Lactose-assimilating yeasts with high fatty acid accumulation uncovered by untargeted bioprospecting

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Abstract

Bioprospecting can uncover new yeast strains and species with valuable biotechnological and industrial traits, such as the capacity to convert different carbon sources in industrial side- and waste streams into bioproducts. In this study, we conducted untargeted bioprospecting in tropical West Africa, collecting 1996 isolates and determining their growth in 70 different environments. While the collection contains numerous isolates with the potential to assimilate several cost-effective and sustainable carbon and nitrogen sources, we focused on characterizing strains capable of growing on lactose, the main carbon source in the abundant side stream cheese whey from dairy industries. Our screening identified 30 different species capable of growing on lactose in both synthetic complete yeast medium and whey, with 20 of these also capable of growth in lactose-containing minimal medium. The lactose-assimilating yeast collection contains both ascomycetes and basidiomycetes, and includes species known to be lactose-assimilating, species not previously known to grow on lactose, and candidates for new species. Observed differences in growth profiles and ratios of intra- and extracellular lactase activities suggest that the yeasts use a range of different strategies to metabolize lactose. Notably, several basidiomycetes, including *Apiotrichum mycotoxinivorans*, *Papiliotrema laurentii* and *Moesziomyces antarcticus*, which accumulated up to 40% of their cell dry weight, proving that they can convert lactose into a bioproduct of significant biotechnology interest. Our findings highlight the value of bioprospecting of underexplored regions to discover yeast strains with substantial biotechnological potential, which may aid the transition to a circular bioeconomy.

Introduction

Yeasts have emerged as key players in biotechnology and molecular and evolutionary biology (Gaikani et al., 2024; Hittinger et al., 2015; Tullio, 2022). Tens to hundreds of thousands of yeast species are expected to exist in nature, of which only a tiny fraction (< 3000) has been identified so far, and only a handful of these have been extensively characterized (Boekhout et al., 2022). By identifying and characterizing more yeast species and strains, we can broaden our understanding of yeast biodiversity and their impact in nature. In addition, the natural biodiversity of yeasts represents a largely untapped resource for industrial applications, where the yeasts can be used as cell factories to produce fermented foods and beverages and synthesize proteins, lipids, and other metabolites of industrial interest (Binati et al., 2021).

Yeasts exist in almost all known natural biomes and are particularly prevalent on the surface or within fruits, plant exudates, soil, insects, rotting wood, and tree bark (Segal-Kischinevzky et al., 2022). Yeasts also interact with humans as beneficial agents in fermentation and as commensal microbes in the human microbiota. In some cases, they may also act as opportunistic pathogens, especially posing a threat to individuals with weakened immune systems (Banerjee, 2009). Whereas the yeast biodiversity of North America, Europe, and parts of Asia is somewhat well mapped, plenty of niches and geographical locations in the global south remain only superficially explored. These include biodiversity hotspots in tropical Africa and South America that likely hold many new yeast species and lineages with ecologically or industrially interesting traits (Boekhout et al., 2022; Rosa et al., 2023)

Novel yeast strains and species can be found through bioprospecting – the systematic and organized search for useful products derived from bioresources (Hosseini et al., 2022). Untargeted bioprospecting aims to collect every microbe that grows on non-selective plates in the laboratory, while targeted bioprospecting

collects microbes that grow well in a select environment, often of industrial interest. Robotics and high-throughput screening have begun to speed up key steps in bioprospecting, but creating a genetically diverse strain collection is still time-consuming and labor-intensive. Beyond strain isolation, bioprospecting requires pure-streaking of colonies to obtain monocultures and DNA-sequencing of isolates to determine their taxonomic position. To achieve the latter, genomic regions that vary extensively between isolates, but that are flanked by conserved sequences, such as the Internal Transcribed Spacer (ITS) region or the D1/D2 domain of rDNA, are often sequenced by targeted short read sequencing and matched to known species sequences in online databases (Leaw et al., 2006). Once established, the resulting strain collections and screening platforms can be used extensively and repeatedly to screen for different combinations of desired characteristics, and ultimately identifying yeast cell factories for transformation of inexpensive and renewable substrates into a spectrum of essential biochemicals (Geijer et al., 2022; Karim et al., 2020).

To work as a cell factory, the yeast should be easy to store, maintain, and cultivate and be fast and efficient in converting available substrates to desired products without the requirements of expensive additives in the culture medium (Gohil et al., 2021). Currently, industrial yeast bioconversion processes are most often carried out by domesticated strains of the baking, brewing and wine yeast *Saccharomyces cerevisiae* (Kavšček et al., 2015). Domesticated *S. cerevisiae* ferments simple 6-carbon sugars, such as glucose, exceedingly well, while using a variety of widely available nitrogen, phosphor, and sulfur compounds to support its growth, and needs only a few externally added complex nutrients, such as vitamins. Unfortunately, *S. cerevisiae* cannot naturally grow on many carbon sources that are abundant in industrial side- and waste streams, including monosaccharides such as xylose, arabinose, agricultural fructose, disaccharides such as cellobiose and lactose, as well as more complex oligo- and polysaccharides such as starch, cellulose, and hemicelluloses. For example, the inability of *S. cerevisiae* to metabolize lactose restricts its use in valorizing cheese whey consisting of 4.5-5% lactose, proteins, fatty acids, and minerals. With a global, annual production of 100 million tons, whey constitute a substantial by-product of the dairy industry with significant potential as a raw material for bioprocessing (Zotta et al., 2020). The ability to cleave the β-1,4 glucosidic bond between lactose's glucose and galactose moieties using lactases (normally β-galactosidases) and assimilate the breakdown products is relatively rare in the fungal kingdom. For example, among a panel of 332 sequenced ascomycetous yeasts, less than 10% could grow on this carbohydrate (Shen et al., 2018). Among the known lactose-assimilating yeasts, “dairy” yeasts from the *Kluyveromyces* genus have been carefully characterized (Ortiz-Merino et al., 2018)d *lactis* and *K. marxianus* are the predominant species employed in current whey bioconversion processes (Bilal et al., 2022; Tesfaw et al., 2021). Other lactose-assimilating yeasts remain poorly explored but may have considerable potential for commercialization by broadening the range of metabolites that can be produced from whey.

One such class of industrially interesting metabolites is lipids, which can be used in society as fuels, chemicals, and food ingredients. Lipids usually make up around 6 to 8% (w/w) of the cell dry weight (Schulze et al., 2014), whereas yeasts that accumulate more than 20% of cell dry weight in lipids are collectively referred to as oleaginous yeasts (Salvador López et al., 2022). Oleaginous yeast species such as *Yarrovia lipolytica, Rhodotorula toruloides*, and *Cutaneotrichosporon oleaginosus* are highly proficient lipid producers and are currently being characterized and developed into cell factories for the production of lipids and various lipid derivatives. The lipids accumulated in these oleaginous yeasts consist predominantly of triacylglycerols (TAGs), which are stored in lipid bodies within the cell. TAG production from sugar is typically stimulated when

carbon is in excess and another nutrient, such as nitrogen, is growth-limiting (Grillitsch et al., 2011). Importantly, yeast species and strains differ in the types and amounts of TAGs they produce, and the TAG profiles also depend on the carbon source and cultivation conditions used (Brandenburg et al., 2021). Thus, it is of great interest to identify and develop new cell factories that efficiently convert carbon sources in abundant industrial side and waste streams into lipids.

In this study, we used untargeted bioprospecting to discover evolutionary diverse yeasts in tropical West Africa, one of the most species-rich but, in terms of yeast, least explored regions (Rosa et al., 2023). Our comprehensive collection of 1996 isolates underwent rigorous high-throughput screening to accurately measure the capacity of each isolate to grow on a broad spectrum of substrates and in conditions capturing the effect of various industrial stress factors. While the collection contained many isolates with a diverse potential of assimilating cheap and sustainable substrates, we particularly focused on characterizing those capable of converting lactose and found several basidiomycetes that could produce high amounts of lipids from this carbon source. Our approach illustrates how unbiased bioprospecting of the tropics can be used to tap the unexplored natural yeast biodiversity to identify isolates with industrially promising properties.

