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Evaluation of Preservation Techniques of Microorganism Resources in the MAFF Genebank

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Abstract

In the central bank of the microorganisms section in the Ministry of Agriculture, Forestry and Fisheries (MAFF) Genebank in Tsukuba, 14,836 strains of fungi, bacteria, yeasts and others are preserved by various methods including cryopreservation and freeze-drying. To evaluate preservation techniques for these microorganisms, the viability of the preserved microorganisms was examined at regular intervals. Almost all tested strains, except for 5 strains out of 2,334 strains, of yeasts, bacteria and Actinomycetes were preserved well in freeze-dried form, indicating that freeze-drying is suitable for their preservation. As for fungi and Oomycetes, 6,578 strains out of 6,681 tested strains (98.5%) and 264 (63.8%) of 414, respectively, survived one-year preservation in the vapor phase of liquid nitrogen. The details on survival of the preserved fungal strains were as follows: 99.0% (1,107 strains/1,118 tested strains) survival in Ascomycota, 96.1% (1,552/1,615) in Basidiomycota, 98.6% (73/74) in Zygomycota, and 99.3% (3,846/3,874) in anamorphic fungi. Thus, cryopreservation is excellent for preservation of most fungus strains, but is not suitable for preservation of many Oomycetous strains.

Discipline: Genetic Resources
Additional key words: cryopreservation, culture collection, freeze-drying

Introduction

It is important that microorganism resources are preserved in a physiologically and genetically stable state. Therefore, frequent subculturing on a slant is not recommended. Subculturing may also lead to contamination. The major methods that give stable preservation are freeze-drying, L-drying (drying from the liquid state), cryopreservation (in the vapor phase of liquid nitrogen or in a deep freezer) and subculture under mineral oil[1,4,6,13]. The methods used for preservation depend on the microbial species. Freeze-drying is suitable for preservation of bacteria, Actinomycetes, yeasts, and spores of fungi. Cryopreservation is applicable to most microorganisms. In these techniques, cryoprotectants and growth conditions are also important for successful preservation.

The microorganisms section of the Ministry of Agriculture, Forestry and Fisheries (MAFF) Genebank was established in 1985 and consists of a central bank and 12 sub-banks (as of 2004) mainly located in Tsukuba[10]. The central bank is placed under the National Institute of Agrobiological Sciences (NIAS). The central bank preserves mainly fungal, oomycetous, bacterial, yeast and actinomycetous strains using cryopreservation and freeze-drying techniques. The preserved microorganisms are tested for viability at regular intervals and the data of viability tests have been input into a database. These data are useful to select a preservation technique suitable for a new deposited strain if the data are summarized in an appropriate table format. In this article, we describe viability of bacteria, Actinomycetes, yeasts, fungi and Oomycetes after one-month preservation or one-year preservation to evaluate the preservation techniques employed in the central bank of the MAFF Genebank.

Materials and Methods

1. Microorganisms

Microbial strains tested are listed in alphabetical order of generic names in Tables 1 (yeasts, bacteria and Actinomycetes) and 2 (fungi and Oomycetes). The culture media and growth conditions used for preparing stocks and testing viability depend on species or strains.

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2. Freeze-drying method and revival of freeze-dried cells

A freeze-drying technique was employed for preservation of bacteria, Actinomycetes and yeasts. Cells on a slant culture were suspended in 10% skim milk with 1.5% monosodium glutamate. This cryoprotectant was autoclaved at 115ºC for 15 min, kept in the autoclave overnight, and then autoclaved again at 110ºC for 10 min. The suspension was dispensed to Pyrex ampoules, frozen at –40ºC overnight and freeze-dried under a vacuum lower than 10 mT with a vacuum freeze dryer (Dura-DryμP, FTF Systems Inc., NY). After freeze-drying, ampoules were sealed and cut with a gas burner. The vacuum in ampoules was tested with a Tesla coil. Ampoules keeping a vacuum were preserved at 5ºC.

