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Isolation and Characterization of a *Saccharomyces Cerevisiae* Strain from Olive Mill Wastewater for Efficient Bioethanol Production

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The selection of efficient yeast strains with robust tolerance to complex waste substrates is a key to effective bioethanol production. This study aims to identify yeasts strains with significant ethanol production capability and tolerance to polyphenols, isolated from olive oil wastewater (OMW). Morphological identification included colony observations, where strains exhibited characteristic colony forms, sizes, and textures. Fermentability tests using Durham tubes at 30℃ for 72 h assessed CO_2 production, while yeast tolerance was evaluated by exposing the strains to Sabouraud medium supplemented with increasing concentrations of OMW (10%, 25%, 50%, 75%, and 100% v/v). Ethanol production was monitored across these concentrations, revealing that strain Y17, identified as *Saccharomyces cerevisiae* through ITS rRNA sequencing, achieved concentrations ranging from 0.5 g/l at 4 h to 11.3 g/l at 72 h, with a corresponding yield of 0.45 g ethanol/g glucose. These results demonstrate the strain's significant tolerance and productivity under high polyphenol conditions. This work provides novel insight into the ability of a native *S. cerevisiae* strain to ferment undiluted, untreated OMW efficiently, without the need for detoxification or supplementation. The findings suggest that *S. cerevisiae* derived from OMW has strong potential for bioethanol production from industrial waste byproducts, supporting sustainable biofuel applications and offering a viable solution for waste management in olive oil-producing regions.

Keywords: ***Saccharomyces cerevisiae* Y17, olive oil wastewater, polyphenol tolerance, bioethanol production, phenolic inhibitors, industrial waste valorization**

Introduction

The global demand for sustainable and renewable energy sources has recently fueled interest in bioethanol production as a cleaner and environmentally friendly alternative to traditional fossil fuels [1]. Bioethanol is usually produced by fermenting sugars and starches using different microorganisms and among these, yeasts, such as *S. cerevisiae*, are commonly used due to their high fermentation capacity and high tolerance for ethanol [2]. Traditional bioethanol production used to rely on carbohydrates rich feedstocks such as sugarcane, corn, and other crops, which is considered one of the main food resources and may lead to a competition about this resource and agricultural land use and environmental sustainability [3–5].

Agro-industrial by-products, such as olive oil wastewater

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(OMW), represent a promising substrate for bioethanol production. Generated in large quantities during olive oil extraction, OMW is a significant by-product in olive-producing countries. While its high organic matter and sugar content offer potential for microbial growth and fermentation [6, 7], OMW also contains inhibitory compounds such as polyphenols and fatty acids, which limit its utility in bioethanol processes [8, 9]. These polyphenols, known for their antimicrobial properties, disrupt microbial cell membranes and metabolic pathways, reducing fermentation efficiency [10, 11]. Managing OMW poses both environmental and industrial challenges: its polluting nature requires sustainable disposal strategies, while its chemical complexity demands robust microbial strains capable of tolerating inhibitory conditions. Identifying such strains could transform OMW from an environmental liability into a resource, enhancing bioethanol yields and advancing circular economy practices in olive-oil-producing regions globally [12]. To efficiently transform OMW into a substrate for bioethanol production, it is essential to identify or develop yeast strains with enhanced tolerance to multiple stressors, including high polyphenol concentrations and ethanol itself [13, 14]. Yeasts capable of withstanding both high-polyphenol and high-ethanol environments while maintaining fermentation efficiency would be able to harness the sugars in OMW despite its complex, inhibitory composition. This requires yeast strains with adaptations at the cellular and molecular level, including robust membrane structures that resist ethanol-induced damage, efficient detoxification mechanisms to neutralize polyphenols, and resilient metabolic pathways to sustain productivity [15]. Developing or selecting such strains is crucial for realizing the potential of waste-based bioethanol production [16]. These specialized strains could transform OMW from an environmental pollutant into a renewable resource, supporting sustainable energy goals and offering a novel solution to waste management in regions like Algeria, which produces 80,000–91,000 tons of olive oil annually, generating up to 2.2 million m^3 of wastewater [17, 18].

While previous studies have investigated the fermentation of OMW [17, 19], many relied on pretreatment steps, substrate dilution, or commercial strains to overcome the inhibitory effects of polyphenols [20, 21]. This study provides novel insight into the ability of a native *S. cerevisiae* strain, isolated directly from OMW, to efficiently ferment untreated OMW without detoxification or external supplementation. This approach reduces processing costs and highlights the potential of indigenous yeast strains adapted to harsh environmental conditions.

