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Identification of *Mucor circinelloides* from environmental soil samples

A short study

Figure 1. The location where the soil sample weas taken.

Introduction:

Fungi can be found everywhere, although most common in soils, and have a wide variety of roles across ecosystems yet they are surprisingly seldom discussed. In this short study, a soil sample was taken with the goal of trying to establish a monoculture to be able to identify a species of fungi from that location. Fortunately, this was possible and the identification of *Mucor circinelloides* from the soil sample was made with the help of phylogenomic methods.

Sample location:

The soil sample was taken 2022-11-09 at 59°51'50.3"N 17°38'11.7"E which is along the northernmost parts of the railway walking path in central Uppsala. The soil itself was taken from right next to a deteriorating tree stump with visible mushroom bodies growing out of the wood (*Figure 1).* The strip of soil is quite narrow with the railway on one side and a parking lot on the other and the soil was quite dense and mud-like. The walking path is also often used by both cyclists and pedestrians.

Materials and methods:

After the soil sample was collected, they were spread onto two agar plates with different growth mediums, one containing PDA (Potato dextrose agar) and the other containing YEPD (Yeast extract – peptone - dextrose). These plates were incubated for 3 days before a colony was selected and transferred to new plates using a sterile loop. After another 2 days of incubation at 30 ºC another selection was made in the hopes of getting a monoculture. Once a monoculture was established, spores were taken and added to a Chelex-solution. The samples were placed on a heating block at 95 ºC for 15 minutes before being centrifuged at 10'000G for 1 minute, leaving a supernatant that could be used for PCR. After adding ITS1 and ITS4 primers the PCR could be done.

The genetic sequences produced from the PCR could then be used to create a phylogenetic tree. This was done by first cleaning up the sequences and creating a consensus using Assseq. This consensus was then put in BLAST in NCBI and the top 25 hits were together with a reference list and the consensus was combined. This file could then be aligned with MAFFT and then processed with IQTREE to create a "treefile" which could be uploaded to iTOL to form the actual phylogenetic tree.

