

# A preliminary investigation on lipid accumulation by *Metschnikowia pulcherrima*

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## Introduction

**Oleaginous yeasts**, producing intracellular lipids for over 20% of dry cell weight, can be key **alternative sources for food and feed** production [1]. *Metschnikowia pulcherrima* can have an industrial potential as an oleaginous species, being able to grow on different substrates, tolerating low pH and temperature [2,3]. This study aimed to investigate the **reliability of qualitative methods**, potentially suitable for high-throughput screening of a large collection of *M. pulcherrima* strains for lipids accumulation, as well as to evaluate the **lipid accumulation with alternative low-cost carbon sources** that transforms waste into high value-added products.

## Material and methods

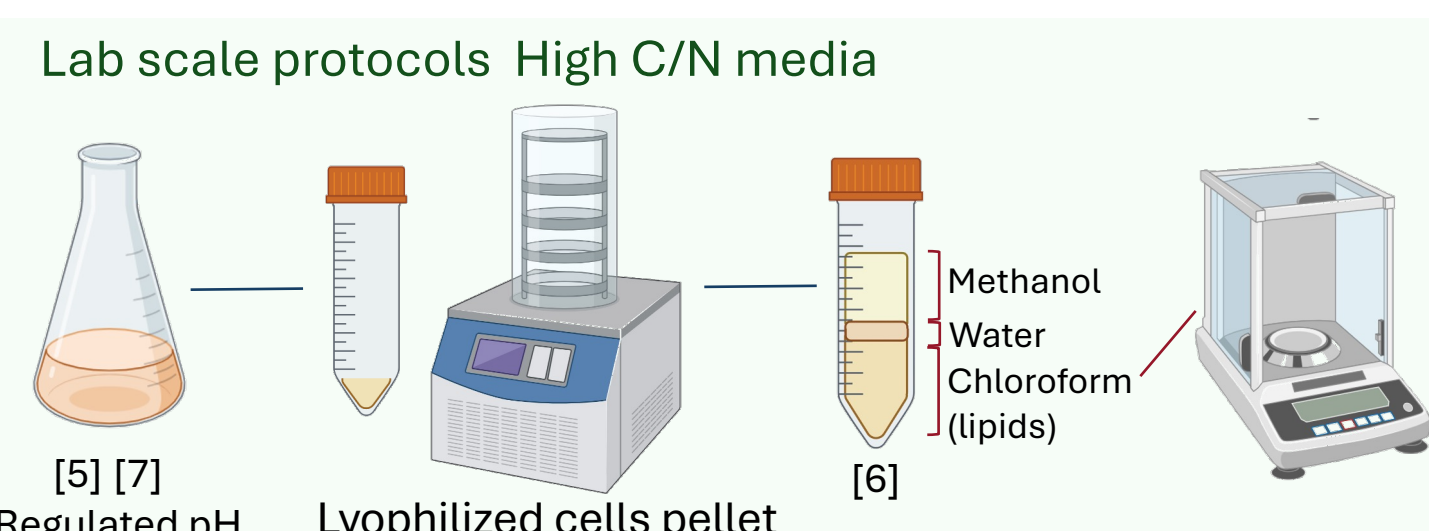
### Test dataset: 10 *Metschnikowia pulcherrima* strains

- *M. pulcherrima* VUCC500
- 9 type strains of the former species of *pulcherrima* clade [4]
  - 7 strains tested for lipid accumulation in literature [5]
  - 2 strains tested for the first time

