METHODS FOR THE CULTURE CONSERVATION OF EDIBLE AND MEDICINAL FUNGI

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ABSTRACT
The maintenance and conservation of strains of microorganisms such as bacteria, yeast, and fungi are vital for the advancement and development of various life science areas, impacting studies in genetics, biodiversity, bioprospecting, biotechnology, medicine, veterinary, environment, food security, nutrition, among others. The preservation of this biological material has achieved the safeguarding of industrial potential and the possibility of researching new functions and the use for the benefit of humanity. Various culture preservation methods have been developed over the years, such as sterile distilled water, cryopreservation, freeze-drying, sub-culture, and sterile mineral oil; these allow storing strains of various microorganisms under appropriate conditions and for long periods. For the case of edible and medicinal fungi, the most used conservation methods are cryopreservation and sterile distilled water, guaranteeing the stability of the characteristics of these fungi, their viability, and their biological potential over time; however, the need to continue evaluating different methods and applications in this type of fungus persists.

Keywords: basidiomycetes fungi, fungi strains, culture methods, preservation methods, mushrooms

INTRODUCTION
Microbial collections are considered centers of biological resources, maintaining strains of different microorganisms in optimal conditions for research, biotechnological, and industrial applications (Hu et al., 2014; Sharma et al., 2017). In order to store under controlled conditions and for a specific time such organisms, it is necessary to apply preservation methods that ensure the viability, availability, and safeguarding of all phenotypic, genotypic, and potential characteristics industrial of the preserved strains (Henao et al., 2006; Ryan et al., 2004; Morales et al., 2010; Montesinos Matías et al., 2015). For several years different culture conservation methods have been developed for the maintenance and conservation of microorganisms such as bacteria and fungi, like freeze-drying (lyophilization), cryopreservation, sub-culture (periodic transfer), sterile distilled water, mineral oil layer, drying on filter paper, drying in soil, sand, silica gel, among others (García et al., 2000; Ryan et al., 2004; Weng et al., 2005; Gatto, 2010). These methods allow that biological material to be available and stable for varying periods; however, the choice and application of one method or other depends on the type of microorganism to be preserved, the time they wish to keep preserved, the resources, laboratory procedures, and staff in charge (Rico et al., 2004; Smith et al., 2012). In Table 1, the main conservation methods of fungi can be seen along with their advantages and disadvantages.

In general, studies for the maintenance of microbial collections have shown the effects of methods over a given time on the phenotypic, genotypic, feasibility, stability, purity, and reproductive capacity of the strains mainly in filamentous fungi, yeasts, and bacteria of importance for the industry, medicine, agriculture, and the environment. However, the development and research of preservation methods for basidiomycetes have not been studied as extensively as in other types of microorganisms, despite the importance that these fungi have in nature, because they are responsible for cycles nutrient replacement, contributing to increased soil fertility; as well as the different secondary metabolites with properties of biotechnology interest and applications in different industry sectors (Cortés et al., 2013; Ladislav Homolka, 2014; Eichlerová et al., 2015). Therefore, this document's objective is to make available to the scientific community a review of the main works carried out on the conservation of basidiomycetes fungi, with an emphasis on edible and medicinal fungi.

Table 1 Advantages and disadvantages of the main fungi culture methods

| Method                  | Advantage                                      | Disadvantages                                      | Reference                  |
|-------------------------|------------------------------------------------|---------------------------------------------------|---------------------------|
| Sterile distilled water | Decreases the tendency to pleomorphism by some species. | It is simple, safe, and inexpensive, does not require personnel specialized. | Burdsall et al. (1994)    |
|                         | It maintains viability, purity, and stability for extended periods. | The strains can degenerate during storage time. | Bueno et al. (1998)       |
|                         | It also allows to preserve fungi that cannot be preserved by liquid nitrogen or freeze-dried. | Conservation times are variable and will depend on the fungus to be preserved. | Martínez et al. (2009)    |
|                         | Has been successfully used to preserve basidiomycetes. |                                                   | Fernández et al. (2013)   |
| Cryopreservation         | Is a reliable method for long-term storage of microorganisms. | It is considered an expensive technique due to the requirement of space and specialized | Hu et al. (2014) S.K. Singh (2017) Prakash et al. (2013) |
There is a low risk of changes in the viability and genotype of the microorganisms. The method is used for the long-term preservation of fungi. This method applies to a broad spectrum of fungi. Equipment. It requires a bioprotectant to avoid excessive dehydration of the cells. Need a constant supply of liquid nitrogen. 

