Studies on Improved Methods of Mushroom Germplasm Conservation

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To determine best method(s) of mushroom germplasm conservation, 11 edible mushroom strains belonging to six genera *viz., Agaricus, Pleurotus, Auricularia, Lentinula, Morchella* and *Volvariella* were preserved using different short-, medium- and long term storage methods. Of short term preservation methods the longest survival of all the test strains was recorded in the treatment wherein the test strains were preserved on wheat grain spawn in the test tubes at 4°C. Out of 12 medium-term storage methods, higher recoveries are being made from four treatments wherein test strains were preserved in either liquid paraffin at room temperature or 4°C or in glycerol (20%) at room temperature or in the form of mycelial disc or spawn multiplied on wheat grains. However, *Lentinula edodes* could not survive in liquid paraffin at room temperature. *Morechella esculenta* could survive for 1 year and 4 months as mycelial disc in liquid paraffin at 4°C and in glycerol (20%) at room temperature. *Volvariella volcacoa* is being recovered only from one treatment wherein the fungus was preserved as mycelial disc in liquid paraffin at room temperature. In long-term liquid nitrogen preservation methods higher recoveries from liquid nitrogen preservation are being made for more than 20 months.

Key words: ***Agaricus, Auricularia,*** **Conservation, Cryopreservation,** ***Lentinula,*** **Mushroom,** ***Morchella, Pleurotus,*** **Preservation,** ***Volvariella***

Successful mushroom production depends upon the proper maintenance of pure culture spawn that is capable of providing fruiting bodies of high productivity with excellent flavour, palatable texture, colour and is pest and disease resistant. Maintenance of vigour and genetic characteristics of pure mycelium is main objective of strain preservation. Further, strain improvement of cultivated mushrooms and domestication of wild mushroom demands a well-planned system for the maintenance, preservation and availability of genetic diversity (Chang and Miles, 1989). There are various methods of maintenance and preservation of mushroom culture and culture collection centre should adopt more than one method to preserve them. These mushrooms might be of academic, industrial, medicinal or of horticultural importance.

Since there is no satisfactory method to check and evaluate the quality of spawn by rapid on-the-spot examination, a method of preserving selected strains which have been thoroughly tested and proved desirable is of primary importance. If no degenerative changes were to take place during the preparation or maintenance of mushroom cultures and of spawn, then the preservation of mushroom cultures would be a relatively simple, routine process. Unfortunately, it is not true. Degeneration of culture, or of the spawn produced by the culture, refers to the loss of desired qualities by changes that result in such things as slow development, poor rate of survival and low level of productivity (Chang and Miles, 1989; Stadelman, 1986).

Frequent subculturing, storage under mineral water, lyophilization and preservation in ultra low temperatures in liquid nitrogen at (–140°C to –196°C) are known methods of preservation for spore brearing cultures of microbes (Buell *et al.,* 1947; Boeswinkel, 1976; Smith and Onions, 1983; Kirsop and Doyle, 1991). Mushrooms are invariable stored as mycelial culture because spores of heterothallic or secondary homothallic species are produced through a sexual process and have genetic difference and may not always result in fruiting (Petersen, 1995). Since mushrooms are stored as mycelial cultures and in absence of hardy structures like double walled spores, cultures becomes susceptible to sudden change in temperatures and pressure (Franks, 1981). Therefore, attempts were made to develop protocols for slow cooling and rapid thawing of mushroom mycelium.

Materials and Methods

To determine best method(s) of mushroom germplasm conversation, eleven edible mushrooms strains *viz., Agaricus bisporus* (strain S-11 and U-3), *Agaricus bitorquis* (NCB-13), *Pleurotus flabellatus* (P1-50), *P. sajor-caju* (P1-10-A), *P. ostreatus* (P1-20), *P. sapidus* (P1-40), *Auricularia polytricha* (OE-4), *Lentinula edodes* (OE-9), *Morchella esculenta* (ME-2) and *Volvariella volvacea* (OE-12) were preserved using different short, medium- and long-term storage methods.

