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DEVELOPMENT OF CRYOPRESERVATION TECHNIQUES FOR PRESERVING FUNGI

TASNIM BINTI MAZLAN

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DEVELOPMENT OF CRYOPRESERVATION TECHNIQUES FOR PRESERVING FUNGI

By

TASNIM BINTI MAZLAN

175115

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CERTIFICATION

The project entitled **"Development of Cryopreservation Techniques for Preserving Fungi"** is prepared by Tasnim binti Mazlan and submitted to the Faculty of Agriculture in partial fulfilment of the requirement of PRT 4999 (Final Year Project) for the award of a degree in Bachelor of Agricultural Science.

Student's name:

Tasnim Binti Mazlan

(175115)

Student's signature:

……..

Certified by:

…….

Dr. Tan Geok Hun

Project Supervisor,

Department of Agriculture Technology,

Faculty of Agriculture,

Universiti Putra Malaysia.

Date: …….

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ABSTRACT

In recent years, tremendous increase in human population causes pressure on the forest and land resources. This condition in turn caused a decrease in the population of medicinal and aromatic plant species. Some animal species are also on the verge of extinction besides plants that are greatly threatened. For this matter, cryopreservation can be used as an effective means to conserve the germplasms of such species. Cryopreservation is a process in which living biological material is frozen and stored at low temperatures (-80^0C). However, this low temperature is not a favourable condition for microbes and germplasms because this process will cause cell damage, protein denaturations and membrane injury towards the microbes. Therefore, a suitable cryoprotectant agent is needed in order to protect the microbes from being damage during the preservation process. Cryoprotectant agent acts like an antifreeze; they lower the freezing temperature and increase viscosity. Thus, the most effective cryoprotectant agent between glycerol, sucrose and honey will be studied and identified at the end of this research. Ten different species of fungi were used in this experiment for the preservation. One hundred microlitre of each fungus was cultured in Potato Dextrose Agar (PDA) media. Following after that, those fungi was tested and preserved by using different cryoprotectant agent with different percentage of concentration which are 10%, 15% and 20% of glycerol, sucrose and honey relatively. The survival of the fungi was observed after one month of preservation. Randomized Completely Block Design factorial (RCBD) was used in this experiment to evaluate the normal percentage and cost effective of cryoprotectant agent used. Glycerol is predicted to be the most effective cryoprotectant agent.

ABSTRAK

Marcapada ini, dengan peningkatan yang sangat besar dalam populasi manusia menyebabkan tekanan ke atas hutan dan tanah sumber meningkat. Keadaan ini seterusnya menyebabkan penurunan dalam populasi spesies tumbuhan ubatan dan beraroma. Sesetengah spesies haiwan juga di ambang kepupusan selain tumbuh- tumbuhan yang sangat terancam. Disebabkan oleh perkara ini, krioperservasi boleh digunakan sebagai satu cara yang terbukti berkesan untuk memelihara satu- satu spesis tersebut. Pengawetan secara krio adalah salah satu proses di mana hidupan dibekukan dan disimpan pada suhu rendah (-80^0C). Walau bagaimanapun, suhu rendah ini bukanlah satu keadaan yang digemari oleh mikrob dan germplasma kerana proses ini akan menyebabkan kerosakan sel dan kecederaan membrane terhadap mikrob dan germplasma. Oleh itu, ejen perlindungan krio yang sesuai diperlukan untuk melindungi mikrob daripada mengalami kerosakan semasa proses pemeliharaan. Ejen perlindungan krio ini bertindak seperti bahan antibeku yang menurunkan suhu dan peningkatan kelikatan beku. Oleh itu, ejen perlindungan krio yang paling berkesan antara gliserol, sukrosa dan juga madu dikaji dan dikenalpasti pada akhirs kajian ini. Sepuluh spesis kulat yang berlainan digunakan dalam ujikaji ini dan 100µL setiap kulat dipelihara dalam media potato (PDA). Seterusnya, kulat diuji dan dipelihara dengan menggunakan ejen krio perlindungan yang berbeza dengan peratusan yang berbeza kepekatan iaitu 10%,15% dan juga 20% gliserol, sukrosa dan juga madu. Ketahanan kulat diuji selepas satu bulan pemeliharaan. Rawak sepenuhnya (RCBD) digunakan dalam eksperimen ini untuk menilai ejen krio perlindungan terbaik dan ketahanan kulat di dalam ejen krio perlindungan.

CHAPTER 1
INTRODUCTION

Fungus is a living organism in kingdom Fungi (Christian, N. 2014). It is the most important eukaryotic microorganisms on the planet. It produces spores and is reproducing by sexual and asexual reproduction (Nakasone et, al. 2004). Hyphae are the main mode of vegetative growth in most fungi, except for yeast. Unlike the cell walls of plants that contain cellulose, fungi's cell wall contains of chitin (Blackwell, M. and Spatafora JW 2004). Since fungus act as a great decomposers for dead organisms, they play an important roles in agriculture. They are capable to colonize a wide range of living and dead tissues, including plants, wood and paper products, agricultural plant residues, and live or dead animal tissues. Besides that, fungi are vitally important for the growth of crops in agriculture through the development of mycorrhizal associations (Buchanan and Gibbons, 1974). In food chains, plants are at the top of the chain and with limited growth of plant will cause all animal and human life in starvation. Fungus as an edible food such as mushroom are cultivated for sale worldwide.

Maintaining fungi in its stable, viable and pure condition are important but can be very difficult (Buell and Weston. 1947). It is necessary to use appropriate growth techniques to ensure stability of microorganisms for research and development. It is very important to retain all characteristics of the fungi throughout the storage process. Temperatures also play an important role in fungi's growth. Due to these difficulties, preservation is important to keep all the characteristics and morphological of the fungi.

Fungi requires appropriate techniques to ensure the viability and stability of every morphological conditions of fungi after being preserved (Castellani, A. 1939). Thus, it is important to choose a suitable preserving techniques to preserve fungi.

There are two types of preservation which are short term preservation and long term preservation. Short-term preservation is usually simple, inexpensive and widely used. Usually, the short-term preservation is used for the cultures that are constant in used. This method are usually simple and inexpensive because specialized equipment is not required. For long-term preservation, there are a lot of ways to preserve culture which are oil overlay freezing, sclerotization, immersion in distilled water, freezing method, freezing in liquid nitrogen and many more (Chandler 1994).

However, this study only discuss on freezing method at -80^{0}C. Freezing methods are versatile and widely applicable and most fungi can be preserved with or without cryoprotectant agent. Cryopreservation at -80^{0}C is the method recommended and used by American Type Culture Collection (1991) to keep the fungi characteristics and viability for many months or years. However, according to Elliot, T. J. (1976), low temperature affects the fungi growth rates, metabolic activity and in some cases may cause the fungi to enter dormancy. Therefore, cryoprotectant agent is used to protect the fungi during preservation and to ensure the viability and stability of the fungi.

Since this preservation is a long and expensive process, it is essential to make sure that the whole process is a success and the characteristics of the fungi is remained in it's pure condition so not to repeat all the process once again (Nakasone, *et al.*, 2004). Choosing a right cryoprotectant may reduce the cell damage and also may reduce the cost of the whole process.

Thus, the present study was conducted with the following objectives:

i. To optimize preservation technique by using different cryoprotectant agent which are glycerol, sucrose and honey with different percentages.
ii. To screen potential fungi which can tolerate to the cryoprotectant solution at different rates.

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