Freeze-drying

The maintenance of the culture is low cost. The method is applied to a wide range of microorganisms. The method can be complicated and requires specialized equipment.

Sub-culture

Useful for small collections of fungi that do not require long-term storage. It is a friendly method for laboratories with limited resources. It has been used in different fungi, remaining viable for 20 years or more. It requires periodic verification to assess contamination.

Sterile mineral oil

It is a friendly method for laboratories with limited resources. It has been used in different fungi, remaining viable for 20 years or more. It requires periodic verification to assess contamination.

CULTURE CONSERVATION METHODS

Sterile distilled water (Castellani method)

It is a widely used technique for conserving microorganisms such as filamentous fungi, yeasts, and some bacteria due to the high percentages of viability obtained over time (García et al., 2000). As seen in Figure 1, the method consists of suspending agar discs or blocks with the colonies of the microorganism in tubes or vials with sterile distilled water, and these are sealed by screw caps, rubber or cotton caps and stored at room temperature (between 20 and 25 °C) or in cold storage between 4 and 5 °C (Castellani, 1963; Nakasone et al., 2004; S.K. Singh, 2017). It is the most preferred method for the maintenance of fungal culture because decreases the tendency to pleomorphism by some species, prevents the attack of mites, is simple, safe, and inexpensive, does not require personnel specialized and maintains viability, purity, and stability for extended periods; also can be applied in fungi that cannot be preserved by liquid nitrogen or freeze-dried (Burdall et al., 1994; Bueno et al., 1998; Fernández et al., 2013; Martínez et al., 2009).

In 1963 Aldo Castellani, in his publication “Further researches on the long viability and growth of many pathogenic fungi and some bacteria in sterile distilled water”, showed to the scientific community the progress made by the application of its method, which consisted of preserving strains of filamentous fungi, yeasts, and some bacteria in test tubes with sterile distilled water (8 to 10 mL) properly capped, kept at room temperature for different periods (one year or more), and subsequently recovered in test tubes with dextrose agar to assess the efficiency of the method. The results obtained in their studies showed that these strains, after several years, maintained high percentages of viability, average growth, and no changes in their morphology (Castellani, 1963). McGinnis et al. (1974) used Castellani’s method in their preservation studies, stating that it is a simple, inexpensive, and reliable technique, since its application for the maintenance of 417 isolates of filamentous fungi, yeasts, and aerobic actinomycetes for a period between 12 to 60 months, showed that 93 % (389) of cultures survived in perfect conditions to storage in sterile distilled water. Hartung de Capriles et al. (1999) evaluated the preservation of 594 fungal strains for a period of 1 to 20 years by this method, 62 % of the preserved strains (368) were viable and maintained their main morphological characteristics; also 90 % of the strains of different species preserved for 20 years were viable. Bueno et al. (1998) preserved 26 strains of the genera and species: Aspergillus niger, Aspergillus candidus, Fusarium sp, Fusarium moniliforme, Mucor griseocyanum, Syncephalastrum sp, Trichoderma sp, Trichoderma harzianum, and Trichoderma koningii using the method of preservation in sterile distilled water, they demonstrated that the strains of these organisms had a 100 % viability for two years, did not have contamination with bacteria or mites, no changes were observed with the naked eye apparent changes and in macroscopic characteristics in any of the culture studied. Similar results are evident in other studies, such as the one conducted by Panizo et al. (2005), assessing the feasibility, purity, morphological, macroscopic, and microscopic stability of 411 fungi, preserved in distilled water and sterile mineral oil for 3 to 48 years. Statistical analysis showed that of the 170 yeasts evaluated, 100 % were kept viable, pure, and morphologically stable by the two preservation methods of 241 filamentous fungi, 100 % evaluated pure and viable, while 95.4% were kept pure and viable by both methods used. On the other hand, in the research conducted by Fernández et al. (2013), in which the feasibility, purity, and stability of the main morphological and physiological characteristics of 240 strains of different fungal species belonging to the collection of pathogenic fungal cultures of the Institute of Tropical Medicine "Pedro Kouri” preserved in sterile distilled water for a period of 15 to 20 years, it was obtained that 80 % of the culture was kept in a viable state and without contamination.