Pure cultures of all the 11 strains were raised using tissue culture method. The cultures were conserved using different methods. For short-term storage methods, six

treatments on which pure cultures were raised on wheat extract agar (WEA) culture medium in test tubes (Singh, *et al.*, 2000) and preserved and T1 = room temperature, T2 = at 4°C, T3 = at –10°C, T4 = pure culture spawn raised on wheat grains in test tubes and stored at room temperatures, T5 = at 4°C and T6 = at –10°C, Observations on effect of short term preservation methods was recorded after every two months from the date of preservation.

For medium-term storage methods, pure cultures were first raised on wheat extract agar (WEA) mediums in petriplates and then 5 mm mycelial disc were cut and preserved in sterile distilled water (SDW), liguid paraffin (LP) or glycerol at room temperature, 4°C and –10°C. Twelve treatments were compared for per cent recovery of different mushroom strains at fixed interval of 4 months. Medium term storage treatments were as under:

T1 = Mycelial disc in SWD at room temp.
T2 = Mycelial disc in SWD at 4°C
T3 = Mycelial disc in SWD at room temp.
T4 = Mycelial disc in LP at 4°C.
T5 = Mycelial disc in glycerol (20%) at room temp.
T6 = On wheat grains in SDW at room temp.
T7 = On wheat grains in SDW at 4°C.
T8 = On wheat grains in LP at room temp.
T9 = On wheat grains in LP at 4°C.
T10 = On wheat grains in glycerol (20%) at 4°C.
T11 = On wheat grains in SDW at –10°C.
T12 = On wheat grains in glycerol at (20%) –10°C.

In long term preservation treatments, all the 11 test strains were preserved both as mycelial disc (5 mm) and fungus multiplied on wheat grain spawn and preserved in cryovials containing glycerol as cryoprotectant in liquid nitrogen at –196°C following the principle of slow cooling and rapid thawing. Cryovials were first placed for 4 h at –20°C and then for 6 h at –70°C in a mechanical freezer before plunging into liquid nitrogen at –196°C. Recoveries were made by placing the cryovials in water blanks each containing 50 ml SDW maintained at 37°C for rapid thawing. The cultures were retrieved after six months.

Results and Discussion

The results of short-term preservation are presented in Table 1. The data exhibits that the test mushroom strains survival for larger periods in the form of spawn as compared to preservation in the form of pure cultures raised on WEA medium. The longest survival of all the preserved strains for 1 year 8 months was recorded in the treatment T5 where in the test strains were preserved on wheat grain spawn in the test tubes at 4°C. The longer survival of mushroom mycelium on wheat grain spawn as compared to culture medium can be attributed to the fact that the mycelium invaded the soft wheat grains and remained concealed inside and nutrients were available for longer period whereas, culture medium dried upon prolonged storage. Whereas, prolonged storage at –10°C without cryopetectant could have destroyed the soft and tender mycelium.

The result of per cent recovery of different mushroom strain are presented vide Table 2. The data exhibits that the higher recovery of most of the strains is being made for more than 1 year and 8 months from treatment T3, T4, T5 and T9 wherein test strains were preserved in either liquid paraffin at room temp., 4°C or in glycerol (20%) at room temperature either in the form of mycelial disc or spawn multiplied on wheat grains. However, *Lentinula edodes* (OE-9) is being retrieved from only three treatments T4, T5 and T9. *Morchella esculenta* could survive up to 1 year 4 months in treatments T4 and T5. *Volvariella volvacea* (OE-12) was recovered from T3 treatment, wherein the fungus was preserved as mycelial disc in liquid paraffin at room temperature.

In medium-term storage higher recoveries in treatments T3, T4 T5 and T9 can be attributed to the fact that in liquid paraffin and glycerol the mycelial disc and mycelium on wheat grain spawn remained disconnected with atmospheric oxygen. Whereas in SDW, most of the test strains over-grew water surface and formed a mycelial mat which dried later.

