Fungal Isolation – *Barnettozyma sp.*

Diversity and Evolution of Microbial Eukaryotes
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Introduction

The aim of this report is to identify a fungal species isolated from a chosen environment. Through making cultures, extracting DNA, sequencing and the construction of a phylogenetic tree, the isolated fungi is identified on genus level. It is thought to be an organism within the *Barnettozyma* genus. A genus containing yeast from the phylum Ascomycota.

Sample collection

The sample was collected on the eleventh of November 2024. It was collected next to a tree behind Sernanders väg 1, 752 61 Uppsala (59.849734°N, 17.592366°E). Figure 1 shows the exact location, where the sample has been collected. The sample consists of a mixture of soil, rotting wet leaves and twigs.

Figure 1: Location of sample collection; patch of soil with leaves and twigs behind Sernanders väg 1, 752 61 Uppsala

Methods

Isolation:

fungi from the sample were isolated through the use of both agar plates and liquid media. In this case, the isolated fungi was found in the liquid culture. The liquid media consists of YEPD (yeast extract peptone dextrose) and chloramphenicol. For the isolation, 1ml of YEPD + chloramphenicol was added to a 1.5 ml Eppendorf tube in addition to a small soil particle from the sample, using a sterile scoop. This Eppendorf tube was then incubated for 3 days at 30°C. After incubation, 25 µl of the bottom of the tube was pipetted on to an agar plate consisting of YEPD + chloramphenicol. Afterwards, a sterile loop was used to spread the sample out and obtain single colonies. The plate was incubated for 3 days at 30°C.

Both filamentous fungi and yeast were present after incubation. Using a sterile loop, the yeast was restreaked for single colonies on another plate consisting of YEPD + chloramphenicol and incubated for 3 days at 30°C. Only the yeast was present on this plate, as can be seen in figure 2. The yeast had a milk-like colour and formed light-blocking streaks. The yeast was restreaked again on to another YEPD + chloramphenicol plate and incubated for 3 days at 30°C. This resulted in clean, single colonies.

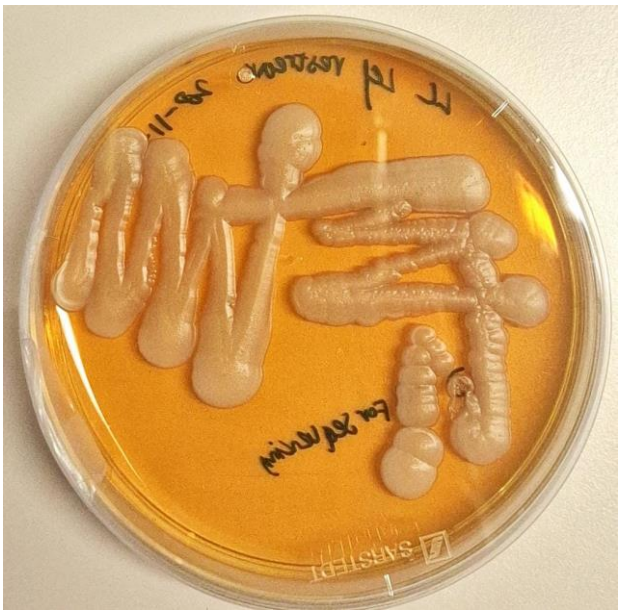

Figure 2: Isolated yeast on YEPD-plate. The colonies have a milky colour and are so dense that light cannot penetrate.

Observation:

After isolation, slides were prepared to observe the morphology of the isolated yeast (figure ?). Individual cells were spherical or elongated and had a diameter of approximately 4µm, but the sizes differ greatly. Some cells appear to be budding, while others are solitary.

