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A Cryopreservation Method of *Scizophyllum commune* Local Strains

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Abstract

Schizophyllan commune isolates were collected from rubber plantations in Selangor and Perak area in Malaysia. Spawns were prepared using three different grains (wheat, corn and paddy). The grains were incubated for 7 days at room temperature. After the grains were fully covered with mycelia, they were place into a cryo-vial with three different treatments, 10% glycerol, sterile distilled water and no cryoprotectants. All of the treatments were frozen in liquid nitrogen for 7 days followed by thawing (cooling) at 30°C for 30 min. The grains were inoculated onto PDA agar to observe the viability (rate of survival) of the seeds. The radius of each grain with different treatments was measured. Results showed that all treatments showed 100% viability after 48 hours and wheat with no cryoprotectants showed the highest growth rate. This indicated that the grains have mycelial protective properties. In this study, a cryopreservation process was simplified as opposed to the conventional method in which 10% glycerol was used as the cryoprotectant. This study promotes an alternative method for cryopreservation which is more economical as no cryoprotectants were required.

Introduction

Cryopreservation is a process of freezing a cell culture whereby the activity of the cell will be paused for a certain time. The freezing process is done at a very low temperature in a liquid nitrogen chamber (tank). The purpose of halting the cell is to maintain the cell culture for long term storing without contamination. The conventional methods of maintaining the cell stock culture is time consuming and may lead to contamination as the cells are being exposed to unstable conditions. Rather than that, the cells are only limited for a certain time and have to be continuously re-cultured. Cryopreservation of plant-like mushrooms needs a carrier or planting material to carry the mycelia. Spawn is the term for the carrier which may be regarded as the seeds of a higher plant.

In order to avoid crystallisation and cell damage, cryoprotective solutions that are known as cryoprotectants are used. In this study, *Schizophyllum commune* isolates were cryopreserved using 10% glycerol and sterile distilled water. The experiment also eliminates the use of cryoprotectants to test whether the seeds can survive without a cryoprotectant agent. The choice of cryoprotectant is dependent upon the type of cells to be preserved. Glycerol and dimethyl sulfoxide (DMSO) have been widely chosen as cryoprotectant agents. Glycerol is a small, poly-hydroxylated solute with a high solubility in water, and a low toxicity during short-term exposure to living cells (Fuller *et al.*,2004). However, DMSO was excluded from this study because of the higher toxicity

of the solution even though it is more penetrating and commonly used as a cryoprotectant agent for complex cells (Simione, 1998).

Materials and methods

Strains

Fruiting bodies of *S. commune* were collected from Perak and Selangor. Pure cultures were obtained on potato dextrose agar (PDA). The cultures were stored on agar slants at 4°C.

Spawn preparations

Spawn (seed that carries mycelia) was prepared using three different grains which are corn, paddy and wheat. The grains were soaked for 24 hours and were left to dry for 10 minutes at room temperature. Gypsum (1% w/w), were added to maintain the pH of the grains. The grains were packed in polyethylene bags (100g per bag) with caps and were autoclaved at 121°C for 15 min for sterilisation purpose. Three discs (± 0.7cm in diameter) of mycelia (7 days old) were inoculated into the sterile packed grains and were placed in the dark for 2 weeks to allow the mycelia to completely cover the grains.

Cryoprotectant preparations

Three different treatments of cryoprotectant were tested in this experiment. The treatments were T1 (10% glycerol v/v), T2 (sterile distilled water) and T3 (without cryoprotectant). T1 and T2 were pipetted into the cryo-vials (1.5ml per vial). All the vials including T3 were autoclaved at 121°C for 15 min. Each vial was filled with 10 seeds that have been covered with mycelia (10 seeds each for 10 vials). The vials were gently shaken to make sure that the cryoprotectant covers all the seeds and were left at room temperature for 60 minutes before being transferred into a liquid nitrogen tank for a week.

Thawing and Inoculation

Thawing is a process of cooling down the frozen seeds in the vial. The vials were taken out from the liquid nitrogen tank and thawed into a water bath at 30°C for 30 minutes. Once thawed, the vials were sterilised with alcohol (70% v/v) and left to dry for 10 minutes. The seeds from the vial were placed onto Potato dextrose agar media (PDA). Each plate was inoculated with 5 seeds to test the viability of the seeds. The growth rates of the seeds were recorded starting from day one untill day 9.

Results and Discussion

The results were obtained after 24 h incubation time. Positive results were recorded when short and tiny mycelia appeared around the seeds. Table 1 shows the percentage of seed viability from different types of seeds and different cryoprotectants.

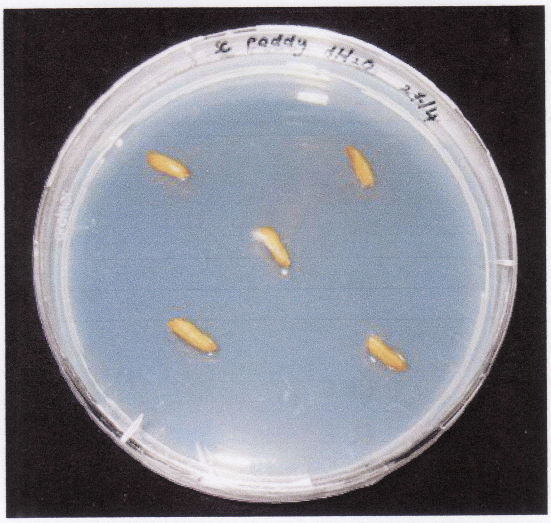

Figure 1: Viability observation of *Schizophyllum commune* on agar plates.

Table 1: Viability of seeds

Grain	Percent of survival
Treatment 1	
Corn	100%
Paddy	100%
Wheat	100%
Treatment 2	
Corn	100%
Paddy	100%
Wheat	100%
Treatment 3	
Corn	100%
Paddy	100%
Wheat	100%

The growth rate of *S. commune* was measured by the radius of the colonies formed by the mycelia on day 3, 6 and 9. The mean radius from 5 plates was recorded. Table 2 shows the comparison of the average diameter (cm) from the mycelia forming colonies of *S. commune* with different cryoprotectant treatments.

Table 2: Growth rate of *S.commune*

Grain	Day 3	Day 6	Day 9
Treatment 1			
Corn	0.87	3.03	4.00
Paddy	0.70	3.07	4.00
Wheat	1.00	3.10	4.07
Treatment 2			
Corn	1.50	2.43	3.67
Paddy	0.70	3.03	3.67
Wheat	1.03	2.47	4.07
Treatment 3			
Corn	0.60	3.10	4.00
Paddy	0.57	3.07	3.67
Wheat	2.00	3.47	4.17

Table 1 (viability of seeds) shows that 100% seeds survive in three treatments (T1, T2 and T3). Mata and Perez (2003) reported that the recovery rate of five edible mushrooms (*Lentinula boryana, Lentinula edodes, Pleurotus djamor, Pleurotus pulmonarius, and Volvariella volvacea*) was 96.8% when treated with glycerol or DMSO and 99.2% without cryoprotectants. In this study, the results demonstrate that *S. commune* showed the highest survival rates when all of the seeds with all treatments were covered by mycelia.The seeds can survive because the grain itself plays a role as a carrier and also a protector to the mycelia. Wheat with no cryoprotectants showed the highest growth rate of *S. commune* afterthe cryopreservation process. This study promotes an alternative method for cryopreservation which is more economical as it does not need any cryoprotectants.

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