

## EVALUATION OF THE GELATIN TECHNIQUE FOR THE PRESERVATION OF PHYTOPATHOGENIC FUNGI

### AVALIAÇÃO DA TÉCNICA DA GELATINA PARA PRESERVAÇÃO DE FUNGOS FITOPATOGÊNICOS

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**ABSTRACT:** The preservation methods for fungi have great importance in *ex situ* collections, representing important biological heritage, useful for mycologists and plant pathologists in several scientific works. However, there is a lack of studies for a suitable and efficient preservation method for the different groups of fungi. Although, the most appropriate is the one that maintain, even after long periods, the original characteristics of culture: viability, sporulation and pathogenicity, excluding mutations and undesirable contamination. The choice will depend of the laboratory infrastructure, microorganism, objectives, preferences and knowledge of the researcher. We conducted this study inside the Laboratory of Mycology and Plant Protection (LAMIP) in UFU (Universidade Federal de Uberlândia), localized in Uberlândia (MG), Brazil. The objective was to evaluate the gelatin preservation method (17 cultures), never used before for phytopathogenic fungi. Other classical methods were concomitantly evaluated, such as sterile soil (68 cultures), resistant structures (*Sclerotinia sclerotiorum*) in 4°C (10 strains) and mineral oil (31 cultures). We examined the time for maintaining the viability, sporulation and colonization in host tissues preserved in different dates. The gelatin method remained viability in 10 cultures; this method is suitable for preservation of the genera and species: *Colletotrichum* spp., *Septoria* spp., *Fusarium* spp., *F. moniliforme* var. *subglutinans*, *Macrophomina* spp., *Phomopsis* spp. and *Verticillium* spp. The viability remained in 38 strains of sterile soil, three of mineral oil, and one strain of sclerotia reached a maximum preservation time in 4°C of four years.

**KEYWORDS:** Colonization. Pathogenicity. Preservation *ex situ*. Sporulation. Viability.

#### INTRODUCTION

Historically, the preservation of microorganisms began with the development of microbiology from the second half of the nineteenth century. Currently, the importance of preserving microorganisms has been observed in several relevant institutions as the American Type Culture Collection (ATCC), USA; the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), Germany; the International Mycological Institute (IMI), England; the Centraalbureau voor Schimmelcultures (CBS), Netherlands and the Japanese Collection of Microorganisms (JCM), Japan (URUBURU, 2003). According to January 2011 data, 585 culture collections in 68 countries were registered in the World Database Centre for Microorganisms (WFCC) and the number of fungi in these collections was calculated at 506,333 species (KARADUMAN et al., 2012).

These fungi cultures collections preserves a broad diversity of species, useful in discoveries in other areas such as biotechnology, functional, comparative and evolution genomics, bioprocesses and novel products (BOUNDY-MILLS et al., 2016). Therefore, they should be available for the reproducibility of the results found by past searches

and should keep unchanged; however, the storage of these microbial cultures is difficult and time consuming, needing the refinement of various techniques (KARADUMAN et al., 2012; SMITH; RYAN, 2012). A storage method that preserves culture viability is essential for research, into the modes of action of these pathogens (BASKARATHEVAN et al., 2009).

The preservation techniques can be divided according to the maximum time in which the cultures may remain without being handled: (a) short-term methods: periodic transfer; (b) medium-term methods: mineral oil, sterile water, -20°C, silica gel desiccant, soil and filter paper; (c) long-term methods: lyophilization, cryopreservation at -80°C and liquid nitrogen (-196°C). The chances of success of the method increases transferring the sample preserved in the culture media, with inoculation in original host every five years, for improvement of the routine work. This procedure reactive physiology of the fungus and can ensure maintenance of the original features for a further period of preservation in the laboratory; notwithstanding, there is a possibility of contamination and alteration of cultures (PIRES et al., 2012).

The maximum duration of storage varies with each method and with the species preserved, but generally revolves in 10 years or less. The existence of a method that is able to preserve viability, sporulation and pathogenicity of as many genera and species as possible, will be more reliable, and more preferably, but isolated no technique has been successfully applied to all fungi (ABD-ELSALAM et al., 2010; APARECIDO; CAMILO, 2013). Several protocols have been developed and applied, however, most have not proven fully effective, because of the peculiarities of the cultures and even the characteristics of techniques; therefore it comes to explaining the combination of two or more methods in order to ensure the best recovery (GREEN, 2008).

The development of more suitable methods for the preservation of microorganisms is of great importance to the culture collections and routine works. The varieties of methods reflect the lack of one satisfactory and convenient method for all organisms and perhaps the rugged individualism of research workers (ALFENAS; MAFIA, 2016). Despite the immense importance of microbial preservation, the research related to this area needs more attention. There are many possibilities of research available in the area of microbial preservation, but there is little interest in the optimization of preservation methods and the preserved cultures are currently limited to the culture collections or bioresource centers (BOUNDY-MILLS et al., 2016).