The results of long-term liquid nitrogen preservation methods indicate higher recoveries from liquid nitrogen preservation in the form of wheat grain spawn as compared to mycelial disc preservation. All the test strains are being preserved satisfactorily in liquid nitrogen on wheat grain spawn for 1 year and 10 months (Table 3). The treatments with higher recoveries in liquid nitrogen on wheat grain spawn for 1 year and 10 months (Table 3). The treatments with higher recoveries in liquid nitrogen on wheat grain spawn can be attributed to the fact that the test fungi remained protected being concealed inside the grains and could have sustained slow cooling pressures as the test cultures were submerged in glycerol

Table 1. Effects of short-term preservation methods on survival of edible mushrooms

Mushroom Strain	Treatment	Per cent recovery		Period of survival	Remarks
		First recovery (%)	Last recovery (%)		
S-11	T_1	100	30	12	
	T_2	100	40	14	
	T_3	90	20	12	
	T_4	100	60	14	
	T_5	100	30	20	Survival Continued
	T_6	100	10	20	Survival continued
U_3	T_1	100	30	12	
	T_2	100	20	14	
	T_3	100	40	10	
	T_4	100	30	18	
	T_5	100	50	20	
	T_6	100	10	20	
NCB-13	T_1	100	30	12	
	T_2	100	50	14	
	T_3	80	20	10	
	T_4	100	30	18	
	T_5	100	50	20	Survival continued
	T_6	100	40	20	Survival continued
P1-20	T_1	100	30	12	
	T_2	100	20	14	
	T_3	90	40	8	Survival continued
	T_4	100	20	10	
	T_5	100	50	20	
	T_6	100	30	20	
P1-50	T_1	100	30	20	Survival continued
	T_2	100	10	14	
	T_3	80	20	10	
	T_4	100	20	16	
	T_5	100	50	20	
	T_6	100	20	20	Survival continued
P1-40	T_1	100	20	12	
	T_2	100	20	14	
	T_3	100	10	8	
	T_4	100	20	18	Survival continued
	T_5	100	50	20	
	T_6	100	80	20	
P1-10A	T_1	100	20	12	Survival continued
	T_2	100	30	14	
	T_3	100	80	8	
	T_4	100	20	12	Survival continued
	T_5	100	50	20	
	T_6	100	30	20	
OE-4	T_1	100	50	12	Survival continued
	T_2	100	30	14	
	T_3	100	70	8	
	T_4	100	30	16	
	T_5	100	30	20	Survival continued
	T_6	100	20	20	
OE-9	T_1	100	40	10	Survival continued
	T_2	100	30	12	
	T_3	90	10	8	
	T_4	100	20	14	
	T_5	100	70	20	
	T_6	100	30	20	Survival continued
ME-2	T_1	100	30	10	Survival continued
	T_2	100	20	12	
	T_3	90	60	8	
	T_4	100	50	12	
	T_5	100	20	12	
	T_6	100	50	12	Survival continued
OE-12	T_1	100	30	10	Survival continued
	T_2	0	0	0	
	T_3	0	0	0	
	T_4	100	30	14	
	T_5	100	10	16	
	T_6	100	20	18	

Table 2. Effects of medium-term preservation methods on survival of edible mushrooms

