

Article

Preliminary Screening of Non-Conventional Yeasts for Olive Mill Wastewater Valorization

Gabriella Siesto ^{1,2}, Rocchina Pietrafesa ^{1,*}, Antonio Caporusso ³, Giorgia La Rocca ¹, Grazia Alberico ¹, Vito Valerio ³ and Angela Capece ^{1,2}

¹ Department of Agricultural, Forest, Food and Environmental Sciences, University of Basilicata, Via dell'Ateneo Lucano 10, 85100 Potenza, Italy; gabriella.siesto@unibas.it (G.S.); giorgia.larocca@unibas.it (G.L.R.); grazia.alberico@gmail.com (G.A.); angela.capece@unibas.it (A.C.)

² Spin-off StarFlinn S.r.l.s., Department of Agricultural, Forest, Food and Environmental Sciences, University of Basilicata, Via dell'Ateneo Lucano 10, 85100 Potenza, Italy

³ ENEA C.R. Trisaia, Laboratory for Processes and Technologies for Biorefineries and Green Chemistry, Italian National Agency for New Technologies, Energy and Sustainable Economic Development (ENEA), S.S. 106 Jonica, 75026 Rotondella, Italy; antonio.caporusso@enea.it (A.C.); vito.valerio@enea.it (V.V.)

* Correspondence: rocchina.pietrafesa@unibas.it; Tel.: +39-0971205585

Abstract

Olive mill wastewater (OMWW) is a highly polluting agro-industrial effluent characterized by elevated organic load, low pH, and high concentrations of phenolic compounds responsible for its phytotoxicity and dark coloration. In this study, 41 non-conventional yeast strains belonging to the University of Basilicata Yeast Collection (UBYC), were tested for both the oleaginous potential traits and OMWW detoxification capacity in comparison to two commercial oleaginous controls, *Yarrowia lipolytica* ATCC 46483 and *Lipomyces tetrasporus* Li-0407. Primary screening in synthetic medium under nitrogen-limited conditions revealed widespread intracellular lipid accumulation. Quantitative analysis showed lipid contents above 20% (*w/w*) in some strains, with *Candida tropicalis* AII122 (33.3%) and *Pichia manshurica* ML-3 (29.4%) exhibiting the highest values in synthetic medium. The cultivation of eight selected strains in synthetic medium supplemented with 15% (*v/v*) of OMWW reduced intracellular lipid accumulation, with the highest value of 6.48% for the 2R1 strain. Levels of phenol reduction and color removal were highly different among all the analyzed strains, and *C. tropicalis* AII122 achieved the highest phenolic reduction and decolorization ability. These findings demonstrate that indigenous non-conventional yeasts represent a source of natural biodiversity, supporting sustainable waste valorization strategies based on the use of selected microorganisms within a circular bioeconomy framework.

Keywords: olive mill wastewater; non-conventional yeasts; lipid accumulation; phenolic compounds; decolorization; circular bioeconomy



Academic Editor: Alessia Tropea

Received: 28 February 2026

Revised: 1 April 2026

Accepted: 5 April 2026

Published: 8 April 2026

Copyright: © 2026 by the authors.

Licensee MDPI, Basel, Switzerland.

This article is an open access article distributed under the terms and conditions of the [Creative Commons Attribution \(CC BY\)](https://creativecommons.org/licenses/by/4.0/) license.

1. Introduction

Olive mill wastewater (OMWW) is widely recognized as one of the most problematic agro-industrial effluents produced by the olive oil industry, particularly in Mediterranean countries where olive cultivation plays a major economic and cultural role [1]. Large volumes of OMWW are generated annually (between 7 and over 30 million m³) during olive oil extraction, creating significant environmental management challenges due to their seasonal production and high pollutant load [2–4]. OMWW is characterized by elevated chemical oxygen demand (COD), high organic content, low pH (typically 3–6),

dark coloration, and significant concentrations of phenolic compounds [5]. These phenolics are primarily responsible for the effluent's phytotoxicity, antimicrobial activity, and resistance to biodegradation, which contribute to its persistence in soils and aquatic environments [4,6,7]. Among the major phenolic compounds identified in OMWW, hydroxytyrosol, tyrosol, oleuropein derivatives, and other polyphenols are the main compounds that contribute both to the effluent's toxicity and its coloration [8]. They inhibit microbial growth, interfere with enzymatic activities and reduce the efficiency of traditional wastewater treatment processes. Therefore, the reduction in total phenolic content and decolorization of OMWW are widely regarded as key indicators of effective detoxification, reduced environmental pollution and improved remediation [9,10]. Biological treatment strategies, based on the use of microorganisms, have gained attention as environmentally sustainable alternatives to physicochemical approaches [11,12]. Within this context, yeasts are promising candidates for OMWW detoxification due to their metabolic versatility, physiological robustness, and tolerance to inhibitory compounds. Several yeast species have been shown to grow in OMWW-based media, reduce phenolic concentrations, and decrease color intensity, mitigating the effluent's inhibitory effects [13,14]. Beyond their role in bioremediation, many yeast species, particularly oleaginous yeasts, can accumulate intracellular lipids, commonly referred to as single-cell oils (SCOs), under conditions of carbon excess and nitrogen limitation. These lipids are mainly stored as triacylglycerols and display fatty acid profiles comparable to those of vegetable oils, making them attractive for food, feed, and biotechnological applications [15–17]. Integrating OMWW detoxification with microbial lipid production represents a challenging strategy within a circular bioeconomy framework, allowing for the transformation of a problematic waste stream into value-added products [15].

Among oleaginous yeasts, *Yarrowia lipolytica* is one of the most extensively studied species due to its high metabolic flexibility, capacity to utilize diverse carbon sources, and production of extracellular enzymes such as lipases. Its growth on OMWW contributes to reductions in organic load and phenolic content, highlighting its potential for combined bioremediation and biomass valorization [16,18]. Similarly, *Lipomyces tetrasporus* is widely used as a reference oleaginous yeast due to its high lipid accumulation potential [16]. However, focusing exclusively on well-characterized model species may limit the discovery of strains with enhanced or complementary traits. Non-conventional yeasts represent a largely untapped microbial resource. Several non-conventional yeasts, including species of *Candida*, *Pichia*, *Metschnikowia*, *Hanseniaspora*, *Cutaneotrichosporon*, and *Debaryomyces*, have demonstrated promising performance in lipid accumulation and OMWW valorization [15,19]. Despite growing interest in yeast-mediated OMWW treatment, systematic studies combining detoxification (phenol reduction and decolorization) with oleaginous potential screening, particularly focusing on indigenous non-conventional yeasts, remain limited. Addressing this gap is essential to identify multifunctional strains capable of coupling environmental remediation with the production of value-added microbial biomass.

In this context, the present study aimed to screen 41 non-conventional yeast strains for their ability to accumulate intracellular lipids and produce lipase enzyme, in comparison with two collection oleaginous yeasts (*Yarrowia lipolytica* ATCC 46483 and *Lipomyces tetrasporus* Li-0407). Eight selected strains were further evaluated for their capacity to detoxify OMWW through decolorization and total phenolic compound reduction while simultaneously accumulating microbial lipids. This integrated approach provides new insights into the potential use of non-conventional yeasts for sustainable OMWW management within a circular bioeconomy.

2. Materials and Methods

2.1. Yeast Strains

In this study, forty-one non-conventional yeasts belonging to the University of Basilicata Yeast Collection (UBYC) (Potenza, Italy) and two collection strains *Yarrowia lipolytica* ATCC 46483 (DSM 8218, DSMZ, Germany) and *Lipomyces tetrasporus* Li-0407 (DSM 70314, DSMZ, Germany), used as positive controls, were analyzed for their oleaginous properties (Table 1).

Table 1. Origin of the non-conventional yeasts tested in this study.