For reviving freeze-dried cells, they were re-suspended in 100 μL of water. The cell suspension was transferred onto an appropriate agar plate medium, such as standard agar or potato peptone glucose agar, and was incubated under conditions where the cells could grow well. Visible growth of the microbial colonies on the plate was assessed as successful preservation.

3. Cryopreservation and revival of frozen cells

Cryopreservation of fungi and Oomycetes and revival of the frozen cells were described previously[7,9]. Briefly, discs (6 mm diam.) were cut out of an agar plate on which mycelia were growing. Five or ten discs were transferred into a plastic vial with a screw cap containing 1 mL of 10% (w/w) glycerol. After cold-hardening in a refrigerator at 5ºC for two to three days and freezing in a deep-freezer at –70ºC for two to three days, the vial was moved to an atmosphere of liquid nitrogen at –165ºC.

For reviving frozen cells, a vial containing frozen cells was thawed quickly in a water bath at 30 to 37ºC. The discs were put on an appropriate agar plate medium, such as potato dextrose agar or V8 agar, and were incubated. Visible growth of fungal colonies from 80% or more of the discs on agar plate medium was assessed as successful preservation.

4. Data processing

Data of viability tests have been stored in the database in the MAFF Genebank and were output in text data. The data were converted to tables showing the numbers of successfully preserved strains of each genus by a Windows application, seizan.exe (http://www.gene.affrc.go.jp/micro/)[8].

Results and Discussion

1. The MAFF Genebank and preserved microorganisms

The microorganisms section of the MAFF Genebank (http://genebank.affrc.go.jp/micro/) is one of the culture collections in Japan. It has extensively collected agrobiological microorganism resources, mainly phytopathogenic fungi and bacteria, and held 20,472 strains in 2003. The section is also characteristic in collecting a wide variety of microorganism, from viruses to cultured insect cells. The section consists of the central bank and 12 sub-banks. The central bank (NIAS), which was activated in 1989, held 14,836 microbial strains in 2003. The sub-banks hold species which are difficult to maintain in the central bank; such as animal pathogenic microorganisms, nematodes and so on.

At regular intervals and before distributing in the case of fungal strains, preserved samples are tested for viability and the records of the tests are input to the database of the MAFF Genebank[3]. The viability data have been accumulated since 1985. There were 27,877 bits of stored viability data in 2003. The viability data consist of scientific name, MAFF accession number, beginning date of preservation, date of test, and result of test in percentage (e.g., “Aeromonas caviae”, “118260”, “1990/10/01”, “1990/10/29”, 100). These data are difficult to see in this format whether a new deposited strain could be successfully preserved by our preservation method. So, we summarized the data in a table format showing species, the number of successfully preserved strains for one month and that for one year (Tables 1 and 2).

2. Freeze-drying of yeasts, bacteria and Actinomycetes

The freeze-drying method is routinely applied for the preservation of yeasts, bacteria and Actinomycetes in the central bank. Ampoules which enclose freeze-dried cells are easy to handle in laboratories and easy to send to customers because there is no need of a freezer to keep them. The results of freeze-drying of yeasts, bacteria and Actinomycetes are summarized in Table 1.

One hundred one yeast strains were preserved for one year, and 99.0% (100/101) of the preserved strains could be preserved successfully. Only a *Candida* strain, which was not identified to species, failed to survive for one month. The strain is preserved by cryopreservation in a deep freezer.

Among 1,996 bacterial strains, 1,992 strains survived one-year preservation. Three *Lactobacillus curvatus* strains and one *Pseudomonas fluorescens* strain could not be preserved, so they are preserved by cryopreserva-

tion at –40ºC.

As for Actinomycetes, 237 tested strains, most of which belong to *Streptomyces*, could survive one-year preservation.

