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CRYOPRESERVATION OF OOMYCETOUS FUNGI IN LIQUID NITROGEN

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Summary

Liquid nitrogen storage was examined as a means of safe, long-term preservation of oomycetous fungi in the Institute for Fermentation, Osaka (IFO). Agar discs with mycelium of the fungal culture were frozen at a constant cooling rate in a programmable freezer. In a preliminary study, eight strains of Phytophthora and two strains of Pythium were successfully preserved for two years by using 10% glycerol or 10% dimethylsulfoxide (DMSO) as a cryoprotectant. The viability of 171 strains of oomycetous fungi preserved in liquid nitrogen was examined. Immediately after freezing, 165 strains were recovered. After six months storage, 164 strains survived. The eight strains that were lost or partly survived in the first recovery test (four strains each of Phytophthora and Pythium) were used to examine the methods for agar disc preparation and thawing. It was found that the agar discs derived from the central part of a precultured fungal colony were stored more successfully than those from edge of the colony. Thawing the frozen agar discs at 30 C for 5 min yielded better results than thawing at 40 C for 3 min.

Keywords: cryopreservation, liquid nitrogen, Oomycetes.

Oomycetes (Mastigomycotina) have been preserved by subculturing (1, 6, 12), storing in water (6) or in liquid paraffin (1), or by freezing in an ultra-low freezer at -80 C (11, 13) or in liquid nitrogen at -196 C (1, 2, 3, 4, 5, 8, 10, 11). The Institute for Fermentation, Osaka (IFO) maintains oomycetous cultures mainly by subculturing, but also by storage in liquid paraffin and freezing at -80 C. However, about 10% of strains are not successfully recovered at each routine subculturing at three-month intervals. Freezing storage at -80 C has not given satisfactory results (13). Storage in water or liquid paraffin involves higher risk of contamination by other organisms or changes in fungal properties during storage. For safe, long-term preservation, the cryopreservation of filamentous fungi in liquid nitrogen has been widely employed. We examined the application of this method to the oomycetous strains deposited in IFO.

Materials and Methods

Three experiments were carried out.

Experiment 1. Eight strains of Phytophthora and two of Pythium (Table 1) were cultured on medium no. 1 (see below) at 24 C for 4 to 12 days. Agar discs containing mycelium were removed from the edge of the fungal colony with plastic tubes 8 mm in diameter. Two agar discs were soaked in 1 ml of each of the following three cryoprotectants in a cryotube (Nunc, 3-66656): 10% glycerol, 10% dimethylsulfoxide (DMSO), and 10% polyethylene glycol (PEG). Eighteen tubes were prepared for each strain (six for each cryoprotectant). After precooling at 5 C for 30 min, the tubes were frozen in a programmable freezer (Taiyo Sanso Ltd., CM-2) at the cooling rate 1 C/min until -40 C and at 2 C/min from -40 to -80 C (Fig. 1). This programmable freezer monitors temperature both in one freezing tube and the freezing chamber, and controls the temperature according to the cooling program by automatically supplying liquid nitrogen to the freezing chamber. This system is possibly able to reduce the injurious effect on frozen cultures caused by latent heat generated at the freezing point. Frozen tubes were submerged in liquid nitrogen and stored for up to 2 years. Recovery tests were carried out at immediately

