Identification of fungi from environmental sample

A Short Study

Ellen Claassen

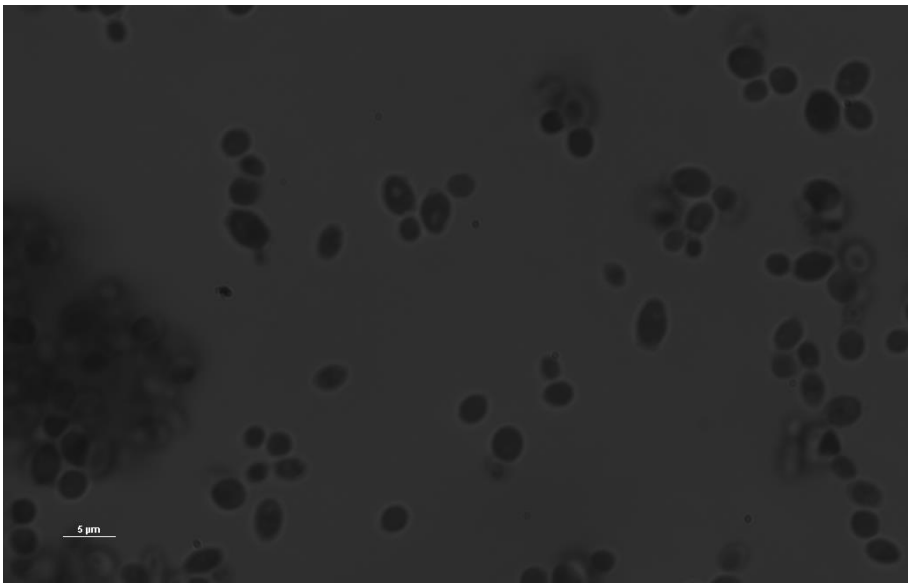
Introduction

Fungi are extremely diverse organisms that can be found living in most spaces ranging from natural environments like forests to the bathroom tiles in our home and even on our skin as well. They can take many different shapes during their life cycle and be both unicellular and multicellular. Mold and fruiting bodies of fungi are often easier to identify, and it's usually only when unicellular fungi form larger colonies that they can be identified by a trained eye.

Aim

To see what kind of microbial fungi there are in our surrounding an experimental study was designed to isolate a specie for identification. A sample was taken from a rotten apple hanging from a tree, location ///crisp.trousers.taken (What3words). In lab the sample was prepared for growth on a medium containing potato dextrose agar (PDA). The resulting growth contained different kinds of yeast that were restreaked several times until pure cultures had formed.

Morphological observations of growth on PDA plate; Smell resembling sour apple, colour white/milkish, forms round glossy bulging spheres on the medium. Using a dissecting microscope it was possible to see semi spherical unicellular yeast bodies (Figure 1). The size ranging from a little bellow 5 µm to cells half that size or even smaller. Any identification of specie based of morphology, besides being yeast, was difficult to deduce.



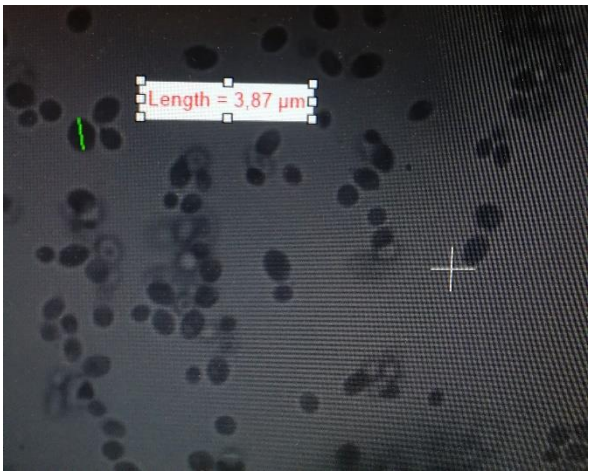


Figure1: Pictures of the yeast sample taken under microscope.