Thus, new methods of preservation, and even adaptations of methods used for other microorganisms should be sought, citing as an example the gelatin method used for the preservation of phytopathogenic bacteria by Takatsu (1985). Thereby, this study aimed to evaluate the gelatin preservation method (17 cultures), never tested for phytopathogenic fungi and other classical methods: sterile soil (68 cultures), resistant structures (*Sclerotinia sclerotiorum*) in 4°C (10 fungi strains) and mineral oil (31 cultures), examining the time maintaining the viability, sporulation and colonization in host tissues preserved in different dates.

## MATERIAL AND METHODS

### Gelatin method

All preserved fungal cultures tested, are inside the Laboratory of Mycology and Plant Protection (LAMIP), in UFU (Universidade Federal de Uberlândia), Uberlândia (MG), Brazil. The

evaluation of the preserved culture developed from August to December, 2014. The procedure used to the gelatin method was an adaptation of the method of drying bacteria (TAKATSU, 1985) – there was no similar method in literature related for phytopathogenic fungi species.

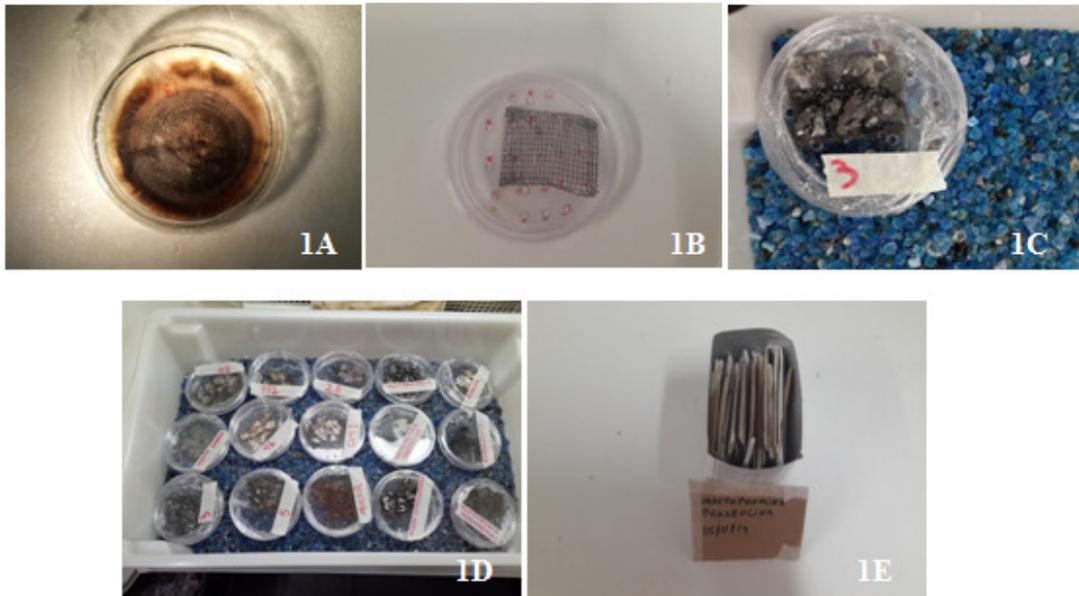
The culture was developed on PDA media to fully cover the Petri dish (90 mm) (Figure 1A) for seven days, and the mycelium, covered successively with dextrose (5%) and bacteriological gelatin (5%) for one minute (each), promoting soaking of the mycelium and removing the excess. The mycelium was divided into approximately 1 cm<sup>2</sup> fragments with a sterilized scalpel; these fragments were inserted under aluminum screens (4 cm<sup>2</sup>) inside plastic Petri dishes, previously perforated to allow gas exchange with silica gel (Figures 1B and 1C). The Petri dishes, containing cultures fragments, were kept inside a plastic container with sterile silica (8 mesh) at its base (Figure 1D), properly sealed, preventing gas exchange with the atmosphere.

The sterilization of the plastic container and Petri dishes performed out in sodium hypochlorite solution 2.5% and 70% alcohol, exposed to ultraviolet light for 20 minutes in laminar flow. After drying for a period of 15 days, the culture fragments inserted in sterile paper envelopes and kept in refrigerator at 4°C (Figure 1E). In the Figure 2, we can observe a simplified diagram for the method.