For actinomycetes fungi, the U.S. Department of Agriculture’s “Forestry Research Center” conducted a study in which they preserved 151 species of wood degrading fungi for periods of up to 7 years in sterile distillate water; obtaining
that 94% of the preserved strains were feasible and growth rates were not affected by the evaluation preservation method; validating that this storage technique has several advantages compared to storing in slanted agar test tubes, such as the long periods during which the isolate can be stored without the need to do periodic transfers, reduced consumption of culture media and increased genetic stability (Burdass et al., 1994). Similarly, Croun et al. (1999) preserved 35 basidiomycetes fungi that inhabit tropical woods using conservation methods in sterile distilled water, cryopreservation in liquid nitrogen and freeze-dried. As a result, 57% of the strains stored in sterile distilled water at 4 °C survived for 2.5 months, while at 15 °C, 92% of the strains survived for ten months, maintaining viability without presenting changes in morphological or biochemical characteristics for both cases. Diogo et al. (2005) came to a similar conclusion when assessing the efficacy for the conservation of 43 species of fungi in sterile distilled water for 12 months; they tested the average growth, viability, and sporulation capacity of the strains under study. Richter et al. (2010) managed to conserve stable and viable 14 isolates of basidiomycetes (12 species) fungi for 18 years in tubes with inclined agar and sterile distilled water.

Maia et al. (2012) used as substrate rice and sterile distilled water preservation solution to conservation of the basidiomycetes Agaricus brasiliensis, Pleurotus ostreatus, Pleurotus sajor-caju, and Lentinula edodes. The rice-based substrate in distilled water at room temperature was the most effective method for preserving Agaricus brasiliensis, with a recovery of 100% of the preserved strains after 12 months; this fungus cannot be stored at low temperatures. Similarly, Karaduman et al. (2012); preserved strains of Schizophyllum commune using 12 different preservation methods for one year, using as response variables the rate of mycelial recovery, mycelial growth, biomass, and enzymatic activity of recovered culture. They obtained that mycelium preserved on agar blocks in sterile distilled water at 4 °C and in glycerol (15 %) at 20 °C were the best conservation methods for this type of fungi, and the strains stored on wheat seeds colonized with mycelium in sterile distilled water at 4 °C and on agar blocks in sterile distilled water at 20 °C, were the alternative methods to preserve Schizophyllum commune.

Palacio et al. (2014) assessed the effect of conservation on sterile distilled water, cryopreservation with glycerol at 10 % temperature of -20 °C and -80 °C, and freeze-dried, of basidiomycete fungi Agaricus blazei, Ganoderma lucidum, Grifola frondosa, and Pleurotus pulmonarius over 12 months. Response variables were feasibility, growth kinetics, biomass production, and polysaccharides. The results obtained after 12 months of conservation, determined that the best method was distilled water at 24 °C, as it achieved the highest percentage of recoverability (83.3 %), followed by the cryopreservation method at -80 °C, where 75 % was recovered, without affecting biomass production and polysaccharides; however, for specific cases like Pleurotus pulmonarius suggest cryopreservation at -80 °C. García-Garcia et al. (2014) evaluated sterile distilled water in the medicinal fungus Humphreyea cofeaeata, using filter paper discs inoculated with the fungus, guaranteeing high viability of the culture for 18 months, without visible morphological changes, contamination by bacteria or other fungi. As observed, sterile distilled water has allowed the conservation of different culture of edible and medicinal fungi between 2.5 months and 48 years, depending on the type of fungus and the conditions of applying the technique.