Species	Strain Code	Source
<i>Pichia kudriavzevii</i>	2-21, 4-16, 2R17, 4-22	Grape must
<i>Hanseniaspora osmophila</i>	ND1	
<i>Metschnikowia pulcherrima</i>	1P1, 1RP2, 1RP9, 1SPR14, 1SPR1, 2-1, 2-7, 2-13, 2R1, 4-2, 4-15, 4R1, 4R-11	
<i>Metschnikowia pulcherrima</i>	AII 37, AII 68, AII 136, AII 181, AII 49	Honey bees
<i>Candida tropicalis</i>	AII 122	
<i>Meyerozyma caribbica</i>	AII 171	
<i>Pichia kluyveri</i>	AII 110	
<i>Lachancea kluyveri</i>	AII 184	
<i>Moniliella pollinis</i>	AII 40	
<i>Cutaneotrichosporon curvatus</i>	AII 176	Cheese
<i>Yarrowia lipolytica</i>	RB3, K, M, C, A	
<i>Pichia nakasei</i>	D, RB1	
<i>Debaryomyces hansenii</i>	RB5, PD 12-1, PD 49-1	
<i>Candida zeylanoides</i>	PD 36-2	Olive mill wastewater
<i>Pichia manshurica</i>	ML-3	
<i>Yarrowia lipolytica</i> ATCC 46483 (DSM 8218)	Y.l.	Control
<i>Lipomyces tetrasporus</i> Li-0407 (DSM 70314)	L.t.	

The yeast strains were maintained at $-20\text{ }^{\circ}\text{C}$ in Yeast Peptone Dextrose broth (YPD, 20 g/L Glucose, 20 g/L Peptone, 10 g/L Yeast Extract, Oxoid, Hampshire, UK) supplemented with 50% glycerol (Merk, Darmstad, Germany) as a protective agent until further analysis.

2.2. Primary Screening for Lipid Accumulation Capacity

To evaluate the intracellular lipids or single cell oils (SCOs) accumulation, the 41 strains were pre-cultured in 20 mL Yeast Malt Broth medium (YMB, yeast extract 3 g/L; malt extract 3 g/L; peptone 5 g/L and glucose 10 g/L, Oxoid, Hampshire, UK) and incubated at $28\text{ }^{\circ}\text{C}$ for 48 h with shaking at 150 rpm.

For each strain, an amount of the pre-culture corresponding to about 1×10^7 cells/mL was transferred into 50 mL Glycerol Yeast Peptone medium (GYP, glycerol 4% (*w/v*) Merk, Darmstad, Germany; yeast extract 1% (*w/v*); peptone 1% (*w/v*) Oxoid, Hampshire, UK) and incubated at $28\text{ }^{\circ}\text{C}$ under agitation (150 rpm). After 120 h, the yeast cells were collected by centrifugation at $4700 \times g$ for 10 min (Eppendorf Centrifuge 5920 R, Hamburg, Germany) and washed three times with NaCl (0.85% *w/v*, Merk, Darmstad, Germany) to remove the culture medium residues [20].

The harvested yeasts biomass fixed on the microscopic slide surface was stained with Sudan Black B solution (3 g/L) prepared in ethanol (70% *v/v*; Merk, Darmstad, Germany), and observed under the ECLIPSE 50i microscope (Nikon, Tokyo, Japan), using 100X objective with direct light to detect the presence of lipid droplets in the cells [21].

2.3. Evaluation of Lipase Activity

The strains were tested for their ability to produce lipase enzyme. To determine the production of lipase enzyme, a qualitative test was performed on Tributyrin Agar medium (TBA), containing 15 g/L of bacteriological agar, 3 g/L of yeast extract, 5 g/L of peptone (Oxoid, Hampshire, UK), and tributyrin 1% *v/v* (Merk, Darmstadt, Germany), added to MgSO_4 (5.0 mM, Merk, Darmstadt, Germany), following the method of Carrazco-Palafox et al. [22].

An aliquot (40 μL) of a fresh 24 h yeast culture was spotted in wells (diameter 0.6 cm) on TBA, and the plates were incubated at 28 °C for 48 and 72 h. The presence of lipolytic activity was evaluated on the basis of the halo size (mm) developed around the inoculated wells.

2.4. Accumulation and Extraction of SCOs from Yeast Cells in Synthetic Medium

The strains were pre-cultured in 100 mL Erlenmeyer flasks, containing 20 mL of YMB medium and incubated in a shaker incubator at 150 rpm, 28 °C for 24 h. An aliquot of pre-inoculum (1×10^7 cell/mL) was transferred into 100 mL of a nutrient culture media containing glucose 20 g/L, peptone 0.22 g/L (Oxoid, Hampshire, UK), glycerol 40 g/L and trace elements, namely KH_2PO_4 0.55 g/L, KCl 0.425 g/L, CaCl_2 0.125 g/L, MgSO_4 0.125 g/L, FeCl_3 0.0025 g/L, MnSO_4 0.0025 g/L (Merk, Darmstadt, Germany). According to previous work [23], 0.18 g/L of yeast extract was added in order to obtain the initial C/N ratio at 60 g/g. After 144 h of incubation (stationary phase) at 28 °C, the yeast biomass was harvested by centrifugation at 4700 rpm for 10 min, washed twice with NaCl solution (0.85% *w/v*, Merk, Darmstadt, Germany), dried overnight at 105 °C and then determined gravimetrically.

To determine the intracellular lipid content, the extraction of lipids was performed following the protocol reported by Thancharoen et al. [21], with some modifications. Briefly, the dried pellet collected from 1 g of biomass was added to 10 mL HCl solution (4 M, Merk, Darmstadt, Germany) and incubated at 60 °C for 2 h in order to disrupt the cell membranes. Then, 20 mL of hexane:methanol mixture (1:1 ratio, Merk, Darmstadt, Germany) was supplemented in the hydrolyzed cell biomass and stirred at room temperature for 3 h. The upper organic phase containing lipids was collected using a syringe and evaporated under vacuum at 40 °C in a rotary evaporator until a constant weight was achieved. Total lipid content was then quantified gravimetrically. Recovery yields, verified using the certified reference material BCR 163 (beef–pork fat blend), were approximately 98%. Lipid content was expressed as the percentage of extracted lipids on the dry biomass.

2.5. Evaluation of Selected Strain Performance in Olive Mill Wastewater-Supplemented Medium

Eight yeast strains, selected on the basis of previous steps, were analyzed in terms of growth ability in YMB supplemented with pasteurized olive mill wastewater (OMWW) (heated to 90 °C for 10 min) at different concentrations (15%, 25%, 50%, *v/v*). At this aim, 5 mL of OMWW medium was inoculated with the cell suspension of each strain and incubated at 28 °C for 48 h. Cell growth was assessed by calculating the difference between the optical density value (at 600 nm) recorded at the beginning and at the end of the experiments [24].

On the basis of cell growth, 15% OMWW addition was selected for further growth and lipid accumulation experiments. After 144 h of incubation, the cell biomass was harvested by centrifugation and analyzed for lipids content according to the protocol previously described.

The obtained supernatant was used to evaluate the OMWW decolorization capacity of the selected strains and the total polyphenol content. To evaluate OMWW decolorization, the absorbance of the supernatant was measured at 395 nm using the spectrophotometer

Spectrostar^{nano} (BMG LABTECH, Ortenberg, Germany) following the method of Sayadi and Ellouz [9].

The determination of the total polyphenol content in the supernatant was carried out following the Folin–Ciocalteu method (FC) as reported by Lindner et al. [7], with some modifications.

Specifically, 0.2 mL of Folin–Ciocalteu reagent (Merk, Darmstadt, Germany) and 1.8 mL of deionized water were added to 0.2 mL of supernatant previously centrifugated ($4700 \times g$ for 5 min), stirred and kept in the dark for five minutes. Then, 2 mL of Na_2CO_3 (7%, Merk, Darmstadt, Germany) and 0.8 mL of deionized water were added to the samples, vortexed and kept in the dark for 90 min.

Total polyphenol content was spectrophotometrically determined at $\lambda = 765$ nm in microplates, using the calibration curve prepared with gallic acid solutions at known concentrations.