Method for genetic identification

After successful PCR the sample was sent for sequencing with 18S markers (this was an error in the lab procedure, the correct marker to use for fungi would have been the ITS region). The forward and reverse sequences were observed in SnapGene software (www.snapgene.com), from which a consensus sequence was derived. The program automatically cut away bits at the start and the end of the sequence that had low resolution for nucleotide base certainty. From The BLAST Sequence Analysis Tool (Tom Madden, 2003) with settings: exclude environmental sequences and limit to sequence from type material, the top 42 results with regions of similarity to the consensus sequence were downloaded. Also, the BLAST search was edited to search for the top 5 hits with *Saturnispora* (taxid:29834), these were downloaded as well and used as an outgroup in the phylogenetic tree later. The alignments between all the sequences were checked using MAFFT multiple sequence alignment program (Katoh,K *et.al* 2002) with the setting: Adjust direction according to the first sequence (accurate enough for most cases). The result showing that there is an alignment although rather short due to the length of the consensus sequence. Lastly the aligned FASTA file was uploaded on Interactive Tree Of Life (iTOL) (https://itol.embl.de), with settings: Branch lengths ignore and bootstrap1 text. The tree was re rooted with the clade *Saturnispora zaruensis* and *Saturnispora hagleri* as an outgroup.

Results

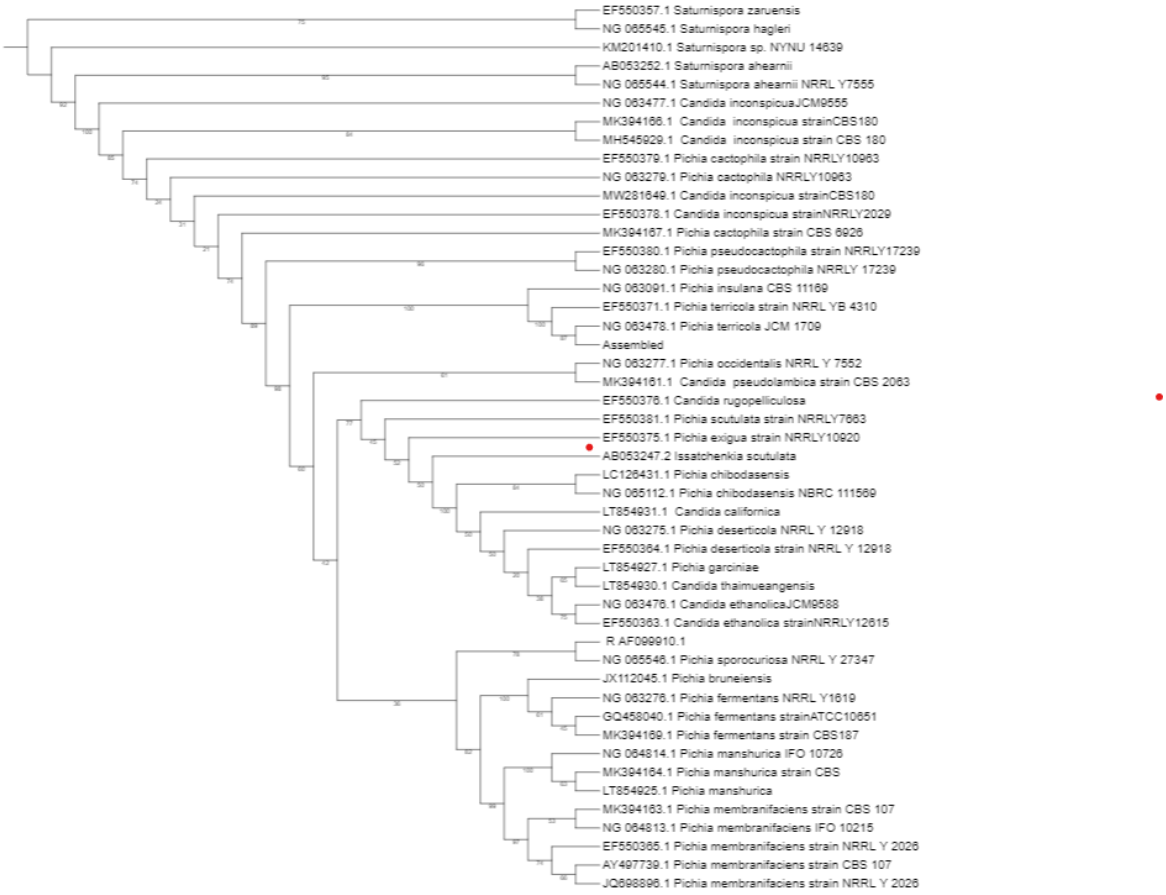


Figure 2: Phylogenetic tree of the top 42 blast results with *S. zaruensis* and *S. hagleri* at as the outgroup.

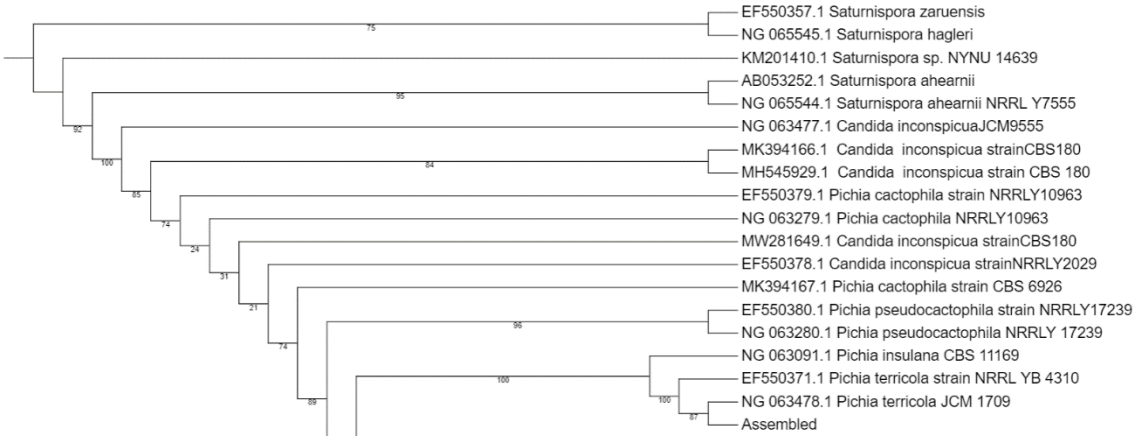


Figure 3: Zoomed in picture of the phylogenetic tree.