Cryopreservation

Cryopreservation is a technique that allows a cell suspension to be stored at a temperature equal to or below the freezing point. Depending on the temperature, this method of preservation can be classified into ordinary freezing (-5 to -20 °C), ultra-cold freezing (-50 to -80 °C), and freezing with liquid nitrogen (-150 to -196 °C) (Hernández et al., 2014). It is a long-term preservation method that allows storage microorganisms between one year and twenty years; it also guarantees the viability, purity, and genetic stability of the stored strains. It is one of the most recommended techniques for conserving fungi that cannot be freeze-dried (Nakasone et al., 2004; Ladislav Homolka, 2014). The method consists of previous growth of the culture in Petri dishes with potato dextrose agar or malt extract agar. Portions of the colonized agar are cut and transferred to cryovials with a cryoprotectant; they are brought to containers at low temperature and subsequently stored in cryo-box at -70 °C (S.K. Singh, 2017). Several factors affect cryopreservation effectiveness, like microorganisms, cell composition, growth medium, and freezing/storage characteristics (figure 2).

Cryopreservation has some disadvantages such as high cost of the equipment required, energy cost, the need to maintain a constant supply of nitrogen (when freezing with liquid nitrogen) or cryoprotectants to maintain the temperature as well same specific conditions for the transport of the strains (Arenclibia et al., 2008). Also, it is necessary to use a cryoprotective because physicochemical phenomena during freezing can affect cell viability. Major cryoprotectants include glycerol, dimethylsulfoxide, skim milk, inositol, glucose, lactose, and sucrose (Rico et al., 2004). Danell et al. (2002) evaluated five protocols for the preservation of strains of the ectomycorrhizal fungus Cantharellus cibarius in liquid nitrogen using different cryoprotectants, cooling rates, and times of incubation; as a result, they found that the best preservation protocol was the one that used sorbitol and dimethyl sulfoxide at a slow freezing rate of 0.3°C min⁻¹ after 72 h and six days, these remained viable and showed no morphological or physiological changes. Kitamoto et al. (2002); preserved 66 strains of fungi, including Oomycota, Zygomycoota, Ascomycota, Basidimycota, and mycetes by rapid freezing at -85 °C with glycerol and ethylene glycol as cryoprotectors at different concentrations; as well as these fungi were preserved using wood sawdust with 65 % moisture. The results obtained in this study showed that with 10 % of glycerol cryoprotectant, strains remain viable and stable for up to 10 years even after defrosting the strains at room temperature and subjecting them to processes of alternating freezing and thawing.

Regarding studies concerning the conservation of edible fungi, the researchers Ohmusa et al. (1996); evaluated the effect of three cryopreservation protocols on the yields of fruiting bodies of the Fissellinia velutipes after having preserved two strains (FMC224 and FMC225) at three temperatures (-20, -85 and -196 °C), and with three cryoprotectants (glycerol, dimethylsulfoxide, and polyethylene glycol) for seven years. Lara et al. (1998) evaluated the effect of conservation in liquid nitrogen on the production of carpophores of six fungal strains of Pleurotus spp, stored for 15 days at -196 °C where the variables of response were: time in days of appearance of the primordia, number mushroom crops obtained, biological efficiency and size of fruiting. As a result, they observed variability in the yields of crops obtained, size of fruiting, and biological efficiency of the strains (55 to 106 %) of the strains, however, the cryopreserved strains did not show morphological differences concerning the control.