The results were reported as the reduction percentage of phenolic compounds in comparison to the control sample:

$$\% \text{ Phenol Reduction} = \frac{(\text{TP}_c - \text{TP}_s)}{\text{TP}_c} \times 100$$

where

TP_c = Total polyphenol content in the control

TP_s = Total polyphenol content in the sample.

2.6. Analytical Methods

Reducing sugars (glucose, fructose, xylose, arabinose, mannose) were determined using an HPIC DX 300 chromatographic system (Dionex, Sunnyvale, CA, USA) equipped with a Nucleogel Ion 300 OA column (Macherey–Nagel, Düren, Germany) operating at 60°C with 10 mM H_2SO_4 solution as a mobile phase and a flux of 0.4 mL/min. The detector used was a Shodex RI101 refractive index detector (ECOM, Chrastany u Prahy, Czech Republic). Each analysis was performed in triplicate. All the quantifications were performed through the external standard technique.

2.7. Data Analysis

All the experiments were carried out in duplicate and the results were analyzed by Paleontological Statistics (PAST) software 4.09 [25]. One-Way Analysis of Variance (ANOVA), followed by Tukey's HSD test, were performed, and differences with p values < 0.05 were considered statistically significant.

3. Results and Discussion

3.1. Chemical Composition of Olive Mill Wastewater

Olive mill wastewater (OMWW) exhibits a highly variable composition, influenced by several factors including the oil extraction process, the degree of fruit ripeness, and the olive cultivar. It is generally characterized by an acidic pH and contains substantial amounts of sugars, lipids, proteins, and phenolic compounds [26,27]. Because of this compositional variability, a comprehensive biochemical characterization of OMWW is essential prior to its use as a cultivation medium for the production of microbial metabolites (Table 2).

The values obtained in the present work are consistent with those of the reference literature [24,28].

Table 2. Biochemical parameters of OMWW.

Parameters	Value (In Present Work)	Value from Reference [28]	Value from Reference [24]
pH	4.7	5.4	5.1
Total reducing sugars (g/L)	9.9	9.4	10.1
Phenolic compounds (g/L)	5.1	3.4	4.8
Lipids	-	0.76%	-

3.2. Qualitative Screening of Yeast Strains for SCOs Accumulation

The SCOs accumulation ability of the forty-one strains was investigated using the Sudan Black B technique. Although this technique did not provide precise information on intracellular lipid content, it proved to be a useful preliminary screening method for assessing the ability of some microorganisms to accumulate lipid droplets as carbon storage. Microorganisms were cultivated under conditions of high carbon availability and nitrogen limitation, in accordance with the reference literature [29–31]. Results showed that many yeasts were able to accumulate lipids, although at different levels. The presence of blue-stained lipid cytoplasmic inclusions, observed by the microscope analysis (Figure 1A,B), was correlated to the ability of each strain to accumulate lipids.

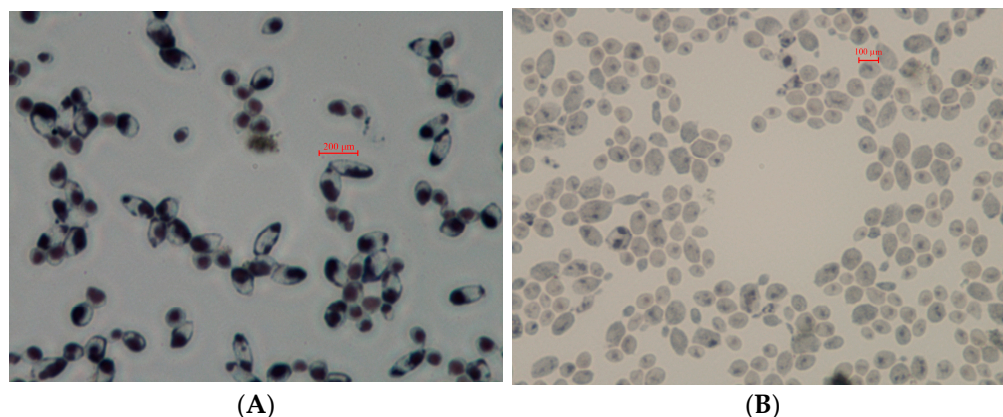


Figure 1. Cells stained with Sudan Black B of potential oleaginous (A) and non-oleaginous yeasts (B) observed with optical microscope at 100X magnification. The (A) refers to cells of *Lipomyces tetrasporus*, while the strain K (*Yarrowia lipolytica*) is reported in (B).

In particular, on the basis of the presence of lipid droplets in the yeast cells, the tested strains were arbitrarily classified into two groups:

- Group 1, characterized by lipid droplets inside some cells, which include the following strains: K (*Y. lipolytica*); AII176 (*Cut. curvatus*); 1RP9; 2-13; 4-2; 4-15; 4R11 (*M. pulcherrima*); 4-22 (*P. kudriavzevii*); and RB1 (*P. nakasei*).
- Group 2, composed of strains containing oil droplets in all the cells. These strains were the following: 1P1, 1RP2, 1SPR1, 1SPR14, 2-1, 2-7, 2R1, 4R1, AII 37, AII 68, AII 136, AII 181, AII 49 (*M. pulcherrima*); M, C, A, RB3 (*Y. lipolytica*); PD 36-2 (*C. zeylanoides*); AII 122 (*C. tropicalis*); RB5, PD 12-1, PD 49-1 (*D. hansenii*); ND1 (*H. osmophila*); AII184 (*L. kluyveri*); AII 40 (*Mon. pollinis*); AII171 (*Mey. caribbica*); 2-21, 4-16, 2R-17 (*P. kudriavzevii*); AII110 (*P. kluyveri*); ML-3 (*P. manshurica*); and D (*P. nakasei*). As expected, the control strains (*L. tetrasporus* and *Y. lipolytica*) showed a high intracellular lipid accumulation ability. The strains included in group 2 can be considered as potential lipid producers.

3.3. Quantitative Production of SCOs

The Sudan Black technique provides partial information on the lipid accumulation capacity of the tested yeast strains [32] without detailed insight into the cellular lipid

content of the yeasts. In order to confirm the results obtained by qualitative screening and select the potential oleaginous strains, lipid production was evaluated among the strains grown in nitrogen limited conditions (C/N = 60 g/g) to favor intracellular lipid accumulation over biomass production. Figure 2 summarizes the main results.

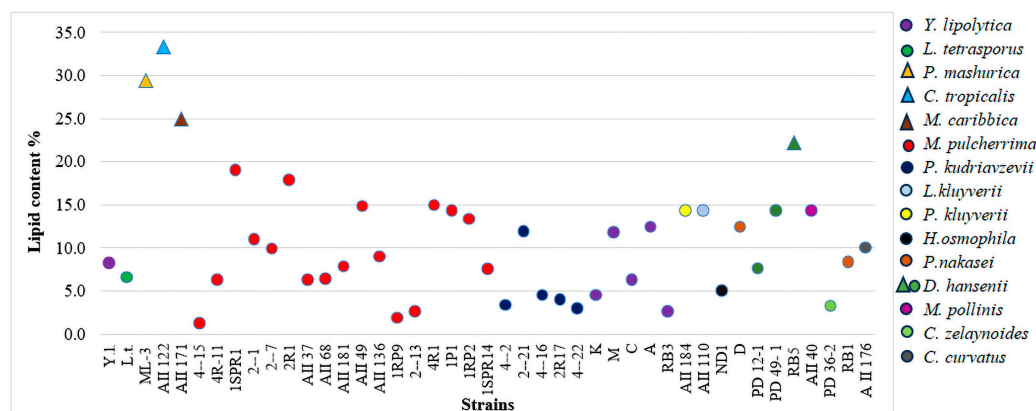


Figure 2. Lipid content (%) in the strains grown in nitrogen limited conditions. Triangles indicate the strains showing the highest lipid content.