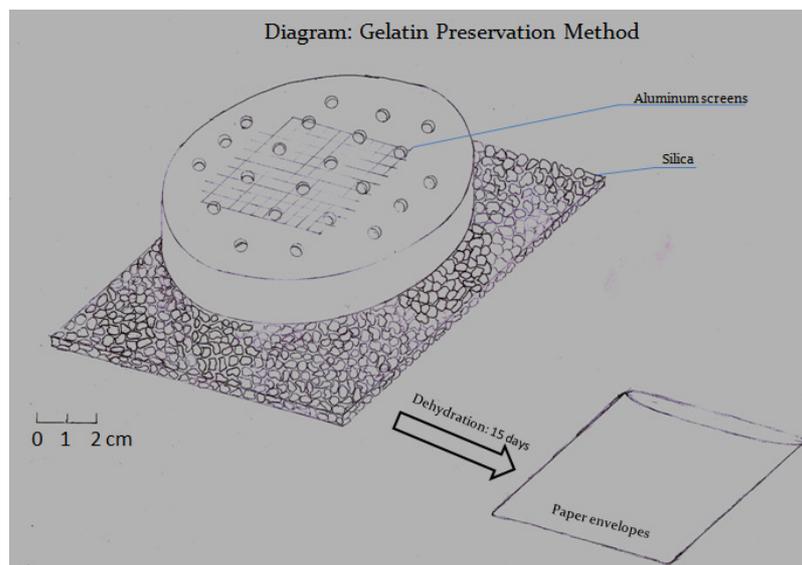
In LAMIP, 17 cultures of genera and species preserved in the gelatin method: *Colletotrichum* spp., *Cladosporium* spp., *Septoria* spp., *Fusarium* spp., *F. moniliforme* var. *subglutinans*, *Cylindrocladium* spp., *Macrophomina* spp., *Phomopsis* spp. and *Verticillium* spp. obtained in different hosts tissues and in different dates (Table 1).

### Sterile soil, Mineral oil and 4°C

Were preserved inside LAMIP, 68 cultures of genera and species in the sterile soil method: *Fusarium* spp., *F. solani*, *F. oxysporum*, *F. oxysporum* f. sp. *lycopersici*, *F. oxysporum* f. sp. *vasinfectum*, *M. phaseolina*, *Rhizoctonia* spp., *R. solani*, *S. sclerotiorum*, *Sclerotium rolfsii* and *Verticillium* spp. obtained in different hosts tissues and in different dates (Table 2). Preserved as sclerotia, the lab had ten fungi strains of *S. sclerotiorum*, stored in paper envelopes inside domestic refrigerator (4°C). Different hosts provided the fungi strains, with different preservation dates (Table 2).



**Figure 1.** Procedures for the gelatin method for fungi preservation.



**Figure 2.** Simplified diagram for the gelatin method for fungi preservation.

**Table 1.** Fungi preserved in the Laboratory of Mycology and Plant Protection using the gelatin method.

Code	Genera and especie	Host	Entry date
LAMIP 132A	<i>Colletotrichum</i> spp.	<i>Passiflora</i> sp.	08/10/1999
LAMIP 132B	<i>Cladosporium</i> spp.	<i>Passiflora</i> sp.	08/10/1999
LAMIP 132C	<i>Colletotrichum</i> spp.	<i>Passiflora</i> sp.	03/03/1998
LAMIP 133A	<i>Septoria</i> spp.	<i>Passiflora</i> sp.	08/18/1998
LAMIP 133B	<i>Septoria</i> spp.	<i>Passiflora</i> sp.	02/10/1999
LAMIP 134	<i>Colletotrichum</i> spp.	<i>Coffea arábica</i>	08/18/1998
LAMIP 134A	<i>Colletotrichum</i> spp.	<i>C.arabica</i>	09/06/2000
LAMIP 134B	<i>Colletotrichum</i> spp.	<i>C.arabica</i>	01/01/1999
LAMIP 134C	<i>Colletotrichum</i> spp.	<i>C.arabica</i>	01/01/1999
LAMIP 135	<i>Fusarium</i> spp.	<i>Passiflora</i> sp.	03/03/1998
LAMIP 138	<i>Fusarium</i> spp.	<i>Zingiber officinale</i>	04/26/2002
LAMIP 140	<i>Fusarium</i> spp.	<i>Gossypium hirsutum</i>	06/21/2004

LAMIP 167	<i>F. moniliforme</i> var. <i>subglutinans</i> ,	<i>Ananas comosus</i>	06/01/2008
LAMIP 168	<i>Cylindrocladium</i> spp.	N.I. *	06/08/2008
LAMIP 169	<i>Macrophomina</i> spp.	N.I.	06/04/2008
LAMIP 170	<i>Phomopsis</i> spp.	N.I.	04/06/2008
LAMIP 171	<i>Verticillium</i> spp.	N.I.	06/04/2008

\*N.I.: No Information

**Table 2.** Fungi preserved in the Laboratory of Mycology and Plant Protection using the sterile soil, mineral oil and 4°C methods.

