Szimonetta Boli
Uppsala University

Fungus isolation from environmental sample

Summary

Different fungal species are found almost everywhere around us and in every type of environment. In fact, most of these species play a significant role in maintaining a well-functioning ecosystem. Therefore, the goal of this study was to identify a fungus from an environmental sample and to construct a phylogenetic tree which would reveal relationships between the identified *Trichoderma* genus and other division within the fungal kingdom.

Collecting fungal sample

The soil sample used in this study was collected on the 10th of November 2024 from a forest ground in Sala, at the position 59,91864°N, 16,58106°E. There were several chopped up tree trunks and branches at this location, which were believed to be "dead wood" in the process of decomposition. The ground was mainly covered by grass and moss, as well as autumn leaves, which was removed in order to collect a bit of the soil underneath. A few visible fungal fruiting bodies were noticed on the dead wood, which explains the exact location of the sampled area, in between two of these trees (Figure 1).

Figure 1. Location of the sampled area. The exact spot where the sample was collected is shown with a red circle.

Method – Isolation of a pure fungal culture

Using aseptic technique, a small amount of the collected sample was placed on two agar plates enriched with different components, one with yeast extract peptone dextrose (YEPD) and one with potato dextrose (PDA). In addition, both plates contained chloramphenicol which is an antibiotic used to ensure the growth of fungal cultures. Parafilmed plates were incubated for 3 days at 30°C. A liquid culture with YEPD was prepared and incubated in the same way for yeast species, but because of a miscommunication chloramphenicol was left out of this medium. Therefore, and also because of no yeast growth, a choice in between the two agar plates were made for continued restreaking in order to achieve a pure fungal culture (Figure 2). In this case a small area from the YEPD plate was chosen and restreaked 4 times, with 2 day intervals.

The most recently restreaked plate was used for DNA extraction, which was done by boiling a small number of cells in a solution called Chelex-100. This solution was then placed in a heating block at 95°C for 15 minutes, then the solution was pelleted, and the supernatant could be used in a PCR reaction. In order to amplify the internal transcribed spacer (ITS) region the primers used in the PCR reaction were the ITS-1 forward primer and the ITS-4 reverse primer, which is specific for identifying fungal species. The PCR product was then run on agarose gel to determine if the amplification of the DNA sequence was successful. The electrophoresis separated also the PCR product after molecular size, which in this case was around 400 bp. A successful electrophoresis indicated that the DNA sequence could be purified with the help of ExoSap and could be sent away for sequencing.

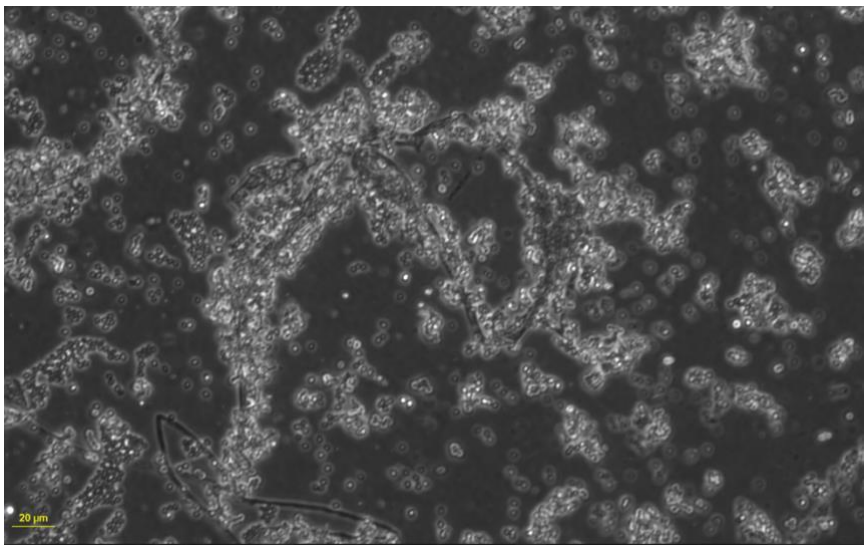

Figure 2. Pure culture under a compound microscope displays some fungal structures(hyphae) and the abundance of spores.

