Microbiol. Cult. Coll. June 2000. p. 13-22 Vol. 16, No. 1

Preservation of Fungi in an Atmosphere over Liquid Nitrogen after Uncontrolled Freezing

Toshirou Nagai*, Aika Ideno, Michiyo Tsuge, Chie Oyanagi, Masaomi Oniki, Kouichi Kita***, Mitsuo Horita, Takayuki Aoki, Takao Kobayashi and Kenichi Tsuchiya**

National Institute of Agrobiological Resources, Ministry of Agriculture, Forestry and Fisheries, Tsukuba, Ibaraki 305-8602, Japan

In the Genebank of the Ministry of Agriculture, Forestry and Fisheries in Japan, 5,406 (95.7%) of 5,651 maintained fungal strains (466 genera and 1,182 species), including strains of oomycetes, were well stored in an atmosphere over liquid nitrogen at −165°C after uncontrolled freezing, showing that this method is applicable to preservation of most of fungal strains. Percentages of strains stored successfully for 1 year within Oomycota, Zygomycota, Ascomycota, Basidiomycota, and anamorphic fungi, were 59.3% (233/393 strains tested), 98.3% (58/59), 98.8% (881/892), 95.4% (1,100/1,153), and 99.4% (3,134/3,154), respectively. Strains of *Achlya, Dictyuchus* (Oomycota), *Volvariella* (Basidiomycota), and *Pseudoseptoria* (anamorphic fungi) could not be stored using this method. Successful preservation depends on the species and strains of fungi *sensu lato*.

Key words : cryopreservation, fungi

In many culture collections, fungal strains are maintained using several methods, such as freeze-drying, oil storage, and cryopreservation (7, 8). The Genebank of the Ministry of Agriculture, Forestry and Fisheries (MAFF) in Japan has collected about 9,000 fungal isolates (including oomycetes) which are stored mainly in an atmosphere over liquid nitrogen after uncontrolled freezing, because this provides stable preservation without the need for complicated handling. Frozen strains have been tested for their viability at intervals, and data concerning viability of stored strains have been accumulated. In this report, viability after one-year storage of the preserved strains belonging to Oomycota, Zygomycota, Ascomycota, Basidiomycota, and anamorphic fungi, is summarized. The subdivisions are used according to Hawksworth et al. (1) ; however, "fungi" include oomycetes in this report.

Fungal strains tested are listed in alphabetical order of generic names (Table 1). The culture medium used for preparing stocks and testing viability was potato dextose agar (Difco), for most of the species. V8 juice agar (9) was used for oomycetes. V8 juice agar contained 20% of the supernatant of commercial V8 juice (Campbell Soup Company, Camden, NJ), which was obtained by centrifugation of a mixture of the juice and 1.5% $CaCO_3$ (9).

Preservation of fungi was performed according to the method of Miki and Kubomura (3), with some modifications. Fungal strains were grown on the agar media for 1-2 weeks at 20°C. Agar disks (6mm dia., 70μl) with spores and/or mycelia were cut off from a plate using gas-sterilized plastic straws. Five or ten disks were soaked in 1ml of 10% glycerol in a 2-ml plastic vial. The vial was allowed to stand at

* Corresponding author : E-mail ; nag@affrc.go.jp
** Present address : National Center for Seeds and Seedlings, Agriculture, Forestry and Fisheries Research Council, Ministry of Agriculture, Forestry and Fisheries, Tsukuba, Ibaraki 305-0852, Japan
*** Present address : Hokkaido National Agricultural Experiment Station, Ministry of Agriculture, Forestry and Fisheries, Sapporo, Hokkaido 062-8555, Japan

5°C for 2 days, for cold-hardening, and then at −60°C in an electrical deep freezer for 2 days. Finally, the vial was transferred to an atmosphere over liquid nitrogen at −165°C.

