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Isolation and assessment of highly sucrose-tolerant yeast strains for honey processing factory's effluent treatment

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Abstract

Wastewater from many food and beverage manufacturers is enriched in organic content, and it must therefore be treated before being discharged to comply with the strict environmental regulations to protect the final water quality. Concretely, the honey processing wastewater, that remains in holding tanks until is disposal, is a rich source of sugars and this high level of organic material will degrade the water quality if not treated properly provoking an imbalance in the ecosystem. There are different strategies for an adequate treatment of this wastewater effluent to obtain a sustainable usage. One of the techniques that is more cost-effective and environmental friendlier than chemical procedures used for water remediation, is the use of microorganisms (including algae, fungi, yeasts, or bacteria). Given that they are fast-growing, robust, and metabolically diverse, yeast strains are often used for wastewater treatment. In this work, we have studied the potential for bioremediation of non Saccharomyces yeast isolated from a honey processing wastewater generated by an Argentine exporting company. The inoculation of these yeast strains to the existing flora in the honey wastewater yielded a better improvement in the treatment yield. These results suggest that these strains display a promising role could for optimizing bioremediation strategies in industrial wastewater treatment processes.

Keywords Honey effluent, Yeast, Wastewater treatment, Sucrose-tolerance, Bioremediation, Sustainable process

Introduction

Nature-based solutions encompass various actions that society can undertake to, among other objectives, effectively ensure water security, thereby offering benefits for biodiversity. These solutions are directly linked to the Sustainable Development Goals (SDGs) (World Health Organization 2015). Consequently, integrated water management and wastewater treatment can yield greater advantages compared to conventional waste treatment methods (UN 2015).

The wastewater discharged by honey processing companies (HPW) encompasses a wide range of constituents, mostly simple sugars, resulting in elevated levels of Total Carbon (TC), Total Organic Carbon (TOC),

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Chemical Oxygen Demand (COD) and Biochemical Oxygen Demand (BOD). It also content traces of Nitrogen, leading to typical C:N ratios over 150 (Malik et al. 2022; Combarros-Fuertes et al. 2019; da Silva et al. 2016; Zamora and Chirife 2006). Moreover, HPW could include effluents from the cleaning of industrial equipment and trucks associated with the honey processing facility. In the present study, HPW is gathered and stored in holding tanks next to the industrial building until it can be adequately managed for final disposal according to Argentinean legislation, incurring related expenses. Hence, it becomes imperative to implement an effective treatment approach to prevent environmental contamination within our specific context.

Yeast treatment technology can serve as a valuable tool in mitigating the adverse impact of industrial honey wastewater. Extensive research has demonstrated that yeast exhibits adaptability to various types of wastewater and extreme conditions, displaying remarkable resilience and resistance to cleaners, disinfectants, acids, osmotic pressure resulting from a high carbohydrate content, and other factors (Qiu et al. 2019). Additionally, yeast metabolism facilitates the conversion of organic matter into non-toxic and nutrient-rich individual organic compounds (Yang and Zheng 2014). Non Saccharomyces yeast could be especially well suited for honey processing wastewater since it can thrive in waters with high levels of organic substances and suspended solids (SS), reducing the oil content of wastewater by up to 100 times (Nicula et al. 2023).

The bioremediation potential of non Saccharomyces yeast was investigated by Wang et al. (2018), who identified several advantages over other microorganisms. These advantages include their high adaptability to diverse environments without the need for nutrient supplements. Some non Saccharomyces yeast can also thrive in non-sterile conditions, similar to the conventional activated sludge process dominated by bacteria. Furthermore, a significant enhancement in treatment efficiency was observed when mixed yeast strains were employed, thereby augmenting bioremediation capacity.

An even more promising approach to the treatment was described by Ling et al. (2014), and involves the combination of yeast and microalgae for wastewater treatment in a distillery plant. This co-culture strategy offers significant advantages, with microalgae releasing oxygen for yeasts while consuming nitrogen and phosphorus from the wastewater. Yeasts in return, provide carbon dioxide for photosynthesis, primarily aiding in the absorption of organic matter.

In the current study, we investigate the biotechnological potential of non Saccharomyces yeast isolated from HPW generated by an Argentine honey exporting company. This development could offer a solution for treating this challenging wastewater.