The bootstrap1 values at the nodes leading up to the consensus sequence named “Assembled”, are not all well supported, a lot of them < 90 % or even < 50% (Figure 3). This indicates that there are several ways that the organisms can be phylogenetically placed in the tree. A possible reason for this is that the 18S region may be inadequate to see genetic diversity between the genera. However, the positioning of the “Assembled” suggests that it could be *Pichia terricola*, the closest node branches have higher certainty percentages (100% and 87%). This coincides with the results from BLAST that showed a 98.81% Per. Identity between the consensus sequence and *Pichia terricola* strain NRRL YB-4310 . The alignment view shows four locations, two in the middle and two at the end of the consensus

sequence that differs, if the consensus strand would have been longer there would probably be more sites that differ. These changes in nucleotide bases can be explained by the presence of some other bands, that were seen in the chromatograph. Worth mentioning also is that there is a difference in results between bootstrap1 and bootrstrap0, the latter having a clade certainty of 0% at the closest node to “Assembled”.

Pichia terricola also called “budding yeast” at NCBI BLAST website, has been reported sampled from soil, grape berries and fruits (ALL-RUSSIAN COLLECTION OF MICROORGANISMS, 2023). This supports the possibility of finding *Pichia terricola* in rotten apples as well, which is where the yeast in this study was sampled.

Conclusion

Even though the sequencing results were a bit problematic, resulting in a short consensus sequence and that there were underlying bands in the chromatograph, there is a potential match and supporting motivations that the sampled yeast could be *Pichia terricola.* For a start its positioning in the phylogenetic tree, the percent identity on BLAST and that it was sampled where this specie has previously been found reported.

Sources:

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