After 1 month (0-2 months) and 1 year (9.6-14.4 months), a vial containing frozen fungus was thawed quickly in a water bath, either at 30°C (5) or at 37°C (3), for 5 min. All disks were placed onto suitable agar for each strain and incubated at the appropriate growing temperature. Viability was defined as the percentage of disks on which visible growth was observed, and viability of more than 80% was assessed as successful preservation. A computer software ; seizan. exe (4), was used to process vivability data.

Among 5,651 fungal strains of 466 genera and 1,182 species, 5,503 strains (97.4% of total strains) were recovered successfully after storage for 1 month and 5,406 strains (95.7%) after 1 year (Table 1). Percentages of strains stored successfully for 1 year within Oomycota, Zygomycota, Ascomycota, Basidiomycota, and anamorphic fungi, were 59.3% (233/393 tested strains), 98.3% (58/59), 98.8% (881/892), 95.4% (1,100/1,153), and 99.4% (3,134/3,154), respectively. While most of the fungal strains could be stored by the uncontrolled freezing method, some strains could not be preserved by this method. No strains of the genera *Achlya* (Oomycota, 15 strains tested), *Dictyuchus* (Oomycota, 6 strains), *Volvariella* (Basidiomycota, 1 strain), and *Pseudoseptoria* (anamorphic fungi, 1 strain) survived one-year storage (Table 1). These strains could be stored by controlled freezing (5). Suitable conditions for preservation of strains that are not stored successfully by uncontrolled freezing are under investigation, and therefore these strains are currently maintained by subculturing on agar slants occasionally with oil overlay.

Though species of *Phytophthora* and *Pythium* are known to have low survival after long term storage (6), some strains of the genera were found alive. Successful preservation depended on species or strains. Some strains of *Phytophthora irregulare* (24 surviving strains/28 strains tested) and *Pythium sylvaticum* (8/10) survived well, whereas all the examined strains of *Py. oedochilum* and *Py. vexans* did not survive one-year storage. The detailed results of the species stored unsuccessfully are given in Table 2.

Table 1. Number of fungal strains surviving by the cryopreservation

Fungal genus	Tested[a]	1 M[b]	1 Y[b]
Oomycota			
Achlya	15	1	0
Aphanomyces	6	2	2
Dictyuchus	6	0	0
Phytophthora	104	88	78
Plectospira	1	1	1
Pythium	255	177	149
Saprolegnia	2	2	2
Thraustotheca	4	1	1
Zygomycota			
Absidia	3	3	3
Choanephora	2	1	1
Cunninghamella	2	2	2
Gongronella	2	2	2
Mortierella	33	33	33
Mucor	8	8	8
Rhizopus	7	7	7
Syncephalastrum	2	2	2
Ascomycota			
Acrospermum	12	12	12
Amphiporthe	1	1	1
Amphisphaeria	1	1	1
Apiospora	3	3	3
Arenariomyces	17	17	17
Ascocalyx	5	5	5
Botryosphaeria	30	30	30
Bulgaria	1	1	1
Calonectria	13	13	13
Calycellina	1	1	1
Ceratocystis	2	2	2
Ceriosporopsis	39	39	39
Chaetomium	22	22	22
Ciborinia	2	2	2
Cistella	19	19	16
Claviceps	7	7	7
Corollospora	33	33	33
Cryphonectria	46	46	46
Cryptodiaporthe	3	3	3
Cucurbidothis	1	1	1
Daldinia	1	1	1
Diaporthe	40	40	40
Diaporthopsis	1	1	1
Diatrype	4	4	4
Diatrypella	1	1	1
Didymella	14	14	14

Table 1. continued.