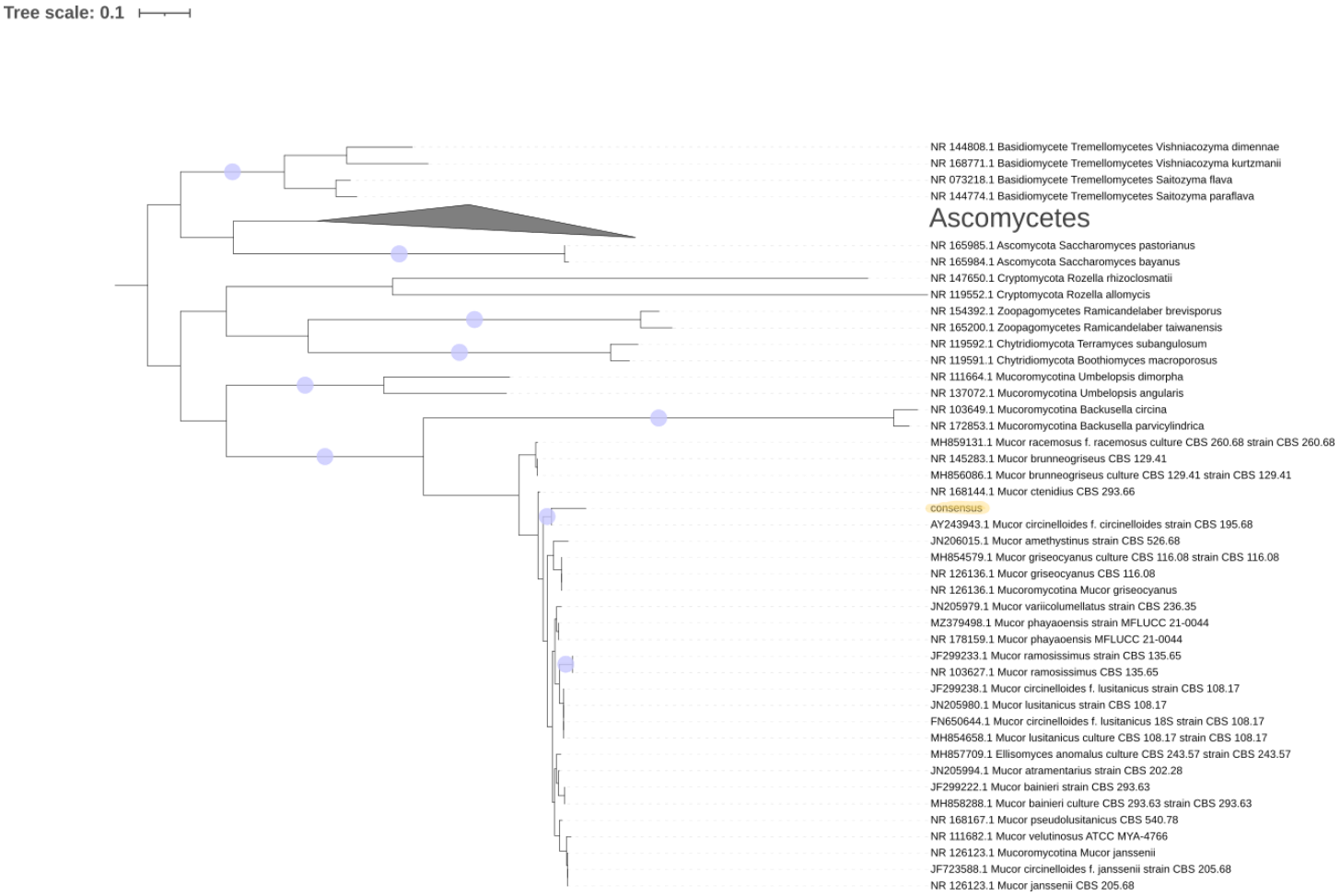

Figure 2. Phylogenetic tree containing consensus sequence from environmental sample highlighted in yellow, 25 top hits from BLAST (names have been shortened) and fungi from reference data-file, blue circles showing nodes calculated with bootstrap tests.

Species identification:

Both by the above 99.6% percentage identity to several references of the same species in BLAST and by looking at the phylogenetic tree and finding a statistically reliable node (*Figure 2*) it is possible to conclude that the fungal sample is of the species *Mucor circinelloides*.

Mucoralean fungi reproduce both asexually and sexually. Both forms of reproduction lead to sporangium but the asexual ones create sporangiospores whilst the sexual reproduction is done via the fusion of hyphae of the opposite mating type forming zygospores which will create sporangium of sexual meiospores. The germination of zygospores can take a long time however leading to asexual spores being more prevalent when speaking about infections in humans and animals. Colonies are usually a grey-brown colour with long branches holding up the sporangiospores. The sporangia are spherical and are usually between 20-80 µm in diameter where the sporangiospores themselves are ellipsoidal and can be smaller (3.5 – 5 µm) or a slightly bigger (4.5 – 7 µm) in size. The sexual zygospores are a more red/brown to dark-brown colour an can be up to 100 µm in diameter (University of Adelaide. 2022). This further supports *Mucor circinelloides* as the identified species as sporangia was measured to have a diameter of approximately 71 µm (*Figure 3*) and sporangiospores were all measured to be around 5-7 µm big (*Figure 4*).

One thing to note is that only the PDA culture resulted in usable genetic data and therefore, only the PDA fungi is presented in this report

Mucor circinelloides is a ubiquitously distributed fungus which can be found in soil, air and food and it is an opportunistic human pathogen. Several fungi of the Mucorales order can cause human infections, the general term now being mucormycosis. The infection can take hold in the blood, spaces inside the brain, in the lungs, the gastrointestinal tract, sinuses and soft tissues.