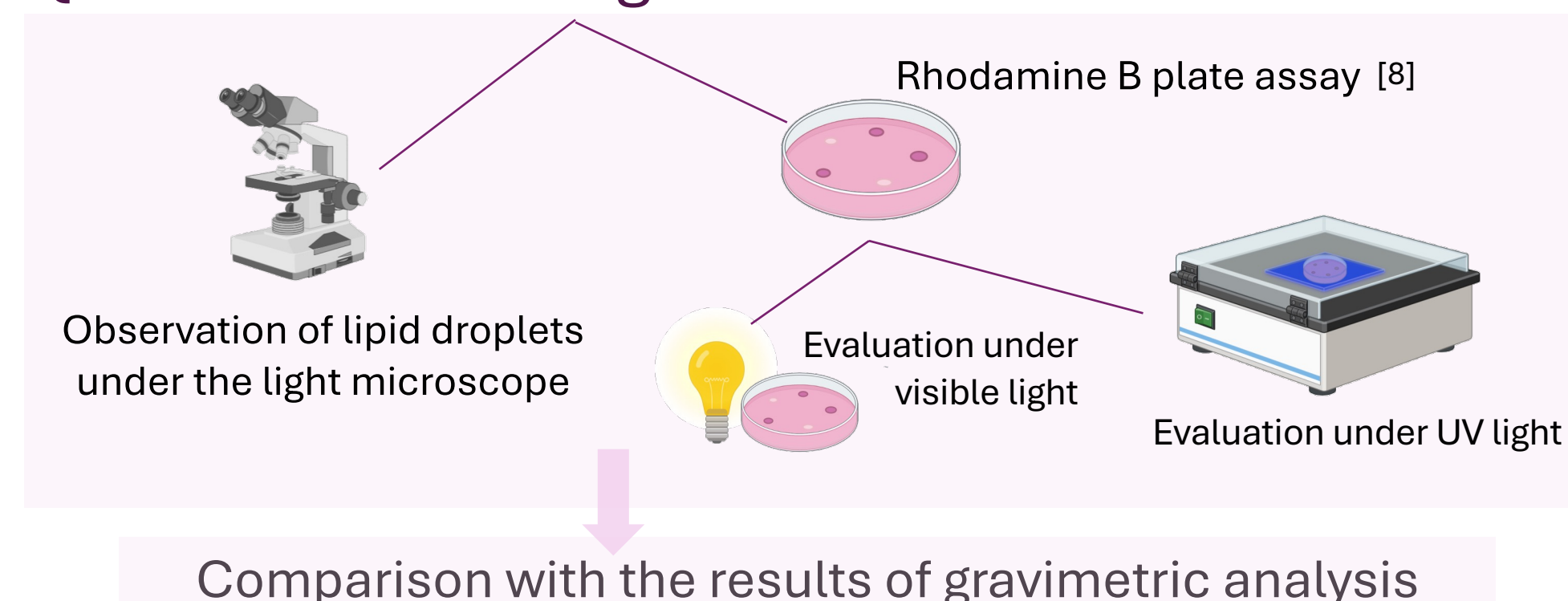
### Carbon sources tested

Glucose  
Selection of 3 strains  
Volatile fatty acids (VFAs)  
Acetic acid  
VFAs from anaerobic digestion of pomace

### Gravimetric quantification



### Qualitative screenings



Evaluation of a reliable screening protocol and possible lipid accumulator candidates

Identification of a potential candidate for development

Two permeates resulting from the anaerobic digestion of pomace, the main by-product of the wine production chain. Mix1 (predominantly acetic acid) and Mix2 (predominantly butyric acid, with a complete profile up to caproic acid).

## Results

*M. pulcherrima* strains were grown at lab scale in a high C/N medium, and lipid droplets were observed under the microscope. The lipid accumulation was tested with a qualitative plate method evaluated on both visible and UV light (Figure 1).

While qualitative protocols did not robustly reveal the oleaginous behavior (Table 1), quantitative data firstly revealed one novel strain, **VUCC500**, as the **highest lipid accumulator with glucose as a carbon source among the test dataset** (Figure 2).

