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Survey of nonconventional yeasts for lipid and hydrocarbon biotechnology

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Abstract: Nonconventional yeasts have an untapped potential to expand biotechnology and enable process development necessary for a circular economy. They are especially convenient for the field of lipid and hydrocarbon biotechnology because they offer faster growth than plants and easier scalability than microalgae and exhibit increased tolerance relative to some bacteria. The ability of industrial organisms to import and metabolically transform lipids and hydrocarbons is crucial in such applications. Here, we assessed the ability of 14 yeasts to utilize 18 model lipids and hydrocarbons from six functional groups and three carbon chain lengths. The studied strains covered 12 genera from nine families. Nine nonconventional yeasts performed better than *Saccharomyces cerevisiae*, the most common industrial yeast. *Rhodotorula toruloides*, *Candida maltosa*, *Scheffersomyces stipitis*, and *Yarrowia lipolytica* were observed to grow significantly better and on more types of lipids and lipid molecules than other strains. They were all able to utilize mid- to long-chain fatty acids, fatty alcohols, alkanes, alkenes, and dicarboxylic acids, including 28 previously unreported substrates across the four yeasts. Interestingly, a phylogenetic analysis showed a short evolutionary distance between the *R. toruloides*, *C. maltosa*, and *S. stipitis*, even though *R. toruloides* is classified under a different phylum. This work provides valuable insight into the lipid substrate range of nonconventional yeasts that can inform species selection decisions and viability of lipid feedstocks.

Keywords: Hydrocarbon utilization, Substrate toxicity, Nonconventional yeast

Introduction

There are over 1,500 recognized species of yeast (Lachance, 2006). These unicellular fungi thrive in diverse environments, from households to extreme ecosystems, meaning they encode diverse metabolic capabilities (Péter et al., 2017; Selim et al., 2020). However, only a small percentage of yeasts have been characterized. A deeper exploration of nonconventional yeast complex phenotypes could be transformative in product development, bioprocessing, food science, and biotechnology (Binati et al., 2021; Gao et al., 2019; Yaguchi et al., 2017, 2018; Yamakawa et al., 2020).

Microbes can play a critical role in the development of a circular economy. The economic viability and sustainability of the biorefinery-type approach require utilization of a wide variety of substrates and flexibility in product identity. Yeast and bacteria are generally viewed as favorable industrial organisms because of their fast growth, ease of process scale-up, and lack of dependence on climate and soil quality (Ageitos et al., 2011; Thorwall et al., 2020). Yeasts are often viewed as preferable to bacteria because of their resistance to phage infection, large repertoire of valuable metabolites, and, in some contexts, their improved tolerance of industrial conditions (Kim et al., 2020). There has been interest in using yeasts for the valorization of wastes for both environmental and economic benefits, with lipids and lipid-like molecules serving as an important group of feedstocks and products in this space. One of the most common examples is the production of fatty acids by *Yarrowia lipolytica* using various waste feedstocks (Gao et al., 2020; Lopes et al., 2021).

Lipids and hydrophobic hydrocarbons are important classes of molecules of biological and industrial relevance. They are the core of the oil and oleochemical industries and there is a dire need to develop new methods for their production and transformation. For example, microbial processes are being used to address environmental and societal problems in the palm oil industry. Palm oil is commonly used in household products, but the harvesting of palm oil trees has caused massive deforestation and societal consequences (Busch et al., 2022; Kadandale et al., 2019).The ongoing development of microbial processes to produce palm oil provides a preferable source of these molecules (Abeln & Chuck, 2021; Whiffin et al., 2016).

There has been substantial enthusiasm regarding the microbial production of lipids, especially by oleaginous yeasts (Bao et al., 2021; Gosalawit et al., 2021). Even though lipid uptake and biotransformation of these molecules are key to applications such as bioremediation, pollution control of fat, oil, and grease (FOG), and upcycling of thermally degraded plastics (Karim et al., 2021; Mihreteab et al., 2019), curation of microbial organisms with innate capacity for lipid consumption is limited. In this study, we explore the lipid substrate range of nonconventional yeasts and identify promising strains for utilization of various classes of lipid and lipid-like molecules.

Materials and Methods

Strains and Culture Conditions

Nonpathogenic yeast species with reported metabolic pathways for lipid and lipid-like molecules were selected and obtained from the Unites States Department of Agriculture (USDA) Agricultural Research Service (ARS) Culture Collection (Northern Regional Research Laboratory). We included

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Table 1. Yeast Strains Studied Here and Their Corresponding USDA ARS and ATCC Strain Designations

Species	Family	Strain designation (NRRL Y-)	ATTC no.
Candida boidinii	Pichiaceae	2332	18810
Candida maltosa	Debaryomycetaceae	17677	28140
Kazachstania exigua	Saccharomycetaceae	12640	10599
Kazachstania unispora	Saccharomycetaceae	1565	N/A
Kluyveromyces marxianus	Saccharomycetaceae	8281	N/A
Metschnikowia pulcherrima	Metschnikowia	7111	18406
Pichia membranifaciens	Pichiaceae	2026	26288
Rhodotorula toruloides	Sporidiobolaceae	1091	10788
Saccharomyces cerevisiae	Saccharomycetaceae	12632	18824
Scheffersomyces stipitis	Debaryomycetaceae	7124	58376
Wickerhamomyces anomalus	Phaffomycetaceae	366	8168
Wickerhamomyces subpelliculosus	Phaffomycetaceae	1683	16766
Yamadazyma philogaea	Debaryomycetaceae	7813	28319
Yarrowia lipolytica	Dipodascaceae	63746	20460

ATCC, American Type Culture Collection; NRRL, Northern Regional Research Laboratory.