Results

Untargeted bioprospecting of Nigerian yeasts

To extensively sample a previously underexplored tropical region, we performed the first country-wide yeast bioprospecting of Nigeria, sampling all states except Jigawa, with most yeast isolates originating from Sokoto, Taraba and Ogun (number of isolates (*n*) = 128, 126 and 116 respectively) (Fig. 1A, B). We collected biological samples from presumed yeast-rich niches, such as fruits and fermented beverages, but also included samples from barks, herbs, soils, and waterways (Fig. 1C). Samples were transferred to agar plates with a cultivation medium formulated to promote yeast growth while inhibiting bacterial growth. From these plates, we selected and pure-streaked smooth, yeast-like colonies. The work resulted in a collection of 1996 isolates from > 300 distinct biological samples. While most isolates are yeasts, some bacterial strains are naturally resistant to antibiotics and may have bypassed our antibacterial-based selection process.

Isolates display diverse growth profiles

To enable high-throughput growth characterization, we recovered frozen isolate stocks in 1536 colony arrays on agar plates. We transferred the recovered colonies first to preculture plates and then to experimental plates for measurement of population sizes at dense intervals during 2–3 days of growth in 70 environments (Fig. 2A). These environments were chosen to capture the strains' abilities to use carbon and nitrogen sources and to tolerate stresses such as high osmolarity and low pH that can be present during industrial bioprocessing.

We measured population sizes by automated scanning of the light transmitted through each colony, transformed the pixel intensities into cell counts, and extracted the cell doubling times as a proxy for fitness from > 700,000 growth curves (Fig. 2B) (Zackrisson et al., 2016). The geographic origins (Nigerian state) and the category of source material (e.g., fruits) the strains were isolated from did not group into larger clusters

(Fig. 2B). This suggests that the source habitats harbor diverse sets of microorganisms and that phenotypically similar species are present across the range of sampled sources and locations.

A hierarchical clustering (Fig. 2B) categorized the isolates into three major groups based on growth profiles: a fast-growing group of 1675 strains (83.9%) with generally fast growth across environments, a hexose-preferring group of 239 (12.0%) strains that thrive if supplied with hexoses, but struggle when using pentoses, sugar alcohols, and complex polysaccharides as carbon and a small, slow-growing group of 77 isolates (3.85%) with mostly poor growth across environments. Thus, in agreement with (Opulente et al., 2024), we see that many isolates consistently performed well whereas others consistently performed poorly, arguing against a the “jack of all trades, master of none” trade-off among yeasts. Figure 2C illustrates growth curves underlying doubling time estimates.

A limitation of the division into three supergroups is that it hides the considerable variation in growth in different environments, including the remarkable number of strains capable of fast growth. For example, many strains achieved substantial (< 8h doubling time), moderate (< 5h) or even fast (< 3h) growth in environments where *S. cerevisiae* typically struggle, such as on the carbon sources arabinose, lactose, and xylose, and on cysteine and lysine as sole nitrogen sources (Fig. 2D). In fact, 13.6% of the strains achieved fast growth (< 3h doubling time) in each of these environments except in cysteine where only 66 strains achieved fast growth. Untargeted bioprospecting of underexplored regions can therefore uncover strains that grow well in many environments of potential industrial interest.

Identification of yeasts growing on lactose agar medium

Lactose-rich whey is currently an under-utilized industrial side stream that can be valorized through microbial conversion into a range of metabolites of societal interest. That 27.4% of our Nigerian strains achieved fast (< 3h doubling time) lactose growth (Fig. 2D) is thus of particular interest to investigate further, especially given that *i)* our bioprospecting was untargeted and did not include lactose-rich niches such as dairy farms and products, and *ii)* lactose-assimilation is considered a rare trait in yeast.

To ensure pure monocultures for further downstream analysis, we streaked the fastest lactose-growing strains on separate synthetic complete (SC) agar plates containing 2% lactose, and isolated colonies expanded from individual cells. Of the ~ 400 selected lactose-growing strains, 203 strains maintained growth throughout 2–3 re-streaking passages. From these, we extracted the total DNA, amplified and sequenced their internal transcribed spacer (ITS) regions, and matched the resulting ITS sequences with sequenced type yeasts in the NCBI BLAST database, which revealed 30 unique lactose-utilizing yeast species from both the *Ascomycota* and *Basidiomycota* phyla (Table 1). A handful of bacterial isolates were also identified and removed from the lactose-assimilating dataset, underscoring that the collection is highly enriched for, but does not exclusively contain, yeasts. Although most of the ITS sequences aligned perfectly or nearly perfectly with those of known yeasts, 57 of our lactose-growing strains exhibited less than the 98.41% identity threshold suggested by Vu and colleagues to delineate yeast species (Vu et al., 2016). Of these, 27 isolates exhibited no ITS type-strain match with a similarity higher than 96% to any known species, indicating that they may be candidates for new species (Table 1). However, for simplicity, we call all isolates by the species names of their closest BLAST-hits.

More than half (*n* = 18) of the 30 lactose-utilizing yeast species were isolated multiple times, with the most common being *Candida pseudointermedia* (57 isolates), followed by *Meyerozyma caribbica* (45), *Candida*

intermedia (19), *Candida tropicalis* (12), and *Yarrowia lipolytica* (10). For each of these species, the lactose-assimilating strains were isolated from a wide variety of geographic origins and source materials (Table 1). Thus, these strains are not merely genetic clones, and lactose growth is likely to be a prevalent property in the species and not restricted to strains from a particular region or habitat. We note that while lactose assimilating isolates of many of these species have been identified before, 17 of our species are not listed as lactose-utilizing on the http://theyeasts.org/ webpage, which provides the most up-to-date and accurate taxonomic and phenotypic information on published yeasts (Boekhout et al., 2022). For the yeasts listed as negative for lactose assimilation, we did a literature search and confirmed that most of these species have indeed not previously been shown to grow on lactose, even if some of the described strains had been isolated from dairy products. However, strains of *Apiotrichum mycotoxinivorans*, *Kodamaea ohmeri*, *Kurtzmaniella quercitrusa* and *Candida orthopsilosis* have been shown to grow on lactose (Apolo et al., 2023; Khaled et al., 2013; Sharma et al., 2018; Vicente et al., 2019) and are therefore marked with -* in Table 1. We can conclude that the dataset contains a range of evolutionary distinct species, where some have been previously shown to assimilate lactose and others have not, as well as several potentially new species.

Table 1
Lactose assimilating strains. *Strain(s) shown to grow on lactose, although http://theyeasts.org/ states “no lactose growth”, n.a. = not available.

