Polyphasic Identification of a Yeast Isolate with Dye Decolourisation Ability

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Abstract

The textile industry generates large volumes of effluents with toxic, coloured, surfactant and chlorinated compounds and salts, thus transforming wastewater into one of the main sources of water pollution[1,2].The wastewaters are characterized by fluctuations in parameters such as the BOD, COD, pH, colour and salinity[1]. Dyes, visible in concentrations lower than 1 ppm, can cause toxic and mutagenic effects to the aquatic habitat[3], thus it is relevant the optimization of the wastewater treatment processes to reduce the environmental impact.

LIVST11 is a yeast isolate from wastewater with the ability to decolourise several dyes. Physiological, biochemical and molecular biology assays were performed currently placing it in the genus Candida. Molecular biology assays are still underway to identify the strain to the species level. The intracellular and extracellular extracts both presented dye decolourisation abilities, however the extracellular extract showed a better decolourisation. The enzymatic characterization tests of the fractions obtained by FPLC is currently underway.

Methods

Morphological characterization

The yeast LIVST11 was grown for 72 hours on Yeast Medium Agar (YM), and Malt Extract Agar at 25ºC and the macroscopic and microscopically characteristics were properly described. The detection of pseudohyphae and true hyphae was performed microscopically with yeast grown on Rice Agar. The strain was grown for 48 h on CHROMagar *Candida*, Biggy Agar and Tetrazolium reduction medium for isolation and differentiation.

Physiological and biochemical characterization

The strain was inoculated in API ID 32 C strip including miniaturized assimilation tests. Furthermore, urea hidrolysis and nitrogen and lysine assimilation was tested. Further fermentation tests are underway.

DNA extraction, ITS amplification and sequencing

The total DNA was extracted from 48 h cultures grown on YM with UltraClean® Microbial DNA Isolation Kit. The internal transcribed spacer ITS was amplified with universal primers ITS-4 (5'-TCCTCCGCTTATTGATATGC-3') and ITS-5 (5'-GGAAGTAAAAGTCGTAACAAGG-3'), and the 26S rDNA was amplified with universal primers NL-1 (5'-GCATATCAATAAGCGGAGGAAAAG-3') and NL-4 (5'-GGTCCGTGTTTCAAGACGG-3'). Sequencing was performed at Macrogen Inc, Korea.

Decolourisation ability in liquid media

Dye decolourisation tests were performed in microplates with 24 wells using 500 µL of NDM supplemented with 100 mg/L of Blue Supra Everdirect dye and inoculated with 3% v/v of the yeast. Microplates were incubated at 25ºC with 100 rpm during 72 h. Sampling was performed every 24h and 100 µL aliquots was centrifuged at 14 000rpm. The decolourisation of the supernatant was quantified by spectrophotometric measurements.

Enzymatic characterization of intracellular and extracellular extract

The presence and activity of intracellular oxidoreductase, azoreductase and tyrosinase, and extracellular enzymes laccase, manganese dependent peroxidase (MnP), manganese independente peroxidase (MiP), lignin peroxidase, veratryl alcohol oxidase (VAO), fractions obtained by FPLC.

Conclusions

The strain LIVST11 presumptively belongs to *Candida* genus, however the physiological, biochemical and molecular biology identification tests don't allow classification to the specie level. Further biochemical and molecular biology methods are being performed to obtain species level classification.

The yeast LIVST11 is able to quickly and effectively decolourise the Blue Supra Everdirect after 24 hours of incubation. Further decolourisation ability tests with other dyes are underway.

Results

Morphological characterization

Characteristics	Colony Morphology on Yeast Medium Agar
Texture	Fluid
Colour	White
Surface	Butyrous
Elevation	Flat
Margin	Entire

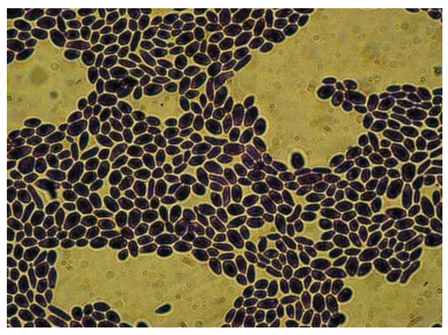

Figure 1 – Cell morphology of yeast strain grown in malt extract agar.

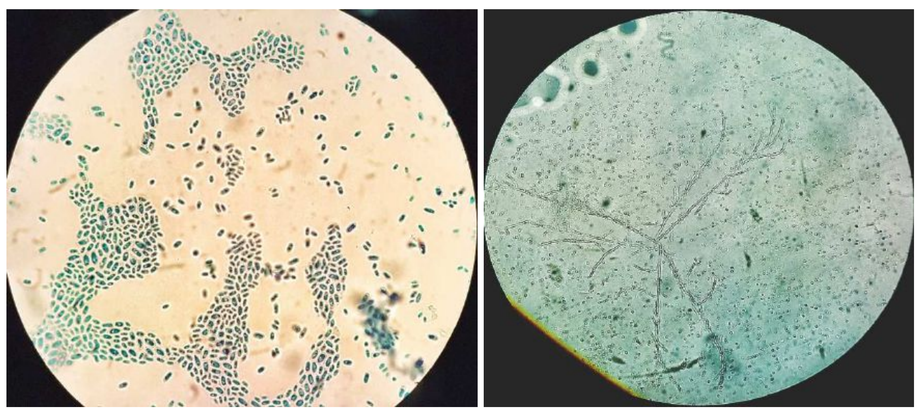

Figure 2 - Microscopic observation of cells with 7 and 10 days of growth on rice agar, respectively.

The strain LISVT11 was examined microscopically (Figure 1). The shape of the cells is oval and they are aggregated in clusters. This strain has asexual reproduction and multilateral budding and formation of pseudohyphae (Figure 2). The yeast grown on CHROMagar *Candida* has white colonies, on Biggy Agar has brown colonies and on Tetrazolium reduction medium has pale pink colonies.

Physiological and biochemical characterization

According with API ID 32 C strip the identification was *Candida norvegensis* (98%). The strain LIVST11 assimilates nitrate and lysine and doesn't hydrolysis urea. The yeast *Candida norvegensis,* according to Bishop *et al.* (2008) also has white colonies on CHROMagar *Candida*[4].

DNA extraction, ITS amplification and sequencing

The highest homology for ITS and 26S rDNA obtainded was *Saccharomycete* sp. and *Candida cabralensis,* respectively. This strain has branch support for the same genera (*Candida*) for both amplicons with species of the same genera for both markers. These results are corroborated by the work carried out by Jorges (2016).

Decolourisation ability in liquid media

This strain presents decolourisation of Blue Supra Everdirect dye (Figure 3). The decolourisation was tested after 24 h. The decolourisation ability is currently under study

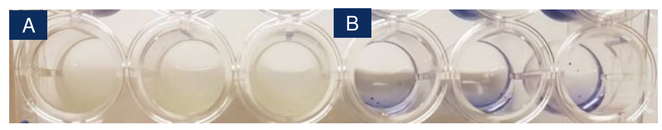

Figure 3 – Decolourisation ability in liquid medium with Blue Supra Everdirect after 24 hours. **A** – Dye decolourisation with yeast **A**; **B** – Control

Enzymatic characterization of Intracellular and extracellular extract

The enzymatic characterization tests of the fractions with intracellular and extracellular extract obtained by FPLC is currently underway. For the intracellular extract, different methods of disruption are being tested in order to improve the enzymatic characterization.

References

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