Therefore, we seek to isolate and identify yeast strains from OMW with the ability to tolerate the conditions posed by the OMW. Specifically, to identify strains with tolerance to polyphenols, given their inhibitory effects on fermentation. Through morphological and molecular characterization, including ITS rRNA sequencing, the most productive strains were selected for further evaluation. Our objective was to assess these yeasts bioethanol production potential across a gradient of OMW concentrations, ultimately identifying candidates suitable for sustainable bioethanol production from OMW.

Materials and Methods

Sample Collection and Preparation

Three samples (5 L each) of OMW were collected from the Ennakhla facility, located in Medjadja, in the province of Chlef, Algeria. The samples were stored immediately after the decantation process to ensure minimal contamination and were stored in sterile containers at 4℃ until use. Prior to experimentation, the samples were pretreated by filtering through a fine mesh to remove large debris and then centrifuged at 5,600 × *g* for 10 min to separate solid residues. The supernatant was retained and used as the primary substrate for yeast isolation and subsequent analyses. For tolerance testing, the OMW was diluted with distilled sterilized water to achieve concentrations of 10%, 25%, 50%, 75%, and 100% (v/v).

To characterize the composition of the OMW, physicochemical analyses were carried out. The pH was measured (digital pH meter, Hanna Instruments), while chemical oxygen demand (COD) and biochemical oxygen demand (BOD_5) were determined according to standard methods recommended by the American Public Health Association [22]. Acidity was measured by titration with 0.1 M NaOH using phenolphthalein as an indicator. Nitrite concentrations (NO_2^-) were quantified using the colorimetric method with Griess reagent, following the APHA protocol. Total polyphenol content was deter-

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mined using the Folin-Ciocalteu method, as described by [23]. Reducing sugars were quantified as glucose equivalents using the DNS method. Therefore, Table 1 reports total reducing sugars as glucose equivalents rather than individual sugar types [24].

Yeast Isolation and Culturing

Yeast strains were isolated from the collected OMW. Samples were serially diluted in sterile saline solution (0.9% NaCl) to concentrations of 10^{-1} to 10^{-6}. Aliquots (100 µl) of each dilution were spread onto Sabouraud Dextrose Agar plates (Merck KGaA, Germany) (composed of 40 g/l dextrose; 10 g/l peptone; 20 g/l agar and 1 L sterile distilled water), adjusted to pH 5.4 and supplemented with chloramphenicol (0.05 g/l) to inhibit bacterial growth. The plates were incubated at 30℃ for 48 h to allow colony formation.

Distinct colonies were selected based on morphological characteristics such as size, shape, color, and texture. Each colony was streaked onto fresh Sabouraud Dextrose Agar plates to ensure purity. For cell morphology, a loopful of each isolate was stained with methylene blue and suspended in sterile distilled water. The stained cells were examined under a light microscope at X400 magnification to observe cell size, shape, budding patterns, and viability. The purified isolates were maintained at 4℃ on Sabouraud agar slants and periodically sub-cultured to preserve viability.

As a reference strain, a commercial baker's yeast (*S. cerevisiae*, Saf-instant®, France) was used for comparative fermentation experiments. The yeast was rehydrated in sterile distilled water according to the manufacturer's instructions and inoculated under the same conditions as the native Y17 strain for 72 h fermentations in 100% OMW.

Fermentability Testing

The fermentative capability of the isolated yeast strains was assessed using the Durham's tube method described by Reiner [25]. Each isolate was inoculated into test tubes containing 10 ml of Sabouraud Dextrose Broth supplemented with 1% (w/v) glucose. A Durham tube was placed inverted in each test tube to capture carbon dioxide (CO_2) produced during the fermentation.

The test tubes were incubated at 30℃ for 48 h under anaerobic conditions. Gas accumulation in the Durham tubes was recorded as a positive indication of fermentation. Isolates demonstrating positive CO_2 production were selected for further evaluation of ethanol production.

Assessment of Yeast Tolerance to OMW Polyphenols

The ability of isolates to tolerate polyphenols, contained in OMW, supernatant was assessed using Sabouraud medium supplemented with increasing concentrations of OMW (10%, 25%, 50%, 75%, and 100% v/v). Only strains that demonstrated positive results in the fermentability tests were selected for this assessment. The selected yeast isolate was inoculated into 10 ml of the respective medium at a density of 1×10^7 CFU/ml (determined by serial dilution and plate counting) and incubated at 30℃ for 48 h under anaerobic conditions.