Genera and specie	Number of cultures tested		
	Sterile soil	Mineral oil	4°C
<i>Fusarium</i> spp.	8	4	-
<i>F. solani</i>	2	1	-
<i>F. oxysporum</i>	9	-	-
<i>F. oxysporum</i> f. sp. <i>lycopersici</i>	19	-	-
<i>F. oxysporum</i> f. sp. <i>vasinfectum</i>	5	-	-
<i>F. moniliforme</i>	-	1	-
<i>Macrophomina phaseolina</i>	1	-	-
<i>Rhizoctonia</i> spp.	13	-	-
<i>R. solani</i>	1	-	-
<i>Sclerotium rolfsii</i>	4	-	-
<i>Sclerotinia sclerotiorum</i>	2	-	10
<i>Verticillium</i> spp.	4	-	-
<i>Cercospora zea-maydis</i>	-	2	-
<i>Diaporthe phaseolorum</i>	-	3	-
<i>Pyricularia</i> spp.	-	3	-
<i>Colletotrichum</i> spp.	-	5	-
<i>C. gloeosporioides</i>	-	3	-
<i>Phyllosticta</i> spp.	-	1	-
<i>Phoma</i> spp.	-	1	-
<i>Cylindrocladium</i> spp.	-	1	-
<i>Drechslera oryzae</i>	-	2	-
<i>Phomopsis</i> spp.	-	2	-
<i>Ramularia</i> spp.	-	1	-
<i>Rhynchosporium</i> spp.	-	1	-
Total	68	31	10

Were preserved 31 cultures in mineral oil of genera and species *Cercospora zea-maydis*, *Diaporthe phaseolorum*, *Pyricularia* spp., *Colletotrichum* spp., *C. gloeosporioides*, *Fusarium* spp., *F. moniliforme*, *F. solani*, *Phyllosticta* spp., *Phoma* spp., *Cylindrocladium* spp., *Drechslera oryzae*, *Phomopsis* spp., *Ramularia* spp. and *Rhynchosporium* spp. obtained in different hosts tissues and in different dates (Table 2).

#### Evaluation of the viability

For evaluation of the fungi viability, were used test tubes (12 x 120 mm) containing inclined potato dextrose agar (PDA) and six repetitions for each specie (repetition equals a tube). In each tube was inserted 3.5 mL of PDA (pH 5.5) with antibiotics (0.5 mg/L): chloramphenicol,

streptomycin and ampicillin, to minimize bacterial contamination. Small fragments (approximately 0.2 mm<sup>2</sup>) inserted into the tube from the use of previously sterilized tweezers. After the inoculation, the tubes were sealed with hydrophobic cotton and kept in an acclimatized chamber (20 ± 2°C), with photoperiod of 12 hours, and viability assessed daily from the confirmation of mycelia growth on a stereomicroscope (40 x) for a period of 15 days, with confirmation by an light microscope (400 x).

For fungi viability detection, it was necessary to verify mycelial growth inside at least one of the tubes. When no mycelial growth was observed or there was development of fungal contaminants in all tubes, we used the Water Agar (WA) 2%, pH 5.5 in test tubes (3.5 mL per tube);

three repetitions used for each specie (repetition equals a tube).

The evaluation of the viability of *S. sclerotiorum* strains occurred in semi-selective medium Neon-S (NAPOLEÃO et al., 2006). For disinfection, the sclerotia kept one minute in distilled water, one minute in 50% ethanol and three minutes in 1% sodium hypochlorite, and the excess removed using distilled water for one minute, and placed in tubes containing 3.5 mL of medium Neons. The tubes sealed with hydrophobic cotton and kept in a growth chamber BOD at 20°C in the dark for a period of 15 days, and the viability assessed only in the last day, with the formation of sclerotia and the characteristic change of color of the semi-selective medium.

### Evaluation of the sporulation and colonization of host tissues



**Figure 3.** Sterile wooden sticks and *Colletotrichum* spp. mycelium growing on PDA media.

The disinfestation of tissues made through passage for one minute in each solution: 1.5% sodium hypochlorite, ethanol 50% and distilled water. Inoculation consisted in use of fungal structures presented in young and pure colonies, with help of sterilized scalpel and tweezers. The fruits/rhizomes inoculated and the control, were kept in climate chamber ( $20 \pm 2^\circ\text{C}$ ), with 12 hours photoperiod and in sealed plastic bags for seven days.

