitol, semicarbazide, sodium thioglycollate and thiourea were found to be remarkably effective, and adenosine, cystine, cytidine, dulcitol, erythritol, glutathione, thiomalic acid, urea and xylitol to have a weak protective effect. In dried cells of *recA*$^+$ strains, adonitol, cysteine and thiourea not only increased survival but also prevented induction of mutation. Thiourea completely precluded mutation at a concentration of 30 mM.

L-Drying has been used for preservation of various bacterial strains in the IFO culture collection, and almost all strains, except such special bacteria as marino-spirilla (3, 4), are expected to survive semi-permanently. On the other hand, several researchers have reported that dryness induces mutation in microbial cells (1, 2, 5, 11, 12), and we have also found that, in a small fraction of bacteria, mutation occurs during storage of the dried cultures at elevated temperatures (10). The frequency of mutation was found to be dependent on the temperature, with mutation occurring more frequently at higher temperature, although it did not occur at temperatures lower than 5 C and its frequency did not exceed 10^{-7} at elevated temperatures.

Even this low frequency of mutation, however, is undesirable for the maintenance of the original characters of organisms. Consequently we conducted a study to improve the method of L-drying by preventing mutation during storage of dried culture even at higher temperature. Compounds were found which protect L-dried culture from mutation when they were added to suspending medium for drying.

Materials and Methods

Bacteria used. *Escherichia coli* F3297 (B/r *arg*$^-$ *recA*$^-$) was employed as an indicator in screening protective agents. *E. coli* F3303 (B/r *trp*$^-$) and F3295 (B/r *arg*$^-$ *uvrA*$^-$) were used to determine mutation frequency of reversion from auxotroph to prototroph.

Media. IFO 203 medium contained 1% polypeptone, 0.3% dehydrated yeast extract, 0.2% glucose, 0.3% glycerol, 2% condensed liver extract, 0.2% NaCl, and

1.5% agar. Minimal medium was composed of 0.7% K_2HPO_4, 0.3% KH_2PO_4, 0.1% ammonium sulfate, 0.1% NaCl, 1% monosodium glutamate, 0.01% $MgSO_4 \cdot 7H_2O$, a trace of vitamin mixture, and 1.5% agar.

Difco nutrient broth supplemented with 0.2% yeast extract and 0.5% glucose (NYG medium) was used for rehydration of dried cultures and for assay of viability.

Preparation of L-dried culture. Basal suspending medium (BSM) was composed of 0.1 M potassium phosphate buffer (pH 7.0) and 3% monosodium glutamate. Test compounds were sterilized by filtration through Millipore filter and added to BSM. Cells harvested from a slope culture on IFO 203 medium incubated at 28 C for 2 days were suspended in BSM at a density of about 2×10^9/ml and mixed with an equal volume of the BSM supplemented with a test compound. The cell suspension was dried *in vacuo* without freezing according to the method described by Iijima and Sakane (6).

Viability of L-dried culture. Viability was determined immediately after drying and after an accelerated storage test at an elevated temperature, 37 C, by the procedure previously reported (10). Viability is expressed as the percentage of colony forming units (CFU) relative to the initial suspension before drying.

Mutation frequency. A portion of rehydrated culture was inoculated into 50 ml of fresh NYG medium and incubated at 28 C. After 20 hr the cells were harvested, washed twice, and resuspended in 2 ml of the minimal medium. The resuspension was divided into two portions, of which one was used for counting the number of viable cells and the other plated on selective minimal agar medium. Reverse mutation from auxotroph to prototroph was scored by counting revertant colonies appearing on the minimal medium after incubation for 48 hr at 37 C. Reversion frequency was expressed as the number of revertants per 10^8 viable cells.

Results and Discussion

Effects of suspending media

Cells of strains F3297 and F3303 were each suspended in pure distilled water, in solution of 3% monosodium glutamate (MSG), in solution of 3% MSG and 1% polyvinylpyrrolidone (PVP), in phosphate buffer containing 3% MSG, and in phosphate buffer containing 3% MSG and 1% PVP, and L-dried. Survival values and reversion frequencies of these dried cells were determined after drying and after preservation for 2 weeks at 37 C. The results are shown in Table 1. In *recA*$^-$ strain F3297, the survival value fell sharply during preservation, but increase of mutation was not found. In *recA*$^+$ strain F3303, on the other hand, the survival value was relatively high while the reversion frequency was increased in all suspending media. It is known that dehydration of cells of *E. coli* causes single strand breakage in their chromosomal DNA (1, 2). This breakage is repaired by a post-replication repair system involving the *recA*$^+$ gene, a system lacking in F3297 but effective in F3303.

Table 1. Survival value and reversion frequency of *E. coli* F3297 (*arg*$^-$, *recA*$^-$) and F3303 (*trp*$^-$, *recA*$^+$) dried in various suspending media.

Suspending media	After drying				After preservation for 2 weeks at 37 C			
	% Survival		⊿RF[4]		% Survival		⊿RF	
	F3297	F3303	F3297	F3303	F3297	F3303	F3297	F3303
Distilled water	0.63	7.9	0	17.1	0.001	0.18	NT[5]	3.7*
3% MSG[1]	48	66	0	4.6	0.24	15	0*	51.1
3% MSG+1%PVP[2]	26	48	0	6.1	0.13	9.6	0*	58.7
Pb+3% MSG[3]	59	73	0	0.1	1.8	52	0	19.5
Pb+3% MSG,1% PVP	35	47	0	0.5	0.48	18	0	26.2

1) MSG: monosodium glutamate. 2) PVP: polyvinylpyrrolidone. 3) Pb, 0.1 M phosphate buffer. 4) RF: reversion frequency expressed as the number of revertants to prototroph per 10^8 CFU; ⊿RF, increase in reversion frequency. 5) NT: not tested.
* Unreliable, because survival value is too small to evaluate reversion frequency.
CFU in cell suspension of F3297 and F3303 before drying is 9.2×10^9/ml and 1.5×10^{10}/ml respectively. Spontaneous background of RF is 1.2 and 4.3 respectively.

The sharp fall in the survival value of F3297 was considered to be the result of damage remaining unrepaired after rehydration. In *recA*$^+$ strain, the survival value was much higher, as damage was repaired.

It was assumed that the difference in the logarithm of the survival value between F3303 and F3297 in the same suspending medium correlates with the capacity to repair the damaged DNA in F3303. In other words, the degree of restoration of viability by DNA repair is probably equivalent to the difference in the logarithm of the survival value between *recA*$^-$ and *recA*$^+$ strains. Lesion of DNA is considered to be one origin of mutation. Consequently it was also assumed that the degree of restoration of viability correlated with the frequency of reverse mutation. Reversion frequencies in the different suspending media are plotted against the degree of restoration in Fig. 1. The data obtained immediately after drying show a linear relationship, indicated by line A. The data after 2 weeks of preservation at 37 C also show a linear relationship, indicated by line B. Thus the second assumption is borne out. The presence of two lines, A and B, implies a difference in the kind of DNA lesion or in the mechanism of induction of mutation between revertants found immediately after drying and after preservation.

When distilled water was used as suspending medium, the survival value of F3303 fell to 0.63% and appreciable mutation was found even before preservation. This suggests that dehydration without a protectant damages the DNA immediately. When phosphate buffer containing MSG was used, both F3303 and F3297 survived drying well and gave few revertants. But while phosphate and MSG each protected DNA from injury during drying, they did not prevent damage of DNA during preservation of dried cells, because F3297 showed a low viability and F3303 an appreciable frequency of revertants after preservation.

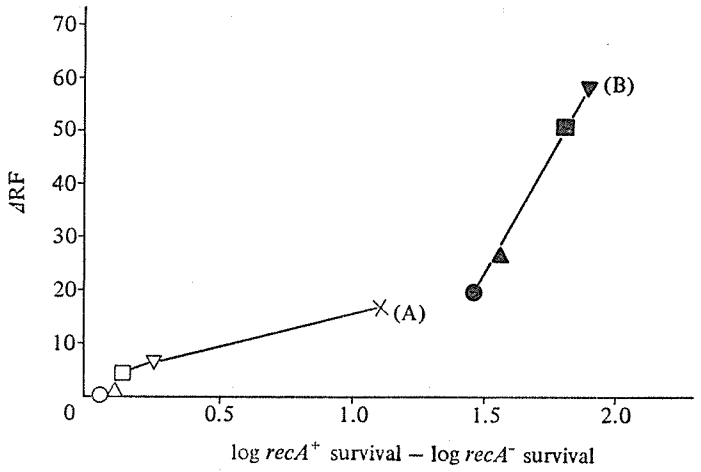

Fig. 1. Relationship between *recA*$^+$-dependent restoration of viability and reversion frequency.

The curves are the plots of ΔRF of F3303 (*recA*$^+$) against the logarithm of the survival value of F3303 divided by that of F3297 (*recA*$^-$) in the same suspending medium. Open symbols (A) indicate values immediately after drying and closed symbols (B) after preservation at 37 C for two weeks. Suspending media: distilled water (×), 3% monosodium glutamate (□), 3% monosodium glutamate with 1% polyvinylpyrrolidone (▽), phosphate buffer containing 3% monosodium glutamate (○), and phosphate buffer containing 3% monosodium glutamate and 1% polyvinylpyrrolidone (△).

Protectant screening

If cells are dried and preserved under conditions such that their DNA escapes damage, mutation should not be induced in the dried cells. Such conditions might also increase the survival of dried cells of *recA*$^-$ strains, even on preservation at elevated temperature. We therefore sought protective compounds that would considerably increase the viability of dried cells of the *recA*$^-$ strain F3297 when added to the basal suspending medium (BSM). Sixty-nine compounds were screened. In nine separate screening experiments, the survival values of F3297 in BSM was $45\pm5\%$ immediately after drying and $0.75\pm0.25\%$ after preservation for 4 weeks at 37 C.

Table 2 lists the effects of the test compounds added to BSM as the viability of F3297 relative to that in BSM without additive. The 24 compounds listed in Table 3 were found effective in protecting the dried cells against loss of viability.

Of the thiol compounds, cysteine was the most effective, while all the other thiol compounds were effective to some extent. The SH radical is thus considered to be involved in the protection. SH compounds such as cysteine have also been reported to reduce the frequency of mutation induced by alkylating agents (8).

Of the carbohydrates, adonitol, D-arabitol, L-arabitol, and xylitol, all of which are isomers of sugar alcohol with 5 carbons were most effective. Polyols with 4 and 6 carbons, namely erythritol, dulcitol, mannitol, and sorbitol, gave viabilities of only

Table 2. Effect of additives to suspending medium on survival of *E. coli* F3297 after drying and preservation.

Additive	Concentration (mM)	Relative ratio of survival	
		After drying	After preservation
Amino acids			
Arginine	30	1.2	1.8
Asparagine	30	0.96	0.67
Citrulline	30	1.0	1.8
Creatine	30	1.1	2.1
Cysteine	30	1.3	16
Cystine	30	1.3	8.5
Lysine	30	0.35	0.37
Methionine	30	0.77	0.81
Tryptophan	30	0.97	1.7
Sugars and Sugar alcohols			
Adonitol	100	1.2	15
D-Arabitol	100	1.2	11
L-Arabitol	100	1.6	11
Dulcitol	100	1.4	5.5
Erythritol	100	1.2	3.3
Galactose	100	0.65	<0.01
Glucose	000	1.1	<0.01
Glycerol	100	1.3	0.04
Inositol	100	1.1	0.38
Lactose	100	0.46	0.17
Mannitol	100	0.88	2.1
Ribose	100	1.3	<0.01
Sorbitol	100	1.0	2.3
Xylitol	100	1.4	8.8
Nucleic acid-related compounds			
Adenine	20	1.3	1.8
Adenosine	20	1.0	4.1
AICA[1)]	20	1.2	18
Allantoin	20	0.95	0.74
Cytidine	20	1.1	4.2
Cytosine	20	1.6	3.6
Guanosine	20	1.0	1.9
SAICAR[2)]	20	1.0	0.20
Thymidine	20	1.3	3.5
Thiol compounds			
Dithioerythritol	1.5	1.5	13
Dithiothreitol	1.5	1.4	11
Glutathione	30	1.4	4.4
Thioglycollate	15	1.4	12

Table 2 (continued)

Additive	Concentration (mM)	Relative ratio of survival	
		After drying	After preservation
2-Thioadenine	5	1.0	0.47
6-Thioguanine	5	1.2	1.9
Thiomalic acid	30	1.2	4.6
2-Thiouracil	5	0.58	0.69
Amines			
Acetamide	30	0.91	1.2
Agmatine	20	1.3	0.94
2-Aminothiazol	5	1.5	1.4
Benzamide	30	0.82	3.6
Cadaverine	20	1.2	2.2
Diaminomethane	30	0.91	0.51
Ethylenediamine	30	0.68	0.58
Histamine	20	0.79	2.0
Hydroxylamine	20	0.11	<0.01
Nicotinamide	20	0.10	0.15
Putrescine	20	0.72	1.9
Semicarbazide	20	1.7	12
Spermidine	10	1.1	1.4
Spermine	10	1.2	1.3
Thiourea	20	1.5	21
Urea	20	1.2	9.8
Others			
Calcium lactate	100	0.26	0.20
Dimethyl sulfoxide	30	0.15	0.33
EDTA-2Na[3]	1	1.5	0.95
Nitrilotriacetic acid	1	1.6	0.53
Pyridoxal	15	0.097	<0.01
2-Pyrrolidone	100	0.005	<0.01
Rongalit[4]	15	0.56	0.07
Sodium ascorbate	15	0.97	<0.01
Sodium azide	5	0.63	0.48
$CoCl_2 \cdot 6H_2O$	0.5	1.5	0.046
$CuSO_4 \cdot 5H_2O$	0.5	1.5	0.26
$FeSO_4 \cdot 7H_2O$	0.5	1.4	0.47
$MgSO_4 \cdot 7H_2O$	0.5	1.2	0.041

1) AICA, 4-amino-5-imidazolcarboxamide
2) SAICAR, 5-amino-4-imidazol-N-succinocarboxamide riboside
3) EDTA-2Na, ethylenediaminetetraacetic acid disodium salt
4) Rongalit, formaldehyde sodium sulfoxylate

Table 3. Compounds enhancing survival on preservation of *E. coli recA*$^-$.

Thiol compounds:
Cysteine, cystine, dithioerythritol, dithiothreitol, glutathione, sodium thioglycollate, 6-thioguanine and thiomalic acid.
Sugar alcohols:
Adonitol, D- and L-arabitol, dulcitol, erythritol, mannitol, sorbitol and xylitol.
Carbamide compounds:
Semicarbazide, thiourea and urea.
Nucleic acid-related compounds:
Adenosine, AICA, cytidine, guanosine and thymidine.

about 30% of those obtained with the 5 carbon polyols. Glycerol, which has three carbons, gave no effect. In contrast, sugars such as ribose, glucose and lactose gave lower survival values than BSM alone. Both the nature of the polyol and the molecular size of 5 carbons thus appear significant for the ability to protect.

Of the amine compounds, urea, thiourea and semicarbazide were remarkably effective, but diaminomethane, acetamide and polyamines showed no effect, even though they are similar in structure to the former three carbamides. The structure $-N-C(=O)-N<$ might be significant for protection. It has been reported that thiourea, which is a potent scavenger of hydroxyl radical, prevents DNA nicking caused by γ-irradiation, but that urea does not (7).

Ribosides of purine and pyrimidine had a weak protective effect, which may be due to a structural analogy to a DNA component. The strongly protective action of AICA seems to work through a different mechanism from that of ribosides, probably through a similar mechanism to the carbamides.

Cooperative protection

Table 4 shows the viability of *recA*$^-$ strain F3297 when the cells were dried in distilled water, phosphate buffer and BSM each supplemented with an indicated protectant. Only thioglycollate gave a higher survival of 2.9% in the absence of phosphate and/or glutamate, while the other 7 protectants did not alone have an estimable effect. Adonitol and sorbitol were remarkably effective in phosphate buffer. Cysteine and thioglycollate of the thiol compounds, and thiourea and semicarbazide of the carbamide compounds, were effective only in phosphate buffer containing glutamate. Cytidine and AICA were less effective in phosphate buffer than in BSM. The protectants thus required glutamate and phosphate to display their full capacity to prevent loss of viability in the dried *recA*$^-$ cells.

Optimum concentration of protectants

The effect of concentration of three protectants, adonitol, cysteine, and thiourea, on recovery of viability was examined. From the results presented in Fig. 2, the optimum concentration of the three protectants was 30–100 mM, 3–30 mM, and 10–30 mM, respectively.

Table 4. Survival value of *E. coli recA*$^-$ dried in various suspending media.

Additive	% Survival		
	Basal suspending media		
	DW[1]	Pb[2]	Pb+MSG[3]
None	0.0003	0	1.7
Cysteine, 30 mM	0	0.53	17
Thioglycollate, 30 mM	2.9	6.14	18
Adonitol, 100 mM	0.0010	28	20
Sorbitol, 100 mM	0.0060	11	14
Thiourea, 30 mM	0.22	1.4	32
Semicarbazide, 30 mM	0	0.46	6.9
Cytidine, 30 mM	0.47	5.2	10
AICA, 30 mM	0	2.3	18

1) DW: distilled water. 2) Pb: phosphate buffer. 3) Pb+MSG: phosphate buffer containing 3% monosodium glutamate
Colony forming units before drying number 9.1×10^8 per ampoule.
The survival values were determined after preservation for 3 weeks at 37 C.

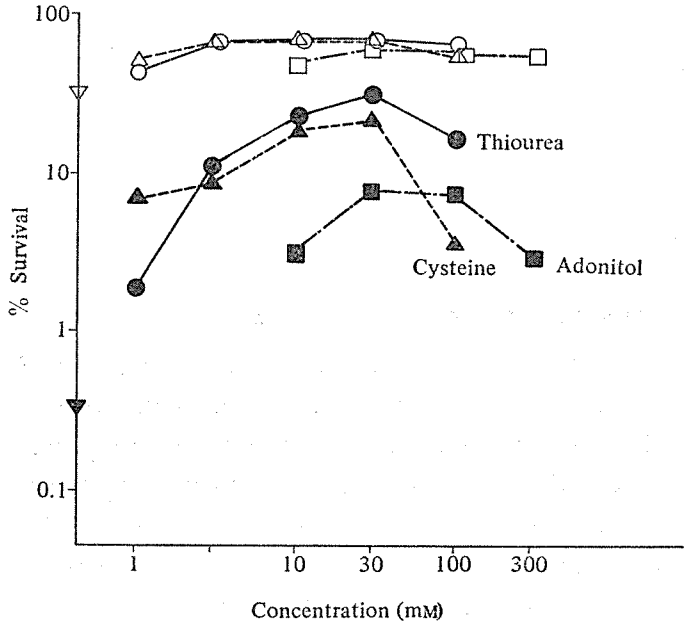

Fig. 2. Influence of additive concentration in suspending medium on survival of *E. coli recA*$^-$.
Open symbols, survival immediately after drying; closed symbols, survival after preservation for 2 weeks at 37 C; circles, thiourea; triangles, cysteine; squares, adonitol; and inverted triangles, BSM.

Preincubation of cells with the protectant

Before drying, cells were incubated in BSM with 30 mM cysteine, 30 mM thiourea, or 100 mM adonitol at 28 C for 60 min, and washed with BSM. The washed

cells were resuspended in BSM with or without the same protectant, then L-dried. Viabilities of the dried cells are presented in Table 5. The cells dried without protectant after treatment with the protectant gave equivalent survival values to cells dried without treatment. The increase of survival value resulted only from drying in BSM with one of the protectants. It was thus concluded that pretreatment of cells with protectant had no effect.

Table 5. Effect of preincubation with adonitol, cysteine or thiourea on survival of L-dried *E. coli recA*$^-$.

Additive	Preincubation for 60 min.	Addition to suspending medium	% Survival	
			After drying	After preservation[2]
None[1]	−		47	1.9
	+		52	0.92
Cysteine, 30 mM	−	+	62	15
	+	+	75	16
	+	−	54	2.6
Adonitol, 100 mM	−	+	51	16
	+	+	48	13
	+	−	53	3.7
Thiourea, 30 mM	−	+	53	32
	+	+	67	29
	+	−	60	1.4

1) BSM
2) 37 C for 4 weeks

Effect of addition of protectant to rehydration medium

Cells of the *recA*$^-$ strain F3297 stored at 37 C after drying in BSM without protectant were rehydrated in NYG medium supplemented with cysteine, thiourea or adonitol, and their viabilities were compared with that of cells rehydrated only with NYG. As Table 6 shows, the addition of the protectants to the rehydration medium failed to enhance the recovery of viable cells. Addition of cysteine to NYG rather produced a harmful effect, a decrease of viability to 10% of that in normal rehydration with NYG. These results suggest that the protective action of the three protectants takes place in the process of L-drying or during preservation.

Prevention of mutation by addition of protectant

To examine whether the protectants against loss of viability of L-dried cells of *recA*$^-$ strain reduce the mutation frequency in *recA*$^+$ wild-type strain after accelerated storage, the three representative protectants, cysteine, thiourea, and adonitol, which were chosen from the 24 compounds listed in Table 3, were examined for their effect on reversion frequencies after storage. Two *recA*$^+$ and auxotrophic mutant strains, F3303 and F3295, were L-dried in the BSM with or without adonitol (100 mM), cys-

Table 6. Effect of cysteine, adonitol or thiourea in rehydration medium on survival of L-dried *E. coli recA*$^-$.

Additive	Concentration (mM)	Viability recovered (CFU $\times 10^{-5}$ per ampoule)	Ratio
None[1)]		274	1.0
Cysteine	2	253	0.92
	20	27	0.10
Thiourea	3	265	0.97
	30	289	1.05
Adonitol	10	258	0.93
	100	271	0.99

1) NYG medium

Table 7. Changes in survival value and frequency of reversion to prototroph in L-dried culture of *E. coli* F3295 (*arg*$^-$, *uvrA*$^-$) and F3303 (*trp*$^-$) on addition of cysteine, thiourea or adonitol to suspending medium

Strain	Additive	Before drying		After drying		After preservation at 37 C for 5 weeks	
		Viability (CFU/amp)	RF[1)]	% Survival	ΔRF	% Survival	ΔRF[2)]
F3303	None	3.7×10^9	3.5	78	1.8	38	24.5
	Cysteine, 30 mM			83	1.4	48	14.7
	Thiourea, 30 mM			87	2.2	75	1.7
	Adonitol, 100 mM			75	0.9	64	4.7
F3295	None	1.5×10^9	5.0	70	0	5.4	48.6
	Cysteine, 30 mM			92	0	23	20.6
	Thiourea, 30 mM			91	0.9	65	3.5
	Adonitol, 100 mM			75	3.7	25	13.0

1) RF: Reversion frequency per 10^8 cells.
2) ΔRF: Increase of reversion frequency per 10^8 cells.

teine (30 mM), or thiourea (30 mM). Viability and reversion frequencies determined after drying and after storage at 37 C are presented in Table 7. In both strains, the viability immediately after drying was 70–90% of that before drying and no reversion was found. In F3303, the three compounds increased viability and reduced the frequency of revertants after storage in comparison with BSM alone: cysteine increased viability 1.3 times and reduced reversion to 1/3; adonitol increased vaibility 1.7 times and reduced reversion to 1/5; and thiourea increased viability twice and remarkably reduced reversion to 1/14. In strain F3295, cysteine increased viability 4.3 times and reduced reversion to 2/5; adonitol increased viability 4.6 times and reduced reversion to 1/4; and thiourea increased viability 12 times and considerably reduced reversion to 1/14. It is apparent that the three protectants prevent DNA damage and, as a result, reduce the frequency of mutation. The effect is the greatest with thiourea,

addition of which results in the highest survival value and almost completely prevents the induction of reversion.

The fact that the three compounds screened as agents which protect dried cells of *E. coli recA*$^-$ strain from death also, as might be expected, prevented the induction of mutation, supports the hypothesis that lesion of DNA during preservation of L-dried cells is a causual event of death in the *recA*$^-$ strain and of mutation in the *recA*$^+$ strain.

In other bacteria, L-drying must similarly damage the DNA, which will be repaired after rehydration, perhaps resulting in mutation. Addition of such protectants as adonitol, cysteine, and thiourea seems likely also to suppress DNA injury and prevent the induction of mutation in these dried cultures. We decided, therefore, to add both 100 mM adonitol and 3 mM cysteine to the basal suspending medium for preparing the L-dried cultures of all bacterial strains in the IFO collection. Thiourea was excluded from general use because its inhibitory action on the growth of some bacteria. A preliminary report on the marked effect of these additions on some bacteria sensitive to ultraviolet irradiation has been made in another paper (9).

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PRESERVATION OF YEAST CULTURES BY L-DRYING: VIABILITIES OF 1710 YEASTS AFTER DRYING AND STORAGE

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Summary

One thousand seven hundred and ten yeast strains belonging to 385 species in 46 genera were dried *in vacuo* from a liquid state and preserved. Their viabilities after drying and after accelerated storage at 37 C were determined. The rate of decrease in viability of a dried culture during storage was constant and the survival value after 30 days at 37 C roughly corresponded to that after 5 years at 5 C. Decimal reduction time in loss of viability during long-term preservation at 5 C was estimated for each strain. The majority of the L-dried strains (about 91%) can be expected to survive for at least 50 years on storage at 5 C.

