Research Article

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A straightforward technique for preserving the cultures of several commercial mushrooms.

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ABSTRACT

Maintaining cultures in a viable and stable state for extended periods of time without sacrificing their physiological, phenotypic, or genetic characteristics is the primary goal of culture preservation (Chang & Miles, 2004). The most popular technique for storing mushroom cultures for a brief length of time involves keeping the culture tubes either in a refrigerator (5–8°C) for an average of 3–4 months or at ambient temperature (28–35°C) for a duration of 1-2 months. This technique requires repeated subculturing, which increases the risk of degeneration and contamination. The goal of this study was to provide a low-cost, straightforward technique for long-term viable mushroom culture preservation.

It was investigated whether different mushroom cultures could be kept on sorghum (Jowar) grain at low temperatures (5–8°C in a refrigerator). The outcome unequivocally demonstrated that the mushroom cultures could be kept for more than a year at low temperatures on sorghum grain without risk of contamination or changes in growth or morphology. The capacity to preserve milky mushroom (Calocybe indica) and some isolates of reishi mushroom (Ganoderma lucidum) cultures—which cannot be kept at low temperatures—was the method's greatest benefit.

Keywords : Basidiomycete – Calocybe indica – Ganoderma – grain – preservation

INTRODUCTION

An essential component of a good mushroom cultivating process is appropriate culture preservation.

Maintaining cultures in a viable and stable state for extended periods of time without losing their physiological, phenotypic, or genetic characteristics is the primary goal of culture preservation (Chang & Miles 2004).

The highest shelf life of isolates in culture tubes kept at ambient temperature was 6–12 months, but isolates kept in refrigeration could last up to 5 years (Stamets 2000).

The interval between subculturing varied depending on the species, although it was typically 3–12 months (Chang & Miles 2004). These reports indicated a relatively lengthy interval between subculturing; however, in India, room temperature storage (28–35°C) required very frequent subculturing (25–30 days) since the medium dries up after this time.

Degenerative issues and pollution result from this. This is the reason it is standard procedure to store culture tubes in a refrigerator. Even with this procedure, further subculturing is necessary, but at longer intervals (3–4 months).

Furthermore, certain isolates of Ganoderma lucidum, a commercial medicinal fungus, and cultures of Calocybe indica and Variella volvacea, edible mushrooms, cannot be kept in a refrigerator.

According to Croan et al. (1999), the majority of tropical basidiomycete isolates cultured on Malt Extract Agar (MEA) were not viable after two months at 4°C or six months at 8–10°C. The IIHR mushroom culture repository is essential to the provision of genuine cultures and spawn to South Indian mushroom growers and units that produce mushroom spawn. The goal of the current study was to create a cost-effective, time-saving, contamination-free storage technique that would provide a viable, physiologically and morphologically intact mushroom culture following preservation.

Calocybe indica, Pleurotus florida, P. sajor–caju (edible oyster mushrooms), and five Ganoderma lucidum isolates were employed in the investigation. The grains of sorghum/jowar (Sorghum vulgare) were rinsed and thoroughly cleansed with enough water. They were then cooked in a grain boiler created at the Indian Institute of Horticultural Research, Bangalore, for two hours. The boiling grains were combined with 4% (four grams per hundred grams) of chalk powder (calcium carbonate) to absorb excess moisture, eliminate starch-induced stickiness, and make the grains flow freely.

Filling the vials with grains to two-thirds of their volume, flat bottom screw-capped vials (15 ml capacity, Borosil) were then sealed. The vials were autoclaved for two hours at 121oC and 15 pounds. Following the autoclaving process, the vials were let to cool.

A 5 mm diameter mycelial plug was placed in the center of 90 mm diameter sterile culture plates containing 1.5% (w/v) Malt

Extract Agar (MEA-Himedia) to support the subcultures of different mushroom species. The plates were then incubated at the required temperature (30 and 35ºC) in the dark. Following growth, actively growing edges of cultures were used to cut 5 mm mycelial discs using a sterile cork borer. The cooled vials received four discs per vial, which were transferred aseptically.

The inoculation vials were maintained at the appropriate temperature in a BOD incubator. Every day, the growth was recorded, and after the grains had fully colonized the tubes, they were moved to a refrigerator (7–10 days).

MEA culture media was used to examine the retrieval growth. The grains were removed from the refrigerator and allowed to come to room temperature (25–30ºC) every two months. Ten grains were added to the medium for each isolate. The ability of vegetative hyphae to sprout on MEA plates was used to determine the viability of the isolates both before and after preservation. We computed and compared the growth rate.

Outcomes

Using this technique, every evaluated species could be recovered in a viable state even after a year in storage (Table1). The average mycelial growth of the examined species was the same before and after a year of storage. The culture tubes were all free of microbiological contamination. The cultures were easily revived, and as with freshly grown mycelium, mycelial development started from the grain in a matter of 24 to 48 hours (Fig. 1).

These isolates' mycelial characteristics didn't change. When stored on medium at 5–8°C (refrigerator), the milky mushroom and some Reishi isolates could not be recovered; however, isolates could be successfully preserved on sorghum grain.

Talking

Under ideal growth conditions of temperature, aeration, and humidity on artificial media, tropical mycelial isolates typically grew well.

After being stored in distilled water at 4°C, these tropical isolates either did not grow at all or did so very weakly. According to Croan (2000), tropical isolates of Basidiomycete were less stable in colder temperatures than temperate isolates, and thus presented challenges for both short- and long-term preservation. Since mycelial growth is most optimal at 30 to 35°C, all of the cultures employed in this investigation were tropical (Table 2). Additionally, these cultures lost viability after a month of storage, therefore they could not be kept in a refrigerator. Therefore, the viability of the C. indica and G. lucidum isolates would have been impacted by the low temperature storage.

In cryopreservation, grain medium outperformed agar media in terms of mushroom strain preservation, as reported by Wang et al. (1990).

During cryopreservation, mycelium grown on wheat grains exhibited a higher degree of genetic stability in comparison to mycelial discs of synthetic media (Singh et al. 2004). The likely cause of the greater survival of mushroom mycelium on sorghum grain could be either the intrinsic properties of the grain or the protection provided by the grain to the soft and tender mycelium, which was hidden inside the grain and may have sustained cooling (Franks 1981, Grout & Morris 1987, Singh et al. 2004).

be enabling improved mushroom mycelium colonization and, thus, improved survival.

The job of preserving cultures becomes challenging because to generative changes and contamination (Chang & Miles 2004). Maintaining the isolates by repeated transfers on a synthetic medium requires a lot of work and accelerates genetic drift. Non-sporulating and non-chlamydosporulating basidiomycetes' mycelial forms were unable to resist lyophilization (Rybnikar 1995, Tan et al. 1994). While cryopreservation is an option for these tropical basidiomycetes, the process requires a lot of labor and expensive liquid nitrogen that needs to be constantly replenished.

Because regular subculturing is not necessary for mycelial preservation on sorghum grains at low temperatures, there is less risk of contamination and quality degradation. This approach is also low-cost, low-maintenance, and requires less room. Future research may find that the efficacy of the medications in the foetus and infant is influenced by the presence of women and a plasma concentration in the foetus that is one-third of the mother level [35]. In order to objectively monitor compliance and other relevant parameters before implementing a treatment plan, it is crucial to measure plasma medication concentrations systematically [37]. When applied prospectively, these could be able to reduce the discrepancy between the predicted and actual results of pregnancy in human PT.

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