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Diversity and Evolution of Microbial Eukaryotes
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Fungal identification: *Fusarium foetens*

Introduction

The course Diversity and Evolution of Microbial Eukaryotes highlights the outstanding diversity of protists and fungi. These organisms can be found in various environments and play key roles in countless ecosystems. This study gives a glimpse of the existing fungal diversity in terrestrial habitats. Here, a fungal specimen was isolated from a soil sample and cultured in laboratory conditions. Genetic analysis permitted the identification of the species and the description of its phylogeny.

Sampling

On 7 November 2024, a soil sample was collected from the top of the Håga mound (Hågahögen, also known as Kung Björns hög) in Uppsala, Sweden (Figure 1). More precisely, the sampling was done at location 59.8373798, 17.5868603. A generous amount of soil from a preexisting pit was collected.

Figure 1: Sampling location.

Culturing

A small portion of the soil sample was plated on two types of culture media: yeast extract peptone dextrose (YEPD) agar with added chloramphenicol and potato dextrose agar (PDA) with added chloramphenicol. The plates were sealed with parafilm and left to incubate at 30°C for several days. A fungal specimen from the YEPD plate was selected for restreaking and further study. After several days of incubation, one of the newly formed colonies was restreaked to establish a pure culture. However, the isolation of single colonies was unsuccessful and the agar plate contained some contaminants. Nevertheless, DNA extraction was performed by boiling fungal cells in Chelex-100. The sample was then prepared for PCR. The ITS-1 (forward) and ITS-4 (reverse) primers were used: these primers amplify the internal transcribed spacer (ITS) region and are often used in fungal identification. Gel electrophoresis of the PCR product showed two distinct bands, suggesting a contaminated sample. The PCR product was purified using ExoSap and prepared for sequencing.

Bioinformatic analysis

After sequencing, the resulting chromatograms were examined using SnapGene. The quality of the reverse sequence was poor, however, the forward sequence was still suitable for analysis. The sequences were trimmed and aligned. The consensus sequence was then input into BLAST to search for similar database entries, excluding “Uncultured/environmental sample sequences” and including only “Sequences from type material”. The fungal sample had a 100% similarity to the species *Fusarium foetens*. 24 more highly similar sequences, all from fungi belonging to the genus *Fusarium*, were downloaded and aligned with sequences from a reference data set and the consensus sequence. The alignment was completed with the help of

MAFFT software. IQTREE was then used to generate a phylogenetic tree, which was visualized using iTOL. The tree was rerooted with the genus *Rozella* as the outgroup. The determined phylogeny of the selected fungus confirms that it belongs to the genus *Fusarium* and supports the species to be *F. foetens* (Figure 2).