L-drying has successfully been used for preservation of bacterial strains (1, 3, 4). We have also attempted to apply L-drying to yeast preservation, and reported on a suitable suspending medium for yeast and the viabilities of 149 strains dried with the suspending medium (2). Of the 149 strains, the survival values of 141 were thought high enough for them to survive over 10 years when stored at a low temperature (5 C). L-drying is considered to be a simple and reliable method for preserving a large number of yeast cultures for a long term. Subsequently, all of the 1710 named yeast strains collected in the IFO have been subjected to L-drying. This paper deals with their viabilities in an accelerated storage test at 37 C and the decimal reduction time in viability at 5 C, which was extrapolated from the reduction rate at 37 C.

Materials and Methods

The yeast strains used have been maintained by serial transfer on potato-yeast extract-glucose agar medium since they came to the IFO.

YM medium was used in pre-culture for drying and in determination of viability.

Phosphate buffer (0.1 M, pH 7.0) containing 5% monosodium glutamate, 5% lactose and 6% polyvinylpyrrolidone (PVP) was used as the suspending medium for drying.

Preparation of L-dried culture and determination of viability were carried out as reported previously (2).

Twenty ampules of dried cultures were prepared for each strain: two ampules were immediately rehydrated and examined for viable count; 4 ampules were kept

at 37 C for an accelerated storage test and examined after 60 days; the remaining 14 ampules were stored at a lower temperature, 5 C, for a long-term preservation.

Results and Discussion

Fig. 1 shows the reduction of survival values of the dried cells in 6 randomly chosen strains. The reduction in logarithm of the survival value is almost linear. A similar linear decline of viability was found in the other yeast strains. Freeze-dried and L-dried cultures of bacteria also show a linear decline during preservation (4, 5). The straight line indicates that the degradation rate is constant during storage in L-dried culture. The reduction rate varied from strain to strain.

The viabilities of dried cultures of 42 yeast strains preserved for 5 years at 5 C were determined and compared with the values estimated 5 years ago for the same dried cultures after accelerated storage for 30 days at 37 C. The data are presented graphically in a correlation map (Fig. 2), which reveals a fairly good correlation between the values after 5 years at 5 C and after 30 days at 37 C. The correlation coefficient is 0.91. The two values compared are equivalent with the limit of experimental error; the survival value after accelerated storage for X days statistically corresponds to the value after 60 times X days in preservation at 5 C. We may thus predict the prospective viabilities of dried cultures stored at a lower temperature from the data obtained in the accelerated test.

A total of 1710 yeast strains representing 385 species in 46 genera were L-dried. Their viable counts before drying and their survival values immediately after drying and after accelerated storage at 37 C are arranged by genera and species in taxonomical order in Table 1. On the assumption that the survival value after 60 days of preservation at 37 C sufficiently corresponds to the value after 10 years at 5 C, and that the reduction of survival at 5 C is linear, the decimal reduction time at 5 C (D-value), that is, the time required for viability to decline tenfold, was calculated for each yeast by the following equation: $D=10/(\log A-\log B)$, where A is survival after drying and B is survival after 60 days at 37 C. D-values are given in the 6th column of Table 1.

Six strains of *Saccharomyces exiguus, Leucosporidium scottii, Candida bogoriensis, C. slooffii, Cryptococcus hungaricus* and *Torulopsis psychrophila* did not survive the drying process. Further investigation into the drying conditions for these strains is required to obtain viable dried cultures.

Fifty-five strains showed very low viabilities of less than 3% after drying. Seventeen of the 55 strains survived well during storage at 37 C, showing lower degradation rate. These should be able to survive with more than 10^4 cells per ampule for at least 40 years. They belong to *Nadsonia commutata, Kluyveromyces marxianus, Pichia angophorae, Saccharomyces bayanus, S. uvarum, Leucosporidium antarcticum, L. stokesii, Cryptococcus albidus, C. dimennae, C. hangaricus, Rhodotorula rubra* and *Trichosporon cutaneum* var. *antarcticum.*

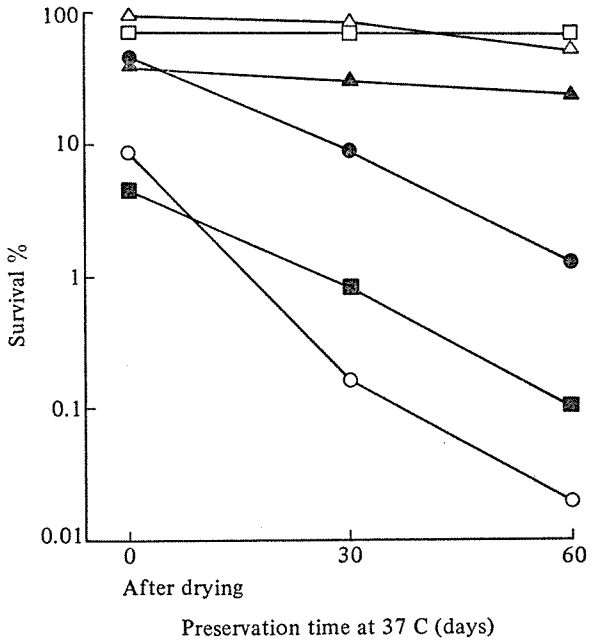

Fig. 1. Decline in survival value of L-dried cultures during accelerated storage at 37 C.
Ambrosiozyma monospora IFO 1965 (○), *Botryoascus synnaedendrus* IFO 1604 (△), *Candida brumptii* IFO 1452 (□), *Debaryomyces melissophilus* IFO 1901 (●), *Leucosporidum frigidum* IFO 1851 (■), *Saccharomyces cerevisiae* IFO 1952 (▲).

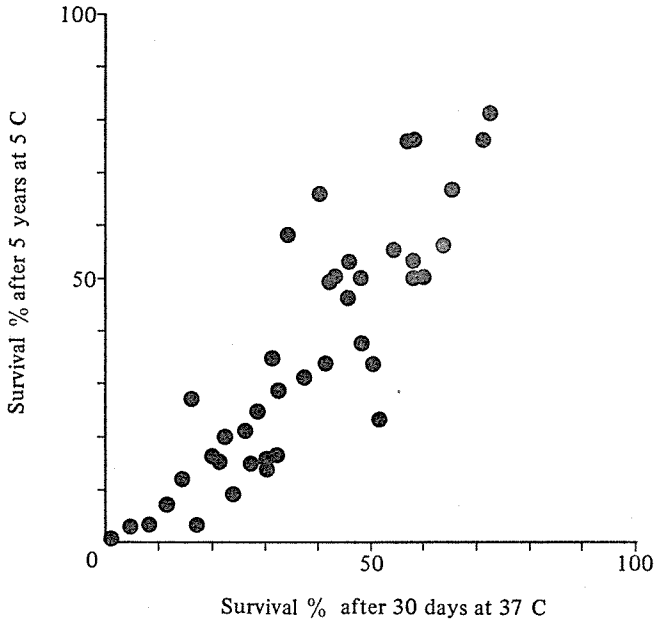

Fig. 2. Correlation between survival values of the L-dried cells after 30 days of accelerated storage at 37 C and after 5 years of preservation at 5 C in 42 yeasts.

Table 1. Viability of 1710 yeasts after L-drying and after preservation of the dried cultures.

	Number of strains dried	Viable count before drying (10^7 CFU/ampule)	Survival value (%) after preservation at 37 C for		D-value[1)] at 5 C (years)
			0	60 days	
ASCOIDEACEAE					
Dipodascus albidus	1	0.1	6.5	0.2	13
ENDOMYCETACEAE					
Endomyces magnusii	2*	0.3, 0.3	29, 88	8.2, 62	12, 65
E. reessii	1*	1.6	16	5.3	20
SACCHAROMYCETACEAE SCHIZOSACCHAROMYCETOIDEAE					
Schizosaccharomyces japonicus var. *japonicus*	1**	0.6	2.7	0.5	13
	3	0.3 – 0.5	16 – 22	5.0 – 25	19 – 48
S. japonicus var. *versatilis*	2	0.2, 0.4	8.9, 36	8.9, 34	402,
S. malidevorans	1	1.3	36	32	500
S. octosporus	2**	4.2, 9.0	1.3, 10	0.1, 0.8	8, 9
	1	0.3	16	15	214
S. pombe	25	0.6 – 4.0	13 – 67	12 – 60	>33
SACCHAROMYCETOIDEAE					
Ambrosiozyma cicatricosa	1*	1.0	2.3	0.05	6
A. monospora	2*	0.4, 5.9	0.6, 8.8	0, 0.02	<4
A. philentoma	1	3.1	1.2	0.05	7
Arthroascus javanensis	2	3.3, 6.1	1.2, 17	0.01, 3.7	4, 14
Botryoascus synnaedendrus	1*	32	70	65	338
Citeromyces matritensis	3	1.6 – 6.2	17 – 43	6.2 – 28	22 – 168
Debaryomyces cantarellii	4	1.3 – 8.5	11 – 44	3.5 – 27	19 – 48

D.	*castellii*	1	2.4	5.4	2.1	24
D.	*coudertii*	2	4.7, 11	13, 46	0, 0.01	0, 2
D.	*formicarius*	1	3.6	55	20	22
D.	*hansenii*	1**	2.7	23	2.1	6
		63	0.9 – 22	23 – 81	5.7 – 40	12 – 152
D.	*marama*	3	10 – 15	44 – 84	11 – 33	17 – 25
D.	*melissophilus*	2	8.7, 15	40, 44	1.2, 5.6	6, 11
D.	*nepalensis*	2	6.2, 12	45, 63	12, 14	15, 17
D.	*phaffii*	1	6.1	12	6.1	34
D.	*tamarii*	1	1.9	14	0	0
D.	*vanriji*	2	1.5, 4.3	29, 31	18, 20	44, 60
D.	*yarrowii*	1	3.5	61	18	19
Dekkera bruxellensis		1	2.2	4.2	0	0
D.	*intermedia*	1	5.4	19	4.3	15
Hansenula anomala var. *anomala*		39	1.3 – 23	12 – 50	4.1 – 38	15– 126
H.	*anomala* var. *miso*	1	9.0	30	29	114
H.	*anomala* var. *schneggii*	3	1.0 – 7.1	32 – 47	24 – 38	38 – 88
H.	*beckii*	3*	2.6 – 5.7	45 – 57	25 – 40	40 – 87
H.	*beijerinckii*	9	4.3 – 11	7.9 – 38	4 – 22	28 – 441
H.	*bimundalis* var. *americana*	2	14, 15	27, 36	26, 36	>471
H.	*bimundalis* var. *bimundalis*	2	12, 16	37, 41	23, 33	41, 222
H.	*californica*	5	8.9 – 19	34 – 47	16 – 44	27 – 62
H.	*canadensis*	4	9.6 – 12	35 – 47	34 – 44	>249
H.	*capsulata*	7	1.0 – 21	10 – 59	1.6 – 43	12 – 57
H.	*ciferrii*	1**	1.0	11	0.8	8
		4	3.8 – 13	24 – 40	9.2 – 30	23 – 80
H.	*dimennae*	1**	8.5	28	0.9	6
		5	10 – 24	26 – 41	14 – 30	33 – 626

Table 1. (continued-1)

	Number of strains dried	Viable count before drying (10^7 CFU/ampule)	Survival value (%) after preservation at 37 C for		D-value at 5 C (years)
			0	60 days	
Hansenula dryadoides	1	5.9	17	1.4	9
H. fabianii	6	4.1 – 29	37 – 83	17 – 75	26 – 226
H. glucozyma	1	6.9	15	15	>300
H. henricii	2	1.1, 4.1	25, 43	1.4, 13	10, 34
H. holstii	5	12 – 16	30 – 56	22 – 48	>66
H. jadinii	2	1.4, 10	23, 47	10, 44	26, 68
H. minuta	3	14 – 24	26 – 32	14 – 22	39 – 73
H. mrakii	3	7.4 – 9.7	19 – 26	19 – 21	>80
H. muscicola	1	17	56	36	52
H. nonfermentans	1	25	32	23	65
H. petersonii	2	9.7, 12	16, 34	16, 28	113
H. philodendra	1	27	65	10	12
H. polymorpha	8	15 – 39	33 – 57	18 – 49	24 – 156
H. saturnus var. *saturnus*	13	5.2 – 8.9	5 – 44	3.7 – 33	24 – 544
H. saturnus var. *subsufficiens*	2	1.2, 1.8	15, 18	4.2, 9.7	17, 36
H. silvicola	3	12 – 19	34 – 37	23 – 33	47 – 268
H. subpelliculosa	1	6.7	14	6.5	28
H. sydowiorum	1	9.3	76	43	20
H. wickerhamii	1	27	63	33	19
H. wingei	11	4.9 – 15	30 – 55	21 – 49	62 – 199
Hormoascus platypodis	1	3.2	17	3.7	14
Hyphopichia burtonii	1	1.2	55	31	41
Kluyveromyces drosophilarum	1	7.5	26	16	50
K. fragilis	6	2.7 – 17	5.6 – 35	1.1 – 20	14 – 82
K. lactis	7	5.0 – 18	33 – 47	14 – 34	25 – 102

K.	*marxianus*	1**	3.8	1.3	0.8	47
		12	3.7 – 9.4	10 – 43	4.6 – 20	24 – 579
K.	*phaffii*	3	3.3 – 6.8	3.8 – 17	0.1 – 1.1	6 – 9
K.	*polysporus*	1	12	1.3	0.1	8
K.	*thermotolerans*	6	2.3 – 8.0	36 – 75	30 – 51	27 – 308
Pachysolen tannophilus		1	34	33	7.9	16
Pichia abadieae		1	47	47	32	58
P.	*amethionina* var. *amethionina*	1	9.9	49	23	31
P.	*amethionina* var. *pachycereana*	1	3.6	51	26	34
P.	*angophorae*	1	2.7	3.0	0.9	19
P.	*besseyi*	1	3.2	19	10	22
P.	*bovis*	3	9.5 – 13	19 – 56	8.1 – 39	21 – 63
P.	*cactophila*	1	26	65	45	53
P.	*castillae*	1	48	58	21	22
P.	*cellobiosa*	1	8.7	24	10	26
P.	*chambardii*	2	2.7, 4.5	19, 20	0.7, 6.0	7, 19
P.	*dispora*	6	5.2 – 12	21 – 64	16 – 45	29 – 83
P.	*etchellsii*	2	10, 13	44, 50	24, 38	38, 84
P.	*farinosa*	21	4.8 – 26	17 – 63	3.4 – 34	14 – 88
P.	*fermentans*	1**	6.7	10	0.2	10
		3	1.9 – 8.4	12 – 85	13 – 62	54 – 74
P.	*fluxuum*	5	3.7 – 11	8.5 – 55	3.3 – 24	14 – 34
P.	*guilliermondii*	1	2.5	10	4.9	29
P.	*haplophila*	2	12, 13	37, 61	26, 33	38, 66
P.	*heedii*	3	15 – 22	43 – 48	20 – 30	26 – 182
P.	*kluyveri*	2	3.2, 11	56, 56	41, 47	71, 132
P.	*kudriavzevii*	2	4.6, 10	35, 47	19, 34	25, 1139
P.	*media*	1	2.8	43	42	>300
P.	*membranaefaciens*	5**	2.7 – 12	19 – 36	0.4 – 2.1	5 – 8
		27	1.1 – 11	16 – 64	10 – 48	17 – 178

Table 1. (continued-2)

	Number of strains dried	Viable count before drying (10^7 CFU/ampule)	Survival value (%) after preservation at 37 C for		D-value at 5 C (years)
			0	60 days	
Pichia mucosa	1	1.6	58	31	36
P. naganishii	1	33	56	51	63
P. norvegensis	1	16	48	30	62
P. ohmeri	1**	25	23	1.0	7
	7	6.6 – 17	22 – 46	8.7 – 19	16 – 37
P. opuntinae var. *opuntinae*	3	8.2 – 14	38 – 42	10 – 31	17 – 80
P. opuntinae var. *thermotolerans*	3	11 – 25	27 – 45	11 – 30	25 – 50
P. pastoris	2	13, 19	26, 40	18, 26	28
P. pijperi	4	1.5 – 13	33 – 66	26 – 58	34 – 104
P. pinus	1**	4.6	3.1	0.004	3
	3	3.5 – 9.3	52 – 80	15 – 30	16 – 23
P. polymorpha	2	3.2 – 3.5	4.6, 4.7	0.7, 0.9	12, 14
P. pseudopolymorpha	2	5.9, 6.3	8.3, 9.5	2.1, 3.5	15, 26
P. quercuum	3	14 – 20	24 – 46	8.6 – 34	22 – 56
P. rabaulensis	1	14	53	46	162
P. rhodanensis	2	1.6, 14	24, 54	18, 42	80, 93
P. saitoi	3	5.8 – 7.0	23 – 56	12 – 31	29 – 40
P. salictaria	1	10	30	24	107
P. sargentensis	1	24	18	10	41
P. scolyti	3*	15 – 36	62 – 82	29 – 45	22 – 38
P. scutulata var. *exigua*	1	5.2	16	7.7	29
P. scutulata var. *scutulata*	1	6.1	23	18	81
P. spartinae	2	6.3, 23	20, 54	7.0, 36	14, 56
P. stipitis	1**	11	5.9	0.6	7
	2	9.5, 15	2.6, 5.7	0.7, 1.0	13, 17
P. strasburgensis	2	6.1, 12	24, 54	17, 53	>71

P.	*terricola*	1**	7.5	42	2.2	10
		3	5.3 – 16	51 – 74	32 – 52	48 – 86
P.	*toletana*	4	4.1 – 12	26 – 74	8.8 – 30	14 – 219
P.	*trehalophila*	1	9.3	15	9.1	41
P.	*vini* var. *melibiosi*	1	17	71	22	20
P.	*vini* var. *vini*	1**	11	53	1.6	6
		5	2.2 – 3.1	47 – 81	19 – 43	26 – 53
P.	*wickerhamii*	1	1.5	33	26	98
Saccharomyces	*amurcae*	1	7.3	35	24	65
S.	*bailii*	29	1.3 – 11	10 – 80	1.3 – 52	10 – 202
S.	*bayanus*	1**	4.3	1.4	1.7	>300
		16	0.5 – 6.8	7.4 – 59	5.7 – 52	20 – 197
S.	*beticus*	1	0.8	21	15	72
S.	*bisporus* var. *bisporus*	13	3.6 – 10	26 – 65	13 – 58	>19
S.	*bisporus* var. *mellis*	9**	1.2 – 8.6	24 – 88	0 – 5.6	3 – 10
		1	0.9	71	35	32
S.	*capensis*	1	5.0	23	20	47
S.	*castellii*	1	6.6	24	13	39
S.	*cerevisiae*	3**	2.5 – 6.0	9.6 – 44	0.2 – 2.3	4 – 26
		99	0.8 – 8.6	6.2 – 76	3.3 – 56	10 – 288
S.	*chevalieri*	17	1.1 – 9.3	9.9 – 70	6.5 – 68	19 – 886
S.	*cordubensis*	1	2.4	34	30	49
S.	*coreanus*	2	2.5, 7.3	31, 37	24, 33	95, 185
S.	*dairensis*	3**	5.7 – 7.2	5.1 – 38	0.02– 1.9	4 – 8
		2	2.6, 21	20, 21	4.6, 14	12, 76
S.	*delbrueckii*	10	5.7 – 20	21 – 52	13 – 46	29 – 62
S.	*diastaticus*	14	2.0 – 6.5	20 – 56	14 – 50	18 –
S.	*eupagycus*	1	11	46	34	73
S.	*exiguus*	9**	1.8 – 9.3	0 – 22	0 – 6.7	<18
		1	6.8	41	37	245

Table 1. (continued-3)

		Number of strains dried	Viable count before drying (10^7 CFU/ampule)	Survival value (%) after preservation at 37 C for		D-value at 5 C (years)
				0	60 days	
Saccharomyces fermentati		3	7.7 – 9.9	44 – 77	34 – 60	89 – 99
S.	*florentinus*	1**	10	78	2.1	6
		5	5.1 – 10	65 – 82	12 – 53	12 – 64
S.	*gaditensis*	1	3.2	60	58	648
S.	*globosus*	7	2.3 – 6.9	13 – 32	2.9 – 19	12 – 47
S.	*heterogenicus*	2	1.1, 1.6	6.1, 23	5.7, 12	35, 339
S.	*hienipiensis*	1	3.9	33	29	202
S.	*hispanica*	1	2.3	36	35	>300
S.	*inconspicuus*	1	5.2	45	39	158
S.	*inusitatus*	1	1.4	8.6	3.9	29
S.	*italicus*	3	3.5 – 7.9	23 – 56	21 – 50	>220
S.	*kloeckerianus*	1**	2.3	11	4.1	21
		5	1.6 – 11	27 – 41	15 – 26	28 – 123
S.	*kluyveri*	4	2.6 – 7.4	25 – 62	10 – 51	27 – 115
S.	*microellipsodes*	3	8.4 – 12	36 – 55	17 – 38	22 – 62
S.	*montanus*	6	5.7 – 16	41 – 61	15 – 58	22 – 817
S.	*mrakii*	1	9.6	31	19	47
S.	*norbensis*	1	4.0	31	25	61
S.	*oleaceus*	1	4.4	56	47	130
S.	*oleaginosus*	2	3.5, 5.4	28, 36	23, 32	56, 531
S.	*pretoriensis*	2	5.7, 7.5	24, 26	8.5, 18	20, 89
S.	*prostoserdovii*	1	7.0	35	22	53
S.	*rosei*	18	0.6 – 19	21 – 65	13 – 65	>31
S.	*rouxii*	13**	1.0 – 4.3	7.9 – 30	0.1 – 0.9	4 – 6
		62	0.2 – 7.5	7.1 – 70	1.4 – 43	10 – 64

S.	*saitoanus*	7	4.3 – 14	21 – 60	6.9 – 45	20– 274
S.	*servazzii*	1	6.4	72	39	38
S.	*telluris*	5	0.5 – 2.0	0.8 – 11	0 – 2.6	<15
S.	*transvaalensis*	1	0.5	13	5.6	27
S.	*unisporus*	11	5.5 – 18	25 – 70	6.0 – 28	9 – 50
S.	*uvarum*	1**	2.2	1.4	0.7	33
		26	0.6 – 6.2	12 – 60	4.8 – 54	17 – 98
S.	*vafer*	4	5.3 – 15	47 – 55	39 – 49	65 – 343
Saccharomycopsis capsularis		2	0.2, 8.1	8.9, 44	0.8, 3.1	6, 8
S.	*crataegensis*	2	4.5, 5.9	63, 63	42, 45	29, 40
S.	*fibuligera*	6*	1.7 – 9.6	26 – 70	9.7 – 45	23 – 77
S.	*lipolytica*	1*,**	2.8	46	3.6	9
		25(10*)	1.3 – 8.2	30 – 100	12 – 100	>19
S.	*malanga*	1	3.2	79	57	36
S.	*vini*	1	2.9	47	35	77
Schwanniomyces alluvius		1	7.0	22	8.8	24
S.	*castellii*	1	13	18	7.1	23
S.	*occidentalis*	3	2.8 – 17	1.6 – 12	0.1– 5.3	8 – 37
S.	*persoonii*	1	7.0	15	9.6	41
Stephanoascus ciferrii		1**	10	33	7.3	15
		3	2.4 – 11	44 – 65	22 – 55	35 – 196
Wickerhamiella domercqii		1	56	73	23	20
Wingea robertsii		1	4.5	37	20	38
NADSONIOIDEAE						
Hanseniaspora guilliermondii		1	4.0	26	9.6	22
H.	*occidentalis*	2	14, 16	38, 46	8.6, 25	13, 30
H.	*osmophila*	4	3.4 – 18	15 – 45	7.8 – 35	30 – 105
H.	*uvarum*	5	4.3 – 18	25 – 79	10 – 63	25 – 217
H.	*valbyensis*	3	2.5 – 17	34 – 49	23 – 42	7 – 75

Table 1. (continued-4)

	Number of strains dried	Viable count before drying (10^7 CFU/ampule)	Survival value (%) after preservation at 37 C for		D-value at 5 C (years)
			0	60 days	
Hanseniaspora vineae	1	5.8	30	8.2	17
Nadsonia commutata	2	0.5, 0.7	0.8, 2.8	0.5, 2.8	48,
N. elongata	2	0.3, 0.5	10, 20	3.5, 8.0	21, 25
N. fulvescens	1	0.4	11	10	175
Saccharomycodes ludwigii	2**	0.9, 4.2	1.7, 14	0.1, 0.1	4, 8
	8	0.9 – 5.6	13 – 38	2.2 – 24	12 – 57
Wickerhamia fluorescens	1	3.4	29	15	36
LIPOMYCETOIDEAE					
Lipomyces lipofer	2	0.5, 1.0	0.5, 3.2	0, 0.2	0, 25
L. starkeyi	2	0.5, 1.9	3.2, 8.0	0.1, 0.5	5, 12
SPERMOPHTHORACEAE					
Metschnikowia biscuspidata	1	10.3	8.7	3.6	26
M. pulcherrima	5	2.8 – 6.8	12 – 49	8.9 – 36	18 – 523
M. reukaufii	2	1.2, 1.9	12, 19	10, 11	34, 300
Nematospora coryli	2	0.5, 0.5	0.3, 1.8	0, 0.2	0, 10
BASIDIOMYCETOUS YEASTS					
Aessosporon salmonicolor	1	2.1	12	2.8	15
Filobasidium capsuligenum	2	0.4, 4.7	18, 89	4.7, 16	7, 248
F. floriforme	3	0.6 – 2.9	23 – 63	10 – 56	30 – 197
F. uniguttulatum	1**	6.1	37	4.2	10
	3	6.4 – 8.9	44 – 59	12 – 47	17 – 101
Leucosporidium artarcticum	3	0.7 – 5.4	0.03 – 0.4	0 – 0.3	<80
L. frigidum	2	1.6, 3.1	0.8, 4.4	0.1, 0.2	6, 16