Materials and methods

Yeast isolation and identification

Effluent Feedstock (residual honey water, RHW) was collected from a honey processing company, coordinates −34.8753, −58.5250, between September 2022 and July 2023. The yeast strains obtained and isolated were deposited in Instituto Nacional de Tecnología Agropecuaria (INTA)-Centro Regional Mendoza-San Juan, Argentina gene bank (register CoMIM4424, CoMIM4425, CoMIM4426 corresponing to H1, H2, H3, resp.).

Yeast isolation

Yeast isolation was carried out via serial dilutions, combining cultures in liquid Yeast Extract Beef (YEB) medium supplemented with ampicillin (10 mg/L) and streptomycin (10 mg/L) at pH 5.5 (adjusted with 1 N HCl), followed by cultures on agarized (1% agar-agar) YEB media (Pincus et al. 2007; Echeverrigaray et al. 2021; Cruz et al. 2022; Elías Leonardo et al. 2022). Initially, 10 ml of the sample were taken and inoculated into 90 ml of liquid YEB medium. The liquid culture was incubated for 72 h at 28 °C and 100 rpm until yeast growth in the medium became evident by turbidity. From this liquid culture, serial dilutions (10^{-1} to 10^{-4}) were prepared and triplicates were plated on agarized YEB medium supplemented with antibiotics. These plates were then incubated at 28 °C until colonies developed. Different yeast morphotypes were selected from each plate and used for subsequent rounds of liquid cultures and isolation through serial dilutions until plates with a single morphotype were obtained. Pure strains were preserved in 20% glycerol at −80 °C. Among the yeast strains obtained from the isolation, three (H1 to H3) were selected primarily for their growth rate in liquid medium, and these yeasts were utilized throughout the remainder of the study.

Total DNA extraction

Total DNA was extracted from a biomass sample of each colony (500 mg) using a tissue homogenizer (Polytron PT 10-35, Kinematica) in three cycles at 28,000 rpm for 20 s on ice, following the DNeasy Plant Mini Extraction Kit protocol (Qiagen) as per the manufacturer's instructions. The integrity of genomic DNA was assessed through agarose gel electrophoresis, and the quantity and purity of DNA were determined by measuring the absorbance at 260 nm, along with absorbance ratios at 260/280 and 260/230, using a Thermo Scientific™ Multiskan™ GO Spectrophotometer.

Yeast identification and phylogenetic analysis

The ITS1, 5.8S, ITS2, and D1/D2 of the 26S rDNA region were amplified with ITS1 and NL4 primers (White et al. 1990; O'Donnell and Cigelnik 1998). Sequencing was performed by Macrogen Inc., South Korea, after consensus sequences generation by BioEdit 7.7 software (Hall 1999) sequences were compared using the National Center for Biotechnological Information (NCBI) BLAST tool (Altschul et al. 1990). The dataset under analysis comprises sequences from the three isolates under study and sequences from 39 previously identified organisms from related species and genera, *Candida silvatica* clade was chosen as outgroup (Table 1). Alignment of the dataset was carried out using the MUSCLE algorithm within MEGA11 (Tamura et al. 2021), concatenated, and trimmed using GBlocks (Kalyaanamoorthy et al. 2017). Phylogenetic analyses were conducted using PhyloSuite v2.0 (Zhang et al. 2020; Xiang et al. 2023). To assess the robustness of our data Maximum Likelihood (ML), and Bayesian Inference (BI) approaches were employed for phylogenetic inference. Maximum Likelihood phylogenies were inferred using IQ-TREE v2.2.0 (Nguyen et al. 2015) under the model automatically selected by IQ-TREE ('Auto' option in IQ-TREE) for 20,000 ultrafast bootstraps (Minh et al. 2013), as well as the Shimodaira–Hasegawa–like approximate likelihood-ratio test (Guindon et al. 2010). Bayesian Inference phylogenies were inferred using MrBayes 3.2.7a (Ronquist et al. 2012) under GTR+I+G+F model (2 parallel runs, 5000000 generations), in which the initial 25% of sampled data were discarded as burn-in. The visualization of the phylogenetic trees was carried out in iTOL v6 (Letunic and Bork 2007). Clades were considered strongly supported when either PP or BS≥ 0.95 (Hyde et al. 2013).

Experimental design and measurements

Bioprocesses were conducted in 250 mL Erlenmeyer flasks containing 50 mL of a 2% V:V solution of *C. ethanolica*·strain H3, which were fed with YEB, RHW, or sterile RHW. The sterile RHW was obtained through autoclaving at 0.1 MPa for 20 min using an Arcano 80 L® Chamberland equipment. The cultures were maintained at 28° ± 2 °C with agitation at 100 rpm.