### RESULTS

Ten cultures preserved by the gelatin method, identified as viable, amid growing PDA and maintained the capacity of sporulation, except the genera *Septoria* spp. (Table 3). The fungi promoted colonization on the tested tissues, such as

The sporulation, regardless of the number of spores found, assessed from observation under a light microscope (400 x), except for the isolates of *S. sclerotiorum* (sporulation occurs only in teleomorphic phase) and *Rhizoctonia* spp. (Class: Agaricomycetes - no production of spores). We observed the formation of sclerotia in *S. sclerotiorum* strains. To confirm the colonization, was conducted an inoculation in living host tissues as fruits and rhizomes, through the tissue perforation with sterile wooden sticks fragments, with the presence of growing mycelium (ALFENAS and MAFIA, 2007). The method consisted, in using sterile wooden sticks in Petri dishes containing PDA media. The culture that grew up in PDA, covered the wooden sticks, and after seven days used to inoculate the tissues (Figure 3).

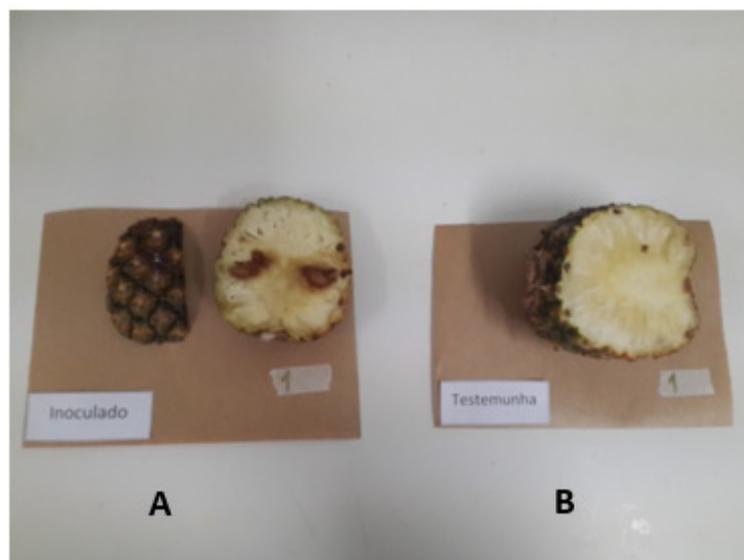
*F. moniliforme* var. *subglutinans*, in fruit of *Ananas comosus* (Figure 3), except *Phomopsis* spp. and *Verticillium* spp. (unknown hosts).

Of the 68 cultures preserved in sterile soil, 38 remained viable: five cultures of *Fusarium* spp. (maximum 13 years and 40 days), five cultures of *F. oxysporum* (maximum 18 years and 328 days), 16 cultures of *F. oxysporum* f. sp. *lycopersici* (maximum 25 years and 186 days), five cultures of *F. oxysporum* f. sp. *vasinfectum* (maximum 15 years and 328 days), one culture of *F. solani* (12 years and 343 days) and one culture of *S. rolfsii* (20 years and 33 days) producing sclerotia.

**Table 3.** Viable fungi observed in PDA media using the gelatin method for preservation.

Code	Genera and specie	Sporulation	Colonization	Preservation time
LAMIP 132C	<i>Colletotrichum</i> spp.	+	<i>Passiflora</i> sp	16 years and 40 days
LAMIP 133A	<i>Septoria</i> spp.	-	<i>Passiflora</i> sp	15 years and 241 days
LAMIP 133B	<i>Septoria</i> spp.	-	<i>Passiflora</i> sp	15 years and 62 days
LAMIP 135	<i>Fusarium</i> spp.	+	<i>Passiflora</i> sp	16 years and 40 days
LAMIP 138	<i>Fusarium</i> spp.	+	<i>Zingiber officinale</i>	11 years and 354 days
LAMIP 140	<i>Fusarium</i> spp.	+	<i>Gossypium hirsutum</i>	9 years and 296 days
LAMIP 167	<i>Fusarium moniliforme</i> var. <i>subglutinans</i> ,	+	<i>Ananas comosus</i>	5 years and 321 days
LAMIP 169	<i>Macrophomina</i> spp.	+	<i>Glycine max</i>	5 years and 318 days
LAMIP 170	<i>Phomopsis</i> spp.	+	N.I. *	6 years and 11 days
LAMIP 171	<i>Verticillium</i> spp.	+	N.I.	5 years and 318 days

(-) No; (+) Yes; \* (N. I.) No information



**Figure 3.** Fruit of *Ananas comosus* colonized by *Fusarium moniliforme* var. *subglutinans*, preserved using the wooden sticks method to inoculation. **A.** Colonized. **B.** Control.

