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Evolution and Diversity of microbial eukaryotes: 1BG235 17429

Fungal Isolation – *Mucor circinelloides*

sample area

The fungi soil-sample was collected on November 27th 2024 underneath a rowan tree in Håga Uppsala (estimated coordinates: 59.845604 °N, 17.5973759 °E). The sample was taken just below surface level (Figure 1) in a 50 mL falcon tube.

Figure 1: The rowan tree which the soil sample was collected underneath from

Isolation method

A sterile plastic spoon was used to take small amounts of soil sample and spread it out on two agar plates, one containing agar + yeast extract – peptone – dextrose (YEPD) + chloramphenicol (antibiotic) and the other containing potato dextrose agar (PDA) + chloramphenicol (antibiotic). The plates where than sealed with parafilm and incubated for 3 days at 30°C. After incubation the plates were observed under microscope and the PDA plate were chosen for further isolation, a single fungi colony was taken from the plate and restreaked on another PDA plate. It was than incubated at 30°C. It was restreaked again (Figure 2) and once again incubated at 30°C. The pure culture was used for DNA extraction and was prepared for PCR. 200 μL of 5% Chelex-100 solution was

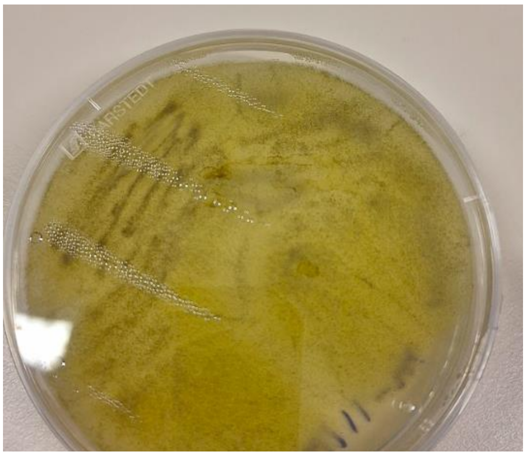

Figure 2: Pure culture of fungi on a PDA plate, created through multiple reastreaks

pipetted in an Eppendorf tube. A loop was sterilized in the flame and was dipped into the Chelex. The pure culture was touched on the conidia with the Chelex-loop and was transferred into the 200 µL Chelex Eppendorf tube. The tube was vortexed for 10 seconds and was than boiled on a heating block at 95 °C for 15 minutes. It was than vortexed again and finally it was centrifuged at 10000xG for 1 minute. The fungi DNA was used for a mastermix with the following parameters: 12.5 µL GoTaq, 2.5 µL forward primer (ITS1), 2.5 µL reverse primer (ITS4), 2.0 µL DNA and 5.5 µL nuclease free water, for a total volume of 25 µL. A control was also created but with 7.5 µL nuclease free water and no DNA. The PCR tube was out in a PCR machine. The next day 10 µL of the PCR product was gel electrophorized, another 10 µL of the PCR product was placed into an Eppendorf tube and 4 µL ExoSap was added and was incubated at 37 °C for 15 minutes and then incubated at 80 °C for 15 minutes and was finally labelled and sent for sequencing.

Bioinformatic analysis

The sequence that was sent back was put into the program SnapGene. The forward and reverse primer sequences was aligned, and non-overlapping sequences was removed. The sequences were saved as a FASTA file and put into NCBI BLAST to determine what species it could be. The top 25 answers were saved as a FASTA complete sequences file and was out together with a reference file with the main lineages of fungi. The file was than put into MAFFT online version to align the sequences according to the top sequences received from the BLAST. The resulting FASTA file was put into IQTREE to create a .treefile which was put into iTOL so a phylogenetic tree over the sequences was created (Figure 3).

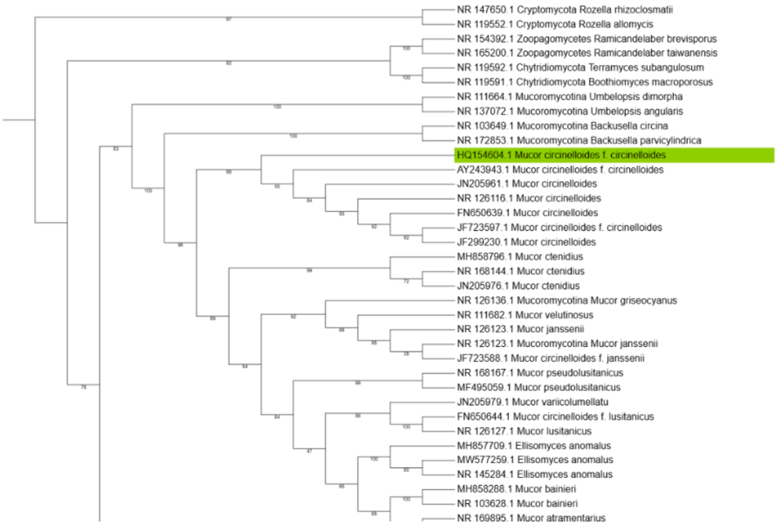

Figure 3: A picture over the phylogenetic tree, the group highlighted in green is the top result from BLAST, the tree was rooted from roozelids since they are the furthest related to the other species and lest likely to have on the plate

