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Diversity and Evolution of Microbial Eukaryotes
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HT23

Identifying fungi - Fusarium

Location and collection:
The sample was collected from Gottsunda dagvatenparken, where surface water from the city is collected and cleaned before it makes its way into Mälaren (Uppsala kommun, 2022). For this 50 mL falcon tube was used. The soil sample was collected from the muddy ground underneath a maple tree, where plant roots closer to the surface also became a part of the sample. The sampling was performed on the 7th of November 2023.

Figure 1: The area from which the samples were taken, the soil sample was collected from underneath a small area with trees.

Isolation
The collected soil was used in order to isolate a pure fungal culture. This was done by first streaking the soil on a YEPDA plate made out of yeast extract, peptone, dextrose agar and chloramphenicol. The antibiotic was used to prevent the growth of bacteria. This first streaking did not give rise to an isolated fungal culture, instead many different fungal cultures were present. Afterwards a second streaking was performed which did lead to a clean fungal culture. The fungal culture used for isolation formed circles with white borders and gray middle. Other types of culturing media, PDA plates and a liquid YEPDA, were also used but were not used for further analysis.

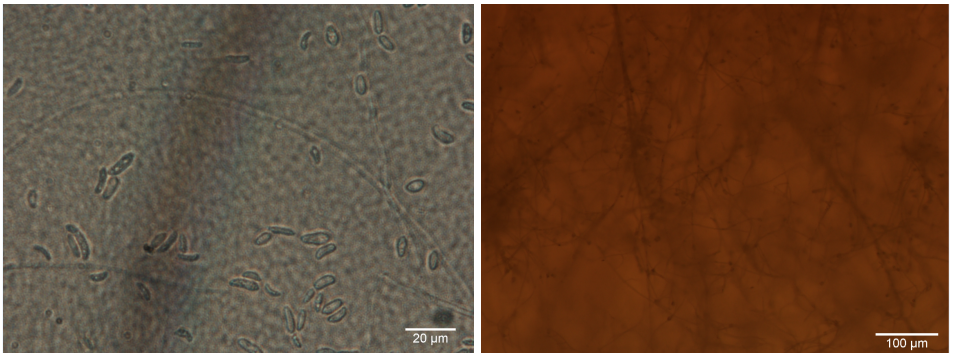

Figure 2: The structure of the fungus. Figure to the left shows asexual spores, the conidia, which have an elongated oval shape. This picture was taken without color. Figure to the right shows the hyphae.

DNA extraction and PCR
After making sure that the isolated culture was pure, DNA extraction was performed. This was done by first lysing the cells with Chelex-100 solution. The solution with conidia and chelex-100 was first vortexed for 10 seconds and placed in a heating block at 95 °C for 15 min. Thereafter the heated sample was centrifuged at the speed of 10 000 xG for 1 min and the supernatant collected for PCR

analysis. The collected supernatant was used to prepare the final PCR solution, here forward and reverse primers, GoTaq and nuclease free water were added. The solution containing fungal DNA was amplified with PCR. Afterwards both a positive and a negative control were prepared and then run on an agarose gel in order to find out if the PCR process was successful. Once this was done the PCR products were cleaned with ExoSap. The sample was first kept at 37 °C for 15 min and then at 80 °C for 15 min. The sample was then sent for sequencing.

Bioinformatic analysis

After the results from sequencing were obtained, bioinformatic analyses were performed. First a consensus sequence was created with the use of SnapGene. This sequence was then put into BLAST and the first 25 matches and the database of fungal sequences were used to create a FASTA file with assembled sequences. For this MAFT was used. The assembled sequences were then used to create a phylogenetic tree, see the appendix.

Species description

Once the consensus sequence was put into BLAST, a 100% identity match was obtained, although this was not the result with the highest query cover. The 100% identity match is an anamorph of Fusarium solani, Nectria haematococca. This means that the cultured fungus is Fusarium solani. This is a common phytopathogenic soil fungus (Yoneyama & Natsume 2010). This fungus can also cause opportunistic infections in humans, causing conditions such as keratitis and mycotoxicosis (Kosmidis & Denning 2017).

References:

Kosmidis C, Denning DW. 2017. 189 - Opportunistic and Systemic Fungi. I: Cohen J, Powderly WG, Opal SM (red.). Infectious Diseases (Fourth Edition), s. 1681-1709.e3. Elsevier,

Nguyen L.-T., Schmidt H.A., von Haeseler A., and Minh B.Q. (2015) IQ-TREE: A fast and effective stochastic algorithm for estimating maximum likelihood phylogenies. Mol. Biol. Evol., 32:268-274.

Yoneyama K, Natsume M. 2010. 4.13 - Allelochemicals for Plant–Plant and Plant–Microbe Interactions. I: Liu H-W (Ben), Mander L (red.). Comprehensive Natural Products II, s. 539–561. Elsevier, Oxford.

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Appendix:

The phylogenetic tree showing the closest relationship with Nectria haematococca. On BLAST the assembled sequence showed a 100.00 % similarity with this species.