

Lab 1 Report – *Mucor* sp.

Introduction :

Fungi is one of the most successful evolutionary group of eukaryotes existing. Some of them are well known from the public due to the large mushroom they form, the use of yeast in alcoholic beverage, in fermentation, in cheese etc. Some are pathogenic for humans but others are necessary for our crops to grow. They are spread in every environment, and are much more abundant than metazoans. However, their diversity is largely unknown as most of them are under filamentous form and they are also largely under studied. The goal of this practical was to isolate fungi from environmental samples and establish a pure colony using cell biology techniques and bioinformatic analyses to identify them.

Sampling :

The water and soil samples were collected at Ekebydammen the 11th of November at position 59°50'42"N 17°35'48"E. The soil sample was collected at the bottom of a lime tree, in a clayey and hard soil, below dead leaves (Figure 1).



Figure 1: Collecting points of earth sample. Circled in red, the precise point of collect

Methods :

Working in sterile environment, a small amount of soil was placed on two agar plates, one with YEPD, the other with PDA both containing chloramphenicol, an antibiotic. After 3 days of incubation at 30°C, some grey and whitish, mold like structures were growing on both plates. From one sample on the PDA medium, a first attempt of selection was performed on a new agar plate. Three more days of incubation revealed that the first attempt to isolate a single colony failed, probably because too much extract was spreaded on the agar plate. A second attempt led to more convincing results (Figure 2). Some extremities of moldy

structures were pulled out and observed under the microscope with a cotton blue staining. Pictures were taken (Figure 4 and 5).



Figure 2: Fungal plate used for DNA extraction

Once a pure culture was obtained, a DNA extraction was performed in Chelex-100. For the following PCR reaction, ITS1 was the forward primer and ITS4 the reverse one. ITS stands for Internal Transcribed Spacer, a region between the small sub-unit ribosomal RNA and the large subunit ribosomal RNA genes that is commonly used for fungi identification. PCR products were separated on an agarose gel according to the size of the fragments. Once controlled with electrophoresis on gel, the PCR products were sent to sequencing.

Identification :

To begin the identification, the nucleotide sequences must be cleaned to generate a consensus sequence. The clean sequence is 577 bp. Then, using the BLAST tool from NCBI, we can load similar sequences to the one we collected. The consensus sequence showed a 98.68 to 99.12% similarity with the species *Mucor circinelloides*. The reference sequences, the consensus sequence and the similar ones found from BLAST were aligned in MAFFT. Once all the sequences aligned, a phylogenetic tree was generated using the software IQTREE, and iTOL to visualize it (Figure 3). This tree was rooted with Cryptomycota as the outgroup. High bootstrap values (98.4) at the node giving rise to both Muromycotina and the genus *Mucor* tell us with strong evidences that our sequence belong to a member of this genus. However, closer to the leaves of the tree, we can see low value (0), meaning the monophyly of *Mucor circinelloides* is not supported and so that we can't place our sequence as belonging to this species.

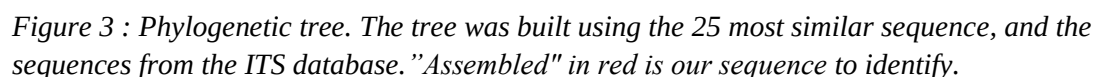


Figure 3 : Phylogenetic tree. The tree was built using the 25 most similar sequence, and the sequences from the ITS database. "Assembled" in red is our sequence to identify.

The genus : *Mucor* sp.

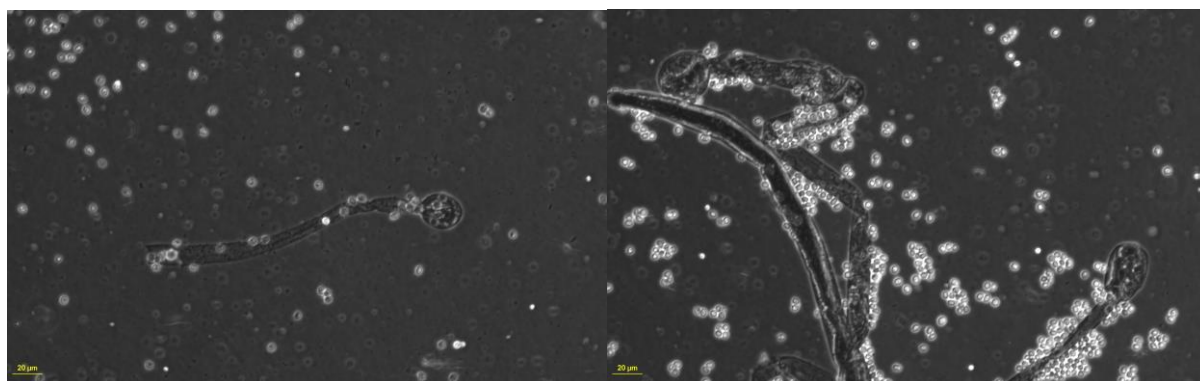


Figure 4 and 5 : *Mucor* sp. observed under the microscope. The dark structures are sporangiophores producing zygosporangia in white

In fungi, the phylum Zygomycota is itself divided into trichomycetes and zygomycetes. Zygomycetes contain the genus *Mucor* with about 3,000 species (Karimi and Zamani). These species are found in the soils, digestive systems, plants. Some species can be at the origin of rare fungal infections called zygomycosis. They have sexual and asexual reproduction where specialized structures called sporangiophores produce sporangiospores (Figure 4 and 5).

With the pictures taken with the microscope, the size of the sporangiophores was estimated to be roughly 200 µm and the spores less than 10 µm. At macroscopic scale, the fungi look like grey mold of 1 cm maximum approximately.

Studies performed on *Mucor indicus* by Karimi and Zamani showed that the species can also reproduce like yeasts do, by budding. This species is under studied for its production of a variety of metabolites (Karimi and Zamani) such as ethanol, fermented food including beer and soy beans in Asia, chitosan, a polymer from the fungal cell wall with several applications in medicine and industry, and a source of food for farming fish.

References :

Karimi, Keikhosro, and Akram Zamani. "Mucor Indicus: Biology and Industrial Application Perspectives: A Review." *Biotechnology Advances*, vol. 31, no. 4, Jan. 2013, pp. 466–81. <https://doi.org/10.1016/j.biotechadv.2013.01.009>.

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