Fungal genus	Tested[a]	1 M[b]	1 Y[b]
Discostroma	1	1	1
Dothidea	2	2	2
Endothia	1	1	1
Epichloe	10	10	10
Eudarluca	2	2	2
Eurotium	2	2	2
Gaeumannomyces	10	10	10
Gibberella	2	2	2
Glomerella	33	33	33
Gnomonia	1	1	1
Graphostroma	6	6	6
Gremmeniella	9	9	8
Grovesinia	4	4	4
Guignardia	38	38	38
Gyromitra	1	1	1
Halbania	1	1	1
Halosarpheia	15	15	15
Halosphaeria	18	18	17
Halosphaeriopsis	30	30	30
Hypomyces	11	11	11
Hypoxylon	13	13	13
Khuskia	1	1	1
Lachnellula	7	7	7
Leptosphaeria	1	1	1
Leptosphaerulina	6	6	6
Leucostoma	2	2	2
Lophiosphaerella	1	1	1
Lophodermium	2	2	2
Marinospora	9	9	9
Massarinula	1	1	1
Melanconis	8	8	8
Microascus	1	1	1
Mixia	1	1	1
Monascus	14	14	14
Monilinia	10	10	10
Monosporascus	2	2	2
Morchella	3	3	2
Mycosphaerella	28	28	28
Nectria	13	13	13
Neocosmospora	2	2	2
Ophiostoma	15	15	15
Ophiovalsa	3	3	3
Pezicula	4	4	4
Peziza	4	3	1
Phaeosphaerella	1	1	1
Phyllachora	1	1	1
Plagiosphaera	1	1	1

Table 1. continued.

Fungal genus	Tested[a]	1 M[b]	1 Y[b]
Plectosphaera	6	6	6
Pleospora	4	4	4
Pseudovalsa	5	5	5
Pseudovalsella	1	1	1
Pyrenophora	2	2	2
Remispora	25	25	24
Rhizina	4	4	4
Rosellinia	25	25	25
Rosenscheldiella	1	1	1
Sarea	20	20	20
Sclerotinia	45	45	44
Septotinia	4	4	4
Sordaria	1	1	1
Sphaerulina	1	1	1
Talaromyces	1	1	1
Taphrina	9	9	9
Thielavia	2	2	2
Torpedospora	20	20	20
Trichocoma	1	1	1
Trichoscyphella	4	4	4
Valsa	32	32	32
Valsella	1	1	1
Venturia	7	7	7
Xylaria	1	1	1
Basidiomycota			
Abortiporus	2	2	2
Agaricus	6	6	6
Agrocybe	3	3	3
Amanita	2	2	1
Amylostereum	2	2	2
Anthracophyllum	3	3	3
Antrodia	11	11	11
Antrodiella	3	3	3
Armillaria	56	54	54
Asterophora	3	3	3
Auricularia	16	14	12
Bjerkandera	5	5	5
Bondarzewia	1	1	1
Botryobasidium	1	1	1
Calocera	4	4	4
Calvatia	4	4	4
Ceratobasidium	49	46	37
Cerrena	1	1	1
Chlorophyllum	1	1	1
Clavicorona	1	1	1
Clitocybe	2	2	2
Collybia	6	6	6

Table 1. continued.

Fungal genus	Tested[a)]	1 M[b)]	1 Y[b)]
Coniophora	1	1	1
Coprinus	10	10	9
Crinipellis	1	1	1
Cryptoporus	3	3	3
Cylindrobasidium	1	1	1
Cyptotrama	2	2	2
Dacrymyces	5	5	5
Dacryopinax	3	3	3
Daedalea	4	4	4
Daedaleopsis	3	3	3
Datronia	2	2	2
Dictyopanus	6	6	6
Diplomitoporus	2	2	2
Ditiola	1	1	1
Eichleriella	10	10	8
Erythricium	18	17	17
Exidia	1	1	1
Exidiopsis	1	1	1
Exobasidium	22	22	22
Favolaschia	1	1	1
Femsjonia	3	3	3
Filoboletus	1	1	1
Fistulina	5	5	5
Flammulina	13	13	13
Fomes	3	3	3
Fomitopsis	9	9	9
Galerina	1	1	1
Ganoderma	23	22	21
Gloeophyllum	4	4	4
Gloeoporus	2	1	1
Graphiola	4	4	4
Grifola	8	8	7
Gymnopilus	7	7	7
Hapalopilus	1	1	1
Hebeloma	5	5	4
Helicobasidium	12	12	11
Hericium	7	7	7
Heterobasidion	3	3	3
Hydnochaete	3	3	3
Hygrophoropsis	1	1	1
Hymenochaete	2	2	2
Hypsizygus	1	1	1
Inonotus	11	11	10
Irpex	4	4	4
Ischnoderma	4	2	2
Junghuhnia	2	2	2
Kuehneromyces	2	2	2

Table 1. continued.