L.	*gelidum*	1	1.1	0.2	0.06	19
L.	*nivalis*	2	0.2, 2.2	1.4, 4.8	0.2, 0.9	52, 77
L.	*scottii*	5**	1.0 – 18	0 – 36	0 – 2.9	0 – 16
		5	1.6 – 3.8	10 – 29	6.3 – 12	22 – 45
L.	*stokesii*	1	5.9	1.1	0.4	22
Rhodosporidium bisporidiis		2	8.5, 9.2	11, 13	2.8, 5.0	16, 22
R.	*capitatum*	1	0.7	16	10	48
R.	*dacryoidum*	2	5.4, 6.4	57, 58	41, 56	66, 548
R.	*diobovatum*	1**	4.6	67	4.6	8
		3	3.8 – 4.7	50 – 69	14 – 39	18 – 92
R.	*infirmo-miniatum*	7	1.6 – 8.5	7.0 – 40	5.0 – 19	27 – 932
R.	*malvinellum*	2	6.4, 8.4	13, 41	1.7, 12	10, 18
R.	*sphaerocarpum*	3	0.5 – 5.4	18 – 55	13 – 37	28 – 192
R.	*toruloides*	15	0.1 – 20	8.4 – 37	3.5 – 31	37 – 748
Sporidiobolus ruinenii		1	2.3	5.2	2.5	28
SPOROBOLOMYCETACEAE						
Bullera alba		3	0.4 – 1.1	11 – 23	3.9 – 9.1	13 – 43
Sporobolomyces gracilis		2	1.2, 1.4	17, 35	10, 20	39, 46
S.	*pararoseus*	1**	0.8	7.4	0.1	5
		6	0.4 – 2.0	4.4 – 32	2.2 – 21	11 – 56
S.	*puniceus*	1	0.9	61	50	122
S.	*roseus*	7	0.4 – 1.4	3.4 – 27	2.1 – 10	11 – 58
CRYPTOCOCCACEAE						
Brettanomyces abstinens		1	3.3	18	1.2	8
B.	*anomalus*	2	5.7, 7.8	17, 26	5.4, 7.7	14, 28
B.	*bruxellensis*	3	5.4 – 7.9	1.3 – 18	0 – 0.6	<6
B.	*claussenii*	1	11	11	1.8	12
B.	*custersianus*	1	5.1	4.1	0.2	7

Table 1. (continued-5)

	Number of strains dried	Viable count before drying (10^7 CFU/ampule)	Survival value (%) after preservation at 37 C for		D-value at 5 C (years)
			0	60 days	
Brettanomyces custersii	1	4.6	20	5.1	16
B. intermedius	1	2.2	3.3	0	<1
B. lambicus	3	5.1 – 6.8	5.3 – 16	1.3 – 2.0	9 – 23
B. naardenensis	1	17	27	4.2	12
Candida acutus	1	9.1	86	69	104
C. albicans	24	1.2 – 14	11 – 55	10 – 47	13 – 2217
C. australis	6	5.1 – 7.5	7.0 – 23	0.1 – 3.2	5 – 11
C. boidinii	2	3.4, 9.3	10, 31	0.5, 9.2	7, 18
C. bogoriensis	1	0	0	0	
C. boleticola	1	21	9.5	7.8	116
C. brassicae	1	4.5	50	40	99
C. brumptii	5	7.5 – 18	25 – 99	7.8 – 52	19 – 47
C. buinensis	1	20	13	0.6	7
C. butyri	1	33	44	12	17
C. cariosilignicola	1	21	72	66	274
C. catenulata	2	6.1, 11	33, 33	34, 36	>300
C. citrea	1	19	48	19	25
C. conglobata	2	5.8, 7.2	27, 49	19, 22	29, 64
C. curiosa	1	2.9	13	2.9	15
C. curvata	4	2.4 – 5.2	2.5 – 10	0.6 – 1.2	9 – 16
C. deformans	1	2.3	33	17	34
C. diddensii	1**	15	1.1	0.03	6
	4	12 – 32	19 – 52	12 – 31	22 – 48
C. diffluens	6	2.4 – 10	12 – 39	4.8 – 13	11 – 31
C. diversa	2	0.9, 23	0.2, 37	0, 8.3	0, 15

C.	*fragicola*	1	12	31	24	89
C.	*glaebosa*	1	16	31	30	396
C.	*guilliermondii* var. *carpophila*	1	21	44	56	>300
C.	*guilliermondii* var. *guilliermondii*	16	10 – 46	13 – 68	6.7 – 4.8	21 – 65
C.	*guilliermondii* var. *japonica*	5	17 – 35	19 – 43	8.3 – 19	23 – 39
C.	*humicola*	4	4.6 – 8.0	5.2 – 30	1.0 – 3.5	10 – 28
C.	*hydrocarbofumarica*	1	13	66	57	155
C.	*inositophila*	1	20	24	12	36
C.	*intermedia*	2	7.9, 12	26, 36	3.1, 5.5	9, 15
C.	*ishiwadae*	1	29	31	18	44
C.	*kefyr*	2	3.3, 7.2	11, 12	3.3, 6.0	17, 34
C.	*krissii*	1	14	12	8.8	68
C.	*krusei*	14	3.2 – 17	17 – 57	15 – 49	>28
C.	*lambica*	3	3.4 – 6.7	6.9 – 25	5.2 – 24	>81
C.	*langeronii*	1	12	31	15	33
C.	*lusitaniae*	1	23	47	25	36
C.	*macedoniensis*	2	3.7, 9.2	13, 14	8.1, 8.4	37, 50
C.	*maltosa*	4	2.7 – 7.8	19 – 47	17 – 46	51 – 835
C.	*marina*	1	10	27	15	40
C.	*melinii*	2	9.1, 14	23, 40	21, 33	122, 262
C.	*membranaefaciens*	7	13 – 24	17 – 35	10 – 22	22 – 180
C.	*mesenterica*	2**	1.5, 7.4	21, 35	0.2, 1.1	4, 6
		6	1.1 – 5.8	20 – 76	6.1 – 33	13 – 27
C.	*mogii*	3	5.4 – 29	37 – 55	6.9 – 19	13 – 452
C.	*norvegensis*	5	2.7 – 17	31 – 67	5.3 – 44	12 – 56
C.	*oregonensis*	1	13	59	40	57
C.	*parapsilosis*	9	6.7 – 18	29 – 57	20 – 34	28 – 114
C.	*pararugosa*	2	5.1, 11	31, 59	14, 28	29, 30
C.	*placentae*	1	5.6	50	12	16

Table 1. (continued-6)

	Number of strains dried	Viable count before drying (10^7 CFU/ampule)	Survival value (%) after preservation at 37 C for		D-value at 5 C (years)
			0	60 days	
Candida pseudointermedia	1	7.2	24	16	40
C. pseudotropicalis	23	4.5 – 19	5.1 – 30	1.1 – 28	11 – 448
C. quercuum	1	22	44	27	49
C. ravautii	4	8.9 – 26	20 – 62	9.5 – 28	19 – 45
C. rugopelliculosa	1	12	23	15	54
C. rugosa	4	4.1 – 20	31 – 62	13 – 44	28 – 269
C. sake	6	5.2 – 15	16 – 44	5.3 – 24	15 – 140
C. salmonicola	1	10	22	7.3	20
C. santamariae	1	4.1	16	9.3	39
C. shehatae	1	6.6	8.6	2.3	15
C. silvae	1	16	46	40	176
C. slooffii	1	0.08	0	0	
C. solani	3	4.5 – 10	16 – 21	10 – 15	43 – 54
C. sorboxylosa	1	8.6	52	42	74
C. stellatoidea	3**	4.1 – 7.8	14 – 52	1.1 – 3.5	6 – 10
	1	5.9	68	40	56
C. succophila	1	27	43	19	28
C. tenuis	4	1.7 – 16	7.7 – 30	2.1 – 14	11 – 185
C. terebra	3	2.7 – 19	35 – 65	18 – 30	18 – 109
C. tropicalis	25	1.2 – 19	11 – 66	6.1 – 32	19 – 386
C. tsukubaensis	1	0.06	72	4.7	8
C. utilis	12	4.7 – 15	10 – 37	9.0 – 33	>25
C. valida	1**	2.1	52	3.9	8
	4	1.8 – 9.5	44 – 64	27 – 36	33 – 58
C. vini	3	6.0 – 11	6.4 – 17	4.9 – 5.4	18 – 93

C. zeylanoides	2**	14, 15	17, 33	0, 3.4	<10
	3	6.0 – 10	23 – 63	19 – 48	86 – 123
Cryptococcus albidus var. *aerius*	1**	2.8	5.7	1.3	8
	2	2.1, 4.2	23, 27	8.8, 17	20, 74
C. albidus var. *albidus*	24	0.3 – 10	1.9 – 62	0.5–61	17 – 283
C. albidus var. *diffluens*	2	0.7, 0.9	42, 57	44, 47	>115
C. ater	1	1.2	41	32	66
C. cereanus	1	9.2	60	42	64
C. dimennae	1	0.7	1.8	0.3	12
C. flavus	1**	1.3	2.5	1.8	70
	4	1.3 – 10	6.2 – 36	2.7 – 10	18 – 27
C. hungaricus	2**	0.1, 0.9	0, 1.3	0, 0.4	0, 19
	2	0.3, 0.4	3.9, 21	1.1, 5.2	13, 16
C. kuetzingii	1	10	23	14	44
C. lactativorus	1	17	18	10	37
C. laurentii var. *flavescens*	10	0.5 – 5.0	15 – 71	4.8 – 56	19 – 548
C. laurentii var. *laurentii*	5	0.02 – 8.0	13 – 55	4.4 – 45	22 – 368
C. laurentii var. *magnus*	3	0.4 – 0.5	33 – 60	24 – 51	>14
C. luteolus	2	0.6, 1.1	17, 23	14, 16	63, 130
C. macerans	2	1.3, 1.5	4.7, 20	7.4, 16	>22
C. melibiosum	1	8.5	47	41	35
C. neoformans	6	1.1 – 7.8	13 – 41	9.3 – 43	39 – 220
C. skinneri	1	0.1	53	32	15
C. terreus	2	0.8, 1.6	10, 12	6.5, 8.9	>45
Kloeckera africana	4	3.1 – 5.2	19 – 23	5.2 – 22	17 – 435
K. apiculata	9	1.9 – 9.2	12 – 81	4.1 – 28	13 – 719
K. corticis	4	0.9 – 6.0	12 – 31	9.7 – 23	>42
K. javanica var. *javanica*	6	0.7 – 4.3	22 – 52	2.9 – 31	11 – 65
K. javanica var. *lafarii*	2	3.1, 5.4	13, 26	13, 22	>126

Table 1. (continued-7)

	Number of strains dried	Viable count before drying (10^7 CFU/ampule)	Survival value (%) after preservation at 37 C for		D-value at 5 C (years)
			0	60 days	
Kloeckera jensenii	1	3.2	33	8.4	16
Rhodotorula aurantiaca	3	2.8 – 4.4	47 – 58	40 – 48	>63
R. fukazawaensis	1	15	25	18	67
R. glutinis var. *dairensis*	3	10 – 15	11 – 41	10 – 20	21 – 607
R. glutinis var. *glutinis*	3**	0.3 – 3.8	4.9 – 13	0 – 3.6	0 – 36
	18	0 9 – 29	8.4 – 42	5.1 – 38	13 – 310
R. glutinis var. *rufusa*	1	8.3	30	19	51
R. glutinis var. *salinalia*	1	5.3	53	31	43
R. graminis	2	0.4, 2.9	21, 32	8.8, 14	17, 55
R. lactosa	3	1.9 – 3.3	23 – 51	26 – 49	126 – 556
R. marina	4	5.1 – 17	27 – 49	20 – 50	>42
R. minuta var. *minuta*	4	5.5 – 14	39 – 68	35 – 48	52 – 324
R. minuta var. *texensis*	7	3.3 – 19	27 – 55	26 – 41	46 – 173
R. pallida	1	10	32	29	295
R. psychrophila	1	0.4	40	38	323
R. rubra	1**	0.1	0.7	0	<1
	47	1.8 – 20	7.4 – 53	6.5 – 48	38 – 462
Selenozyma intestinalis	2	0.6, 3.5	30, 32	6.3, 10	13, 20
S. peltata	1	32	31	14	28
Sterigmatomyces elviae	2	7.0, 9.7	39, 63	29, 45	69, 79
S. halophilus	1	5.4	56	35	49
S. indicus	1	16	26	19	72
Syringospora albicans	1	19	47	38	108
S. claussenii	1	3.2	40	19	30

Torulopsis apis var. *galacta*		1	11	58	28	32
T.	*auricolariae*	1	6.3	10	8.6	97
T.	*bovina*	7	1.1 – 2.8	0.3 – 6.3	0 – 0.2	0 – 12
T.	*candida*	8	1.2 – 30	4.1 – 57	2.4 – 34	11 – 42
T.	*cantarellii*	1	4.5	49	38	98
T.	*colliculosa*	5	3.8 – 28	22 – 65	11 – 44	35 – 61
T.	*ernobii*	1	13	30	12	26
T.	*etchellsii*	3	4.6 – 10	31 – 55	0.2 – 2.7	4 – 7
T.	*fructus*	1	13	33	0.1	3
T.	*glabrata*	3	23 – 32	39 – 70	21 – 31	40 – 59
T.	*gropengiesseri*	1	7.3	26	11	26
T.	*haemulonii*	1	14	51	37	70
T.	*halonitratophila*	3	9.3 – 25	4.6 – 55	0 – 1.8	0 – 6
T.	*halophilus*	1	8.0	50	15	20
T.	*holmii*	2	3.2, 3.5	1.9, 5.8	0.1, 0.2	5, 10
T.	*inconspicua*	2	2.8, 38	34, 36	17, 29	33, 105
T.	*ingeniosa*	1	2.9	4.6	0.7	12
T.	*lactis-condensi*	2	2.2, 2.7	44, 59	11, 13	15, 17
T.	*magnoliae*	3	13 – 36	30 – 44	8.2 – 21	13 – 41
T.	*mannitofaciens*	1	8.9	20	10	36
T.	*maris*	1	23	12	9.0	68
T.	*musae*	1	16	35	3.1	9
T.	*nitratophila*	1	23	29	18	47
T.	*nodaensis*	1	11	52	8.0	12
T.	*pintolopesii*	4	1.1 – 3.4	6.5 – 9.9	0.04– 1.6	4 – 13
T.	*pinus*	2	18, 23	7.1, 13	0.1, 0.7	5, 7
T.	*psychrophila*	2	1.9, 3.6	0, 0	0, 0	
T.	*sonorensis*	1	7.6	56	34	46
T.	*sorbophila*	1	36	67	36	37

Table 1. (continued-8)

	Number of strains dried	Viable count before drying (10^7 CFU/ampule)	Survival value (%) after preservation at 37 C for		D-value at 5 C (years)
			0	60 days	
Torulopsis stellata	8	0.5 – 10	38 – 82	9.0 – 54	15 – 205
T. versatilis	1**	2.2	18	1.4	8
	3	6.4 – 15	33 – 42	6.8 – 16	12 – 24
Trichosporon brassicae	1*	5.5	39	18	29
T. capitatum	1*	0.6	91	46	34
T. cutaneum var. *autarcticum*	1*	4.9	2.7	2.1	91
T. cutaneum var. *cutaneum*	11*	3.1 – 14	8.9 – 100	5.6 – 100	>19
T. pullulans	1*	4.9	47	33	67
Trigonopsis variabilis	4	22 – 52	20 – 31	3.2 – 6.1	10 – 15

1) D-value: Estimated decimal reduction time of viability in preservation at 5 C.
*: The cells were harvested from liquid culture incubated on reciprocal shaker for 2 days at 24 C.
**: The strain(s) which showed especially low viability after drying and/or after preservation are presented separately from the others.

In contrast, the remaining 38 of the 55 strains showed a further rapid decrease in viability during storage. Their D-values at 5 C are less than 10 years and the viable count will fall below 10^4 cells per ampule in at most 15 years. The present investigation should be followed up to improve method of L-drying to increase the recovery rate in these yeasts. These yeasts belong to the following species:

Ambrosiozyma cicatricosa, A. monospora, A. philentoma, Arthroascus javanensis, Saccharomycodes ludwigii, Kluyveromyces polysporus, Lipomyces lipofer, Pichia pinus, P. stipitis, Saccharomyces exiguus, S. telluris, Schwanniomyces occidentalis, Nematospora coryli, Schizosaccharomyces octosporus, Leucosporidium antarcticum, L. frigidum, L. gelidum, L. nivalis, Brettanomyces intermedius, Candida curvata, C. diddensii, C. diversa, Rhodotorula glutinis, R. rubra, Torulopsis bovina, T. holmii.

The ability of a strain to survive well during L-drying did not necessarily reflect its ability to remain viable during storage. The data should be interpreted in two aspects: the decrease of survival during the drying process and during storage.

One hundred and eight of 1658 strains that remained moderately or highly viable after the drying, rapidly lost viability during storage, showing D-values of less than 10 years. The viable counts of these dried cultures will fall to 10^4 within at most 25 years of preservation at 5 C. The L-dried cultures of these yeasts should be checked and renewed after about a decade even at 5 C.

The majority of yeasts listed in the table, namely 1547 strains (about 91%), can be expected to survive for at least 50 years on storage at 5 C, since they showed high viabilities and large D-values.

Generally the decrease in viability during L-drying and during storage each varied from strains to strain in a species. In the following yeasts, however, almost all strains in a genus or in a species showed very low survival values:

Genera; *Ambrosiozyma, Arthroascus, Lipomyces, Nematospora, Leucosporidium, Brettanomyces.*

Species; *Saccharomycopsis capsularis, Nadsonia commutata, Debaryomyces couderтii, Saccharomyces exiguus, S. telluris, Candida curvata, Torulopsis bovina, T. etchellsii, T. halonitratophila, T. holmii, T. pintolopesii, T. pinus, T. psychrophila.*

Yeasts which produce filamentous cells, osmotolerant yeasts, and psychrophilic yeasts seem most apt rapidly to lose viability during L-drying and/or preservation.

We believe that the dried cultures for maintenance and for distribution should have more than 10^4 viable cells per ampule. The time to renew the stored dried cultures can be estimated from their initial viable counts and D-values at 5 C in the table.

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PRESERVATION OF ISP STRAINS OF ACTINOMYCETES BY L-DRYING

Tatsuo YOKOYAMA and Isamu ASANO

Summary

ISP strains of Actinomycetes were dried directly from the liquid state *in vacuo* (L-drying). Of the 154 strains tested, all of which belong to the so-called ISP 2nd group in Japan, 147 strains survived with a wide range of colony forming units after 1, 3 or 13 months of storage at 3 C; six strains gave no colony forming units from the dried samples with negligible growth; one strain was not viable after 13 months of storage. In total 18 strains showed very poor viabilities and need further improvements in their culturing and drying procedures.

Additional trials of the L-drying of 22 selected strains were made with 5 different media for pre-drying cultures and subsequent recovery tests on the same media. These strains failed to survive or survived only barely in the preceding L-drying tests with only one kind of medium for each strain. Of the 22 strains tested, 18 survived comparatively well on at least one of the 5 media after 60 days of storage at either 3 C or 37 C; 3 strains survived only poorly; and 1 strain was not viable on any of the media after 60 days of storage at 3 C.

For the long-term preservation of Actinomycete strains, lyophilization (freeze-drying) or freezing and preservation in a frozen state are probably the most effective methods currently used (2, 5–8). However, so far as the Actinomycete strains are concerned, little has been reported on the suitability for them of drying from the liquid state *in vacuo*, namely, L-drying (1, 3, 4).

This paper reports the results of our trial application of L-drying to the long-term preservation of Actinomycete strains.

Materials and Methods

Actinomycete strains. The ISP (International Streptomyces Project) strains listed in the IFO List of Cultures, 6th edition (1978) were employed. These strains, listed in Table 1, consist of 154 strains of so-called 2nd group of ISP strains, which have been deposited by the Society for Actinomycetes, Japan (SAJ) since 1973. The 22 strains which are marked with an asterisk were also used for the evaluation of suitable media for L-drying.

Names of taxon for these strains are those given by the ISP Committee of the SAJ when these strains were deposited. The Approved Lists of Bacterial Names (1980) is not referred to in this paper.

Media. The media used for culturing the strains before and after L-drying and

after a given month of storage were as follows:

Medium No. 227 (ISP Medium No. 2)

Yeast extract	4 g	Distilled water	to 1 liter
Glucose	4 g	Agar	20 g
Malt extract	10 g		pH 7.3

Medium No. 228 (Bennett's agar)

Yeast extract	1 g	Distilled water	to 1 liter
Beef extract	1 g	Agar	20 g
N.Z. Amine, type A*	2 g		pH 7.3
Glucose	10 g		

* Sheffield Chemical Co., San Ramon, USA.

Medium No. 231

Same as Medium No. 228, but with maltose instead of glucose as carbon source.

Medium No. 8 (Oatmeal agar)

Oatmeal	50 g	Agar	20 g
Distilled water	to 1 liter		pH 5.6

YS Medium

Starch*	10 g	Agar	20 g
Yeast extract**	2 g		pH 7.2
Distilled water	to 1 liter		

* Inu Mark (Koso Chemical Co., Ltd., Tokyo, Japan).

** Oriental Yeast (Oriental Yeast Kogyo Co., Ldt., Tokyo, Japan).

Before drying, each strain was slant-cultured on a given medium for 10 days (7 days for some strains) at 28 C (37 C for some strains). Plate culture in a plastic Petri-dish of 9 cm diam was used for the subsequent viability tests of all strains and for culture of the 22 selected strains, which were incubated for 14 days at 28 C. Growth was indicated by the numbers 0 to 4 according to the degree of development of aerial mycelium as defined in the footnote of Table 1.

Suspending medium. Potassium phosphate buffer, 0.1 M, pH 7.0, containing monosodium glutamate at a final concentration of 3% was used for preparing propagule suspensions.

Drying procedure. The procedure for drying *in vacuo* is essentially the same as that described by Iijima and Sakane (3, 4). Aerial mycelium and spores on cultures were scraped off and suspended in the suspending medium to give a suspension of approximately 10^8 to 10^9 colony forming unit (CFU) per ml. Portions of 0.1 ml of these propagule suspensions were poured into a test tube (110×8 mm) and dried *in*

vacuo for 1 hr. The dried samples were stored at 3 C and 37 C.

Rehydration and viability measurement. On opening the sealed tubes, 0.5 ml of sterile distilled water was added to the dried samples, which were then allowed to stand for 20 min to facilitate homogenous resuspension. After dilution of 1: 50 and 1: 500 with sterile distilled water, 0.1-ml portions of the suspension were plated on a given medium in a Petri-dish for CFU estimation. Viability was indicated according to the overall growth of visible colonies: ++, very heavy (approximately 10^6 CFU per plate or more); +, heavy (approximately 10^4 to 10^6 CFU per plate); and +, moderate (approximately 10^2 to 10^4 CFU per plate).

When the CFU count on a plate was below 100, the actual count was recorded. A CFU count of 100 per plate was approximately equivalent to 10^4 propagules per ml in the rehydrated suspensions diluted to 1: 50.

Results

Table 1 shows the results of the viability tests just before and after L-drying and after 1, 3 and 13 months of storage at 3 C.

Table 2 shows the results of the L-drying tests to evaluate media for pre-drying cultures and subsequent viability tests, together with the viability tests after 60 days of storage at 3 C and 37 C.

Discussion and Conclusion

As Table 1 shows, 119 of 154 strains tested survived satisfactorily after 13 months of storage at 3 C. The viabilities of 31 strains, including *Streptomyces bikiniensis* IFO 13350, *S. salmonicida* IFO 13393, *S. vinaceus* IFO 13425, *S. rosa* IFO 13436, *S. flavochromogenes* IFO 13443 and *S. colombiensis* IFO 13454, were excellent. Of these, *S. vinaceus* IFO 13425 and *S. flavochromogenes* IFO 13443 showed slightly better growth after 13 months of storage than after 3 months. The other 4 of the 6 representative strains mentioned above showed highest viabilities after 1 and 3 months of storage. The growth index of all these strains was 4, excepting that of for *S. flavochromogenes* IFO 13443, which was 1.

Seven strains showed high viabilities after 3 months of storage, followed by a slight decline after 13 months of storage: *Streptomyces thermoviolaceus* subsp. *thermoviolaceus* IFO 13387, *S. rishiriensis* IFO 13407, *S. nigellus* IFO 13408, *S. pactum* IFO 13433, *S. aspergilloides* IFO 13461, *Actinomyces oligocarbophilus* IFO 13478 and *Streptomyces bambergiensis* IFO 13479.