Saccharomyces cerevisiae as the baseline strain. The strains were grown in media consisting of 10 g/l yeast extract, 20 g/l peptone, 0.4 g/l adenine hemisulfate, and 20 g/l dextrose (YPAD). Each of the organisms listed in Table 1 was able to reach OD_{600} >2 when grown in BD Difco yeast nitrogen base (YNB) without amino acids containing 2% wt/vol dextrose at 30°C within 48 hr.

Model Compounds

Six functional groups were selected to represent the substrate scope: monocarboxylic acids, dicarboxylic acids, alcohols, alkanes, alkenes, and esters. For biological relevance, the selection scope was limited to saturated and even chain length molecules with preference for functionalities located at the first carbon (Table 2). The compiled molecule identifiers and physiochemical properties for the selected model compounds can be found in Tables S4 and S5.

Characterization of Molecule Utilization

Every model compound was tested for utilization by inoculating each yeast in YNB minimal media with the corresponding compound provided as the sole carbon source. Precultures were grown from single colonies in 250 ml baffled flasks with 50 ml YPAD to mid-exponential phase and washed three times with minimal media to remove any residual carbon source by centrifugation at 4415 × *g* for 5 min and resuspending in YNB without amino acids or a carbon source. For each strain, culture tubes with 3 ml of YNB without amino acids and 0.05 M of the focal model compound were prepared. The pH of the chemically defined media was 5.4 ± 0.2. Media containing 0.05 M glucose as carbon source was included as a control. Additionally, for each model compound a negative control (noninoculated) was also prepared. All the conditions were prepared in triplicate. All the cultures were inoculated to an initial OD_{600} of 0.1 with the washed precultures and incubated for 48 hr at 30°C with 250 rpm agitation in a MaxQ 6000 incubated shaker (Fig. 1). Negative controls were also incubated in the same condition. Growth was monitored via the measurement of OD_{600} (Cary 50 Bio UV-Visible Spectrophotometer). The use of the OD_{600} value to compare growth across organisms was validated by measurement of dry cell weight (Table S1).

Genomic DNA Isolation and Phylogenetic Analysis

Cells were cultured in YPAD broth overnight at 30°C and 250 rpm. An aliquot of 1 ml was harvested from each culture, equivalent to ∼100 mg of wet cell mass, and pelleted. Genomic DNA was isolated using a Wizard® Genomic DNA Purification Kit (Promega, USA). The final DNA pellet was dissolved in 50 µl deionized autoclaved water. In order to acquire the 16S rRNA sequence, a high-fidelity DNA polymerase (AccuPrime™ Pfx DNA Polymerase, Invitrogen, USA) was used and PCR was performed with amplification primers for the ITS1 and ITS2 regions. A ReliaPrep™ DNA Clean-Up and Concentration System (Promega, USA) was used to purify PCR products, which were then sequenced in both directions using ITS1 and ITS2 primers and a BigDye Terminator v3.1 cycle sequencing kit with the Applied Biosystems 3730xl DNA Analyzer at the Iowa State University DNA Facility (Toju et al., 2012). A standard nucleotide BLAST analysis optimized for highly similar sequences (megablast) was performed with BLAST+ 2.12.0 against the nucleotide collection last updated on December 1, 2021, to confirm organism identity. All positions containing gaps and missing data were eliminated (complete deletion option). Sequences were aligned using the MUSCLE algorithm version 5 from the alignment tools of MEGA 11 and a phylogenetic tree was generated using the neighbor-joining method (Edgar, 2004; Saitou & Nei, 1987; Tamura et al., 2021). A total of 93 nucleotides were analyzed for homology. A bootstrap analysis of 1,000 replications was used to test the robustness of all analyses.

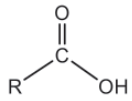
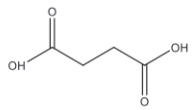
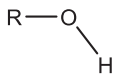
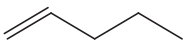
Statistics

Growth data from the final OD_{600} was normalized with the following formula:

$$\text{Normalized OD} = \frac{\text{Average OD}_{600}\text{ by yeast X in compound Y} - \text{Average OD}_{600}\text{ of compound Y blanks}}{\text{Average OD}_{600}\text{ of yeast X in glucose}}.$$

This equation mitigates variability in culture conditions between batches and accounts for differences in total growth. The heat map visualization was prepared using the natural