Although some microbial strains could not be preserved by the freeze-drying method, it was excellent in preserving almost all strains of yeasts, bacteria and Actinomycetes, as reported previously[1]. The MAFF Genebank continues to preserve the freeze-dried cells, and it might demonstrate the results of longer freeze-dried preservation elsewhere.

3. Cryopreservation of fungi and Oomycetes

In the MAFF Genebank, fungal and oomycetous cells are preserved using cryopreservation, in which the cells in 10% glycerol are preserved in the vapor phase of liquid nitrogen after pre-freezing at –80ºC. This is because mycelia are difficult to preserve by a convenient freeze-drying method. However, spores of some spore-forming species such as *Aspergillus* spp. can be freeze-dried. Freezing in a deep freezer is one choice; 93.5% of tested Basidiomycetous strains preserved were reportedly alive after one-year preservation or more[2].

Among 6,681 fungal strains, 6,631 (99.3%) and 6,578 (98.5%) strains survived one-month preservation and one-year preservation, respectively (Table 2). The value of one-year preservation was nearly equal to those of one-month preservation, so whether a strain could be preserved by this method might be determined in one month or less. The survey for longer preservation is under investigation. Percentages of strains surviving one-year preservation in each division were 99.0% (1,107 strains/1,118 tested strains) in Ascomycota, 96.1% (1,552/1,615) in Basidiomycota, 98.6% (73/74) in Zygomycota and 99.3% (3,846/3,874) in anamorphic fungi. These values are compatible with the ones investigated in 2000[9].

In the preservation of Oomycetes, 72.5% (300/414) and 63.8% (264/414) strains could be preserved successfully for one month and one year, respectively (Table 2). Oomycetous strains are difficult to preserve by cryopreservation as known previously[12]. Our results also show low viabilities of Oomycetes, but whether cells can be preserved by this method depended on species or strains. Interestingly, a recent article said that some *Phytophthora* strains had survived 6 to 23-year storage in water[5], suggesting a very simple and effective preservation method for Oomycetes.

The cells are frozen under uncontrolled conditions in the MAFF Genebank, that is, they are only frozen in a deep freezer before transferring to the atmosphere of liquid nitrogen. Recently, we began to use Cryo 1ºC Freezing Containers (Nalge Nunc International Corp., NY) for the strains which were difficult to preserve by cryopreservation mentioned above. In this container, samples are able to be frozen at a rate of –1ºC/min in a deep freezer. Although the best method to freeze cells is controlled freezing, this requires an expensive programmable freezer[11]. Therefore, cells that can not survive cryopreservation are reluctantly maintained by subculture at the present time.

Table 1. The number of strains of freeze-dried yeasts, bacteria and Actinomycetes which survived after one month or one year of preservation in freeze-dried form

Genus	No. of strains	Preservation term		Percentage[c)]
		1 month[a)]	1 year[b)]	
Yeasts	**101**	**100**	**100**	**99.0**
Candida	16	15	15	93.8
Debaryomyces	1	1	1	100.0
Galactomyces	1	1	1	100.0
Hansenula	4	4	4	100.0
Hyphopichia	1	1	1	100.0
Pichia	11	11	11	100.0
Pseudozyma	1	1	1	100.0
Rhodotorula	1	1	1	100.0
Saccharomyces	55	55	55	100.0
Torulaspora	1	1	1	100.0
Zygosaccharomyces	9	9	9	100.0
Bacteria	**1,996**	**1,994**	**1,992**	**99.8**
Aeromonas	1	1	1	100.0
Agrobacterium	137	137	137	100.0
Alcaligenes	1	1	1	100.0
Arthrobacter	2	2	2	100.0
Azorhizobium	6	6	6	100.0
Azospirillum	32	32	32	100.0
Bacillus	175	175	175	100.0
Bradyrhizobium	263	263	263	100.0
Burkholderia	2	2	2	100.0
Corynebacterium	36	36	36	100.0
Enterococcus	3	3	3	100.0
Erwinia	218	218	218	100.0
Escherichia	1	1	1	100.0
Flavobacterium	1	1	1	100.0
Herbaspirillum	1	1	1	100.0
Janthinobacterium	1	1	1	100.0
Lactobacillus	41	39	38	92.7
Lactococcus	3	3	3	100.0
Leuconostoc	14	14	14	100.0
Methylobacterium	1	1	1	100.0
Methylosinus	2	2	2	100.0
Micrococcus	5	5	5	100.0
Mycetocola	3	3	3	100.0
Ochrobactrum	7	7	7	100.0
Pediococcus	10	10	10	100.0
Proteus	1	1	1	100.0
Pseudoalteromonas	1	1	1	100.0
Pseudomonas	698	698	697	99.9
Ralstonia	2	2	2	100.0
Rhizobium	68	68	68	100.0
Streptococcus	4	4	4	100.0
Xanthomonas	256	256	256	100.0
Actinomycetes	**237**	**237**	**237**	**100.0**
Nocardia	4	4	4	100.0
Nocardiopsis	2	2	2	100.0
Streptomyces	231	231	231	100.0