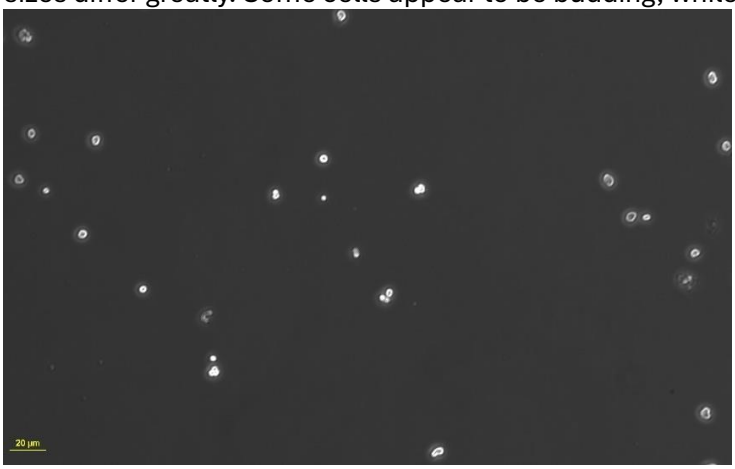

Figure 3: Isolated yeast under the microscope. Solitary and budding yeast can be observed. Individual cells have a diameter of approximately 4 µm.

DNA extraction, amplification and quality assessment:

DNA was extracted with the help of Chelex-100. After adding the yeast to 200 µl Chelex, the sample was placed in a heating block at 95◦C for 15 minutes. The samples were then spun down and the supernatant was used in the following PCR reaction. The primers used for the PCR reaction were ITS1 and ITS4, which amplify the internal transcribed spacer region between the small-subunit rRNA and the large-subunit rRNA. To assess the quality of the PCR products, agarose gel electrophoresis was done. This resulted in a single clear band. The PCR product was cleaned using ExoSap and send for sequencing.

Fungi2g identification

After receiving the sequences, The program SnapGene (Llc, n.d.) was used to assess the quality of the forward and reverse sequences. Both these sequences were combined to make the

consensus sequence. The consensus sequence was trimmed down to optimize the quality of the sequencing results. A BLAST (Sayers et al., 2021) search was done using this sequence. The top 25 results of this search were primarily *Barnettozyma* species, but some *Candida* species were given as well. The consensus sequence, the top 25 BLAST results were combined with a provide ITS database, containing sequencing results of the ITS region of different species throughout the fungal kingdom. All these sequences were aligned using the software MAFFT (Rozewicki et al., 2019). A phylogenetic tree of these alignments was constructed with IQ-TREE (Nguyen et al., 2015). To visualize the tree iTOL (Letunic et al,. 2006) was used, resulting in figure 4.