Table 2. Lipids and Lipid-Like Molecules Characterized in This Study

Functional group	Carbons	Common name	CAS number	Melting point (°C)
Carboxylic acids	14	Myristic acid	544-63-8	54
	18	Stearic acid	57-11-4	70
	22	Behenic acid	112-85-6	80
Dicarboxylic acids	14	Tetradecanedioic acid	821-38-5	127
	18	Octadecanedioic acid	871-70-5	125
	22	Docosanedioic acid	505-56-6	128
Alcohols	14	1-Tetradecanol	112-72-1	37
	18	1-Octadecanol	112-92-5	56
	22	1-Docosanol	661-19-8	72
Alkanes	14	n-Tetradecane	629-59-4	6
	18	n-Octadecane	593-45-3	28*
	22	n-Docosane	629-97-0	43
Alkenes	14	1-Tetradecene	1120-36-1	-12
	18	1-Octadecene	112-88-9	17.5
	22	1-Docosene	629-97-0	38
Esters	14	Lauryl acetate	112-66-3	1.3
	18	Methyl heptadecanoate	1731-92-6	30

Note. Melting point values were obtained from either the supplier or from PubChem.
[a]These melting points were reported in PubChem, but the compounds remained solid during experimentation at 30°C.

logarithm of normalized OD values in Prism 9. Mean comparisons were completed with one-way ANOVA and *p*-values obtained from the all-pair Tukey Kramer test in JMP Pro 16. All measurements were performed in triplicate. Error propagation calculations yielded the standard deviation of normalized and summed values.

Results and Discussion

The ability of our focal yeast species to utilize each model compound as sole carbon source was characterized by production of biomass as indicated by OD_{600} (Fig. 2). As expected, none of these model compounds fully dissolved in our culture media. N-tetradecane, 1-tetradecene, 1-octadecene and lauryl acetate formed a second liquid phase that was less dense than the aqueous media; the other compounds were present as solid particles. Both the secondary liquid phase and the solid particles persisted throughout the course of growth monitoring. To support comparison across species, the maximum observed OD in each condition was normalized relative to the maximum observed OD on glucose for that organism.

Yeasts Broadly Utilize Lipid and Lipid-Like Molecules as Sole Carbon Source

The ability of each yeast species to utilize individual model compounds is presented in Fig. 2a, with a threshold of 0.05 normalized OD being applied as the minimum signal to represent growth. For one organism, *Kluyveromyces marxianus*, we did not observe growth above background on any of the model compounds. The other 13 strains were observed to utilize at least one (6%) of the model compounds, though with various degrees of versatility. *Rhodotorula toruloides* was the most versatile yeast studied, with utilization of 11 (61%) distinct model compounds.

This versatility is a testament to biological complexity and the range of metabolic adaptations. Summation of the normalized OD values for each species across all model compounds further quantifies this versatility not just in terms of the number of model

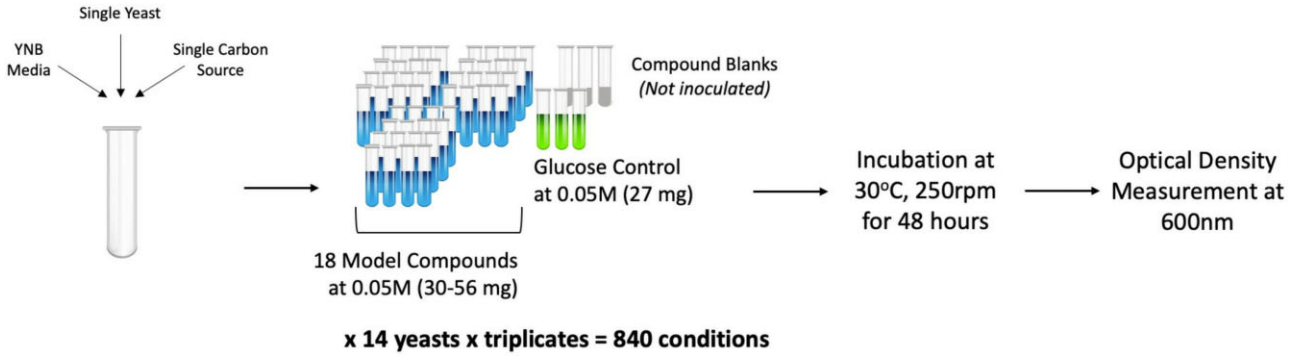


Fig. 1. Methodology workflow used to characterize model compound utilization. Each culture tube had YNB without amino acids (minimal media), and a single carbon source, and was inoculated with a single yeast with a starting OD_{600} of 0.1. In total, 18 model compounds were tested as single carbon source per organism, as well as a glucose control without model compounds and a no-cell control for each model compound to use as a blank.