(continued)

a): The number of strains that survived after one-month preservation.
b): The number of strains that survived after one-year preservation.
c): The number of strains that survived after one-year preservation / the number of tested strains × 100.

Table 2. The number of strains of fungi and Oomycetes which survived after one month or one year of preservation in the vapor phase of liquid nitrogen

Genus	No. of strains	Preservation term		Percentage[c]
		1 month[a]	1 year[b]	
Fungi	**6,681**	**6,631**	**6,578**	**98.5**
Ascomycota	**1,118**	**1,115**	**1,107**	**99.0**
Halosphaeria	18	18	17	94.4
Morchella	3	3	2	66.7
Peziza	5	5	3	60.0
Rosellinia	30	29	27	90.0
Scleroderris	9	9	8	88.9
Sclerotinia	49	49	48	98.0
Thermoascus	2	0	0	0.0
Others*	1,002	1,002	1,002	100.0

*Others include successfully preserved species in Ascomycota as below (numbers of tested strains are shown in parenthesis).

Acrospermum (12), *Amphisphaeria* (1), *Apiospora* (4), *Apiosporopsis* (1), *Arenariomyces* (17), *Ascocalyx* (5), *Aulographum* (1), *Biscogniauxia* (3), *Botryosphaeria* (23), *Botryotinia* (1), *Bulgaria* (1), *Byssochlamys* (1), *Calonectria* (17), *Camarops* (2), *Ceratocystis* (4), *Ceriosporopsis* (39), *Chaetomium* (26), *Ciborinia* (1), *Cistella* (21), *Claviceps* (11), *Cochliobolus* (27), *Corollospora* (34), *Cryphonectria* (13), *Cryptodiaporthe* (5), *Cucurbidothis* (1), *Cyclodothis* (1), *Daldinia* (2), *Diaporthe* (40), *Diaporthopsis* (1), *Diatrype* (5), *Diatrypella* (2), *Dicarpella* (1), *Didymosphaeria* (1), *Discostroma* (1), *Dothidea* (2), *Endothia* (34), *Entonaema* (1), *Epichloe* (10), *Eudarluca* (2), *Eurotium* (2), *Gaeumannomyces* (1), *Gibberella* (60), *Glomerella* (42), *Gnomonia* (1), *Graphostroma* (6), *Grovesinia* (5), *Guignardia* (49), *Gyromitra* (1), *Halbania* (1), *Halosarpheia* (15), *Halosphaeriopsis* (20), *Helotium* (1), *Hypomyces* (13), *Hypoxylon* (53), *Khuskia* (1), *Lachnellula* (3), *Leptosphaeria* (1), *Leptosphaerulina* (4), *Leucostoma* (2), *Lophodermium* (2), *Marinospora* (9), *Massarinula* (1), *Melanconis* (8), *Microascus* (1), *Monascus* (14), *Monilinia* (13), *Monosporascus* (2), *Morenoella* (1), *Mycosphaerella* (52), *Nectria* (20), *Nemania* (1), *Neocosmospora* (3), *Ophiobolus* (9), *Ophionectria* (1), *Ophiostoma* (16), *Ophiovalsa* (3), *Pezicula* (4), *Phaeosphaerella* (1), *Phillipsia* (1), *Phyllachora* (1), *Physalospora* (1), *Plagiosphaera* (2), *Plectosphaera* (4), *Pleospora* (6), *Pseudoplea* (3), *Pseudovalsella* (6), *Pyrenophora* (13), *Remispora* (25), *Rhizina* (4), *Rosenscheldiella* (1), *Sarea* (20), *Septotinia* (4), *Sordaria* (1), *Sphaerulina* (1), *Stromatinia* (2), *Talaromyces* (1), *Taphrina* (10), *Thielavia* (2), *Torpedospora* (20), *Trichocoma* (1), *Trichoscyphella* (8), *Valsa* (33), *Valsella* (1), *Venturia* (7), *Xylaria* (8)

