

## *Mucor circinelloides* - a short study



Figure 1: the location where the soil sample was collected. More exact it was inside of the groove underneath a tree.



Figure 2: To the left – a picture of the sporangium from the University of Adelaide. To the right – a picture of the fungus found in Uppsala.

### Summary

During the course 'The diversity and evolution of microbial eukaryotes' a small study have been made on what we can find in soil and freshwater samples from different places in and outside of Uppsala. This report covers the soil sample with the aim of growing one species of fungi in lab and find out what species it is. Luckily, a result was given, and the species was *Mucor circinelloides*, a common fungus that can be pathogenic to mostly animals, but also humans. It is a fast growing fungus which produces large numbers of spores quickly.

### The location of the sample

The soil sample for the fungi were taken at 59°50'41.27"N 17°36'4.52"E, 4th of November 2021. These coordinates mark the location of a grove in a green area in the skirts of Uppsala, Sweden(see figure 1). The grove is not at all large, but approximately 20 trees. The trees were mainly deciduous. At the time point of the sample, 3<sup>rd</sup> of November, the soil was very damp and covered in yellowed leaves. The sample was taken at an approximate depth of 10 cm.

### The procedure and the isolated sample

After the samples were collected, a few soil particles were placed on two different agar plates; one with yeast extract peptone dextrose(YEPD) agar + chloramphenicol, and one with potato dextrose agar(PDA) + chloramphenicol. They were then incubated for 3 days.

After 3 days one of the two plates was chosen for further studies, in this case, the sample on the PDA plate. Some part of the fungi were then isolated further on another plate with the PDA media, and was then incubated for a few more days. The fungus was observed under a microscope where measurements were done(see figure 3). One sample was coloured and one was not, and on the picture of the uncoloured one(figure

3), it is seen that the sporangiophores have the diameter of 0,0535 mm. Other traits for this fungus is the huge amount of spores it was able to produce in just a few days. The plate was quickly covered in a white "fluff" where the sporangiophores were large enough to see with the naked eye. Under the "fluff" it had a dark grey, almost black colour.

### The phylogenetic tree

To find out which species that was found, a phylogenetic tree was created. To be able to do this, different programs were used. Earlier a PCR was done before the samples was sent for sequencing. For the fungi we used the following primers: ITS-1 for forward, and ITS-4 for reverse.

The following steps briefly describes the interpretation of the sequencing chromatograms:

1. Assseq – the chromatograms were visually inspected. Unfortunately, the forward sequence was not good enough, therefore only the reverse sequence was cleaned and saved as consensus.
2. BLAST in NCBI – the consensus was blasted in NCBI, giving that it with over 99% similarities was the fungus *Mucor circinelloides*. The top 25 hits was downloaded. All settings were default, except marking the "limit to sequences from type material" box.
3. Notepad – The consensus, the top 25 hits and the reference file were combined in notepad. All spaces was exchanged with underscore("\_") for it to work further. There were in total 78 sequences in the file.
4. MAFFT – the file with all sequences was uploaded to MAFFT. All settings was left default.

5. iQtree – the Fasta-file from was then uploaded to iQtree. This was also run with the default settings.
6. Figtree – Visualised the tree from the Tree-file downloaded from iQtree. Different settings was changed to make the tree easy to read. The consensus was marked with a colour.

In the tree it is clearly shown that the fungus is a *Mucor circinelloides*.

***Mucor circinelloides* – species description**

Overall the genus *Mucor* contains a few species, today around 50 known. Not only is this fungus widespread, it is also infectious with most infections reported in the genus. It can survive in a temperature up to 37 degrees, and is pathogenic for mostly animals, but also humans. This specific species is not hard to find and is common in nature. The sporangiophores are hyaline and mostly sympodial branched and often have the size between 4.5-7 x 3.5-5 micrometres. The sporangia have a

spherical shape and can be between 20-80 micrometres in diameter. Overall chlamydospores are absent. The zygospores, on the other hand, can only be produced by compatible mating types, with a reddish-brown to dark-brown colour. They(the zygospores) are spherical with stellate spines and can be as big as 100 micrometres.

One thing that is interesting with this specific species of *Mucor*, is that it differ from the rest of the genus in its formation of short, branched sporangiophores which bearers brown sporangia and the fungus ability to assimilate ethanol and nitrates.

**Sources:**

Mycology | University of Adelaide.  
”Mucor”. Åtkomstdatum 27 december 2021.

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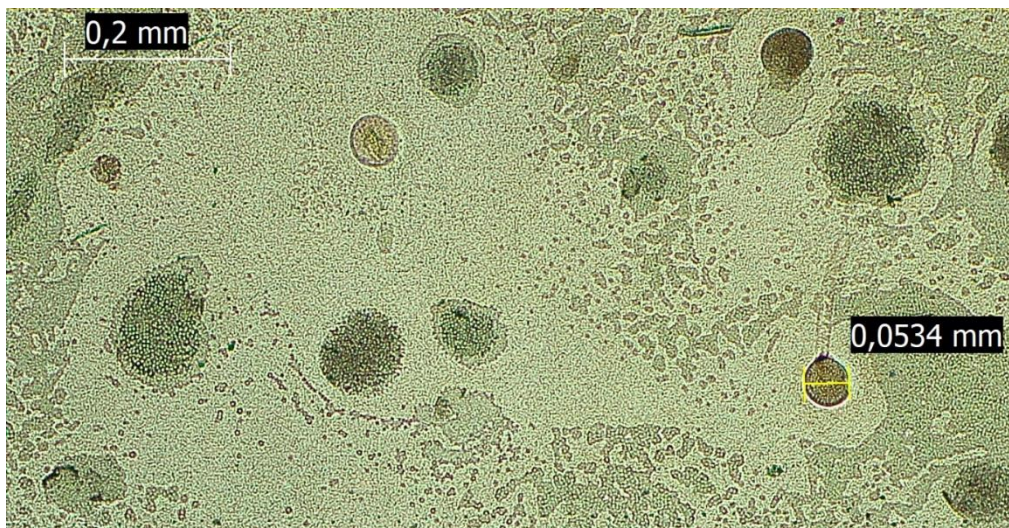


Figure 3: A picture of sporangia and spores with measurement.