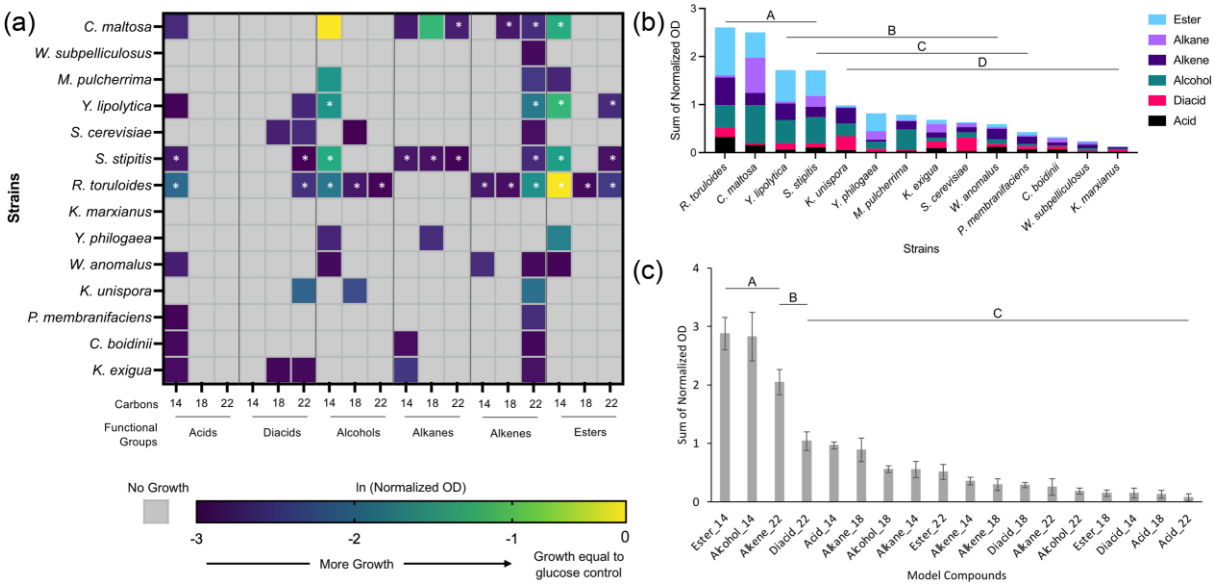


Fig. 2. Yeast species studied here show broad utilization of the model lipid molecules across functional groups as well as selectivity across chain length. (a) Observed maximum OD_{600} relative to the OD_{600} on glucose with normalized values below 0.05 shown as "no growth" in this subfigure only. Asterisks, shown only for *C. maltosa*, *Y. lipolytica*, *S. stipitis and R. toruloides* indicate carbon source utilization previously unreported in the literature. (b) Total observed growth, normalized by glucose, for each strain, and (c) total normalized growth for each model compound. In (b) and (c), columns not connected by the same letter are significantly different ($p < 0.05$, Tukey–Kramer test).

compounds used as sole carbon source but also in the total amount of growth supported (Fig. 2b). Overall, four species stand out: *R. toruloides*, *Candida maltosa*, *Y. lipolytica* and *Scheffersomyces stipitis*, with the first two growing significantly more than the bottom 10 strains within this group of 14. *Rhodotorula toruloides* and *Y. lipolytica* have garnered interest for lipid production and for their ability to use low-cost feedstocks and renewable sources, while exhibiting high tolerance to common contaminants in those feedstocks (Qi et al., 2020; Yu et al., 2020). *Scheffersomyces stipitis* is also intriguing in the bioethanol space for being a natural xylose fermenter (Song et al., 2022). On the other hand, while *C. maltosa* has been studied for potential applications involving utilization of phenols and alkanes, it remains undercharacterized relative to *Y. lipolytica*, *S. stipitis*, and *R. toruloides* (Chrzanowski et al., 2008; Schlüter et al., 2019).

Our findings reaffirm the potential of *R. toruloides*, *C. maltosa*, *Y. lipolytica*, and *S. stipitis* for utilization of this class of molecules while also reporting new carbon sources among these four yeasts. The novelty of these findings was determined by queries using the organism names as provided in Table 1 as well as prior names (Table S2). Model compounds were described using both the names provided in Table 2 and synonyms (Table S3). Specifically, 11 novel carbon sources were identified for *R. toruloides*, 9 for *S. stipitis*, 4 for *Y. lipolytica*, and 4 for *C. maltosa* (Fig. 2a).

In addition to exploring which organisms are best suited for utilization of these model compounds, we are also interested in exploring which model compounds are most amenable to microbial utilization (Fig. 2c). Similar to the observation of a small number of high-performing organisms, three model compounds were observed to broadly support microbial growth: lauryl acetate (C14 ester), 1-tetradecanol (C14 alcohol), and 1-docosene (C22 alkene). Thus, within this relatively limited set of 14 organisms and 18 compounds we identified four high-performing organisms and three high-performing compounds. These top performers do not possess any obvious similarities—the top four organisms do not correspond to specific family groupings and the top three compounds are within different functional groups and have varying chain lengths. The lack of distinguishing features