Fungal genus	Tested[a)]	1 M[b)]	1 Y[b)]
Laccaria	3	3	2
Lactarius	3	3	3
Laeticorticium	1	1	1
Laetiporus	12	12	12
Laetisaria	1	1	1
Lampteromyces	10	10	10
Lanopila	1	1	1
Lentinellus	1	1	1
Lentinula	8	8	8
Lentinus	8	8	8
Lenzites	3	3	3
Lepiota	3	3	3
Lepista	6	5	5
Leucoagaricus	4	3	3
Leucocoprinus	3	3	3
Leucopaxillus	3	3	2
Linderia	1	1	1
Loweporus	6	6	6
Lycoperdon	1	1	1
Lyophyllum	12	12	11
Macrolepiota	6	6	6
Marasmius	20	20	20
Microporus	1	1	1
Mundkurella	4	4	4
Mycena	9	9	9
Mycoacia	2	2	2
Mycoleptodonoides	5	5	5
Naematoloma	13	13	13
Neolentinus	7	7	7
Nia	10	10	10
Oligoporus	6	6	6
Omphalotus	2	2	2
Oudemansiella	16	16	14
Oxyporus	1	1	1
Panaeolina	2	2	2
Panaeolus	2	2	2
Panellus	10	10	10
Panus	3	3	3
Paxillus	3	3	3
Pellicularia	1	1	1
Perenniporia	10	10	10
Phaeolepiota	3	3	2
Phaeolus	5	5	5
Phellinus	51	51	51
Phlebia	4	4	4
Phleogena	33	33	33
Pholiota	18	18	17

Table 1. continued.

Fungal genus	Tested[a)]	1 M[b)]	1 Y[b)]
Phyllotopsis	1	1	1
Piptoporus	8	8	8
Platygloea	12	12	12
Pleurocybella	5	5	5
Pleurotus	43	42	42
Plicaturopsis	2	2	2
Polyporellus	5	5	5
Polyporus	18	18	17
Poria	1	1	1
Protodaedalea	2	2	1
Psathyrella	1	1	1
Pseudoclitocybe	1	1	1
Pseudocolus	2	2	2
Pseudomerulius	1	1	1
Psilocybe	5	5	4
Pulcherricium	2	2	2
Pycnoporus	8	8	8
Ramaria	1	1	1
Rhodophyllus	1	1	1
Rhodotus	1	1	1
Rigidoporus	7	7	6
Ripartitella	1	1	1
Russula	1	1	1
Schizophyllum	6	6	6
Schizopora	2	2	2
Sebacina	15	15	15
Serpula	2	2	2
Sparassis	4	4	4
Sporisorium	5	5	5
Steccherinum	3	3	3
Stereum	7	7	7
Strobilurus	3	3	3
Stropharia	3	3	3
Suillus	5	4	4
Thanatephorus	1	1	1
Tilletia	3	3	3
Tinctoporellus	2	2	2
Trametes	22	22	22
Tremella	2	2	2
Trichaptum	6	6	6
Tricholoma	87	87	83
Typhula	28	28	28
Tyromyces	1	1	1
Ustilago	21	20	20
Volvariella	1	1	0
Waitea	20	20	20
Wolfiporia	3	3	3

Table 1. continued.