Species	Division	Lactose assimilating*	Isolates (n)	Sources	States (n)	Sequence identity (%)
Apiotrichum mycotoxinivorans	*Basidiomycota*	-*	5	Citrus fruits (4), water (1)	5	99.87 ± 0.30
Candida intermedia	*Ascomycota*	+	19	Various fruits (18), water (1)	9	95.75 ± 1.27
Candida orthopsilosis	*Ascomycota*	-*	1	Coconut	1	99.31
Candida palmioleophila	*Ascomycota*	-	5	Banana (2), Pineapple (2), Orange (1)	1	99.82 ± 0.00
Candida parapsilosis	*Ascomycota*	-	1	Coconut	1	100
Candida pseudocylindracea	*Ascomycota*	+	1	Water Apple	1	95.97
Candida pseudointermedia	*Ascomycota*	+	57	Various fruits (40), Various tree barks (8), Cassava (2), Soils (2), Waters (2), Coconut (1), Caterpillar (1)	15	99.39 ± 1.07
Candida tropicalis	*Ascomycota*	+	12	Various fruits (6), Various nuts (4), Carrot (1), Water (1)	6	97.28 ± 2.60
Cutaneotrichosporon curvatum	*Basidiomycota*	-	1	Papaya	1	99.78
Cyberlindnera fabianii	*Ascomycota*	-	1	Tree bark	1	99.64
Debaryomyces nepalensis	*Ascomycota*	+	4	Coconut (1), Water (1), Lime (1), Sugarcane (1)	3	98.48 ± 2.58
Kodamaea ohmeri	*Ascomycota*	-*	2	Bark of orange tree (1), Pineapple (1)	2	98.48 ± 0.03

Species	Division	Lactose assimilating*	Isolates (n)	Sources	States (n)	Sequence identity (%)
Kurtzmaniella quercitrusa	*Ascomycota*	-*	1	Tangerine	1	96.82
Meyerozyma caribbica	*Ascomycota*	-	45	Various fruits (25), Various tree barks (6), Cucumbers (6), Coconuts (3), Soils (3), Honeycombs (2), Tree Exudate (1)	13	99.73 ± 0.34
Meyerozyma carpophila	*Ascomycota*	-	3	Avocado (1), Banana (1), Tomato (1)	3	100.00 ± 0.00
Meyerozyma guilliermondii	*Ascomycota*	-	3	Bark of orange tree (2), Coconut (1)	2	99.39 ± 0.38
Moesziomyces antarcticus	*Basidiomycota*	+	1	Lemon	1	96.73
Papiliotrema flavescens	*Basidiomycota*	+	2	Soil	1	100.00 ± 0.00
Papiliotrema laurentii	*Basidiomycota*	+	2	Guava (2)	1	97.65 ± 0.04
Papiliotrema rajasthanensis	*Basidiomycota*	+	1	Eggplant (1)	1	99.75
Pichia kudriavzevii	*Ascomycota*	-	5	Citrus fruits (2), Coconuts (2), Water (1)	4	92.38 ± 5.51
Rhodosporidiobolus ruineniae	*Basidiomycota*	-	2	Velvet tamarind (2)	2	98.73 ± 0.74
Rhodotorula mucilaginosa	*Basidiomycota*	-	2	Lime (2)	1	96.89 ± 3.35
Trichosporon asahii	*Basidiomycota*	+	7	Various fruits (4), Various vegetables (3)	4	99.64 ± 0.63
Trichosporon insectorum	*Basidiomycota*	+	1	Soil	1	100
Ustilago sparsa	*Basidiomycota*	n.a.	1	Lemon	1	97.89

Species	Division	Lactose assimilating*	Isolates (n)	Sources	States (n)	Sequence identity (%)
Vishniacozyma taibaiensis	*Basidiomycota*	-	1	Lemon	1	99.78
Yamadazyma mexicana	*Ascomycota*	+	1	Cassava	1	99.81
Yarrowia galli	*Ascomycota*	-	4	Pineapple (2), Avocado (1), Coconut (1)	3	97.06 ± 0.89
Yarrowia lipolytica	*Ascomycota*	-	10	Various fruits (10), Coconut (1)	6	98.58 ± 0.08

Yeast isolates display robust yeast biomass production in cheese whey

To evaluate whether the 203 isolates capable of growth on SC lactose agar plates could also use liquid whey as a growth substrate, we prepared fresh whey using a cheese-making kit. The whey was filter-sterilized to eliminate contaminating microorganisms and then used as the sole growth substrate for the strains in liquid microscale cultivations, without addition of any supplementary nutrients.

Almost all isolates (94.6%) exhibited robust growth (mean doubling time of 2.17 h) in whey (Fig. 3A). However, while lactose is the dominant carbon and energy source in cheese whey, small amounts of other sources of carbon and energy may also be present and may help support growth (Osorio-González et al., 2022). Thus, to ensure that the recorded growth was accompanied by lactose consumption, we analyzed the lactose content of the whey before and after three days of microscale liquid cultivation (Fig. 3B). We found that especially the basidiomycetous yeasts *Moesziomyces antarcticus, Ustilago sparsa, Papiliotrema laurentii* and *Papiliotrema rajasthanensis* had consumed all, or almost all, the available lactose. Other yeast species, including the ascomycetes *C. (pseudo)intermedia*, displayed a more moderate consumption, averaging 27.6%. For these species, there was substantial variation in lactose consumption among different strains. However, many species with good growth on whey failed to consume substantial amounts of the lactose available and must thus have achieved their growth primarily by catabolizing other carbon sources present in the whey. This may not necessarily reflect an inability to use lactose in whey but could potentially indicate suppression of lactose utilization by the presence of a preferred carbon source, similar to the glucose repression of galactose assimilation in *S. cerevisiae* (Simpson-Lavy and Kupiec, 2019).

To track the fate of the carbon consumed by the yeast isolates, we also measured the extracellular accumulation of the lactose hydrolysis products glucose and galactose, as well as a variety of small carbon compounds that often serve as end products of carbon catabolism. Overall, we found small but detectable levels of glucose, galactose, galactitol, succinate, and glycerol in multiple species. *Trichosporon asahii*, *Trichosporon insectorum* and *Cutaneotrichosporon curvatum* produced approx. 0.5 g/L of fumarate, and moderate amounts (0.5–2.3 g/L) of ethanol were produced by some isolates of *Apiotrichum mycotoxinovorans*, *Candida orthopsilosis*, *Candida palmioleophila*, *Candida (pesudo)intermedia*,

Debaryomyces nepalensis, *Meyerozyma caribbica*, *Yamadazyma mexicana*, *Yarrowia galli* and *Yarrowia lipolytica* (Fig. 3C). However, in general, the carbon contained in these small carbon compounds was negligible, indicating that most of the carbon consumed had been channeled into yeast biomass and/or carbon dioxide (not measured). Thus, modifications to either the whey composition, the operational conditions or the yeast strains themselves may be necessary to optimize the production of these and other relevant metabolites.

Yeast isolates differ in their capacities to grow on and hydrolyze lactose

The discrepancy between growth and consumption of lactose in whey for some of the strains prompted us to test whether the yeasts can assimilate lactose in the absence of other carbon sources. We precultured strains in SC glucose and then transferred them to Minimal Medium (MM) supplemented with lactose only, and tracked growth of populations as the change in optical density in microscale (250 µL). The resulting growth curves revealed a variety of growth capacities, ranging from very fast growth (e.g. *A. mycotoxinivornas*, *C. (pseudo)intermedia* and *P. laurentii*) to no detectable growth at all (e. g. *M. carribica*, *T. insectorum* and *Yarrowia galli*) (Fig. 4A). Moreover, some yeasts, including individual isolates of *M. carribica and Y. lipolytica*, displayed a long lag phase in liquid MM lactose, which may mean that the recorded doubling time does not reflect their maximal growth rate. Also, we cannot rule out the possibility that the absence of growth may actually reflect lag phases longer than the 72-hour time frame of the experiment. Nonetheless, we found a substantial overlap between poor lactose consumption in liquid whey and poor growth in liquid MM lactose. Of the 17 species with an average lactose consumption of less than 10% in whey, 10 did not grow in liquid MM lactose, while the remaining species showed growth in only one or two out of several strains.