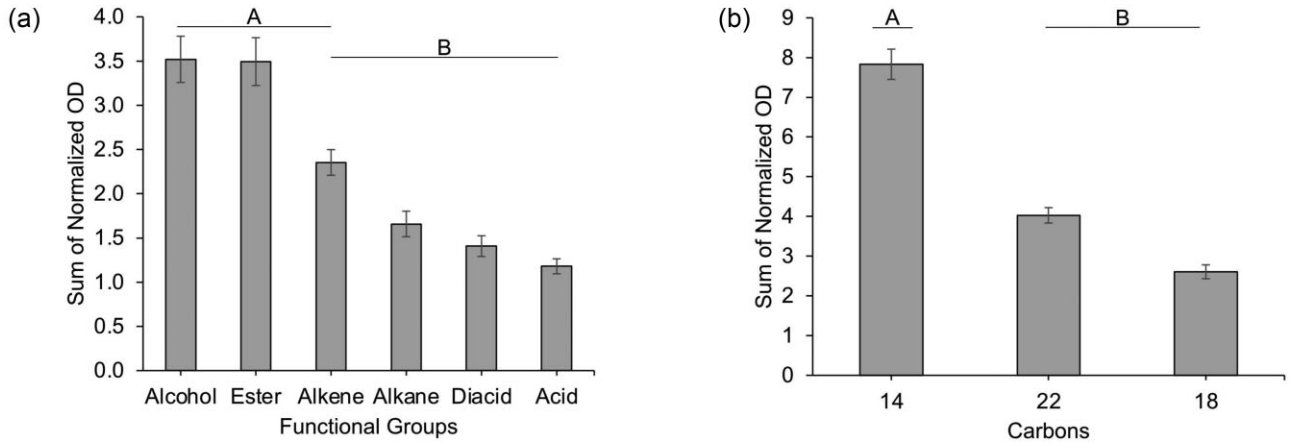


Fig. 3. (a) Total summed growth, normalized by glucose, for each of the three molecules in each functional group across all species. (b) Total summed growth, normalized by glucose, for the six molecules with similar carbon chain lengths across all species. Columns not connected by the same letter are significantly different ($p < 0.05$ in the Tukey–Kramer test).

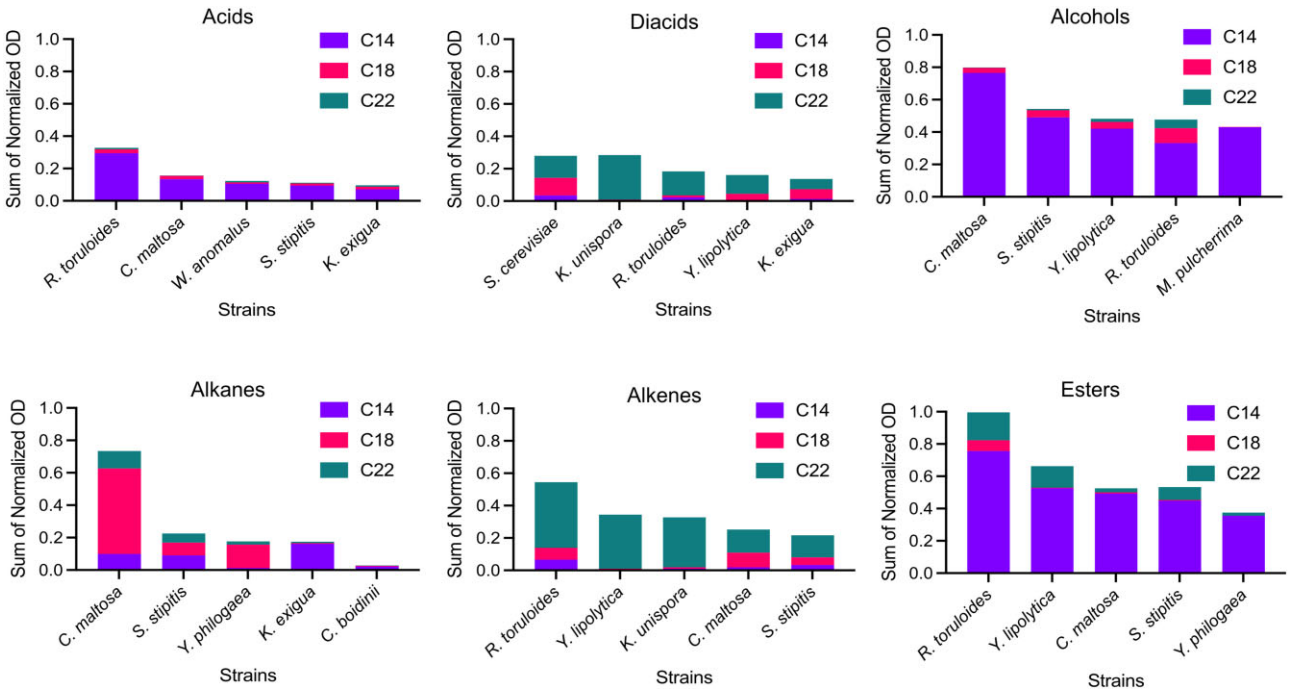


Fig. 4. Chain length preference varies according to molecule type. Data are shown only for the five organisms with the highest summed growth within each functional group and organisms are ordered according to this value.

among these preferred model compounds motivated further exploration of substrate preferences with this set of organisms.

Functional Groups and Carbon Length Influence Lipid-Molecule Utilization

Having observed that the three most highly utilized molecules each belong to a different functional group, we parsed growth data according to molecule type and chain length (Fig. 3). Similar to our finding that tetradecanol and lauryl acetate were the top growth-supporting model compounds, the alcohol and ester functional groups had the highest summed growth. Also, across chain lengths, 14-carbon molecules support the highest summed growth.

However, this bulk analysis of functional groups and chain lengths obscures differences among the functional groups. Consideration of the normalized growth of each organism on each model compound (Fig. 4) shows that while C14 is more supportive of growth for acids, alcohols, and esters, within the diacid and alkene groups C22 is the most growth supportive.