Growth was monitored visually by observing turbidity and by measuring optical density at 600 nm (OD_{600}) using a spectrophotometer. Tolerance was determined based on the ability of the yeast strains to grow and maintain viability at higher OMW concentrations. Significant growth was defined as an OD_{600} increase of ≥ 0.15 from the initial inoculum ($OD_{600} = 0.05$) after 48 h, indicating measurable biomass production in 100% OMW.

DNA Extraction and ITS rRNA Sequencing

The most promising yeast isolate was subjected to molecular identification using 5.8S-Internal Transcribed Spacer (ITS) rRNA region sequencing. Genomic DNA was extracted from fresh yeast cultures using the protocol detailed by Fazio and his collaborators [26]. PCR was performed in a final volume of 50 µl using a Thermal Cycler 2720 (Applied Biosystems, USA); the reaction mix consisted of 25 µl of DreamTaq™ Green PCR Master Mix 2X (Thermo Fisher Scientific, USA), 2 µl of primers ITS1 (5′ TCC GTA GGT GAA CCT GCG G 3′) and ITS4 (5′ TCC TCC GCT TAT TGA TAT GC 3'), 16 µl of ultrapure water (DNAse/RNAse free) and 5 µl of previously obtained DNA template. The amplification program was: initial denaturation at 95℃ for 15 min, 35 cycles of 1 min at 95℃ for denaturing, annealing at 54℃ for 2 min, extension for 2 min at 72℃, and a final extension at 72℃ for 10 min. ITS-PCR products were visualized on 1.5% TBE 1X agarose gel (Lonza, Switzerland) stained with 3 µl of GelRed stain (Biotium, USA) under UV transilluminator (Axygen, Gel Documentation System).

Amplified products were subjected to Restriction Fragment Length Polymorphism (RFLP) using *HaeIII* and *HinfI* (Thermo Fisher Scientific) enzymes. Restriction mixtures were incubated at 37℃ for 2 h and then analysed on 2.0% TBE 1X agarose gel at 100 V for 3 h. After comparing the RFLP profiles, a representative isolate for each cluster was selected for sequencing. In detail, amplified products were purified using the Qiaquick PCR purification kit (Qiagen, Germany) and sequenced using the same PCR primers with Sanger method performed by an external service (Eurofins Genomics, Italy).

The ITS rRNA gene sequence of the selected strain Y17 was deposited in the GenBank database [27]. To determine the closest strains, the sequence was submitted to BLAST analysis using the National Center for Biotechnology Information (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Twelve type strains were selected for this analysis, in addition to an outgroup (Torulaspora globosa CBS 764). ITS rRNA sequences were aligned with MUSCLE [28].

The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model [29]. The tree with the highest log likelihood (-1566.20) was retained. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.5515)). The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 49.09% sites). The analysis involved 13 nucleotide sequences. All positions containing gaps and missing data were eliminated. There was a total of 632 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 [30].

Ethanol Productivity Measurement

The ethanol production capacity of the selected yeast strains was evaluated in batch fermentation experiments. Yeast cells were inoculated into 1000 ml fermentation flasks containing 700 ml of OMW as a fermentation medium. The flasks were equipped with an exhaust system allowing gas to escape through a one-way valve fitted with a 22-micron filter to prevent contamination. Additionally, a second outlet was incorporated to facilitate sampling while minimizing the risk of contamination. Fermentation was carried out at 30℃ for 72 h under continuous shaking at 150 rpm.

Samples were collected at predetermined time intervals: 0, 3, 12, 24, 48, and 72 h. At each time point, pH, optical density at 600 nm (OD_{600}), and glucose concentration were measured to monitor fermentation progress.

Glucose concentration was determined using the 3,5-dinitrosalicylic acid (DNS) method, where reducing sugars react with DNS to produce a reddish-brown color. The absorbance at 540 nm was measured and compared against a glucose calibration curve to quantify glucose levels [24].

The ethanol in the fermentation broth was first distilled using a rotary evaporator (rotavapor) to separate it from other components. Ethanol concentration was then determined using the permanganate method, which involves oxidation of ethanol by potassium permanganate ($KMnO_4$) in an acidic medium. The reduction of $KMnO_4$ from purple to colorless was monitored, and ethanol concentration was quantified by comparing against a calibration curve prepared using standard ethanol solutions (Geies and Abdelazim, 2021; Zhang *et al*., 2019).