Mushroom Strain	Recovery Date	Per cent recovery of different mushroom strains											
		T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}	T_{11}	T_{12}
S-11	First recovery	100	40	100	100	100	0	60	20	60	60	0	0
	Last recovery	40	20	100	100	20	0	60	20	40	20	0	0
	Survival (months)	12	16	20	20	16	0	8	16	16	12	0	0
	Remarks	-	-	Contd.	Contd.	-	-	-	-	-	-	-	-
U-3	First recovery	80	20	100	100	80	0	60	0	60	60	0	0
	Last recovery	20	20	100	100	20	0	20	0	60	20	0	0
	Survival (months)	12	8	20	20	16	0	12	0	16	16	0	0
	Remarks	-	-	Contd.	Contd.	-	-	-	-	-	-	-	-
NCB-13	First recovery	100	100	100	100	100	0	80	0	100	100	0	0
	Last recovery	20	40	60	80	40	0	40	0	40	40	0	0
	Survival (months)	12	20	20	20	20	0	12	0	16	16	0	0
	Remarks	-	Contd.	Contd.	Contd.	Contd.	-	-	-	-	-	-	-
Pl-20	First recovery	100	100	100	100	100	100	100	100	100	60	20	0
	Last recovery	40	80	80	100	100	60	20	40	80	60	20	0
	Survival (months)	16	20	20	20	20	8	16	12	20	8	8	0
	Remarks	-	Contd.	Contd.	Contd.	Contd.	-	-	-	Contd.	-	-	-
Pl-50	First recovery	60	100	100	100	100	60	100	80	100	100	60	0
	Last recovery	60	40	80	100	60	60	20	20	80	60	60	0
	Survival (months)	8	20	20	20	20	8	20	12	20	12	8	0
	Remarks	-	Contd.	Contd.	Contd.	Contd.	-	Contd.	-	Contd.	-	-	-
Pl-40	First recovery	100	100	100	100	100	100	100	100	100	100	0	0
	Last recovery	20	40	100	100	100	20	20	40	60	20	0	0
	Survival (months)	16	20	20	20	20	16	16	12	20	16	0	0
	Remarks	-	Contd.	Contd.	Contd.	Contd.	-	-	-	Contd.	-	-	-
Pl-10A	First recovery	100	100	100	100	100	0	100	80	100	100	0	20
	Last recovery	20	60	80	100	100	0	20	20	80	80	0	20
	Survival (months)	16	20	20	20	20	0	20	12	20	12	0	0
	Remarks	-	Contd.	Contd.	Contd.	Contd.	-	Contd.	-	Contd.	-	-	-
OE-4	First recovery	60	100	100	100	100	100	40	0	100	100	0	0
	Last recovery	40	40	100	80	100	80	40	0	40	40	0	0
	Survival (months)	12	20	20	20	20	8	8	0	20	16	0	0
	Remarks	-	Contd.	Contd.	Contd.	Contd.	-	-	-	Contd.	-	-	-
OE-9	First recovery	100	60	100	100	100	100	100	100	100	0	0	0
	Last recovery	40	10	40	20	80	80	20	40	60	0	0	0
	Survival (months)	12	20	16	20	20	8	16	12	20	0	0	0
	Remarks	-	Contd.	-	Contd.	Contd.	-	-	-	Contd.	-	-	-
ME-2	First recovery	0	100	100	80	80	0	0	100	60	0	0	0
	Last recovery	0	40	40	20	40	0	0	20	40	0	0	0
	Survival (months)	0	12	16	20	20	0	0	12	8	0	0	0
	Remarks	-	-	-	Contd.	Contd.	-	-	-	-	-	-	-
OE-12	First recovery	0	100	100	40	100	0	0	100	100	0	0	0
	Last recovery	0	20	80	40	20	0	0	80	60	0	0	0
	Survival (months)	0	16	20	16	16	0	0	12	12	0	0	0
	Remarks	-	-	Contd.	-	-	-	-	-	-	-	-	-

Table 3. Effects of long-term liquid nitrogen preservation methods on survival of edible mushrooms.

Mushroom Strain	Treatment	Percent recovery		Period of survival	Remarks
		First recovery (%)	Last recovery (%)		
S-11	T_1	80	60	20	Survival continued
	T_2	100	100	20	Survival continued
U-3	T_1	70	60	20	Survival continued
	T_2	90	90	20	Survival continued
MCB-13	T_1	80	70	20	Survival continued
	T_2	90	90	20	Survival continued
P1-20	T_1	100	100	20	Survival continued
	T_2	100	100	20	Survival continued
P1-50	T_1	100	100	20	Survival continued
	T_2	100	100	20	Survival continued
P1-40	T_1	100	100	20	Survival continued
	T_2	100	100	20	Survival continued
OE-4	T_1	90	90	20	Survival continued
	T_2	100	100	20	Survival continued
OE-9	T_1	0	0	—	Survival continued
	T_2	100	100	20	Survival continued
ME-2	T_1	0	0	—	Survival continued
	T_2	60	60	20	Survival continued
OE-12	T_1	0	0	—	Survival continued
	T_2	90	80	20	Survival continued

0 = (No survival)

T_1 = Fungus preserved in glycerol as mycelial disc.

T_2 = Fungus preserved Glycerol multiplied in wheat grain spawn.

which is a known cryoprotectant, (glycerol) have also given protection to exposed soft mycelial culture disc to a great extent but still during slow cooling cell wall could have been damaged due to change of water present inside the cell wall into ice which eventually ruptured the tender cell wall. Although little metabolic activity takes places below –70°C, re-crystalization of ice crystal growth can cause damage during storage. The volume occupied by water increases by 10% when water crystalizes to form ice. This puts the cell under mechanical stress (Grout and Morris, 1987, Franks, 1981). This explains the reason for less recovery of test strains when preserved as mycelial disc under liquid nitrogen.

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