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## Letter to the Editor

### A comparison of preservation methods for *Trichoderma harzianum* cultures

#### Comparación de métodos de preservación para cultivos de *Trichoderma harzianum*

Dear Editor:

To eliminate problems encountered by serial transfers of stock cultures, several methods for long-term preservation have been developed<sup>2</sup>. Most methods slow metabolism of microorganisms by storage at 4 °C and/or mineral oil coating, thereby limiting oxygen uptake by the cell, decreasing metabolic activities, and increasing storage time.<sup>3</sup> One storage method using cellulose filter paper has been little used in mycological collections.<sup>1–3</sup> In the present study, we conducted experiments to evaluate several of these methods, including the cellulose filter paper technique, to establish a simple and reliable preservation method for the maintenance of stock *Trichoderma harzianum* strains isolated from soil cultivated with horticultural crop.

Ten fungal isolates of *T. harzianum* were selected for estimating the effect of different preservation methods on viability over time. The following preservation methods were compared: (1) distilled water stasis, (2) mineral oil, (3) silica gel, and (4) cellulose filter paper. Five replicates of the same *T. harzianum* strain were used for each storage method. For the distilled water stasis method, sterile screw-cap glass tubes measuring 1.5 × 15 mm<sup>2</sup> containing 4 ml of sterile distilled water were used. The sporulating cultures on potato dextrose agar (PDA) were cut into small blocks (approximately 5 mm<sup>2</sup>) and placed into the tubes that were then kept at 4 °C. Cultures were recovered from storage by aseptically transferring a block of the inoculum from the water onto a fresh plate of 9 ml of PDA. For storage in mineral oil, heavy mineral oil (Sigma Chemical Co., St. Louis, MO) double sterilized by autoclaving at 24 h intervals was used. Slants (100 × 10 mm) containing PDA and sporulating culture of fungi were covered with 5 ml of mineral oil. Cultures were recovered from mineral oil storage by removing small blocks of the stored culture and transferring the blocks to fresh PDA.

For storage with silica gel, uncolored, 200 mesh size, grade 40 silica gel (Fisher Scientific, Fair Lawn, NJ) was used after sterilization in an oven at 160 °C for 6 h. Glass tubes (100 × 10 mm<sup>2</sup>) and caps were sterilized. Fungal spores were harvested by scraping the surface of a culture maintained in PDA with a sterile loop, and spore suspensions were made with sterilized distilled water (1 × 10<sup>8</sup> spores/ml). One hundred microliters of each suspension was placed into each tube containing 5 g of silica gel that had been kept at 4 °C. The silica gel was mixed with the suspension by rotating the tubes several times until the crystals were easily separated. Then the tubes were maintained at

4 °C. Cultures were recovered by aseptically placing a few crystals onto fresh PDA. In the cellulose filter paper method, this substrate was used for fungal colonization. Ten sterilized cellulose filter paper strips measuring 20 × 10 mm<sup>2</sup> were put into each culture of *T. harzianum*. After the filter papers were well-colonized by the fungus, they were dried at room temperature for 5–7 days and stored in sterilized envelopes at –20 °C.<sup>1</sup>

The viability of all isolates was evaluated when the storage began as well as after 3, 6, 12, 18, and 24 months of storage. The fungal subcultures were evaluated on the basis of whether or not they grew after the different storage regimes at each time interval, and if they kept their original morphologic characteristics.

All strains tested survived up to 24 months of storage with the methods 1, 2, and 4, but with method 3, 6 strains survived only to 12 months and 3 only up to 18 months. All strains recovered on PDA agar after 24 months storage in each of the methods used were evaluated for preservation of their original macroscopic and microscopic features. Strains recovered from materials stored in distilled water, mineral oil, and cellulose filter paper kept the original macroscopic characteristics. Assessment of the microscopic structures observed in microcultures<sup>4</sup> indicated no significant changes between isolates retained by the different methods tested.<sup>3</sup>

This is the first report of the use of cellulose filter paper as a method of preservation of *T. harzianum* strains. We recommend the use of this method for *Trichoderma* strains preservation because it is simple, inexpensive, the least labor intensive, and requires very little refrigeration for the storage of the isolates.

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