Mata et al. (2003) used five strains of edible fungi (Lentinula boreyana, Lentinula edodes, Pleurotus djamor, Pleurotus pulmonarius, and Volvariella volvacea) inoculated on sorghum seeds to evaluate three protocols for one week; in the first protocol, the sorghum seeds inoculated with the fungi were preserved in cryovials using glycerol and dimethylsulfoxide as cryoprotectants, in the second protocol distilled water was used, and in the third protocol without cryoprotectant. Once the storage time elapsed, the strains were recovered on Petri dishes. The viability, purity, growth rate, and macro and microscopic characteristics of the strains were evaluated, 96.8 % of the strains stored by the three protocols successfully recovered while retaining all their morphological characteristics. However, only strains preserved without cryoprotectant achieved a recovery percentage of 99.2 %, showing that it is suitable for the conservation of basidiomycete fungi and could handle large quantities of commercial fungal strains at reduced production costs. Other research conducted by S. K. Singh, Upadhyay, Kamal, et al. (2004) evaluated the effect of cryopreservation on the survival, yield, and genetic stability of 11 the edible fungus Agaricus bisporus, Agaricus bitorquis, Pleurotus flabellatus, Pleurotus species sajor-caju, Pleurotus ostreatus, Pleurotus sapidus, Agrocybe arbutacea, Lactarius deliciosus, Marasmius melíaceus, and Volvariella volvacea, preserved on wheat grains in cryovials with 15% glycerol at -196 °C. The results showed that 9 of the 11 strains conserved did not show significant changes in viability, biological efficiency, the weight of fruiting bodies, and genetic stability.

Ladislav Homolka et al. (2006); developed a new method for the conservation of 442 strains of basidiomycete fungi of different species; by the cryopreservation of the fungal mycelium using agricultural grade perlites as

![Figure 2 Main factors that affect the effectiveness of cryopreservation. Adapted from Hübule (2003).](image-url)
support. The cultures were stored in cryotubes (1.8 mL) for 48 h up to 3 years in liquid nitrogen; after this time, the ability to maintain laccase production, growth, and the macro and microscopic characteristics of the conserved strains were evaluated. They obtained that 100 % of the strains maintained the viability, purity, morphological characteristics, and growth rate of the microorganism during the evaluated period. In 2007, these same authors evaluated the capacity of 50 strains of basidiomyce fungi preserved by the pellet protocol to survive three successive cycles of freezing and thawing, obtaining that in the first cycle, 100 % of the strains evaluated kept viability, purity, macro, and microscopic characteristics, showing a viability of 96 % as well as macroscopic characteristics of 96 %. The strains survived while maintaining the same characteristics mentioned above. Therefore, they assured that with this cryopreservation method, the difficulties caused by the interrupted supply of liquid nitrogen or electrical energy during storage could be overcome without affecting the preserved culture's survival and quality (Ladislav Homolka et al., 2007).

In a study on the vitality and genetic stability of mycelium in 15 species of white-rot fungi, different cryopreservation protocols were evaluated at -80 °C and lyophilization. These included variables such as culture medium, cryoprotectants, time, number of fractions, and origin of the samples. The results showed that it is possible to perform adequate conservation of the basidiomyce strains by these techniques; however, in lyophilization, morphological changes occurred in two isolates of Ganoderma adspersum, something that did not occur with freezing (Voyron et al., 2009). The researchers Ladislav Homolka et al. (2010) conserved 30 strains of basidiomyce fungi on pearls in liquid nitrogen for ten years, and they found that in most cases, the viability for lyophilization was 75 % while the viability remained stable, as was the enzymatic activity of preserved fungi. On the contrary, in the study conducted by Kaur et al. (2011) on conservation of strains of edible fungi Agaricus bisporus and Pleurotus florida at different temperatures (25 °C to 35 °C and -20 °C to -196 °C) in 10 % glycerol, showed that the viability and recovery of the strains are affected by storage conditions after six months.