Species identification

To identify the cultured species a phylogenetic approach was used after receiving the chromatographs from the DNA sequencing, which used Sanger technology. Both the forward and reverse sequenced was analysed in SnapGene. In this application the sequences were trimmed for quality and aligned to generate a consensus sequence, which was later used in BLAST, a function in NCBI that finds similarities between biological sequences. The consensus sequence was thereby compared to a database of the most similar sequences found

in other species. The cultured fungus had 100.0% similarity to several species. Some of them being *Trichoderma rifaii* and *Trichoderma camerunense*, found in the division of Ascomycota and the class of Sordariomycetes. The top 25 BLAST hits were downloaded as a FASTA file and by using a text editor these sequences were combined with a provided reference set of fungus sequences and the consensus sequence. This way a large dataset of 78 sequences was obtained, which was later used in a website called MAFFTA to align all sequences. AliView was used to visually check if the alignment was successful, if so, the alignment was saved and used as an input in IQTREE to generate a phylogenetic tree. The generated tree file could be visualized with the help of iTOL where the tree was rerooted for an easier identification of the sequenced and assembled fungus (Figure 3.).

The cultured species showed a strong phylogenetic relationship to other species from the genus *Trichoderma* and to the phylum Ascomycota. Therefore, the sequenced DNA is believed to originate from the *Trichoderma spp*. group of fungi. However, it is important to mention that low bootstrap values in the phylogenetic tree indicates low statistical support and a somewhat uncertain relationship between species, most likely because of the strong similarities. Although strong similarities were observed in BLAST and in the tree, it is still hard to determine the exact species because of the studied ITS region. Two species can show high similarities in the ITS region while being completely different elsewhere in the genome.