In particular, AII122 and ML-3, identified as *Candida tropicalis* and *Pichia manshurica*, respectively, exhibited the highest lipid contents (33.3% and 29.4% of CDW). These values fall in the range typically reported for oleaginous yeasts, which are conventionally defined as microorganisms able to accumulate more than 20% of intracellular lipids under appropriate culture conditions. Previous studies have shown that *C. tropicalis* is a versatile oleaginous yeast of great scientific and industrial interest. Dey P. and Maiti [33] demonstrated that *C. tropicalis* showed a maximum lipid production at C:N 150:1, at which the lipid yield was 58.6% with glucose as the carbon source and ammonium sulfate as the nitrogen source.

The literature data on *Pichia manshurica* are very limited, as this species is not traditionally classified among the model oleaginous yeasts. However, a recent work [34] indicates that selected strains, isolated from sugarcane bagasse, may accumulate lipids in the range of 25–40% under optimal conditions. In this context, the 29.4% lipid content observed for strain ML-3 suggests a promising metabolic capability to produce lipids under fine-tuned conditions.

Strains AII171 (*Meyerozyma caribbica*) and RB5 (*Debaryomyces hansenii*) also showed lipid contents of 25.0% and 22.2%, respectively.

Mey. caribbica has mainly been investigated for its biocontrol activity and fermentative versatility [35], while reports on its ability to accumulate lipid are comparatively limited. Therefore, the 25% lipid content observed in strain AII171 indicates a noteworthy storage capacity under the tested conditions.

Similarly, *D. hansenii* is widely recognized for its halotolerance, osmotolerance, and application in food fermentations [36] rather than for high lipid production. Although lipid accumulation in this species is usually reported at moderate levels, strain-dependent variability has been described, particularly under stress or nutrient-imbalanced conditions. The lipid content recorded for RB5 (22.2%) suggests that, under the cultivation parameters adopted in this study, this strain was able to redirect carbon flux toward storage lipid biosynthesis beyond its structural requirements.

Finally, the other tested strains, including 1SPR14, 1RP2, 1P1, 4R1, AII49, 2R1 and 1SPR1 (identified as *Metschnikowia pulcherrima*), accumulated lipids in a range between 6.25% and 19.05%. Although these values do not classify them as strictly oleaginous, moderate lipid accumulation has reported. *M. pulcherrima* is a species commonly recognized

for its biocontrol activity [37], pigment production [38], oenological applications [39] and intracellular polysaccharides accumulation [40] rather than for high lipid yields. Therefore, its lipid biosynthetic capacity, even if limited compared to highly oleaginous yeasts, may represent an additional interesting trait for this species.

Moderate lipid accumulation was also recorded for strains M, A, D, AII110 and 2-21 (11.8–14.3%). Such values are consistent with non-oleaginous or weakly oleaginous yeasts, where lipid accumulation remains primarily associated with structural membrane synthesis. Finally, strains belonging to *Hanseniaspora osmophila* (ND1), *Cutaneotrichosporon curvatus* (AII176), and *Pichia nakasei* (RB1) exhibited lipid contents below 10%. While *C. curvatus* is recognized in the literature as a highly oleaginous species [41], the low lipid content observed in strain AII176 suggests that cultivation parameters, strain-specific variability, or nutrient balance likely limited storage lipid biosynthesis under the adopted experimental setup.

Overall, these findings highlight that lipid accumulation is not only species-dependent but also influenced by strain-level metabolic variability and culture conditions.

3.4. Determination of the Lipase Activity

To assimilate exogenous lipids, microorganisms must produce lipases that hydrolyze triglycerides into free fatty acids, which can then be taken up by the cells [42]. As OMWW contains variable quantities of olive oil, it can be used as a substrate by yeast for the production of lipolytic enzymes. This step is fundamental for *ex novo* lipid synthesis, a pathway in which yeasts accumulate lipids starting from hydrophobic substrates, such as oils and fats. Although the OMWW analyzed in this study did not contain lipids (Table 2), our aim was to submit different strains to a wide screening for different characteristics, potentially useful for OMWW valorization.

In contrast, during *de novo* lipid synthesis, yeasts utilize hydrophilic carbon sources, including polysaccharides and glycerol, which are metabolized through central carbon metabolism and the fatty acid biosynthetic pathway to generate lipid bodies.

Lipase activity was evaluated in TBA medium using the well diffusion assay. The formation of a clear zone (mm) around the inoculated wells indicated lipid hydrolysis. Analyses were performed after two different incubation times, 48 and 72 h. Figure 3 shows that the lipolytic activity was expressed at different levels by the tested yeasts. In most of the strains, this activity had already been exhibited after 48 h of incubation, similarly to the control strains (*L. tetrasporus* and *Y. lipolytica*), while few strains showed this activity only after 72 h of incubation. This was the case of almost all *Pichia kudriavzevii* strains (with the exception of 4-22), as well as the AII 171, AII 40, and 4-2 strains. However, in the majority of strains, lipase activity increased with incubation time, with the highest values found after 72 h of incubation. Conversely, in some strains (RB3, AII 122, PD36-2, AII 184, 4R1, AII 168, 1P1, 2-7) this activity was not affected by incubation time. The highest lipolytic activity was found in three strains (M, RB5 and D) after 72 h, with the diameter of the clear zone being 10, 9 and 6 mm respectively. Finally, lipase activity was lacking in two strains, ND1 and ML-3.

The ability to produce lipases has been extensively studied and well-documented for *Y. lipolytica* [43,44] and *D. hansenii* [44,45] strains. For instance, Papagora et al. [45] investigated and optimized lipase production by *D. hansenii* isolated from dry-salted olives. Their study demonstrated that sugar and lipid concentrations, as well as pH, are key factors influencing the production of highly active lipases. Under optimized conditions, lipase activity reached 7.44 U/mL, corresponding to an increase of approximately 2.3-fold compared to non-optimized conditions. Furthermore, other studies have shown that the lipolytic activity of *D. hansenii* is strongly influenced by the type of fatty acids present in the culture medium, with

comparable to or higher than those recorded in YMB, indicating effective adaptation to low OMWW concentrations. In particular, strains D and AII110 showed markedly enhanced growth at 15% OMWW, with statistically significant differences compared to higher concentrations. Conversely, 50% OMWW generally resulted in reduced biomass accumulation, confirming the inhibitory impact of high content of phenolic compounds in OMWW used in this study (Table 2).

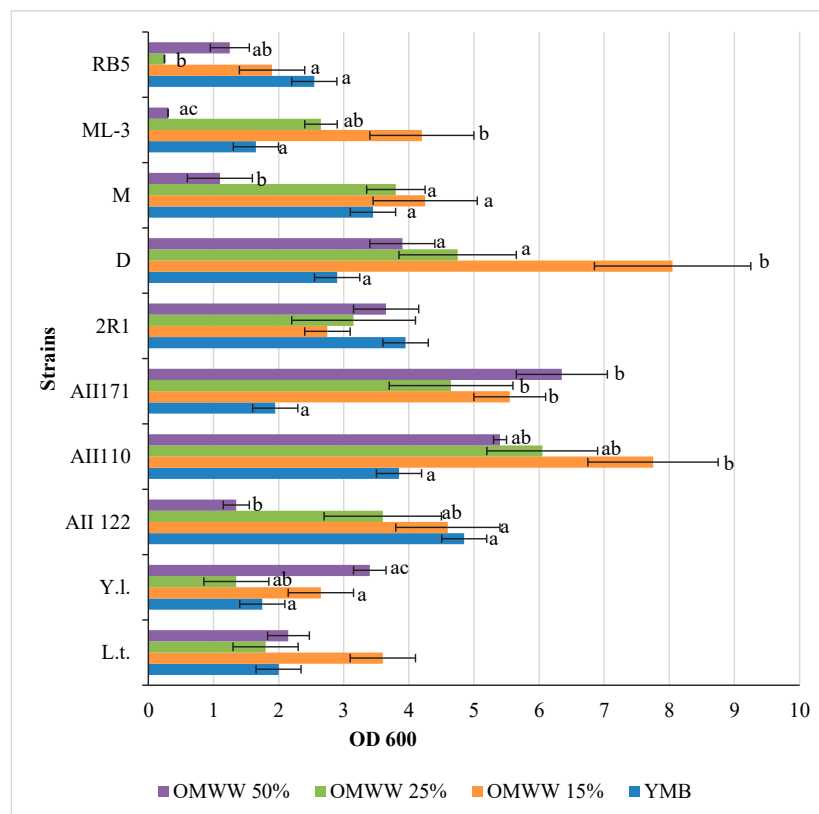


Figure 4. OD values at 600 nm (expressed as $Abs_{48h} - Abs_{0h}$) by the yeast strains grown in OMWW (15%, 25%, 50%, v/v) in comparison to YMB medium without OMWW addition. Letters (a, b, ab, ac) indicate significant differences among treatments within each strain ($p < 0.05$).