Twelve of the 119 strains showed low viabilities throughout the tests and their growth indices were also low: *Streptomyces fungicidicus* IFO 13340, *S. caeruleus* IFO 13344, *S. xantholiticus* IFO 13354, *S. listeri* IFO 13360, *S. natalensis* IFO 13367, *S. phaeofaciens* IFO 13372, *S. glomeroaurantiacus* IFO 13380, *Streptoverticillium parvisporogenes* IFO 13394, *Streptomyces spectabilis* IFO 13424, *S. albolongus* IFO 13465,

Table 1. Viability of ISP strains of Actinomycetes before and after L-drying.

	Strain	Name of taxon	Temperature for pre-drying and recovery incubation	Incubation period (day)	Pre-drying and recovery medium	Viability[2]					
						Growth index[1]	Before drying	Just after drying	After 1 month of storage at 3 C	After 3 months of storage at 3 C	After 13 months of storage at 3 C
	IFO 13340 (ISP 5020)	Streptomyces fungicidicus	28	10	231	0	++	+	+	+	++
*	" 13341 (" 5027)	" thioluteus	28	10	228	0	6	3	1	0	3
	" 13342 (" 5602)	" humifer	28	10	227	0	+++	+++	+++	+++	+++
*	" 13343 (" 5086)	Streptoverticillium fervens	28	10	227	2	22	9	7	1	5
*	" 13344 (" 5103)	Streptomyces caeruleus	28	10	228	2	++	+	+	0	+
	" 13345 (" 5104)	" sulphureus	28	10	228	2	++	++	++	++	++
	" 13346 (" 5108)	" cyaneus	28	10	231	4	+++	++	++	++	++
	" 13347 (" 5110)	" viridochromogenes	28	10	231	4	+++	+++	+++	+++	+++
	" 13348 (" 5196)	" zaomyceticus	28	10	231	4	+++	++	++	++	++
	" 13349 (" 5209)	" violatus	28	10	231	4	++	++	++	++	++
	" 13350 (" 5235)	" bikiniensis	28	10	228	4	+++	+++	+++	+++	+++
*	" 13351 (" 5237)	" viridoflavus	28	10	231	0	+	+	60	20	64
	" 13352 (" 5239)	" viridifaciens	28	10	227	4	++	++	++	++	++
	" 13353 (" 5243)	" viridosporus	28	10	231	2	++	++	++	++	+++
	" 13354 (" 5244)	" xantholiticus	28	10	227	0	++	++	+	++	+
*	" 13355 (" 5266)	" horton	28	10	231	0	14	18	1	0	41
*	" 13356 (" 5269)	" sclerotialus	28	10	227	0	10	4	4	7	5
	" 13357 (" 5270)	" flavisclerotiicus	28	10	231	0	++	++	++	++	+
	" 13358 (" 5271)	" purpurogeniscleroticus	28	10	227	0	++	++	+	++	+
	" 13359 (" 5280)	Actinomyces viridiviolaceus	28	10	231	3	+++	+++	+++	+++	+++
	" 13360 (" 5297)	Streptomyces listeri	28	10	231	0	++	+	+	+	+
*	" 13361 (" 5301)	" minutiscleroticus	28	10	227	0	++	++	++	++	11
	" 13362 (" 5302)	Chainia nigra	28	10	231	0	+++	+++	++	++	++
	" 13363 (" 5303)	Streptomyces roseiscleroticus	28	10	227	2	80	30	30	23	31
*	" 13364 (" 5336)	Streptoverticillium kashmirense	28	10	231	2	0	0	0	0	0

	IFO 13365 (ISP 5347)	Streptomyces odorifer	28	10	228	0	++	8	50	60	+
	" 13366 (" 5350)	" galtieri	28	10	228	2	++	++	++	++	+++[F]
	" 13367 (" 5357)	" natalensis	28	10	228	2	++	+	+	+	+
*	" 13368 (" 5358)	" novaecaesareae	28	10	227	0	+	+	+	+	57
	" 13369 (" 5361)	" anulatus	28	10	231	2	++	++	++	++	++
	" 13370 (" 5365)	" thermophilus	37	7	227	3	+++	+++	+++	+++	+++
*	" 13371 (" 5366)	" flavofungini	28	10	227	0	60	0	0	0	1
	" 13372 (" 5367)	" phaeofaciens	28	10	228	0	+	+	+	+	+
	" 13373 (" 5381)	" viridis	28	10	231	4	+++	+++	+++	+++	+++
	" 13374 (" 5384)	" chromogenus	28	10	231	2	++	++	++	++	+
	" 13375 (" 5392)	" griseoluteus	28	10	231	3	++	++	++	++	++
	" 13376 (" 5419)	" caesius	28	10	227	3	++	++	++	++	++
	" 13377 (" 5420)	" chryseus	28	10	231	3	60	60	24	30	82
	" 13378 (" 5421)	" coelescens	28	10	227	4	+++	++	++	++	+++
	" 13379 (" 5476)	" spadicis	37	7	227	4	+	+	15	33	26
	" 13380 (" 5429)	" glomeroaurantiacus	28	10	228	0	++	+	+	+	+
	" 13381 (" 5430)	" griseoaurantiacus	28	10	231	0	+++	++	++	+++	+++
	" 13382 (" 5431)	" helvaticus	24	10	231	0	++	++	++	++	+
	" 13383 (" 5432)	" indigocolor	28	10	231	4	+++	++	+++	+++	+++
	" 13384 (" 5433)	" lazureus	28	10	227	4	+++	++	++	++	+++
	" 13385 (" 5434)	" lividans	28	10	227	4	+++	+++	+++	+++	+++
	" 13386 (" 5441)	" nobilis	28	10	231	2	++	13	80	27	13
	" 13387 (" 5443)	" thermoviolaceus subsp. thermoviolaceus	37	7	227	0	+++	+++	+++	+++	++
	" 13388 (" 5445)	" subrutilus	28	10	228	4	++	++	++	++	++
	" 13389 (" 5449)	" diastatochromogenes	28	10	227	2	+	+	+	24	28
	" 13390 (" 5454)	" viridogenes	28	10	228	0	+++	++	++	++	+++
	" 13391 (" 5459)	" willmorei	28	10	227	3	+++	+++	+++	++	+++
	" 13392 (" 5465)	Actinomyces alborubidus	28	10	231	4	+++	++	++	+++	+++
	" 13393 (" 5472)	Streptomyces salmonicida	28	10	228	4	+++	+++	+++	+++	+++
	" 13394 (" 5473)	Streptoverticillium parvisporogenes	28	10	228	2	++	+	+	+	+

Table 1. (continued)

Strain	Name of taxon	Temperature for pre-drying and recovery incubation	Incubation period (day)	Pre-drying and recovery medium	Viability[2]					
					Growth index[1]	Before drying	Just after drying	After 1 month of storage at 3 C	After 3 months of storage at 3 C	After 13 months of storage at 3 C
* IFO 13395 (ISP 5475)	Streptomyces atrofaciens	28	10	227	0	60	62	17	46	42
" 13396 (" 5477)	" tenebrarius	28	10	227	4	##	#	#	##	##
" 13397 (" 5478)	" albaduncus	28	10	231	2	+	+	#	#	84
" 13398 (" 5479)	" cirratus	28	10	227	4	##	##	##	##	##
" 13399 (" 5480)	" galbus	28	10	231	4	##	##	##	##	##
" 13400 (" 5481)	" galilaeus	28	10	231	2	#	#	#	#	#
" 13401 (" 5482)	" iakyrus	28	10	227	2	#	+	+	+	87
" 13402 (" 5483)	" luteogriseus	28	10	231	2	+	16	16	20	+
" 13403 (" 5485)	" coriofaciens	28	10	228	2	##	#	#	#	##
" 13404 (" 5486)	" werraensis	28	10	227	2	#	#	#	#	+
" 13405 (" 5487)	" lavenduligriseus	28	10	227	4	##	##	##	#	##
" 13406 (" 5488)	" roseogriseus	28	10	227	2	##	##	#	#	#
" 13407 (" 5489)	" rishiriensis	28	10	228	2	##	##	##	##	#
" 13408 (" 5490)	" nigellus	28	10	227	2	##	##	##	##	#
" 13409 (" 5491)	" versipellis	28	10	231	2	#	#	#	#	#
" 13410 (" 5492)	" albulus	28	10	227	4	#	#	#	#	##
" 13411 (" 5494)	" capoamus	28	10	231	2	#	85	54	20	45
" 13412 (" 5496)	" diastaticus	28	10	231	2	#	#	#	#	#
" 13413 (" 5499)	" griseochromogenes	28	10	231	4	##	#	#	#	#
* " 13414 (" 5500)	" kanamyceticus	28	10	228	0	4	0	0	0	0
" 13415 (" 5501)	" mediterranei	28	10	227	0	##	##	##	#	##
" 13416 (" 5503)	" morookaensis	28	10	228	4	##	#	#	#	#
" 13417 (" 5504)	" showdoensis	28	10	228	4	##	##	##	#	#
" 13418 (" 5505)	" tuirus	28	10	231	4	##	##	##	##	##
" 13419 (" 5506)	" prasinosporus	28	10	231	2	+	+	+	57	72

	IFO 13420 (ISP 5507)	Streptoverticillium griseoverticillatum	28	10	227	3	++	++	++	++	++
	" 13421 (" 5508)	Streptomyces naraensis	28	10	227	2	+++	+++	+++	+++	+++
	" 13422 (" 5509)	" luteoreticuli	28	10	228	4	++	++	++	++	+
	" 13423 (" 5511)	" ostreogriseus	28	10	227	2	+++	+++	+++	+++	+++
	" 13424 (" 5512)	" spectabilis	28	10	231	1	+	+	+	+	+
	" 13425 (" 5515)	" vinaceus	28	10	231	4	+++	+++	+++	+++	+++
*	" 13426 (" 5517)	" erythraeus	28	10	228	2	50	0	0	0	62
	" 13427 (" 5518)	" gedanensis	28	10	227	0	++	++	++	++	++
*	" 13428 (" 5520)	" tropicalensis	28	10	228	3	0	0	0	0	0
	" 13429 (" 5526)	" avidinii	28	10	227	3	+++	+++	+++	++	+++
	" 13430 (" 5527)	" ardus	28	10	231	3	60	44	43	34	49
	" 13431 (" 5528)	" canarius	28	10	227	3	++	++	++	++	+
	" 13432 (" 5529)	" moderatus	28	10	231	4	++	++	++	++	94
	" 13433 (" 5530)	" pactum	28	10	231	2	+++	+++	+++	+++	++
	" 13434 (" 5531)	" pallidus	28	10	227	3	+++	+++	+++	+++	+++
	" 13435 (" 5532)	" regalis	28	10	228	2	++	++	++	++	++
	" 13436 (" 5533)	" rosa	28	10	231	4	+++	+++	+++	+++	+++
	" 13437 (" 5534)	" cyaneogriseus	28	10	231	4	++	++	++	++	++
	" 13438 (" 5535)	" anandii	28	10	227	0	++	++	++	++	++
*	" 13439 (" 5536)	" roseoflavus	28	10	231	0	60	30	1	1	10
	" 13440 (" 5537)	" saprophyticus	28	10	231	3	+++	+++	+++	+++	+++
	" 13441 (" 5539)	" durhamensis	28	10	231	3	+++	+++	+++	+++	+++
	" 13442 (" 5540)	" acidoresistans	28	10	227	3	+++	+++	+++	+++	+++
	" 13443 (" 5541)	" flavochromogenes	28	10	231	1	+++	+++	+++	+++	+++
	" 13444 (" 5545)	" chrestomyceticus	28	10	231	3	++	++	++	++	+
	" 13445 (" 5546)	" nogalater	28	10	231	4	+++	+++	+++	+++	+++
*	" 13446 (" 5547)	" steffisburgensis subsp. steffisburgensis	28	10	228	0	10	2	1	2	7
	" 13447 (" 5550)	" katrae	28	10	231	4	+++	++	++	++	++
	" 13448 (" 5551)	" regensis	28	10	227	3	+++	+++	+++	+++	+++
	" 13449 (" 5552)	" omiyaensis	28	10	227	4	+++	+++	+++	+++	+++

Table 1. (continued)

	Strain	Name of taxon	Temperature for pre-drying and recovery incubation	Incubation period (day)	Pre-drying and recovery medium	Viability[2]					
						Growth index[1]	Before drying	Just after drying	After 1 month of storage at 3 C	After 3 months of storage at 3 C	After 13 months of storage at 3 C
	IFO 13450 (ISP 5553)	Streptomyces mirabilis	28	10	231	3	[F]	##	##	##	##
*	" 13451 (" 5554)	" avellaneus	28	10	228	3	0	0	0	0	10
	" 13452 (" 5555)	" libani	28	10	231	3	##	##	##	##	##
	" 13453 (" 5557)	" alni	28	10	231	0	##	##	+	##	##
	" 13454 (" 5558)	" colombiensis	28	10	231	4	##	##	##	##	##
	" 13455 (" 5559)	" graminofaciens	28	10	227	3	##	##	##	##	##
	" 13456 (" 5560)	" tauricus	28	10	227	1	##	##	##	##	+
	" 13457 (" 5561)	Actinomyces cretaceus	28	10	227	4	##	##	##	##	##
	" 13458 (" 5562)	Streptomyces griseosporeus	28	10	227	2	##	##	##	##	##
	" 13459 (" 5563)	" violaceoniger	28	10	228	3	##	##	##	##	54
	" 13460 (" 5564)	" bluensis	28	10	231	2	##	##	##	##	##
	" 13461 (" 5565)	" aspergilloides	28	10	227	3	##	##	##	##	##
	" 13462 (" 5566)	" pulcher	28	10	231	1	##	##	##	##	##
*	" 13463 (" 5567)	" paraguayensis	28	10	228	0	##	##	+	+	21
	" 13464 (" 5568)	" lusitanus	28	10	231	2	##	##	##	##	##
	" 13465 (" 5570)	" albolongus	28	10	227	1	##	+	##	+	+
*	" 13466 (" 5571)	Streptoverticillium orinoci	28	10	227	2	42	5	1	3	0
	" 13467 (" 5572)	Streptomyces gannmycicus	28	10	231	4	##	##	##	##	##
	" 13468 (" 5573)	" thermodiastaticus	37	7	231	4	##	##	##	##	##
	" 13469 (" 5575)	" xanthocidicus	28	10	231	4	##	##	##	##	##
	" 13470 (" 5576)	" takataensis	28	10	227	4	##	##	##	##	##
	" 13471 (" 5577)	Streptoverticillium septatum	28	10	227	2	##	##	##	##	##
	" 13472 (" 5578)	Streptomyces hygroscopicus	28	10	227	4	##	##	##	##	+
	" 13473 (" 5579)	" thermonitrificans	37	7	231	3	##	##	##	##	##
	" 13474 (" 5585)	" tetanusemus	28	10	231	2	##	##	##	##	##

	IFO 13475 (ISP 5586)	Streptomyces hydrogenans	28	10	231	0	++	++	++	++	+++
	" 13476 (" 5587)	Streptoverticillium ladakanus	28	10	227	2	++	++	++	++	+
	" 13477 (" 5588)	Streptomyces neyagawaensis	28	10	227	2	+++	+++	+++	++	++
	" 13478 (" 5589)	Actinomyces oligocarbophilus	28	10	227	2	+++	+++	+++	+++	++
	" 13479 (" 5590)	Streptomyces bambergiensis	28	10	231	3	+++	+++	+++	+++	++
	" 13480 (" 5591)	Actinomyces ochroleucus	28	10	227	1	++	++	++	++	++
	" 13481 (" 5592)	Streptomyces peruviensis	28	10	231	4	++	++	++	++	+
*	" 13482 (" 5593)	" fulvissimus	28	10	231	0	70	16	1	2	6
	" 13483 (" 5594)	" ochraceiscleroticus	28	10	227	0	+++	++	++	++	++
	" 13484 (" 5595)	" olivaceiscleroticus	28	10	231	0	++	++	++	++	+
	" 13485 (" 5596)	" poonensis	28	10	231	0	++	+	+	+	+
	" 13486 (" 5597)	" violens	28	10	231	0	+++	+++	+++	+++	+++
	" 13487 (" 5598)	" bacillaris	28	10	228	1	+++	+++	+++	++	+++
*	" 13488 (" 5599)	" longispororuber	28	10	231	0	25	11	3	1	11
	" 13489 (" 5600)	" luteolutescens	28	10	227	0	++	++	++	++	+++
*	" 13490 (" 5603)	" caespitosus	28	10	227	3	8	3	0	0	5
	" 13491 (" 5604)	Streptoverticillium eurocidicum	28	10	227	2	+	+	+	+	+
	" 13492 (" 5605)	Streptomyces tumuli	28	10	231	0	+++	+++	+++	+++	+++
	" 13493 (" 5606)	" alanosinicus	28	10	231	4	++	++	++	++	+

* Strains used to evaluate media for pre-drying cultures and viability tests.

1) 4: Very vigorous aerial mycelium (AM). 3: Vigorous AM. 2: Rich AM. 1: Few AM. 0: No AM.

2) CFU was counted with rehydrated samples diluted to 1:50: +++, very heavy growth (approximately 10^6 CFU per plate or more); ++, heavy growth (approximately 10^4 to 10^6 CFU per plate); +, moderate growth (approximately 10^2 to 10^4 CFU per plate). CFU counts below 100 are indicated by the actual numbers on a plate.

[F]: Fungal contamination.

Table 2. Viability of 22 selected ISP strains of Actinomycetes before and after L-drying.

IFO No.	ISP No.	Growth index * in pre-drying culture on IFO medium No.					Viability** just before L-drying on IFO medium No.					Viability** just after L-drying on IFO medium No.					Viability after 60 days of storage** at 3 C on IFO medium No.					Viability after 60 days of storage** at 37 C on IFO medium No.				
		227	228	231	8	YS	227	228	231	8	YS	227	228	231	8	YS	227	228	231	8	YS	227	228	231	8	YS
13341	5027	0	0	0	0	0	+	+	++	50.5	31.5	12	19	0.5	3	18.5	0	50.5	10	4	0.5	0	3	0	0	0
13343	5086	4	3	4	1	3	+	+	+	+	++	29.5	+	36.5	+	++	37	23.5	+	+	++	17	9	80.5	+	++
13344	5103	2	—	0	—	2	0	0	++	0	++	0	0	+	0	+	+	0	++	0	++	7.5	0	++	0	++
13351	5237	1	1	1	0	1	+	+	+	++	73.5	+	+	25.5	+	17	74	43	50.5	++	15.5	+	33	16	18.5	+
13355	5266	0	0	0	0	0	++	37	+++	++	+++	++	17.5	++	++	++	++	11.5	+++	++	+	+	3	++	+	++
13356	5269	0	0	0	0	0	+	+	+	+++	+++	67.5	+	+	+++	+++	73.5	7.3	+	+++	+++	50	+	+	+++	++
13361	5301	2	1	1	2	2	++	++	++	+++	+++	++	++	++	+++	+++	+	+	++	+++	+++	+	+	++	+++	+++
13364	5336	0	0	4	0	1	3.5	0.5	3.5	16.5	1	0	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0
13368	5358	0	0	1	0	0	+	++	+	+	+	+	+	+	4.5	57	+	+	+	5	25	60.5	81.5	+	4.5	27
13371	5366	0	0	0	0	0	+	++	++	+	+	1.5	31.5	72	16	15.5	1	11.5	88	28	14.5	0	8.5	13.5	1.5	9
13395	5475	1	1	2	3	3	++	++	+++	+++	+++	+	++	+++	++	+++	++	++	+++	++	+++	++	+	+++	++	+++
13414	5500	1	1	1	1	1	++	++	++	+++	+++	++	++	++	+++	+++	++	++	+	+++	+++	++	+	+	+++	+++
13426	5517	3	3	3	2	2	+++	++	16	+++	+++	++	0	0	++	29.5	+++	+	0.5	++	1	++	7	8	++	++
13428	5520	4	3	3	3	2	9.5	5.5	0.5	53	0	1.5	0.5	0	28	1	1.5	0.5	0	0.5	0	2.5	0	0	3.5	0
13439	5536	3	3	3	2	3	++	++	++	+++	++	++	+	+	++	+	++	++	+	++	51	++	++	+	++	+
13446	5547	1	0	1	1	1	+	57	++	++	++	98	26.5	+	+	+	87	12	++	++	+	23	5	+	+	+
13451	5554	4	3	2	2	3	++	+	+	++	+	67.5	13	14.5	24.5	44.5	+	20.5	14.5	0	30	2	0	3	0	1
13463	5567	2	1	0	0	2	++	++	+	+++	+++	++	++	83	+++	+++	++	+	81	+++	++	++	+	52	+++	++
13466	5571	2	2	0	2	0	+	72	+	++	+	32	14	81.5	++	88.5	8.5	10	+	++	88.5	1.5	1	7.5	++	20
13482	5593	0	0	2	0	1	+	+++	+	++	++	2	++	55	+	+	+	++	46.5	+	90	34.5	++	20.5	+	+
13488	5599	0	0	2	0	1	++	++	+	+	+	+	55	43.5	15.5	+	++	+	+	17	+	79	17	46.5	8.5	67.5
13490	5603	4	4	3	2	3	35	27.5	+	+	28	15.5	18.5	+	+	8	19.5	15	+	+	4	4	7	53.5	+	11

* 4: Very vigorous aerial mycelium (AM). 3: Vigorous AM. 2: Rich AM. 1: Few AM. 0: No AM.

** CFU was counted with rehydrated samples diluted to 1:50:

+++, very heavy growth (approximately 10^6 CFU per plate or more); ++, heavy growth (approximately 10^4 to 10^6 CFU per plate);

+, moderate growth (approximately 10^2 to 10^4 CFU per plate). CFU counts below 100 are indicated by the actual numbers on a plate.

All strains were incubated for 14 days at 28C. Viability was tested on the same medium as used for pre-drying cultures.

S. poonensis IFO 13485 and *Streptoverticillium eurocidicum* IFO 13491.

Another 14 strains showed somewhat reduced viabilities after 13 months of storage, although their viabilities were high after 1 or 3 months of storage: *Streptomyces flaviscleroticus* IFO 13357, *S. purpurogeniscleroticus* IFO 13358, *S. chromogenus* IFO 13374, *S. helvaticus* IFO 13382, *S. werraensis* IFO 13404, *S. luteoreticuli* IFO 13422, *S. canarius* IFO 13431, *S. chrestomyceticus* IFO 13444, *S. tauricus* IFO 13456, *S. hygroscopicus* IFO 13472. *Streptoverticillium ladakanus* IFO 13476, *Streptomyces peruviensis* IFO 13481, *S. olivaceiscleroticus* IFO 13484 and *S. alanosinicus* IFO 13493. The growth indices of these strains varied from 0 to 4.

Thirty-five of the 154 Actinomycete strains tested showed unfavourable results. In particular, 6 strains were not viable either just after L-drying or after 1 and 3 months of storage: *Streptoverticillium kashmirense* IFO 13364, *Streptomyces flavofungini* IFO 13371, *S. kanamyceticus* IFO 13414, *S. erythraeus* IFO 13426, *S. tropicalensis* IFO 13428 and *S. avellaneus* IFO 13451. Of these, *S. kashmirense* IFO 13364, *S. tropicalensis* IFO 13428 and *S. avellaneus* IFO 13451 were not viable even just before drying. These 6 strains showed no or very scant viability after 13 months of storage. It is clear, therefore, that L-drying is not suitable for these 6 strains. The growth indices of these 6 strains were variable, i.e., 0 for 2 strains, 2 for 2 strains and 3 for 2 strains; *S. tropicalensis* IFO 13428 and *S. avellaneus* IFO 13451, which were not viable before and just after L-drying, had a growth index of 3.

In addition to these strains, *Streptoverticillium orinoci* IFO 13466 was not viable after 13 months of storage, even though it was viable after 1 and 3 months. Similarly, *Streptomyces thioluteus* IFO 13341, *Streptoverticillium fervens* IFO 13343, *Streptomyces sclerotialus* IFO 13356, *S. steffisburgensis* subsp. *steffisburgensis* IFO 13446, *S. fulvissimus* IFO 13482 and *S. caespitosus* IFO 13490 showed very poor viabilities after 13 months of storage.

A total of 18 strains showed very poor viabilities throughout the experiments, namely, the 7 just mentioned, together with *Streptomyces horton* IFO 13355, *S. roseiscleroticus* IFO 13363, *S. odorifer* IFO 13365, *S. chryseus* IFO 13377, *S. nobilis* IFO 13386, *S. atrofaciens* IFO 13395, *S. luteogriseus* IFO 13402, *S. capoamus* IFO 13411, *S. ardus* IFO 13430, *S. roseoflavus* IFO 13439 and *S. longispororuber* IFO 13488. Of these, *S. horton* IFO 13355 showed very low viability after 1 and 3 months of storage, but had slightly better viability after 13 months. The growth indices of these strains also varied, although almost half had indices of 0. The viabilities of these strains were thus independent of their growth indices.