Fungal genus	Tested[a)]	1 M[b)]	1 Y[b)]
Xeromphalina	10	10	10
Xerula	2	2	2
Xylobolus	3	3	3
Anamorphic fungi			
Acremonium	5	5	5
Acrodictys	1	1	1
Albophoma	1	1	1
Alternaria	65	65	65
Apiocarpella	2	2	2
Arthrinium	17	17	17
Arthrobotrys	3	3	3
Arthrographis	2	2	2
Ascochyta	13	13	13
Aspergillus	78	78	78
Asperisporium	2	2	2
Asterosporium	1	1	1
Aureobasidium	11	11	11
Bartalinia	1	1	1
Beauveria	12	12	12
Beltrania	1	1	1
Bipolaris	94	94	94
Botryodiplodia	2	2	2
Botryotrichum	3	3	3
Botrytis	31	31	31
Camarosporium	1	1	1
Camposporium	1	1	1
Catinula	1	1	1
Cephaliophora	2	2	2
Cephalosporium	4	4	4
Cercospora	74	74	72
Cercosporella	3	3	3
Cercosporidium	9	9	9
Chalara	2	2	2
Chalaropsis	1	1	1
Chromelosporium	3	3	3
Chrysosporium	1	1	1
Cladorrhinum	3	3	3
Cladosporium	61	61	61
Codinaea	1	1	1
Colletotrichum	172	172	172
Coniella	8	8	8
Coniothyrium	1	1	1
Corynespora	43	43	43
Coryneum	1	1	1
Cristulariella	2	2	2
Cryptosporiopsis	10	10	10
Curvularia	42	42	42

Table 1. continued.

Fungal genus	Tested[a]	1 M[b]	1 Y[b]
Cylindrocarpon	17	17	17
Cylindrocladiella	3	3	3
Cylindrocladium	30	30	30
Cylindrosporium	1	1	1
Cytophoma	1	1	1
Cytospora	18	18	18
Dactylaria	1	1	1
Dematophora	4	4	4
Dendrodochium	1	1	1
Dendryphion	1	1	1
Dinemasporium	1	1	1
Diplodia	7	7	7
Diplodina	2	2	2
Discosia	4	4	4
Discula	1	1	1
Doratomyces	1	1	1
Dothiorella	8	8	8
Dothistroma	2	2	2
Drechslera	50	50	50
Duosporium	2	2	2
Embellisia	1	1	1
Endothiella	1	1	1
Epicoccum	17	17	17
Exserohilum	15	15	15
Fulvia	11	11	11
Fusarium	633	633	633
Geosmithia	1	1	1
Gliocladium	10	10	10
Gloeocercospora	11	11	11
Haprosporella	1	1	1
Harzia	5	5	5
Helicomyces	1	1	1
Helminthosporium	4	4	4
Hendersonula	1	1	1
Humicola	11	11	11
Hyalodendron	1	1	1
Hymenula	1	1	1
Hyphodiscosia	1	1	1
Irpicomyces	1	1	1
Kabatiella	2	2	2
Lasiodiplodia	16	16	16
Leptochlamys	1	1	1
Leptographium	2	2	2
Macrophoma	20	20	20
Macrophomina	17	17	17
Mammaria	1	1	1
Mariannaea	3	3	3

Table 1. continued.

Fungal genus	Tested[a]	1 M[b]	1 Y[b]
Marssonina	5	5	5
Melanconium	7	7	7
Metarhizium	9	9	9
Microdochium	30	30	30
Monacrosporium	4	4	4
Monilia	4	4	4
Monochaetia	2	2	2
Monocillium	1	1	1
Monostichella	1	1	1
Monotosporella	1	1	1
Myceliophthora	1	1	1
Mycocentrospora	2	2	2
Mycovellosiella	2	2	2
Myrothecium	11	11	11
Myxosporium	1	1	1
Naranus	1	1	1
Nigroporus	1	1	1
Nigrospora	31	31	31
Nodulisporium	3	3	3
Nomuraea	3	3	3
Ochroconis	5	5	5
Oedocephalum	1	1	1
Oidiodendron	10	10	10
Paecilomyces	11	11	11
Papulaspora	3	3	3
Paracercospora	2	2	2
Penicillium	9	9	9
Periconia	5	5	5
Pestalotia	17	17	17
Pestalotiopsis	107	107	107
Phaeoisariopsis	3	3	3
Phaeoseptoria	4	4	4
Phialomyces	1	1	1
Phialophora	17	17	17
Phloeospora	2	2	2
Phoma	40	40	40
Phomopsis	84	84	84
Phyllosticta	30	30	30
Pithomyces	6	6	6
Pleiochaeta	2	2	2
Pseudocercospora	74	74	74
Pseudocercosporella	37	37	37
Pseudoseptoria	1	1	0
Pyrenochaeta	30	30	30
Pyricularia	111	111	111
Racodium	1	1	1
Raffaelea	3	3	3