Five cultures identified as viable, only on WA media: two of *Fusarium* spp. (maximum 11 years and 55 days); and three of *Rhizoctonia* spp. (maximum 37 years and 142 days) (Table 4). When preserved in mineral oil, 31 of the tested cultures, only three remained viable, identified in PDA: *Colletotrichum* spp. (11 years and 84 days); *F.*

*moniliforme* (8 years and 168 days) and *Phomopsis* spp. (19 years and 226 days) (Table 4). All cultures maintained sporulation on PDA culture media. Only one fungal strain of *S. sclerotiorum* preserved in 4°C (about four years), remained viable, producing mycelium and resistance structures on semi-selective medium Neon-S (Table 4).

**Table 4.** Maximum viability of fungi cultures preserved by the sterile soil, mineral oil and 4°C methods.

Code	Genera and specie	Storage method	Sporulation	Colonization	Preservation time	Viability in Culture media
LAMIP 044	<i>Fusarium</i> ssp.	Sterile soil	-	<i>Passiflora</i> sp.	13 years and 40 days	PDA
LAMIP 014	<i>F. oxysporum</i>	Sterile soil	+	<i>Musa</i> sp.	18 years and 328 days	PDA
LAMIP 013	<i>F. oxysporum</i> f. sp. <i>Vasinfectum</i>	Sterile soil	+	<i>Gossypium hirsutum</i>	15 years and 66 days	PDA
LAMIP 043	<i>F. solani</i>	Sterile soil	+	<i>Glycine max</i>	12 years and 343 days	PDA
LAMIP 073	<i>Sclerotium rolfsii</i>	Sterile soil	RE*	<i>Capsicum annuum</i>	20 years and 33 days	PDA
LAMIP 068	<i>Rhizoctonia</i> ssp.	Sterile soil	A**	N.I.***	37 years and 142 days	WA
LAMIP 120	<i>Colletotrichum</i> ssp.	Mineral oil	+	<i>Coffea arabica</i>	11 years and 84 days	PDA
LAMIP 161	<i>F. moniliforme</i>	Mineral oil	+	<i>Saccharum officinarum</i>	8 years and 168 days	PDA
LAMIP 050	<i>Phomopsis</i> spp.	Mineral oil	+	<i>Solanum gilo</i>	19 years and 226 days	PDA
LAMIP 175	<i>Sclerotinia sclerotiorum</i>	4°C	RE	<i>Glycine max</i>	Approximately 4 years	Neon-s

\*RE=Resistant structure; \*\* Class. Agaricomycetes (No production of spores); \*\*\*No information

## DISCUSSION

Drying methods are technically simple and do not require expensive equipments. Furthermore, drying is a useful method of preservation for cultures that produce spores or other structures (FONG et al., 2000, NAKASONE et al., 2004). Some fungi can be preserved easily and successfully for many years in dry, sterile soil or sand. Dormancy caused by dryness can take time to develop, however, morphological changes in some fungi have been recorded (NAKASONE et al., 2004).

Many soil fungi develop sclerotia, microsclerotia or other long-term survival propagules in culture as well as in nature. Preserving such structures, usually at 3°-5°C is a good way to preserve fungal strains such as *Magnaporthe*, *Phymatotrichum* and *Cylindrocladium*, species that remain viable from about two to five years (SINGLETON et al., 1992). Example of long preservation in sterile soil is the fungus *V. dahliae* preserved 5 years (MELOUK, 1992). In our study, only six of 68 preserved fungi in sterile soil remained viable.

Bueno (2006) noted that after nine months of evaluation, there was a gradual decrease in the survival of the fungus in the refrigerator temperature

(5°C). The author has developed a methodology for the preservation of *S. sclerotiorum* resistance structures at -20°C and stored such structures into 500 mL vials. After a year of preservation, all maintained their pathogenicity in the developed methodology. Singleton et al. (1992) suggests *Sclerotinia* genera can be preserved by periodic subcultures for a short period. In our study the preservation of *S. sclerotiorum* (strain LAMIP 175) remain viable at 4°C for approximately four years, suggesting that kind of preservation cannot exceed this period.

The mineral oil method advantages reported was the long viability of some fungi, the mites do not penetrate into the cultures and low cost. However, the strains do eventually lose viability, contamination with other fungi and bacteria is an occasional problem, and selection for mutants that can grow under adverse conditions may occur (ALFENAS; MAFIA, 2016).

Various genera was preserved in mineral oil for long periods in several collections, such as *Colletotrichum* spp. remained viable for up to 21 years, *F. solani* (25 years) and *V. dahliae* (2 years) at the Commonwealth Mycological Institute (CMI), UK (SMITH; ONIONS, 1983). In our study only three of 31 cultures remained viable, suggesting that it was not suitable for the preserved fungi.