In another study conducted by Mantovani D’Agostini et al. (2012), developed alternative techniques in the cryopreservation of basidiomyce fungi, evaluating the effect of different substrates such as whole grains of oats, wheat, rice, and millet and cryoprotectives agents such as dimethyl sulfoxide, glycerol, sucrose, glucose, polyethylene glycol and malt extract for the conservation of strains of Pleurotus ostreatus by the cryopreservation method at -20 °C and -70 °C for a period of 1 to 3 years. The results showed that any substrate combined with the cryoprotectants studied effectively preserved mycelium, except for millet grains with polyethylene glycol after three years. Wheat grains combined with any cryoprotectant effectively keep mycelium viable after one year of cryopreservation at -20 °C, and when combined with sucrose or glucose, they are more effective after three years. Echlerová et al. (2015) used two protocols: pearls and plastic straws for the cryopreservation of Pleurotus ostreatus and Trametes versicolor; these strains were frozen at -70 °C at a speed of 1 °C min⁻¹ using 5 % glycerol as a cryoprotectant and then stored in liquid nitrogen for 12 years, in order to assess the viability, growth rate, morphological characteristics, laccase enzyme activity, and decolorization test. As a result of this investigation, they obtained that 100 % of the strains conserved by these methods managed to remain viable, retained their macro and microscopic characteristics, and maintained their enzymatic activity.

Freeze-drying or lyophilization

It is a long-term conservation method that guarantees the genetic stability and viability of the organisms preserved for periods of 10 or more years, also prevents the occurrence of successive generations (Arenchibila et al., 2008). The method stops the microorganism's metabolism and extracts water from the frozen cells by sublimation of the ice under high vacuum conditions (Morales et al., 2010). The culture of the microorganism is carried out in Petri dishes with potato dextrose agar or malt extract agar, then spore or hyphae suspensions are transferred in glass ampoules, these are frozen at -70 °C for 4 to 6 h, then lyophilized and later stored in cold storage or room temperature (S.K. Singh, 2017). The dehydration of the cells, necessary to avoid intracellular crystallization during freezing, is regulated through the cooling rate and depends on the cell size, freezing rate, thickness of the cells (Ladislav Homolka, 2014). The addition of a cryoprotective agent is required to prevent the cells from suffering some damage, such as monosodium glutamate, glucose, sucrose, trehalose, skim milk, inositol; other cryoprotectants such as glycerol, dimethylsulfoxide, 1,2 propanediol, ethylene glycol, ethanol, methanol, polyethylene glycol are used; however most of these are toxic (Arenchibila et al., 2008; Nakasone et al., 2010; Ladislav Homolka, 2014). This method is highly recommended for its convenience for storage and transport of the strains, as they can be stored at room temperature (Pinzón Gutiérrez et al., 2009); however, it is a high-cost technique due to infrastructure and equipment requirements; thus, it cannot be applied in laboratories with limited resources (Gato, 2010; Montesinos Matías et al., 2015). In some cases, the lyophilization of basidiomycetes has been successful, which has increased interest in this method. Tan et al. (1991) evaluated the method in 4 ascomycete strains (Alternaria bataticola, A. diantibolica, Cercospora autensis, and Chaetomium bulbosum) and two strains of basidiomycetes (Coprinopsis sp and Schizophyllum commune); finding that all strains were recovered after the lyophilization process with differences in the survival rate which was lower in basidiomycetes compared to the ascomycetes evaluated; the rate increased in both phylum when incubated in media containing trehalose.

Cronan et al. (1999); applied this method of preservation in Pleurotus ostreatus, P. populorum, and P. pulmonarius, which showed an intense colonization mycelial growth and more effective substrate after preservation compared to non-lyophilized, at cold storage (4 °C), 57 % of the isolates survived for 2.5 months at 15 °C, and 92 % of the isolates survived ten months. S. K. Singh, Upadhyay, Yadav, et al. (2004) worked on this method with 11 strains from Pleurotus ostreatus. After 10 months of storage, the fungi showed a viability of 90.3 % after one year of storage. Van work development on the vitality and genetic fidelity with mycelium of fungi of the white-rot. Voyron et al. (2009) evaluated 12 lyophilization protocols with 15 strains of basidiomycetes; they found that all the isolates maintain the viability in two of the 12 evaluated protocols, the morphological characteristics are maintained in 13 of the 15 strains, and the physiological characteristics were not modified after one month of storage. Palacio et al. (2014) evaluated the viability of 4 strains through 3 conservation methods, finding that under the conditions they applied, lyophilization was inappropriate for the isolates since none was viable after 1, 6, and 12 months of conservation. Recently in a study conducted by Sun et al. (2018), evaluated the effect of the conservation method on the Cordyceps militaris; the results showed that the growth rate of the fungus was not affected by age and sub-culture, maintaining vitality for 12 months.