(continued)

Genus	No. of strains	Preservation term		Percentage[c]
		1 month[a]	1 year[b]	
Basidiomycota	**1,615**	**1,587**	**1,552**	**96.1**
Amanita	3	3	2	66.7
Amauroderma	10	10	9	90.0
Armillaria	56	53	53	94.6
Auricularia	17	16	14	82.4
Ceratobasidium	44	41	39	88.6
Coprinus	13	13	12	92.3
Corticium	46	45	45	97.8
Eichleriella	10	10	8	80.0
Exobasidium	38	35	34	89.5
Ganoderma	28	27	27	96.4
Gloeoporus	5	4	4	80.0
Grifola	9	9	8	88.9
Hebeloma	5	5	4	80.0
Helicobasidium	15	12	11	73.3

(continued)

Table 2. The number of strains of fungi and Oomycetes which survived after one month or one year of preservation in the vapor phase of liquid nitrogen (continued)

Genus	No. of strains	Preservation term		Percentage[c)]
		1 month[a)]	1 year[b)]	
Inonotus	13	13	12	92.3
Ischnoderma	5	3	3	60.0
Laccaria	3	3	2	66.7
Lampteromyces	14	14	13	92.9
Lepista	6	5	5	83.3
Leucoagaricus	5	3	3	60.0
Leucopaxillus	3	3	2	66.7
Lyophyllum	17	17	16	94.1
Naematoloma	38	37	35	92.1
Oudemansiella	20	20	19	95.0
Panellus	30	30	29	96.7
Pholiota	122	122	121	99.2
Pluteus	1	1	0	0.0
Polyporus	24	24	23	95.8
Protodaedalea	2	2	1	50.0
Psilocybe	5	5	4	80.0
Rhizopogon	1	0	0	0.0
Rigidoporus	9	9	8	88.9
Rozites	1	0	0	0.0
Serpula	4	2	2	50.0
Suillus	9	8	7	77.8
Tricholoma	88	88	83	94.3
Ustilago	21	20	20	95.2
Volvariella	1	1	0	0.0
Others*	874	874	874	100.0

*Others include successfully preserved species in Basidiomycota as below (numbers of tested strains are shown in parenthesis).

