A simple method for culture conservation of some commercial mushrooms

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The main objective of culture preservation is to store cultures in viable and stable form for long periods without losing genotypic, phenotypic and physiological traits (Chang & Miles, 2004). The most common method of short-term storage of mushroom culture is storing the culture tubes at room temperature (28–35ºC) for a period of 1–2 months or in refrigerator (5–8ºC) for an average period of 3–4 months. This method necessitates frequent sub culturing leading to the problems of contamination and degeneration. The objective of the present study was to develop an inexpensive and simple method to preserve mushroom cultures in a viable state for an extended period. The possibility of storing various mushroom cultures on sorghum (Jowar) grain at low temperature (5–8ºC in refrigerator) was explored. The result clearly showed that the mushroom cultures could safely be stored at low temperatures on sorghum grain free from contamination for more than one year without any growth and morphological changes. The most significant advantage of this method was its suitability to conserve milky mushroom (*Calocybe indica*) and some isolates of reishi mushroom (*Ganoderma lucidum*) cultures, which cannot be stored at low temperatures.

Key words – Basidiomycete – *Calocybe indica* – *Ganoderma* – grain – preservation

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Introduction

Proper culture preservation is an integral part of the successful mushroom cultivation. The main objective of culture preservation is to store cultures in viable and stable form for long periods without losing genotypic, phenotypic and physiological traits (Chang & Miles 2004). Isolates in culture tubes stored at room temperature had a maximum life of 6–12 months, whereas those kept under refrigeration survived for five years or more (Stamets 2000). The time gap between subculturing varied with species and in general, was in the range of 3–12 months (Chang & Miles 2004). These reports stated comparatively long period between subculturing, however in India, storage at room temperature (28–35ºC) necessitated very frequent subculturing (25–30) days as the medium dries after this period). This leads to contamination and degenerative problems. Due to this reason storage of culture tubes under refrigerated condition is the regular practice. Even this practice requires frequent subculturing but at extended intervals (3–4 months). Added to this, cultures of *Calocybe indica*, *Volvariella volvacea* (commercial edible mushrooms) and some isolates of *Ganoderma lucidum* (commercial medicinal mushroom) cannot be stored under refrigerated condition. Most tropical basidiomycete isolates grown on Malt Extract Agar (MEA) were not viable beyond two months at 4ºC or after six months at 8–10ºC (Croan et al. 1999). Mushroom culture repository at IIHR play a vital role in

supply of authentic cultures and spawn to the mushroom spawn producing units and mushroom growers of South India. The present study aimed to develop a dependable, inexpensive, time saving, contamination free storage method that yields viable, morphologically and physiologically unchanged mushroom culture after preservation.

Methods

Pleurotus florida, *P. sajor–caju* (edible oyster mushrooms), *Calocybe indica* and five isolates of *Ganoderma lucidum* were used for the study. The sorghum/jowar (*Sorghum vulgare)* grains were cleaned and washed thoroughly with sufficient water and then boiled for 2 hours in a grain boiler developed at Indian Institute of Horticultural Research, Bangalore. Chalk powder (calcium carbonate) was mixed with boiled grains @ 4% (4g/100g grain) to absorb extra moisture, remove the stickiness due to starch and make the grain free flowing. The grains were filled in flat bottom screw capped vials (15 ml capacity, Borosil) upto two-thirds of their capacity and capped. The vials were autoclaved at 15 lb at 121ºC for two hours. After autoclaving, the vials were allowed to cool.

The cultures of various mushroom species were subcultured on 1.5% (w/v) Malt Extract Agar (MEA-Himedia) contained in 90 mm diameter sterile culture plates by placing a 5 mm diameter mycelial plug at the center of the plates and were incubated at desired temp (30 and 35ºC) in dark. After attaining growth, mycelial discs of 5 mm were cut using a sterile cork borer from actively growing edges of cultures. Four discs/vial were transferred aseptically to the cooled vials. The inoculated vials were kept in BOD incubator (at the desired temperature). The growth was noted everyday and the tubes were shifted to refrigerator after complete colonization of the grains (7-10 days).

The retrieval growth was tested on MEA culture medium. The grains were taken at an interval of two months from the refrigerator, brought to room temperature (25-30ºC). For each isolate, 10 grains were transferred to the medium. The viability of the isolates before and after preservation was measured by the ability of vegetative hyphae to regrow on MEA plates. The growth rate was calculated and compared.

Results

All the tested species could be retrieved in viable condition through this method even after one year of storage (Table 1).