Sub-culture

It is considered a short-term conservation technique since microbial cultures must be reactivated and renewed periodically to maintain the characteristics that make them important; therefore, these time intervals depend on the type of microorganism to be preserved. Some microorganisms need to be transferred to new culture media after days, weeks, or months (usually in less than a year), while other culture preservation methods managed to conserve for months or years; however, it is one of the most commonly used techniques for basidiomycete fungi since they cannot be easily preserved by other methods (Nakasone et al., 2004; Ladislav Homolka, 2014).

This method consists of periodically transferring the strains of microorganisms to Petri dish with fresh media with fresh culture before the loss of the predecessor culture (Nakasone et al., 2004; Ladislav Homolka, 2014). It is a method that has several drawbacks, including the selection of phenotypic variants (pleomorphic growth) and mutant gene, the loss of pathogenicity, viability or sporulation. It is a universally applicable technique currently used in most low-budget collections or as a second maintenance method. Generally, the storage temperature in this method is ambient; and one way to avoid performing several cultures is cold storage or using wet perlite; which can be an alternative method that has shown excellent results in maintaining strains for up to four years (Nakasone et al., 2004; L. Homolka et al., 2008; Ladislav Homolka, 2014).

Regarding edible fungi, Bermeo Escobar et al. (2020) analyzed the influence of conservation in sterile distilled water, sterile mineral oil, sterile saline, and periodic subculture on the purity and viability of the Pleurotus ostreatus strains, finding that the conservation methods evaluated maintained the characteristics of the microorganism after four months at 4 °C. Yin et al. (2017) identified that the use of subculture of Cordyceps militaris stored for ten days at 23 °C could impact gene strain degeneration, restricting the large-scale production of this medicinal and edible mushroom.

Sterile mineral oil

It is an alternative method commonly used to conserve mycelium or fungi that are not sporulated or have poor sporulation, which is susceptible to lyophilization and freezing; also, when deep freezing is not an option, due to its high cost. With this method, only a portion of the culture viability is maintained for 2 to 3 years at -35 °C or 32 years at room temperature or temperatures between 15 °C and 20 °C (Nakasone et al., 2004; Ladislav Homolka, 2014). It is a method of suppressing evaporation. It consists of completely covering the culture in a tube (after its development in a solid medium), with a layer of sterile mineral oil (sterile petrolatum jelly (1 cm), tubes are stored in a cardboard box at cold storage or room temperature, preventing the evaporation of the water contained in the culture medium and avoiding the increase of osmotic pressure.
Edible and medicinal mushrooms represent an alternative to obtain nutrients or active compounds in the diet, being a possible strategy for reducing food insecurity. Edible and medicinal mushrooms have various industrial applications, this is why evaluation is essential to evaluate other preservation techniques that have been applied mainly for the conservation of filamentous fungi. Sebacina,
on their growth, morphological, enzymatic and genetic characteristics. Fungal Biology, 114(11-12), 929-935. doi: 10.1016/j.fbi.2010.08.009

Homelka, L., Lis, L., & Nerud, F. (2006). Basidiomyce cryopreservation on petri: evaluation of a new method. Cryobiology, 53(2), 446-453. doi: 10.1016/j.cryobiol.2006.02.003

Homelka, L., Lis, L., & Nerud, F. (2007). Basidiomyce cultures on petri survive successfully repeated freezing and thawing in cryovials without subculturing. Journal of Microbiological Methods, 69(3), 529-532. doi: 10.1016/j.mimet.2006.08.003

Hu, X., Webster, G., Xie, L., Yu, C., Li, Y., & Liao, X. (2014). A new method for the preservation of axenic fungal cultures. Journal of Microbiological Methods, 99(1), 81-83. doi: 10.1016/j.mimet.2014.02.009

Hubílek, Z. (2003). Protectants used in the cryopreservation of microorganisms. Cryobiology, 46(3), 205-229. doi: 10.1016/S0011-1286(03)00046-4