Abortiporus (2), *Agaricus* (6), *Agrocybe* (4), *Amyloporia* (1), *Amylostereum* (2), *Anthracophyllum* (3), *Antrodia* (6), *Antrodiella* (1), *Armillariella* (9), *Asterophora* (3), *Bjerkandera* (5), *Bondarzewia* (1), *Botryobasidium* (1), *Calocera* (4), *Calvatia* (4), *Ceriporia* (1), *Ceriporiopsis* (4), *Cerrena* (1), *Chlorophyllum* (1), *Clavicorona* (1), *Clitocybe* (2), *Collybia* (6), *Coniophora* (1), *Coriolopsis* (6), *Coriolus* (9), *Crinipellis* (1), *Cryptoporus* (3), *Cyclomyces* (1), *Cyptotrama* (2), *Dacrymyces* (5), *Daedalea* (11), *Daedaleopsis* (3), *Datronia* (2), *Dictyopanus* (7), *Diplomitoporus* (3), *Ditiola* (1), *Earliella* (2), *Echinoporia* (2), *Elfvingia* (1), *Elmerina* (1), *Erythromyces* (2), *Exidia* (1), *Exidiopsis* (1), *Favolaschia* (1), *Favolus* (3), *Femsjonia* (3), *Fibroporia* (1), *Filoboletus* (3), *Fistulina* (5), *Flammulina* (18), *Flavodon* (1), *Fomes* (5), *Fomitella* (1), *Fomitiporia* (3), *Fomitopsis* (12), *Galerina* (1), *Gloeophyllum* (4), *Grammothele* (2), *Graphiola* (4), *Guepinia* (4), *Gymnopilus* (7), *Gyrodontium* (2), *Hapalopilus* (1), *Hericium* (9), *Heterobasidion* (4), *Hirschioporus* (1), *Hydnochaete* (4), *Hygrophoropsis* (1), *Hymenochaete* (5), *Hyphodontia* (1), *Hypsizygus* (18), *Irpex* (5), *Junghuhnia* (4), *Kuehneromyces* (2), *Lactarius* (2), *Laeticorticium* (1), *Laetiporus* (12), *Laetisaria* (1), *Lanopila* (1), *Laricifomes* (1), *Lentinellus* (1), *Lentinula* (10), *Lentinus* (20), *Lenzites* (3), *Lepiota* (3), *Leucocoprinus* (4), *Linderia* (1), *Loweporus* (3), *Lycoperdon* (1), *Macrolepiota* (6), *Marasmius* (21), *Megasporoporia* (2), *Merulius* (4), *Microporus* (2), *Mundkurella* (4), *Mycena* (10), *Mycoacia* (1), *Mycoleptodonoides* (11), *Neolentinus* (8), *Nia* (10), *Oligoporus* (5), *Omphalotus* (2), *Oxyporus* (2), *Panaeolina* (2), *Panaeolus* (2), *Panus* (5), *Paxillus* (3), *Pellicularia* (1), *Perenniporia* (13)

(continued)

Table 2. The number of strains of fungi and Oomycetes which survived after one month or one year of preservation in the vapor phase of liquid nitrogen (continued)