Table 3 reports the intracellular lipid content and dry cell weight (DCW) of the eight selected strains cultivated in YMB supplemented with 15% (v/v) OMWW. Regarding biomass production, no statistically significant differences were observed among the tested strains compared to *Y. lipolytica*, although strains D, AII110, and ML-3 showed significantly lower DCW than *L. tetrasporus* (8.69 ± 0.28 g/L), which exhibited the highest biomass yield under the tested conditions. Furthermore, for some strains (i.e., RB5 and ML-3) inconsistent values for DCW (Table 3) and OD600 (Figure 4) were found in OMWW 15%; indeed, the RB5 strain resulted in 9.05 g/L DCW (Table 3) and 1.9 OD600 approximately (Figure 4), while the ML-3 resulted in 6.73 g/L DCW and 4.0 OD600. These results might be due to the use of two methods to evaluate the growth performance. Optical density evaluation is a fast method for the quantification of biomass yield, while direct measurements of DCW are generally more accurate than OD-based estimations.

Overall, intracellular lipid accumulation was consistently low in all strains, ranging between approximately 1% and 6%. These values are far below the threshold commonly adopted to define oleaginous microorganisms, namely 20% of dry cell weight [15]. The highest lipid contents were detected in AII110 ($4.6 \pm 0.07\%$), AII171 ($4.47 \pm 0.72\%$), and 2R1 ($6.48 \pm 0.27\%$), which were significantly higher than those of the control strains, yet

remain quantitatively modest. Such low percentages most likely correspond to structural membrane lipids rather than the accumulation of storage triglycerides.

Table 3. Intracellular lipid accumulation (%) and dry cell weight (DCW, g/L) of selected yeast strains grown in OMWW (15% v/v).

Strain	Lipid Production (%)	DCW (g/L)
RB5 (<i>Debaryomyces hansenii</i>)	2.88 ± 0.06	9.05 ± 0.04
ML-3 (<i>Pichia manshurica</i>)	1.84 ± 0.05	6.73 ± 0.13 (#)
M (<i>Yarrowia lipolytica</i>)	2.78 ± 1.47	8.31 ± 0.14
D (<i>P. nakasei</i>)	2.71 ± 0.52	5.43 ± 0.73 (#)
2R1 (<i>Metschnikowia pulcherrima</i>)	6.48 ± 0.27 (#,*)	8.30 ± 0.36
AII 171 (<i>Meyerozyma caribbica</i>)	4.47 ± 0.72 (#,*)	8.13 ± 0.20
AII 110 (<i>P. kluyverii</i>)	4.60 ± 0.07 (#,*)	6.11 ± 0.06 (#)
AII 122 (<i>Candida tropicalis</i>)	2.68 ± 0.15	9.86 ± 0.24
Y.l. (<i>Y. lipolytica</i>)	1.80 ± 0.36	7.54 ± 1.93
L.t. (<i>Lipomyces tetrasporus</i>)	1.67 ± 0.02	8.69 ± 0.28

Data are expressed as mean ± SD values of two independent experiments. In each column, strains significantly different (Tukey's test, $p < 0.05$) from L.t. are indicated by (#) and those significantly different from Y.l. by (*).

Comparable results were reported by Arous et al. [48], who observed lipid contents ranging from 2.8% to 5% in yeasts cultivated on undiluted OMWW. Under those conditions, biomass composition was characterized by a high protein fraction (up to 39%), indicating a metabolic direction for cellular growth and protein synthesis rather than lipid storage.

In contrast, other investigations have reported substantially higher lipid production from OMWW [49]. For example, *L. starkeyi* and *Y. lipolytica* were shown to reach lipid contents of approximately 25% when OMWW was supplemented with additional carbon sources [50]. Similarly, Sayin et al. [51] reported 41% of lipid accumulation in *Y. lipolytica* cultivated on diluted OMWW.

In the present work, the limited lipid production could be explained by a metabolic imbalance induced by OMWW. The presence of phenolic compounds and other inhibitory molecules can impose significant physiological stress. Therefore, the limited lipid accumulation observed in this study is likely due to substrate inhibition rather than an unfavorable nutrient balance. Under such conditions, cells likely redirect metabolic energy toward stress-protective mechanisms, medium detoxification, rather than intracellular lipid production. Taken together, these findings highlight the intrinsic complexity of inducing lipid synthesis in complex wastewaters such as OMWW. They also confirm the high interstudy variability reported in the literature, which is strongly influenced by strains, wastewater composition, and process parameters.

Indeed, the two control strains (*L. tetrasporus* and *Y. lipolytica*), previously tested in different substrates and culture conditions [52], gave lipid yield higher than levels found in this study. This might be due to the severe effect of process parameters, such as substrate, temperature, pH and dissolved oxygen (DO), on yeast lipid production [53]. As a consequence, further characterization of the indigenous yeasts showing lipid production higher than control strains, such as AII 110, AII 171 and 2R1, are necessary in order to evaluate the possibility of increasing lipid production by optimizing fermentative conditions.

3.6. Effect of the Potential Oleaginous Yeasts on OMWW-Based Medium Decolorization and Polyphenol Content Reduction

Olive mill wastewaters (OMWWs) represent a polluting liquid effluent, mainly due to their elevated concentration of phenolic compounds. Numerous studies have demonstrated that the toxicity of OMWWs is largely associated with their phenolic fraction [54,55]. In

fact, polyphenols represent the main class of phytotoxic compounds present in this waste and seem to be responsible for the dark color of OMWWs [24].

Therefore, the identification of microorganisms capable of valorizing these effluents is of particular interest, as it offers a dual advantage: the detoxification of the wastewater and the simultaneous production of value-added bio-based products. The capacity of several microorganisms to metabolize phenolic compounds is well-established. For instance, *Candida tropicalis* ATCC 750, *Rhodotorula* spp., and *Y. lipolytica*, have been reported to metabolize phenolic compounds present in OMWWs [24,49,56]. The ability of fungi to break down phenolic compounds is correlated to the secretion of enzymes, such as the extracellular oxidases (ligninolytic enzymes) laccase, lignin peroxidase and peroxidase, although the secretion of these enzymes is strain-dependent and influenced by culture conditions [57].

Table 4 reports the percentage of phenol reduction and the corresponding medium discoloration for each tested strain. In general, the values of decolorization in comparison to the non-inoculated sample ranged between 1.04 and 41.78%, while the range of reduction in phenolic compounds was between 11.60 and 40.35%.

Table 4. Percentage of decolorization and reduction in phenolic compounds by selected yeasts grown in OMWW-based medium.

Strains	Phenolic Compounds Reduction (%)	Decolorization (%)
RB5 (<i>Debaryomyces hansenii</i>)	20.42 ± 2.02 (#,*)	26.39 ± 1.94
ML-3 (<i>Pichia manshurica</i>)	22.95 ± 0.46 (#,*)	17.25 ± 0.52 (#,*)
M (<i>Yarrowia lipolytica</i>)	34.17 ± 1.43	22.22 ± 1.22 (*)
D (<i>P. nakasei</i>)	23.20 ± 1.64 (#,*)	18.98 ± 2.76 (*)
2R1 (<i>Metschnikowia pulcherrima</i>)	15.51 ± 0.71 (#,*)	3.47 ± 0.02 (#,*)
AII 171 (<i>Meyerozyma caribbica</i>)	37.07 ± 3.11	1.04 ± 0.16 (#,*)
AII 110 (<i>P. kluyverii</i>)	11.6 ± 0.14 (#,*)	25.00 ± 0.33
AII 122 (<i>Candida tropicalis</i>)	40.35 ± 3.05 (*)	41.78 ± 1.80 (#,*)
Y.I. (<i>Y. lipolytica</i>)	31.90 ± 2.85	29.97 ± 0.49 (#)
L.t. (<i>Lipomyces tetrasporus</i>)	32.53 ± 2.32	24.07 ± 0.89 (*)

Data are expressed as mean ± SD values of two independent experiments. In each column, strains significantly different (Tukey's test, $p < 0.05$) from L.t. are indicated by (#) and those significantly different from Y.I. by (*).