Within each functional group, we identified the five organisms with the highest summed normalized growth. For alcohols, esters, and alkenes, each of the four organisms identified as top-performing organisms among all model compounds were also among the best for the functional group with the exception of *Y. lipolytica* for acids. For acids, diacids, and alkanes, *Y. lipolytica* was not among the five best performers. *Yamadazyma philogaea*, *Kazachstania unispora*, and *Kazachstania exigua* showed some evidence of high performance among discrete functional groupings: *Y. philogaea* on esters and alkanes, *K. unispora* on diacids and alkenes, and *K. exigua* on acids, diacids, and alkanes. These results emphasize the idea that some organisms can be considered generalists with a

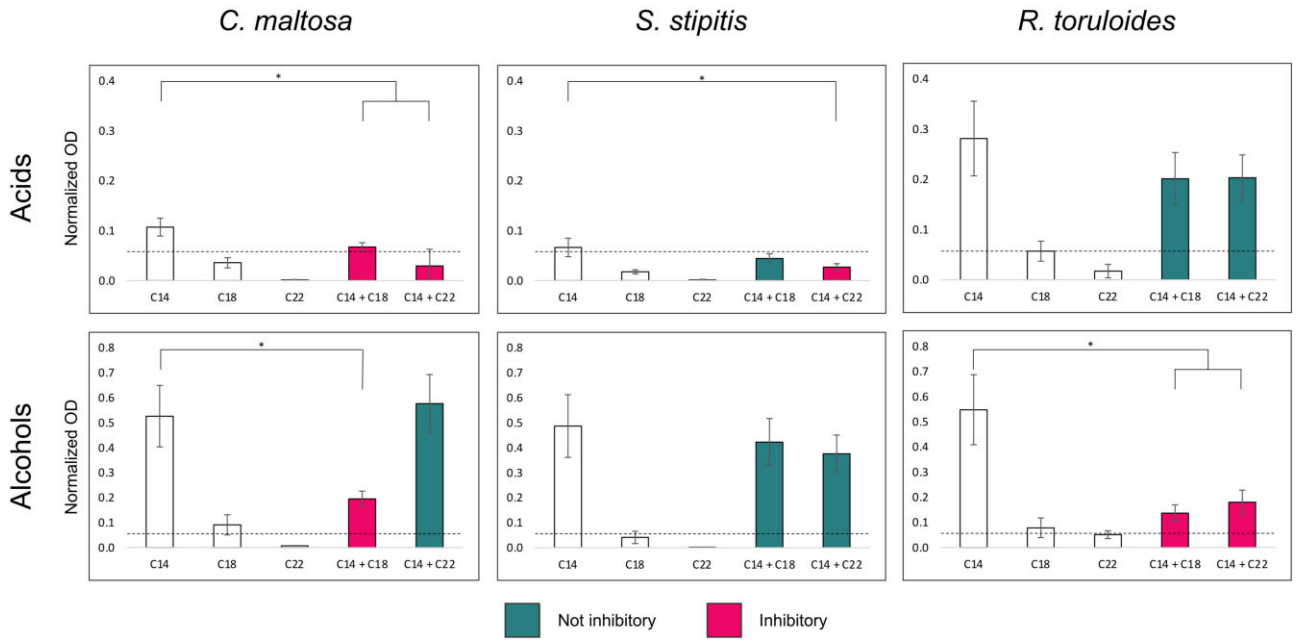


Fig. 5. Inhibition assessment of non-growth-supporting fatty acids and fatty alcohols. Inhibition is determined based on significant differences relative to the 14-carbon molecules for each organism and functional group. A significant difference between two conditions is represented by an asterisk ($p < 0.05$ in the Tukey–Kramer test).

broad range of substrates, while others are more specialized and can utilize compounds that are poorly utilized by the generalist species.

Carbon Chain Length Variations Cause Species-Specific Toxicity

An ideal microbial cell factory can utilize all available substrates. Here, we have identified generalist organisms that use many of our model compounds, but no one organism utilized all of the compounds. Engineering of an organism for utilization of any given substrate requires understanding of the nature of the deficiency for substrate utilization. The deficiency could be due to the native genetic chassis of the organism, in that one or more of the proteins required for funneling the molecule into central metabolism are either not encoded by the organism or are not expressed. The deficiency could be due to process conditions such that the molecule in question has limited bioavailability, such as when the molecule has very low solubility in the growth media. A final possible reason for the deficiency is inhibition of microbial activity due to toxicity of the molecule.

Failure to encode or express the necessary proteins could be determined by omics analysis, assuming that the proteins associated with utilization of these compounds have been thoroughly annotated. The possibility of toxicity of any molecule X can be explored using growth analysis similar to that described earlier. If the lack of microbial growth on X is due to its toxicity, then addition of X to a condition that would normally support growth would negatively impact growth. If no reduction in growth is observed, then inhibition by X can be eliminated from consideration.

The top three generalist species were each observed to robustly utilize C14 monoacids and C14 alcohols, but poorly utilized the 18- and 22-carbon molecules. To assess the possible toxicity of the poorly-utilized compounds, they were provided in combination with the C14 molecule, while maintaining the same total model compound concentration of 0.05M.