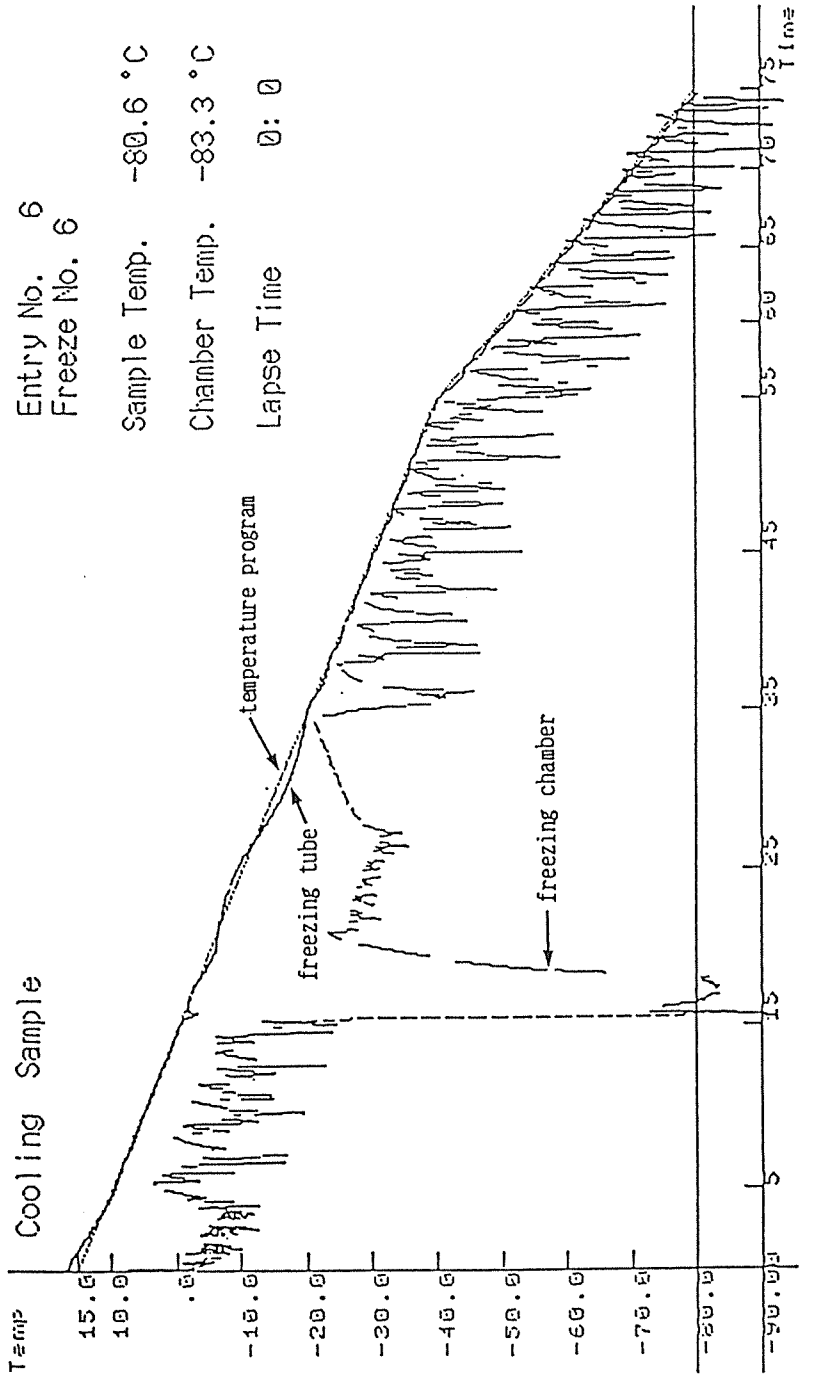

Fig. 1. Temperature change during freezing with programing freezer.

after freezing, and 1 month, 6 months, 12 months, and 24 months after freezing. For thawing the cultures, the lower half of the cryotubes were immersed in water at 40 C and agitated for 3 min. The thawed agar disks were incubated on agar plates of medium no. 1 at 24 C for one week.

Experiment 2. One hundred and seventy-one strains of oomycetous fungi preserved in IFO (24 species, 88 strains of Phytophthora; 26 species, 79 strains of Pythium; 1 species, 1 strain of Saprolegnia; 1 species, 3 strains of Aphanomyces) (Table 2) were examined for their suitability for cryopreservation in liquid nitrogen. All strains were precultured on the appropriate agar media at a suitable temperature (see Table 2 and below for the medium contents). Agar discs for storage were prepared in the same manner as in Exp. 1. Two discs were put in a cryotube containing 0.7 ml of 10% glycerol. Four tubes were prepared for each strain. After freezing with the programmable freezer, the tubes were stored in liquid nitrogen. Survival was examined immediately after freezing and at six months after freezing.

Experiment 3. The eight strains (four strains of each Phytophthora and Pythium) which had been lost completely or partly survived in the first recovery test at Exp. 2 and three strains of Aphanomyces iridis were used for the following examination. From the precultured colony, ten agar discs each were removed from the edge and the central part of the colony and used to prepare five tubes for each strain. Freezing was carried out in the same manner as in Exp. 2. Frozen discs were recovered three days after freezing. In the recovery test, the tubes were thawed either at 40 C for 3 min or at 30 C for 5 min.

The agar media used for preculturing fungal strains and for recovery tests were as follows:

Medium No. 1. Potato sucrose agar

200 g potato, 20 g sucrose, 20 g agar, 1000 ml distilled water, pH 5.6.

Medium No. 8. Oatmeal agar

50 g oatmeal, 20 g agar, 1000 ml distilled water

Medium No. 12. Semi-solid CMSA

20 g cornmeal, 3 g agar, 1000 ml half-strength seawater, pH 7.0-

7.5.

Medium No. 14. Corn fishmeal extract agar

20 g corn fishmeal mixture (feed for birds), 20 g agar, 1000 ml distilled water, pH 6.5.

Preparation of these media is detailed in the IFO List of Cultures, Appendix 2.