The ability to grow in MM lactose was next correlated with the expression of lactases, which cleave the lactose glucosidic bond. Many yeasts including *Kluyveromyces* are known to import lactose and break the lactose intracellularly (Lane and Morrissey, 2010), while others such as *M. antarcticus*, secrete lactases and thus break the lactose glucosidic bond extracellularly (Nascimento et al., 2024) with the resultant monomers being imported and catabolized. To determine both the degree and localization of lactase activities, we harvested end-points (72h) of the microscale MM lactose liquid cultures, separated cells from the extracellular liquid and used a β-galactosidase activity assay to measure the lactase activity in both fractions. This revealed a range of different intra- and extracellular lactase activities (Fig. 4B). The isolates with no, or almost no, lactase activity (< 1 absorbance at 420 nm) were all isolates that failed to grow on lactose, and all isolates with substantial lactose growth also displayed some lactase activity (> 1 absorbance at ABS420). However, the capacity to break down lactose and convert it into yeast biomass (growth) was far from uniform. For example, the numerous *M. carribica* strains, which almost uniformly failed to grow in liquid MM lactose, displayed a range of (intracellular) lactase activity levels. In terms of lactase localization, the majority of the strains displayed preferentially intracellular breakdown of the glucosidic bond. However, interestingly, we note that many of the fastest lactose-growing isolates, including *M. antarcticus*, *U. sparsa* and *A. mycotoxinivorans* and some, but not all, of the isolates of *C. pseudointermedia*, had both intra- and extracellular lactase activities. The results suggest that the lactose metabolic strategies vary substantially, both between and within species.

We hypothesized that the inability of some strains to grow in liquid MM lactose, despite having detectable lactase activities and despite growing on both whey and SC lactose agar, could be explained by the absence of externally supplied amino acids in MM. To clarify this, we streaked a subset of isolates that did not grow in

liquid MM lactose (*M. carribica and M. guilliermondii, Y. lipolytica, Y. galli, C. tropicalis, T. insectorum* and *K. ohmeri*) on MM agar plates supplemented with either glucose or lactose. We also included the auxotrophic *S. cerevisiae* lab strain BY4741, which cannot grow on MM due to the absence of uracil, histidine, leucine, and methionine, as a negative control. All strains, except *S*cBY4741, grew on MM glucose plates (Fig. 4C), strongly suggesting that they are prototrophs on glucose medium. On MM lactose plates, we found *T. insectorum* to grew well, although it completely failed to grow in liquid MM lactose, showing that its lactose growth depends on the mode of cultivation. *M. carribica, M. guilliermondii, Y. lipolytica, Y. galli, C. tropicalis, T. insectorum* and *K. ohmeri* formed microcolonies visible to the naked eye on MM lactose agar (Fig. 4D). As these strains formed much larger colonies on SC lactose agar (Fig. S1), this result suggests that their lactose growth is dependent on the media composition.

During our re-streaking of isolates on SC lactose agar, we lost approximately 50% of the original 400 lactose-growing isolates identified on the colony arrays agar plates used for initial growth phenotyping of the whole collection (Fig. 2). Because some isolates were subsequently found to secrete lactases (Fig. 4B), we hypothesized that the loss of these isolates could, at least partly, be explained by that they are not truly lactose assimilating, but rather that they grew in the initial arrays using extracellular lactases and their monomeric breakdown products produced by adjacent colonies on the shared nutrient medium on the agar plates. To explore this hypothesis, we set up a feeding experiment, where one *A. mycotoxinivorans* strain with high levels of external lactase activity was placed in the middle of an MM lactose agar plates and then surrounded by yeast isolates displaying poor or no growth when cultivated alone on this medium. *T. insectorum*, which autonomously produces intracellular lactases on MM lactose agar, was used as a positive control. Indeed, the presence of *A. mycotoxinivorans* allowed slow but evident growth of all cells close to the feeder strain (Fig. 4E). While this communal aspect of yeast lactose growth bears some resemblance to natural microbial consortia, we note that it could serve as a confounding factor for the identification of lactose-growing strains in experimental setups where the isolates share the environment, such as agar plates. Likely, this holds true also for other carbon sources for which the degradation partially or completely takes place extracellularly, including plant biomass polymers such as cellulose and different hemicelluloses.

Overall, there seem to be multiple species and strains in our dataset for which the capacity to grow on lactose is influenced by the cultivation system (liquid/solid) and media composition. We conclude that caution is needed when assigning a strain with the ability to grow on lactose.

Basidiomycetous Yeasts convert lactose into stored lipids

To function as a future cell factory, a yeast strain must not only assimilate a substrate efficiently but must also convert it into product(s) of industrial interest. A literature search showed that several of the identified yeasts, particularly the basidiomycetous species, have been reported as oleaginous yeasts, i.e., they can produce and accumulate lipids to > 20% of the dry cell weight.

We therefore investigated the ability of 14 isolates, selected for their robust lactose-assimilation, to accumulate fatty acids from lactose under nitrogen-limited conditions (carbon to nitrogen (C/N) ratio 50), again using liquid MM lactose as it is completely devoid of additional carbon sources and gives us full control of the C/N ratio. We first conducted 250 µL miniature cultures, tracking growth well into the stationary phase where most of the lipids are typically produced and stored (Rostron and Lawrence, 2017), and identified 120

hours as an appropriate time point for sampling (Fig. 5A). We then cultured the strains in 30 mL shake flask cultures (*n* = 3) for 120 h, extracted the total lipids from harvested and freeze-dried cells, and measured their lipid content relative to cell dry weight (Bligh and Dyer, 1959) (Fig. 5B). Over half (n = 8) of the 14 tested strains demonstrated substantial lipid production and accumulation (exceeding 20% of their total dry weight). The basidiomycetous strains previously recognized as oleaginous (strain names in blue in Fig. 5B), did indeed show high lipid content. To the best of our knowledge, lipid production from lactose has not been previously demonstrated for most of these species. This holds true also for *A. mycotoxinivorans*, the most prolific lipid producer found in our screen, which accumulated 40% of its cell dry weight in lipids. We also note that some isolates, particularly the isolate of *C. curvatum*, accumulated relatively high lipid titers (1.6 g/L) despite not achieving a particularly high growth yield on lactose. This suggests that the carbons from lactose were channeled into lipids rather than biomass (Fig. 5A-B). Of the species whose isolates failed to produce high lipid levels (< 20% dry weight), none were previously known as oleaginous. We conclude that some of our best lactose-assimilating yeasts, including isolates of *A. mycotoxinivorans*, *Papiliotrema laurentii*, *Moesziomyces antarcticus* and *C. curvatum*, have the capacity to effectively channel the lactose into lipids and thus demonstrating high cell factory potential.

Discussion

Untargeted yeast bioprospecting uncovers isolates of potential industrial use

Tropical rainforests and other species-rich biomes can be found in Asia, Australia, Africa, Central and South America, Mexico, and many Pacific Islands. These biomes cover less than 20% of the Earth's land area but may contain up to 50% of the planet's biodiversity (Dinerstein et al., 2017; Rosa et al., 2023). Numerous studies have been carried out in Central America, South America, and Asia (Barros et al., 2023; Cadete et al., 2012; Guamán-Burneo et al., 2015), while Africa and Australasia remain underexplored sources of novel microorganisms, such as yeasts (Rosa et al., 2023). As these biomes are under threat due to deforestation (Hoang and Kanemoto, 2021) and environmental changes, it is important to catalog their microbial biodiversity before it is lost.

We used an untargeted bioprospecting approach in the sense that we isolated yeasts on agar plates with a rich nutrient medium and glucose as carbon source, allowing the growth of a wide range of yeast species, while deselecting for bacteria by including three antibiotics in the medium. Admittedly, using a single isolation growth medium, we likely missed many species that requires substantially different nutrients, pH, temperature, osmolarity, or other factors to grow and that could have been identified through e.g., a metagenomic study. However, the single isolation condition approach served an important purpose: all the isolated strains could subsequently be cultivated together on the same media plates and compared in an unbiased fashion. This makes it easy to extend the phenotypic characterization to encompass more environments of industrial interest. And while the cultivation of yeast as colonies on shared nutrient medium opens for strain-strain interactions which can influence growth estimates, it probably better resembles the natural growth mode of most yeasts.