Analytical methods

Cell growth was assessed by turbidimetric measurements at an optical density of 600 nm (using a SHIMADZU Spectrophotometer). Biomass was determined by measuring fresh weight (FW) and dry weight (DW) after 72 h at 70 °C or until a constant weight was achieved. Total sugar concentration was determined as described by Dubois et al. (1951, 1956) in the culture medium. The total soluble protein content was evaluated with bovine serum albumin as a standard according to Bradford (1976). Biomass extracts were obtained by cell disruption using a homogenizer (Polytron PT 10-35, Kinematica) and adding cold phosphate-buffered saline (0.24 g KH2PO4/L, 1.44 g Na2HPO4/L, 0.2 g KCl/L, 8 g NaCl/L, pH 7.0–7.2), which also contained 10 µg leupeptin/mL. The extract was then centrifuged at 14,000*g* for 20 min at 4 °C, and the supernatant was used for analytical tests.

Stoichiometric parameters were determined using Fermentool software (Marconi et al. 2014). Kinetic cell growth was estimated using the formula:

$$\mathrm{dx/(dt)} = \mu * \mathrm{X}.$$

where x represents the biomass obtained at time (t), and µ is the specific growth rate. The duplication time was calculated as ln(2)/µ.

Biomass yield with respect to substrate consumed was calculated as:

$$\mathrm{Yx/s} = \mathrm{dx/ds}.$$

where s represents the substrate consumed.

Volumetric productivity (P) was also calculated as follows:

$$\mathrm{P} = \mathrm{dx/dt} * 1/(\mathrm{vol.})$$

where x represents the biomass obtained in a volume (vol.) over a unit of time (t).

Statistical analysis

Physicochemical and microbiological analyses were conducted both at the beginning and at the end of the bioprocess, with analytical determinations performed in triplicate. The results were assessed using ANOVA with a post-hoc Tukey test for multiple comparisons or the Kruskal-Wallis test for non-normally distributed variables, all analysed using Infostat software (Tukey 1953; di Rienzo et al. 2013).

Kinetic studies were carried out using the 'Fermentertootool' (https://www.fermentertool.com/en/).

Results

Samples were collected from holding tanks (15 m^3) where effluents were stored until they could be properly disposed of. From the isolation and selection scheme, three yeast strains were selected primarily based on their morphology and growth rate. These strains were employed in further experiments and cryopreserved in glycerol 20% at – 80 °C.

Molecular identification

The PCR amplification yielded a 990-base-pair product comprising 582 base pairs from the D1/D2 domain of

Table 1 Internal transcribed spacer (ITS) and the large subunit (LSU) sequences from previously identified species and specimens used in the phylogenetic study together with their GenBank accession numbers