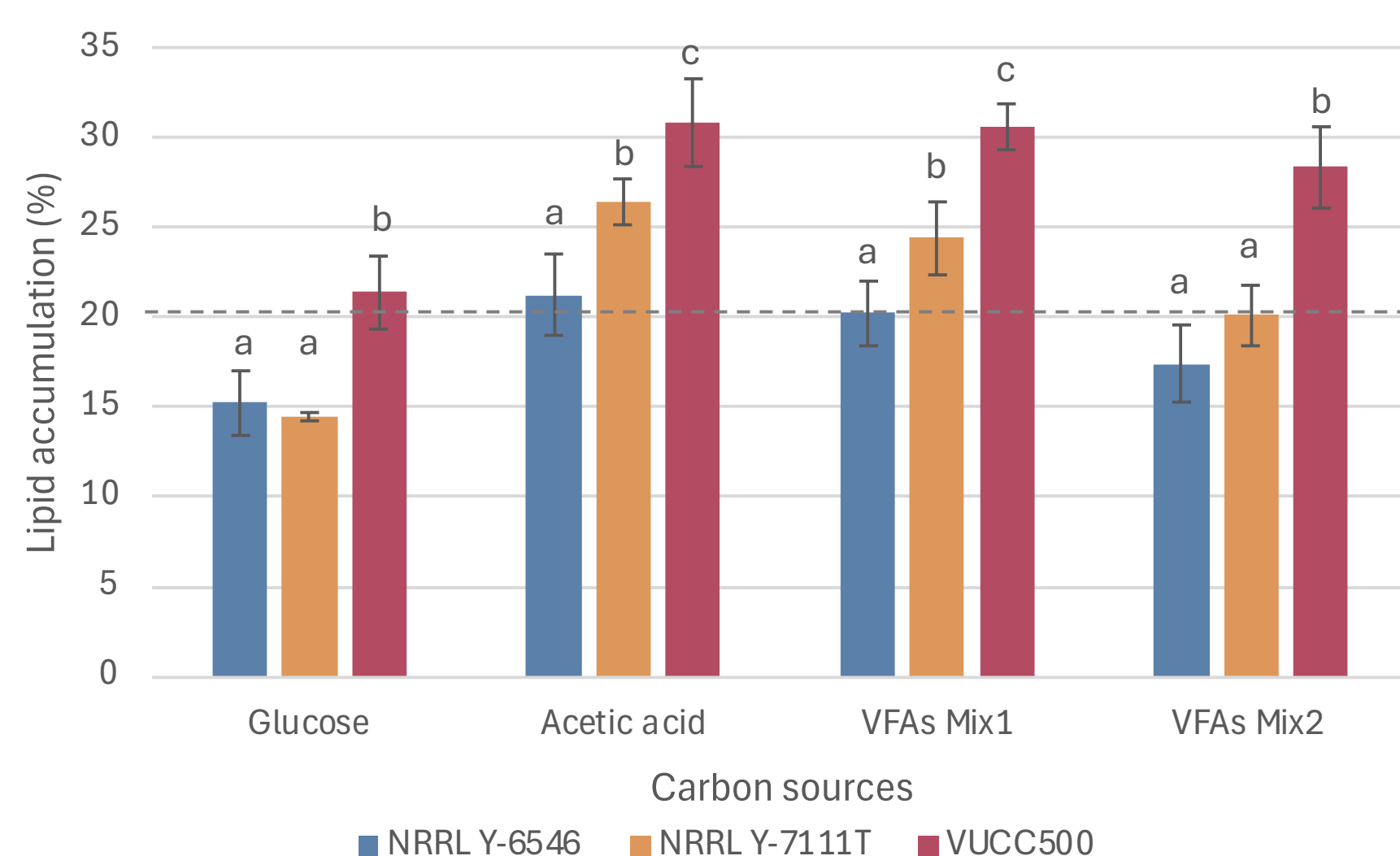


**Figure 2** Results of lipid accumulation of selected strains base on glucose as carbon source on acetic acid and the two VFAs mixes. t-test comparisons were performed within each series. Letters indicate significant differences within the same series only ( $p < 0.05$ ).

### Selected strains

- *Metschnikowia pulcherrima* NRRL Y-6546
- *Metschnikowia pulcherrima* NRRL Y-7111<sup>T</sup>
- *Metschnikowia pulcherrima* VUCC500

Three strains were selected from tests with glucose: VUCC500, the type strain already used in literature [7], and the second highest accumulator.

The **lipid accumulation increased** for all strains selected in preliminary tests with **both acetic acid and low-cost alternative carbon sources** (Figure 2).

**Table 1** Results of the Rhodamine B plate screening protocol [8]. Accumulation was evaluated based on the presence of lipid droplets in all cells and on colour intensity (grey -, violet +, purple ++).

| 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 <sup>T</sup> |            |               |          |
|                                  | VUCC500                  |            |               |          |
|                                  | NRRL Y-6064              |            |               |          |
|                                  | NRRL Y-48710             |            |               |          |
|                                  | CBS 10357                |            |               |          |
|                                  | NRRL Y48712              |            |               |          |

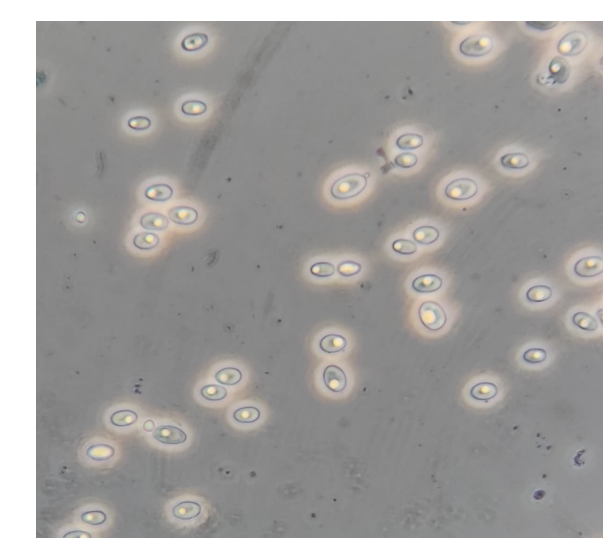


**Figure 1** *M. pulcherrima* VUCC500 under optical microscope after lipid accumulation

## Conclusions and perspectives

- The strain VUCC500 consistently resulted the highest accumulator in the test dataset
- Since its genome has been sequenced, further analyses will focus on safety-related aspects following EFSA guidelines
- Further studies are necessary to define reliable protocols for a fast qualitative screening of *M. pulcherrima* strains

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