Lactose growth capacity found in a diverse set of yeasts

Although we did not sample any lactose-rich environments, we found several yeast species from both the *Ascomycota* and *Basidiomycota* phyla that could grow on this carbon source. Lactases, the collective name of β-galactosidases and (less commonly) β-glucosidases active on lactose, are found in several glycoside hydrolase (GH) families that differ in structure, substrate specificity, catalytic mechanism, and optimal working conditions (such as pH and temperature), contributing to their functional diversity (Movahedpour et al., 2022). Most likely, these enzymes initially evolved to degrade ubiquitous plant biomass-derived oligo- and disaccharides rather than lactose, which is a relatively scare carbon source outside dairy farms. We hypothesize that upon extended exposure to lactose, yeasts that possess these enzymes with basic levels of activity towards lactose may evolve more efficient lactases over time. Supporting this hypothesis, we found strains exhibiting lactase activity but no lactose growth, and substantial variability in the lactose-growth capacity among different strains of the same species. However, obtaining a comprehensive understanding of lactose metabolism across various species and strains would necessitate genome sequencing and detailed molecular characterization of their lactose catabolic traits, which falls outside the scope of this study.

Lactose metabolism has been studied in detail in *Kluyveromyces* yeasts, where lactose is imported across the cell membrane via a *LAC12*-encoded lactose permease and subsequently hydrolyzed by a *LAC4*-encoded lactase (a β-galactosidase from GH family 2) (Schaffrath and Breunig, 2000). The *LAC12* and *LAC4* genes are co-regulated and sit next to each other in the genome, forming a metabolic gene cluster (the *LAC* cluster) (Varela et al., 2019). In contrast, little is known about how lactose is hydrolyzed and taken up by most other yeasts. For example, some of the best lactose-growing yeasts in our dataset, *A. mycotoinivorans, P. laurentii* and *C. curvatum*, have not yet been thoroughly characterized. Interestingly, these fast-growing basidiomycetous yeasts exhibited both intra- and extracellular lactase activities, and the GH families of these enzymes remain unknown. Future research includes characterizing these lactases, which may possess different properties in terms of optimal pH and temperature, substrate specificity, and end-product inhibition, compared to lactases from *Kluyveromyces* (GH2) and *Aspergillus* (GH1) currently dominating in industrial production of lactose-free dairies.

Surprisingly, we did not isolate any lactose-assimilating *Kluyveromyces* strains in our collection. Instead, the most frequently sampled species were *C. intermedia* and its sister species *C. pseudointermedia,* They belong to the *Metschnikowia* family of ascomycetous yeasts, and are, to the best of our knowledge, the only species in this family that display robust lactose growth (Peri et al., 2023). Our previous work on *C. intermedia* shows that this species possesses both the conserved *LAC* cluster and an additional gene cluster, the *GALLAC* cluster, which is essential for its lactose growth (Peri et al., 2023). The exact phylogenetic relationship between *C. intermedia* and *C. pseudointermedia* has not yet been determined, and whether *C. pseudointermedia* also possesses a *GALLAC* cluster remains to be elucidated. We also isolated multiple strains of *M. carribica* and the closely related yeast *M. guilliermondii.* In contrast to *C. (pseudo)intermedia*, the isolated *Meyerozyma* strains did not display significant lactose-consumption in whey nor robust growth in liquid MM lactose medium (although they grew on SC lactose agar plates). Genomic analysis of the type strains of both *M. carribica* and *M. guilliermondii* shows that they possess the conserved *LAC* cluster, confirming their genetic prerequisites for lactose metabolism (unpublished results). Follow up studies will focus on elucidating the relationship between the ability to grow on lactose, the genetic setup, and the composition of the growth medium, both for these *Meyerozyma* strains and for other strains displaying inconsistent lactose-growth within the collection.

Basidiomycetous yeasts – future cell factories for conversion of lactose into lipids?

Our study identified several oleaginous basidiomycetous strains capable of efficiently converting lactose into lipids. Currently, yeast lipids are mostly produced from single mono- and disaccharides, hydrolysates, and glycerol, accounting for around 70% of the feedstocks used. In contrast, whey accounts for less than one percent of the feedstocks used, although it is a sizable, available, and cost-competitive (Abeln and Chuck, 2021). This underutilization of whey can be attributed, in part, to the limited metabolic capacity of known oleaginous yeasts to metabolize lactose. In this way, our findings open new possibilities for the use of whey for lipid production in the future.

To date, over 160 yeast species have been described as having the ability to store more than 20% (w/w) of their dry weight as lipids, and thereby termed oleaginous. Literature is dominated by *Cutaneotrichosporon oleaginous, Rhodotorula toruloides* and *Y. lipolytica*, while many other of the oleaginous yeasts are still poorly characterized (Abeln and Chuck, 2021). Neither *C. oleaginous* nor *R. toruloides* were found in our screen, but we identified ten strains of *Y. lipolytica*. However, they did not readily consume lactose in whey, nor did they grew well in MM lactose, consistent with literature stating that *Y. lipolytica* is a poor lactose-grower (McKay, 1992). Instead, we found several basidiomycetous, oleaginous yeasts, including *A. mycotoxinivorans* (formerly *Trichosporon mycotoxinivorans*), *P. laurentii*, *M. antarcticus* and *C. curvatum* (formerly *Apiotrichum curvatum*), which all readily assimilated lactose in whey and produced high levels (25-40%) of lipids.

While we used MM lactose for lipid production, as this allowed us to set a defined, high carbon to nitrogen ratio to promote lipid biosynthesis and accumulation, we see no reason why cheese-whey could not serve as a substrate for these yeasts. In fact, optimizing the cultivation conditions in terms of temperature, feeding strategy, and medium composition, including fine-tuning the C/N to perfectly fit the needs of individual species and strains, holds promise to significantly enhance lipid titers and yields (Lei et al., 2024). Moreover, genetic engineering can enable the construction of lipid-overproducing strains and/or strains in which b-oxidation and lipid turnover are abolished, ensuring that produced lipids are accumulated rather than consumed (Szczepańska et al., 2022). We hope this study can help basidiomycetous yeasts gain the visibility they deserve for their superior lactose-to-lipid conversion capacities and cell factory potential.

Conclusions and Future Perspectives

In conclusion, bioprospecting is important for understanding the natural yeast biodiversity. The resulting strain collections can be used to identify novel yeasts with cell factory potential and lead to development of new and improved industrial applications. In our dataset of lactose-assimilating yeasts, we find multiple species with superior lactose growth that have not previously been acknowledged for this trait. We also found several strains that do not match known species particularly well, suggesting that they are new species. However, a more extensive combination of phenotypic profiling, DNA barcoding, and multi-locus phylogenetics would be essential to robustly delineate and describe these novel yeast species (Ryan et al., 2024). Additionally, we show that some strains produce high amounts of lipids from lactose, which can be further improved in by extensive process optimization and strain development.

Methods

Collection of source material and yeast isolation

Nigerian Yeast Collection: Different yeast source materials were collected from environments and geographical regions across Nigeria (Fig. 1). Samples were collected in sterile plastic bags in the morning (16-20 °C) and transported to the lab within 24 hours. One unit of source material was mixed with nine units of sterile water to create a stock solution. Immediately after mixing, the solution was tenfold diluted in sterile water. From the dilutions, 100 µL was spread on sterile Saccharomyces Sensu Stricto Enrichment Medium (SSEM) (Liti et al., 2017), containing yeast extract (3 g/L), malt extract (3 g/L), bacto peptone (5 g/L), glucose (10 g/L), and chloramphenicol (200 mg/L) and 2% agar. The plates were cultured at 25-28 °C for 24-72 hours because most yeasts are mesophilic (Kurtzman et al., 2011). Single colonies were pure streaked, and their colony morphology was analyzed. Selected strains were transferred to SSEM agar in 96-well plates and transported to Sweden for further phenotyping and storage. To keep only yeast strains, the final strain collection was further selected on chloramphenicol (200 mg/L), ampicillin (100 mg/L) and gentamycin (20 mg/L).

