Experimental Isolation – *Mucor sp.*

Abstract

Fungi are essential organisms in many processes on earth, from decomposition and nutrient cycling, to direct human uses in industry (Paul and Joshi, 2022). This study aimed to isolate and identify a fungal species from an environment using morphological and phylogenetic identification techniques. A fungi belonging to the genus *Mucor* was successfully isolated and identified, and a phylogenetic tree constructed.

Sampling

A solid substrate sample was obtained on 11/11/2024 in Uppsala, Sweden at the position 59.850°N, 17.595°E. The sample was taken from a compost pile, containing leaf litter, twigs and larger logs (Figure 1). It was observed that the sample material had moss and lichen growth.

Culture

To obtain colonies to observe initial fungi growth originating from the sample, the solid material was first incubated in liquid media. A small amount of the sample substrate was placed in an Eppendorf tube with yeast extract-peptone-dextrose (YEPD) liquid media and incubated for 3 days (Note: this liquid media contained no antibiotic and so may have contained bacterial growth). This resulted in a white liquid forming at the bottom of the tube, which was plated and streaked on a YEPD + antibiotic agar plate. This technique is used to enrich for yeast species. However, after the plate was incubated for 2 days, the observed fungal growth was filamentous, which is atypical for yeast. A re-streak of this white growth was performed to obtain pure culture (Figure 2), and fungal structures were taken to be observed under a microscope.

Figure 2: Fungi re-streak agar

Description

In order to observe fungal structures under the microscope, slides were prepared using tape to fix aerial hyphae, and placed on a glass slide. Cotton blue stain was used to stain the fungal cell wall and provide greater contrast to identify fungi microscopic features. Figure 3 shows some of the structures observed, including a partial hyphae, intact sporangia (fruiting bodies) and individual spores. The sporangia was an estimated 20μm in diameter, and a large number of spores were observed.

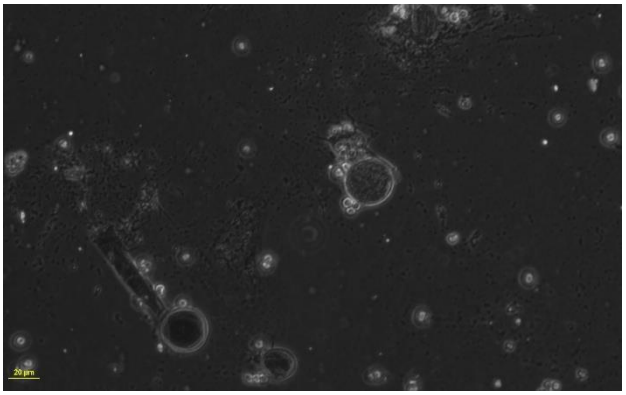

Figure 3: Fungal features under a microscope

DNA Sequencing and Phylogenetics

To further investigate the fungal species, DNA amplification and sequencing was performed. DNA was extracted using the Chelex method, and amplified by PCR. Specific primers used for amplification were chosen, ITS1 as the forward primer and ITS4 as the reverse primer. These amplify the internal transcribed spacer (ITS) region, which is commonly used to identify fungi. PCR products

were run on an agarose gel and produced a visible band, confirming the PCR was successful. The PCR product was then cleaned using ExoSap, forward and reverse primers added (separately) before being sent away for Sanger sequencing. This returns a chromatogram which was analysed for accuracy using SnapGene (2024). The sequences were trimmed for quality, and ambiguous bases were checked over. The reverse sequence was reverse complemented, and both sequences were aligned to create a consensus sequence. This was then used as a query with BLAST in NCBI (2024) to look for highly similar sequences, which produced a 98.84% percentage identity match to the genus *Mucor.* Using the top 25 hits from BLAST, a reference database for fungi and the assembled sequence, a phylogenetic tree was constructed using aligned sequences using MAFFT (Katoh et al, 2002). This tree (Figure 4) , generated using IQTREE (2024) and visualised with iTOL (2024), was used to locate where the identified genus fall and further explore environmental diversity. The constructed tree shows the assembled fungi sequence falls with the *Mucor* genus, with bootstrap values of over 90 supporting this conclusion. However, it is not possible to make a species-level identification, this could be due to the amplicon size falling on the low end of the 400-800 bp size estimate, and so there was not enough sequence to confirm the identity.

