Andreas Andersson
Diversity and Evolution of Microbial Eukaryotes
HT2024

UPPSALA UNIVERSITET

A Fast-growing Fungi from Flogsta - *Geotrichum sp.*

Summary
In this course, environmental samples were taken of both fungi and algae. This report will follow the fungi. The fungal samples were grown on media and studied under the microscope. Following PCR, the samples were sent for sequencing and the sequenced genomes were used to identify the species and build a phylogenetic tree.

Collecting the sample

On the 12th of november 2024 at 10:02, a soil sample was taken from under a birch tree close to Ekebydammen. The exact location is 59.845903°N, 17.598792°E in Ekeby, Uppsala. Close to the trunk, grass and leaves were removed to reveal the dirt. A small sample was collected by scooping up some soil with a 50 mL falcon tube.

Figure 1: A birch tree next to Ekebydammen, where the soil sample was collected.

Isolating a single species from the sample

A small amount of the sample was poured onto two separate agar plates. One with yeast extract (YEPD) agar with chloramphenicol for selection, and one with potato dextrose agar (PDA) and chloramphenicol. These plates were then sealed with parafilm and incubated at 30°C for 3 days. After, sufficient growth on the plates could be seen. A single colony was taken from the YEPD-plate with a sterile loop and swatched on a new YEPD-plate. This was made in order to isolate that single colony. After incubation for 2 days, a colony from the new plate was again restreaked onto a new YEPD-plate, to ensure a pure culture. This colony was also studied under a light microscope with and without blue staining. When a pure culture

was achieved, a small amount of conidia was taken from the culture and mixed with a Chelex-100 solution. The supernatant was then added into a PCR tube with ITS1 and ITS4 primers, GoTaq and nuclease free water. The primers amplify the ITS region, which is a region between the small-subunit rRNA and large-subunit rRNA genes. This region is commonly used to identify fungal species. The PCR products were analyzed using gel electrophoresis. A singular clean band indicated that the DNA has successfully been purified and the DNA sample got sent for sequencing.

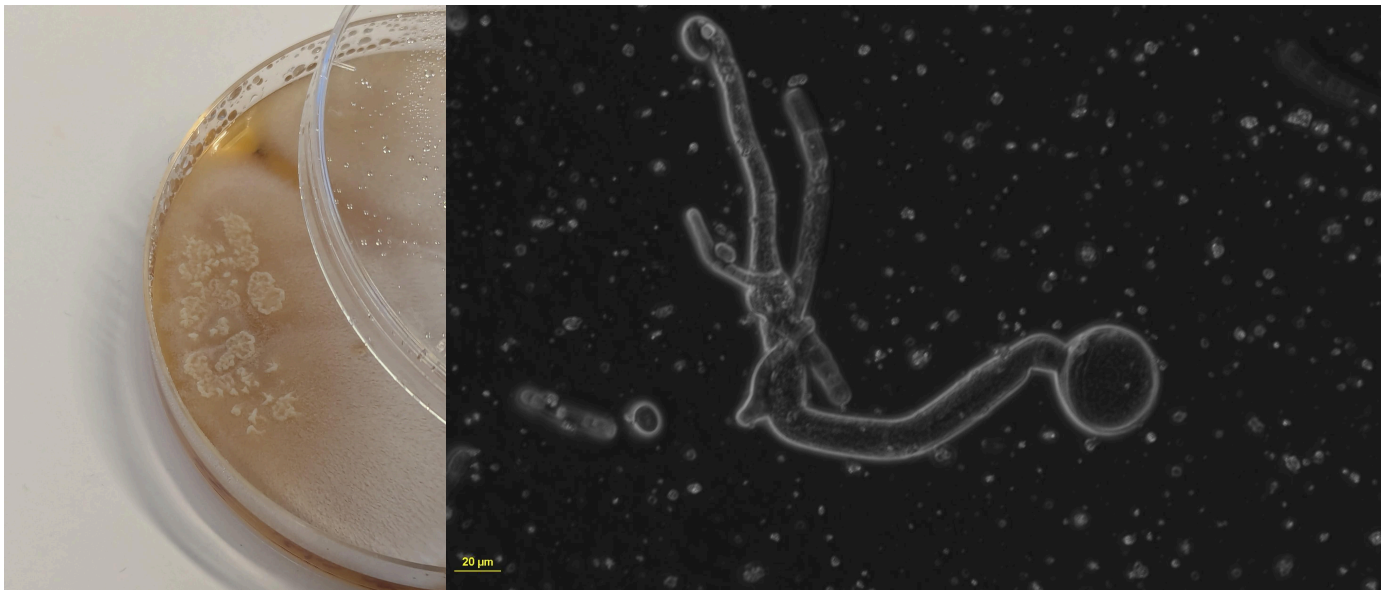

Figure 2: A macroscopic and microscopic view of the fungi. Left: The culture grown on YEPD-plate. Spreading hyphae across the plate, but also forming small structures. Right: A hyphae with a sporangium.

Identifying the fungi

When the sequences came back, they were analyzed as a chromatogram in SnapGene. Bad ends were cut and the forward and reverse sequence were aligned to create a reliable sequence which was then used with BLAST. The species with the most identical sequence were *Geotrichum pandrosion*, being 98.38% identical. The top 25 matches were then downloaded as a .FASTA file and aligned using MAFFT. A new .FASTA file was downloaded from MAFFT and put into AliView to see that all the sequences had aligned correctly. The .FASTA file was uploaded to IQTREE to generate a phylogenetic tree. The tree was later visualized with iTOL.

