

Trichoderma Sp.

Sampling:

The fungus was isolated from a dirt sample taken beneath a pine at 60,2643448 N°, 16,8980704 O° on the 10th of November.

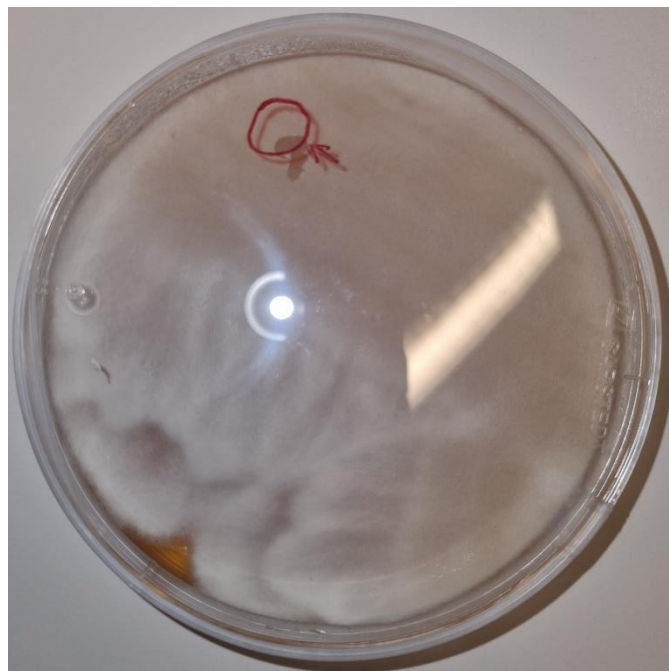
The sample included dirt, pine needles and small pieces of wood from a depth of 0cm to 10cm. The pine was close to a river stream.

Culturing:

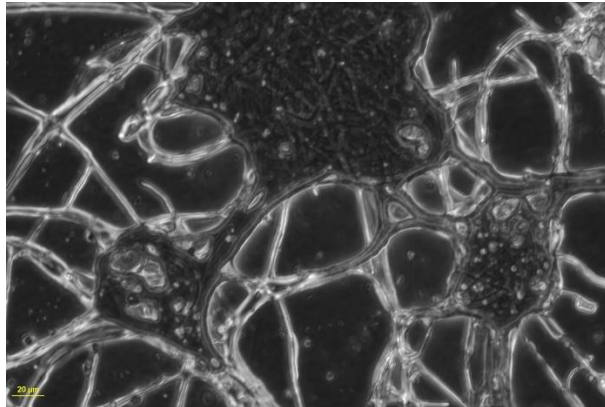
A small amount of the sample was spread on PDA agar plates and incubated for three days at 30 °C. A colony was picked with a loop and restreaked on another PDA agar plate for isolation. The fungus was restreaked a total of four times to obtain a pure culture.

Description:

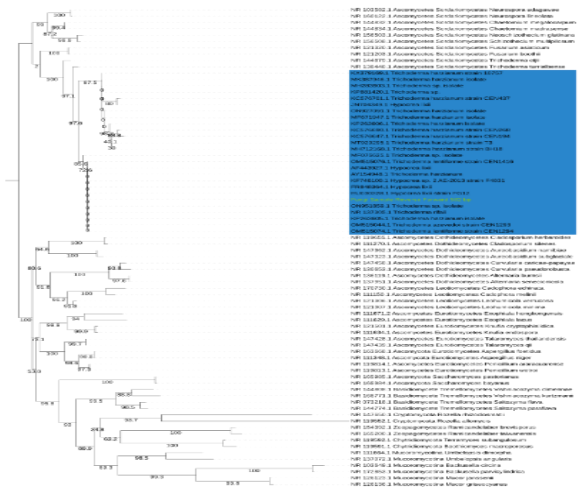
On the first plate a green fungus grew close to a small piece of wood. This one was picked and restreaked. The new plate showed the same darkish green colour at first, but white filaments were visible as well. To make sure there was no contamination, the green part was picked again and was restreaked another time. The same thing happened again. Another restreak of the green fungus led to a homogenous plate of white filamentous fungi.