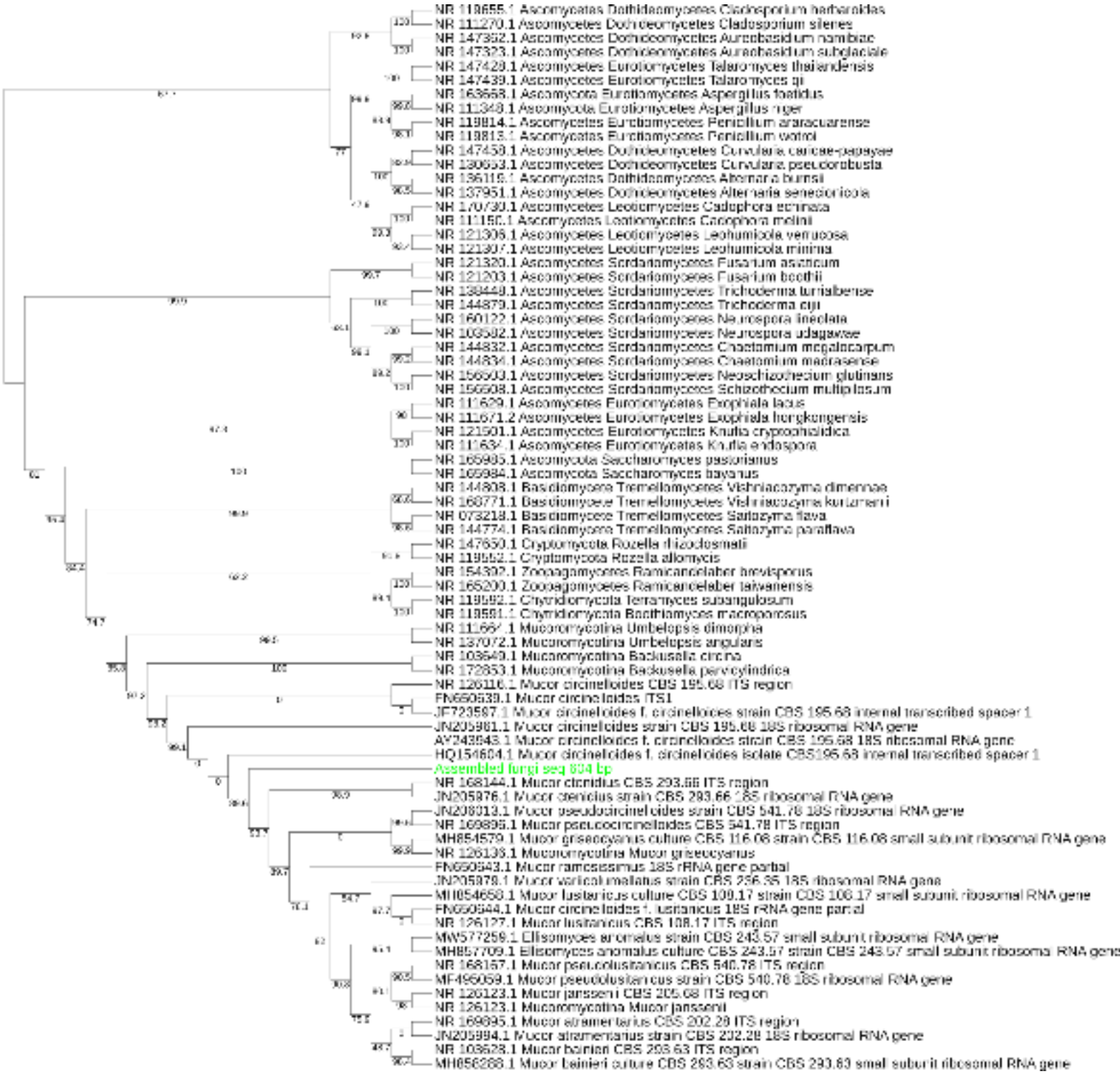

Figure 4: Fungi phylogenetic tree

Mucor sp.

Mucor species grow as hyphae, producing an abundance of spores. There are several known dimorphic species of *Mucor*, particularly the closely related *M. circinelloides,* meaning they are able to grow either as filamentous or yeast-like form under particular conditions. In

an oxygenated environment (like a sealed agar plate), the species grows as a mold, whereas in a low oxygen environment (like that of the sealed Eppendorf tube), they exhibit yeast-like forms. This helps to shed light on the unusual morphological traits observed during the study. *Mucor* species have been investigated for use in research into fungal genetics due to their natural competency (Iturriaga et al, 1992), and as potential candidates for biotechnological purposes (Morin-Sardin et al, 2017).

References

Katoh, K., Misawa, K., Kuma, K.I. and Miyata, T., 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic acids research*, *30*(14), pp.3059-3066.

Iturriaga, E.A., Díaz-Mínguez, J.M., Benito, E.P., Alvarez, M.I. and Eslava, A.P., 1992. Heterologous transformation of Mucor circinelloides with the Phycomyces blakesleeanus leul gene. *Current genetics*, *21*, pp.215-223.

Morin-Sardin, S., Nodet, P., Coton, E. and Jany, J.L., 2017. Mucor: A Janus-faced fungal genus with human health impact and industrial applications. *Fungal Biology Reviews*, *31*(1), pp.12-32.

SnapGene (2024) Available at: https://www.snapgene.com/ (Accessed: 20/12/2024)

NCBI (2024) '*BLAST'* Available at: https://blast.ncbi.nlm.nih.gov/Blast.cgi (Accesssed: 20/12/2024)

Paul, S. and Joshi, S.R., 2022. Industrial perspectives of fungi. In *Industrial Microbiology and Biotechnology* (pp. 81-105). Singapore: Springer Singapore.

IQ TREE (2024) Available at: http://iqtree.cibiv.univie.ac.at/ (Accessed: 20/12/2024)

iTOL (2024) Available at: https://itol.embl.de/ (Accessed: 20/12/2024)