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The species belong to the genus *Geotrichum*. In the phylogenetic tree below it is labeled as *Geotrichum sp.* As the highest percent identity was lower than 100% (98,38), the sample fungi will not be recognized as a specific species but instead a part of the genus *Geotrichum*. Below you can see the phylogenetic tree and the sample fungi labelled in red.

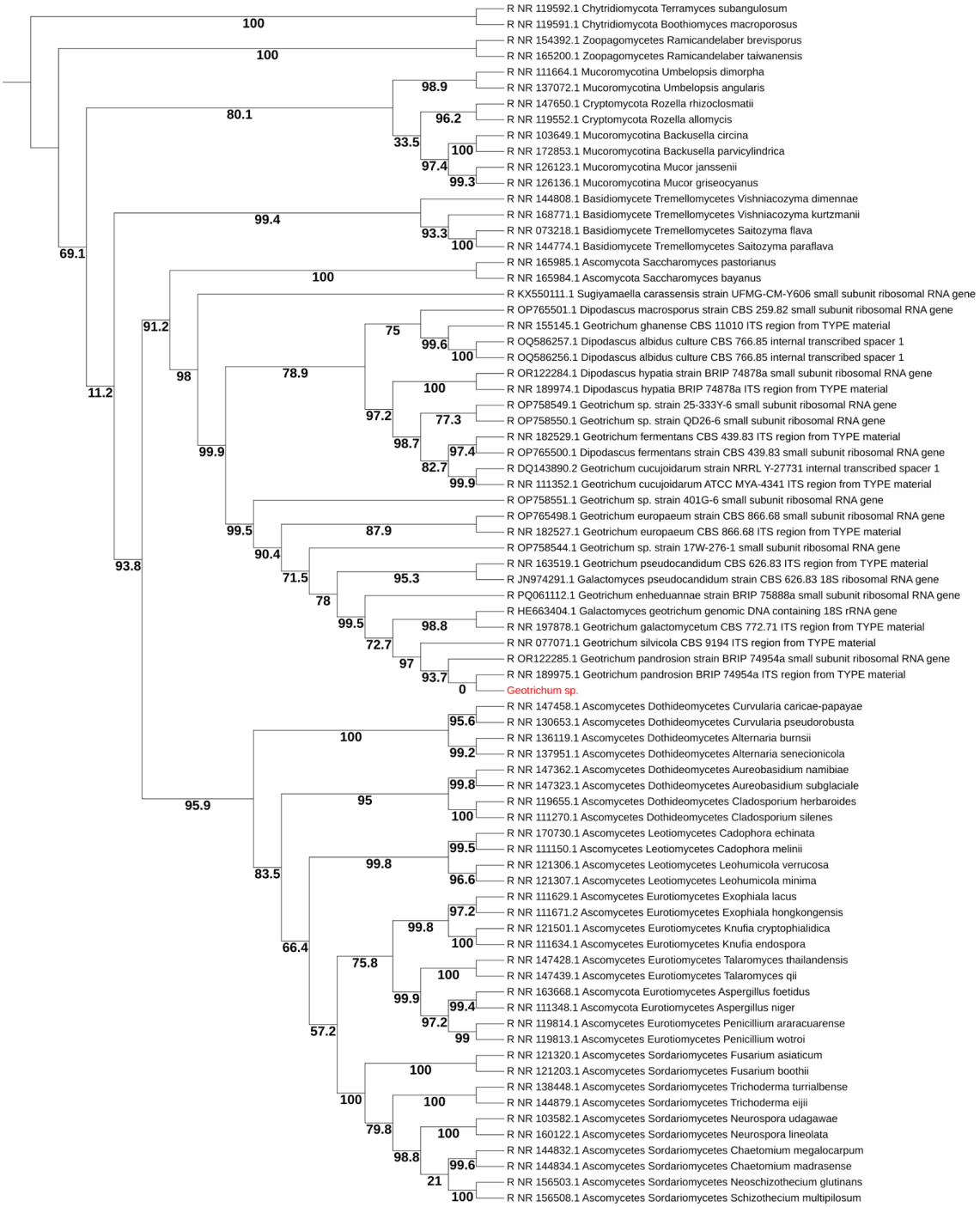

Figure 3: A phylogenetic tree containing large reference groups of the fungal kingdom, the top 25 identity matches from BLAST and the fungal sample species, labelled in red. The numbers are showing the probability of relation.

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The genus *Geotrichum*

Geotrichum is a genus in the order *Saccharomycetales*, division *Ascomycota*. It is a common yeast found in soil, water and air. It is also found in the normal human flora. Members of the *Geotrichum* genus are opportunistic pathogens and may cause infection in immunocompromised patients. They are fast-growing and create white, powdery colonies.

References

1. Aaron A, Vogan J, Mahwash, Javier F G. (2024). *Diversity and Evolution of Microbial Eukaryotes - 1BG235 Lab 1 - Enviromental Isolation.* Uppsala University
2. Doctor Fungus, *Geotrichum Species*. Available at: https://drfungus.org/knowledge-base/geotrichum-species/ (Accessed: 18 June 2024).