The results obtained for the phenolic compounds removal showed statistical differences in all the analyzed strains compared to both control strains, except for the strains AII 171 and M, whereas for the sample inoculated with AII 122, the phenolic reduction (40.35%) was significantly higher than the sample inoculated with Y.I. (31.90%). Finally, the decolorization capacity in the strains ML-3, AII171, 2R1 and AII 122 was significantly different from the values obtained for both control strains. Conversely, no statistical differences were observed for the strains RB5 and AII 110 in comparison to Y.I. and L.t.

The strain AII122 (*C. tropicalis*) showed the highest values for both of the desired characteristics, the reduction in phenolic content (40.35 ± 3.05) and OMWW decolorization (41.78 ± 1.80%). The strain AII 171 (*Mey. caribbica*) led to a significant reduction in total phenolic content (37.07%), but no appreciable decolorization of the olive mill wastewater was observed. This result suggests that *Mey. caribbica* selectively metabolized specific phenolic compounds that do not contribute to the chromatic properties of the OMWW. Indeed, the color of olive mill wastewater is mainly associated with complex and polymerized phenolic structures, which may remain unaffected or be transformed into still chromophoric derivatives [58]. Although no decolorization was observed, this finding is noteworthy, since, to the best of our knowledge, the ability of *Mey. caribbica* to remove phenolic compounds from olive mill wastewater has not been previously reported in the literature. This result confirms the high metabolic versatility and ecological adaptability

of this yeast species, which, over the past two decades, has been identified as a yeast with significant relevance to biotechnology, agriculture, environmental remediation, and food applications [35].

4. Conclusions

This study demonstrated that non-conventional yeasts isolated from different ecological niches represent a valuable resource for the valorization of olive mill wastewater. The preliminary screening using Sudan Black B staining revealed that several strains were able to accumulate intracellular lipids. Quantitative analyses confirmed that some strains exhibited significant oleaginous potential, with lipid contents exceeding 20% of dry biomass. In particular, *Candida tropicalis* AII122 and *Pichia manshurica* ML-3 showed the highest lipid accumulation in synthetic medium, while strains such as *Meyerozyma caribbica* AII171 and *Debaryomyces hansenii* RB5 also displayed promising lipid yields.

When cultivated in OMWW-based medium (15% v/v), the selected strains were able to grow efficiently, confirming their adaptation to this inhibitory substrate. Although overall lipid accumulation remained low (1–6% of DCW), *M. pulcherrima* 2R1 exhibited significantly higher lipid accumulation (6.48% of DCW) compared to the control strains *Yarrowia lipolytica* ATCC 46483 and *Lipomyces tetrasporus* Li-0407. Furthermore, the strain *C. tropicalis* AII122 achieved the highest phenolic compound reduction and color removal, outperforming at least one of the control strains. Overall, the results highlight the feasibility of coupling OMWW detoxification with microbial production, supporting a circular bioeconomy approach. Among the tested strains, *C. tropicalis* AII122 emerged as the most promising multifunctional candidate, combining high biomass production and effective phenolic reduction in OMWW with the highest lipid accumulation observed under synthetic medium conditions. Although lipid production on OMWW remained limited under the tested conditions, the strong detoxification capacity and robust biomass formation displayed by this strain suggest that, through a process optimization, the lipid synthesis on OMWW could be significantly enhanced. These findings provide new insights into the biotechnological approach based on use of indigenous non-conventional yeasts for sustainable OMWW management and open perspectives for further optimization and scale-up studies.

Author Contributions: G.S.: Conceptualization, Data curation, Investigation, Methodology, Writing—original draft, Writing—review and editing. R.P.: Conceptualization, Investigation, Data curation, Formal analysis, Methodology, Software, Writing—original draft. A.C. (Antonio Caporusso): Methodology, Data curation, Writing—review and editing. G.A.: Conceptualization, Investigation, Methodology, Writing—original draft. V.V.: Investigation, Methodology, Validation. G.L.R.: Validation, Writing—review and editing. A.C. (Angela Capece): Conceptualization, Project administration, Supervision, Writing—review and editing. All authors have read and agreed to the published version of the manuscript.

Funding: This study was carried out within the Agritech National Research Center and received funding from the European Union Next-Generation EU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR)—MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4—D.D. 1032 17/06/2022, funding number: CN00000022). This manuscript reflects only the authors' views and opinions; neither the European Union nor the European Commission can be considered responsible for them.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in the study are included in the article; further inquiries can be directed to the corresponding author.

Acknowledgments: The JRU MIRRI-IT (<http://www.mirri-it.it/>, accessed on 15 January 2026) is greatly acknowledged for scientific support.

Conflicts of Interest: Gabriella Siesto and Angela Capece were employed by Spin-off StarFInn S.r.l.s. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. The authors declare that this study received funding from European Union Next-Generation EU. The funder was not involved in the study design, collection, analysis, interpretation of data, the writing of this article or the decision to submit it for publication.