	ITS		LSU
Candida awuaii CBS 11011	NR_151796.1	*Candida awuaii CBS 11011*	NG_058977.1
Candida cabralensis CBS 11679	NR_153279.1	*Candida cabralensis CBS 11679*	NG_055163.1
Candida californica CBS 989	NR_153280.1	*Candida californica CBS 989*	NG_055093.1
Candida ethanolica CBS 8041	NR_077165.1	*Candida ethanolica NRRL Y-12615*	NG_055105.1
Candida inconspicua CBS 180	NR_111116.1	*Candida inconspicua NRRL Y-2029*	NG_055114.1
Candida pseudolambica CBS 2063	NR_153281.1	*Candida pseudolambica CBS 2063*	NG_060822.1
Candida rugopelliculosa CBS 6377	NR_153282.1	*Candida rugopelliculosa NRRL Y-17079*	NG_055112.1
Candida thaimueangensis ATCC MYA-4468	NR_111358.1	*Candida thaimueangensis ATCC MYA-4468*	NG_042471.1
Candida silvatica MUCL 29938	NR_111303.1	*Candida silvatica NRRL Y-7777*	NG_055240.1
Candida ethanolica H1 (this study)	OR966490	*Candida ethanolica H1 (this study)*	OR966490
Candida ethanolica H2 (this study)	OR966491	*Candida ethanolica H2 (this study)*	OR966491
Candida ethanolica H3 (this study)	OR966492	*Candida ethanolica H3 (this study)*	OR966492
Pichia barkeri CBS 7256	NR_153283.1	*Pichia barkeri NRRL Y-17350*	NG_055118.1
Pichia bovicola DMKU MP6-4	NR_182389.1	*Pichia bovicola DMKU MP6-4*	NG_088241.1
Pichia bruneiensis CBS 12611	NR_173357.1	*Pichia bruneiensis CBS 12611*	NG_075177.1
Pichia cactophila CBS 6926	NR_138243.1	*Pichia cactophila NRRL Y-10963*	NG_055115.1
Pichia cecembensis NRRL Y-27985	NR_164078.1	*Pichia cecembensis NRRL Y-27985*	AM159112.3
Pichia cephalocereana CBS 7273	NR_153285.1	*Pichia cephalocereana NRRL Y-17225*	NG_055121.1
Pichia chibodasensis NBRC 111569	NR_153305.1	*Pichia chibodasensis NBRC 111569*	NG_055170.1
Pichia deserticola CBS 7119	NR_077085.1	*Pichia deserticola NRRL Y-12918*	NG_055106.1
Pichia dushanensis CBS 13912	NR_160321.1	*Pichia dushanensis CBS 13912*	NG_064367.1
Pichia eremophila CBS 7272	NR_153287.1	*Pichia eremophila NRRL Y-17224*	NG_055120.1
Pichia exigua CBS 6836	NR_153288.1	*Pichia exigua NRRL Y-10920*	NG_055111.1
Pichia fermentans ATCC 10651	NR_130688.1	*Pichia fermentans ATCC 10651*	GQ458040.1
Pichia garciniae CBS 10758	NR_153289.1	*Pichia garciniae CBS 10758*	NG_058374.1
Pichia gijzeniarum CBS 15024	NR_168173.1	*Pichia gijzeniarum CBS 15024*	NG_068258.1
Pichia insulana CBS 11169	KM252834	*Pichia insulana CBS 11169*	NG_055166.1
Pichia kluyveri CBS 188	NR_138210.1	*Pichia kluyveri NRRL Y-11519*	NG_055122.1
Pichia kudriavzevii ATCC 6258	NR_131315.1	*Pichia kudriavzevii NRRL Y-5396*	NG_055104.1
Pichia manshurica CBS 209	NR_138211.1	*Pichia manshurica IFO 10726*	NG_055078.1
Pichia membranifaciens CBS 107	NR_111195.1	*Pichia membranifaciens NRRL Y-2026*	NG_042444.1
		Pichia nakasei NRRL Y-7686	NG_055119.1
Pichia nanzhaoensis NYNU 178136	NR_173268.1	*Pichia nanzhaoensis NYNU 178136*	NG_079517.1
		Pichia nongkratonensis BCC 11772	NG_055079.1
Pichia norvegensis CBS 6564	MW284513.1	*Pichia norvegensis NRRL Y-7687*	NG_055113.1
		Pichia occidentalis NRRL Y-7552	NG_055110.1
Pichia paraexigua NYNU 178135	NR_173270.1	*Pichia paraexigua NYNU 178135*	NG_079521.1
		Pichia pseudocactophila NRRL Y-17239	NG_055116.1
Pichia rarassimilans CBS:10901	KY104644	*Pichia rarassimilans CBS 10901*	NG_055081.1
Pichia scutulata CBS:6670	KY104645	*Pichia scutulata NRRL Y-7663*	NG_055117.1
Pichia sporocuriosa CBS 8806	NR_153293.1	*Pichia sporocuriosa NRRL Y-27347*	NG_055107.1
Pichia terricola CBS 2617	NR_153294.1	*Pichia terricola* NRRL YB-4310	NG_055108.1
Candida ethanolica H1 (this study)	OR966490	*Candida ethanolica* H1 (this study)	OR966490
Candida ethanolica H2 (this study)	OR966491	*Candida ethanolica* H2 (this study)	OR966491
Candida ethanolica H3 (this study)	OR966492	*Candida ethanolica* H3 (this study)	OR966492

LSU and 408 base pairs from the ITS1-5.8s-ITS2 region. The LSU fragment, from isolates 1, and 2 exhibited a 99.07% identity with *Candida ethanolica* NRRL Y-12,615 (NG_055105.1), while isolate 3 showed a 99.27% identity with the same sequence. For the ITS region, identities of 92.94%, 94.50%, and 92.84% were observed with *Candida ethanolica* CBS 8041 (NR_077165.1), for isolates 1, 2, and 3, respectively.

When comparing the 582 bp sequence in the D1/D2 domains of the three isolates with that of the type species *Candida ethanolica* CBS 8041, three substitutions were

identified in H1 and H3, while H2 exhibited four substitutions (Fig. 1).

