Revival of Oyster mushroom (Pleurotus spp.) strains after mineral oil preservation

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Twenty four strains of oyster mushrooms (*Pleurotus* spp.) were preserved in paraffin oil at room temperature over a period ranging from 1 year and 7 months to 10 years and 2 months. Twenty two strains could be revived by the described method, with an overall revival rate of 90 %. The mycelial morphology and vigour was unaffected after preservation and revival. Six strains had produced asexual spores in vitro before preservation and 4 of them retained these characteristics. Basidioma primordia were produced, in vitro, by 6 of the 22 strains that were revived. Ten years was the optimum period. Storage at about 24 °C did not affect the viability of the strains.

Key words: *Pleurotus* spp., mushrooms, mineral oil preservation, revival, culture characters.

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Jsou popisovány podmínky konzervace kmenů hlív přechovávaných pod parafinovým olejem při pokojové teplotě. Dále jsou popsány metody oživení kultur a jednotlivé znaky kmenů in vitro.

Fungal cultures are preserved by different methods. Optimum cooling rate, age of mycelium, lyophilization and storage under liquid nitrogen at ultra-low temperatures have facilitated long term preservation and high recovery rates. However, the hyphae of cultures belonging to mushrooms (Agaricales, Basidiomycetes) were found to be sensitive to the stress of lyophilization and cryogenic storage. Basidiomycetes cultures require specific cooling rates, cryoprotectants, age of the mycelium and storage temperature to obtain high recovery (Morrison et al. 1988). Preservation at ultra-low temperatures has been successfully employed in mushrooms in recent years (Jong and Davis 1986, Smith and Onions 1983 and Davis 1993). Empirically, cryogenic preservation is the best method for all fungi, however, it is both infrastructural and operational cost-intensive (Heckly 1978).

Alternative methods of preservation are active sub-culturing, storage in mineral oil, water or silica gel, and cryogenic preservation (Li and Chen 1981, Kobayashi 1984, Sathe and Dighe 1987). These result in varying revival rates in mushrooms. In a few cases they have proved to be useful for the retention of viability and other characteristics (Smith 1993).

The optimum recommended period for preservation by storage in mineral oil is 6–8 and 8–10 years. The cultures of three species of *Pleurotus* survived 15 to 30

years preservation in mineral oil. It has been used with a success rate of 85 % in most of the filamentous fungi. However, they had lost the ability to develop the sexual state, various structures and biochemical properties. Since mushrooms are commercially cultivated, their mycelial vigour and the development of reproductive structures are of importance.

MATERIALS AND METHODS

The method of culture preservation has been described earlier (Sathe and Dighe 1987). Each vial contained 3 mycelium-agar punches submerged in paraffin oil. These vials were wrapped in non-absorbent cotton wool, 10 vials of 1 strain formed one set, 4 to 6 such sets were placed in a polyethylene bread box, partitioned by a 20 mm thick polystyrene foam. The bread boxes were stored in a wall cupboard at about 24 °C.

The cultures were revived on Wheat (malt) Extract Agar by the following method: 38 gm of wheat was boiled in 1 litre of water for two hours and allowed to settle overnight. It was filtered through polypropylene (screen printing mesh) fabric. The volume was made up to 1 litre, 17.5 gm of Agar powder was added to it and the pH was adjusted to 5.8. The mixture was melted and 20–25 ml was dispensed in 25 × 150 mm borosilicate glass test tubes, plugged with non-absorbent cotton wool and sterilized at 15 psi for 20 minutes. Agar slopes with ca. 2 square inch surface were prepared. MEA-mycelium punches preserved under paraffin oil were examined and those with a visible mycelium were transferred to the above revival medium. The cultures were incubated at room temperature of about 24 °C and ambient humidity conditions 60–80 %. Mycelial growth was recorded after 1 week and every 3 days thereafter to record development of spores and basidioma primordia.

Source of the strains

42 strains of *Pleurotus* exist in our culture collection, from which 24 representative strains from various sources were selected. These sources were Centraalbureau voor Schimmelcultures, Baarn, The Netherlands (CBS 411.71, 593.82 and 804.85), Building Research Establishment, Princess Risborough, U. K. (BRE-66), Indian Agricultural Research Institute, New Delhi, India (ITCC–1724, 1725, 2572, 3046 and 3049), Punjab Agricultural University, Ludhiana, India (PAU) and forests in a 200 square kilometre area around this laboratory which were surveyed for *Pleurotus* species. The specimens were isolated in pure culture on MEA and identified up to species level ('wild' and from 'local' source of commercial cultivation) as listed in Table 1.