Table 1. continued.

Fungal genus	Tested[a]	1 M[b]	1 Y[b]
Ramichloridium	12	12	12
Ramularia	2	2	2
Rhizoctonia	241	234	227
Rhizosphaera	9	9	9
Rhynchosporium	7	7	7
Robillarda	2	2	2
Sarocladium	1	1	1
Sclerotium	43	43	42
Scopulariopsis	2	2	2
Seimatosporium	2	2	2
Seiridium	12	12	12
Selenophoma	3	3	3
Septogloeum	1	1	1
Septonema	2	2	2
Septoria	16	16	16
Septotis	3	3	3
Sirosporium	2	2	2
Spegazzinia	1	1	1
Sphaceloma	6	6	5
Sphaerellopsis	48	48	48
Sphaeropsis	8	8	8
Sporidesmium	6	6	6
Stachybotrys	2	2	2
Stagonospora	4	4	4
Staphylotrichum	3	3	3
Stemphylium	10	10	10
Stigmina	6	6	6
Strasseriopsis	2	2	2

Table 1. continued.

Fungal genus	Tested[a]	1 M[b]	1 Y[b]
Taeniollela	1	1	1
Tetracladium	2	2	2
Thielaviopsis	3	3	3
Thysanophora	1	1	1
Ticogloea	1	1	1
Torula	4	4	4
Torulomyces	1	1	1
Trichocladium	1	1	1
Trichoderma	28	28	28
Trichothecium	4	4	4
Trinacrium	1	1	1
Tripospermum	1	1	1
Tritirachium	1	1	1
Trochophora	7	7	7
Truncatella	1	1	1
Tubakia	4	4	4
Tubercularia	3	3	3
Ulocladium	6	6	6
Ustilaginoidea	2	1	1
Verticillium	152	152	152
Volutella	2	2	2
Wallemia	1	1	1
Total	5,651	5,503	5,406

[a] Number of strains tested.

[b] Number of strains surviving for a period indicated, 1 month (1M) or 1 year (1Y).

Table 2. Fungal species not showing 100% recovery

Fungal species	Tested[a]	1 M[b]	1 Y[b]	Recovery[c]
Oomycota				
Achlya americana	2	0	0	0
Achlya caroliniana	1	0	0	0
Achlya conspicua	2	0	0	0
Achlya flagellata	6	1	0	0
Achlya klebsiana	1	0	0	0
Achlya sp.	2	0	0	0
Achlya stellata	1	0	0	0
Aphanomyces cladogamus	1	0	0	0
Aphanomyces cochlioides	3	1	1	33
Aphanomyces euteiches	1	0	0	0
Dictyuchus anomalus	2	0	0	0
Dictyuchus carpophorus	1	0	0	0
Dictyuchus pseudoachlyoides	1	0	0	0
Dictyuchus sterile	2	0	0	0

Table 2. continued.