Genus	No. of strains	Preservation term		Percentage[c]
		1 month[a]	1 year[b]	
Phaeolepiota (3)				
Phanerochaete (1)				
Phlebia (1)				
Phylloporia (2)				
Piptoporus (8)				
Pleurocybella (6)				
Plicaturopsis (2)				
Polyporellus (5)				
Porodaedalea (4)				
Pseudoclitocybe (1)				
Pseudomerulius (1)				
Pycnoporus (8)				
Ramaria (1)				
Rhodotus (1)				
Roseofomes (1)				
Sarcodon (1)				
Schizopora (2)				
Sorosporium (5)				
Spongiporus (5)				
Stereum (10)				
Strobilurus (3)				
Thanatephorus (1)				
Tilletia (4)				
Trametes (26)				
Trichaptum (5)				
Typhula (48)				
Waitea (20)				
Xeromphalina (10)				
Xylobolus (7)				
Phaeolus (5)				
Phellinus (63)				
Phleogena (33)				
Phyllotopsis (1)				
Platygloea (12)				
Pleurotus (52)				
Podoscypha (1)				
Poria (6)				
Psathyrella (1)				
Pseudocolus (2)				
Pulcherricium (2)				
Pyrofomes (3)				
Rhodophyllus (1)				
Ripartitella (1)				
Russula (1)				
Schizophyllum (9)				
Sebacina (15)				
Sparassis (5)				
Steccherinum (3)				
Stilbum (1)				
Stropharia (3)				
Theleporus (1)				
Tinctoporellus (4)				
Tremella (2)				
Tricholomopsis (1)				
Tyromyces (3)				
Wolfiporia (3)				
Xerula (2)				
Zygomycota	**74**	**73**	**73**	**98.6**
Choanephora	2	1	1	50.0
Others*	72	72	72	100.0
Absidia (4)				
Cunninghamella (2)				
Gongronella (3)				
Mortierella (37)				
Rhizopus (11)				
Syncephalastrum (2)				
Umbelopsis (5)				
Mucor (8)				
Anamorphic fungi	**3,874**	**3,856**	**3,846**	**99.3**
Cercospora	209	209	207	99.0
Fusarium	776	767	767	98.8
Pestalotiopsis	144	144	143	99.3
Rhizoctonia	258	250	245	95.0
Rhynchosporium	8	7	7	87.5
Selenophoma	4	4	3	75.0
Sphaceloma	7	7	6	85.7
Others*	2,468	2,468	2,468	100.0
Acremoniella (5)				
Acrocylindrium (1)				
Actinopelte (1)				
Alternaria (75)				
Arthrinium (18)				
Arthrographis (2)				
Aspergillus (100)				
Asterosporium (1)				
Bartalinia (1)				
Beltrania (2)				
Botryodiplodia (8)				
Botrytis (49)				
Camarosporium (1)				
Catinula (1)				
Cephalosporium (11)				
Cercosporidium (7)				
Chalaropsis (1)				
Chrysosporium (1)				
Cladosporium (68)				
Colletotrichum (282)				
Coniothyrium (3)				
Coryneum (1)				
Cryptosporiopsis (11)				
Cylindrocarpon (21)				
Cylindrosporium (1)				
Cytospora (17)				
Dactylaria (1)				
Dendrodochium (1)				
Dendryphion (1)				
Dinemasporium (2)				
Diplodina (2)				
Discula (1)				
Acremonium (6)				
Acrodictys (1)				
Albophoma (1)				
Apiocarpella (2)				
Arthrobotrys (3)				
Ascochyta (16)				
Asperisporium (3)				
Aureobasidium (13)				
Beauveria (15)				
Bipolaris (47)				
Botryotrichum (5)				
Camposporium (1)				
Cephaliophora (2)				
Cercosporella (6)				
Chalara (2)				
Chromelosporium (3)				
Cladorrhinum (3)				
Codinaea (1)				
Coniella (8)				
Corynespora (45)				
Cristulariella (2)				
Curvularia (41)				
Cylindrocladium (33)				
Cytophoma (1)				
Dematophora (4)				
Dendrophoma (3)				
Dictyochaeta (1)				
Diplodia (12)				
Discosia (6)				
Doratomyces (1)				

*Others include successfully preserved species in Zygomycota as below (numbers of tested strains are shown in parenthesis).

*Others include successfully preserved species in anamorphic fungi as below (numbers of tested strains are shown in parenthesis).

(continued)

(continued)

Table 2. The number of strains of fungi and Oomycetes which survived after one month or one year of preservation in the vapor phase of liquid nitrogen (continued)