Statistical Analysis

Statistical analysis were performed using GraphPad Prism 10 (Ver. 10.4.1). Statistical differences between the means of the properties were noted and all analyses were made in triplicate (at a significance level of $p < 0.05$).

Specific growth rate (μ) and its standard error (SE) were calculated by linear regression of ln (OD_{600}) versus time between 6 and 24 h. The slope of the regression provided μ, and SE was derived from the residual standard error of the fit.

Results

Physicochemical Characteristics of OMW

The OMW used in this study was analyzed for key

Table 1. Physicochemical characteristics of OMW.

Parameter	Mean ± SD	Range
pH	4.6 ± 0.2	4.3 – 4.7
Chemical Oxygen Demand (COD)	178 ± 5 gO_2 /l	175 – 185
Biochemical Oxygen Demand (BOD_5)	7 ± 0.3 gO_2/l	6.7 – 7.3
Average Acidity (%)	1.65 ± 0.05	1.6 – 1.7
Nitrite	30 ± 2 mg/l	28 – 32
Total Polyphenols	5.77 ± 0.1 g/l	5.6 – 5.8
Glucose concentration	26.0 ± 0.4 g/l	25.6 – 26.4
Total reducing sugars	~29.5 ± 0.7 g/l (as glucose equivalents)	28.8 – 30.2

physicochemical parameters and is summarized in Table 1. The effluent exhibited an acidic pH, high organic load, and a significant concentration of polyphenols, which are known as inhibitors of microbial fermentation.

Reducing sugars were quantified as glucose equivalents using the DNS method. Other sugars such as fructose and sucrose have been reported in OMW [33], but were not analyzed in this study.

Gas Production Assessment

The fermentability test was conducted on 13 yeast strains to evaluate their ability to produce biogas. Among all the strains tested, only Y17 demonstrated positive biogas production, indicating its ability to ferment under these conditions. The other strains (Y02, Y03, Y06, Y07, Y15, Y16, Y18, Y19, Y20, Y21, YP1, and YP2) did not produce any detectable biogas.

Assessment of Yeast Tolerance to OMW Polyphenols

The growth of the selected strain Y17 was evaluated in OMW across varying concentrations (10%, 25%, 50%, 75%, and 100%) over a 48 h incubation period (Table 2). Optical density (OD_{600}) measurements were recorded at different time points to assess yeast growth dynamics and tolerance to increasing OMW levels. As illustrated in Figure 1, Y17 exhibited gradual adaptation, with higher growth observed in diluted OMW (10–50%) compared to more concentrated samples (75–100%), where growth was reduced but still detectable.

Table 2 presents the specific growth rate (μ ± SE), doubling time, and maximum OD_{600} of *S. cerevisiae* Y17

Table 2. Specific growth rates (μ), doubling times (td) and maximum OD_{600} of Y17 at different OMW concentrations.

OMW (%)	M (h^{-1}) ± SE	td (h)	R^2	Max OD_{600}
0	0.037 ± 0.002	18.7	0.98	2.28
10	0.032 ± 0.003	21.7	0.89	1.52
25	0.107 ± 0.005	6.48	0.96	1.32
50	0.083 ± 0.004	8.35	0.94	1.09
75	0.058 ± 0.003	11.9	0.91	0.81
100	0.029 ± 0.002	23.9	0.97	0.47

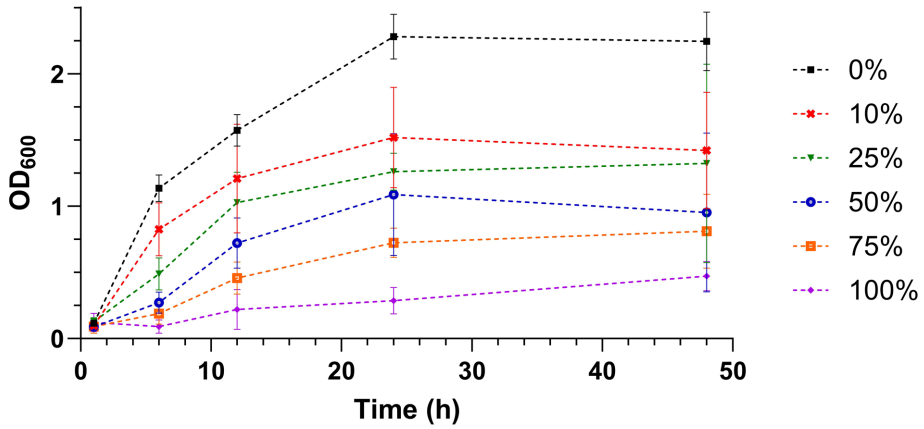

Fig. 1. Growth of Y17 strain in olive oil wastewater at different concentrations over time.