Fungal species	Tested[a)]	1 M[b)]	1 Y[b)]	Recovery[c)]
Phytophthora cactorum	7	6	6	86
Phytophthora capsici	5	2	2	40
Phytophthora infestans	15	11	11	73
Phytophthora melonis	1	0	0	0
Phytophthora nicotianae	35	32	27	77
Phytophthora porri	2	1	0	0
Phytophthora sp.	15	13	10	67
Phytophthora vignae	6	5	4	67
Pythium aphanidermatum	21	14	11	52
Pythium apleroticum	1	0	0	0
Pythium carolinianum	6	3	2	33
Pythium dissimile	1	0	0	0
Pythium dissotocum	7	5	5	71
Pythium echinulatum	5	4	2	40
Pythium elongatum	4	3	2	50
Pythium graminicola	12	10	7	58
Pythium hydnosporum	4	1	1	25
Pythium inflatum	6	4	4	67
Pythium intermedium	9	8	8	89
Pythium irregulare	28	26	24	86
Pythium myriotylum	1	0	0	0
Pythium oedochilum	7	2	0	0
Pythium periplocum	2	1	1	50
Pythium rostratum	9	5	5	56
Pythium sp.	7	4	4	57
Pythium spinosum	23	15	11	48
Pythium splendens	9	3	0	0
Pythium sylvaticum	10	10	8	80
Pythium torulosum	25	18	16	64
Pythium ultimum	27	14	11	41
Pythium vexans	4	0	0	0
Thraustotheca clavata	4	1	1	25
Zygomycota				
Choanephora infundibulifera	2	1	1	50
Ascomycota				
Cistella japonica	19	19	16	84
Gremmeniella abietina	9	9	8	89
Halosphaeria appendiculata	18	18	17	94
Morchella esculenta	3	3	2	67
Peziza ostracoderma	2	2	0	0
Peziza vesiculosa	2	1	1	50
Remispora stellata	16	16	15	94
Sclerotinia sclerotiorum	29	29	28	97
Tricholoma bakamatsutake	10	10	9	90
Tricholoma matsutake	59	59	56	95
Basidiomycota				
Amanita muscaria	1	1	0	0

Table 2. continued.

Fungal species	Tested[a]	1 M[b]	1 Y[b]	Recovery[c]
Armillaria mellea (sensu lato)	41	40	40	98
Armillaria sp.	2	1	1	50
Auricularia auricula	4	4	3	75
Auricularia polytricha	11	9	8	73
Ceratobasidium cornigerum	8	6	6	75
Ceratobasidium setariae	9	9	8	89
Ceratobasidium sp.	22	21	13	59
Coprinus atramentarius	1	1	0	0
Eichleriella leucophaea	10	10	8	80
Erythricium salmonicolor	18	17	17	94
Ganoderma applanatum (sensu lato)	9	8	8	89
Ganoderma lucidum	6	6	5	83
Gloeoporus dichrous	2	1	1	50
Grifola frondosa	8	8	7	88
Hebeloma spoliatum	2	2	1	50
Helicobasidium mompa	12	12	11	92
Inonotus obliquus	4	4	3	75
Ischnoderma resinosum	4	2	2	50
Laccaria laccata	1	1	0	0
Lepista nuda	3	2	2	67
Leucoagaricus rubrotinctus	1	0	0	0
Leucopaxillus septentrionalis	2	2	1	50
Lyophyllum sykosporum	1	1	0	0
Oudemansiella pudens	2	2	1	50
Oudemansiella radicata	7	7	6	86
Phaeolepiota aurea	3	3	2	67
Pholiota lucifera	1	1	0	0
Pleurotus eugrammus	1	0	0	0
Polyporus badius	1	1	0	0
Protodaedalea hispida	2	2	1	50
Psilocybe argentipes	3	3	2	67
Rigidoporus ulmarius	2	2	1	50
Ustilago esculenta	7	6	6	86
Volvariella volvacea	1	1	0	0
Anamorphic fungi				
Cercospora citrullina	2	2	1	50
Cercospora corchori	1	1	0	0
Pseudoseptoria donacis	1	1	0	0
Rhizoctonia solani	160	156	150	94
Rhizoctonia sp. (binucleate)	64	63	62	97
Rhizoctonia sp.	6	4	4	67
Sclerotium rolfsii	29	29	28	97
Sphaceloma sp.	3	3	2	67
Ustilaginoidea virens	2	1	1	50

[a] Number of tested strains.
[b] Number of strains surviving for a period indicated, 1 month (1M) or 1 year (1Y).
[c] Percentage of strains surviving during the 1-year storage.