After trimming, 586 positions from the LSU fragment (191 parsimoniously informative) and 167 positions from the ITS fragment (21 parsimoniously informative) were utilized. The concatenated matrix, consisting of 753 positions, was employed for phylogenetic estimation.

In the phylogenetic analysis, the nucleotide frequencies were as follows: A=23.00%, T=23.30%, C=21.60%, and G=25.00%. The SYM+I+G4 model was chosen based on the Bayesian Information Criterion (BIC), and the estimated value of the shape parameter for the discrete Gamma Distribution was 0.476. The tree length was calculated to be 2.757. For Maximum Likelihood (ML) values estimation, a tree topology was automatically computed, and the final maximum log-likelihood for this computation was −5592.284 (Fig. 2).

Growth kinetics and sugar degradation

A comprehensive analysis of growth kinetics and sugar degradation was conducted in both YEB and RHW media using the *C. ethanolica* H3·strain isolated from RHW, and the dynamics of the study were executed through the Fermentertool application at a constant oxygen transfer rate (see M&M).

The biomass of the three strains increased in both culture media (feed with YEB or RHW wastewater), exhibiting similar growth curves (data not shown). As depicted in Fig. 3, both cultures displayed a well-defined S-shaped growth pattern, featuring an extended lag phase lasting up to 10 h in *C. ethanolica* H3·strain (2% V:V inoculum). Following this, an exponential growth phase occurred, lasting 1.4 d for YEB and 1.26 d for RHW. Ultimately, both cultures entered the stationary phase after 2 days of growth, likely due to nutrient depletion and changes in pH, decreasing from 5.5 to 4.9 for YEB and from 4.3 to 3.7 for RHW.

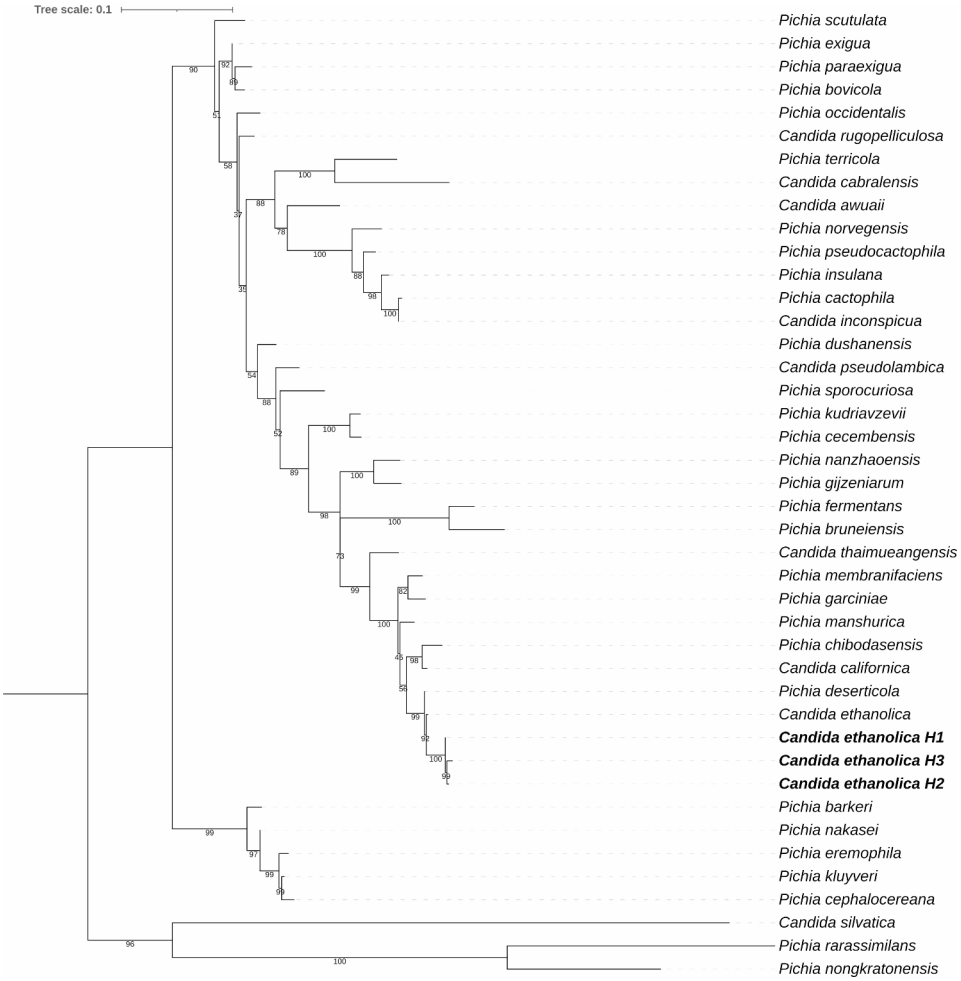

Fig. 1 Maximum Likelihood phylogram based on the concatenated ITS and D1/D2 domain of LSU from rDNA sequences from Candida and Pichia related clades. Bootstrap values (> 50%) are shown below the branches