Lactose Yeast Collection: To isolate wild yeasts utilizing lactose as a sole carbon source, the collection of 1996 strains were pinned to unbuffered SC medium composed of 0.14% Yeast Nitrogen Base (CYN2210, ForMedium), 0.5% NH4SO4, 0.077% Complete Supplement Mixture (CSM; DCS0019, ForMedium), 2.0% (w/v) glucose, and 0.6% (w/v) NaOH, with 2.0% (w/v) agar and 2% lactose. After 7 days of incubation at room temperature, lactose-growing colonies were streaked for single colonies on fresh SC lactose medium. Positive strains were streaked through single colony bottlenecks 2-3 times to ensure pure isolates. Strains were observed microscopically to confirm their identity. The strains were cultured in YPD (Yeast Peptone Dextrose) composed of 1.0% (w/v) Yeast extract, 2.0% (w/v) Peptone and 2.0% (w/v) glucose overnight at 30 °C and stored in 20% glycerol at -80 °C in 96-well microtiter plates.

Species Identification: The ITS region was sequenced using default primer pairs (Macrogen and Eurofins). Species identification was performed by NCBI BLAST against the standard Nucleotide collection and the ITS Fungi type and reference material database. The top hits from both databases were used to determine species identities, and the percentage identities were recorded in Table S1.

Yeast Growth Media and Phenotyping Conditions

Storage and recovery of strains: All yeast strains were stored at -80 °C in 20% glycerol. The yeast collection was recovered and distributed in arrays on solid agar plates by robotic pinning (ROTOR HDA, Singer Instruments, UK). The solid medium Singer Plus plates (Singer Instruments, UK) were cast on a leveled surface with the addition of 50 mL agar medium and air-dried at room temperature for two days before use. Frozen glycerol stocks of yeast strains were recovered on YPD medium. Except during the recovery of frozen glycerol stocks, yeast strains were pre-cultivated on SC medium pH buffered to 5.8 with 1.0% (w/v) succinic acid, and 0.6% (w/v) NaOH, with 2.0% (w/v) agar to solidify the medium. In nitrogen-limiting environments, the background medium was modified to avoid confounding growth on stored nitrogen by replacing CSM with 20 mg/L uracil and reducing the $(NH_4)_2SO_4$ concentration to 30 mg Nitrogen/L.

Growth phenotyping media: Carbon environments were composed of SC medium, with various concentrations or other carbon sources replacing 2.0% glucose. Stress environments were prepared by supplementing SC medium with various compounds while keeping glucose as the sole carbon source at 2.0%. Nitrogen-limiting environments were prepared using a modified SC medium with 20 mg/L uracil instead of CSM and supplemented with various nitrogen sources to 30 mg Nitrogen/L. Carbon environments were prepared using other sugars or concentrations instead of glucose. Liquid cultures and, in a few cases, solid media environments were composed of MM (minimal medium) medium and contained 2.0% agar (w/v), 20 g/L lactose or glucose, 5 g/L ammonium sulfate, 3 g/L potassium phosphate, 1 g/L magnesium sulfate, vitamins, and trace metals as described previously (Verduyn et al., 1990), with pH adjusted to 5.0 using 2 M KOH.

Cheese whey preparation: Cheese whey was prepared fresh using fresh unhomogenized whole milk with a fat content of 3.8-4.5%. A quarter of a rennet tablet (Fromase 50, DSM Food Specialities) was dissolved in 50 mL H_2O. Separately, 7.5 ml citric acid powder was dissolved in 50 mL H_2O. A volume of 4.5 L milk was added to a pan, and the citric acid solution was carefully spread in the milk with a slotted spoon. The solution was slowly heated to 32 °C and removed from the heat source. The rennet solution was added, and the solution was left undisturbed under a lid for 20 minutes. The cheese curds were cut, and the solution was heated to 40 °C. The cheese whey was separated from the curds and filter-sterilized to remove any potential contaminants.

Growth phenotyping in high-throughput

Solid media growth phenotyping: Yeast strains were arranged in colony arrays and individually tracked for growth using the Scan-o-matic system (Zackrisson et al., 2016) version 2.2 (https://github.com/Scan-o-Matic/scanomatic/releases/tag/v2.2). Solid media plates were secured in position by an acrylic fixture, left undisturbed without lids for 48 h in high-resolution desktop scanners (Epson Perfection V800 PHOTO scanners, Epson Corporation, UK) kept inside dark, humid, and temperature-controlled (30 °C) thermostatic cabinets. Colony growth on the plates was recorded by sequential transmissive scanning at 600 dpi resolution, with four plates per scanner, during twenty-minute intervals. Pixel intensity normalization and standardization were performed across different scanners and experimental runs using a transmissive grayscale calibration strip. Each scanned plate had a virtual grid placed across it, with intersections at the center of each colony. Colonies and the surrounding agar medium were segmented, and local background was separated from colony pixel intensities. A pre-defined *Saccharomyces cerevisiae* calibration function, based on spectroscopic and flow cytometer measurements (Zackrisson et al., 2016), was used to convert colony pixel intensities to cell numbers. Population size growth curves were obtained, and growth rates were identified at the steepest slope using a local regression. The growth curves were processed to remove noise, and erroneous curves were manually inspected and excluded from the analysis. Growth phenotypes were extracted as numerical values from all growth curves which passed the quality requirements (Table S2).

Liquid media growth phenotyping: A subset of strains capable of growing on lactose as a carbon source were further analyzed in liquid media. Strains were precultured for 72 h in 96-well microtiter plates. In Growth Profiler 96 well plates, 250 µL fresh media was inoculated with 5 µL saturated starter culture. The plates were mounted in the Growth Profiler 960 (Enzyscreen), and the cultures were imaged at 30-minute intervals, as controlled by the Growth Profiler Control v6.1.0. The increase in culture turbidity was detected by the software

GP960Viewer and reported as green values. Growth curves were analyzed using PRECOG (Fernandez-Ricaud et al., 2016), and the single numeric growth parameters generation time, lag, and yield were extracted.

Detection of metabolites and bioproducts

β-Galactosidase-activity: was detected using the Yeast β-Galactosidase Assay Kit (75768, Thermo Scientific) following the Microplate protocol provided by the manufacturer. After recording the final timepoint, 100 µL of the liquid cultures were transferred to new 96-well plates. The plates were centrifuged at 5000 g for 10 minutes to pellet the cells. The intracellular (INT) and extracellular (EXT) activities were separated by transferring 80 µL of the supernatant from each well to a new plate (EXT), while the remaining cell pellets were washed with 180 µL of distilled water in the original plate (INT). Both plates were centrifuged again at 5000 g for 10 minutes. For the INT plate, the supernatant was discarded, and 70 µL of distilled water was added to the pellets. For the EXT plate, 70 µL of the supernatant was transferred to a new plate (EXT2). Next, 70 µL of a 1:1 mixture of Y-PER reagent and assay buffer was added to each well of both plates (INT and EXT2). The plates were incubated at 37 °C with shaking at 400 rpm. After 15 minutes, the reactions were stopped by adding 56 µL of stop solution to each well. The plates were then mixed for 10 seconds at 400 rpm. The absorbance was measured using a plate reader (FLUOstar Omega) at 420 nm.

HPLC: Yeast cultures were centrifuged in their respective wells of the 96-well GrowthProfiler plates at 5000 g for 10 min. For each sample (n=1), 100 µL of the supernatant was tenfold diluted; cells were removed by passing the 1 mL diluted samples through a syringe filter (0.22 µm polyethersulfone). The processed samples were transferred to HPLC glass vials. The analysis aimed to detect media components and metabolites using an Aminex HPX-87H carbohydrate analysis column (Bio-Rad Laboratories). The injection volume was 5 µL and HPLC column was maintained at 80 °C, and 5 mM H_2SO_4 served as the mobile phase at a constant flow rate of 0.8 mL/min. Chromatogram peaks were treated and integrated using the Chromeleon v6.8 software (Dionex).