Table 1. Performance of *Pleurotus* strains during revival

Species	Strain No.	Source	Period Y, m	Characters on Revival[c]		
				Mycelium	Fruiting	Spores
P. cystidiosus	01	Wild	10, 2	(–)	(–)	(–)
P. cystidiosus	41	Wild	8, 9	++	(–)	+[a]
P. cystidiosus	251	Wild	2, 7	+++	(–)	+++[a]
P. cystidiosus	267	Wild	1, 6	++	(–)	(–)
P. milleri	57	Wild	8, 6	++	(–)	+++[a]
P. dryinus	196	CBS804.85	6, 4	+++	(–)	+[b]
P. eous	253	Wild	2, 7	++++	(–)	(–)
P. eous	263	Wild	1, 7	(–)	(–)	(–)
P. flabellatus	27	ITCC-1724	9, 3	++++	+++	(–)
P. flabellatus	34	Wild	9	+++	(–)	(–)
P. flabellatus	64	RRL	8, 6	++++	(–)	(–)
P. flabellatus	266	Wild	1, 7	++++	+	(–)
P. eryngii	29	ITCC-3046	9, 4	++	(–)	(–)
P. euosmus	145	BRE-66	7, 6	+[d]	(–)	(–)
P. ostreatus	25	ITCC-2572	9, 3	++++	(–)	(–)
P. ostreatus	70	CBS411.71	8, 4	++++	(–)	(–)
P. ostreatus	97	PAU	8, 3	++++	(–)	(–)
P. ostreatus var. floridanus	72	CBS593.82	8, 4	++++	(–)	(–)
P. ostreatus cv. 'florida'	96	PAU	8, 3	++++	(–)	(–)
P. pulmonarius	03	LOCAL	10, 2	++++	+	(–)
P. sajor caju	28	ITCC-1725	9, 3	++++	+++	(–)
P. sajor caju	95	PAU	8, 3	++++	+++	(–)
P. sajor caju	214	ITCC-3049	1, 7	++++	++	(–)
P. shivapurense	49	Wild	8, 8	+	(–)	(–)

[a]– Conidiospores of *Antromycopsis* stage. [b]– Chlamydospores (reddish brown) [c]– Incubated for 3–4 weeks after transfer on Agar slopes for revival. [d]– Recorded after 8 weeks. (–) indicates no revival. +, ++, +++, ++++ indicate relative abundance with + = ±10 mm growth.
Y, m- Correspond to Years and months of preservation.

RESULTS AND DISCUSSION

The agar punch nearest to the meniscus showed maximum growth and revival. Mycelial characters were similar to those observed in culture prior to preservation. Amongst the strains preserved *P. euosmus* (M-145) was the slowest growing strain, which survived preservation retaining this character. *Pleurotus eous* (M-263) could not be revived even after the short period of 1 year and 7 months, probably due to low vigour. *P. cystidiosus* (M-1) could not be revived in the present study. It was, however, found to be viable after 9 years' preservation.

The occurrence of the imperfect stage *Antromycopsis* is characteristic of the strains of the *P. cystidiosus* complex and was produced by 3 of the 4 cultures that revived. Typical reddish brown chlamydospores were produced by the strain belonging to *P. dryinus*. During the above in vitro studies, mushroom primordia were produced by all the strains of *P. pulmonarius*, by 1 out of 6 strains of *P. flabellatus* and by 1 strain of *P. eryngii*.

The isolates of Basidiomycotina survived liquid nitrogen storage for a period ranging from 2–13 years and had a 86–92 % revival rate (Smith 1982). Revival of Basidiomycete cultures after 9 years of cryogenic storage did not affect their radial growth rate or the pattern of the colony (Hwang et al. 1976). The success rate was less favourable at 53 % for agarics and 0 to 100 % for the species of *Pleurotus* following cryogenic storage (Chvostová et al. 1995). The overall success rate for Pleurotus strains was $\geq$ 90 % in this experiment. Von Arx and Schipper (1978) observed failures among the Agaricales, following preservation by the oil-seal method and ten years was found to be the optimum storage period for this method, in general. Similarly, preservation at about 24 °C did not affect the viability of the strains.

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