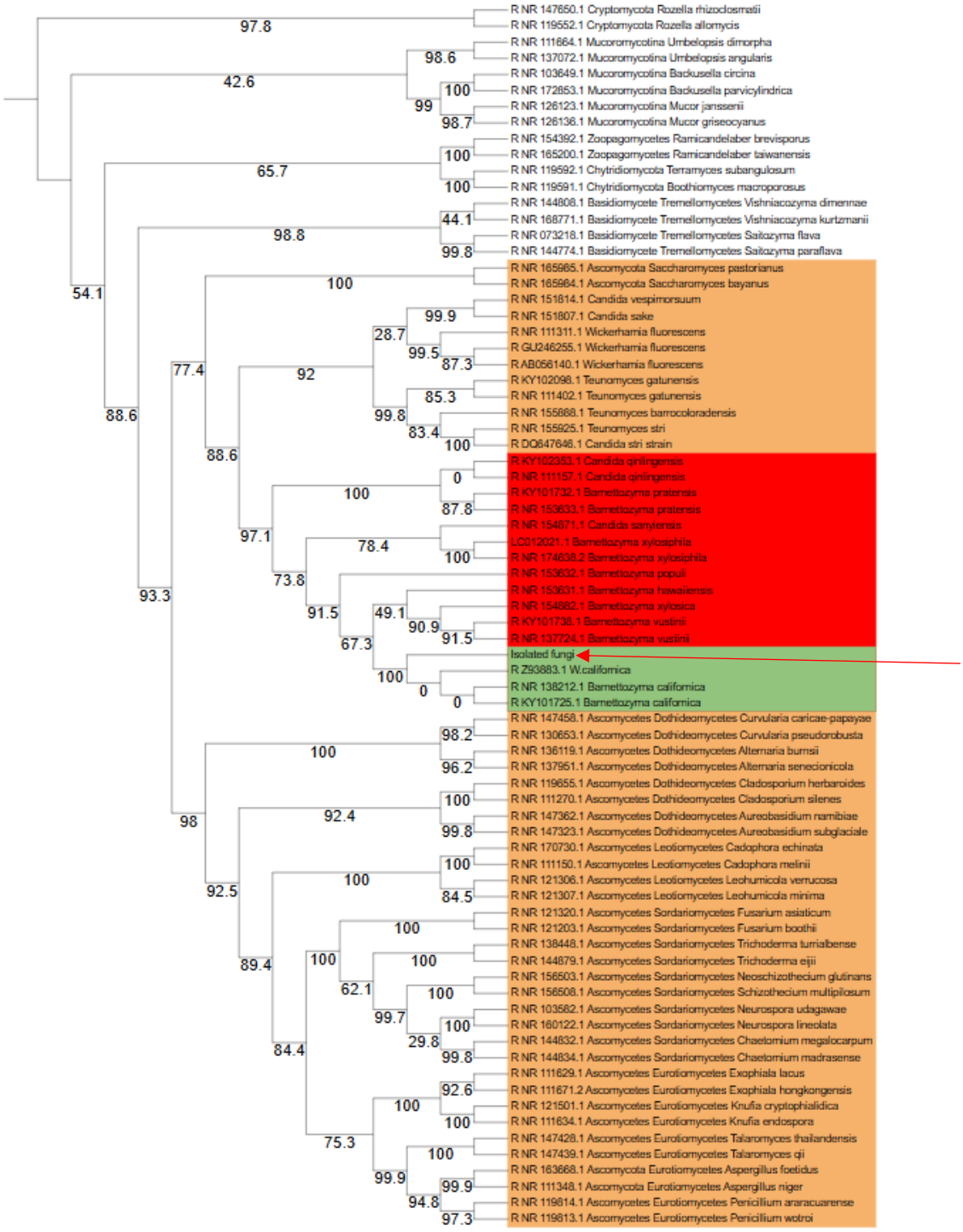

Figure 4: Fungal phylogenetic tree. The isolated fungi is marked by a red arrow. It is part of the phylum Ascomycota. The bootstrap values for it to be part of the Barnettozyma genus are high.

The isolated yeast is thought to be part of the ascomycetes (names in orange). The bootstrap value supporting this is high. Looking further in the tree it is fairly certain that the yeast is part of the *Barnettozyma* genus (names in red and green + several *Candida* species). This is also supported by the high bootstrap values and the results from the BLAST searches. However, the bootstrap values do not support species identification. Therefore no conclusion can be made concerning the precise species.

Barnettozyma sp.

As can be seen the isolated yeast is though to be a part of the *Barnettozyma* species. The cells take on a different range of shapes from ovoid to spherical or elongate. The formation of hyphae has not been observed, but there are observations of pseudohyphae. The species can reproduce either asexually or sexually. In asexual reproduction, budding is multilateral. In sexual reproduction asci can contain one to four ascospores with differing morphologies. The genus appears to be ubiquitous, such is the case with *Barnettozyma californica* (figure 5) , which has been isolated from soil, water, trees and feces (Cletus et al., 2011). In terms of biochemistry, it is observed that a lot of standard carbon sources are not utilized for growth, with the exception of glucose and sucrose. Some can however, utilize nitrate for growth (Fang et al., 2021).

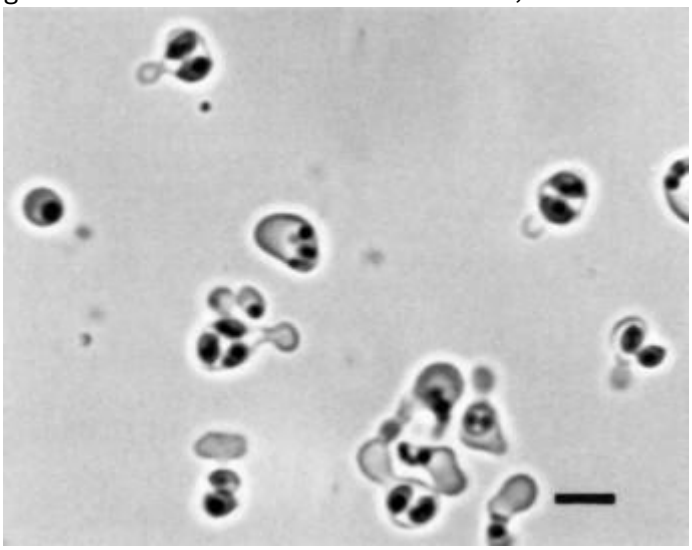

Figure 5: Barnettozyma californica. Asci with asci spores can be observed.

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