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SURVIVAL OF FROZEN CULTURES OF OOMYCETOUS FUNGI AFTER 12 AND 24 MONTHS' STORAGE IN LIQUID NITROGEN

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Summary

The viability of 171 strains of oomycetous fungi preserved in liquid nitrogen was examined serially for two years. Immediately after freezing and after 6 months' storage, 165 strains and 164 strains were recovered respectively. After 12 months' storage, 164 of 168 strains examined were found to have survived. After 24 months' storage, 143 of 149 strains were recovered. Overall, survival rates of more than 96% were achieved in the serial recovery tests.

Keywords: cryopreservation, liquid nitrogen, Oomycetes

The viability of 171 frozen cultures of oomycetous fungi (mainly Phytophthora and Pythium species) maintained in the IFO culture collection were previously examined immediately after freezing and after 6 months' storage in liquid nitrogen (2). In the present report, the survival of the preserved cultures was further examined after 12 months' and 24 months' storage. Survival rates in the serial examinations were evaluated and the effectiveness of freezing storage in liquid nitrogen was discussed for long-term preservation of oomycetous fungi.

Materials and Methods

Strains examined and procedures for preculturing, preparation of agar discs with mycelium, freezing tubes and cryoprotectant, and pro-

grammed freezing were the same as those described for Experiment 2 in the previous report (2).

One handred and seventy-one strains of oomycetous fungi preserved in IFO were used for the examination. All strains were precultured on the appropriate agar media at a suitable temperature one to two weeks. Agar discs containing mycelium were removed from the edge of the fungal colony with plastic tubes 8 mm in diamteter. Two agar discs were put in a cryotube containing 0.7 ml of 10% glycerol. Four tubes were prepared for each strain. The tubes were frozen in a programmable freezer at the cooling rate 1 C/min until -40 C and at 2 C/min from -40 to -80 C. Following the previous examinations immediately after freezing and after 6 months' storage, survival was examined after 12 months' and 24 months' strage. Thawing of frozen cultures was carried out by immersing the tubes in water at 30 C for 5 min. The thawed agar discs were incubated on agar plates of the appropriate media at a suitable temperature for one to two weeks.

Results and Discussion

Viability of the frozen cultures after 12 and 24 months' storage was compiled with the survival data immediately after freezing and after 6 months' storage (Table 1).

After 12 months' storage, 4 of 168 strains, namely Phytophthora sp. IFO 30635, Py. porphyrae IFO 30347, IFO 30800, and IFO 30801, failed to survive. One of the two agar discs in a frozen tube survived in 5 strains: Py. graminicola IFO 31997, Py. periplocum IFO 31933, Pythium sp. IFO 32197, Py. sylvaticum IFO 32198, and Saprolegnia parasitica IFO 8978. After 24 months' storage, 6 of 149 strains, namely, Phy. infestans IFO 9173, Phytophthora sp. IFO 30635, Py. aristosporum IFO 32219, Py. porphyrae IFO 30800, IFO 30801, Py. zingiberum IFO 30817, were completely dead; and 4 strains, Phy. syringae IFO 31089, Phy. infestans IFO 9174, Py. graminicola IFO 31997, Py. porphyrae IFO 30347, were partly lost (Table 1).

In the serial recovery tests, Phytophthora sp. IFO 30635 and Py. porphyrae IFO 30347, IFO 30800, IFO 30801 showed comparatively low viability in liquid nitrogen storage, but there was no strain that was com-

Table 1. Viability of oomycetous fungi immediately after freezing and after storage for 6 months, 12 months, and 24 months in liquid nitrogen.