Genus	No. of strains	Preservation term		Percentage[c]
		1 month[a]	1 year[b]	
Dothiorella (9)				
Dothistroma (2)				
Drechslera (23)				
Duosporium (2)				
Ellisiella (1)				
Embellisia (1)				
Endophragmia (1)				
Endothiella (1)				
Ephelis (14)				
Epicoccum (17)				
Exserohilum (10)				
Fulvia (5)				
Fusicoccum (4)				
Geotrichum (1)				
Gliocladium (12)				
Gloeocercospora (11)				
Gloeosporium (4)				
Gonytrichum (1)				
Haplosporella (1)				
Helicomyces (1)				
Helminthosporium (49)				
Hendersonula (1)				
Heterosporium (3)				
Humicola (14)				
Hyalodendron (1)				
Hyphodiscosia (1)				
Irpicomyces (1)				
Kabatiella (2)				
Lasiodiplodia (17)				
Leptochlamys (1)				
Leptographium (2)				
Leucocytospora (1)				
Macrophoma (22)				
Macrophomina (16)				
Mammaria (1)				
Mariannaea (1)				
Marssonina (5)				
Melanconium (7)				
Metarhizium (10)				
Microdochium (1)				
Microsphaeropsis (1)				
Microsporum (1)				
Monacrosporium (5)				
Monilia (5)				
Monilochaetes (1)				
Monochaetia (2)				
Monocillium (1)				
Monodictys (1)				
Monostichella (2)				
Monotosporella (1)				
Myceliophthora (1)				
Mycocentrospora (2)				
Mycovellosiella (3)				
Myrothecium (28)				
Myxosporium (1)				
Naranus (1)				
Nigroporus (2)				
Nigrospora (31)				
Nodulisporium (3)				
Nomuraea (3)				
Ochroconis (3)				
Oedocephalum (1)				
Oidiodendron (10)				
Ordus (1)				
Ovularia (6)				
Pachybasium (1)				
Paecilomyces (14)				
Papulaspora (4)				
Paracercospora (1)				
Penicillium (12)				
Periconia (5)				
Periconiella (1)				
Pestalotia (50)				
Phaeoisariopsis (6)				
Phaeoseptoria (4)				
Phialomyces (1)				
Phialophora (17)				
Phloeospora (2)				
Phoma (49)				
Phomopsis (91)				
Phyllosticta (34)				
Pithomyces (8)				
Pleiochaeta (1)				
Pseudocercospora (94)				
Pseudocercosporella (42)				
Pyrenochaeta (35)				
Pyricularia (113)				
Racodium (1)				
Raffaelea (3)				
Ramichloridium (12)				
Ramularia (3)				
Rhizosphaera (9)				
Robillarda (2)				
Sclerotium (33)				
Scolecobasidium (2)				
Scolicotrichum (2)				
Scopulariopsis (2)				
Seimatosporium (2)				
Seiridium (14)				
Septogloeum (1)				
Septonema (3)				
Septoria (30)				
Septotis (3)				
Spegazzinia (1)				
Sphaerellopsis (80)				
Sphaeropsis (5)				
Spicellum (3)				
Sporidesmium (5)				
Stachybotrys (2)				
Stagonospora (5)				
Staphylotrichum (3)				
Stemphylium (14)				
Stigmina (6)				
Strasseriopsis (2)				
Taeniolella (1)				
Tetracladium (2)				
Thielaviopsis (3)				
Thysanophora (1)				
Ticogloea (1)				
Torula (5)				
Torulomyces (1)				
Trichocladium (11)				
Trichoderma (32)				
Trichothecium (5)				
Trinacrium (1)				
Tripospermum (1)				
Tritirachium (2)				
Trochophora (7)				
Truncatella (1)				
Tubakia (4)				
Tubercularia (3)				
Ulocladium (6)				
Ustilaginoidea (2)				
Verticillium (165)				
Volutella (2)				
Wallemia (1)				
Wiesneriomyces (1)				
Oomycetes	**414**	**300**	**264**	**63.8**
Achlya	15	1	0	0.0
Aphanomyces	6	2	2	33.3
Dictyuchus	6	0	0	0.0
Phytophthora	114	97	88	77.2
Pythium	266	196	170	63.9
Thraustotheca	4	1	1	25.0
Others*	3	3	3	100.0

*Others include successfully preserved species in Oomycete as below (numbers of tested strains are shown in parenthesis).

Plectospira (1)

Saprolegnia (2)

(continued)

a): The number of strains that survived after one-month preservation.
b): The number of strains that survived after one-year preservation.
c): The number of strains that survived after one-year preservation / the number of tested strains × 100.

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