Bueno (2006) suggest that the sclerotia preservation of *R. solani* produced in sandy-organic substrate was not promising because they did not remain viable after five months of evaluation. The results found in our study contrary this information, since the cultures remained viable in sterile soil for periods of maximum 37 years and 142 days, higher than those found in the literature. The viability of these genera identified only in the WA media. Preserve the sclerotia of *S. sclerotiorum*, after removal from diseased plants or culture media, at temperatures between 2 and 5°C (SINGLETON et al., 1992).

Maragon et al. (2003) proposes an adaptation of a preservation method: the dehydrated gelatin drops method (DGD). Twenty-seven strains of fungi of clinical interest were maintained by this method for a seven-year period at room temperature. None of the strains maintained by DGD were found to be contaminated by bacteria or other fungi and no apparent changes were observed in morphology or macroscopic features. This indicate that other gelatin method, such as the proposed by Takatsu (1985) can be use for vast genera of fungi.

## CONCLUSIONS

The gelatin method was suitable for preservation of the genera and species: *Colletotrichum* spp., *Septoria* spp., *Fusarium* spp., *F. moniliforme* var. *subglutinans*, *Macrophomina* spp., *Phomopsis* spp. and *Verticillium* spp. in an optimal work time for laboratory necessities (minimum 5 years).

It is not an expensive method, easy to reproduce, can be use in laboratories that do not have large financial resources to acquire equipments such as lyophilizer and deep-freezers, enabling the exchange of cultures between research institutions.

The viability remained in 38 cultures of sterile soil, three of mineral oil and sclerotia maximum time of preservation in 4°C was four years (one fungal strain).

## ACKNOWLEDGMENT

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**RESUMO:** Os métodos de preservação de fungos têm grande importância em coleções *ex situ*, representando importante patrimônio biológico, útil para micologistas e fitopatologista como suporte para vários trabalhos científicos. Não existe um método de preservação adequado e eficiente para os diferentes grupos de fungos, entretanto, o mais apropriado é o que mantém, mesmo após longos períodos, as características originais da cultura: viabilidade, esporulação e patogenicidade, excluindo mutações e contaminação indesejável. A escolha dependerá da infra-estrutura laboratorial, microorganismo, objetivos, preferências e conhecimentos do pesquisador. Este trabalho foi realizado no Laboratório de Micologia e Proteção Vegetal (LAMIP) da UFU (Universidade Federal de Uberlândia), localizado em Uberlândia (MG), Brasil. O objetivo foi avaliar o método de preservação da gelatina (17 culturas), nunca testado antes para fungos fitopatogênicos. Concomitantemente foram avaliados métodos clássicos, como solo estéril (68 culturas), escleródios (*Sclerotinia sclerotiorum*) em 4 ° C (10 isolados) e óleo mineral (31 culturas). Avaliou-se a manutenção na viabilidade, esporulação e patogenicidade dos isolados. O método da gelatina manteve a viabilidade em 10 culturas, sendo adequado para a preservação dos gêneros e espécies: *Colletotrichum* spp., *Septoria* spp., *Fusarium* spp., *F. moniliforme* var. *subglutinans*, *Macrophomina* spp., *Phomopsis* spp. e *Verticillium* spp. A viabilidade foi mantida em 38 isolados em solo estéril, três isolados em óleo mineral e um apenas um isolado de escleródio atingiu um tempo máximo de preservação de quatro anos.

**PALAVRAS-CHAVE:** Colonização. Patogenicidade. Preservação *ex situ*. Esporulação. Viabilidade.

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## REFERENCES

- ABD-ELSALAM, K. A.; YASSIN, M. A; MOSLEM, M. A; BAHKALI, A. H.; DE WIT, P. J. G. M.; MCKENZIE, E. H. C.; STEPHENSON, S. L.; CAI, L.; HYDE, K. D. Culture collection, the new herbaria for fungal pathogens. **Fungal Diversity**, Hong Kong, v. 45, n. 1, p. 21-32, 2010. <https://doi.org/10.1007/s13225-010-0063-z>
- ALFENAS, A. C.; MAFIA, R. G. **Métodos em Fitopatologia**. Viçosa. 1ª Ed. UFV. 382p, 2007.
- ALFENAS, A. C.; MAFIA, R. G. **Métodos em Fitopatologia**. Viçosa. 2ª Ed. UFV. 516p, 2016.

