

Valorization of wine-related microbial bioresources for sustainability and innovation: case studies in the agri-food chain

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Background

The aim of the PhD project is the exploration through accurate identification and characterization for possible valorization of wine-related microbial bioresources, also considering safety aspects. These were isolated during vintages from 2015 and 2020 and stored in VUCC-DBT and/or preserved in the strain collection of the Microbiology Laboratory. They belong to 17 genera, and *Metschnikowia pulcherrima* resulted as the most abundant wine-related species. The information will contribute to obtain ing well-characterised bioresources as well as to redefining specific screening protocols for selected biotechnological applications.

Introduction

Among the wine-related yeast species, *M. pulcherrima* is a promising non-conventional yeast explored for different characteristics and bioprotective potential. The strain VUCC500 (VUCC-DBT) resulted in interest for several traits, and its genome is sequenced and available (Troiano *et al.*, 2023). This species can also produce single-cell oils as intracellular lipids for over 20% of its dry cell weight as energy storage. This accumulation is favoured by growth on a high carbon-nitrogen ratio by promoting *de novo* lipid synthesis (Caporusso *et al.*, 2021). Lipid accumulation has been assessed on different strains of the former species of *pulcherrima* clade (Němcová *et al.*, 2021). This case study aims to investigate lipid accumulation by reliable screening procedures and highlights strains with the potential to formulate a feed supplement.

Material and methods

Test dataset

Metschnikowia pulcherrima VUCC500 (VUCC-DBT)

9 type strains of the former species of *pulcherrima* clade (Sipiczki, 2022)

7 strains tested for lipid accumulation in the work of Němcová *et al.*, 2021.

2 strains newly tested for lipid accumulation in this work

NRRL Y-48695
NRRL Y-27615
NRRL Y-27328
NRRL Y-7111^T
NRRL Y-48710
CBS 10357
NRRL Y48712

NRRL Y-6546
NRRL Y-6064

2 positive strains

Lipomyces starkeyi DBVPG 6193
Yarrowia lipolytica DBVPG 6053

VUCC-DBT: Verona University Culture Collection-Department of Biotechnology; NRRL: Agricultural Research Service Culture Collection; CBS: Centraalbureau voor Schimmelcultures; DBVPG: Industrial Yeast Collection University of Perugia.

Lab scale
Glucose as carbon source

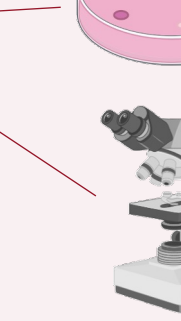
Protocols

Gravimetric quantification



Qualitative screenings

Rhodamine B plate assay



Observation of lipid droplets under the light microscope

Comparison and selection

Identification of a potential candidate for development

Evaluation of a reliable screening protocol and possible lipid accumulator candidates