"Fluctuation" has been reported in the preservation of some strains : i.e., some fungal strains were not revived after storage for a certain period, but could be occasionally revived later after longer storage (2). However, information about the phenomenon was lost on data processing, because Tables 1 and 2 were made based on the longest periods of successful storage for each strain (4). An example of "fluctuation" in our study was *Phytophthora infestans* MAFF 236317, which had 10% viability after 1 month storage, but which showed 80% viability after 1 year. The cause of the "fluctuation" remains to be elucidated. One cause may be experimental error, because repeated examinations often show normal viabilities. Thus, replication of stocks is important to avoid errors or reporting "fluctuation."

Cryopreservation after uncontrolled freezing was shown to be effective and useful for the storage of many fungal strains. This supports the use of the cryopreservation in the MAFF Genebank. Longevity of the strains preserved by the cryopreservation in the Genebank will be surveyed further.

Acknowledgment. The authors wish to thank the personnel of the MAFF Genebank for their assistance.

REFERENCES

1. Hawksworth, D. L., Kirk, P. M., Sutton, B. C. and Pegler, D. N. Ainsworth & Bisby's dictionary of the fungi, 8th ed., CAB International, Oxfordshire (1995).
2. Ito, T. and Yokoyama, T. Further investigation on the preservation of basidiomycete cultures by freezing. IFO Res. Comm. **13** : 69-81 (1987).
3. Miki, N. and Kubomura, Y. Preliminary trials of freeze-preservation of fungi for long term storage. Misc. Publ. Natl. Inst. Agrobiol. Resour. **5** : 1-29 (1993) (in Japanese).
4. Nagai, T. A simple program for summarizing viability of stored resources. submitted for publication.
5. Nishii, T. and Nakagiri, A. Cryopreservation of oomycetous fungi in liquid nitrogen. IFO Res. Comm. **15** : 105-118 (1991).
6. Smith, D. Liquid nitrogen storage of fungi. Trans. Br. Mycol. Soc. **79** : 415-421 (1982).
7. Smith, D. Maintenance of filamentous fungi, *In* Kirsop, B.E. and Doyle, A. (eds.), Maintenance of Microorganisms and Culture Cells : A Manual of Laboratory Methods, 2nd ed., p. 133-159, Academic Press, London (1991).
8. Smith, D. and Onions, A.H.S. The preservation and maintenance of living fungi, 2nd ed., CAB International, Oxfordshire (1994).
9. Tsuchiya, Y. Investigation of preservation methods of plant pathogenic fungi. Jpn. J. Freez. Dry. **32** : 64-69 (1986) (in Japanese).

未制御で凍結した糸状菌類の液体窒素気相中での保存

永井利郎，井手野亜伊香，柘植倫代，小柳千栄，鬼木正臣，喜多孝一，
堀田光生，青木孝之，小林享夫，土屋健一

農林水産省農業生物資源研究所

農林水産省ジーンバンクでは，糸状菌類（卵菌類を含む）を冷却速度を制御しない方法で凍結した後，−165°Cの液体窒素気相中に保存してきた．保存されている 466 属 1,182 種にわたる 5,651 菌株の生残率を調べた結果，1 年間安定に保存できた菌株数は 5,406 株（95.7 %）であった．1 年間の保存が可能であった菌株の割合は，卵菌門に属する菌株では 59.3 %（233/393 供試菌株数），接合菌門 98.3 %（58/59），子嚢菌門 98.8 %（881/892），担子菌門 95.4 %（1,100/1,153），不完全菌類 99.4 %（3,134/3,154）となった．*Achlya*，*Dictyuchus*（卵菌門），*Volvariella*（担子菌門）および *Pseudoseptoria*（不完全菌類）属の糸状菌類には本法は適用できなかった．