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Extraction of fungal hyphae: The Sieving and Sucrose Centrifugation (SSC) method

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Isabelle Elisabeth Metzen¹, Marcel Bucher¹

¹University of Cologne, Institute for Plant Sciences



Isabelle Elisabeth Metzen

University of Cologne

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We use this protocol and it's working

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Abstract

The Sieving and Sucrose Centrifugation (SSC) technique efficiently extracts diverse fungal hyphae from natural soils and preserves attached microbes. The method combines, optimizes, and semi-automates existing methods.

Guidelines

3.3 Measuring hyphal length density

1. For measurement of the hyphal length density (HLD) (fungal hyphae in cm per hyphal compartment), 2 ml of the solution containing the hyphae and spores were put onto a nitrocellulose filter with a pore size of 1.2 μm and fixed in manifold holding system that was connected to a vacuum pump.
2. A vacuum was applied to remove any remaining liquid on the filter.
3. The hyphae and spores on the filters were immersed in an ink solution for 20 min.
4. The ink was aspirated through the vacuum, and the sample on the filter was rinsed with deionised H_2O . Once the sample was dry, the intersections of hyphae with the gridline were counted at 13 sites in each sample under the Leica DM 1000 LED microscope using 10x magnification.
5. Hyphal length density was calculated, based on Tennant, (1975) and Shen et al. (2016).

3.4 DNA extraction

1. 8 ml of the water solution containing hyphae were lyophilized.
2. DNA was extracted from freeze dried hyphae and spores using the SpinKit for soil (MP Biomedicals) according to the manufacturer's protocol, with the modification that homogenization using the Precellys 24 tissue lyzer (Bertin Technologies, Montigny-le Bretonneux, France) was performed out twice, each for 30 seconds at 6200 rpm.

Materials

2.1 Technical equipment:

1. Wet sieving machine (Note 1)
2. Sieving convoy made out of a 630 μm sieve, 400 μm sieve, 315 μm sieve, aeration ring, ring sieve, 40 μm sieve, aeration ring and a collecting bowl (Note 2)
3. Self-priming, single-stage side channel pump (Note 3)
4. Water tubes (Hard PVC spiral 25×3.6m and PVC fabric tube 13×19.8mm)
5. Manometer
6. Freeze dryer associated with a vacuum pump (Note 4)
7. Manifold holding system connected to vacuum pump

2.2 Standard laboratory equipment:

1. Pipette and low retention tips
2. 500 ml beaker
3. 50 ml and 15 ml Microcentrifuge Tubes
4. Spray Bottle
5. Centrifuge
6. Syringes with connected tube (0.5 cm)
7. 1.2 μm nitrocellulose filter
8. Gridline and light microscope (Note 5)
9. Optional: Vortex

2.3 Solutions:

1. 70% sucrose solution
2. H_2O
3. Ink solution (5% ink (Pelikan 4001 royal blue), 5% acetic acid)

Before start

The SSC method was performed in a cold room set to 4°C to ensure DNA quality and potentially RNA quality.

3.1 Semi-automated wet sieving

- 1 Soil samples weighing between 25 and 300 g were placed in a beaker and filled to 400 ml with deionized water at 4 °C. For individual experiments, we recommend using soil samples of similar weight to ensure consistency. The samples were then mixed manually with a spoon to facilitate the detachment of fungal hyphae from dense soil particles.
- 2 Samples were manually placed on the first sieve of the sieving convoy. 4°C-cold water was pumped through a low-pressure water pump into the wet sieving machine that was built of water-blast pipe and the sieving convoy. The water pressure and amplitude were set to 2.5 bar and 75% of the maximum available power setting (corresponds to approximately 2.25 mm oscillation amplitude at a fixed frequency of 50 Hz, as per the specifications of the AS 200 Basic model), respectively. The samples underwent an initial sieving process lasting 8 min.
- 3 The first three sieves with the widest mesh sizes of 630 µm, 400 µm and 315 µm were removed, and the remaining material was sieved for additional 6 min.
- 4 The remaining particles on the 40 µm sieve were collected and transferred into 50 ml microcentrifuge tubes. Samples were kept on ice.

3.2 Sucrose centrifugation

- 5 The volume within each microcentrifuge tube was adjusted to 20 ml by adding deionised 4°C cold H₂O as needed.
- 6 The solutions were shaken by hand to resuspend the particles; alternatively, the samples could be vortexed. Equal volumes of 70% sucrose were then carefully injected beneath the aqueous solution using a syringe fitted with a 7 cm long plastic tube (0.5 cm diameter).
- 7 The samples were then centrifuged (2000 rpm for 10 min, 4°C). Hyphae and spores were collected by a syringe with connected tubing from the interphase of both solutions. The tubing was held at the interface while suction continued until both solutions were completely collected.
- 8 The collected material was placed on a 40 µm sieve, where they were gently washed multiple times with deionized water using a spray bottle and subsequently collected in a 15 ml centrifuge tube. The final volume was adjusted to 10 ml to standardize the volume across all samples for downstream analysis.

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Notes

- 16 Note 1: Vibratory sieve shaker AS 200 Basic (Retsch GmbH, Haan, Germany)
Note 2: The sieving convoy (ISO 3310/1, 200×50 mm, made of stainless steel) was made of a 630 μm sieve, 400 μm sieve, 315 μm sieve, aeration ring, ring sieve, 40 μm sieve, aeration ring and a collecting bowl in descending order. Two aeration rings separated the 315 μm and 40 μm sieve as well as the 40 μm and the collecting bowl to avoid the overflow of water due to pressure.
Note 3: Neptun Classic NCGP-E 110i (Neptun Handels GmbH, Dreieich, Germany)
Note 4: Christ beta 1-8 Lo plus freeze dryer (Christ, Osterode am Harz, Germany) with associated vacuum pump (Vacuubrand, Wertheim, Germany).



Note 5: The intersections of hyphae with the gridline were counted at 13 sites in each sample under the Leica DM 1000 LED microscope using 10x magnification (Leica Camera, Wetzlar, Germany).

Note 6: Autoclaved pipette tips with low retention were used.

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