Total Lipid Extraction: The lipid content was determined from dry cell weight using a protocol adapted from (Bligh and Dyer, 1959). Yeast strains were cultivated in 2% lactose YNB medium with a C/N ratio of 50, and the cultures were incubated at 30 °C for 120 h. In 250 mL shake flasks, strains were inoculated to an OD600 of 0.1 in 30 mL media in triplicate. After incubation, cells were harvested by centrifugation at 3000 g for 5 min, washed twice in double-distilled sterile water, and freeze-dried (Labconco 7753011) for 24 hours. The dry weight of the cells was measured. Cell disruption was achieved by adding 1.5 mL of 2M HCl and incubating at 90 °C and 700 rpm for one hour. Subsequently, each sample received 3 mL of a 1:2 (v/v) chloroform-methanol solution and was mixed at 1000 rpm at 25 °C for 10 min. After that, 1 mL chloroform and 0.8 mL 0.88% (w/v) KCl was added and the samples were mixed at 1000 rpm in 25 °C for 5 minutes, followed by centrifugation at 3000 g for 10 minutes. The lower chloroform phase was transferred to pre-weighed tubes and centrifuged again. Any traces of an upper aqueous phase in the chloroform samples were removed. Solvents were evaporated by flushing N_2 gas for 48 h using an evaporator. Finally, total lipid extracts were weighed, and lipid content was calculated by dividing the lipid weight by the cell dry weight.

Declarations

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Author contributions

Conceptualization: KP, OA, JW, and CG; methodology: KP, VO, IN, KVRP, OA, JW, CG; investigation: KP, VO, IN, KVRP, CO, PN, COn, SO; manuscript preparation: KP, JW and CG. All authors approved the final manuscript.

Availability of data and materials

Available on reasonable request.

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Figures

Figure 1

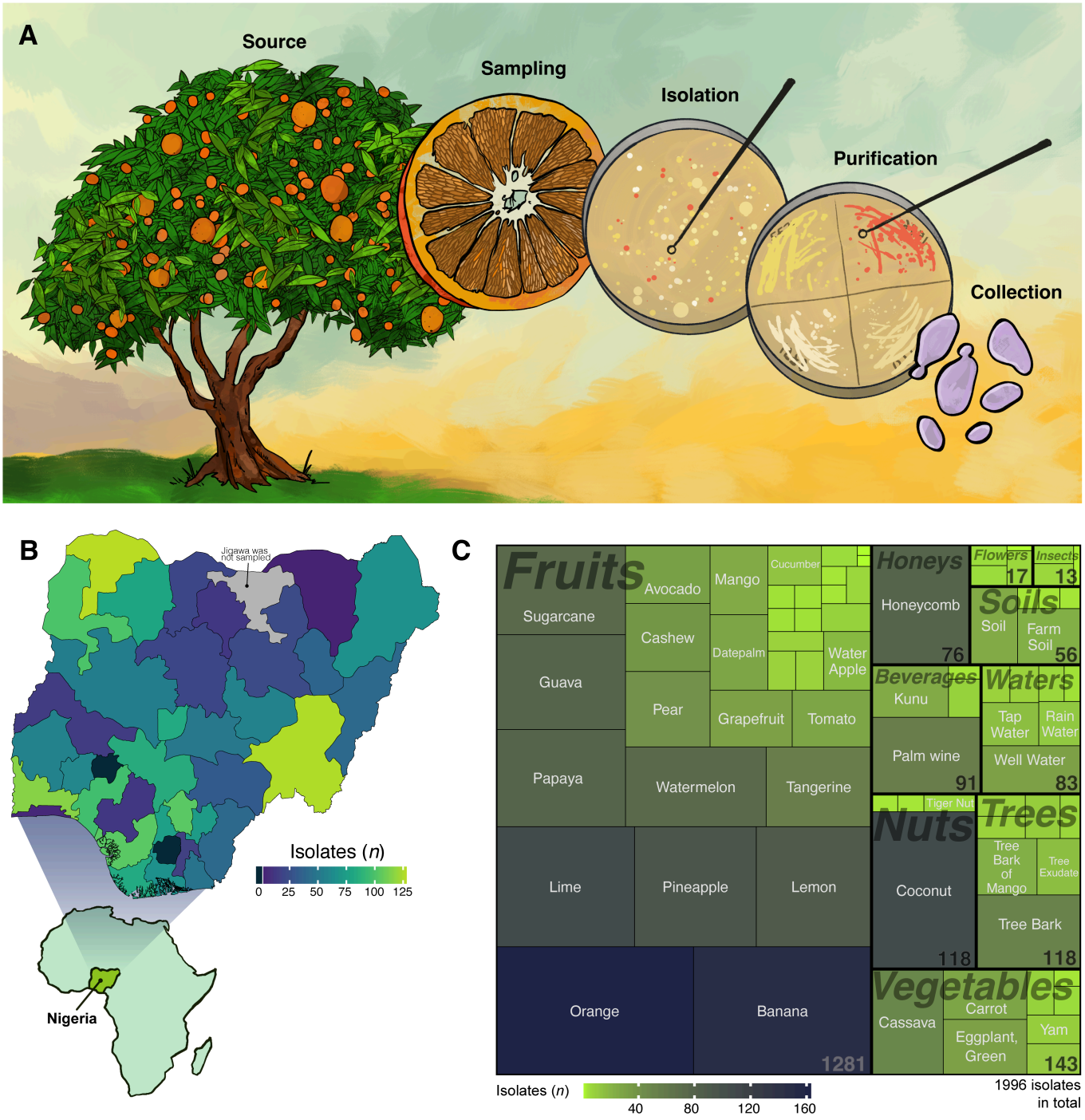

Figure 1

Untargeted bioprospecting in Nigeria. A) Yeast isolation strategy. Samples were collected in sterile plastic bags, transferred to water and spread on SSEM medium. After 24-72h of growth, diverse single colonies were pure-streaked, and further selected on chloramphenicol (200 mg/L), ampicillin (100 mg/L), and gentamycin (20 mg/L) to remove potential bacterial contaminants. The final collection was stored in 20% glycerol at -80 °C. B) Geographic origin of samples. Nigerian states are color-coded according to the number of isolates in the final strain collection. C) Source material of samples. The tree-map chart shows the proportions and types of source materials from which the strains were isolated. Fields are color-coded by the number of isolates (*n*) of

each source material. Numbers in the bottom right corner show the number of samples from each category of sources (e.g., fruits).