Under a microscope the fungus showed hyphae with an approximate width of 10µm and spores of approximately 5µm. The hyphae where quite densely packed in certain regions.



aligned using MAFFT online. The aligned sequences were put into IQTREE which created a phylogenetic tree which was edited in iTOL.

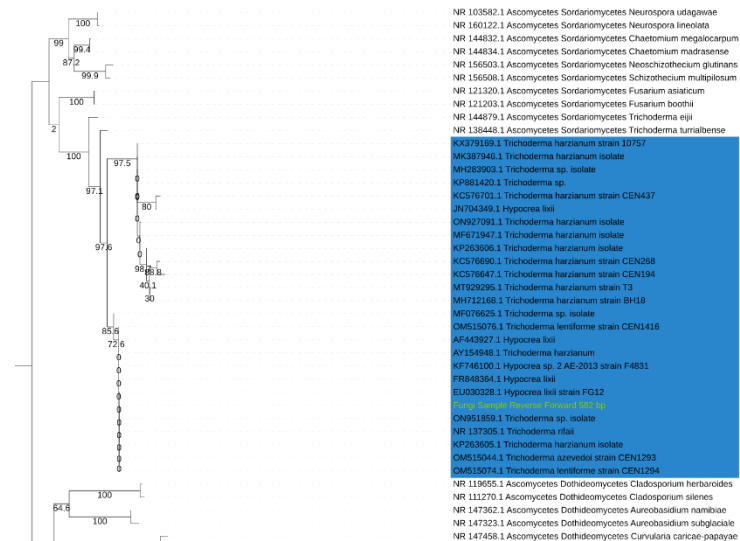


Phylogeny:

Methods:

DNA was extracted by using Chelex-100 solution. A small amount of fungus was added into an Eppendorf tube with 200µl of 5% Chelex-100. The tubes were heated for 15 minutes at 95 C°. Afterwards the sample was centrifuged at 10000g for 1 minute. The supernatant was immediately used for a PCR. The PCR included 1x GoTaq, 1µm the forward primer ITS1, 1µm reverse primer ITS4, 2µl of supernatant. Nuclease free water was added to reach a reaction volume of 25µl. After PCR gel electrophoresis was used to confirm successful DNA amplification of the ITS region. The PCR product was purified with ExoSap at 37 C° for 15 minutes after which ExoSap was inactivated at 80 C° for 15 minutes. The purified PCR product was prepared for Sanger sequencing of the ITS region with the ITS1 and ITS4 primers.

The sequence chromatogram was edited in SnapGene. The cleaned sequence was put into BLAST nucleotide search. The first 25 hits in the BLAST Databank, the sample sequence and the Ref-database-ITS were combined and



Discussion:

In BLAST the sequence led to all the 25 top results having a percent identity of 100%. Included were fungi of the genus *Trichoderma* but different species. Species that appeared often were *Harzianum* but that might just be attributed to them being well studied. In the tree the sample got sorted together with all the other BLAST hits. They branch off other ascomycetes with bootstrap values of 97.6. Further division in the clade itself shows at first still acceptable results with bootstrap values of 97.5 and 87.6 respectively but further distinguishment show no good bootstrap values anymore. The sample gets clustered with multiple *Hypochrea* and different species of *Trichoderma* but only one of them being *Harzianum* while other *Harzianum* sequences were clustered separately. Therefore I would consider the sample being of the genus *Trichoderma* while the specific species remains unknown but probably is not a *Harzianum*.

The genetic data also overlaps with what I was able to observe with *Trichoderma* developing hyphae, them growing green on agar plates but with colour variety and the sample being taken from under a tree, close to its roots.¹

Citations:

- 1) Harman, G. E., Howell, C. R., Viterbo, A., Chet, I., & Lorito, M. (2004). *Trichoderma* species--opportunistic, avirulent plant symbionts. *Nature reviews. Microbiology*, 2(1), 43–56. <https://doi.org/10.1038/nrmicro797>
- 2) https://uppsala.instructure.com/courses/94698/files/7566618?module_item_id=1225025