- APARECIDO, C. C.; PIRES, G. C. C.; FINATTI, D.; CAMILO, C. M. Preservação de micro-organismos a - 80° C. **Biológico**, v. 74, p. 23-29, 2012.
- APARECIDO, C. C.; CAMILO, C. M. Comparação de métodos para a preservação de fungos do gênero *Colletotrichum* em laboratório. **Biológico**, v. 75, p. 17-22, 2013.
- BASKARATHEVAN, J.; JASPERS, M. V.; JONES, E. E.; RIDGWAY, H. J. Evaluation of different storage methods for rapid and cost-effective preservation of *botryosphaeria* species. **New Zealand Plant Protection**, v. 62, p. 234-237, 2009.
- BOUNDY-MILLS, K. L.; GLANTSCHNIG, E.; ROBERTS, I. N.; YURKOV, A.; CASAREGOLA, S.; DANIEL, H-M., GROENEWALD, M.; TURCHETTI, B. Yeast culture collections in the twenty-first century: new opportunities and challenges. **Yeast**, v. 33, 2016.
- BUENO, C. J.; AMBRÓSIO, M. M. Q.; SOUZA, N. L. Preservação de fungos fitopatogênicos habitantes do solo. **Summa Phytopathologica**, v. 32, p. 42-50, 2006. <https://doi.org/10.1590/S0100-54052006000100006>
- FONG, Y. K.; ANUAR, S; LIM, H. P., THAM, F. Y.; SANDERSON, F. R. A modified filter paper technique for long-term preservation of some fungal cultures. **Mycologist**, v. 4, p. 127-130, 2000. [https://doi.org/10.1016/S0269-915X\(00\)80090-7](https://doi.org/10.1016/S0269-915X(00)80090-7)
- GREEN, L. H. **Practical handbook of microbiology**. CRC: London, 2 ed. 874 pp, 2008.
- KARADUMAN, A. B.; ATLI, B.; YAMAÇ, M. An example for comparison of storage methods of macrofungus cultures: *Schizophyllum commune*. **Turkish Journal of Botany**, v. 36, p. 205-212, 2012.
- MARANGON, A. V.; BERTONI, T. A., KIOSHIMA, E. S., FALLEIROS DE PÁDUA, R. A.; VENTURINI, S.; SVIDZINSKI, T. I. Dehydrated gelatin drops: a good method for fungi maintenance and preservation. **The New Microbiologica**, v. 26, p. 305-309, 2003.
- MELOUK, H. A. Verticillium. In: SINGLETON, L. L.; MIHAIL, J. D.; RUSH, C. M. (Ed.). **Methods for research on soilborn phytopathogenic fungi**. St. Paul: The American Phytopathological Society, 1992. P. 175-178.
- NAKASONE, K. K.; PETERSON, S. W, JONG, S. C. **Preservation and distribution fungal cultures**. In: Mueller GM, Bills GF & Foster MS (eds.) *Biodiversity of Fungi*, p. 37-47. Amsterdam: Elsevier Academic Press, 2004. <https://doi.org/10.1016/B978-012509551-8/50006-4>
- NAPOLEÃO, R.; NASSER, L.; Carlos Lopes, C.; CAFÉ FILHO, A. Neon-S, novo meio para detecção de *Sclerotinia sclerotiorum* em sementes. **Summa Phytopathologica**, v. 32, p. 180-182, 2006. <https://doi.org/10.1590/S0100-54052006000200014>
- PASSADOR, M. M.; PIRES, G. C. C.; FINATTI, D.; APARECIDO, C.C.; FIGUEIREDO, M. B. Manutenção da viabilidade e patogenicidade de culturas mantidas na micoteca “Mário Barreto Figueiredo”. **Biológico**, v. 72, p. 51-55, 2010.
- PIRES, G. C. C.; APARECIDO, C. C., FINATTI, D.; Preservação em laboratório de fungos filamentosos por longos períodos de tempo. **Biológico**, v. 74, p. 9-16, 2012.
- SINGLETON, L. L.; MIHAIL, J. D.; RUSH, C. M. **Methods for research on soilborne phytopathogenic fungi**. St. Paul: The American Phytopathological Society, 265 pp., 1992.
- SMITH, D.; ONIONS, A. H. S. A comparison of some preservation techniques for fungi. **Transactions of the British Mycological Society**, v. 81, p. 535-540, 1983. [https://doi.org/10.1016/S0007-1536\(83\)80122-3](https://doi.org/10.1016/S0007-1536(83)80122-3)

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BELOTI, I. F.; JULIATTI, B. C.; JULIATTI, F. C.

SMITH, D.; RYAN, M. Implementing best practices and validation of cryopreservation techniques for microorganisms. **The Scientific World Journal**, Cairo, 2012.

TAKATSU, A. Preservação de bactérias fitopatogênicas pelo método de dessecação. Universidade de Brasília. Departamento de Biologia Vegetal, 5pp., 1985.

URUBURU, F. 2003. History and services of culture collections. **International Microbiology**, v. 6, p. 101-103, 2003. <https://doi.org/10.1007/s10123-003-0115-2>