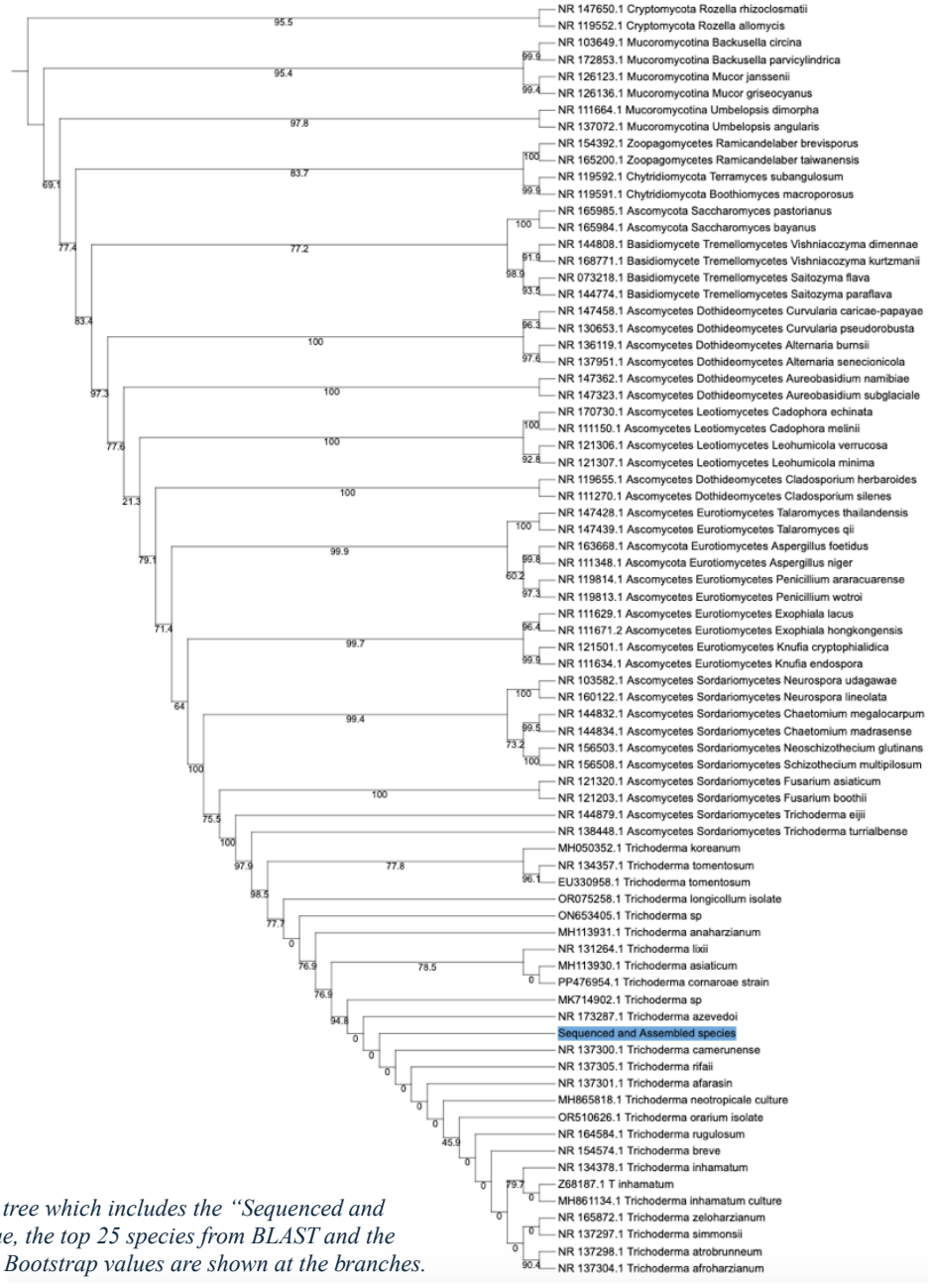

Figure 3. Generated phylogenetic tree which includes the "Sequenced and Assembled species" marked in blue, the top 25 species from BLAST and the reference set of fungus sequences. Bootstrap values are shown at the branches.

Trichoderma spp.

The genus *Trichoderma* belongs to the class Sordariomycetes found within the division of Ascomycota. Fungi from this genus are commonly found in habitats that includes soil and rotting wood (Błaszczyk L. *et al.* 2014). Usually they colonise leaves, roots and grains of plants but they have also been isolated from more unusual environments such as marine bivalves and termites. Species within *Trichoderma* is dividing asexually and tend to produce an abundant number of spores while having a high growth rate. They produce several pigments which can alter the appearance of different species and their spores, some can for instance appear green, yellow, red and even colourless (Błaszczyk L. *et al.* 2014). The sequenced fungus used in this study produced mainly green spores, which were at first white but turned dark green over time (Figure 4).

In addition, this genus has an industrial importance since some *Trichoderma* species have the ability to antagonise plant pathogens. In other words, they are used as biocontrol agent to control and maintain plant diseases. *Thrichoderma harazianuma* is a well-known species used as biocontrol agent in the field of agriculture because they increase the level of carbon dioxide (CO_2) and dioxygen (O_2) utilization by controlling expression of certain genes in plants (Pani S. *et al.* 2021).

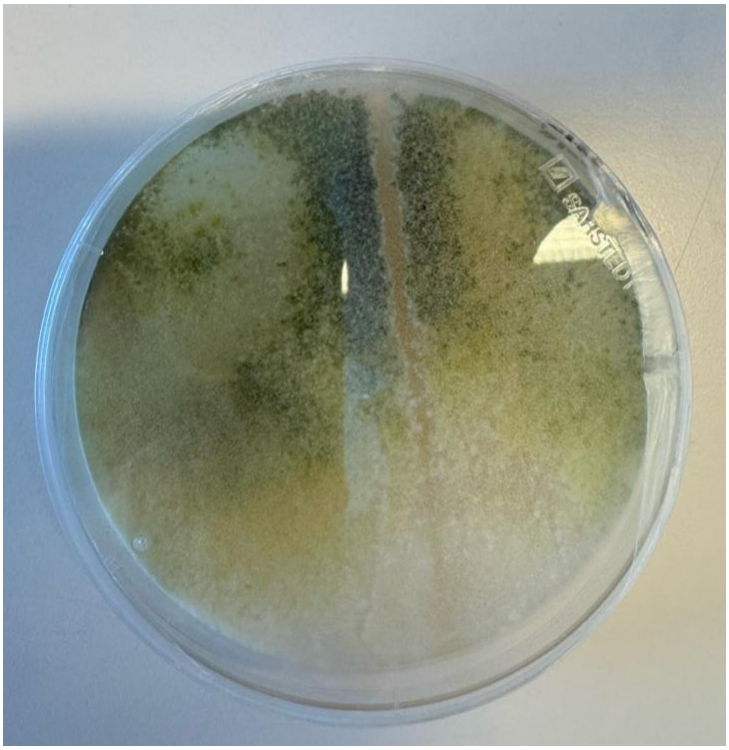

Figure 4. The most recently restreaked plate (2024.12.05) after 3 days of incubation.

References

Błaszczyk L., Siwulski M., Sobieralski K., Lisiecka J., Jędryczka M. (2014). *Trichoderma spp. – application and prospects for use in organic farming and industry*, Journal of plant protection research, 54(4): 309-317

Pani S., Kumar A., Sharma A. (2021). *Trichoderma harzianum: An Overview*, Bull. Env. Pharmacol. Life Sci., 10(6): 32-39

Vogan A., Jamy M., García J. (2021). Diversity and Evolution of Microbila Eukaryotes – 1BG235 Lab 1 – Environmental isolation (Lab 1 manual), Uppsala university, Version 1.0