Results

In Exp. 1, all ten strains survived two years of storage in liquid nitrogen when 10% glycerol or 10% DMSO was used as cryoprotectant, whereas when 10% PEG was used, six of ten strains were not recovered even immediately after freezing (Table 1). Accordingly, Exp. 2 and 3 were carried out with 10% glycerol as cryoprotectant. In Exp. 2, the viability of 171 strains of the oomycetous fungi was examined immediately after freezing and after six months' storage (Table 2). In the first recovery test, six strains failed to survive, namely, Phy. capsici IFO 9752, Phy. palmivora IFO 30812, Phy. vesicula IFO 32216, Py. zingiberum IFO 30818, Py. myriotylum IFO 31022, and Py. periplocum IFO 31933; and only one each of the two agar discs of Phytophthora sp. IFO 30635 and Py. iwayamai IFO 31991 survived. After six months' storage, four strains were completely dead, namely, Phytophthora sp. IFO 30635, Py. porphyrae IFO 30347, 30801, and Py. iwayamai IFO 31991; and only one of the two agar discs each of Phy. vignae IFO 30473, Py. porphyrae IFO 30800, Py. graminicola IFO 31997, and 31998 survived (Table 2). In these tests, Phytophthora sp. IFO 30635 and Py. iwayamai IFO 31991 showed comparatively low viability in liquid nitrogen storage, while there was no strain that was completely dead in the both recovery tests. From the results of Exp. 3 (Table 3), it was found that thawing at 30 C gave higher recovery in most strains than thawing at 40 C. In addition, discs derived from the central part of the colony showed higher survival rate than those from the edge of the colony in most cases. The differences in survival rate were more apparent in the cases of Phy. palmivora IFO 30812 thawed at 40 C, Phytophthora sp. IFO 30635 thawed at 30 C, and the three strains Pythium IFO 31022, 31933, and 30818 thawed at 30 C (Table 3). In contrast, for three strains of A. iridis, IFO 31934, 31935, and 31936, the agar discs from

Table 1. Viability of *Phytophthora* and *Pythium* strains stored in liquid nitrogen for two years using different cryoprotectants.

Species	IFO No.	10% Glycerol						10% Dimethylsulfoxide (DMSO)						10% Polyethylene Glycol (PEG)					
		0M*	1M	3M	6M	12M	24M	0M*	1M	3M	6M	12M	24M	0M*	1M	3M	6M	12M	24M
Phytophthora cactorum	30474	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Phy. capsici	30697	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Phy. combivora	30472	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Phy. nicotianae var. *nicotianae*	4873	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Phy. nicotianae var. *parasitica*	30595	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Phy. palmivora	9755	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	-
Phy. porri	30417	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Phy. vignae	30613	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Pythium butleri	31214	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Py. zingiberum	30817	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	+

+ : viable. - : non-viable. 0M* : immediately after freezing.

Table 2. Viability of oomycetous fungi immediately after freezing and after six months' storange in liquid nitrogen.

Species	IFO No.	0 M*	6 M**	Medium No.	Temp.(C)
Phytophthora cactorum (Lebert & Cohn) Schroter	30474	+ +	+ +	1	24
Phy. cactorum	31084	+ +	+ +	1	24
Phy. cactorum	31151	+ +	+ +	1	24
Phy. cactorum	32191	+ +	+ +	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	+ +	+ +	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	+ +	+ +	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	+ +	+ +	1	24
Phy. megasperma	32174	+ +	+ +	1	24
Phy. megasperma	32175	+ +	+ +	1	24
Phy. megasperma	32176	+ +	+ +	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 2. (continued)

Species	IFO No.	0 M*	6 M**	Medium 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	- -	+ +	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. vesicura Anastasiou & Churchland	32216	- -	+ +	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 2. (continued)

Species	IFO No.	0 M*	6 M**	Medium No.	Temp.(C)
Pythium afertile Kanouse & Humphrey	32195	+ +	+ +	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	+ +	+ +	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	+ -	- -	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	- -	+ +	1	24
Py. porphyrae Takahashi & Sasaki apud Takahashi et al.	30347	+ +	- -	13	24
Py. porphyrae	30800	+ +	+ -	13	24
Py. porphyrae	30801	+ +	- -	13	24
Py. sp.	32197	+ +	+ +	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 2. (continued)

Species	IFO No.	0 M*	6 M**	Medium 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	+ +	+ +	8	24
Py. spinosum	32213	+ +	+ +	8	24
Py. spinosum	32214	+ +	+ +	8	24
Py. sylvaticum Campbell & Hendrix	31942	+ +	+ +	1	24
Py. sylvaticum	31943	+ +	+ +	1	24
Py. sylvaticum	32198	+ +	+ +	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	+ +	+ +	8	24
Py. ultimum	7216	+ +	+ +	8	24
Py. ultimum	7217	+ +	+ +	8	24
Py. ultimum Trow var. ultimum	32210	+ +	+ +	8	24
Py. ultimum	32211	+ +	+ +	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	- -	+ +	1	34
Saprolegnia parasitica Coker	8978	+ +	+ +	1	24
Aphanomyces iridis Ichitani & Kodama	31934	+ +		14	24
Ap. iridis	31935	+ +		14	24
Ap. iridis	31936	+ +		14	24

+ : viable
- : non-viable (Each sign indicates the viability of the each one of two agar discs)
* : immediately after freezing
** : six months after freezing

the edge of the colony recovered well, while all of those from the central part of the colony did not.