Comparison with the results of gravimetric analysis

Results

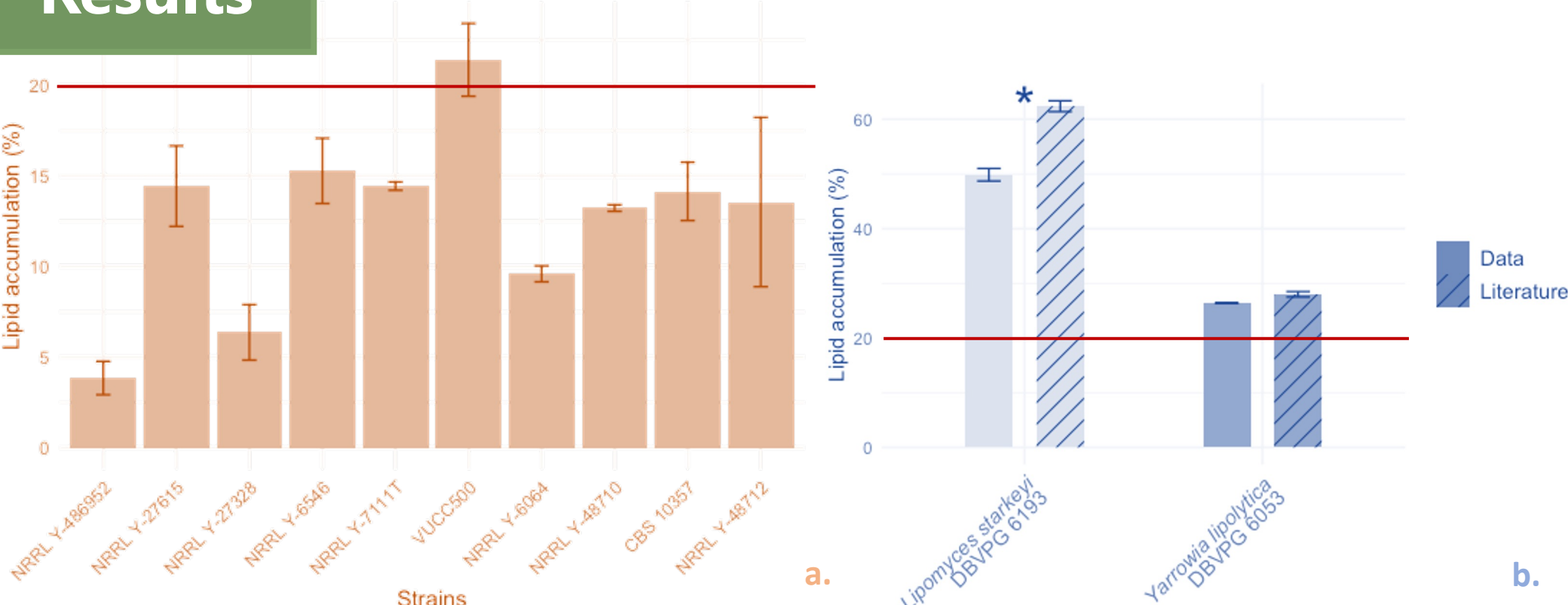


Table 1 Results of the Rhodamine B plate screening protocol by Niehus *et al.* (2018). Accumulation was evaluated based on the presence of lipid droplets in all cells and on colour intensity (-, +, ++).

Species	Strains	Microscope	Visible light	UV light
<i>Metschnikowia pulcherrima</i>	NRRL Y-48695	++	-	-
	NRRL Y-27615	-	-	++
	NRRL Y-27328	++	-	-
	NRRL Y-6546	++	-	+
	NRRL Y-7111 ^T	++	-	+
	VUCC500	+	-	-
	NRRL Y-6064	++	-	+
	NRRL Y-48710	++	-	-
	CBS 10357	++	++	++
	NRRL Y48712	-	-	-

The quantitative method was first assessed and compared with literature data by replicating the same accumulation condition both on positive strains (Filippucci *et al.*, 2016; Lin *et al.*, 2011) and on seven of ten *M. pulcherrima* strains (Němcová *et al.*, 2021). In the latter case, statistical analysis (t-test) did not reveal any significant differences between the data in this study and the literature. Once it was found to be consistent, this method could also be applied to evaluate the most applicable qualitative method to screen a collection for lipid accumulation. Furthermore, quantitative data showed that *M. pulcherrima* VUCC500 could be an interesting SCO strain with a percentage of lipid accumulation of $21,4 \pm 2,0\%$ with glucose as a carbon source (Figure 1).

Qualitative protocols did not robustly reveal the oleaginous behaviour; in fact, by comparing the three qualitative methods, only one strain of the total showed a positive accumulation trait in all three parameters assessed (Table 1).

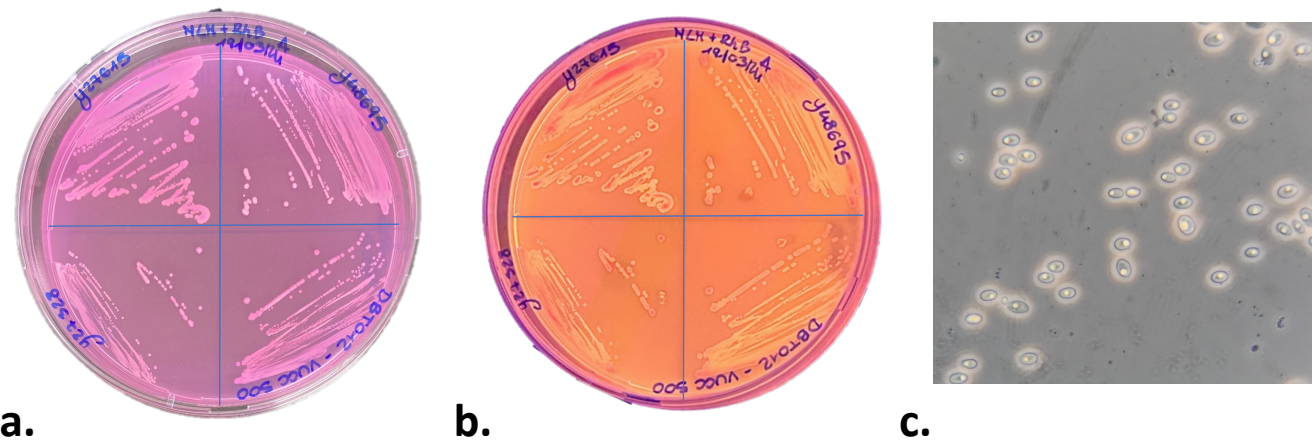


Figure 2 Results of the plate screening with Rhodamine B for *M. pulcherrima* NRRL Y-48695, NRRL Y-27615, NRRL Y-27328 and VUCC500 analysed **a.** under visible light, **b.** under UV light, and **c.** the microorganisms analysed under an optical microscope (VUCC500).

Conclusions

Based on the results obtained, the qualitative method revealed that *M. pulcherrima* VUCC500 produced lipids above 20% of dry cell weight, defined as the threshold for oleaginous microorganisms, by utilizing glucose as a carbon source. In contrast, the qualitative test did not consistently reveal oleaginous behaviour across the dataset.

Next steps

Considering the outcomes so far, it will be necessary to test the accumulation with alternative low-cost carbon sources. On the other hand, to identify an alternative primary screening method to test the entire collection by applying the same setup. Finally, to investigate the potential to formulate a feed supplement from the most promising strain of the collection, further analyses will be focused on safety aspects of this non-QPS species (Larini *et al.*, 2025).

References:

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