Species	IFO No.	0 M[a]	6 M[b]	12M[c]	24M[d]	Med.No.	Temp.(C)
Phytophthora cactorum (Lebert & Cohn) Schroter	30474	+ +	+ +	+ +	+ +	1	24
Phy. cactorum	31084	+ +	+ +	+ +	+ +	1	24
Phy. cactorum	31151	+ +	+ +	+ +	+ +	1	24
Phy. cactorum	32191	+ +	+ +	+ +	ND	1	24
Phy. cactorum	32192	+ +	+ +	+ +	+ +	1	24
Phy. cactorum	32193	+ +	+ +	+ +	+ +	1	24
Phy. cactorum	32194	+ +	+ +	+ +	+ +	1	24
Phy. cambivora (Petri) Buisman	30471	+ +	+ +	+ +	+ +	1	24
Phy. cambivora	30472	+ +	+ +	+ +	+ +	1	24
Phy. cambivora	30714	+ +	+ +	+ +	+ +	1	24
Phy. cambivora	30715	+ +	+ +	+ +	+ +	1	24
Phy. capsici Leonian	8386	+ +	+ +	+ +	+ +	1	24
Phy. capsici	9752	- -	+ +	+ +	+ +	1	24
Phy. capsici	30696	+ +	+ +	+ +	+ +	1	24
Phy. capsici	30697	+ +	+ +	+ +	+ +	1	24
Phy. capsici	30698	+ +	+ +	+ +	+ +	1	24
Phy. capsici	30699	+ +	+ +	+ +	+ +	1	24
Phy. capsici	31400	+ +	+ +	+ +	ND	1	24
Phy. capsici	31402	+ +	+ +	+ +	+ +	1	24
Phy. citricolor Sawada	31017	+ +	+ +	+ +	+ +	1	24
Phy. citrophthora (Smith & Smith) Leonian	31408	+ +	+ +	+ +	+ +	1	24
Phy. citrophthora	31410	+ +	+ +	+ +	+ +	1	24
Phy. colocasiae Raciborski	30695	+ +	+ +	+ +	+ +	1	24
Phy. cryptogea Pethybridge & Lafferty	31411	+ +	+ +	+ +	+ +	1	24
Phy. cryptogea	31412	+ +	+ +	+ +	+ +	1	24
Phy. cryptogea	31622	+ +	+ +	+ +	ND	1	24
Phy. drechsleri Tucker	31085	+ +	+ +	+ +	+ +	1	24
Phy. drechsleri	31153	+ +	+ +	+ +	+ +	1	24
Phy. drechsleri	31154	+ +	+ +	+ +	+ +	1	24
Phy. erythroseptica Pethybridge	31152	+ +	+ +	+ +	+ +	1	24
Phy. fragariae Hickman	31086	+ +	+ +	+ +	+ +	1	24
Phy. infestans (Montagne) de Bary	9173	+ +	+ +	+ +	- -	1	24
Phy. infestans	9174	+ +	+ +	+ +	+ -	1	24
Phy. katsurae Ko & Chang	9753	+ +	+ +	+ +	+ +	1	24
Phy. katsurae	30433	+ +	+ +	+ +	+ +	1	24
Phy. katsurae	30434	+ +	+ +	+ +	+ +	1	24
Phy. katsurae	30435	+ +	+ +	+ +	+ +	1	24
Phy. macrospora (Saccardo) Ito & Tanaka	9049	+ +	+ +	+ +	+ +	1	24
Phy. megasperma Drechsler	31623	+ +	+ +	+ +	+ +	1	24
Phy. megasperma	31624	+ +	+ +	+ +	ND	1	24
Phy. megasperma	32174	+ +	+ +	+ +	+ +	1	24
Phy. megasperma	32175	+ +	+ +	+ +	+ +	1	24
Phy. megasperma	32176	+ +	+ +	+ +	ND	1	24
Phy. megasperma Drechsler var. sojae Hildebrand	31014	+ +	+ +	+ +	+ +	1	24
Phy. megasperma var. sojae	31015	+ +	+ +	+ +	+ +	1	24
Phy. megasperma var. sojae	31016	+ +	+ +	+ +	+ +	1	24

Table 1. (continued)

