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Mushroom Preservation Protocol

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Abstract

Mushrooms are macro-fungi species due to their visible structures. This is one type of genetic resource and a material having immense potential. For example, it used as a source of food and pharmaceutical products. Globally, Mushrooms is consumed and introduced into a part of the daily diet in many countries due to their nutritional component and immune capacity building. To store/deposit mushroom for the next coming generation is vital in many ways such as biotechnological, pathological, taxonomical, environmental, ecological, medical, industrial, systematic and biodiversity applications or studies. This work discusses the mechanisms used to preserve in short and long terms. The actual standard protocols (such as sub-culturing, lyophilization, cryopreservation, inoculation of mycelia in malt extract agar, and inoculation of the spawn on a sterilized substrate like cereal seeds) which are already applied in many culture collection centers and supported with many cryo-biologist. Overall, Chemical, physical and biological methods are addressed and allowed for preservation.

Keywords: Mushroom; Preservation; Factors; Techniques

Introduction

Mushrooms are macro-fungi with visible structures that produce spores otherwise known as fruiting bodies. They offer tremendous applications as they can be used as food and medicines besides their key ecological roles. Numerous species are consumed throughout the world as a delicacy particularly for their specific aroma, texture, and taste [1]. Mushrooms are low in fat, high in complex carbohydrates and protein [2] and they also lack cholesterol and are good sources of vitamins and minerals [3]. Mushrooms have received critical scientific and socio-economic attention in the last few decades. Nowadays, it is increasingly being utilized as an important food product for their crucial role in human health, nutrition, and disease control. Currently, common mushroom genera *(Agaricus spp, Lentinula spp,Auricula spp, Flammulina spp, Volveriella spp, Grifola spp, Pholiota sppand Pleurotus spp*) already cultivated, utilized, processed, identified, characterized, and preserved in different parts of the world [4,5]. In Ethiopia also particularly in the central (Addis Ababa), southern (Kaffa), western (Gumeze and Berta) peoples showed that a little interest and have a small background for mushroom farming and consumption.

Mushrooms are highly perishable food items that tend to lose their unique organoleptic properties immediately after harvesting. Their short shelf-life is mainly explained by the high losses of water vapour that favour dehydration, high respiration and microbial colonization by bacteria or fungi. Furthermore, enzymatic activity and biochemical alteration lead to mushrooms quality losses [6]. The microorganisms most often associated with mushroom spoilage and colonization are gram-negative bacteria especially those belonging to Pseudomonad family such as *Pseudomonas fluorescens* [7]. Preservation is the processes of permanently preserved in metabolically inactive states. In successful storage, the purity, viability, and genomic integrity of the culture should be maintained and the morphological, physiological, and genetic characteristics of the culture should not change. In Ethiopia, no more edible (cultivated) and non-edible (wild) types are not intensively studied and preserved when compared with an Asian and European country. So this paper provides information for inside and outside country researchers to do more and prevent the genetic material loss. More recent research articles have reported regarding to a long-term preservation technique, but all showed that no universal protocol for the preservation of basidiomycetes or mushrooms. That is, vary from one culture collection center from another. To fill the basic information gap this short communication may answer for any confusions. This study aimed to present basic preservation methods which are simple and cheap to implement adequately. Later, it acts a starting point to initiate scholars for ex-situ conservation and accelerate their exploration of mushrooms (Figure 1).

Figure 1: Mushroom.

Advantages of Mushroom

Medicinal value (immune modulating, antitumor, antioxidant, radical scavenging, cardiovascular, antibacterial, antiviral, antihypertensive, antihyperholesterolemic, detoxification, hepatoprotective, antidiabetic activities, anti-inflammatory, disease prevention and cholesterol reduction) for health-promoting effects attributed to their bioactive compounds. But detailed mechanisms of the various health benefits of mushrooms to humans still require intensive investigation. Isolation and characterization of their active ingredients, with mechanism-based potential therapeutic value, remains a challenge [8,9].

Why We Need Mushroom to become Preserved?

- Frequent loss of texture-softening.
- Protein and carbohydrate contents are decreased.
- Formation of brown coloration.
- Depletion of soluble compounds.
- To use in the future in different areas (like for biotechnological, pathological, taxonomical environmental, ecological and industrial, systematic and biodiversity applications or studies).Currently, there is a growing interest in obtaining and studying the biologically active compounds from higher Basidiomycetes.
- To solve morphological change and to minimize the risk of contamination.
- For further maintenance, characterization, and identification of culture, to tackle anthropogenic and natural influence that affect species diversity or abundance.
- To sustain food security and safety because it is quickly spoiled.
- For many years genetic stability, distribution, and commercialization.
- Forever increasing human population and diminishing farm sizes have resulted in declining soil fertility associated land productivity and increasing poverty levels.
- Combating malnutrition, generating income, accessing and benefit sharing.
- Creating new dimensions of sustainable agriculture and forestry (bio-innovations) [10].