References

1. Afonso, I.S.; Duarte, C.; Afonso, M.J.; Ribeiro, A.E.; Amaral, J.S.; Sousa, P.C.; Lima, R.A.; Ribeiro, J.E. Physicochemical characterisation of olive mill wastewaters based on extraction methods and filtration levels. *Clean Technol.* **2025**, *7*, 73. [\[CrossRef\]](#)
2. Ginos, A.; Manios, T.; Mantzavinos, D. Treatment of olive mill effluents by coagulation–flocculation–hydrogen peroxide oxidation and effect on phytotoxicity. *J. Hazard. Mater.* **2006**, *133*, 135–142. [\[CrossRef\]](#) [\[PubMed\]](#)
3. Dermeche, S.; Nadour, M.; Larroche, C.; Moulti-Mati, F.; Michaud, P. Olive mill wastes: Biochemical characterizations and valorization strategies. *Process Biochem.* **2013**, *48*, 1532–1552. [\[CrossRef\]](#)
4. Foti, P.; Romeo, F.V.; Occhipinti, P.S.; Timpanaro, N.; Musumeci, T.; Randazzo, C.L.; Caggia, C. Olive mill wastewater as renewable raw materials to generate high added-value ingredients for agro-food industries. *Appl. Sci.* **2021**, *11*, 7511. [\[CrossRef\]](#)
5. Kavvadias, V.; Doula, M.; Theocharopoulos, S. Long-term effects on soil of the disposal of olive mill waste waters (OMW). *Environ. Forensics* **2014**, *15*, 37–51. [\[CrossRef\]](#)
6. Elmansour, T.E.; Mandi, L.; Ahmali, A.; Hejjaj, A.; Bubba, M.D.; Ouazzani, N. Effect of polyphenols on activated sludge biomass during the treatment of highly diluted olive mill wastewaters. *Water Sci. Technol.* **2020**, *82*, 1416–1429. [\[CrossRef\]](#)
7. Lindner, A.V.; Pleissner, D. Removal of phenolic compounds from olive mill wastewater by microalgae grown under dark and light conditions. *Waste Biomass Valor.* **2022**, *13*, 525–534. [\[CrossRef\]](#)
8. Obied, H.K.; Allen, M.S.; Bedgood, D.R.; Prenzler, P.D.; Robards, K.; Stockmann, R. Bioactivity and analysis of biophenols recovered from olive mill waste. *J. Agric. Food Chem.* **2005**, *53*, 823–837. [\[CrossRef\]](#)
9. Sayadi, S.; Ellouz, R. Roles of lignin peroxidase and manganese peroxidase from *Phanerochaete chrysosporium* in the decolorization of olive mill wastewater. *Appl. Environ. Microbiol.* **1995**, *61*, 1098–1103. [\[CrossRef\]](#)
10. Ramires, F.A.; Durante, M.; Maiorano, G.; Migoni, D.; Rampino, P.; Fanizzi, F.P.; Perrotta, C.; Mita, G.; Grieco, F.; Bleve, G. Industrial scale bio-detoxification of raw olive mill wastewaters by the use of selected microbial yeast and bacterial strains to obtain a new source for fertigation. *J. Environ. Manag.* **2020**, *265*, 110574. [\[CrossRef\]](#)
11. Mateescu, C.; Lungulescu, E.M.; Nicula, N.O. Effectiveness of biological approaches for removing persistent organic pollutants from wastewater: A mini review. *Microorganisms* **2024**, *12*, 1632. [\[CrossRef\]](#)
12. Mishra, T.; Tiwari, P.B.; Kanchan, S.; Keshari, M. Advances in microbial bioremediation for effective wastewater treatment. *Water* **2025**, *17*, 3196. [\[CrossRef\]](#)
13. Bevilacqua, A.; Petruzzini, L.; Corbo, M.R.; Sinigaglia, M. Bioremediation of olive mill wastewater by yeasts—A review of the criteria for the selection of promising strains. In *Applied Bioremediation—Active and Passive Approaches*; Patil, Y.B., Rao, P., Eds.; IntechOpen: London, UK, 2013. [\[CrossRef\]](#)
14. Gonçalves, C.; Lopes, M.; Ferreira, J.P.; Belo, I. Biological treatment of olive mill wastewater by non-conventional yeasts. *Bioresour. Technol.* **2009**, *100*, 3759–3763. [\[CrossRef\]](#)
15. Caporusso, A.; Capece, A.; De Bari, I. Oleaginous yeasts as cell factories for the sustainable production of microbial lipids by the valorization of agri-food wastes. *Fermentation* **2021**, *7*, 50. [\[CrossRef\]](#)
16. Ratledge, C.; Wynn, J.P. The biochemistry and molecular biology of lipid accumulation in oleaginous microorganisms. *Adv. Appl. Microbiol.* **2002**, *51*, 1–51. [\[CrossRef\]](#) [\[PubMed\]](#)
17. Wierzchowska, K.; Szulc, K.; Zieniuk, B.; Fabiszewska, A. Bioconversion of liquid and solid lipid waste by *Yarrowia lipolytica*: Lipase biosynthesis and microbial lipid production. *Molecules* **2023**, *28*, 1187. [\[CrossRef\]](#)
18. Ledesma-Amaro, R.; Nicaud, J.-M. Metabolic engineering for expanding the substrate range of *Yarrowia lipolytica*. *Trends Biotechnol.* **2016**, *34*, 798–809. [\[CrossRef\]](#)
19. Donzella, S.; Serra, I.; Fumagalli, A.; Pellegrino, L.; Mosconi, G.; Scalzo, R.L.; Compagno, C. Recycling industrial food wastes for lipid production by oleaginous yeasts *Rhodospiridiobolus azoricus* and *Cutaneotrichosporon oleaginosum*. *Biotechnol. Biofuels Bioprod.* **2022**, *15*, 51. [\[CrossRef\]](#)
20. Planonth, S.; Chantarasiri, A. The oleaginous yeast *Pichia manshurica* isolated from *Lansium domesticum* fruit in Thailand and its fatty acid composition of single cell oil. *Biodiversitas* **2022**, *23*, 801–809. [\[CrossRef\]](#)

21. Thancharoen, K.; Malasri, A.; Leamsing Korn, W.; Boonyalit, P. Selection of oleaginous yeasts with lipid accumulation by the measurement of Sudan Black B for benefits of biodiesel. *Int. J. Pharma Med. Biol. Sci.* **2017**, *6*, 53–57. [\[CrossRef\]](#)
22. Carrazco-Palafox, J.; Rivera-Chavira, B.E.; Ramírez-Baca, N.; Manzanares-Pérez, J.; Nevárez-Moorillón, G.V. Improved method for qualitative screening of lipolytic bacterial strains. *MethodsX* **2018**, *5*, 68–74. [\[CrossRef\]](#)
23. Caporusso, A.; Casiello, M.; De Leo, V.; Perna, A.; Catucci, L.; D'Accolti, L.; Capece, A.; De Bari, I. Low impact methods for microbial lipids production for third generation biofuels and biobased polyurethanes. *J. Environ. Chem. Eng.* **2025**, *13*, 117375. [\[CrossRef\]](#)
24. Dias, B.; Lopes, M.; Ramôa, R.; Pereira, A.S.; Belo, I. *Candida tropicalis* as a promising oleaginous yeast for olive mill wastewater bioconversion. *Energies* **2021**, *14*, 640. [\[CrossRef\]](#)
25. Hammer, Ø.; Harper, D.A.T.; Ryan, P.D. PAST: Paleontological statistics software package for education and data analysis. *Palaeontol. Electron.* **2001**, *4*, 9.
26. Russo, E.; Spallarossa, A.; Comite, A.; Pagliero, M.; Guida, P.; Belotti, V.; Caviglia, D.; Schito, A.M. Valorization and potential antimicrobial use of olive mill wastewater (OMW) from Italian olive oil production. *Antioxidants* **2022**, *11*, 903. [\[CrossRef\]](#)
27. Issa, A.; El Riachy, M.; Bou-Mitri, C.; Doumit, J.; Skaff, W.; Karam, L. Influence of geographical origin, harvesting time and processing system on the characteristics of olive-mill wastewater: A step toward reducing the environmental impact of the olive oil sector. *Environ. Technol. Innov.* **2023**, *32*, 103365. [\[CrossRef\]](#)
28. Ayadi, I.; Gargouri, A.; Guerfali, M. Sustainable Valorization of Olive Mill Wastewater Via Oleaginous Yeasts: Single-Cell Oil Production and Effluent Detoxification. *Waste Biomass Valor.* **2026**, *17*, 439–455. [\[CrossRef\]](#)
29. Robles-Iglesias, R.; Naveira-Pazos, C.; Fernández-Blanco, C.; Veiga, M.C.; Kennes, C. Factors affecting the optimisation and scale-up of lipid accumulation in oleaginous yeasts for sustainable biofuels production. *Renew. Sustain. Energy Rev.* **2023**, *171*, 113043. [\[CrossRef\]](#)
30. Poontawee, R.; Lorliam, W.; Polburee, P.; Limtong, S. Oleaginous yeasts: Biodiversity and cultivation. *Fungal Biol. Rev.* **2023**, *44*, 100295. [\[CrossRef\]](#)
31. Lei, Y.; Wang, X.; Sun, S.; He, B.; Sun, W.; Wang, K.; Chen, Z.; Guo, Z.; Li, Z. A review of lipid accumulation by oleaginous yeasts: Culture mode. *Sci. Total Environ.* **2024**, *919*, 170385. [\[CrossRef\]](#)
32. Pan, L.; Yang, D.; Shao, L.; Li, W.; Chen, G. Isolation of the Oleaginous Yeasts from the Soil and Studies of Their Lipid-Producing Capacities. *Food Technol. Biotechnol.* **2009**, *47*, 215–220.
33. Dey, P.; Maiti, M.K. Molecular characterization of a novel isolate of *Candida tropicalis* for enhanced lipid production. *J. Appl. Microbiol.* **2013**, *114*, 1357–1368. [\[CrossRef\]](#) [\[PubMed\]](#)
34. Legodi, L.M.; Moganedi, K.L.M. Selection of oleaginous yeasts isolated from sugarcane bagasse as the potential producers of single cell oils and other metabolites. *Ann. Microbiol.* **2023**, *73*, 21. [\[CrossRef\]](#)
35. de Souza Miranda Muynarsk, E.; dos Santos, A.A.; Rüntzel, C.L.; Orozco Colonia, B.S.; de Oliveira Penha, R.; Vieira Marques, B.B.; Grunig Humberto da Silva, D.; Alves, S.L., Jr. From discovery to application: Twenty years of *Meyerozyma caribbica*. *FEMS Yeast Res.* **2026**, *26*, foag010. [\[CrossRef\]](#) [\[PubMed\]](#)
36. Navarrete, C.; Estrada, M.; Martínez, J.L. *Debaryomyces hansenii*: An old acquaintance for a fresh start in the era of the green biotechnology. *World J. Microbiol. Biotechnol.* **2022**, *38*, 99. [\[CrossRef\]](#)
37. Canonico, L.; Agarbati, A.; Galli, E.; Comitini, F.; Ciani, M. *Metschnikowia pulcherrima* as biocontrol agent and wine aroma enhancer in combination with a native *Saccharomyces cerevisiae*. *LWT-Food Sci. Technol.* **2023**, *181*, 114758. [\[CrossRef\]](#)
38. Kántor, A.; Hutková, J.; Petrová, J.; Hleba, L.; Kačániová, M. Antimicrobial activity of pulcherrimin pigment produced by *Metschnikowia pulcherrima* against various yeast species. *J. Microbiol. Biotechnol. Food Sci.* **2015**, *5*, 282–285. [\[CrossRef\]](#)
39. Morata, A.; Loira, I.; Escott, C.; del Fresno, J.M.; Bañuelos, M.A.; Suárez-Lepe, J.A. Applications of *Metschnikowia pulcherrima* in Wine Biotechnology. *Fermentation* **2019**, *5*, 63. [\[CrossRef\]](#)
40. Karayannis, D.; Papanikolaou, S.; Vatistas, C.; Paris, C.; Chevalot, I. Yeast Lipid Produced through Glycerol Conversions and Its Use for Enzymatic Synthesis of Amino Acid-Based Biosurfactants. *Int. J. Mol. Sci.* **2023**, *24*, 714. [\[CrossRef\]](#)
41. Di Fidio, N.; Minonne, F.; Antonetti, C.; Raspolli Galletti, A.M. *Cutaneotrichosporon oleaginosus*: A Versatile Whole-Cell Biocatalyst for the Production of Single-Cell Oil from Agro-Industrial Wastes. *Catalysts* **2021**, *11*, 1291. [\[CrossRef\]](#)
42. Beopoulos, A.; Chardot, T.; Nicaud, J.-M. *Yarrowia lipolytica*: A model and a tool to understand the mechanisms implicated in lipid accumulation. *Biochimie* **2009**, *91*, 692–696. [\[CrossRef\]](#) [\[PubMed\]](#)
43. Colacicco, M.; Ciliberti, C.; Agrimi, G.; Biundo, A.; Pisano, I. Towards the physiological understanding of *Yarrowia lipolytica* growth and lipase production using waste cooking oils. *Energies* **2022**, *15*, 5217. [\[CrossRef\]](#)
44. da Silva, J.L.; Sales, M.B.; de Castro Bizerra, V.; Nobre, M.M.R.; de Sousa Braz, A.K.; da Silva Sousa, P.; Cavalcante, A.L.G.; Melo, R.L.F.; Gonçalves De Sousa Junior, P.; Neto, F.S.; et al. Lipase from *Yarrowia lipolytica*: Prospects as an Industrial Biocatalyst for Biotechnological Applications. *Fermentation* **2023**, *9*, 581. [\[CrossRef\]](#)
45. Papagora, C.; Roukas, T.; Kotzekidou, P. Optimization of extracellular lipase production by *Debaryomyces hansenii* isolates from dry-salted olives using response surface methodology. *Food Bioprod. Process.* **2013**, *91*, 413–420. [\[CrossRef\]](#)

