Comparison and Validation of Cryopreservation Methods of *Candida* Genus Fungi: A Brief Review

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Cryopreservation is a freezing technique that prevents the microorganism's genetic material from carrying out possible reactivations. This method commonly requires cryoprotectants to minimize the possible damage that freezing can cause. The current article seeks to evaluate the efficiency and viability of cryopreservation of fungi from the genus *Candida*, aiming at the best technique. The method was based on searching articles related to the analyzed theme and their ratifications. Thus, with the bibliographic survey, it was possible to conclude the most executable method among the three most used.
Keywords: Cryopreservation Methods. *Candida*. Cryoprotectants.

Introduction

Cryopreservation is fundamental for laboratory research in microbiology, as it keeps microorganisms preserved, allowing the possibility of storing biological material for an extended period until it is necessary to use it [1]. However, the preservation method varies according to the type of microorganism used, which implies no option of standardizing a method for general use. Therefore, it is necessary to analyze the advantages and disadvantages of each type of cryopreservation for different biological materials to determine which is the best for each situation.

Currently, the most used technique is cryopreservation in the ultra-freezer –80°C; being necessary to use cryoprotectants due to the low temperatures. Cryoprotectants are substances added to the medium to prevent severe damage to microorganisms by freezing and thawing them [1]. The protective substance selection depends on which part of the cellular needs protection: external, which not penetrates the cell, or internal, which penetrates the cell [2].

Candida is responsible for 80% of fungal infections in the hospital environment and contributes significantly to bloodstream infections [3]. In addition, these microorganisms have an extensive habitat, like the digestive mucosa and the vaginal mucosa, and can cause infections in the mucous membranes, skin, and systemic tissues [4].

The present study aims to evaluate the efficiency of *Candida*'a genus fungi cryopreservation and compare the methods most used in 3 articles from the current literature.

Materials and Methods

We searched different *Candida*'s genus fungi cryopreservation methods using databases (Google Scholar, Scielo, Pubmed, and Mendeley). After that, we did a spreadsheet with the data collected, filtering about microorganisms, cryopreservation method, and cryoprotectant. Finally, we chose the 3 most relevant articles ("Maintenance of yeast by freezing at – 20°C" by Silva and colleagues (2008) [5], "Update on fungal conservation methods applied to microbiological collections" by Gadêlha and colleagues (2022) [6], and "Evaluation of preservation methodologies for the maintenance of microorganisms of the *Phylum Ascomycota* and yeasts of the genus *Candida* belonging to the Collection of Microorganisms of Medical Interest of INPA" by Oliveira and colleagues [4] (2015).

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Literature Review and Discussion

Cryopreservation is the preservation of microorganisms through freezing at low temperatures, aiming to keep the DNA stable for possible reactivation, with several methods to be carried out, each according to the type of fungus used. Because these are icy environments, cryoprotectants are usually necessary to protect cell structures, reducing the damage that freezing can cause. The formation of intracellular ice crystals, the same as the method, is selected according to the feasibility of the study material. Table 1 presents the microorganisms used, the methods, and the cryoprotectants.

So, reviewing different methods in the same microorganisms allows us to know which technique is more advantageous to be applied according to viability, purity, and growth analysis. Table 1 shows the results of the four different techniques on the same microorganism.

Conclusion

We have evidenced that cryopreservation is a viable preservation technique for *Candida* in the three methods. Furthermore, there were methods with better performances than others. Thus, according to the results indicated in the methods used, we concluded that direct cryopreservation at –70° C proved more efficient and viable since it obtained high growth and purity and was simple to perform and easy to store.

Table 1. Researched articles, methods, and results found.

Article	Method	Results
Yeast maintenance by freezing at –20°C	Cryopreservation at –20°C preceded by 7 days of refrigeration, using flasks containing brain-heart infusion broth with 20% glycerol, incorporated with sterile beads [5].	• High fungal growth; • High conservation; • High recovery; • Easy execution; • Ease of storage [5].
Update on fungal conservation methods applied to microbiological collections	Cryopreservation at –20 °C preceded by 24 hours of refrigeration at 5°C, using cryotubes containing glycerinated broth, seeded with yeast, incorporated with sterile beads [6].	• High fungal growth; • High conservation; • High recovery; • Easy execution; • Ease of storage [6].
Update on fungal conservation methods applied to microbiological collections	Cryopreservation at –80 °C preceded by 24 hours of refrigeration at 5°C and another 24 hours in a freezer at –20°C, using tubes of penicillin containing Sabouraud agar with activated charcoal and performed the fungal culture.	• Medium fungal growth; • Media conservation; • Media recovery; • Easy execution; • Ease of storage [6].
Evaluation of preservation methodologies for the maintenance of microorganisms of the *Phylum Ascomycota* and yeasts of the genus *Candida* belonging to the Collection of Microorganisms of Medical Interest at INPA	Direct cryopreservation at –70° C, using sterile 2 mL microtubes with 0.8 mL of sterile distilled water, 0.05 mL of DMSO dimethylsulfoxide (cryoprotectant), 0.1 mL of glycerol (cryoprotectant), 10 mg of beads (2mm, with orifice) sterile and 100 mg of biomass of fungal isolates [4].	• High fungal growth; • High conservation; • High recovery; • Easy execution; • Ease of storage [4].

Source: Authors.

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