grown in OMW at varying concentrations, using a common 6–24 h exponential window. The specific growth rate declined as OMW concentration increased, from 0.037 h^{-1} in the control to 0.029 h^{-1} in 100% OMW. Intermediate OMW levels (25–50%) supported the highest growth rates (0.083–0.107 h^{-1}).

Yeast Strain Identification and Phylogenetic Analysis

The retained yeast strain, designated Y17, was initially selected based on its strong fermentation performance. Morphological observations confirmed typical yeast cell features, while definitive identification was achieved through ITS rRNA gene sequencing, which classified the isolate as *S. cerevisiae*. Members of the genus *Saccharomyces* are known for their facultative anaerobic metabolism and ability to efficiently ferment a wide range of sugars, making them valuable for bioethanol production [34]. Under aerobic conditions, they are effective at transforming sugars into energy, biomass and CO_2, while under anaerobic conditions, they use them in alcohol fermentation [35].

5.8S-Internal Transcribed Spacer (ITS) rRNA sequencing is generally used for yeast identification. The phylogenetic tree estimating the degree of relatedness between the isolate the strain Y17 and its similar sequences from closely linked yeast is shown in Figure 2. Results of ITS rRNA sequences analysis revealed that the strain Y17 has a great similarity with *Saccharomyces* species. ITS rRNA sequence of the starin Y17 has been deposited in the GenBank database, accession number is: PQ566690.

The phylogenetic analysis indicates that of the strain Y17 is clustered together with *S. cerevisiae* CBS 1171, *S. paradoxus* CBS 432 and *S. cariocanus* NRRL 27337 in the green clade (Fig. 2). Analysis managed with ITS rRNA fragments showed a high similarity between the yeast strains Y17 and *S. cerevisiae* CBS 1171 (98.68%).

Fermentation Results with 100% OMW

Fermentation of OMW at 100% concentration was carried out using both the native *S. cerevisiae* strain Y17 (Fig. 3A) and a commercial yeast strain (Fig. 3B), under identical conditions. The performance of each strain was evaluated over 72 h by monitoring glucose consumption, ethanol production, and pH variation (Fig. 3).

At the start of fermentation (t = 0), glucose concentration was 26.0 ± 0.4 g/l and pH was 4.8 ± 0.1 for both strains. Ethanol was absent in both systems. After 12 h, Y17 produced 2.2 ± 0.2 g/l ethanol, while the commercial strain produced only 1.2 ± 0.1 g/l ethanol.

By 48 h, ethanol production in Y17 reached 8.5 ± 0.4 g/l, with glucose levels dropping to 6.3 ± 0.2 g/l. In con-