Table 1 *In vitro* mycelial growth of various mushroom isolates before and after storage

Isolate	Growth (mm/day) before storage	Growth (mm/day) after 1 year
Pleurotus florida	14.67	14.87
Pleurotus sajor–caju	15.23	15.12
Calocybe indica	9.50	9.89
Ganoderma lucidum (G–8)	16.6	16.8
G. lucidum (G–17)	18.0	18.3
G. lucidum (G–34)	13.8	13.7
G. lucidum (G–35)	19.7	20.0
G. lucidum (G–40)	17.5	17.46

There was no difference between the average mycelial growth of tested species before and after storage for one year. None of the culture tubes had microbial contamination. Revival of cultures was easy and the mycelial growth commenced from the grain within a period of 24–48 hours as with freshly growing mycelium (Fig. 1).

The mycelial characters of these isolates remained unchanged. The milky mushroom and some isolates of Reishi mushroom could not be retrieved after storage at 5–8°C (refrigerator) when it was stored on medium, however, isolates could successfully be prerved on sorghum grain.

Dicussion

Tropical mycelial isolates generally grew well under optimal growth conditions of temperature, aeration and humidity on artificial media. These tropical isolates either grew poorly or not at all after storage in distilled water at 4°C. Croan (2000) reported that cold temperature was detrimental to tropical basidiomycete isolates and tropical isolates were problematic with respect to both short and long term preservation as compared to temperate isolates. All the cultures used in this study were tropical as they showed optimum mycelial growth at 30–35°C (Table 2).

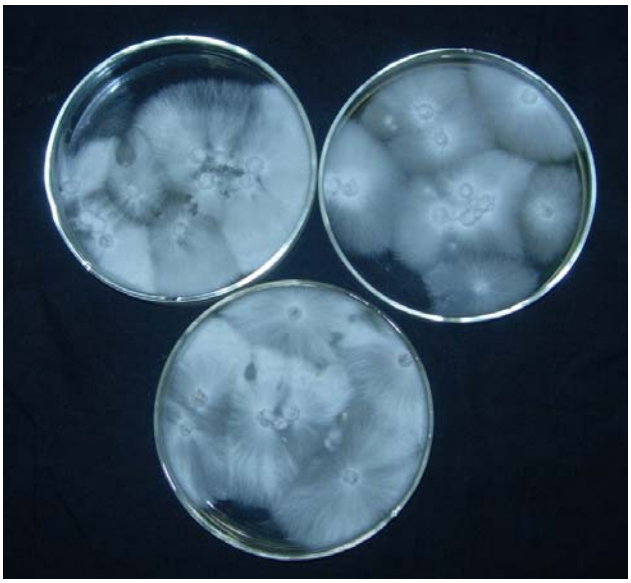

Fig. 1 – Retrieval of mycelial growth from preserved sorghum grain.

Table 2 Temperature regimes for *in vitro* growth of various mushroom species tested

Isolate	Temperature range (ºC)	Optimum temperature (ºC)
Pleurotus florida	15–35	30
Pleurotus sajor–caju	15–35	30
Calocybe indica	20–35	30
Ganoderma lucidum (G-8)	20–35	30
G. lucidum (G-17)	20–35	30
G. lucidum (G-34)	20–35	30
G. lucidum (G-35)	15–30	30
G. lucidum (G-40)	20–35	35

Further these cultures could not be stored in the refrigerator as they lost viability within one month of storage. Thus the storage under low temperature would have affected the viability of the isolates of *C. indica* and *G. lucidum*. According to Wang et al. (1990), in cryopreservation mushroom strains were better preserved on grain media than on agar media. Mycelium multiplied on wheat grains were genetically more stable as compared to mycelial discs of synthetic media during cryopreservation (Singh et al. 2004). The probable reason for the better survival of mushroom mycelium on sorghum grain may be either because of the protection given by the grain to the soft and tender mycelium, concealed inside the grain and thus could have sustained cooling (Franks 1981, Grout & Morris 1987, Singh et al. 2004) or the innate quality of the grain may be allowing better colonization of the mushroom mycelium and thereby better survival.

Due to generative changes and contamination, preservation of cultures becomes a difficult task (Chang & Miles 2004). Maintaining, the isolates by frequent transfer on an artificial medium is labour intensive and increases genetic drift. Mycelial forms of non sporulating and non chlamydosporulating basidiomycetes could not withstand lyophilisation (Rybnikar 1995, Tan et al. 1994). Eventhough these tropical basidiomycetes can be preserved through cryopreservation, liquid nitrogen is expensive, needs constant resupply and moreover the procedure is labour intensive. Mycelial preservation on sorghum grains at low temperatures does not require frequent subculturing and thus reduces the chances of contamination and quality degeneration. In addition this method is inexpensive; requires less space and is maintenance free. Hence this method is very useful for mushroom culture preservation. However, storage beyond 12 months should be tested to exploit the exact potential of the method. The method is species specific and may be standardized for individual species.

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