Species	IFO No.	0 M[a]	6 M[b]	12M[c]	24M[d]	Med.No.	Temp.(C)
Phytophthora melonis Katsura	31413	+ +	+ +	+ +	+ +	1	24
Phy. melonis	31414	+ +	+ +	+ +	+ +	1	24
Phy. melonis	31415	+ +	+ +	+ +	+ +	1	24
Phy. nicotianae van Breda de Haan var. nicotianae	4873	+ +	+ +	+ +	+ +	1	24
Phy. nicotianae van Breda de Haan var. parasitica (Dastur) Waterhouse	30595	+ +	+ +	+ +	+ +	1	24
Phy. nicotianae var. parasitica	30716	+ +	+ +	+ +	+ +	1	24
Phy. nicotianae var. parasitica	30810	+ +	+ +	+ +	+ +	1	24
Phy. nicotianae var. parasitica	30811	+ +	+ +	+ +	+ +	1	24
Phy. nicotianae var. parasitica	31018	+ +	+ +	+ +	+ +	1	24
Phy. nicotianae var. parasitica	31019	+ +	+ +	+ +	+ +	1	24
Phy. nicotianae var. parasitica	31020	+ +	+ +	+ +	+ +	1	24
Phy. nicotianae var. parasitica	31021	+ +	+ +	+ +	+ +	1	24
Phy. nicotianae var. parasitica	31416	+ +	+ +	+ +	+ +	1	24
Phy. nicotianae var. parasitica	31419	+ +	+ +	+ +	+ +	1	24
Phy. nicotianae var. parasitica	31423	+ +	+ +	+ +	+ +	1	24
Phy. nicotianae var. parasitica	31425	+ +	+ +	+ +	+ +	1	24
Phy. palmivora (Butler) Butler	9755	+ +	+ +	+ +	+ +	1	24
Phy. palmivora	30285	+ +	+ +	+ +	+ +	1	24
Phy. palmivora	30812	- -	+ +	+ +	ND	1	24
Phy. palmivora	30813	+ +	+ +	+ +	+ +	1	24
Phy. palmivora	31428	+ +	+ +	+ +	+ +	1	24
Phy. porri Foister	30416	+ +	+ +	+ +	+ +	1	24
Phy. porri	30417	+ +	+ +	+ +	+ +	1	24
Phy. porri	30418	+ +	+ +	+ +	+ +	1	24
Phy. sp.	30635	+ -	- -	- -	- -	1	24
Phy. sp.	30636	+ +	+ +	+ +	+ +	1	24
Phy. sp.	30637	+ +	+ +	+ +	+ +	1	24
Phy. sp.	30638	+ +	+ +	+ +	+ +	1	24
Phy. sp.	30639	+ +	+ +	+ +	+ +	1	24
Phy. sp.	30640	+ +	+ +	+ +	+ +	1	24
Phy. sp.	30641	+ +	+ +	+ +	+ +	1	24
Phy. sp.	30642	+ +	+ +	+ +	+ +	1	24
Phy. syringae Klebahn	31087	+ +	+ +	+ +	+ +	1	24
Phy. syringae	31088	+ +	+ +	+ +	+ +	1	24
Phy. syringae	31089	+ +	+ +	+ +	+ -	1	24
Phy. vignae Purss	30473	+ +	+ -	+ +	+ +	1	24
Phy. vignae	30613	+ +	+ +	+ +	+ +	1	24
Phy. vignae	31026	+ +	+ +	+ +	+ +	1	24
Phy. vignae	31027	+ +	+ +	+ +	+ +	1	24
Phy. vignae	31028	+ +	+ +	+ +	+ +	1	24
Phy. vignae	31029	+ +	+ +	+ +	+ +	1	24

Table 1. (continued)