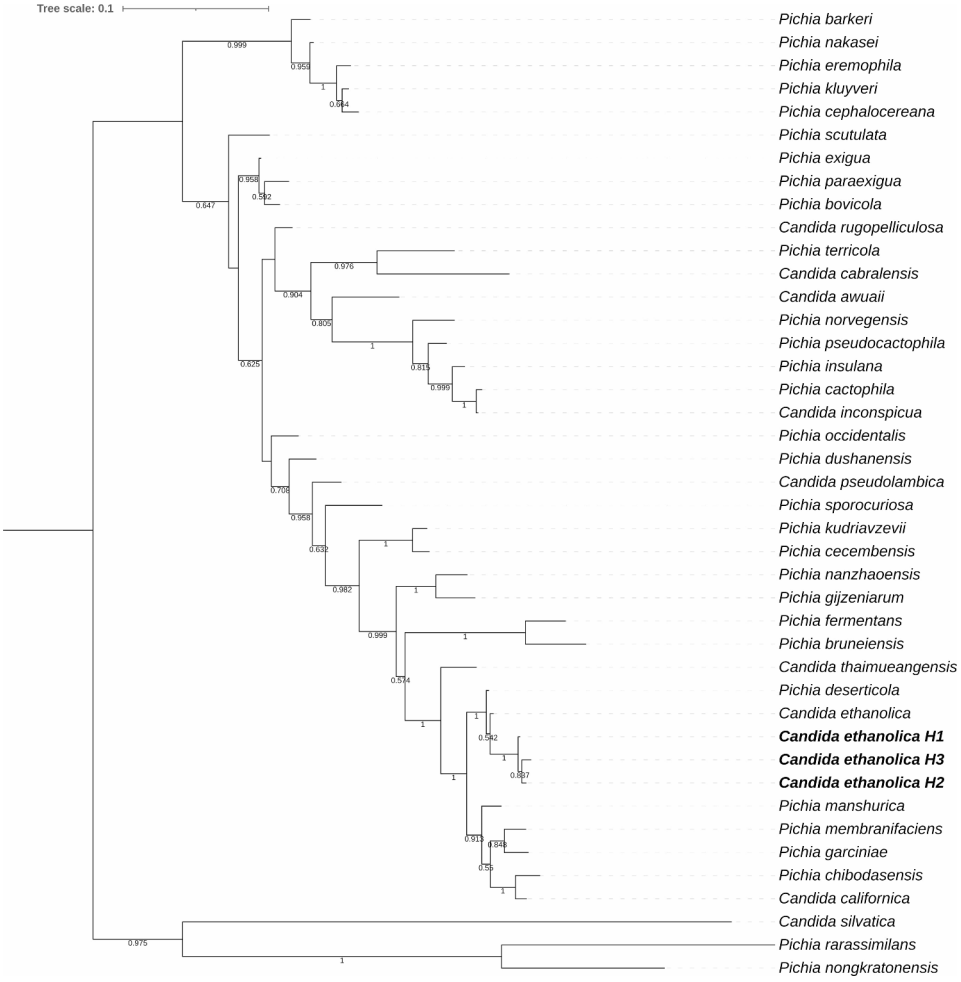

Fig. 2 Bayesian phylogram based on the concatenated ITS and D1/D2 domain of LSU from rDNA sequences from Candida and Pichia related clades. Bayesian posterior probabilities (> 0.50) are shown below the branches