46. Takaç, S.; Sengel, B.S. Extracellular lipolytic enzyme activity of a newly isolated *Debaryomyces hansenii*. *Prep. Biochem. Biotechnol.* **2010**, *40*, 28–37. [\[CrossRef\]](#)
47. Fickers, P.; Marty, A.; Nicaud, J.-M. The lipases from *Yarrowia lipolytica*: Genetics, production, regulation, biochemical characterization and biotechnological applications. *Biotechnol. Adv.* **2011**, *29*, 632–644. [\[CrossRef\]](#)
48. Arous, F.; Azabou, S.; Jaouani, A.; Zouari-Mechichi, H.; Nasri, M.; Mechichi, T. Biosynthesis of single-cell biomass from olive mill wastewater by newly isolated yeasts. *Environ. Sci. Pollut. Res.* **2016**, *23*, 6783–6792. [\[CrossRef\]](#)
49. Laribi, A.; Zieniuk, B.; Bouchedja, D.; Elmechta, L.; Becila, S. Valorization of olive mill wastewater via *Yarrowia lipolytica*: Sustainable production of high-value metabolites and biocompounds—A review. *Fermentation* **2025**, *11*, 326. [\[CrossRef\]](#)
50. Dourou, M.; Kancelista, A.; Juszczak, P.; Sarris, D.; Bellou, S.; Triantaphyllidou, I.-E.; Rywinska, A.; Papanikolaou, S.; Aggelis, G. Bioconversion of Olive Mill Wastewater into High-Added Value Products. *J. Clean. Prod.* **2016**, *139*, 957–969. [\[CrossRef\]](#)
51. Sayın, B.; Polat, Z.; Kaban, G. Enhanced Lipid Yield from Olive-Mill Wastewater by *Yarrowia lipolytica* NRRL YB-423. *J. Agric. Prod.* **2025**, *6*, 32–40. [\[CrossRef\]](#)
52. Caporusso, A.; De Bari, I.; Liuzzi, F.; Albergo, R.; Valerio, V.; Viola, E.; Pietrafesa, R.; Siesto, G.; Capece, A. Optimized Conversion of Wheat Straw into Single Cell Oils by *Yarrowia lipolytica* and *Lipomyces tetrasporus* and Synthesis of Advanced Biofuels. *Renew. Energy* **2023**, *202*, 184–195. [\[CrossRef\]](#)
53. Abeln, F.; Chuck, C.J. The history, state of the art and future prospects for oleaginous yeast research. *Microb. Cell Fact.* **2021**, *20*, 221. [\[CrossRef\]](#)
54. Rharrabti, Y.; El Yamani, M. Olive Mill Wastewater: Treatment and Valorization Technologies. In *Handbook of Environmental Materials Management*; Hussain, C., Ed.; Springer: Cham, Switzerland, 2019; pp. 1659–1686. [\[CrossRef\]](#)
55. El Hassani, F.Z.; El Karkouri, A.; Errachidi, F.; Merzouki, M.; Benlemlih, M. The impact of olive mill wastewater spreading on soil and plant in arid and semi-arid areas. *Environ. Nanotechnol. Monit. Manag.* **2023**, *20*, 100798. [\[CrossRef\]](#)
56. Ghilardi, C.; Sanmartin Negrete, P.T.; Rodríguez Gutiérrez, G.; Monetta, P.M.; Arroyo López, F.N.; Hornero-Méndez, D.; Carelli, A.A.; Borroni, V. Influence of olive mill waste phenolic compounds levels on carotenoid production by *Rhodotorula* spp. *Process Biochem.* **2022**, *120*, 275–286. [\[CrossRef\]](#)
57. Crognale, S.; D’Annibale, A.; Federici, F.; Fenice, M.; Quarantino, D.; Petruccioli, M. Olive oil mill wastewater valorization by fungi. *J. Chem. Technol. Biotechnol.* **2006**, *81*, 1547–1555. [\[CrossRef\]](#)
58. Sayadi, S.; Allouche, N.; Jaoua, M.; Aloui, F. Detrimental effects of high molecular-mass polyphenols on olive mill wastewater biotreatment. *Process Biochem.* **2000**, *35*, 725–735. [\[CrossRef\]](#)

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.