# Polyphasic Identification of a Yeast Isolate with Dye Decolourisation Ability

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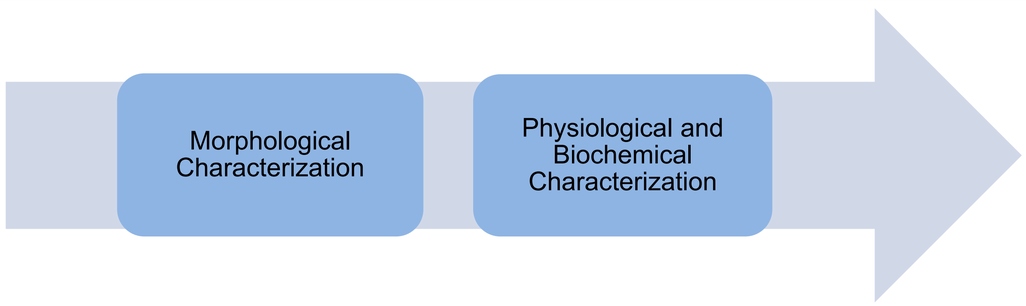
## Introduction

Lately, environmental problems have become increasingly critical and more frequent, mostly due to the increased population growth and industrial activity, namely the textile industry. The textile industry has left a huge footprint in the waters of the planet reflecting on a serious environmental problem of modern society [3].

The present research work focused on the LIVST11 yeast isolate polyphasic identification, which was isolated from wastewater and possesses the ability to decolourise several dyes. LIVST11 isolate was morphologically, physiologically and biochemically characterised thru classical approaches and chromogenic media. In addition, genotypic identification was performed thru sequencing of relevant genes, as well as whole genome sequencing and phylogenetic analysis.

## Methods

### Phenotypic Identification




### Genotypic Identification

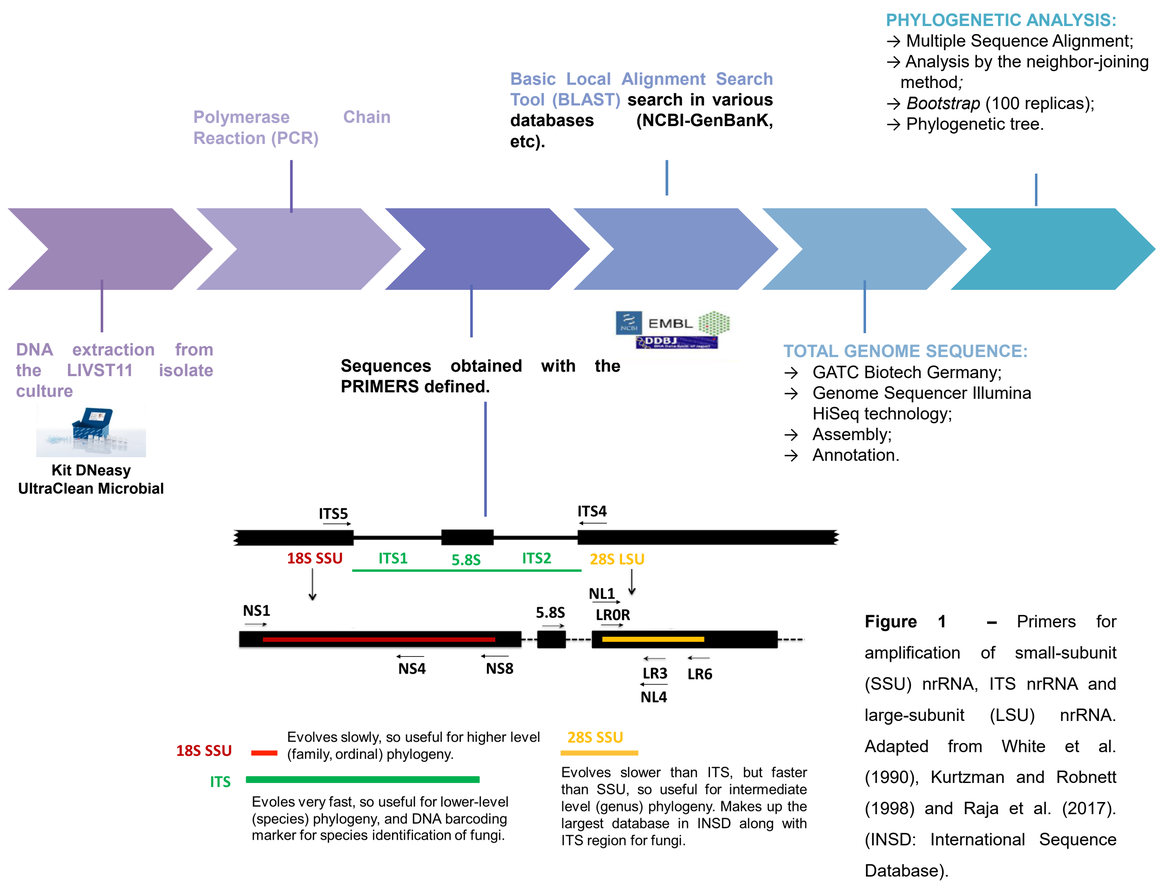



**Figure 1 –** Primers for amplification of small-subunit (SSU) nrRNA, ITS nrRNA and large-subunit (LSU) nrRNA. Adapted from White et al. (1990), Kurtzman and Robnett (1998) and Raja et al. (2017). (INSD: International Sequence Database).