Eleven of 35 strains that showed good viabilities after drying and periods of storage tended to show very poor viabilities after 13 months of storage: *S. minutiscleroticus* IFO 13361, *S. novaecaesareae* IFO 13368, *S. albaduncus* IFO 13397, *S. iakyrus* IFO 13401, *S. moderatus* IFO 13432, *S. violaceoniger* IFO 13459 and *S. paraguayensis* IFO 13463; or after 1 or 3 months of storage: *Streptomyces viridoflavus* IFO 13351, *S. spadicis* IFO 13379, *S. diastatochromogenes* IFO 13389 and *S. prasinosporus* IFO 13419. Of these, *S. moderatus* IFO 13432, *S. violaceoniger* IFO 13459 and *S. minutiscleroticus* IFO 13361, which showed excellent viabilities after 1 and 3 months

of storage, showed a marked decline in their viabilities after 13 months. The growth indices of these 11 strains varied from 0 (4 strains) to 4 (2 strains). The marked decline in viability of *S. moderatus* IFO 13432, which showed a growth index of 4, and *S. violaceoniger* IFO 13459, which showed a growth index 3, is not explainable in terms of growth index, since a similar result was found in *S. paraguayensis* IFO 13463, which showed a growth index of 0. Thus the growth indices do not reflect the viabilities of individual strains.

As shown in Table 2, the viabilities of the 22 selected strains just after L-drying varied to some extent, but most showed good results. This is a remarkable contrast to the preceding experimental results shown in Table 1. For instance, 6 of the 22 strains tested showed no viability just after L-drying in the preceding test with only one kind of medium, but, with the exception of *Streptoverticillium kashmirense* IFO 13364, showed good viabilities in this experiment, which employed 5 kinds of media.

Although the viabilities of the strains differed to some extent according to the kind of medium, most of the strains tested showed good viability on at least one of the 5 media. *S. kashmirense* IFO 13364 showed no viability after drying on any of the media and is considered impossible to preserve by L-drying without improvement of the method.

The viabilities of the 22 strains were tested after 60 days of storage both at 3 C and 37 C. As Table 2 shows, *Streptoverticillium kashmirense* IFO 13364 and *Streptomyces tropicalensis* IFO 13428 showed little or no viability irrespective of the medium.

On the other hand, the choice of medium had a remarkable effect on the viabilities of other strains, particularly sensitive strains to L-drying. For example, the viability of *Streptomyces erythraeus* IFO 13426 was excellent on medium No. 227 and medium No. 8, but remarkably poor on the other 3 media. Similarly, *S. viridoflavus* IFO 13351, *S. paraguayensis* IFO 13463, *Streptoverticillium orinoci* IFO 13466 and *Streptomyces caespitosus* IFO 13490 survived with good viability on medium No. 8 and *S. caeruleus* IFO 13344 and *S. atrofaciens* IFO 13395 survived vigorously on medium No. 231 and YS medium. Medium No. 8 and YS medium were also found to be favourable for *Streptomyces kanamyceticus* IFO 13414, *S. sclerotialus* IFO 13356 and *S. minutiscleroticus* IFO 13361.

Two strains of the Actinomycetes, *Streptoverticillium kashmirense* IFO 13364 and *Streptomyces tropicalensis* IFO 13428, could not be preserved by L-drying; the traditional preservation methods such as serial transfer and freezing in a mechanical refrigerator must therefore be used until further improvement of L-drying is achieved. For such strains as *Streptomyces thioluteus* IFO 13341, *S. flavofungini* IFO 13371 and *S. avellaneus* IFO 13451, which gave low CFU counts after drying and after 60 days of storage, the use of heavy spore or propagule suspensions sufficient to give a high CFU value in viability tests before and after drying is recommended.

The effect of storage temperature in accelerated storage tests was compared at 3 C and at 37 C, but no significant effect on the viability of the selected strains was found after 60 days except in *S. horton* IFO 13355 and *S. longispororuber* IFO 13488.

It should, however, be stressed that storage at a lower temperature such as at 3 C is considered favourable for the long-term preservation of any microorganism.

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PRESERVATION OF BASIDIOMYCETE CULTURES BY FREEZING

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Summary

Basidiomycete stock cultures maintained at the Institute for Fermentation, Osaka (IFO) were stored in a mechanical refrigerator at −80 C. The cultures have survived with 93.5% viability for the total of 940 strains tested after one year of storage.

Keeping stock cultures in the frozen state at −80 C is practically useful, because of its simple procedure and high viability. This method is especially effective for the long-term preservation of the nonsporulating fungi such as mycelial basidiomycete cultures.

To avoid frequent serial transfer which may induce the degeneration or mutation of microorganisms, various long-term preservation methods have been developed. Dispersal of spores in soil, freezing in a mechanical refrigerator, freeze-drying, mineral oil sealing and freezing in liquid nitrogen have commonly been used. Almost all of microorganisms are known to be preservable by these methods, but not all of these methods are economically or technically practical. For instance, some strains which do not produce any kind of spores are difficult to preserve except by the mineral oil, deep freezing and liquid nitrogen methods. Although Carmichael (1, 2), Hamilton and Weaver (3) and Kramer and Mix (4) used deep freezing for certain sporulating fungi, it would be advantageous to find a simpler and more satisfactory procedure to preserve these fungi in mycelial form.

In preliminary tests, we found that direct freezing in a mechanical refrigerator at −80 C followed by preservation in a frozen state, even with repeated freezing and thawing procedures, is effective as much the same as controlled-rate freezing at 1 deg/min and is practically useful for nonsporulating fungi (unpublished).

This paper reports a simplified method for the long-term preservation of basidiomycete cultures which could not be preserved by freeze-drying and L-drying methods.

Materials and Methods

A total of 940 strains of species belonging to Basidiomycotina in the Institute for Fermentation, Osaka (IFO) Culture Collection of Fungi were subjected to freezing at −80 C. These strains were inoculated on a Petri dish (90 mm diam) containing 15 ml of suitable medium (mainly PSA and Matsutake media) and incubated for 3 weeks at a given temperature until vigorous vegetative mycelium covered the surface of the agar

plates. Some strains took more than 3 weeks to develop a satisfactory mycelial growth. After an adequate period of incubation, agar mycelial disks of 6 mm in diam were removed from the plates with a sterilized cork borer. Six disks were placed in a Cryotube (Nunc 1076–1A, 38×12.5 mm) containing one ml of 10% glycerol in water, and two tubes for each strain were stored in a deep freezer (Sanyo refrigerator MDF–390 AT) at −80 C. After one year of storage, tubes were removed from the freezer and allowed to thaw rapidly in a water bath at 40 C. Two thawed disks were removed from the tube and inoculated onto a slant of the same medium as used for precultures, then incubated for 3 weeks at a given temperature. Tubes with remaining disks were refrozen in a mechanical refrigerator for use in further tests.

Results and Discussion

Table 1 shows the number of strains used, the number of viable strains and the proportion of survivors (%) of these strains, which are grouped according to the taxonomic rank of the order. Of a total of 940 strains tested, consisting of 135 genera and

Table 1. Viability of strains of basidiomycete fungi after freezing at −80 C for one year.

Order	Number of strains	Number of viable strains	Proportion of survivors (%)
Aphyllophorales	396	393	99.2
Agaricales	427	377	88.3
Lycoperdales	5	3	60.0
Nidulariales	2	2	100.0
Phallales	2	2	100.0
Hymenogastrales	7	5	71.4
Sphaerobolales	2	2	100.0
Ustilaginales	27	27	100.0
Tremellales	42	42	100.0
Auriculariales	11	7	63.6
Dacrymycetales	1	1	100.0
Anamorphs of Basidiomycotina	18	18	100.0
Total	940	879	93.5

325 species, 879 strains were recovered in this experiment. This corresponds to an overall proportion of survivors of 93.5%. Sixty-one strains did not survive, including 3 strains of Aphyllophorales, 50 of Agaricales, 2 of Lycoperdales, 2 of Hymenogastrales and 4 of Auriculariales. Strains of Aphyllophorales, Ustilaginales and Tremellales could be preserved by this procedure without any problems, but some strains of Agaricales were not viable after one year of storage. It is difficult to estimate the viabilities of the strains in the order rank, because we tested only a limited number of strains and species of Lycoperdales, Nidulariales, Phallales, Hymenogastrales, Sphaerobolales, Auriculariales and Dacrymycetales. Anamorphs of Basidiomycotina survived well, and those

Table 2. Basidiomycete fungi preserved by freezing at −80 C for one year.

Species	Number of strains tested	Number of viable strains
Aphyllophorales		
Aleurodiscus aurantius	1	1
Aleurodiscus cerussatus	1	1
Aleurodiscus disciformis	1	1
Aleurodiscus roseus	1	1
Amylostereum areolatum	1	1
Aporpium caryae	1	1
Auriscalpium vulgare	1	1
Bjerkandera adusta	3	3
Clavicorona pyxidata	1	1
Coniophora puteana	1	1
Coriolus brevis	1	1
Coriolus consors	7	7
Coriolus elongatus	1	1
Coriolus fibula	1	1
Coriolus hirsutus	10	10
Coriolus pargameus	2	2
Coriolus pubescens	1	1
Coriolus unicolor	1	1
Coriolus versicolor	13	13
Corticium caeruleum	1	1
Corticium catonii	2	2
Corticium fuciforme	1	1
Corticium galactinum	2	2
Corticium gramineum	1	1
Corticium lundellii	1	1
Corticium rolfsii	32	32
Cryptoderma pini	1	1
Cryptoporus volvatus	3	3
Cyclomyces fuscus	1	1
Cymatoderma elegans	1	1
Daedalea dickinsii	5	5
Daedalea malicola	1	1
Daedaleopsis styracina	2	2
Daedaleopsis tricolor	3	3
Echinodontium japonicum	2	2
Echinodontium taxodii	1	1
Exobasidium bisporum	2	2
Exobasidium camelliae	1	1
Exobasidium gracile	5	5
Exobasidium japonicum	4	4
Exobasidium pieridis-ovalifoliae	1	1

Table 2 (continued)

Species	Number of strains tested	Number of viable strains
Exobasidium reticulatum	2	2
Exobasidium shiraianum	1	1
Exobasidium symploci-japonicae	1	1
Exobasidium yoshinagai	1	1
Exobasidium spp.	5	5
Favolus arcularius	4	4
Fistulina hepatica	2	2
Fomes fomentarius	3	3
Fomitopsis annosa	1	1
Fomitopsis insularis	2	2
Fomitopsis pinicola	1	1
Fuscoporia obliqua	1	1
Ganoderma applanatum	6	6
Ganoderma lucidum	3	3
Gloeophyllum sepiarium	2	2
Gloeophyllum striatum	4	4
Gloeophyllum trabeum	3	3
Gloeophyllum ungulatum	2	2
Grifola frondosa	8	8
Gyrodontium versicolor	1	1
Hapalopilus croceus	2	2
Hericium coralloides	1	1
Hirschioporus abietinus	3	3
Hymenochaete tabacina	1	1
Inonotus dryadeus	1	1
Inonotus mikadoi	2	2
Irpex lacteus	4	4
Laetiporus sulphureus	8	8
Laetiporus versisporus	4	4
Laurilia sulcata	1	1
Lenzites betulina	6	6
Merulius tremellosus	3	3
Mycoleptodonoides pergameneum	3	3
Onnia orientalis	1	1
Pellicularia filamentosa	17	17
Pellicularia filamentosa f. sp. *microsclerotia*	2	2
Pellicularia filamentosa f. sp. *sasakii*	31	31
Pellicularia filamentosa f. sp. *solani*	12	12
Pellicularia filamentosa f. sp. *timsii*	1	1

Table 2 (continued)

Species	Number of strains tested	Number of viable strains
Pellicularia flavescens	1	1
Pellicularia praticola	2	2
Peniophora mutata	1	1
Peniophora pubera	1	1
Phaeolus schweinitzii	3	3
Phellinus linteus	1	1
Phellinus pomaceus	1	1
Pistillaria micans	1	1
Pistillaria setipes	1	1
Polyporellus brumalis	1	1
Polyporellus picipes	2	2
Polyporus sulphureus	1	1
Poria aurantiofibrillosus	1	1
Poria cocos	2	2
Porodisculus pendulus	1	1
Protodaedalea hispida	2	2
Punctularia atropurpurascens	5	5
Pycnoporus cinnabarinus	4	4
Pycnoporus coccineus	16	16
Ramaria botrytis	1	0
Ramaria flaccida	1	0
Serpula lacrymans	4	4
Spongiporus sinuosus	1	1
Stereum annosum	1	1
Stereum bicolor	1	1
Stereum frustulosum	3	3
Stereum hirsutum	1	1
Stereum roseum	2	2
Stereum spectabile	1	1
Stereum subpileatum	1	1
Stereum taxodii	1	1
Thanatephorus cucumeris	30	29
Trametes albida	5	5
Trametes cubensis	1	1
Trametes gibbosa	2	2
Trametes kusanoana	1	1
Trametes orientalis	3	3
Trametes serialis	1	1
Trechispora raduloides	1	1
Tyromyces caesius	1	1
Tyromyces palustris	5	5
Tyromyces ptychogaster	1	1
Veluticeps angularis	2	2

Table 2 (continued)

Species	Number of strains tested	Number of viable strains
Agaricales		
Agaricus bisporus	5	5
Agaricus campestris	3	3
Agrocybe cylindracea	2	2
Agrocybe paraecox	2	2
Amanita aspera	1	1
Amanita citrina	3	2
Amanita muscaria	1	0
Amanita pantherina	1	0
Amanita rubescens	1	0
Amanita spissa	1	0
Amanita spissacea	1	1
Amanita sp.	1	1
Armillariella mellea	5	5
Armillariella tabescens	2	2
Clitocybe acromelalga	1	1
Clitocybe clavipes	1	0
Clitocybe nebularis	1	0
Clitocybula sp.	1	1
Collybia butyracea	1	1
Collybia confluens	1	1
Collybia cookei	2	2
Collybia peronata	1	1
Collybia tuberosa	1	1
Collybia sp.	1	1
Conocybe lactea	1	1
Conocybe tenera	1	0
Coprinus angulatus	1	1
Coprinus atramentarius	1	1
Coprinus bilanatus	1	1
Coprinus cinereus	6	6
Coprinus cinereus f. sp. *microsporus*	3	3
Coprinus comatus	2	2
Coprinus disseminatus	3	3
Coprinus echinosporus	3	1
Coprinus filamentifer	1	0
Coprinus fissolanatus	1	1
Coprinus friesii	1	1
Coprinus lagopides	1	1
Coprinus lagopus	4	3
Coprinus macrocephalus	1	1
Coprinus neolagopus	1	1

Table 2 (continued)

Species	Number of strains tested	Number of viable strains
Coprinus ochraceo-velatus	1	1
Coprinus phlyctidosporus	3	3
Coprinus pseudolagopus	1	0
Coprinus radians	2	2
Coprinus radiatus	1	0
Coprinus rhizophorus	1	1
Coprinus stercorarius	2	1
Coprinus spp.	3	2
Cortinarius cinnamomeus	1	1
Crinipellis stipitaria	1	1
Filoboletus manipularis	1	1
Flammulina velutipes	24	24
Galerina fasciculata	1	1
Gymnopilus aeruginosus	3	3
Gymnopilus spectabilis	2	2
Hebeloma crustuliniforme f. sp. *microspermum*	1	1
Hebeloma radicosum	2	2
Hebeloma spoliatum	4	2
Hebeloma vinosophyllum	3	3
Hygrophoropsis aurantiaca	1	1
Kuehneromyces mutabilis	1	1
Laccaria laccata	1	1
Laccaria proxima	2	1
Lactarius chrysorheus	1	1
Lampteromyces japonicus	8	8
Lentinus edodes	28	27
Lentinus lepideus	7	7
Lepiota bresadolae	2	2
Lepista irina	1	1
Lepista luscina	1	1
Lepista nuda	7	5
Lepista personata	1	1
Lepista sordida	4	4
Leucoagaricus excoriatus	1	1
Leucoagaricus naucinus	3	2
Leucocoprinus birnbaumii	1	1
Leucocoprinus luteus	7	7
Lyophyllum anthracophilum	2	2
Lyophyllum decastes	2	2
Lyophyllum fumosum	1	1
Lyophyllum gibberosum	2	2

Table 2 (continued)

Species	Number of strains tested	Number of viable strains
Lyophyllum leucopaxilloides	1	1
Lyophyllum shimeji	1	0
Lyophyllum tylicolor	4	4
Lyophyllum transforme	2	1
Lyophyllum ulmarium	3	3
Macrolepiota mastoidea	1	0
Macrolepiota procera	1	1
Macrolepiota rhacodes	2	2
Marasmius purpureostriatus	1	1
Marasmius siccus	1	1
Mycena crocata	2	2
Mycena haematopoda	1	0
Mycena luteopallens	1	1
Naematoloma fasciculare	3	3
Naematoloma sublateritium	4	4
Omphalotus olearius	2	2
Oudemansiella mucida	1	1
Oudemansiella radicata	2	2
Panaeolina rhombisperma	2	2
Panaeolus cambodginiensis	1	1
Panaeolus sphinctrinus	1	1
Panellus serotinus	3	3
Panellus stypticus	3	3
Panus conchatus	2	2
Panus rudis	3	3
Phaeolepiota aurea	5	0
Pholiota adiposa	3	3
Pholiota aurivella	3	3
Pholiota carbonaria	3	3
Pholiota lenta	3	3
Pholiota lubrica	1	1
Pholiota nameko	4	4
Pholiota spumosa	1	1
Pholiota terrestris	2	2
Pleurocybella lignatilis	1	1
Pleurocybella porrigens	2	2
Pleurotus cornucopiae	2	2
Pleurotus cystidiosus	16	16
Pleurotus ostreatus	12	12
Pleurotus sajor-caju	2	2
Pleurotus salmoneo-stramineus	2	1
Psathyrella candolleana	3	2

Table 2 (continued)

Species	Number of strains tested	Number of viable strains
Psathyrella velutina	3	2
Pseudohiatula ohshimae	1	1
Psilocybe argentipes	2	2
Psilocybe cubensis	2	2
Psilocybe cyanescens	1	1
Psilocybe fasciata	5	5
Psilocybe merdaria	1	1
Psilocybe subaeruginascens	2	2
Psilocybe subcaerulipes	1	1
Rhodophyllus hirtipes	1	0
Schizophyllum commune	9	9
Strobilurus stephanocystis	3	2
Strobilurus tenacellus	1	1
Stropharia aeruginosa	1	1
Stropharia rugosoannulata	3	3
Suillus tomentosus	1	0
Tricholoma bakamatsutake	2	2
Tricholoma fulvocastaneum	11	9
Tricholoma matsutake	26	20
Tricholoma ponderosum	1	1
Tricholoma robustum	4	4
Tricholoma sp.	12	10
Volvariella speciosa	1	0
Volvariella volvacea	2	2
Volvariella sp.	1	0
Xeromphalina cauticinalis	2	2
Xerula pudens	2	2
Lycoperdales		
Calvatia craniiformis	4	3
Pisolithus tinctorius	1	0
Nidulariales		
Cyathus stercoreus	1	1
Cyathus striatus	1	1
Phallales		
Kobayasia nipponica	2	2
Hymenogastrales		
Limnoperdon incarnatum	7	5
Sphaerobolales		
Sphaerobolus stellatus	2	2
Ustilaginales		
Doassansia horiana	2	2
Graphiola phoenicis	3	3

Table 2 (continued)

Species	Number of strains tested	Number of viable strains
Leucosporidium scotii	2	2
Neovossia danubialis	1	1
Tilletia caries	1	1
Tilletialia anomala	1	1
Ustilago antherarum	1	1
Ustilago cynodontis	3	3
Ustilago esculenta	1	1
Ustilago kusanoi	1	1
Ustilago maydis	2	2
Ustilago nuda	1	1
Ustilago onumae	1	1
Ustilago rabenhorstiana	1	1
Ustilago shiraiana	2	2
Ustilago violacea	4	4
Tremellales		
Fibulobasidium inconspicum	4	4
Pseudohydnum gelatinosum	1	1
Sporidiobolus johnsonii	2	2
Tremella aurantia	2	2
Tremella brasiliensis	4	4
Tremella encephala	5	5
Tremella foliacea	6	6
Tremella fuciformis	3	3
Tremella mesenterica	11	11
Tremella samoensis	2	2
Tremella subanomala	2	2
Auriculariales		
Auricularia auricula-judae	4	4
Auricularia mesenterica	1	0
Auricularia polytricha	1	1
Auricularia sp.	1	0
Helicobasidium mompa	3	1
Helicobasidium sp.	1	1
Dacrymycetales		
Femsjonia luteo-alba	1	1
Anamorphs of Basidiomycotina		
Ptychogaster corruscans	1	1
Ptychogaster cubensis	1	1
Rhizoctonia solani	15	15
Sporotrichum dimorphosporum	1	1
Total	940	879

strains with oidia or other kind of spores may easily be stored by this procedure for a long time.

Table 2 shows the number of strains tested and the number of viable strains for each species. A total of 37 strains in 9 species of *Coriolus*, 40 in 7 species of *Corticium*, 23 in 14 species of *Exobasidium* and 66 in 7 species of *Pellicularia*, all of which belong to Aphyllophorales, were alive, and these species are considered to be preserved satisfactorily by this method. In addition, 35 strains in 8 species of *Tremella*, which show yeast forms on agar medium, could also be stored. Of the Agaricales, 10 strains in 8 species of *Amanita* and 56 in 6 species of *Tricholoma* did not survive as well as those in other genera of the same order. It is uncertain why strains of these two genera did not survive well. However, the growth of these strains in preculture was slow and poor even under the best known conditions. Therefore, more suitable conditions should be found for the mycelial growth of these strains. None of 5 strains of *Phaeolepiota aurea* survived, and this species was considered very sensitive to the freezing by this procedure. Only one of 30 strains of *Thanatephorus cucumeris* and one of 28 of *Lentinus edodes* were not recovered. This is presumably because of a difference between the strains.

A total of 325 species of the basidiomycete fungi were tested by this method. However, it is hard to conclude whether the discrepancies found in the proportion of survivors in each species reflect the characteristics of the species, because the number of the strains tested was not so many for all species.

Finally, 61 strains in 45 species did not survive by this procedure. Therefore, the following points should be verified to establish a satisfactory procedure for the long-term preservation of these sensitive strains in a stable condition.

1. It is necessary to find more suitable media to secure vigorous vegetative mycelium in prefreezing cultures, as these strains generally develop poor and restricted colonies on culture media.
2. It is necessary to improve the medium for checking viabilities, as we used only a limited medium for survival tests.
3. It is necessary to evaluate the cryoprotective agents effective for these strains, as we used 10% glycerol in water exclusively.

The authors wish to thank Dr. K. Tubaki, Professor of University of Tsukuba, for reviewing the manuscript and giving valuable suggestions.

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IFO Res. Comm. **11**,
71-76, 1983 (March)

DESCRIPTIVE CATALOGUE OF IFO FUNGUS COLLECTION VIII.

In the routine work of identification of fungi newly isolated in Japan and in checking the list of fungi preserved in the IFO culture collection for published records of their occurrence in Japan, many fungi have been found to be taxa either new to Japan or obscurely or insufficiently described. In some cases, the first record of a fungus in Japan gives only the name of a taxon, without an adequate description of the species concerned. The object of this series is to provide descriptions of the fungi preserved or newly deposited in the IFO culture collection and/or in the IFO herbarium and to contribute to the knowledge of the fungal flora of Japan.

New taxa will be described in original papers or submitted to other mycological journals. Authors of the descriptions of these fungal taxa are shown in brackets.

77. Aspergillus carneus (v. Tiegh.) Blochwitz (Fig. 1, A & B) Hyphomycetes Thom & Raper, A Manual of the *Aspergillus*. p. 201 (1945); Raper & Fennell, The genus *Aspergillus*. p. 564 (1965).

Syn. *Sterigmatocystis carnea* v. Tiegh., Bull. soc. bot. France **24**: 103 (1877), *Aspergillus carneus* Blochwitz, Ann. Mycol. **31**: 81 (1933), *Aspergillus aureofulgens* Luppi Mosca, Allionia **19**: 33 (1973), *Aspergillus carneus* (v. Tiegh.) Blochwitz var. *curvatus* B.S. Mehrotra & Basu, Nova Hedwigia **27**: 601 (1976).

Colonies on Czapek agar growing rapidly, reaching a diameter of 5 cm in 10 days at 24 C, velvety or floccose to funiculose, at first white to pale pink, later becoming vinaceous fawn with white margin, plane and irregularly lobed at the margin, blue-green sectors sometimes observed; aerial mycelium white to pale yellow; exudating pale pink soluble pigment into the medium in most strains; reverse various shades, luteous, yellow-brown, dark blue-green or red-brown. Conidial heads pink to vinaceous fawn, loosely columnar. Conidiophores long, straight, smooth, hyaline to pale yellow, 400–700 (–1000)×4.5–5.5 μm; vesicles subglobose to pyriform, concolor with conidiophores, 13–16(–18)×8–10(–13) μm; metulae pale fawn, clavate, 8–10×2.5–3.0 μm; phialides pale fawn, elliptical, 5–8×1.5–2.5 μm. Conidia globose, smooth to finely rough, hyaline to slightly pink, 2.5–3.0 μm.

Growth is good at 37 C.