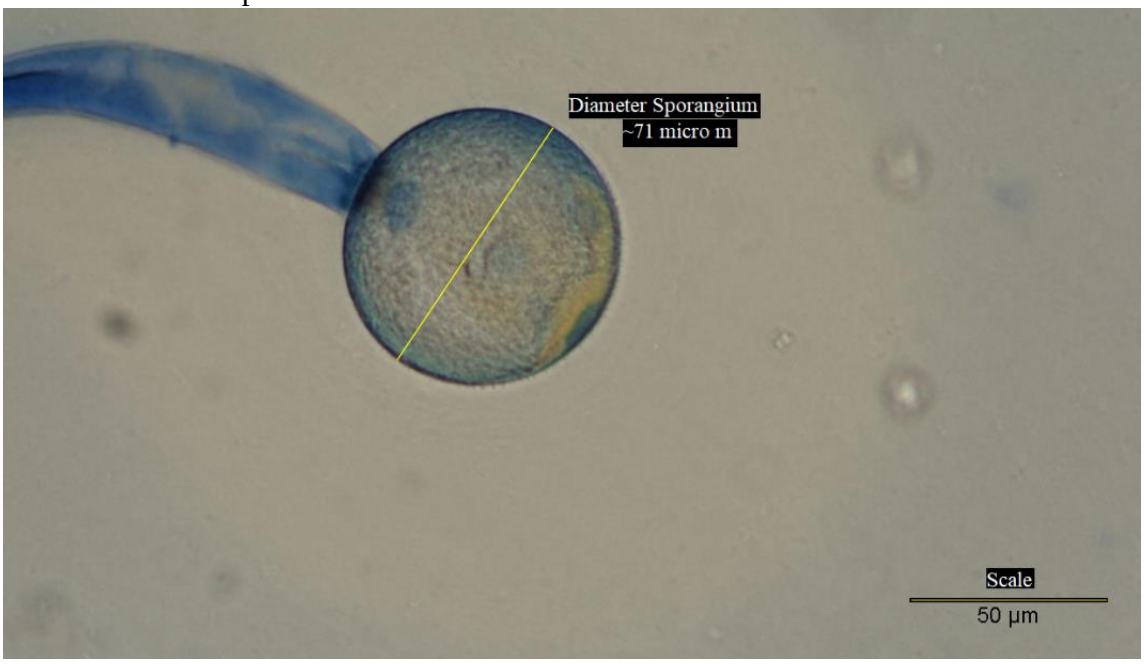

Figure 3. Sporangia stained with cotton blue at 20 times magnification with Scale and Diameter measured in ImageJ and labelled.

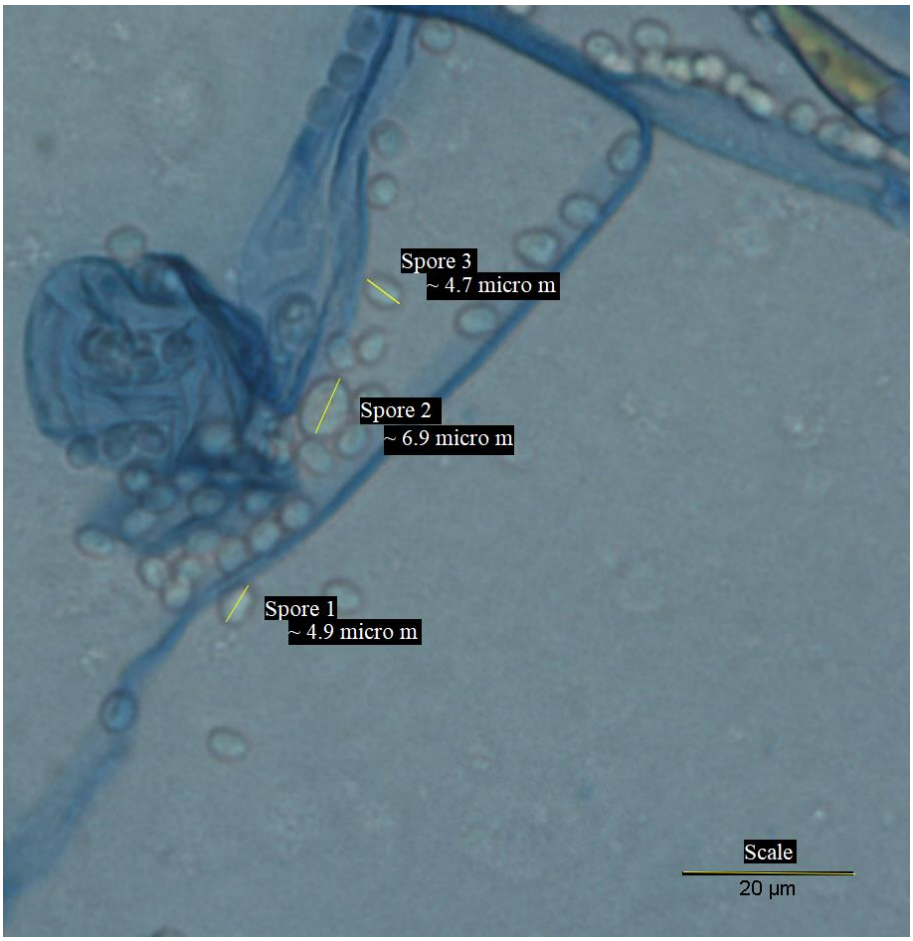

Figure 4. Burst sporangia stained with cotton blue at 40 times magnification with Scale and sporangiospores (Spore 1, Spore 2 and Spore 3) measured in ImageJ and labelled.

Sources:

Mendoza L, Vilela R, Voelz K, Ibrahim A, Voigt K, Lee SC. 2014. Human Fungal Pathogens of Mucorales and Entomophthorales. Cold Spring Harbor perspectives in medicine, pp. 1–33.

Mucor. University of Adelaide: https://www.adelaide.edu.au/mycology/fungal-descriptions-and-antifungal-susceptibility/zygomycota-pin-moulds/mucor. Accessed 14 December 2022.