## Conclusions

- The results obtained from the phenotypic and genotypic identification of LIVST11 isolate support its inclusion on the genera *Candida*, with the exception of 18S amplicon, with all results confirming the classification in the *Ascomycota* phylum.
- The analysis of the yeast LIVST11 genome confirmed the previous results and added data that might support a new species definition. The LIVST11 isolate presented the highest homology (99.79%) with *C. cabralensis* with amplicon ITS using genome data. The evolutionary relationship was inferred using Neighbor-joining method and might indicate a closely related species to *C. cabralensis*, or a perhaps a different strain of that same species. However, at the phenotypic level, conflicted evidence was obtained that reinforces that LIVST11 isolate may not belong to *C. cabralensis*, such as the ability of LIVST11 to ferment several carbohydrates that is not described for *C. cabralensis*.
- LIVST11 isolate seems to be a new yeast species of *Candida* genera.

## References

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## Results

### Phenotypic Identification

**Table 1 –** Phenotypic characterization of the LIVST11 isolate.

| | ISOLADO DE LEVEDURA LIVST11 |
|---|---|
| **Growth temperature range** | 25-30 ºC |
| **YM characteristics** | Colonies are flat, butyrous, white and smooth |
| **Reproduction** | Asexual by formation of pseudohyphae |
| **Nitrogen Source** | Nitrate, nitrite and lysine. |
| **Fermentation of carbohydrates** | **Positive:** D-glucose, sucrose, dextrose, maltose, D-galactose, xilose, trealose<br>**Negative: I**nulin and lactose |
| **Assimilation of carbon compounds** | **Positive:** D-glucose, glucosamine, esculina and ferric citrate (variable)<br>**Negative:** D-Galactose, D-Saccharose, N-Acetyl-Glucosamine, Lactic Acid, L-Arabinose, D-Cellobiose, D-Rafinose, D-Maltose, D-Trehalose, Potassium 2-Ketogluconate, Methyl-αD- Glucopyranoside, D-Mannitol, D-Lactose, Inositol, D-Sorbitol |
| **Germinative Tube** | Positive after 2h |
| **Urea hydrolysis** | Not hydrolysed |
| **Cycloheximide** | Negative |

### Genotypic Identification

**Table 2 –** Results obtained by BLAST search through NCBI-GenBanK. LIVST11 isolate identification using NS1-NS4, ITS5-ITS4, NL1-NL4, LR0R-LR6 and ITS5-LR3 sequence regions obtained from the genome sequencing.

| Region | Primers | Description | Query cover (%) | E value | Homology (%) |
|---|---|---|---|---|---|
| 18S | NS1-NS4 | *P. insulana* | 96 | 0.0 | 100 |
| | | *P. terricola* | 99 | 0.0 | 97.64 |
| ITS | ITS5-ITS4 | *Candida* sp. | 80 | 0.0 | 100 |
| | | *Candida cabralensis* | 92 | 0.0 | 99.79 |
| D1/D2 28S | NL1-NL4 | *Candida* sp. | 96 | 0.0 | 100 |
| | | *C. cabralensis* | 95 | 0.0 | 99.82 |
| 28S | LR0R-LR6 | *C. rugopelliculosa* | 96 | 0.0 | 93.06 |
| | | *P. terricola* | 96 | 0.0 | 93.05 |

Through the data analysed by the Nucleotide BLAST tool on the NCBI web site (Table 2), the strain LIVST11 presented different homology matches for all sets of primers. The highest homology (99.79%) was with *C. cabralensis* with amplicon ITS (DNA Barcode).

Genetically, the alignment between LIVST11 isolate ITS region and the sequence of *C. cabralensis* obtained from GenBank showed that they differ only in one nucleotide. Moreover, the alignment between LIVST11 and the sequence of *P. terricola* and *P. fermentans* obtained from GenBank differ in various nucleotides. The physiology of LIVST11 is very different *from C. cabralensis* and from *P. terricola* and *P. fermentans*. The main difference between LIVST11 strain and *C. cabralensis*, *P. terricola* and *P. fermentans* is in the carbohydrate's fermentation. Also, carbon assimilation, nitrogen compounds assimilation and the reproduction are different between these species and LIVST11 isolate.

In the ITS region phylogenetic tree (figure 2), it is possible to verify that the branches that support the LIVST11 clade have strong bootstrap values (bootstrap value > 95%). These results show that LIVST11 is close to *C. cabralensis*, being the bootstrap values sufficiently strong to support the different branches (bootstrap value > 95%).

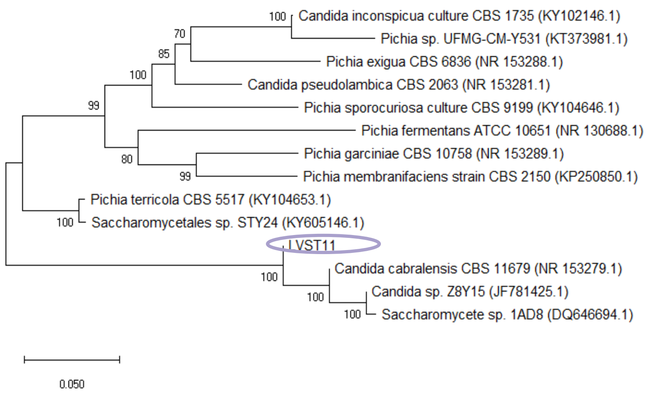


**Figure 2 -** Phylogenetic tree calculated from neighbour-joining analysis of the ITS region with ITS5-ITS4 primers. The tree is presented with bootstrap support.

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