Factors Affecting Preservation

Several factors affect the effectiveness of the preservation process: lack of good laboratory practice and viability test, strain type or quality, selection of temperature, composition of the growth and preserve media, time storage, and concentration of cryo-conservation/freeze-drying protecting agent (like glycerol, dimethyl sulfoxide, bovine albumin serum, skimmed milk, ethylene glycolsorbitol, and trehalose or myo-inositol). Few microorganisms can survive after the preservation process without a protecting agent; these agents can provide a longer storage time, avoiding cellular injuries [11,12]. However, it attacked by pathogens and pests dominantly. Therefore, the storage of microbial cultures is difficult and time-consuming. In order to guarantee viability for these microorganisms, various procedures have been carried out allowing the management of strains. In another word, evaluation of conservational methods and strain viability must be performed always.

Short and Long-Term Types of Mushroom Preservation Techniques

Each species needs compatible and distinctive alternative techniques for their active, pure and viable physiology in terms of color, texture, and taste. Preservation protocols are applied to store cultures in viable and stable form for long periods without losing genotypic, phenotypic and physiological traits. Some of them listed below.

1. Continuous sub-culturing but it has its own limitation at all. Several studies demonstrated that storage of fungi by sub-culturing may induce antigenic changes, phenotypic and genetic alterations as well as attenuation of virulence. Sub-culturing was used conventionally for regular activity. In most cases, it leading to the problems of contamination and degeneration. It is difficult for storing large numbers of fungal cultures. It is time-consuming. Then, it does not prevent genetic and physiological changes.
2. Inoculation of mycelia in malt extract agar then store in sterile distilled water at +4^{o}C [13]. 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.
3. Lyophilization and cryopreservation (freezing at -80°C and storage in liquid nitrogen [-196°C]). Cryoprotectants are of two types: penetrating agents such as glycerol and dimethyl sulfoxide (DMSO), which readily pass through the cell membrane and protect intracellularly and extracellularly, and non-penetrating agents such as sucrose, lactose, glucose, mannitol, sorbitol, dextran, polyvinyl-pyrrolidone, and hydroxyethyl starch, which exert their protective effect external to the cell membrane. Cryopreservation is a process where cells or whole tissues are preserved by cooling to low sub-zero temperatures by adding cryoprotectants which act as antifreeze by lowering the freezing temperature and increasing the viscosity. Cryopreservation of cultures is considered as the most acceptable method of preservation.
4. At +4°C and 20^{o}C indifferent concentration of glycerol by using two different methods (slant culture and slice cut method) with Agar plug components [14].
5. Inoculation of the spawn on a sterilized substrate (seeds).Cereal grains (hard endosperm wheat, medium hard endosperm wheat or hard endosperm rye) plus malt extract agar at -70ºC or 4^{o}C. The use of glycerol as a cryoprotective solution at -70ºC. Over the years, researchers have developed practical, effective, and ingenious methods of preserving fungi or mushroom on various organic substrate such as wood chips, cereal grains, straw, filter paper, insect and plant tissues [10,14,15].
6. Potassium sorbate (0.1%) and citric acid (4%) extended the shelf life of mushroom for 24 days [16].
7. 30% brine solution. At a temperature range become 25-30^{0}c for 6 up to 7 days [17].
8. Drying Techniques it includes low heat air blow (*LHAB*, Anjaad *TM*), sun drying (*SD*) and gas laboratory oven (LO) drying, solar dryer, vibro dryer, vacuum dryer, and hot air oven. Drying seems to be an effective approach to extend shelf life. This is widely reported worldwide [18,19].

Conclusion

Mushroom has a nutritional value which serve as a functional/staple food globally. When coming to inoculation of the spawning substrate (seeds), it has a potential impact and profitable outcome. However, Periodic serial subculture is not crucial because gradually DNA and chromosomal mutation rate become high. Generally, this kind of traditional way is unsupported and unsafe. The present study was revealed that the paper indeed a good success (sources) of information.

Recommendation

Further target full and advanced cryo-biological technologies must introduce through experimental trials on the area. This paper emphasis for other researchers and governmental bodies should formulate in-situ type conservation practice to pass the genetic material for the next generation and to save nature.

Conflict of Interest

The authors declare no conflict of interest

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