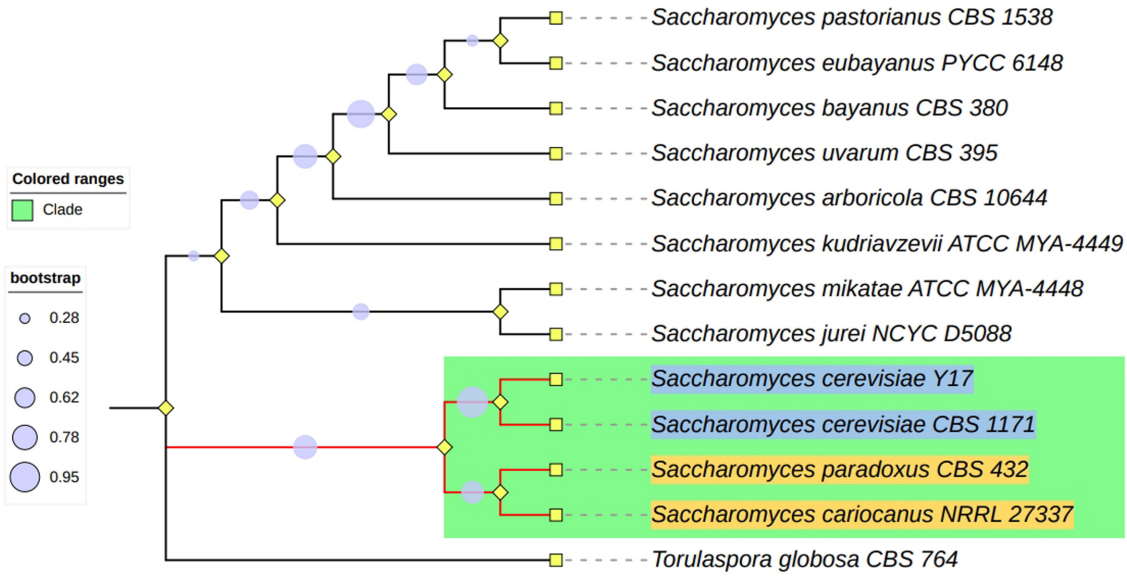

Fig. 2. Maximum likelihood (ML) phylogenetic tree generated from the 5.8S-ITS sequence of the selected strain Y17 (shaded in blue) and its closest type strains. Numbers on branches represent bootstrap values. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. *Torulaspora globosa* CBS 764 served as an outgroup.

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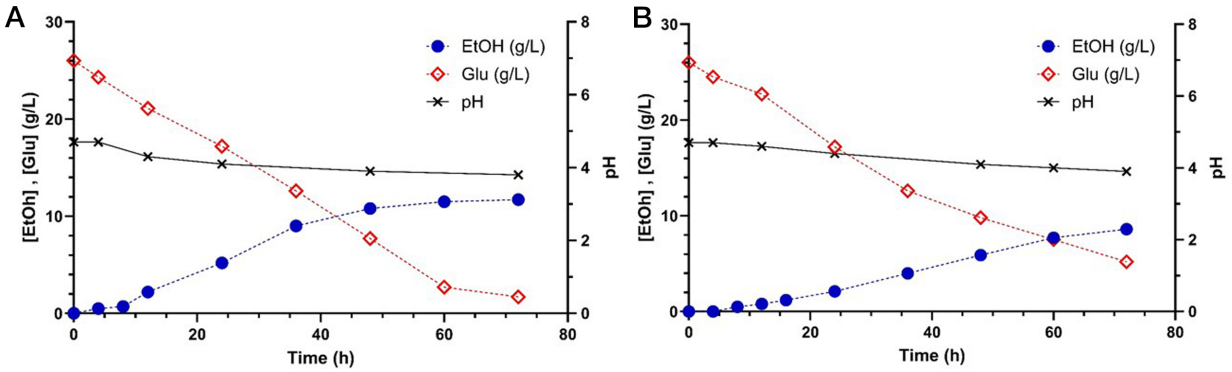

Fig. 3. Comparative fermentation profiles of native strain Y17 (A) and commercial *S. cerevisiae* (B) in 100% olive oil wastewater (OOW) over 72 h.

trast, the commercial strain produced 6.0 ± 0.4 g/l ethanol and retained a higher glucose concentration of 9.0 ± 0.5 g/l. At the end of fermentation (72 h), Y17 achieved a maximum ethanol concentration of 11.3 ± 0.5 g/l and reduced glucose to 1.0 ± 0.1 g/l. The commercial yeast reached a lower ethanol concentration of 8.6 ± 0.4 g/l and left 5.2 ± 0.2 g/l residual glucose.

The pH in both systems decreased progressively throughout the fermentation. Y17 showed a sharper pH drop, reaching 3.8 ± 0.1 at 72 h, compared to 3.9 ± 0.1 for the commercial strain.

The ethanol yield of Y17 was calculated at 0.45 g/g glucose consumed, while the commercial strain achieved a slightly lower yield of 0.41 g/g. These results confirm the superior fermentative capacity and environmental tolerance of the Y17 strain in untreated OMW conditions, with better sugar utilization and ethanol productivity than the commercial counterpart.

Discussion

Physicochemical characteristics of the OMW analyzed in this study are consistent with values commonly reported for similar agro-industrial effluents. The pH measured at 4.6 ± 0.2 falls within the typical acidic range (4.5–5.5) described in the literature [36–38]. The chemical oxygen demand (COD: 178 ± 5 gO_2/l) and biochemical oxygen demand (BOD_5: 7 ± 0.3 gO_2/l) resulted in a high COD/BOD_5 ratio (>25), indicating the presence of poorly biodegradable organic compounds. This finding aligns with those of Gueboudji *et al.* [39] and with our previous work on similar OMW collected from the same region of Chlef, Algeria, where high COD and polyphenol content were also observed [19].