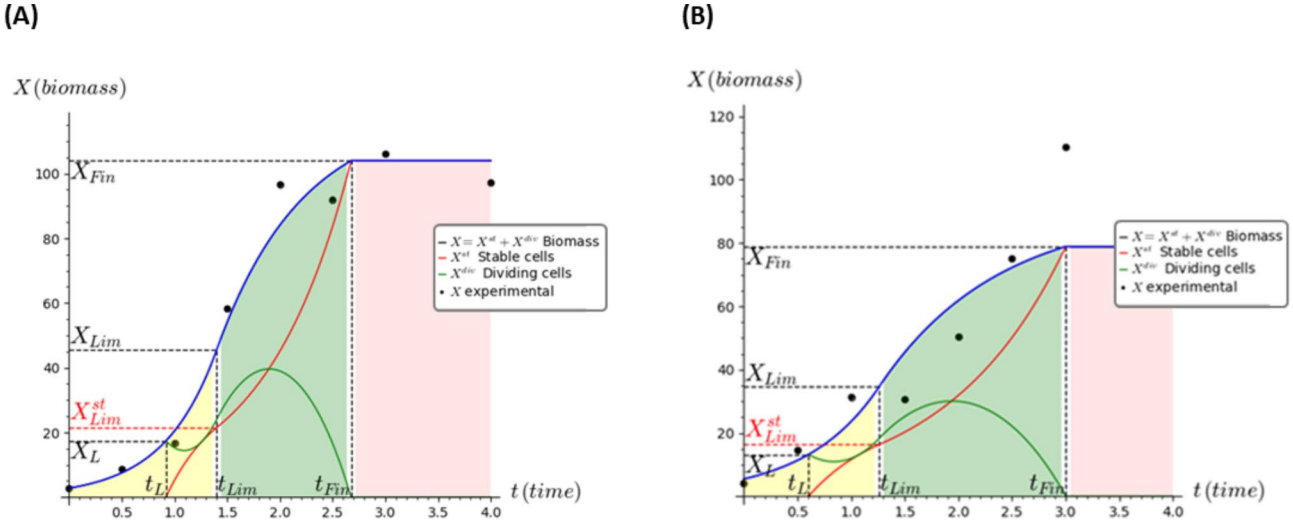

Fig. 3 Growth of *C. ethanolica* H3·strain (2% V:V inoculum) measured as DW (g/L) of biomass (X) in batch culture feed with YEB culture media (**A**) or RHW wastewater (**B**) for 4 d. Blue line is obtained from the average of triplicate experimental data

Significant differences were noted in the experimental coefficients μ and dt between YEB (1.902 and 0.77 days, respectively) and RHW (0.898 and 0.36 days, respectively), consistent with the results generated by the applied model. Furthermore, significant differences ($P<0.05$) were observed in terms of P, with YEB (48.33 g/L·day) surpassing RHW (36.75 g/L·day) when employing a 2% (V:V) inoculum size. Both culture media show significant differences in their substrate composition, attributable to their respective components. YEB media is supplemented with mannose at a concentration of 5 g/L, while RHW has a complex composition with an unspecified sugar mixture at a concentration of 1.5 g/L.

Figure 4 illustrates the dynamics of substrate consumption over 5 days in a batch culture employing RHW as the feeding medium. Additionally, two variations of RHW were investigated: RHW as control, or RHW sterilized, and RHW inoculated with *C. ethanolica* H3·strain (2% V:V). In the initial 24 h, RHW+H3 exhibited a higher substrate consumption rate, exceeding the other treatments by 40%. By the third day, substrate levels had decreased by half in RHW+H3 and by 40% in RHW. On the contrary, in sterile RHW media, without any bacteria or yeast, no significant diminish were observed.

Discussion

Molecular identification of the isolates allows to identify as *Candida ethanolica* CBS 8041 (NR_077165.1). According to Kurtzman and Robnett (1998), yeasts with up to three nucleotide substitutions in this region could be considered co-specific strains, whereas strains with six or more substitutions usually represent different species. Consequently, isolates were identified as *Candida ethanolica* H1, H2, and H3. Both Bayesian and Maximum Likelihood trees demonstrate strong alignment in our study. The chosen outgroup, *Candida silvatica* clade, consistently separated in both phylogenetic reconstructions, affirming the robustness of our analysis. While the overall tree topologies generally align with prior studies (Kurtzman et al. 2008; Kobayashi et al. 2017), a few discrepancies, such as the relative positions of *Pichia sporocuriosa*, *Candida pseudolambica*, *Candida rugopelliculosa*, *Pichia exigua*, and *Pichia scutulata*, can be attributed primarily to the inclusion of recently described species in our investigation and to the selection of different species in previous works, especially within the *Pichia* clade.