Igual, M., Hueso, C., Yuste, M., & Cazorla, J. (2017). Evaluation of the gelatin technique for the preservation of phytopathogenic fungi. World Journal of Microbiology and Biotechnology, 33(4), 923-932. doi: 10.1007/s11277-017-2711-6

Iqbal, S., Ashfaq, M., Humayun Malik, A., Inam-ulhaq, Safiullah Khan, K., & Mathews, P. (2017). Isolation, preservation and revival of Trichoderma viride in culture media. Journal of Entomology and Zoology Studies, 5(3), 1640-1646.

Johnson, G. C., & Martin, A. K. (1992). Survival of Wood Fungous Cultures. Fungal Biology, 80(10), 532. doi: 10.1580/975514.1992.1201780

Rico, M., Piattoni, C., Gonzalez, C., Monela, R., Latorre, M., & Lurá, M. (2004). Viabilidad de cepas fúngicas conservadas mediante diferentes métodos. FAIBICIB, 8, 163-172. doi: 10.14409/fabicib.v8i1.744

Richter, D. L., Kangas, L. C., Smith, J. K., & Laks, P. E. (2010). Comparison of effectiveness of wood decay fungi maintained by annual subculture on agar and stored in sterile water for 18 years. Canadian Journal of Microbiology, 56(3), 268-271. doi: 10.1139/W10-001

Ryan, M. J., & Smith, D. (2017). Fungal genetic resource centres and the genomic challenge. Mycological Research, 108(12), 1351-1362. doi: 10.1016/j.mycres.2014.02.009

Singh, S. K. (2017). Ex situ Conservation of Fungi: A Review Developments in Fungal Biology and Applied Mycology (pp. 20). Singapore: Springer Nature Singapore

Singh, S. K., Upadhyay, R. C., Kamal, S., & Tiwari, M. (2004). Mushroom cryopreservation and its effect on survival, yield and genetic stability. Cryo Letters, 25(1), 23-32.

Singh, S. K., Upadhyay, R. C., Yadav, M. C., & Tiwari, M. (2004). Development of a novel lyophilization protocol for preservation of mushroom mycelial cultures. Current Science, 87(5), 568-570.

Smith, D., & Onions, A. H. S. (1983). A comparison of some preservation techniques for fungi. Transactions of the British Mycological Society, 81(3), 535-540. doi: 10.1006/JMIM.2001.1739801223

Sun, H., Hu, T., Guo, Y., & Liang, Y. (2018). Preservation affects the vegetative growth and fruiting body production of Cordyceps militaris. World Journal of Microbiology and Biotechnology, 34(4), 1-10. doi: 10.1007/s11277-018-2550-4

Sun, H., Hu, T., Guo, Y., & Liang, Y. (2018). Preservation affects the vegetative growth and fruiting body production of Cordyceps militaris. World Journal of Microbiology and Biotechnology, 34(4), 1-10. doi: 10.1007/s11277-018-2550-4

Vehera, D., & Rodriguez, O., De la Torre, J., Martínez, R., Pérez, R., & González, G. (2009). Conservación de cepas de.phi útilipes. Revista de la Sociedad Venezolana de Microbiología, 25(1), 35-40.

Pinzón Gutiérrez, Y. A., Bustamante, S. L., & Buitrago, G. (2009). Evaluación de métodos para la conservación de hongos fitopatógenos del fráme (Dioscorea sp.). Revista Colombiana de Biotecnología, 11(2), 8-18.

Prakash, O., Nimonkar, Y., & Shouche, Y. S. (2013). Practice and prospects of microbial preservation. FEMS Microbiology Letters, 339(1), 1-9. doi: 10.1111/j.1574-696x.2013.07331.x

Palacio, A., Gutiérrez, Y., Rojas, D., Atehortúa, L., & Zapata, P. (2014). Viabilidad de Basidiomyce fungal strains under different conservation methods: cryopreservation vs. freeze-drying processes. Actualidades Biológicas, 36(100), 13-21.