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We are still developing and optimizing this protocol

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Fungal Culture Long-term Storage (Dry Filter Paper Technique) V.2

Anuar Morales[1]

[1]Cornell University

Nimalka M Weerasuriya
Oklahoma State University, USDA Agricultural Research Servic...

DISCLAIMER

This is not an original protocol.

ABSTRACT

Culture collections are expensive to support, as they require special equipment and continuous attention in order to maintain fungal cultures without losing their pathogenicity or virulence. Two examples of these collections are the USDA-ARS Collection of Entomopathogenic Fungal Cultures, with more than 5500 cultures of over 350 species of fungi from 900 hosts. The International Entomopathogenic Bacillus Centre in the Institute Pasteur has nearly 3500 strains of *Bacillus thuringiensis*, the most important bacterium used in biocontrol. Cultures are typically either freeze-dried in a process called lyophilization, or stored in liquid nitrogen at ultra-low temperatures. Both techniques require intense labor and expensive equipment.

At the International Center of Tropical Agriculture (CIAT, in Colombia), expenses were reduced with a novel, reliable and cheap technique. The dry filter paper technique was developed by Rosalba Tobon and Ximena Aricapa in the early 1980s and can be used for preservation of cultures of insect pathogenic and plant pathogenic fungi as well as many molds.

The new technique is not only reliable, it is very inexpensive and easy to use in any laboratory with few resources. CIAT uses this method to store about 500 cultures of insect pathogenic fungi and 1000 cultures of plant pathogenic fungi and bacteria. Evaluations of purity, pathogenicity and virulence were performed on fungi stored between 5 to 10 years. With a few exceptions the fungus was recovered easily and with the same characteristics of pathogenicity and virulence it had when first stored. This technique has been successfully implemented in other institutions with great results. Research studies at CIAT are adapting this methodology to work with bacteria and viruses.

GUIDELINES

International Center of Tropical Agriculture (CIAT, Colombia) uses a cheap technique, the dry filter paper technique developed by Rosalba Tobon and Ximena Aricarpa in the early 1980s and can be used for preservation of cultures of insect pathogenic and plant pathogenic fungi as well as molds.

BEFORE START INSTRUCTIONS

Fungi need to be isolated into pure culture.

Initial Isolation

1 A pinch of pure fungal culture is taken from insect or plant host and added to culture medium in a Petri dish.
Selective media PARP5 can be used or general PDA. Additives include chloramphenicol, lactic acid, ampicillin, streptomycin, etc.

1.1 Grow for 5-10 days prior to storage process at Room temperature

2 Filter papers (1 x 1 cm) cut and sterilized in an autoclave

Storage Process

3 Fume hood: Papers placed on agar surface of new plates with the same or selective medium. Colonies or spores are cut from pure culture and placed on top of each piece.

4 Petri dishes are sealed and placed in an incubator at appropriate growth temperatures. The fungus will grow more slowly on filter paper; approximately 10-15 days to fully colonize.

5 Once spores are formed on filter paper, individual pieces are separated and placed in new dishes **without culture medium**.

5.1 Dishes are placed in the incubator until paper and fungus are completely dried; approximately 20-30 days. If the drying process is too fast, the pathogenicity and virulence may be affected.

6 After drying, 10-12 pieces of paper filter are put in a sterile glassine envelope. Each envelope is labeled, bagged in a plastic bad, and stored in a plastic container at 4 °C or -20 °C

Re-Culturing

7 Fresh culture can be created by using a small piece of filter paper removed from the envelope and placed on fresh medium.