Hab. Paddy field soil: Shakudo, Habikino, Osaka Pref., Aug. 20, 1976, T. Yokoyama XI42–3–10–4 (IFO 30897); Nakatsu-cho, Ibaraki, Osaka Pref., Oct. 17, 1976, T. Yokoyama ZII42–1–5–5 (IFO 30898); Jul. 16, 1978, T. Yokoyama ZIX–1–5–13; Jan. 22, 1979, T. Yokoyama ZXI42–1–5–4 (IFO 30899).

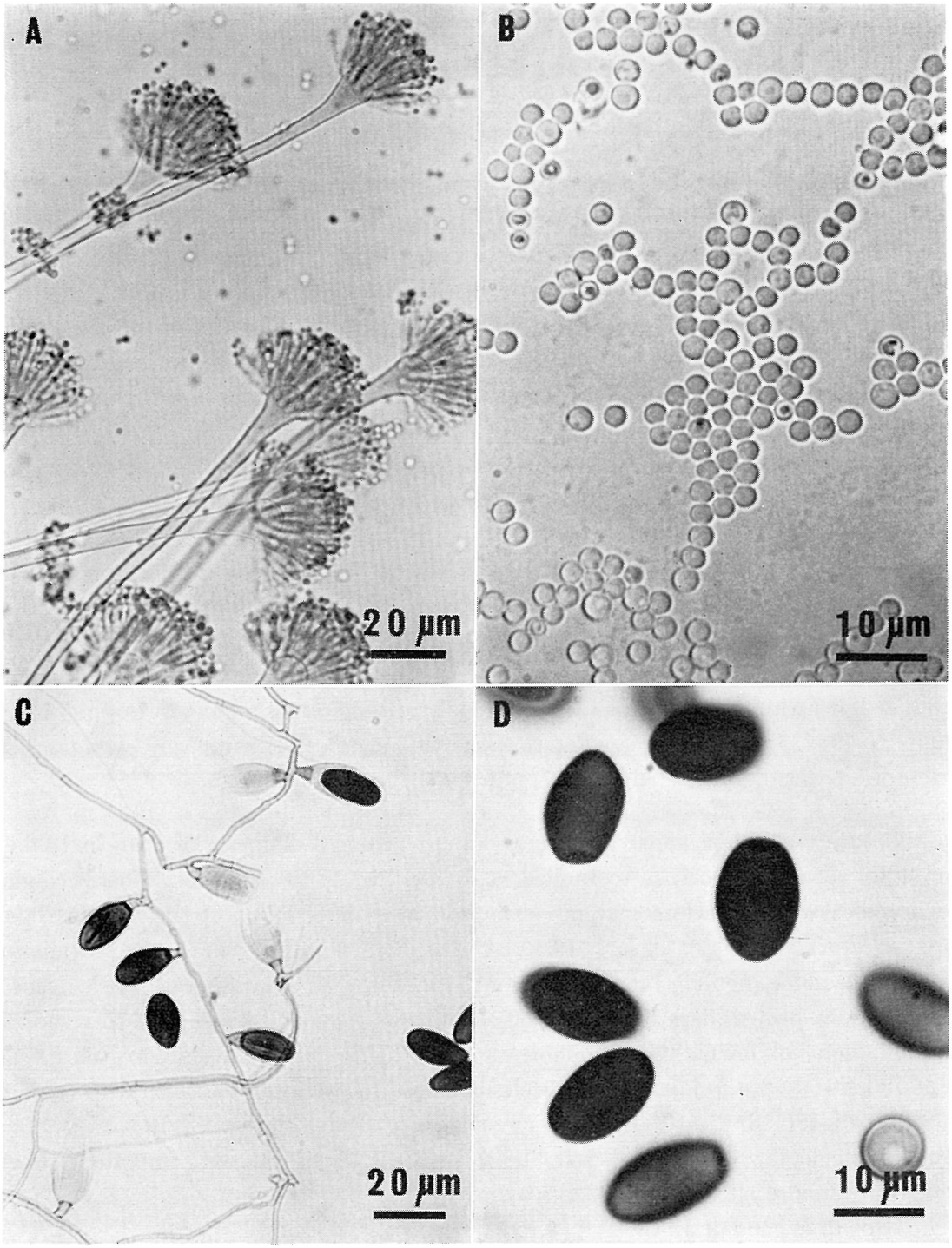

Fig. 1. A & B. *Aspergillus carneus* (IFO 30897). A. Conidial structure. B. Conidia.
C & D. *Conioscypha varia* (IFO 31204). C. Conidial structure. D. Conidia.

Additional strain examined: IFO 5861.

This fungus is characterized by the pale vinaceous to vinaceous fawn shade of conidial heads. Samson (1979) treated *A. aureofulgens* Luppi Mosca as a synonym of *A. carneus* (v. Tiegh.) Blochwitz because yellow sterile hyphal bodies are often produced in fresh isolates. In addition, *A. carneus* (v. Tiegh.) Blochwitz var. *curvatus* B.S. Mehrotra & Basu was included by him in *A. carneus*.

Four strains of this species were isolated from paddy field soils in Osaka. They were confirmed as conspecific with the strain IFO 5861 and agreed well with the description given by Raper & Fennell (1965).

[T. Ito & T. Yokoyama]

78. Conioscypha varia Shearer (Fig. 1, C & D) Hyphomycetes
Mycologia **65**: 128 (1973).
Syn. *Cylicogone regenerans* Van Emden & Veenbaas-Rijks, Acta Bot. Neerl. **22**: 637 (1973).

Colonies on oatmeal agar growing moderately fast, reaching a diameter of 3–4 cm in 2 weeks at 24 C, velvety, yellow-green or dark green to almost black; mycelium immersed and creeping on medium, hyaline to pale yellow, 2–2.5 μm; reverse yellow-green to dark green, almost black at maturity. Distinct conidiophores not observed. Conidiogenous cells hyaline, short-stalked, borne directly and laterally or terminally along the surface of the hyphae, 3–5.5$\times$3–4.5 μm. Conidia formed enteroblastically and singly with percurrent proliferation of the conidiogenous cells. Outer walls remain as cup-like collarette. Conidia irregular in shape, clavate to obovate, sometimes pyriform, pale brown to dark brown at maturity, truncate at the base, 13–20$\times$8–14 μm.

Growth is nil at 37 C.

Hab. Paddy field soil: Shakudo, Habikino, Osaka Pref., May 21, 1978, T. Yokoyama XVIII–1–15–11 (IFO 31201); Nakatsu-cho, Ibaraki, Osaka Pref., Jul. 16, 1978, T. Yokoyama ZIX–1–5–22 (IFO 31204); Jul. 16, 1978, T. Yokoyama ZIX–5–10–17 (IFO 31205); Shakudo, Habikino, Aug. 21, 1978, T. Yokoyama XIX–4–10–20 (IFO 31202) and T. Yokoyama XIX–4–15–6 (IFO 31203); Nose-cho, Toyono-gun, Osaka Pref., Sept. 3, 1978, T. Yokoyama WIX–3–5–28 (IFO 31200); Nakatsu-cho, Ibaraki, Oct. 23, 1978, T. Yokoyama ZX–3–5–11 (IFO 31206).
Additional strains examined: IFO 30671 (ATCC 18833), IFO 30674 (ATCC 22765), IFO 30672 (ATCC 26317), IFO 30673 (ATCC 26504), IFO 30675 (ATCC 26832).

The genus *Conioscypha* was erected by Höhnel (1904) to accomodate a single species, *C. lignicola* Höhnel, which was originally isolated from fallen wood of *Carpinus betulus* L. in Wienerwald, Lower Austria. Shearer (1973) redescribed this fungus and added a second species, *C. varia* Shearer, which was isolated from balsa wood submerged in the Patuxent River in Maryland, USA.

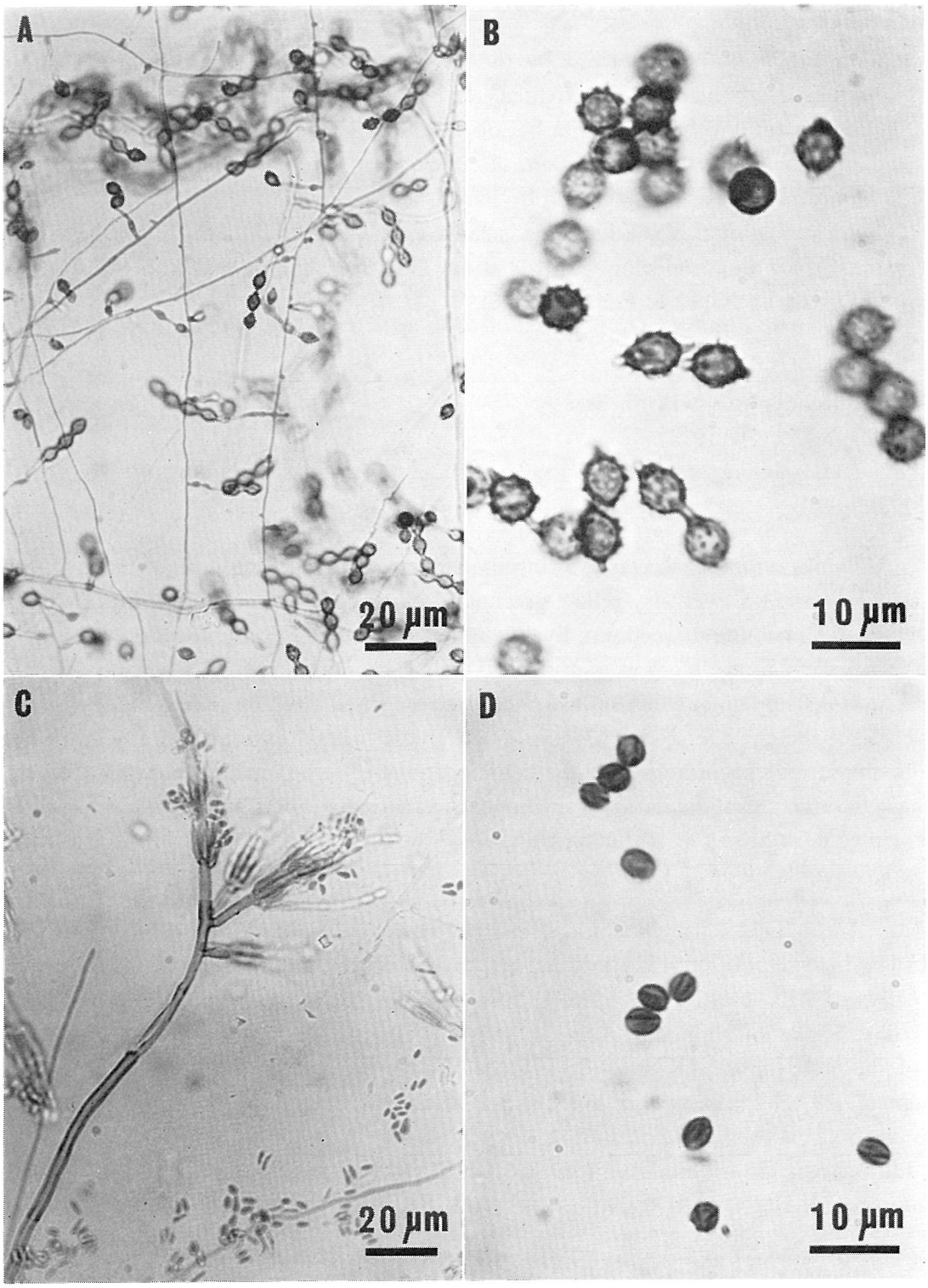

Fig. 2. A & B. *Sagenomella oligospora* (IFO 31208). A. Conidial structure. B. Conidia.
C & D. *Talaromyces leycettanus* (IFO 31191). C. Conidial structure. D. Ascospores.

Jong and Roxon (1975) examined the type strains of *C. varia* and *Cylicogone regenerans* and concluded, based on their morphorogical characteristics, that *C. regenerans* should be treated as a later, facultative synonym of *C. varia*. Matsushima (1975) described a third species, *C. bambusicola* Matsushima, found on *Bambusa multiplex* and *Phyllostachys edulis* in Japan.

We obtained more than 100 isolates of *C. varia* from paddy field soils in Osaka and compared them with the type strain of *C. varia*. The conidial size of our isolates was slightly larger than that of the type strain of *C. varia*, but other morphological characteristics were identical. We, therefore, concluded that our isolates were conspecific with *C. varia*.

[T. Ito & T. Yokoyama]

79. Sagenomella oligospora W. Gams & Luiten apud W. Gams (Fig. 2, A & B) Hyphomycetes
Persoonia **10**: 97 (1978).

Colonies on oatmeal agar growing somewhat restrictedly, reaching a diameter of 2–2.5 cm in 7 days at 28 C, at first white, becoming grey to dark brown; vegetative mycelium velvety to partly floccose, submerged at the margin; exudating pale pink pigment into agar medium; reverse becoming dark brown. Phialides simple and erect on vegetative hyphae, hyaline, smooth-walled, flask-shaped, tapering towards the apex with dark green pigment, 7.5–10×2–3.5 μm, 1–1.5 μm wide at the tip. Conidia catenate, in chains of 5–11 conidia, ellipsoid, thick- and rough-walled, 1-celled, greenish brown, 6–7.5×3–4 μm.

Growth is very little at 37 C.

Hab. Paddy field soil: Hachioji, Ikeda, Osaka Pref., Aug. 5, 1977, T. Yokoyama YV–1–15–15 (IFO 31207) and T. Yokoyama YV–5–5–27 (IFO 31208); Shakudo, Habikino, Osaka Pref., Nov. 21, 1978, T. Yokoyama XX–1–10–33 (IFO 31209).
Additional strains examined: IFO 31210 (CBS 168.74), IFO 31211 (CBS 615.76).

This species is easily distinguishable by its conspicuously brown and rough-walled conidia. Three strains were isolated from paddy field soils in Osaka and agreed well with Gams' description.

[T. Ito & T. Yokoyama]

80. Talaromyces leycettanus Evans & Stolk (Fig. 2, C & D) Eurotiales
Trans. Br. mycol. Soc. **56**: 45 (1971); Stolk & Samson, Studies in Mycology **2**: 1 (1972).
Status anamorphosis: *Paecilomyces leycettanus* (Evans & Stolk) Stolk et al.,
Persoonia **6**: 342 (1971).

Colonies on oatmeal agar growing rapidly at 37 C, reaching a diameter of 7 cm in 7 days, pale buff, becoming greenish yellow, velvety or slightly floccose, raised at cent-

ral areas, producing numerous penicilli; ascocarps produced most abundantly at 28 C; reverse yellow-brown. Ascocarps greenish yellow, globose to subglobose, 75–200 μm in diameter, discrete, usually ripening within 7 days at 28 C; covering hyphae scanty, consisting of irregular networks, but bearing dark brown, crowded hyphae at the base of ascocarps; ascogonial initials pale brown, coiled, septate. Asci hyaline, globose to subglobose, 8–10×8–9 μm. Ascospores ellipsoid, 4–5×2.5–3 μm, ornamented with 2 to 6 longitudinal striations. Conidiophores smooth, pale brown, 50–300 μm long and 2.0–3.5 μm wide; penicilli usually irregular, asymmetrical; metulae smooth-walled, 10–15×2.0–3.5 μm; phialides cylindrical, hyaline, 10–15×2–3 μm. Conidia long-chained, cylindrical to ellipsoid, smooth, pale yellow, becoming yellow-brown in mass, 4–6× 1.5–2 μm. Chlamydospores sometimes produced, globose, smooth, 5 μm in diameter.

Growth is good at 37 C and observed even at 50 C.

Hab. Paddy field soil: Kawasaki, Ryozu, Niigata Pref., May 30, 1981, T. Yokoyama V_5 I42–10–1 (IFO 31191) and T. Yokoyama V_5 I42–15–3 (IFO 31192).
Additional strains examined: IFO 31193 (CBS 398.68), IFO 31194 (CBS 275.70), IFO 31195 (CBS 276.70).

These strains produce ascocarps in abundance on oatmeal agar at 28 C, but the conidial state is formed better at 37 C than 28 C. The present species, originally found by Evans from *Leycett* coal spoil tips in Staffordshire, UK, has not since been rediscovered. Two strains were isolated from paddy field soils in Niigata Prefecture as the second record.

[T. Ito & T. Yokoyama]

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77-80, 1983 (March)

DESCRIPTIVE CATALOGUE OF IFO YEAST COLLECTION IV.

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

27 and 28. **Kluyveromyces thermotolerans** (Philippov) Yarrow

Yarrow, D. 1972. Antonie van Leeuwenhoek **38**: 359.
Nomenclatural synonym. *Kluyveromyces veronae* (Lodder et Kreger-van Rij) van der Walt 1970. *in* The Yeasts, A Taxonomic Study, ed. by J. Lodder, North-Holland Publ. Co., Amsterdam, p. 369.

IFO 10066 and 10067

Growth in glucose-yeast extract-peptone water: After 3 days at 24 C the cells are spheroidal to oval, 2.8–5.0×5.0–8.0 μm; single, in pairs. A sediment is present.

After two weeks at 24 C a sediment and a ring are present.

Growth on YM agar: After 3 days at 25 C the cells are spheroidal to oval, 2.4–6.0×3.5–8.7 μm: single, in pairs, in short chains or small clusters.

After 2 weeks at 24 C the streak culture is cream-colored with fine stripes perpendicularly to the ridge, smooth, low convex, semiglossy. Margine is delicately crenate.

Dalmau plate culture on corn meal agar: Pseudomycelium is developed under anaerobic condition but it is rudimentary. Many asci are produced on pseudohypha after the growth stopped.

Formation of ascospores on corn meal agar and YM agar: The conjugation between independent vegetative cells precedes ascus formation. Gourd-shaped asci result from the conjugation, containing two to four sphaerical ascospores. Most spores contain a refractive globule. They are liberated from the asci promptly when matured.

Fermentation:

Glucose	+	Melibiose	+
Galactose	+	Raffinose	+
Sucrose	+	Inulin	−
Maltose	+	Soluble starch	−
Trehalose	+(slow)	α-Methyl-D-glucoside	+
Lactose	−	Melezitose	+

Assimilation of carbon compounds:

Glucose	+	D-Ribose	—
Galactose	+	L-Rhamnose	—
L-Sorbose	+	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	—
Cellobiose	—	Ribitol	—
Trehalose	+	Galactitol	—
Lactose	—	D-Mannitol	+
Melibiose	+	D-Glucitol	+
Raffinose	+	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	—
Inulin	—	DL-Lactic acid	—
Soluble starch	—	Succinic acid	—
D-Xylose	—	Citric acid	—
L-Arabinose	—	Inositol	—
D-Arabinose	—		

Splitting of arbutin: Absent.

Assimilation of nitrogen compounds:

Potassium nitrate; Absent.

Ethylamine hydrochloride; Positive.

Growth in vitamin-free medium: Absent.

Growth on 50% (w/w) glucose-yeast extract agar: Positive.

Growth at 37 C: Absent.

Cycloheximide resistance: Absent.

Co-Q system: Co-Q 6

G+C content: 44.6–44.9 mol%

IFO 10066 was isolated from the litter-leaf collected at Mt. Daisen, Tottori Pref., on Aug. 3, 1974 (strain No. D-34al); IFO 10067 from a flower at Mt. Ontakesan. Nagano Pref., on Aug. 31, 1976 (No. On-11m3).

K. Kodama[1] isolated 3 strains which closely resemble the type culture of *K. thermotolerans* with the exception of fermentation of melibiose, and regarded them as a form of the species. IFO 10066 and 10067 also differ in fermentation of melibiose from the description of *K. thermotolerans*. Guanine+cytosine content of DNA was found to be 44.6±2%, which dose not significantly differ from 45.0% of the type culture of the species. The strains should be included in *K. thermotolerans* as a biotype, following Kodama's opinion.

[K. Mikata]

1) Kodama, K. 1974. Ascosporogenous yeasts isolated from exudates in Japan (continued), J. Ferm. Technol. 52: 605–613 (in Japanese).

29. Ambrosiozyma monospora (Saito) van der Walt

van der Walt, J.P. 1972. Mycopathologia et Mycologia applicata. **46**: 308.

Nomenclatural synonym. *Endomycopsis monospora* Saito 1932. *in* J. Brewery Sci. **10**: 11

IFO 4841

Growth in glucose-yeast extract-peptone water: After 3 days at 24 C the budding cells are spherical to oval and elongate, 6.3–8.7×7.5–19.2 μm; single, in pairs. Pseudomycelia are present. A floccose sediment is formed.

After one month at 20 C a thick, flocculent sediment occupies a half of the liquid and a broad ring is formed.

Growth on YM agar: After 3 days at 24 C the budding cells are spherical to oval, 4.0–7.5×5.0–10.3 μm; single, in pairs. True mycelium and pseudomycelium are formed.

After one month at 20 C the streak culture is pale-brown to brown colored, moderately tough, raised, dull, finely crinkled surface. The margine is fringed with mycelium.

Dalmau plate cultures on corn meal agar: True mycelium is abundantly formed. True mycelium consists of branched hypha with septa in which single pore bodies of the dolipore type is found (Fig. 1B). Chains and clusters of spherical to short-oval blastospore are formed at the end of side branches and alongside stems. Pseudomycelia also are present.

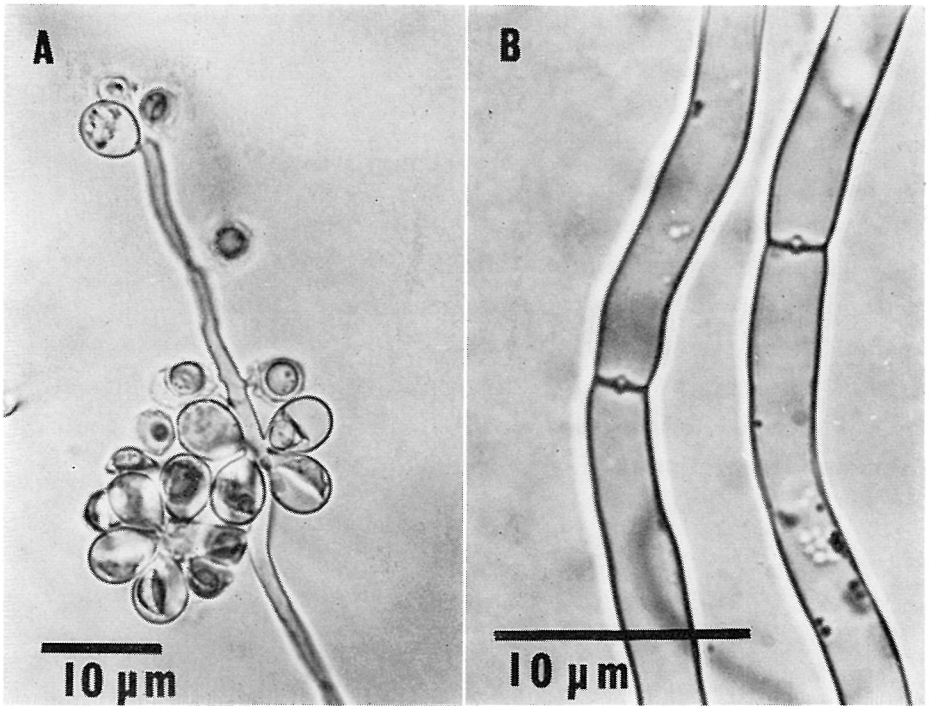

Fig. 1. *Ambrosiozyma monospora* IFO 4841, (A) Asci containing ascospore and (B) hypha with doliporesepta. Dalmau plate culture on corn meal agar after 7 days at 24 C.

Formation of ascospores on corn meal agar and YM agar: The asci are produced singly or in cluster at the tip of ascophore-like side branches and alongside stem-hyphae. (Fig. 1A) The spherical to oval asci are fairly larger than blastospores. One (rarely two) hat-shaped spore is formed per ascus. Most spores are released from the asci. The presence of many spores colors the culture brown.

Fermentation: Only glucose is fermented.

Assimilation of carbon compounds:

Glucose	+	D-Ribose	+
Galactose	−	L-Rhamnose	−
L-Sorbose	−	Ethanol	+
Sucrose	+	Glycerol	+
Maltose	+	Erythritol	+
Cellobiose	+	Ribitol	+
Trehalose	+	Galactitol	−
Lactose	−	D-Mannitol	+
Melibiose	−	D-Glucitol	+
Raffinose	−	α-Methyl-D-glucoside	+
Melezitose	+	Salicin	+
Inulin	−	DL-Lactic acid	+(weak)
Soluble starch	−	Succinic acid	+
D-Xylose	+	Citric acid	+(weak)
L-Arabinose	+	Inositol	−
D-Arabinose	−		

Splitting of arbutin: Positive.

Assimilation of nitrogen compounds:

Potassium nitrate; Absent.

Ethylamine hydrochloride; Positive.

Growth in vitamin-free medium: Absent.

Growth on 50% (w/w) glucose-yeast extract agar: Absent.

Growth at 37 C: Positive.

The strain was obtained from H. Naganishi in 1946 under a name of *Chalara mycoderma*. The characteristics obtained by careful reexamination coincide exactly with the standard description of *A. monospora*.

[K. Mikata & I. Banno]

IFO Res. Comm. **11**,
81-84, 1983 (March)

DESCRIPTIVE CATALOGUE OF IFO BACTERIAL COLLECTION VI.