The total polyphenol concentration of OMW in this study (5.77 ± 0.1 g/l) is within the inhibitory range reported for microbial growth, as OMW typically contains 0.5–8 g/l of phenolics depending on processing conditions [40]. Polyphenols are well known for their antimicrobial activity [10, 23] and studies have shown that even low concentrations (0.1–0.5 g/l) of compounds such as ferulic acid or vanillic acid can impair yeast growth, while levels above 1–2 g/l strongly inhibit most strains [41]. Comparative studies further confirm that inhibitors like vanillin, syringaldehyde, and coumaric acid markedly reduce yeast growth, with *Saccharomyces* species generally more tolerant than non-*Saccharomyces* species [42]. These findings underline the significance of Y17's tolerance, since it was able to grow and ferment efficiently at nearly 6 g/l phenolics.

Microscopic observation of the isolates confirmed typical yeast cell structures with some variation in cell shape and size. These observations were used only for preliminary characterization, while molecular identification was performed to accurately classify the strains.

Despite these morphological variations, fermentability tests using Durham tubes showed that only strain Y17 was able to produce biogas, confirming its ability to carry out fermentation under the tested conditions. This finding highlights Y17's unique metabolic capacity compared to the other isolates, which showed growth but no detectable gas production. Such absence of fermentation in most strains may be linked to limitations of the Sabouraud medium or regulatory mechanisms [13].

High glucose concentrations may have triggered catabolite repression, a phenomenon where yeast preferentially metabolizes glucose over other available substrates, thereby inhibiting the use of alternative carbon sources. Under these conditions, glycolysis and fermentation remain dominant, while the metabolism of other nutrients is repressed [16].

Genetic diversity among the strains likely contributed to variations in fermentative capacity, as some strains may lack the enzymatic machinery needed for efficient sugar conversion under the provided conditions [2, 43]. Previous studies have shown that not all yeast species exhibit equal ability to ferment sugars present in various media, which could account for the observed differences in biogas production [43–45].

The ability of yeast strains to tolerate polyphenols is critical for successful bioethanol production from OMW, which is characterized by its high polyphenolic content. In this study, strain Y17 exhibited significant resilience to increasing concentrations of OMW, showing its potential for fermentation in complex environments. Polyphenols are known for their antimicrobial properties, which can inhibit microbial growth and fermentation efficiency by damaging cell membranes and disrupting essential metabolic processes [8, 10, 46]. Strain Y17's capacity to thrive in high concentrations of OMW suggests that it possesses specific adaptations, such as robust membrane structures and efficient detoxification mechanisms, allowing it to neutralize the inhibitory effects of polyphenols [13, 46]. These results indicate the importance of selecting yeast strains with enhanced polyphenol tolerance for optimizing bioethanol production from agro-industrial by-products.

Phylogenetic analysis of the ITS rRNA sequence (Fig. 2) revealed that strain Y17 clustered within a monophyletic group containing *S. cerevisiae* CBS 1171, *S. paradoxus* CBS 432, and *S. cariocanus* NRRL 27337, showing the closest relationship to *S. cerevisiae* CBS 1171. Based on these results, strain Y17 was identified as *S. cerevisiae*.

These findings coincide with the initial designation of the strain Y17 in the *Saceharomycetaceae* species generated phenotypically. Morphologically, this strain exhibited characteristics consistent with *S. cerevisiae*, including typical cell shapes, budding patterns, and colony morphology observed on Sabouraud Dextrose Agar. The ability of strain Y17 to produce ethanol from OMW shows its potential application in sustainable bioethanol production, transforming a challenging waste product into a valuable energy resource. Prior research has demonstrated that *S. cerevisiae* exhibits remarkable adaptability to stressors like high ethanol concentrations and polyphenolic compounds, which enhances its fermentation capabilities [1, 3]. Furthermore, the strain's inherent metabolic flexibility allows it to efficiently utilize sugars present in complex substrates despite the presence of inhibitory substances [5]. The outcomes from this study not only facilitate to the understanding of yeast diversity in OMW but also highlighted the importance of selecting robust strains capable of thriving in complex substrates for effective bioethanol production.