Discussion

More than 97% of 168 stains of oomycetous fungi were successful after six months storage in liquid nitrogen (Exp. 2). This suggests that cryopreservation in liquid nitrogen is equally as effective for oomycetous fungi as for other fungal groups. Of the unsuccessful strains, some survived freezing but not six months' storage, while others that had shown no survival immediately after freezing recovered well after six months' storage. The former finding suggests that the storage period may affect the viability of frozen cultures; and this possibility will be examined through recovery tests at 12 months and 24 months after freezing. The latter phenomenon suggests that the conditions of the agar discs for freezing, such as the age of the mycelium in the agar disc, the extent of hyphal septation, the presence or absence of oospores or other resting spores, etc., may affect their survival rate. From this point of view, we examined the survival of the agar discs of diffent ages in Exp. 3. In the strains of Phytophthora and Pythium, the agar discs from the central part of the colony (older mycelium) achieved higher survival than those from the edge of the colony (younger mycelium). This phenomenon may correspond to the fact that bacterial cells in the stationary phase are generally more tolerant to freezing or freeze-drying than those in the logarithmic growth phase (9). Aphanomyces iridis, however, showed the opposite result. We supposed more oospores or chlamydospores were produced in older mycelia of Phytophthora and Pythium and in younger mycelia of Aphanomyces than younger and older ones, respectively, but microscopically no clear difference was observed between them. The viability of the preserved cultures seems to depend significantly on the conditions of the agar disc, and requirements may differ from species to species. When preserving a new strain in liquid nitrogen, it will be necessary to examine beforehand the viability of agar discs derived from different parts of the precultured fungal colony, or to neutralize the agar disc effect by, for example, mixing agar discs from different parts of the colony for preservation.

Table 3. Viability of young and old agar discs of some Oomycetes after thawing at different temperaturs.

Species	IFO No.	Thawing at 40 C for 3 min, Agar discs derived from Colony edge					Thawing at 40 C for 3 min, Agar discs derived from Colony center					Thawing at 30 C for 5 min, Agar discs derived from Colony edge					Thawing at 30 C for 5 min, Agar discs derived from Colony center				
Phytophthora capsici	9752	--	--	--	++	+-	--	--	+-	+-	--	+-	+-	++	++	++	++	+-	++	++	++
Phy. palmivora	30812	--	--	--	--	--	++	+-	+-	+-	+-	++	++	++	++	++	++	++	++	++	++
Phy. sp.	30635	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	+-	--	+-	+-	+-
Phy. vesicula	32216	++	--	++	++	--	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
Pythium iwayamai	31991	++	+-	++	+-	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
Py. myriotylum	31022	--	--	+-	+-	--	--	--	--	--	+-	++	++	--	+-	--	++	++	++	++	++
Py. periplocum	31933	--	--	--	--	--	++	--	+-	+-	--	--	--	--	--	--	++	++	++	++	++
Py. zingiberum	30818	+-	+-	--	--	--	--	+-	++	--	--	--	--	--	--	--	++	++	++	++	++
Ahanomyces iridis	31934											++	++	++	++	++	--	--	--	--	--
Ap. iridis	31935											++	++	++	++	++	--	--	--	--	--
Ap. iridis	31936											++	++	++	++	++	--	--	--	--	--

+ : viable. - : non-viable.

The cooling rate for freezing of cultures was set at 1 C/min until -40 C and 2 C/min from -40 to -80 by using the programmable freezer. Optimal cooling rates for the various fungal groups are known to have a wide range. Morris et al. (7) reported the highest recovery after cooling at 5 to 10 C/min with several oomycete strains. However, their study was not conducted using a programmable freezer as was used in this study, so reexamination is necessary. Practically, the cooling rate permitting all strains concerned to survive should be determined before storing. As a cryoprotectant, we found 10% glycerol and 10% DMSO to be effective for liquid nitorgen storage. Further investigation of their optimal concentrations is necessary. As to the thawing procedure, immersing a frozen tube in water at 35 to 40 C has been employed widely (7, 10, 11). However, this study revealed that a temperature of 30 C for 5 min gave better survival than 40 C. Although this phenomenon may depend on the fungus concerned, thawing at the lower temperature could be used for the oomycetous fungi. Further improvement on various points is necessary for the safe, long-term preservation of a wide range of oomycetous cultures.

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