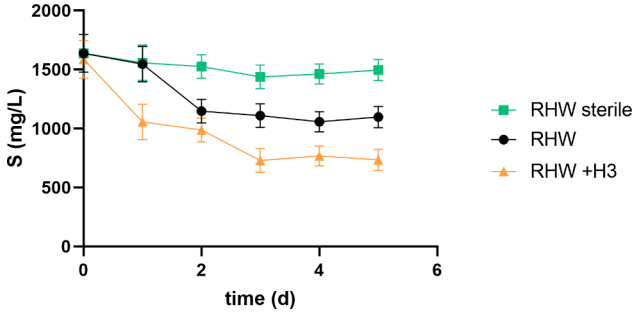

Fig. 4 Substrate consumption measure (S, mg/L) as total sugars in the RHW culture media compared to RHW autoclaved in sterile conditions (RHW sterile) for 20 min and RHW inoculated with 2% V:V inoculum of *C. ethanolica* H3 (RHW+H3). Batch cultures were maintained for 5 d. Error bars correspond to s.d. from the average of triplicate experimental data

The identification of species of biotechnological interest often relies on phylogenetic analyses that involve a limited number of species. In this study, we chose to evaluate phylogenetic relationships exclusively based on type groups. Nevertheless, the three isolates formed a clade with *Candida ethanolica* and *Pichia deserticola*, displaying full statistical support (PP: 1, BS: 100). Thus, they were identified as *C. ethanolica* H1, *C. ethanolica* H2, and *C. ethanolica*H3, since no ascospores were observed during the assay. The status of *Candida ethanolica* and *Pichia deserticola*, being considered sibling species or strains of the same genus is still matter of debate. Despite observable phenotypic differences in their ability to thrive in a vitamin-free medium, current evidence derived from the ITS, LSU, SSU regions, and karyotypic traits only provides light support classification as separate species (Wu and Bai 2005; Kurtzman et al. 2008; Sipiczki 2012). This clade belongs to a larger taxonomic group previously documented by Kurtzman et al. (2008) and Sipiczki (2012), encompassing species such as *Candida californica*, *Pichia chibodasensis*, and *Pichia manshurica* among others.

Fermentertool model differentiated between two main groups of biomasses: dividing cells (Xdiv) and nondividing cells (Xst). The Log model (X) aligned well with the experimental data in both culture media, exhibiting higher accuracy in YEB. Furthermore, the model accurately predicted the proliferation of nondividing cells, as indicated by the growth inhibition phase (GIP), and the decrease in the population of dividing cells, which entered the stationary phase after 40 h. This mirrored the experimental data observed in RHW.

Additionally, the presence of microorganisms and the supplement with yeast were studied (Fig. 4). Higher substrate consumption rate were obtained in the RHW+H3 treatment suggesting the additive effect of native flora and the supplemented yeast. After the third day, the discrepancy suggests the presence of bacteria and yeast in RHW that actively consumes the substrate, albeit at a slower rate compared to the inoculated RHW+H3. In sterile RHW, substrate levels remained constant throughout the assay due to the absence of microorganisms. After 3 days in culture, substrate consumption ceased in agreement with the biomass kinetics previously described. Consequently, the increase in the concentration of the

H3 strain introduced to the existing flora in the RHW resulted in enhanced bioremediation capabilities. These findings provide compelling evidence of the impressive degree of specialization exhibited by yeast strains in their collaborative function.

The study demonstrated a significant improvement in treatment efficacy with the inclusion of an inoculum composed of yeast strains isolated from the same substrate that is to be remedied. The utilization of yeasts, either individually or in combination with other crops or technologies, has the potential to achieve a higher level of efficiency in the treatment of honey wastewater. Finally, future investigations could aim to elucidate mechanism of sucrose tolerance, which could provide key information for optimizing the use of this strain in applications involving high levels of sucrose.

Abbreviations

BI	Bayesian inference
BOD	Biochemical oxygen demand
SS	Suspended solids
RHW	Residual honey water
DW	Dry weight
FW	Fresh weight
GIP	Growth inhibition phase
ITS	Internal transcribed spacer
LSU	Large subunit sequences
ML	Maximum likelihood (ML)
P	Volumetric productivity
SDGs	Sustainable development goals
HPW	Honey processing companies
TC	Total carbon
TOC	Total organic carbon
COD	Chemical oxygen demand
Xdiv	Dividing cells
Xst	Nondividing cells
YEB	Yeast extract beef
NCIB	National Center for Biotechnological Information

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Author contributions
JGSN, PLM, HP designed the project. JGSN, FGD and PLM carried out the experiments. All the authors analyzed the data, discussed and prepared the final report. All authors have read and approved the final manuscript.

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Data availability
The data generated during the study are included in this article.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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