The strains are affected differently by the model compounds. For *R. toruloides*, the 18- and 22-carbon acids were not inhibitory, in that the normalized OD was not changed for C14 + C18 or C14 + C22 relative to C14 only (Fig. 5). In contrast, the 18- and 22-carbon alcohols were inhibitory, as evidenced by the decreased normalized OD. For both functional groups, utilization of the 14-carbon compound in the combined condition is demonstrated by the increased normalized OD relative to growth on only the 18- or 22-carbon molecules. Thus, for this organism, the lack of utilization of longer-chain acids can possibly be attributed to genetic deficiencies, but the lack of utilization of longer-chain alcohols can primarily be attributed to the molecule toxicity.

On the other hand, the fatty alcohols were not inhibitory for *S. stipitis* and fatty acids were inhibitory for *C. maltosa*, completely opposite to *R. toruloides*. These results demonstrate the idiosyncrasies of individual organisms and the dangers of generalizing observations of molecule toxicity, thus emphasizing the benefit of characterizing multiple organisms.

Phylogenetic Analysis Identifies Close Relationships Between Top-Performing Strains

Here, we have compared organisms based on their growth behavior. These observed patterns of substrate utilization are not consistent with taxonomic-based classification (Table 1). We sequenced the ITS2 16S rRNA region and performed phylogenetic analysis to inform the evolutionary relationships between the 14 organisms (Fig. 6). This analysis groups strains based on evolutionary distance, and some of the resulting clades align in terms of their substrate range. For example, *Metschnikowia pulcherrima* and *Y. lipolytica* were both able to utilize alcohols, esters, and alkenes but not alkanes, and these two organisms form a single clade. *Scheffersomyces stipitis* and *C. maltosa* are in the same taxonomic family and their observed substrate ranges were similar, but they are fairly distant in our phylogenetic analysis.

The inclusion of *R. toruloides* in the clade with so many other species and yet excluding *C. maltosa*, *M. pulcherrima*,

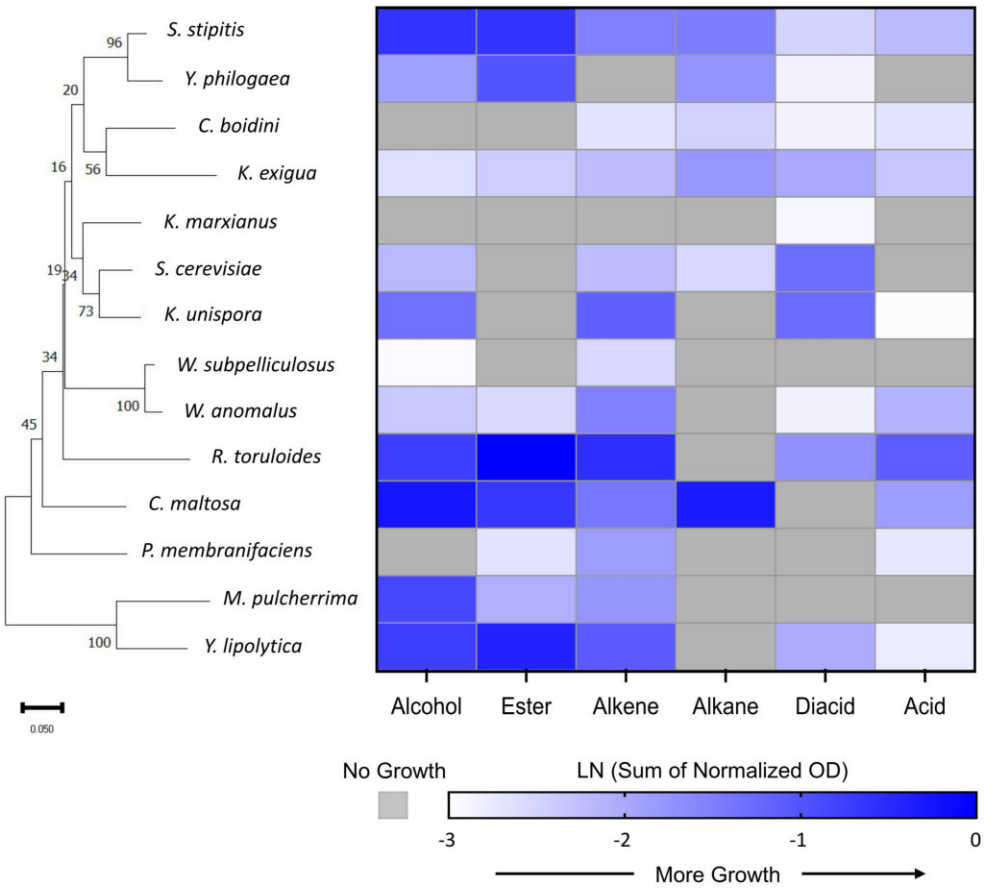


Fig. 6. Phylogenetic analysis groups similarly performing strains in two clusters of the upper and lower branches. The tree reports the percentage of replicate trees from 1,000 replicates in which the associated taxa clustered together in the bootstrap test. Branch lengths represent evolutionary distances.