The purpose of this catalogue is to describe the taxonomic properties of strains which had been misidentified or published invalidly but have since been reclassified in the routine work of reidentification of the IFO bacterial collection. Below, the descriptions of strains are arranged in alphabetical order of the scientific name, and the authors are given in brackets.

66. Lactobacillus helveticus (Orla-Jensen) Bergey et al. 1925
IFO 3809

This strain was obtained under the name of *Lactobacillus bulgaricus* (Orla-Jensen) Rogosa and Hansen, with strain number Sherman 09. It has been employed for assay of orotic acid.* It was identified as *Lactobacillus helveticus* by the following properties:
Cells: Gram-positive rods; non-motile; no spore formation.
Catalase and oxidase are not produced.
Facultatively anaerobic, but prefers anaerobic condition.
Initial pH for growth: Grows between pH 3.8 and pH 5.9, but not between pH 6.6 and pH 7.4.
Temperature relations: Grows between 30 C and 42 C, but not at 20 C or 48 C.
Acid from galactose, glucose, lactose and mannose, weakly from fructose, ribose and sucrose, but not from arabinose, cellobiose, esculin, gluconate, maltose, mannitol, melezitose, melibiose, raffinose, rhamnose, salicin, sorbitol, trehalose and xylose.
No gas from glucose or gluconate.
Homofermentative.
Both L(+)- and D(−)-lactic acids are produced from glucose.
The G+C content of the DNA is 37.0 mol% (Tm).
Orotic acid is required for growth.

[T. Sakane]

67. Leuconostoc lactis Gravie 1960 (Fig. 1)
IFO 12455

This strain was obtained under the name of *Lactobacillus batatas* Kitahara. The bacterial name *Lactobacillus batatas* was not accepted by the Judicial Commision of International Committee on Systematic Bacteriology.** The characters of IFO 12455 agreed with those of the genus *Leuconostoc* described in Bergey's Manual of Determina-

* Wieland, O.P., J. Avener, E.M. Boggiano, N. Bohonos, B.L. Hutchings and J.H. Williams. 1950. J. Biol. Chem. **186**: 737.
** Skerman, V.B.D., V. McGowan and P.H.A. Sneath. 1980. Int. J. Syst. Bacteriol. **30**: 225.

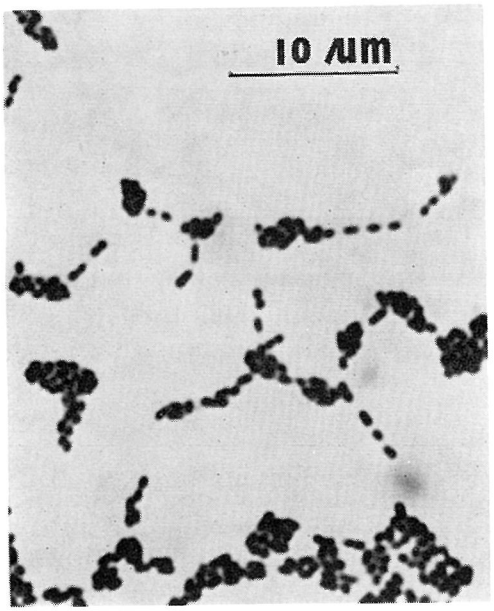

Fig. 1.

tive Bacteriology, 8th edition,* and IFO 12455 was identified as *Leuconostoc lactis* by the following taxonomic properties:

Cells: Cococid to short rods, 0.5 to 0.6 by 0.6 to 1.0 μm, occurring singly, in pairs and short chained (Fig. 1); gram-positive; non-motile; no spore formation.

Agar colonies: Circular, entire, low-convex, smooth, gray.

Facultatively anaerobic.

Catalase and oxidase are not produced.

No dextran is produced from sucrose.

Citrate is not utilized as carbon source.

Temperature relations: Grows between 9 C and 37 C, but not at 45 C.

Initial pH for growth: Grows between pH 5.0 and pH 7.4, but not at pH 3.8.

Growth in NaCl broth: Grows in 4% NaCl, but not in 6% NaCl.

Acid from fructose, galactose, glucose, maltose, melibiose, raffinose, salicin, sucrose and xylose, but not from arabinose, cellobiose, esculin, gluconate, lactose, mannose, mannitol, melezitose, rhamnose, ribose, sorbitol and trehalose.

Heterofermentative.

D(−)-Lactic acid is produced from glucose.

Growth factor requirements: Requires niacin, riboflavin and thiamine; does not require folic acid and pyridoxal.

The G+C content of the DNA is 42.2 mol% (Tm).

[T. Sakane]

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* Garvie, E.I. 1974. Bergey's Manual of Determinative Bacteriology, 8th edition, ed. by R.E Buchanan and N.E. Gibbons, The Williams and Wilkins Co., Baltimore, p. 510.

68. Pediococcus damunosus Claussen 1903
IFO 12220

This strain was obtained under the name of *Gaffkya homari* Hitchner and Snieszko. In Bergey's Manual of Determinative Bacteriology, 8th edition, *G. homari* was treated as a subjective synonym of *Aerococcus viridans* Williams et al.,* but strain IFO 12220 was identified as *Pediococcus damunosus* by the following taxonomic properties:
Cells: Spherical, 0.8 to 1.0 μm in diameter, occurring in pairs, tetrads and irregular clusters; gram-positive; non-motile; no spore formation. Facultatively anaerobic, but prefers anaerobic condition.
Catalase and oxidase are not produced.
Nitrate is not reduced to nitrite.
Gelatin and Tween 80 are not hydrolyzed.
Initial pH for growth: Grows at pH 5.0, but not between pH 7.0 and pH 9.0.
Temperature relations: Grows between 16 C and 30 C, but not 10 C or 37 C.
Acid from fructose, galactose, glucose, mannose and sucrose, but not from arabinose, lactose, maltose, mannitol, sorbitol and xylose.
No gas from glucose.
Homofermentative.
Both L(+)- and D(−)-lactic acids are produced from glucose.

[T. Sakane]

69 and **70. Pseudomonas diminuta** Leifson and Hugh 1954
IFO 13181 and 13182

Both strains had been received under the name of *Achromobacter parvulus* (Conn) Breed, but were reidentified as *Pseudomonas diminuta* by the following properties.

	IFO 13181	IFO 13182
Gram-reaction	negative	negative
Cells: form	rods	rods
size	0.7–1.0 × 0.4 μm	0.7–1.3 × 0.4 μm
Colony color	none	none
Motility	positive	positive
flagellation	single polar flagellum with very short wave length	single polar flagellum with very short wave length
Hugh-Leifson's OF test	oxidative	oxidative
Catalase	strong positive	strong positive
Oxidase	strong positive	strong positive
Urease	negative	negative

* Evans, J.B. 1974. Bergey's Manual of Determinative Bacteriology, 8th edition, ed. by R.E. Buchanan and N.E. Gibbons, The Williams and Wilkins Co., Baltimore. p. 516.

(continued)

	IFO 13181	IFO 13182
Denitrification	negative	negative
Reduction of NO_3 to NO_2	negative	negative
Methyl red test	negative	negative
Voges-Proskauer test	positive	weakly positive
pH in V.P. broth	8.0	8.0
Production of indol	negative	negative
Decarboxylation of arginine	strong positive	negative
lysine	weakly positive	positive
ornithine	positive	positive
Oxidation of gluconate	negative	negative
malonate	negative	negative
Phenylalanine deamination	negative	negative
ONPG test	negative	negative
Decomposition of caseine	positive	positive
tyrosine	positive	positive
Tween 80	strong positive	strong positive
esculine	negative	negative
Hydrolysis of starch	negative	negative
hippurate	positive	positive
cellulose	negative	negative
Gelatin liquefaction	negative	negative
Citrate utilization	negative	negative
Litmus milk: change of pH	alkaline	alkaline
reduction of litmus	positive	positive
coagulation	negative	negative
serum	negative	negative
3-Ketoglucoside test	negative	negative
Aniline blue absorption	negative	negative
Utilization of glucose as carbon source	negative	negative
Requirement for growth	biotin, cystine, pantothenate	biotin, cystine, pantothenate

[M. Takeuchi]

ANNOUNCEMENTS

ACTIVITIES OF ACTINOMYCETES COLLECTION IN IFO

The Institute for Fermentation, Osaka (IFO) has collected cultures of Actinomycetes since 1949. Until 1977, the number of cultures accepted every year averaged about twenty, excluding the 454 ISP (International Streptomyces Project) strains deposited separately in 1969 and 1973. During the three years from 1978, the number of cultures deposited in each year increased to almost 80, because type strains were deposited in IFO in line with a requirment for such strains to be deposited in an authorized culture collection by 1980 in order to gain approval of the species name. Figure 1 shows the number of strains deposited every year and the cumulative total.

Year	Yearly No.	Cumulative total
1949	5	5
1950	2	7
1951	0	7
1952	26	33
1953	25	58
1954	21	79
1955	48	127
1956	0	127
1957	7	134
1958	25	159
1959	3	162
1960	14	176
1961	20	196
1962	0	196
1963	0	196
1964	18	214
1965	24	238
1966	43	281
1967	34	315
1968	17	332
1969	301	633
1970	35	668
1971	16	684
1972	18	702
1973	171	873
1974	12	885
1975	29	914
1976	9	923
1977	18	941
1978	98	1039
1979	75	1114
1980	88	1202
1981	25	1227
1982	17	1244

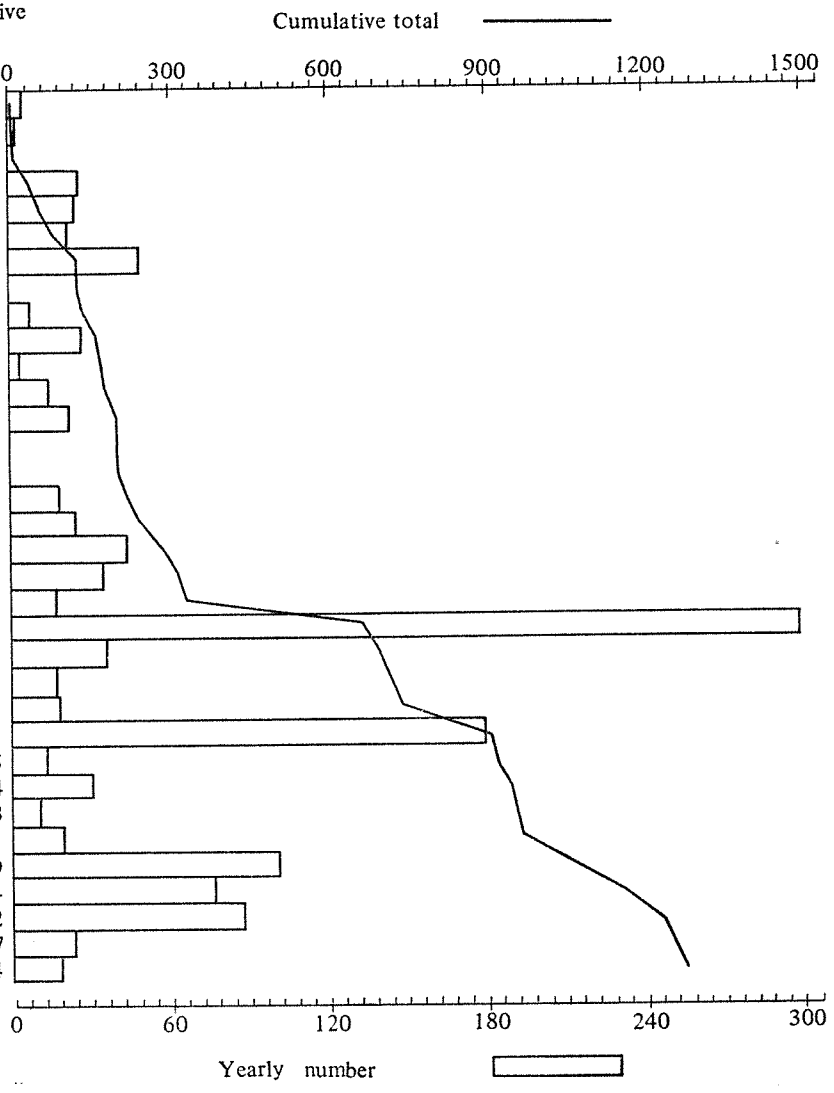

Fig. 1. Changes in number of Actinomycetes deposited in IFO. (Oct. 1, 1982)

Table 1. Number of Actinomycetes deposited, grouped by genus. (Oct. 1, 1982)

Genus	Preserved in IFO			Species of Approved Lists	Species in Bergey's Manual (8 ed.)
	Species	Strain	Species of Approved Lists		
Actinobifida	1	1	0	0	3
Actinomadura	12	13	7	24	0
Actinomyces	25	25	0	6	5
Actinoplanes	23	27	7	9	4
Actinopolyspora	1	1	1	1	0
Actinopycnidium	1	1	1	1	0
Actinosynnema	1	1	1	1	0
Agromyces	1	2	1	1	0
Amorphosporangium	2	2	2	2	1
Ampullariella	8	8	4	4	4
Catenuloplanes	1	2	0	0	0
Chainia	2	3	11	12	0
Dactylosporangium	5	5	2	2	2
Dermatophilus	1	1	1	1	1
Elytrosporangium	1	1	1	3	0
Geodermatophilus	4	4	1	1	1
Intrasporangium	1	1	1	1	0
Jensenia	1	1	0	0	0
Kineosporia	1	2	1	1	0
Kitasatoa	5	5	4	4	4
Microbispora	10	12	8	10	9
Microellobosporia	4	4	4	4	4
Micromonospora	27	32	13	19	16
Micropolyspora	4	5	4	5	8
Microtetraspora	2	2	1	4	0
Nocardia	9	16	5	20	31
Nocardioides	3	5	1	1	0
Nocardiopsis	3	3	1	1	0
Oerskovia	1	1	1	2	0
Pilimelia	1	1	1	2	2
Planobispora	1	2	1	2	2
Planomonospora	1	1	1	4	2
Promicromonospora	1	1	1	1	0
Pseudonocardia	2	2	1	2	2
Rhodococcus	2	6	2	10	0
Rothia	1	2	1	1	1
Saccharomonospora	1	1	1	1	0
Saccharopolyspora	1	1	1	1	0
Spirillospora	1	1	1	1	1
Sporichthya	1	1	1	1	1
Streptoalloteichus	1	1	0	0	0
Streptomyces	527	753	321	376	463
Streptosporangium	14	16	12	15	11
Streptoverticillium	47	65	33	43	40
Thermoactinomyces	5	8	3	5	2
Thermomonospora	4	5	3	4	2
Thermopolyspora	1	1	0	0	0
Total	772	1054	468	609	622

Of the 1244 strains, 84 have been withdrawn because of death or deterioration and 104 (including patented strains) are restricted in their distribution. The remaining 1054 strains are freely distributable and include 230 antibiotic producing strains such as *Streptomyces kasugaensis* and *Streptomyces aureofaciens*.

These 1054 strains are listed in taxonomical groups in Table 1, which gives a similar breakdown of the species of Actinomycetes in the Approved Lists of Bacterial Names (Int. J. Syst. Bacteriol. 30: 225 (1980)) and the names of species adopted in Bergey's Manual (8 ed.). The IFO strains cover 47 genera, including 21 not found in Bergey's Manual (8 ed.). The number of species represented, 772, almost 150 more than the number listed in Bergey's Manual (8 ed.) and they consist of 468 among 609 species of Actinomycetes in the Approved Lists and 304 species not found in this list. It should be mentioned that the IFO stock cultures include ISP strains (453 strains checked by the Society for Actinomycetes, Japan every 4 years) and holotypes (80 species) which are used as references for identification of unknown strains.

Distribution of cultures is one of the most important activities of IFO. For example, about 500 subcultures were distributed in 1981 to research institute, universities, firms etc. not only in Japan but also abroad. A related activity is the accumulation of test results for all distributable strains, which can be supplied on request to the recipients of subcultures. Details of the eight tests carried out in 1981–1982 are given in Table 2. Growth under different conditions of temperature, pH, oxygen content, and hypertonicity, growth in liquid medium, antimicrobial activity estimated by cross-streak method, and enzymatic activities (cellulase and proteinase) are all expressed by numerical indices, which are recorded in a computor and can be recovered promptly when a subculture is dispatched. The computor system is set up so that strains with desired characteristics can be located quickly. If a request is received, for example, for a strain which produces an anti-yeast substance and can grow in conditions in which most microorganisms can not, it requires only about five minutes for the computor to come up with the strain IFO 12177, which grows well at 45 C and pH 8.5 and shows antimicrobial activity to yeast. If a strain is required that produces a useful substance

Table 2. Informations about Actinomycetes strains in IFO. (Oct. 1, 1982)

Test		Checked items
T–1)	Temperature;	17 C, 28 C, 37 C, 45 C.
T–2)	Hypertonic;	growth on the medium containing 12% of carbon source.
T–3)	Antimicrobial activity; (cross streak method)	gram-positive, -negative bacteria, yeast, fungus.
T–4)	Observation of liquid culture;	pellet formation, segmented growth.
T–5)	Oxygen requirement;	aerobic, microaerophile, etc.
T–6)	Cellulase activity;	growth on filter paper.
T–7)	Proteinase activity;	gelatin liquefaction.
T–8)	pH condition;	pH 4.5, pH 7.4, pH 8.5.

Table 3. Examples of use of computer-stored information to locate useful strains

ID	IFO	Med	T-1				T2	T-3					T4	T5	T6	T7	T-8		
			17	28	37	45		B	St	Co	Ca	Pen		O2	Ce	Ge	45	74	85
	Ex-1 grows at 45 C and pH 8.5, inhibits growth of yeast.																		
185	12177	231	3	4	4	4	0	2	2	2	2	1	4	3	0	0	5	4	4
	Ex-2 microaerophilic, grows on hypertonic medium at 37 C.																		
785	13488	231	3	4	4	4	4	2	2	0	1	1	2	2	0	1	1	5	1
	Ex-3 inhibits growth of fungi and shows segmented growth at 45 C. No strain was found.																		
	Ex-4 inhibits growth of both gram-positive bacteria and fungus and grows on hypertonic medium.																		
25	3177	231	2	4	1	0	4	2	2	2	1	2	2	3	0	0	3	3	3
48	3354	231	3	4	3	0	4	2	2	2	1	2	3	3	0	1	5	3	3
57	3363	231	4	4	0	0	4	2	2	2	1	2	3	2	0	0	3	3	3
73	3400	231	3	4	4	0	4	2	2	1	2	2	3	3	0	0	1	1	1
	Eleven strains in total were found.																		

in large-scale fermentation, the computor will come up with the strain IFO 13488, which is microaerophilic and grows well on hypertonic medium at 37 C. The selected cultures are to be sent to the user who requested the search. These two examples are shown with two others in Table 3. Actinomycetes section is preparing to expand the number of items tested.

In sum, the Actinomycetes collection in IFO is expected to serve not only as a type culture collection but also source of strains that produce useful substances. The collection will also help guard the security of private strains which are deposited under contract with depositor.

Taiki KUSAKA

CATALOGUE OF NEWLY ACCEPTED STRAINS

Sep. 1980—Sep. 1982

(ALPHABETICAL)

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

YEASTS

IFO No.	NAME	MATING TYPE
1965	Ambrosiozyma monospora	
1966	Candida bogoriensis	
1967	Candida boidinii	
10035	Candida boidinii	
1969	Candida diddensii	
1970	Candida diddensii	
1971	Candida diddensii	
1972	Candida guilliermondii var. carpophila	
1973	Candida hydrocarbofumaria	
10057	Candida ingens	
1974	Candida langeronii	
1975	Candida maltosa	
1976	Candida maltosa	
1977	Candida maltosa	
1978	Candida maltosa	
1979	Candida marina	
1980	Candida oregonensis	
1981	Candida sake	
1982	Candida santamariae	
1983	Candida shehatae	
10058	Clavispora lusitaniae	a
10059	Clavispora lusitaniae	a
10013	Cryptococcus cereanus	
10028	Debaryomyces formicarius	
1984	Dipodascus albidus	
1986	Hyphopichia burtonii	
10005	Kluyveromyces marxianus	
1985	Kluyveromyces thermotolerans	
10029	Nadsonia commutata	
10030	Nadsonia commutata	
10014	Pichia amethionina var. amethionina	
10015	Pichia amethionina var. pachycereana	
10016	Pichia angophorae	
10017	Pichia cactophila	
1987	Pichia etchellsii	
10018	Pichia heedii	
10019	Pichia heedii	a
10020	Pichia heedii	alpha
10060	Pichia humboldtii	
1988	Pichia kluyveri	
10062	Pichia membranaefaciens	

10021	Pichia opuntiae var. opuntiae	h+
10022	Pichia opuntiae var. opuntiae	h-
10023	Pichia opuntiae var. opuntiae	h-
10024	Pichia opuntiae var. thermotolerans	h+
10025	Pichia opuntiae var. thermotolerans	h-
10026	Pichia opuntiae var. thermotolerans	h-
10061	Pichia sorbitophila	
10006	Pichia stipitis	
10007	Pichia stipitis	
10063	Pichia stipitis	
1989	Pichia vini var. melibiosi	
10064	Pityrosporum pachydermatis	
10032	Rhodosporidium toruloides	A
10033	Rhodosporidium toruloides	A
10034	Rhodosporidium toruloides	a
10052	Rhodotorula acheniorum	
10053	Rhodotorula araucariae	
10054	Rhodotorula araucariae	
1990	Saccharomyces amurcae	
1991	Saccharomyces capensis	
1992	Saccharomyces castellii	
10055	Saccharomyces cerevisiae	
10008	Saccharomyces dairensis	
10009	Saccharomyces dairensis	
1993	Saccharomyces eupagycus	
1994	Saccharomyces hienipiensis	
1995	Saccharomyces hispanica	
1996	Saccharomyces montanus	
1997	Saccharomyces oleaceus	
1998	Saccharomyces oleaginosus	
10010	Saccharomyces uvarum	
10011	Saccharomyces uvarum	
10012	Saccharomyces uvarum	
10036	Saccharomycodes ludwigii	
1999	Sterigmatomyces elviae	
10031	Torulopsis apis var. galacta	
10037	Torulopsis etchellsii	
10001	Torulopsis haemulonii	
10002	Torulopsis ingeniosa	
10003	Torulopsis maris	
10004	Torulopsis nitratophila	
10027	Torulopsis sonorensis	
10038	Torulopsis versatilis	
10056	Torulopsis versatilis	

BACTERIA & ACTINOMYCETES

14182	Actinomadura cremea
14183	Actinomadura cremea subsp. rifamycini
14094	Actinomadura ferruginea
14095	Actinomadura libanotica
14096	Actinomadura libanotica
14102	Actinomadura macra
14098	Actinomadura roseoviolacea
14099	Actinomadura spadix

14097	Actinomadura spiralis
14100	Actinomadura verrucosospora
14106	Actinopolyspora halophila
14130	Alcaligenes sp.
14175	Bacillus alvei
14141	Bacillus amyloliquefaciens
14206	Bacillus licheniformis
14117	Bacillus subtilis
14132	Bacillus subtilis
14133	Bacillus subtilis
14140	Bacillus subtilis
14144	Bacillus subtilis
14191	Bacillus subtilis
14192	Bacillus subtilis
14176	Catenuloplanes japonicus
14177	Catenuloplanes japonicus
14170	Cytophaga marinoflava
14103	Dactylosporangium salmoneum
14104	Dactylosporangium variesporum
14181	Dactylosporangium vinaceum
14126	Erythrobacter longus
14129	Escherichia coli
14195	Escherichia coli
14196	Escherichia coli
14197	Escherichia coli
14180	Hyphomicrobium methylovorum
14136	Microbacterium flavum
14135	Microbacterium lacticum
14137	Microbacterium lacticum
14138	Microbacterium lacticum
14107	Micromonospora carbonacea subsp. aurantiaca
14108	Micromonospora carbonacea supsp. carbonacea
14109	Micromonospora echinospora subsp. ferruginea
14110	Micromonospora echinospora subsp. pallida
14111	Micromonospora grisea
14112	Micromonospora halophytica subsp. halophytica
14113	Micromonospora megalomicea subsp. megalomicea
14114	Micromonospora megalomicea subsp. nigra
14115	Micromonospora rhodorangea
14116	Micromonospora zionensis
14118	Mycobacterium vaccae
14198	Nocardiopsis atra
14201	Nocardiopsis trehalosei
14169	Photobacterium mandapamensis
14159	Pseudomonas alcaligenes
14160	Pseudomonas fluorescens
14161	Pseudomonas maltophilia
14162	Pseudomonas mendocina
14163	Pseudomonas pertucinogena
14167	Pseudomonas pseudoalcaligenes
14164	Pseudomonas putida
14165	Pseudomonas stutzeri
14105	Pseudonocardia fastidiosa
14168	Rhizobium leguminosarum
14193	Salmonella typhimurium

14194	Salmonella typhimurium
14209	Salmonella typhimurium
14210	Salmonella typhimurium
14211	Salmonella typhimurium
14212	Salmonella typhimurium
14147	Streptomyces albulus
14148	Streptomyces fulvoviolaceus
14179	Thermomonospora mesophila
14178	Thermomonospora mesouviformis