Species	IFO No.	0 M[a]	6 M[b]	12M[c]	24[d]	Med.No.	Temp.(C)
Pythium afertile Kanouse & Humphrey	32195	+ +	+ +	+ +	ND	1	24
Py. aphanidermatum (Edson) Fitzpatrick	7030	+ +	+ +	+ +	+ +	8	24
Py. aristosporum Vanterpool	32219	+ +	+ +	+ +	- -	1	24
Py. butleri Subramaniam	31214	+ +	+ +	+ +	+ +	1	24
Py. debaryanum	7211	+ +	+ +	+ +	+ +	1	24
Py. debaryanum Hesse var. pelargonii H. Braun	5919	+ +	+ +	+ +	+ +	8	24
Py. dissotocum Drechsler	32196	+ +	+ +	+ +	ND	1	24
Py. gracile Schenk	30819	+ +	+ +	+ +	+ +	1	37
Py. graminocola Subramaniam	31996	+ +	+ +	+ +	+ +	1	24
Py. graminocola	31997	+ +	+ -	+ -	+ -	1	24
Py. graminocola	31998	+ +	+ -	+ +	+ +	1	24
Py. irregulare Buisman	7220	+ +	+ +	+ +	+ +	8	24
Py. irregulare	30346	+ +	+ +	+ +	+ +	8	24
Py. irregulare	32072	+ +	+ +	+ +	+ +	8	24
Py. irregulare	32073	+ +	+ +	+ +	+ +	8	24
Py. iwayamai S. Ito	31990	+ +	+ +	+ +	+ +	1	24
Py. iwayamai	31991	+ -	- -	+ +	ND	1	24
Py. iwayamai	31992	+ +	+ +	+ +	+ +	1	24
Py. myriotylum Drechsler	31022	- -	+ +	+ +	+ +	1	24
Py. oedochilum Drechsler	7218	+ +	+ +	+ +	+ +	1	24
Py. okanoganense Lipps	31921	+ +	+ +	+ +	+ +	1	24
Py. okanoganense	31922	+ +	+ +	+ +	+ +	1	24
Py. okanoganense	31941	+ +	+ +	+ +	+ +	1	24
Py. paddicum Hirane	31993	+ +	+ +	+ +	+ +	1	24
Py. paddicum	31994	+ +	+ +	+ +	+ +	1	24
Py. paddicum	31995	+ +	+ +	+ +	+ +	1	24
Py. periplocum Drechsler	31933	- -	+ +	+ -	ND	1	24
Py. porphyrae Takahashi & Sasaki apud Takahashi et al.	30347	+ +	- -	- -	+ -	12	24
Py. porphyrae	30800	+ +	+ -	- -	- -	12	24
Py. porphyrae	30801	+ +	- -	- -	- -	12	24
Py. sp.	32197	+ +	+ +	+ -	ND	1	24
Py. spinosum Sawada	7031	+ +	+ +	+ +	+ +	8	24
Py. spinosum	7193	+ +	+ +	+ +	+ +	8	24
Py. spinosum	7194	+ +	+ +	+ +	+ +	8	24
Py. spinosum	7195	+ +	+ +	+ +	+ +	8	24
Py. spinosum	7196	+ +	+ +	+ +	+ +	8	24
Py. spinosum	7197	+ +	+ +	+ +	+ +	8	24
Py. spinosum	7198	+ +	+ +	+ +	+ +	8	24
Py. spinosum	7199	+ +	+ +	+ +	+ +	8	24
Py. spinosum	7200	+ +	+ +	+ +	+ +	8	24
Py. spinosum	7201	+ +	+ +	+ +	+ +	8	24
Py. spinosum	7202	+ +	+ +	+ +	+ +	8	24
Py. spinosum	7203	+ +	+ +	+ +	+ +	8	24
Py. spinosum	7204	+ +	+ +	+ +	+ +	8	24

Table 1. (continued)