In fermentation trials, Y17 achieved a maximum ethanol concentration of 11.3 g/l at 72 h following a typical fermentation profile, correlating with significant glucose consumption (from 26.0 g/l to 1.0 g/l) and a decline in pH from 4.8 to 3.8. The ethanol yield of approximately 0.45 g ethanol per g glucose consumed aligns with the upper end of values reported for untreated or minimally pretreated OMW media, where yields typically range from 0.40–0.51 g/g [47–49]. Furthermore, ethanol concentrations above 10 g/l by 48 h are in line with values reported by Sarris et al., where 12–15 g/l ethanol was produced in shake-flask and batch fermentations under similar conditions.

Importantly, when compared under identical conditions, the commercial Saf-instant® baker's yeast showed lower ethanol production (8.6 g/l) and left higher residual glucose (5.2 g/l), with a slightly reduced yield of 0.41 g/g. The ethanol productivity of Y17 (0.16 $g \cdot l^{-1} \cdot h^{-1}$) was also higher than that of the commercial strain (0.12 $g \cdot l^{-1} \cdot h^{-1}$), further demonstrating the superior fermentation performance of Y17 in untreated OMW. These results indicate that Y17's enhanced activity is likely linked to its environmental adaptation to phenolic-rich conditions. This observation supports findings by Parapouli *et al.* [15], who noted that wild-type *S. cerevisiae* strains often outperform commercial strains in complex or inhibitory substrates.

Interestingly, ethanol production occurred without the need for extensive pretreatment of the OMW, with fermentation completing successfully even as the pH declined from an initial 4.8 to 3.8 by the end of the 72 h

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period. This suggests that *S. cerevisiae* exhibits robust tolerance to acidification and residual phenolics in the OMW, consistent with findings by Nikolaou and Kourkoutas (2018), who reported high fermentation efficiency with immobilized cells in OMW-molasses blends. However, the absence of immobilization in this experiment did not appear to negatively impact ethanol production efficiency, further reflecting the adaptability of Y17 under suboptimal conditions. The slight but gradual decrease in pH during fermentation is typical in OMW systems and likely attributed to organic acid production and yeast metabolism, as noted in studies where pH declines to 3.5–3.8 by the end of fermentation [47].

Moreover, compared to other studies relying on non-*Saccharomyces* yeasts (*e.g.*, *Pichia* or *Candida*) for OMW fermentation, Y17 demonstrated superior productivity and phenolic tolerance [43, 50]. While some non-conventional yeasts show potential for polyphenol detoxification, they often struggle under elevated ethanol concentrations [51–53].

The findings of this study establish *S. cerevisiae* strain Y17 as a promising candidate for direct OMW fermentation, capable of simultaneously overcoming the toxic effects of polyphenols and achieving high ethanol yields. Future work could focus on scaling up fermentation trials, further analyzing Y17's physiological and genetic mechanisms of phenol resistance, and exploring its performance in co-culture systems with microbes capable of preemptive detoxification of OMW phenolics.

Conclusion

This study demonstrated the successful isolation and characterization of a native yeast strain *S. cerevisiae* Y17, from OMW a substrate known for its complex composition, high polyphenol content, and inhibitory nature. Y17 exhibited remarkable tolerance to 100% untreated OMW and strong ethanol production capabilities, reaching 11.3 g/l after 72 h of fermentation.

These findings highlight the adaptability of *S. cerevisiae* Y17 to harsh environmental conditions and its efficiency in glucose utilization while maintaining metabolic activity under acidifying conditions. Notably, Y17 outperformed the commercial *S. cerevisiae* Saf-instant® strain under the same conditions, confirming the potential of wild-type strains for fermenting non-conventional substrates.

Overall, the results support the valorization of OMW as a viable feedstock for sustainable bioethanol production, offering a dual benefit of renewable energy generation and effective waste management. The identification of a robust, naturally adapted strain paves the way for future studies focused on process optimization, scale-up, and integration with detoxification or immobilization strategies. By leveraging agro-industrial byproducts such as OMW, this approach contributes to reducing environmental burdens and supports the development of a circular bioeconomy.

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Author Contributions

Djawad Rouam: Conceptualization, Methodology, Validation, Writing – original draft, review and editing. **Malika Meziane**: Conceptualization, Supervision, Methodology, Validation, Writing –critical review. **Mohammed El Amine Bendaha**: Conceptualization, Supervision, Methodology, Validation, Writing – critical review. **Alessandra Pino**: Conceptualization, Methodology, Validation, Writing – original draft preparation. **Cinzia Lucia Randazzo**: Conceptualization, Methodology, Validation, Writing – original draft preparation.

Data Availability

Data will be made available on request.

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Conflict of Interest

The authors have no financial conflicts of interest to declare.

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