Y. lipolytica, and *P. membranifaciens* was surprising given its taxonomic placement in a distinct phylum (Basidiomycota) from the other organisms (Ascomycota), the highest level of taxonomic organization after kingdom (fungi). These findings could inform strain selection decisions and could help pinpoint conserved mechanisms within short evolutionary distances important for the utilization of these classes of molecules.

Model Compound Utilization Trends Poorly With Molecule Properties

The essential requirement of carbon source utilization is that the organism encode for proteins with a role in uptake of the carbon source (e.g. transporters) and its metabolism. However, physicochemical properties of the substrate likely influence its interaction with the initial steps of the metabolic processing. Therefore, we collated physicochemical properties for our model compounds and compared these values to growth data. This correlation was performed with various levels of categorization, ranging from individual species, aggregated families, and the total normalized sum across all organisms (Table 3). For many of the physical properties, data were available for only a subset of model compounds. For example, the value of the dielectric constant at 30°C was found for only 8 of the 18 compounds. This lack of information diminishes confidence in the assessment of these correlations and their statistical significance.

Microbial uptake and utilization of the model compounds are potentially sensitive to mass transfer limitations. For the screening method used here, it was initially expected that compound utilization would trend with aqueous solubility of the molecule. However, for the 12 model compounds with available data, a significant correlation was not observed. Some species, such as *Y. lipolytica*, are known to produce biosurfactants to overcome those limitations (Soong et al., 2019; Yalçın et al., 2018). Physiological changes have been documented in other species, such as *C. maltosa*, to improve contact with and utilization of hydrophobic carbon sources (Zvonarev et al., 2021). Some of the other strains could be employing similar mechanisms, but uptake mechanisms for hydrophobic substrates are poorly characterized for the yeast species studied here. Understanding these mechanisms could inform strain engineering efforts to possibly reduce the need for surfactants in industrial processes.

The number of heavy atoms in a molecule is calculated as the number of nonhydrogen atoms. For example, myristic acid contains 16 heavy atoms, but the corresponding tetradecanoic diacid contains 18 heavy atoms. Similar to our observation that model compounds containing 14 carbons were generally preferred relative to 18 and 22 carbons, we identified an inverse correlation between the number of heavy atoms and microbial growth. However, this correlation was only significant when compared with the summed growth for the Phaffomycetaceae family (*Wickerhamomyces subpelliculosus* and *Wickerhamomyces anomalus*).

Molecule complexity is calculated according to the Bertz–Hendrickson–Ihlenfeldt formula based on the molecule structure, composition, and symmetry (Bertz, 2002; Hendrickson et al., 2002). For example, 1-tetradecanol has a complexity of 102 and myristic acid has a complexity of 155. We also observed a negative correlation between molecule complexity and microbial growth,

Table 3. Correlation Summary for Model Compound Properties and Yeast Growth

Property	Unit	Range	*n*	Response	Correlation	*p* > *F*
LogP		6–10	9			
XLogP3		4.3–12	18			
H bond donor		0–2	18			
H bond acceptor		0–4	18			
Rotatable bonds		11–21	18			
Polar surface area	Å^2	0–75	18			
Heavy atoms		14–26	18	Phaffomycetaceae	−0.5015	0.0340
Complexity		74–300	18	Phaffomycetaceae	−0.5245	0.0254
Water solubility	mg/ml	8×10^{-10}–0.6	12			
Dielectric constant		2–100	8			
Surface tension	mN/m	26–70	3	*C. maltosa*	0.9993	0.0231
				R. toruloides	0.9993	0.0241
				Top 4 sum	0.9992	0.0256
				Deabryomycetaceae	0.9999	0.0074
Mass density	Kg/m^3	760–900	7			

but as with the number of heavy atoms, this correlation was only significant when restricted to the two members of the Phaffomycetaceae family.

Surface tension of the solid compound at 30°C was found to have a significant, positive correlation with growth across several organism groupings, including the summed behavior of the top four generalist organisms. However, surface tension values were available for only 3 of the 18 model compounds (two alcohols and an alkene) and thus this finding is presented with caution.

Conclusion

Industrial fermentation processes have largely focused on microbial utilization and valorization of naturally produced sugars. However, anthropogenic carbon is steadily increasing and there is an opportunity and need to develop processes for degradation, and possibly valorization, of these molecules (Elhacham et al., 2020). These molecules include lipid and lipid-like hydrocarbons. Here, we selected 18 representative lipid and lipid-like model compounds and assessed their use as sole carbon source by a set of 14 yeasts. Four organisms, *R. toruloides*, *C. maltosa*, *S. stipitis*, and *Y. lipolytica*, were identified as having a superior ability to utilize these compounds and convert them to biomass relative to *S. cerevisiae* and other nonconventional yeasts. Not only did these organisms grow to a higher optical density on these molecules, but they were also able to utilize a larger number of the model compounds. This characterization includes 28 instances of previously unreported viable carbons sources for these four organisms. The absence of utilization of some model compounds could be attributed, at least in part, to molecule toxicity. Our findings highlight the potential of nonconventional yeasts for lipid biotechnology, and we hope that they help inform yeast selections based on the substrate range within this class of molecules.

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Supplementary Material

Supplementary material is available online at *JIMB* (*www.academic.oup.com/jimb*).

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Conflict of Interest

The authors declare no conflict of interest.

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