FUNGI

31139	Acremonium fusidioides
31140	Acremonium fusidioides
31141	Acremonium fusidioides
31196	Acremonium potronii
31197	Acremonium potronii
31198	Acremonium potronii
31199	Acremonium potronii
31155	Acrophialophora levis
31156	Acrophialophora levis
31157	Acrophialophora levis
31158	Acrophialophora nainiana
31099	Agaricus campestris
31100	Agaricus campestris
31188	Alternaria alternata
31189	Alternaria alternata
31226	Alternaria brassicicola
31227	Alternaria brassicicola
31182	Alternaria steviae
31183	Alternaria steviae
31184	Alternaria steviae
31185	Alternaria steviae
31212	Alternaria steviae
31170	Apiosordaria yaeyamensis
31166	Armillariella mellea
31098	Arthrinium japonicum
31125	Aspergillus niger
31221	Aspergillus ochraceus
31222	Aspergillus sydowii
31217	Aspergillus terreus var. aureus
31223	Aspergillus versicolor
31172	Bipolaris coicis
31173	Bipolaris coicis
31174	Bipolaris coicis
31073	Calvatia craniiformis
31066	Chaetomium longirostre
31065	Coemansia erecta
31200	Conioscypha varia
31201	Conioscypha varia
31202	Conioscypha varia
31203	Conioscypha varia
31204	Conioscypha varia
31205	Conioscypha varia

31206	Conioscypha varia	
31162	Coriolus elongatus	
31215	Corticium rolfsii	
31163	Daedalea dickinsii	
31095	Fusarium equiseti	
31096	Fusarium fusarioides	
31224	Fusarium oxysporum f. sp. cucumerinum	
31180	Fusarium oxysporum f. sp. fragariae	
31213	Fusarium oxysporum f. sp. lycopersici	
31097	Fusarium semitectum	
31093	Fusarium solani	
31094	Fusarium solani var. coeruleum	
31147	Ganoderma applanatum	
31225	Gliocladium catenulatum	
31164	Grifola albicans	
31228	Hebeloma vinosophyllum	
31229	Hebeloma vinosophyllum	
31230	Hebeloma vinosophyllum	
31231	Hebeloma vinosophyllum	
31123	Hygrophoropsis aurantiaca	
31160	Isaria atypicola	
31161	Isaria japonica	
31059	Lasiodiplodia theobromae	
31107	Lentinus edodes	
31108	Lentinus edodes	
31109	Lentinus edodes	
31110	Lentinus edodes	
31111	Lentinus edodes	
31112	Lentinus edodes	
31113	Lentinus edodes	
31114	Lentinus edodes	
31115	Lentinus edodes	A1B1
31116	Lentinus edodes	A1B1
31117	Lentinus edodes	A1B2
31118	Lentinus edodes	A1B2
31119	Lentinus edodes	A2B1
31120	Lentinus edodes	A2B1
31121	Lentinus edodes	A2B2
31122	Lentinus edodes	A1BX
31167	Lyophyllum decastes	
31138	Monochaetia monochaeta	
31216	Oudemansiella canarii	
31168	Panellus stypticus	
31071	Penicillium argillaceum	
31128	Penicillium argillaceum	
31148	Penicillium argillaceum	
31072	Penicillium cylindrosporum	
31129	Penicillium cylindrosporum	
31132	Penicillium funiculosum	
31133	Penicillium janthinellum	
31134	Penicillium janthinellum	
31149	Penicillium namyslowskii	
31135	Penicillium piceum	
31130	Penicillium putterillii	
31131	Penicillium putterillii	

31136	Penicillium verruculosum	
31067	Petriella setifera	
31090	Physalospora piricola	
31091	Physalospora piricola	
31092	Physalospora piricola	
31143	Physalospora piricola	
31144	Physalospora piricola	
31145	Physalospora piricola	
31074	Pleurotus cystidiosus	
31075	Pleurotus cystidiosus	
31076	Punctularia atropurpurascens	
31077	Punctularia atropurpurascens	
31165	Pycnoporus cinnabarinus	
31175	Pyricularia oryzae	A
31176	Pyricularia oryzae	A
31177	Pyricularia oryzae	a
31178	Pyricularia oryzae	a
31214	Pythium butleri	
31219	Rollandina capitata	
31207	Sagenomella oligospora	
31208	Sagenomella oligospora	
31209	Sagenomella oligospora	
31210	Sagenomella oligospora	
31211	Sagenomella oligospora	
31146	Scedosporium apiospermum	
31181	Septoria steviae	
31190	Septoria steviae	
31124	Suillus tomentosus	
31218	Synnematomyces capitata	
31069	Talaromyces bacillisporus	
31150	Talaromyces bacillisporus	
31070	Talaromyces emersonii	
31126	Talaromyces emersonii	
31127	Talaromyces emersonii	
31159	Talaromyces emersonii	
31169	Talaromyces emersonii	
31191	Talaromyces leycettanus	
31192	Talaromyces leycettanus	
31193	Talaromyces leycettanus	
31194	Talaromyces leycettanus	
31195	Talaromyces leycettanus	
31060	Thielavia arenaria	
31061	Thielavia arenaria	
31142	Thielavia arenaria	
31062	Thielavia kuwaitensis	
31063	Thielavia minuta	
31137	Trichoderma viride	
31068	Trichophyton concentricum	
31064	Trichophyton violaceum	
31179	Verticillium psalliotae	
31104	Volvariella volvacea	
31220	Westerdykella dispersa	

The List of Deleted Strains from 6th Edition of *IFO List of Cultures*

YEASTS	IFO No.
Candida krusei	0839
Candida krusei	0840
Candida mogii	0437
Candida pseudotropicalis	0617
Candida rugosa	1542
Cryptococcus neoformans	0410
Cryptococcus neoformans	0545
Cryptococcus neoformans	0608
Cryptococcus neoformans	0693
Cryptococcus neoformans	0819
Cryptococcus neoformans	0875
Cryptococcus neoformans	1318
Cryptococcus neoformans	1319
Cryptococcus neoformans	1420
Hansenula suturnus	0810
Rhodotorula glutinis	0898
Rhodotorula marina	1421
Rhodotorula marina	1432
Rhodotorula rubra	0471
Saccharomyces cerevisiae	1136
Sporobolomyces roseus	1105
Torulopsis candida	0863
Torulopsis dattila	0876
BACTERIA	
Clostridium butyricum	3852
Lactobacillus acidophilus	3205
Lactobacillus lactis	12522
Leuconostoc dextranicum	3347
Leuconostoc mesenteroides	12454
Microtetraspora glauca	13916
Pediococcus acidilactici	3884
Pseudomonas aeruginosa	12045
Streptococcus salivarius	3350
Streptococcus thermophilus	3535
Thermomonospora fusca	14049
Thiobacillus thiooxidans	12544
BACTERIOPHAGE	
Bacteriophage l	20022
FUNGI	
Chaetomium subspirale	6592
Choanephora conjuncta	8089
Choanephora trispora	6333

Coccidioides immitis	5960
Coprinus phlyctidosporus	30326
Helotium populinum	9641
Humicola insolens	9737
Linderia bicolumnata	30140
Myxotrichum cancellatum	8950
Onnia orientalis	4935
Phytophthora melonis	9754
Pseudogymnoascus caucasicus	9128
Sporendonema purpurascens	9190
Stagnospora arenaria	7282
Stereum fasciatum	4994
Talaromyces helicus var. helicus	7993
Thielavia terricola	5815

ABSTRACTS 1981-1982

A new disease of sudangrass caused by *Curvularia lunata* and *C. intermedia*

Y. KOMOTO*, N. NISHIHARA** and T. YOKOYAMA

Bull. Chugoku Nat. Agr. Exp. St. Ser. E **17**: 1–15 (1980)

A new leaf spot disease was found on the leaves of Greenleaf, a variety of sudangrass, in Fukuyama, Hiroshima. The isolation of the causal pathogens from diseased plants revealed that two species of fungi belonging to the genus *Curvularia* were virulent to several varieties of sudangrass. From the morphological characteristics of these fungi, one species was identified as *Curvularia lunata* (Wakker) Boedijn and the other species was identified as *C. intermedia* Boedijn, respectively. A new name "*Curvularia* leaf spot disease" was proposed for the disease of sudangrass which was caused by single infection or double infection of these two fungi.

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[in Japanese]

Hyphomycetes from Korean soil. I. The genus *Penicillium* with a teleomorphic state *Eupenicillium javanicum*

K.H. MIN*, S.W. HONG** and T. YOKOYAMA

Korean J. Microbiol. **18**: 91–103 (1980)

A mycological survey was carried out with the soil samples collected in Korea from September, 1978 to December, 1979. Special attention was paid to the fungus genus *Penicillium*. One hundred twenty three isolates, as a result, were obtained from the Korean soils. Among these, sixteen species were identified and described in this paper. Almost all of the fungi reported here are new to Korea. One of them is an ascomycete fungus, *Eupenicillium javanicum* van Beyma which produces abundant pale yellow cleistothecia of 120–150 μm in diam. Ascospores of this fungus were found lenticular with an equatorial furrow as indicated in the previous descriptions.

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Hyphomycetes from Korean soil. II. The genus *Aspergillus* and some other microfungi

K.H. MIN*, S.W. HONG** and T. YOKOYAMA

Korean J. Microbiol. **18**: 104–114 (1980)

Fourteen species of the hyphomyceteous fungi isolated from Korean soils were described. Among these, one species has a teleomorphic state and was identified as *Emericella nidulans* var. *nidulans*, similar to *E. spectabilis* with the exception of size of the conidiophores as well as color and the arrangement of the hülle cells. Four species of the hyphomyceteous fungi, *Chrysosporium pannorum, Doratomyces microsporus, Trichoderma koningii, T. viride,* were reported here for the first time in Korea.

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On the genus *Dendrosphaera* and its conidial state

Y. KOBAYASI* and T. YOKOYAMA

Bull. Nat. Sci. Mus., Tokyo, Ser. B **7**: 15–22 (1981)

Anamorphic state of *Dendrosphaera eberhardtii* Patouillard was found in ascosporic cultures from the fruitbodies which were collected in Iriomote Is., Okinawa. The fungus belongs to *Penicillium herquei* series and a new name of taxon for the conidial state, *Penicillium eberhardtii* Yokoyama, st. nov., was proposed. Sporodochium-bearing stroma of this fungus were also collected in Yakushima Is., Kagoshima, which were covered with the phialides and conidia identical with the ascosporic cultures.

* Department of Botany, National Science Museum, Tokyo.

Properties of the lactose transport system in *Klebsiella* sp. strain CT-I

K. IMAI and B.G. HALL*

J. Bacteriol. **145**: 1459–1462 (1981)

Highly purified [D-glucose-1-^{14}C]lactose has been used to study the transport of lactose by *Klebsiella* sp. strain CT-1. Strain CT-1 transports lactose by a lactose-inducible system that exhibited an apparent K_m of 6 mM lactose and an apparent V_{max} of 140 nmol/min per mg of cell protein. Lactose uptake was inhibited competitively by *o*-nitrophenyl-β-D-galactoside with a K_i value of 8 mM, but was not inhibited by thio-β-methyl-galactoside. D-Glucose, D-mannose, 2-deoxyglucose, and α-methyl-D-

glucoside also inhibited lactose uptake. Phosphoenolpyruvate-dependent hydrolysis of *o*-nitrophenyl-β-D-galactoside and lactose-dependent release of pyruvate from phosphoenolpyruvate by benzene-treated CT-1 cells showed that CT-1 transports lactose by a phosphoenolpyruvate:sugar phosphotransferase system. Correlations between the growth rate of CT-1 on lactose and properties of the transport system indicated that transport is the rate limiting step in utilization of lactose.

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Amino acid sequence of α_k substance, a mating pheromone of *Saccharomyces kluyveri*

Y. SATO*, A. SAKURAI*, N. TAKAHASHI*, Y.-M. HONG*, Y. SHIMONISHI*, C. KITADA**, M. FUJINO**, N. YANAGISHIMA*** and I. BANNO

Agric. Biol. Chem. **45**: 1531–1533 (1981)

The following amino acid sequence was proposed for α_k substance from results obtained by analysis of N-terminal amino acid, sequence analysis according to dausyl-edoman procedure, and FD mass spectrometric analysis:

X-His-Trp-Leu-Ser-Phe-Ser-Lys-Gly-Glx-Pro-Met(O)-Tyr-OH

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Compounds protecting L-dried cultures from mutation

T. SAKANE, I. BANNO and T. IIJIMA

Japan. J. Freez. Dry. **27**: 16–22 (1981)

A study was conducted to make L-dried cultures escape from mutation during preservation. A method was elaborated for selecting compounds which, when added to suspending medium, increase the survival value of L-dried cells of *Escherichia coli* B/r *recA*$^-$ as an indicator, and 59 various compounds were screened. Adonitol, AICA, cysteine, dithioerythritol, semicarbazide, sodium thioglycollate and thiourea were found to be remarkably effective and adenosine, cystine, cytidine, erythritol, glutathione, thiomalic acid, thymidine and urea to have weakly protective effect. In dried cells of *recA*$^+$ strains, adonitol, cysteine and thiourea not only increased survival value but also prevented induction of mutation. Especially thiourea completely precluded mutation at a concentration of 30 mM.

[in Japanese]

Change in the regulation of enzyme synthesis under catabolite repression in *Bacillus subtilis* pleiotropic mutant lacking transketolase

K. SASAJIMA and T. KUMADA

Agric. Biol. Chem. **45**: 2005–2012 (1981)

The regulation of enzyme synthesis has changed in *Bacillus subtilis* pleiotropic mutant lacking transketolase (*tkt*). The *tkt* mutant is hypersensitive to D-glucose repression of the synthesis of D-mannitol catabolic enzymes, such as D-mannitol-1-phosphate dehydrogenase and D-mannitol transport system. D-Gluconate, D-xylose and L-arabinose are also effectors for repression in the *tkt* mutant. In contrast, the synthesis of sorbitol catabolic enzymes, such as sorbitol permease and sorbitol dehydrogenase, are almost insensitive to D-glucose repression. These changes in the regulation of enzyme synthesis seem to be related to some defect in the cell surface structure of the *tkt* mutant by which other pleiotropic properties are also generated.

Derepressed syntheses of sporulation marker enzymes in a *Bacillus* species mutant

A. YOKOTA and K. SASAJIMA

Agric. Biol. Chem. **45**: 2417–2423 (1981)

Sporulation marker enzymes, D-glucose dehydrogenase and L-alanine dehydrogenase were synthesized derepressively by vegetative cells of a *Bacillus* species mutant which was isolated as an improved D-ribose producer. It was also elucidated by electron microscopy that no morphological change concerning sporulation took place during the course of the enzyme syntheses in the mutant strain. The presence of Mn^{2+} and Ca^{2+} in the medium was necessary for the morphological development of sporulation even in the mutant strain. The mechanism of derepressed enzyme syntheses is discussed in relation to regulation of sporulation.

Stepwise loss of metabolism of ε-aminocaproic acid cycric dimer in *Alcaligenes* species D-2

T. FUKUMURA*, M. TAKEUCHI and I. BANNO

Eur. J. Appl. Microbiol. Biotechnol. **14**: 120–124 (1982)

A bacterium which is able to utilize the cyclic dimer of ε-aminocaproic acid (ACA) as a sole source of carbon and nitrogen has been isolated, classified as a member of *Alcaligenes* and tentatively named D-2. The initial step of the ACA cyclic

dimer metabolism in D-2 may be composed of the following three reactions, which are catalyzed by specific enzymes: opening of the ACA cyclic dimer, splitting of the ACA linear dimer and transamination of ACA. By treatment with mitomycin C or ethidium bromide, 2–3% of the D-2 cells lost both ACA cyclic dimer-opening and ACA linear dimer-splitting activities. Slow growth of colonies of this variant strain on ACA agar medium kept at 12±3°C for 4 weeks resulted in the production of a new variant which had lost the ACA transaminase activity as well as the hydrolysis activities. When the parent strain (D-2) was grown slowly on ACA cyclic dimer agar medium in the same way, the ACA transaminase activity alone was lost by about 30% of the colonies. All the variants have been stable during 6 months of culture by successive transfer on agar media. These facts suggest that both the ACA cyclic dimer-opening enzyme and the ACA linear dimer-splitting enzyme are encoded by the same plasmid whereas the ACA transaminase is encoded by a second plasmid.

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Genetics of the lac-PTS system of *Klebsiella*

B.G. HALL*, K. IMAI and C.P. ROMANO*

Genet. Res., Camb. **39**: 287–302 (1982)

The isolation of a temperature sensitive *ptsI* mutant which fails to utilize lactose provides strong evidence that *Klebsiella* strain CT-1 utilizes lactose via a phosphoenol-pyruvate-dependent lactose-phosphotransferase system (PTS-lac). We designate this lactose utilization system *elu* for *evolved lactose utilization*. Analysis of a series of *Lac*$^-$ mutants identifies two genes, *eluA* and *eluB*, whose function is required for lactose utilization by this pathway. The functions specified by these genes are not known, but neither locus specifies the hydrolytic enzyme phospho-β-galactosidase. A mutant of CT-1, strain RPD-2, exhibits a half-maximal growth rate at a lactose concentration 40 fold lower than that of strain CT-1; and it has a K_m for lactose uptake that is 40 fold lower than that of strain CT-1. That mutation defines the locus *eluC*, which is assumed to specify the enzyme II(lac) of the PTS-lactose system. From the observations that (i) cellobiose induces the phospho-β-galactosidase enzyme, (ii) pregrowth in cellobiose dramatically reduces the growth lag when cells are shifted into lactose minimal medium, (iii) *eluB* mutants exhibit a growth lag when shifted into cellobiose minimal medium, and (iv) lactose induces a phospho-β-glucosidase enzyme; we speculate that the phospho-β-glucosidase enzyme is the same enzyme as the phospho-β-glucosidase that normally functions in cellobiose metabolism. We conclude that the original mutation that allowed CT-1 to utilize lactose was a regulatory mutation that permitted inducible expression of the *eluC* gene.

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Preservation of bacteria by L-drying; Improved media for drying and for rehydration

T. SAKANE and I. BANNO

Japan. J. Freez. Dry. **28**: 46–50 (1982)

The agents increasing survival of the seven strains sensitive to desiccation were examined on the medium for drying and for rehydration. Addition of adonitol and cysteine into the suspending medium and addition of $MgSO_4$ into the rehydration medium remarkably increased survival values of 6 strains which were sensitive to UV irradiation. Survival value of remaining one, which is insensitive to UV irradiation, was increased by addition of $MgSO_4$ to the rehydration medium but not by addition of adonitol and cysteine to the suspending medium.

[in Japanese]

Protection of cultures from mutation during preservation: Compounds protecting L-dried cultures from mutation

T. SAKANE, I. BANNO and T. IIJIMA

Japan. J. Freez. Dry. **28**: 77–82 (1982)

Eighty seven various compounds were examined for ability to protect L-dried cells of *Escherichia coli recA*$^-$ from damage of DNA during preservation. Thiol compounds, sugar alcohols, carbamide compounds, purine and pyrimidine ribonucleosides and AICA were found to have protective effect. These compounds were effective when added into the suspending medium for drying, whereas pretreatment or rehydration of the cells with these compounds showed no protection. Addition of these compounds together with both of sodium glutamate and phosphate to the suspending medium brought about the best result.

[in Japanese]

Sex pheromone of α mating type in the yeast *Saccharomyces kluyveri* and its synthetic analogues in relation to sex pheromones in *Saccharomyces cerevisiae* and *Hansenula wingei*

H. FUJIMURA*, N. YANAGISHIMA*, A. SAKURAI**,
C. KITADA***, M. FUJINO*** and I. BANNO

Arch. Microbiol. **132**: 225–229 (1982)

Three analogues of the peptidyl pheromone, α pheromone of *Saccharomyces kluyveri*, synthesized based on the amino acid sequence proposed by Sato et al. (Agric Biol. Chem. 45: 1531–1533, 1981) were tested for both shmoo-inducing and agglutinability-inducing actions. Purified natural α pheromone of the yeast showed the highest activity among the peptides tested. When methionine in the peptides was oxidized, the activity decreased significantly. α Pheromone of *S. kluyveri* induced sexual agglutinability in *a* cells of *Saccharomyces cerevisiae*, and shmoo in *a* cells of *S. cerevisiae* and *S. kluyveri*. *a* Pheromone of *S. kluyveri* had no agglutinability-inducing action on α cells of *S. cerevisiae*. *a* Cells of *S. kluyveri* inactivated only α pheromone of the same species, but *a* cells of *S. cerevisiae* inactivated α pheromones of both *S. cerevisiae* and *S. kluyveri*

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PRESENTATION OF PAPERS AT SCIENTIFIC MEETINGS 1981-1982

Author(s)	Title	Scientific Meeting
K. IMAI & B.G. HALL*1	Improved lactose transport system in a mutant of *Klebsiella* strain CT-1.	Annual Meeting of the American Society for Microbiology, Dallas, USA (March 1981)
K. KATO*2, K. HIROTA*2 & T. YOKOYAMA	On the *Ceratocystis* canker of fig-trees caused by *Ceratocystis fimbriata* Ellis & Halsted.	Phytopathological Society of Japan. Sendai (April, 1981)
H. NASU*3, S. FUJII*3 & T. YOKOYAMA	Studies on the ecology and control of the fly-spec disease of grapes. II. The causal pathogen of the fly-spec disease of grapes, Japanese persimon and apples.	Phytopathological Society of Japan. Sendai (April, 1981)
M. OCHIAI*4, T. SAKUMA*5 & T. YOKOYAMA	Occurrence of the apple stem rot disease caused by *Sclerotium rolfsii* Sacc.	Phytopathological Society of Japan. Sendai (April, 1981)
T. SAKANE, I. BANNO & T. IIJIMA	Compounds protecting L-dried cultures from mutation.	Japanese Society for Research of Freezing and Drying. Tokyo (April, 1981)
K. SASAJIMA & T. KUMADA	Cell surface change in the transketolase mutant of *Bacillus subtilis*: properties of revertant strains.	Agricultural Chemical Society of Japan. Kyoto (April, 1981)
T. ITO, T. YOKOYAMA & Y. HORIE*6	Japanese species of the genus *Acrophialophora* Edward.	Mycological Society of Japan. Niigata (May, 1981)
T. YOKOYAMA & K. MINAMIURA*7	*Ptychogaster rubescens* Boudier and *Rectipilus fasciculatus* (Persoon) Agerer, two basidiomycetes new to Japan.	Mycological Society of Japan. Niigata (May, 1981)
T. IIJIMA, T. SAKANE & I. BANNO	Preservation of bacteria by L-drying.	IVth International Congress of Culture Collection. Brno, CSSR (July, 1981)

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Author(s)	Title	Scientific Meeting
I. BANNO, T. SAKANE & T. IIJIMA	Preservation of bacterial and yeast cultures in Institute for Fermentation, Osaka.	4th International (Korea-Japan) Symposium on Industrial Fermentation. Seoul, Korea (September, 1981)
A. YOKOTA & K. SASAJIMA	Mechanism of formation of a new monosaccharide 1-deoxy-D-altro-heptulose in a transketolase mutant of *Bacillus pumilus*.	Agricultural Chemical Society of Japan. Gifu (October, 1981)
K. IMAI & B.G. HALL*1	Properties of the lactose-PTS in *Klebsiella* sp.	Society of Fermentation Technology, Japan. Osaka (November, 1981)
T. SAKANE, I. BANNO & T. IIJIMA	Compounds protecting L-dried cultures from mutation.	Seminar of Japanese Society for Research of Freezing and Drying. Tsukuba (November, 1981)
H. HONMA*2, H. YAEGASHI*3, T. YOKOYAMA & M. YAMADA*3	Three plant pathogenic fungi isolated from Job's tears seeds.	Phytopathological Society of Japan. Tokyo (April, 1982)
K. IMAI	Chemotaxis toward sodium chloride in *Photobacterium phosphoreum*.	Agricultural Chemical Society of Japan. Tokyo (April, 1982)
K. IMAI, M. TAKEUCHI & I. BANNO	Proper taxonomic positions of gram-positive *Flavobacterium* strains.	Agricultural Chemical Society of Japan. Tokyo (April, 1982)
T. SAKANE & I. BANNO	Preservation of bacteria by L-drying: Improved media for drying and for rehydration.	Japanese Society for Research of Freezing and Drying. Tokyo (April, 1982)
I. BANNO	Isolation of yeasts by enrichment method.	5th Yeast Symposia Japan. Tokyo (May, 1982)
K. MIKATA & I. BANNO	Unknown species of *Pichia* isolated from forest materials.	Mycological Society of Japan. Tokyo (May, 1982)

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*3 National Institute of Agricultural Sciences, Yatabe, Ibaraki.

CORRECTIONS

In the issue of IFO Research Communications No. 10, the following corrections should be made.

Page	Line	Type	Should read
5	3	autolytic-defective	autolysis-defective
7	15	Archbald	Archibald
8	6	Fein, E. F.	Fein, J. E.
12	last	therefore/be	therefore be
17	14	*S. pretriensis*	*S. pretoriensis*
31	2	frequencey	frequency
35	11	semi-premanently	semi-permanently
43	8	*Eremoascus*	*Eremascus*
76	30	Emericella arizonica	Emericella navahoensis

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