Result and Discussion

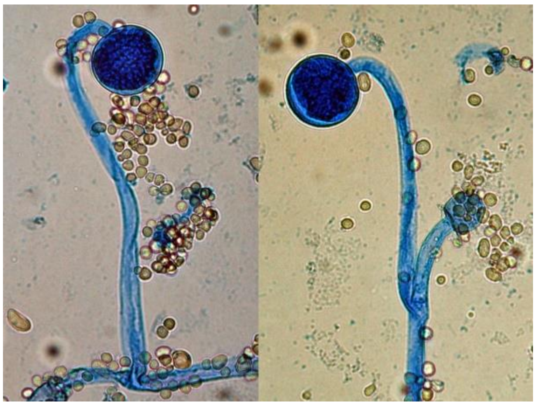

Figure 4: mucor circinelloides sporangium, picture source: The university of ADELAIDE

According to the tree (Figure 3) the species *is Mucor circinelloides*. This is a very plausible result since according to boostrap1 that is used to see the percentage certain that the different clades and nodes are related, it's a 100% on the Mucor clade, however some uncertainties are observed since towards the end of the node the 3 groups of *Ellsomyces anomalus* are found. However, as you move closer to their branch the bootstrap1 values get lower, indicating that there is more uncertainty if they are related, values as low as 64% and even 47% are observed. A boostrap1 value above 90 is regarded as good. Close to the *is Mucor circinelloides* which was received from the BLAST an 88% can be found, however this is deemed to be good enough since it's the branches after that start to become lower indicating that the species after are the ones that have a lot of uncertainty. By comparing figure 4 and 5 it becomes more evident that the group is in fact *mucor circinelloides since they are similar due to the clear visible sporangium. Therefore, the conclusion is made that the species is mucor circinelloides*

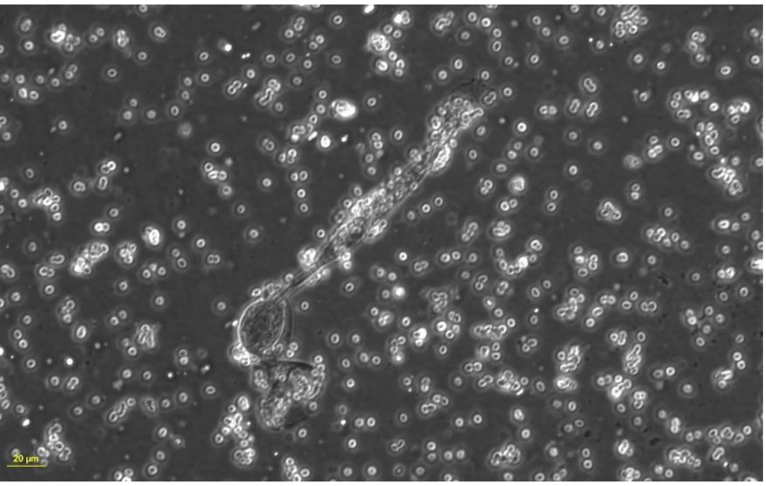

Figure 5: Electron microscopic pictures of the fungi cultivated on the PDA plate, the picture shows a sporangium circa 20 µm big, and the spores seem to be between 1/5 and 1/4 of 20 µm so circa 4 or 5 µm

Mucor circinelloides

M.circinelloides is a mold belonging to the class Mucoromycetes and order Mucorales (Vellanki et.al 2018). It is a model species for genetic modification since its one of the first filamentous fungi to have its entire genome sequenced (Cristiano et.al 2019). It is commonly found in soils, and it is known to cause the disease mucormycosis (Wagner et.al 2020). Mucormycosis mostly affects immunocompromised people and have a variety of symptoms depending on where in the body it takes root; the symptoms include fever, headache, seizures, cough, chest pain, skin lesion, nausea, vomiting and many more (Jorge et.al 2023, WHO). It undergoes both a sexual and an asexual life cycle, in the asexual cycle spores germinate and form mycelia and aerial hyphae, which has a sporangium with asexual spores. In the sexual cycle zygospores are created which are formed by two different hyphae (+ and -) fusing (Charles et.al 2011).

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