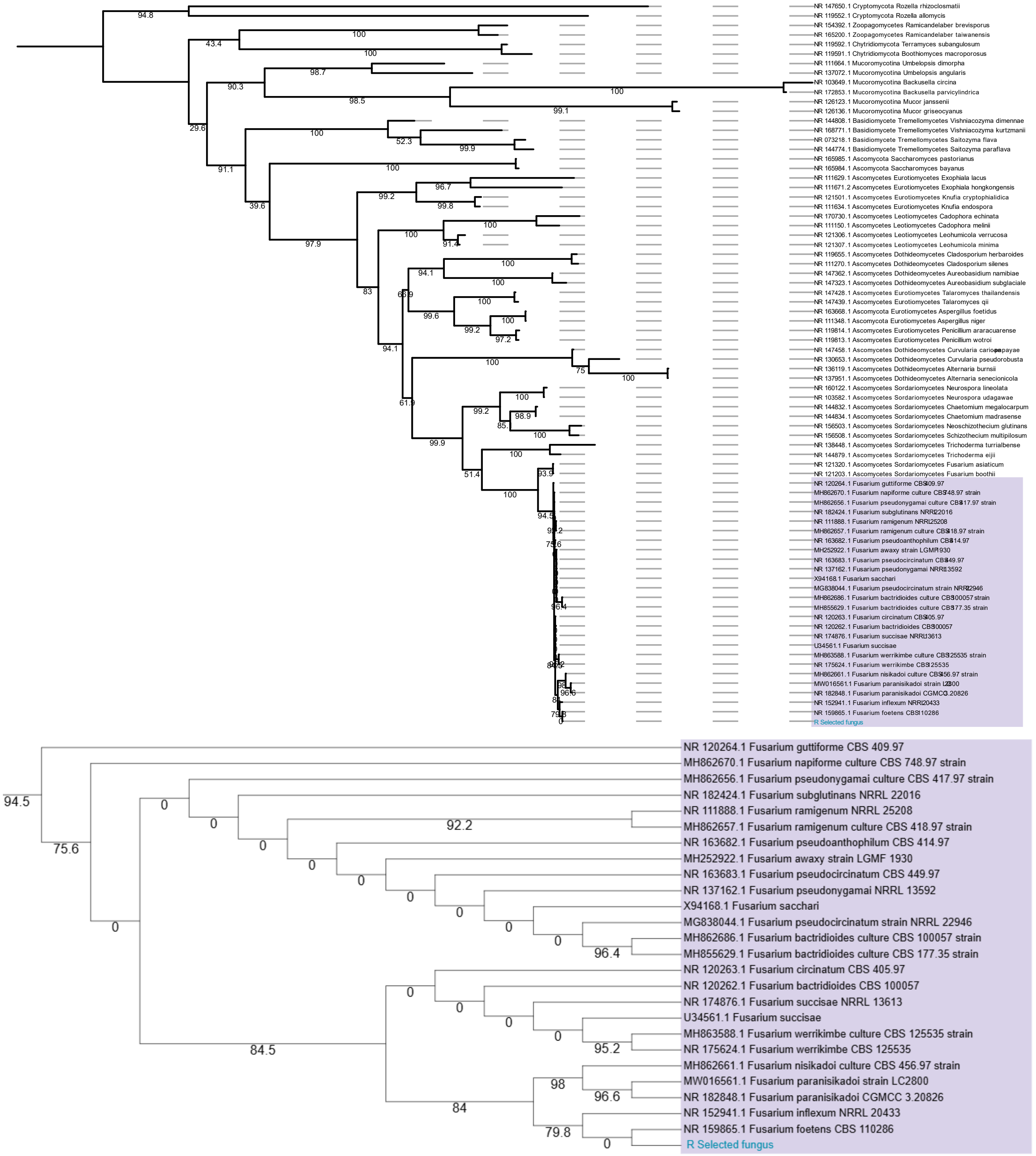

Figure 2: Phylogenetic tree and its close-up containing the selected fungus (marked in blue). The tree is based on 25 highly similar sequences obtained from BLAST and 50 sequences from a reference data set. The genus Fusarium is marked in purple.

Description

The selected fungus, now identified as *F. foetens*, belongs to a group of plant pathogens (Schroers et al., 2004). In the early 2000s, it was discovered that *F. foetens* causes disease in begonia elatior hybrids. *F. foetens* is also a known pathogen of rooibos, potatoes, tomatoes, bell peppers, and cayenne peppers (Liu et al., 2023). The species is an ascomycete (Mahyar Mirmajlessi, 2022) and belongs to the *Fusarium oxysporum* species complex (Joint Genome Institute, 2021). It is morphologically similar to other fungi within the genus (Liu et al., 2023). On YEPD agar, *F. foetens* has a bright white, dense mycelium that grows in tufts (Figure 3). Colonies can exhibit whitish-orange pigmentation at the center. The fungal hyphae can grow in single strands or ropes consisting of a few hyphae (Schroers et al., 2004). *F. foetens* forms both mono- and poly-phialides, and its microconidia form laterally from the fungal hyphae (Liu et al., 2023).

Figure 3: YEPD agar plate with a pure culture of the selected fungal specimen.

References

Mahyar Mirmajlessi, S. (Ed.). (2022). Fusarium - An Overview of the Genus. IntechOpen. https://doi.org/10.5772/intechopen.95213

Joint Genome Institute. (n.d.). *Fusarium foetens NRRL 38302 v1.0*. Mycocosm.jgi.doe.gov. https://mycocosm.jgi.doe.gov/Fusfoe1/Fusfoe1.home.html

Liu, L., Jin, X., Lu, X., Guo, L., Lu, P., Yu, H.-B., & Lv, B. (2023). Mechanisms of Surfactin from Bacillus subtilis SF1 against Fusarium foetens: A Novel Pathogen Inducing Potato Wilt. *Journal of Fungi*, *9*(3), 367–367. https://doi.org/10.3390/jof9030367

Schroers, H.-J., Baayen, R. P., Meffert, J. P., de Gruyter, J., Hooftman, M., & O'Donnell, K. (2004). Fusarium foetens, a new species pathogenic to begonia elatior hybrids (Begonia x hiemalis) and the sister taxon of the Fusarium oxysporum species complex. Mycologia, 96(2), 393–406. https://doi-org.proxy.uba.uva.nl/10.2307/3762070