Species	IFO No.	0 M[a)]	6 M[b)]	12M[c)]	24M[d)]	Med.No.	Temp.(C)
Pythium spinosum Sawada	7205	+ +	+ +	+ +	+ +	8	24
Py. spinosum	7206	+ +	+ +	+ +	+ +	8	24
Py. spinosum	7207	+ +	+ +	+ +	+ +	8	24
Py. spinosum	7208	+ +	+ +	+ +	+ +	8	24
Py. spinosum	7209	+ +	+ +	+ +	+ +	8	24
Py. spinosum	7210	+ +	+ +	+ +	+ +	8	24
Py. spinosum	32212	+ +	+ +	+ +	ND	8	24
Py. spinosum	32213	+ +	+ +	+ +	ND	8	24
Py. spinosum	32214	+ +	+ +	+ +	+ +	8	24
Py. sylvaticum Campbell & Hendrix	31942	+ +	+ +	+ +	+ +	1	24
Py. sylvaticum	31943	+ +	+ +	+ +	+ +	1	24
Py. sylvaticum	32198	+ +	+ +	+ -	ND	1	24
Py. torulosum Coker & Patterson	32166	+ +	+ +	+ +	+ +	1	24
Py. torulosum	32167	+ +	+ +	+ +	+ +	1	24
Py. torulosum	32168	+ +	+ +	+ +	+ +	1	24
Py. ultimum Trow	7212	+ +	+ +	+ +	+ +	8	24
Py. ultimum	7213	+ +	+ +	+ +	+ +	8	24
Py. ultimum	7214	+ +	+ +	+ +	+ +	8	24
Py. ultimum	7215	+ +	+ +	+ +	ND	8	24
Py. ultimum	7216	+ +	+ +	+ +	+ +	8	24
Py. ultimum	7217	+ +	+ +	+ +	+ +	8	24
Py. ultimum Trow var. ultimum	32210	+ +	+ +	+ +	ND	8	24
Py. ultimum	32211	+ +	+ +	+ +	ND	8	24
Py. vanterpoolii V. Kouyeas & H. Kouyeas	31923	+ +	+ +	+ +	+ +	1	24
Py. vanterpoolii	31924	+ +	+ +	+ +	+ +	1	24
Py. vanterpoolii	31925	+ +	+ +	+ +	+ +	1	24
Py. vanterpoolii	32169	+ +	+ +	+ +	+ +	1	24
Py. vanterpoolii	32170	+ +	+ +	+ +	+ +	1	24
Py. vanterpoolii	32171	+ +	+ +	+ +	+ +	1	24
Py. vexans de Bary	7221	+ +	+ +	+ +	+ +	1	24
Py. volutum Vanterpool & Truscott	31926	+ +	+ +	+ +	+ +	1	24
Py. volutum	31927	+ +	+ +	+ +	+ +	1	24
Py. volutum	31928	+ +	+ +	+ +	+ +	1	24
Py. zingiberum Takahashi	30817	+ +	+ +	+ +	- -	1	34
Py. zingiberum	30818	- -	+ +	+ +	ND	1	34
Saprolegnia parasitica Coker	8978	+ +	+ +	+ -	ND	1	24
Aphanomyces iridis Ichitani & Kodama	31934	+ +	+ +	ND	ND	14	24
Ap. iridis	31935	+ +	+ +	ND	ND	14	24
Ap. iridis	31936	+ +	+ +	ND	ND	14	24
Halophytophthora vesicula Anastasiou & Churchland	32216	- -	+ +	+ +	+ +	1	24

+ : viable
- : non-viable (signs indicate the viability of each of two agar discs)
a) : immediately after freezing
b) : 6 months after freezing
c) : 12 months after freezing
d) : 24 months after freezing
ND : No Data

pletely lost in the all recovery tests. The total survival rate in each recovery test was calculated (Table 2) and found to be constant at higher than 96% during two years of storage.

Table 2. The survival rates of frozen oomycetous cultures examined serially for two years.

	Storage period (months)			
	0	6	12	24
Strains examined	171	171	168	149
Strains surviving[a)]	165	164	164	143
Survival rate (%)	96.5	96.0	97.6	96.0

a) : numbers include both completely and partly surviving strains.

More than 96% of 149 strains of oomycetous fungi were succesfully stored for 24 months in liquid nitrogen. This suggests that cryopreservation in liquid nitrogen is equally as effective for oomycetous fungi as for other fungal groups (1, 4, 5). Of the unsuccessful strains, some survived freezing but did not survive or only partly survived 6 months, 12 months, 24 months of storage, while others that had shown no or partial survival immediately after freezing or after 6 months' or 12 months' storage showed good recovery in later tests. The former phenomenon, which was found in 12 strains, may indicate that storage period affects the viability of frozen cultures. On the other hand, the latter was observed for 10 strains and may suggest that the conditions of the agar discs for freezing may affect their survival rate (2, 3), rather than the period of storage. Further studies are necessary to clarify whether the freezing storage of oomycetous fungi in liquid nitrogen is effective for long-term preservation. However, since high survival rates were achieved over at least two years in this examination, it appears that with further improvements, e.g., in preculturing, cryoprotectants, freezing method etc., in addition to those already acheived by the authors in the freezing rate and the freezing tube case (3), liquid nitrogen storage

has the potential to be practically useful for the safe and long-term preservation of a wide range of oomycetous cultures.

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