Figure 2

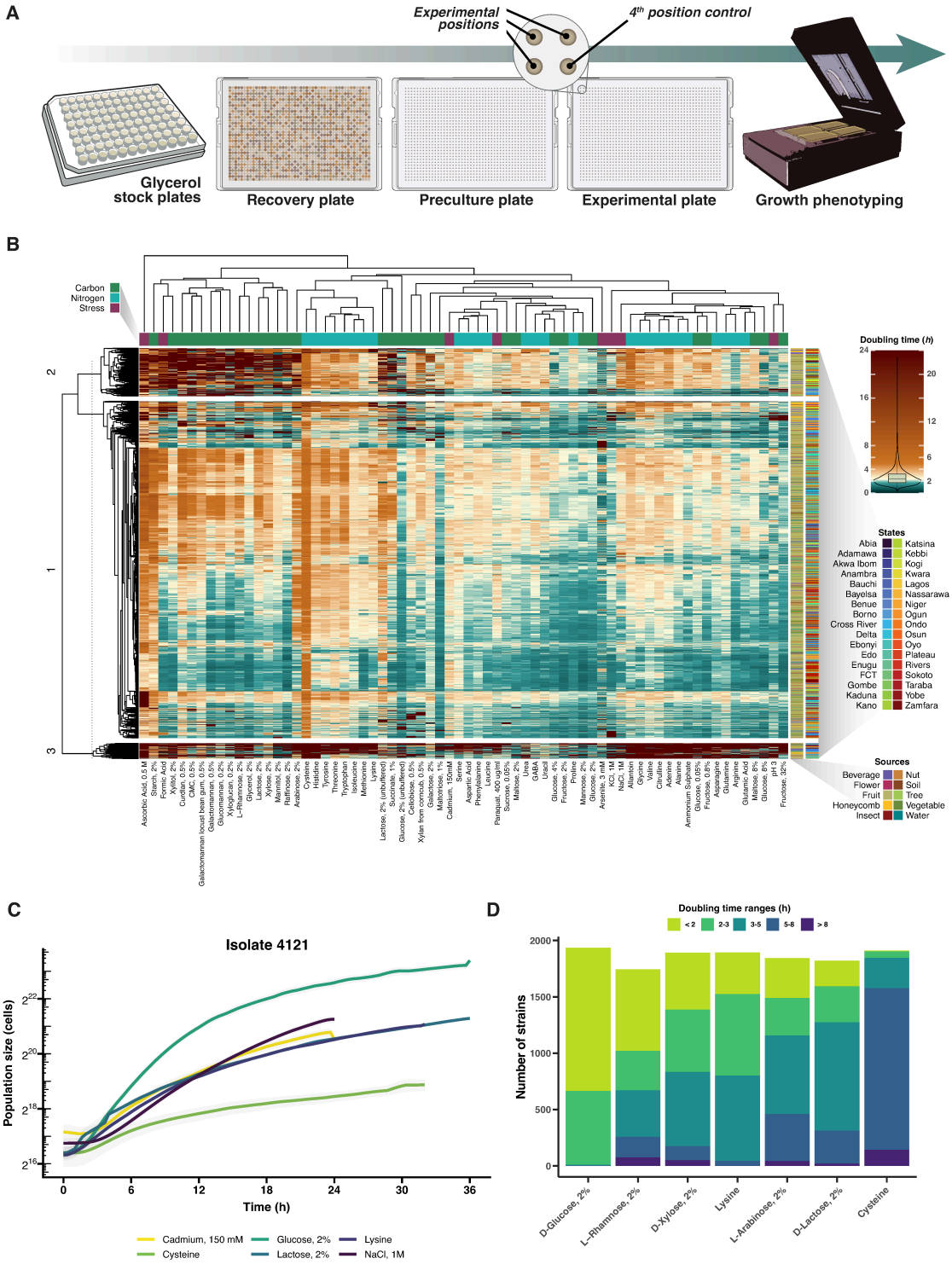

Figure 2

Growth phenotyping of bioprospected strains in high throughput. A) Design of colony growth experiments on agar medium. Isolates were recovered from glycerol stocks and robotically arranged in 1536 colony array grids on recovery (YPD) plates and then transferred first to pre-cultivation plates (SC, 2% glucose) and then

experimental plates (SC with different carbon and nitrogen sources as well as stress factors). B) Heatmap showing the cell doubling time of all isolates (*y*-axis, *n*=1996) in all tested environments (*x*-axis). Isolates and strains were grouped based on similarity in doubling time profiles using hierarchical clustering (Euclidian distance metric, median used for groups). Numbers to the left indicate major group, with the broken line indicating the delineation of these groups. C) Example growth curves of one strain in select environments. The *y*-axis shows cell counts in cell populations. D) Stacked bar plot showing cumulative numbers of yeast isolates with a range of doubling times in glucose and in environments where wildtype *S. cerevisiae* fail to grow.

Figure 3

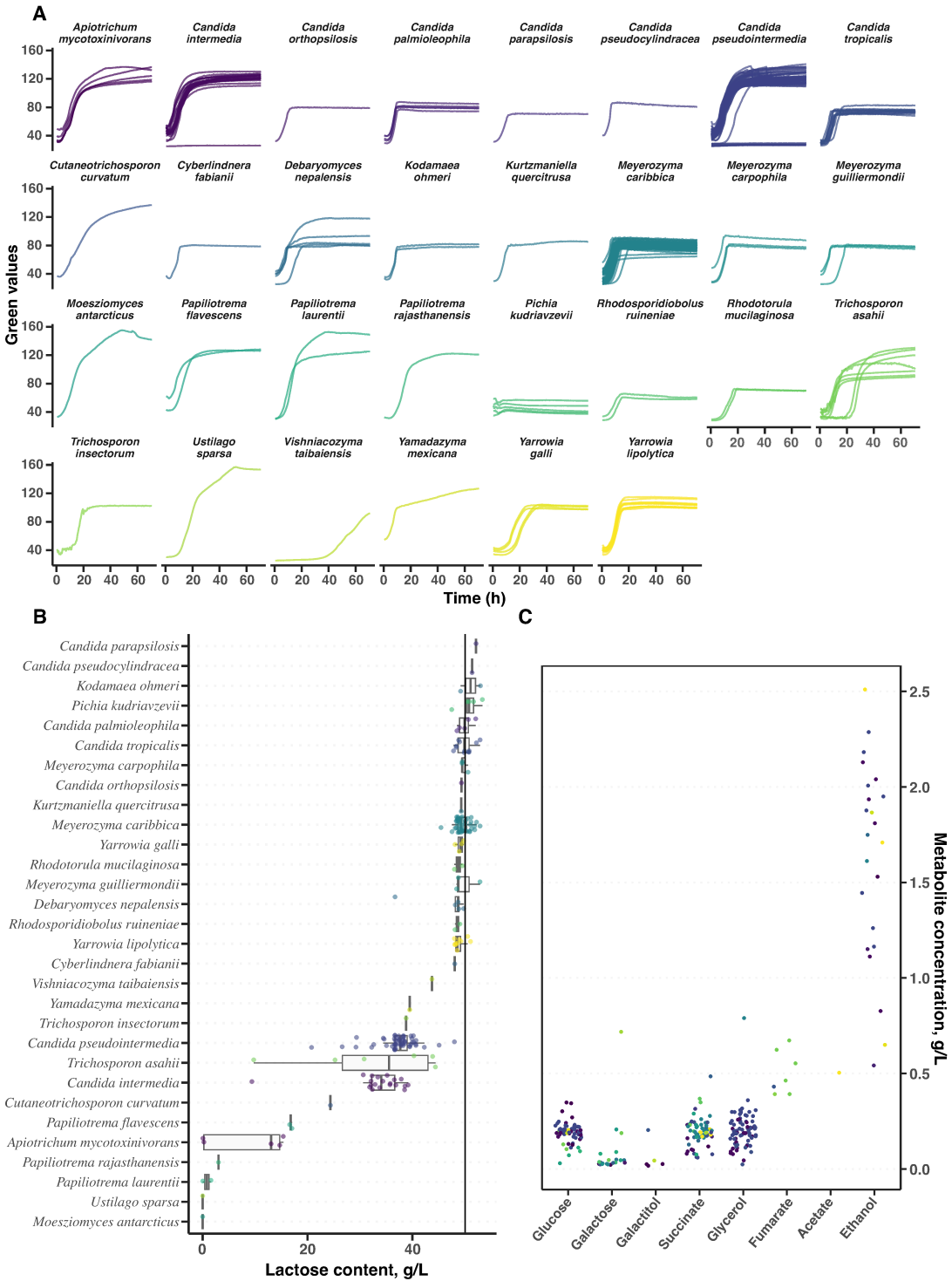

Figure 3

Yeasts growing on lactose in cheese whey. A) Growth curves of strains growing on filter sterilized cheese whey in microscale liquid cultures; samples for HPLC were collected at the endpoints. B) Lactose left in the supernatant after cultivation was determined by HPLC. The lateral line indicates the concentration of lactose in whey before inoculation. C) Traces of other metabolites found in whey after cultivation, color according to species, as in A and B.

Figure 4

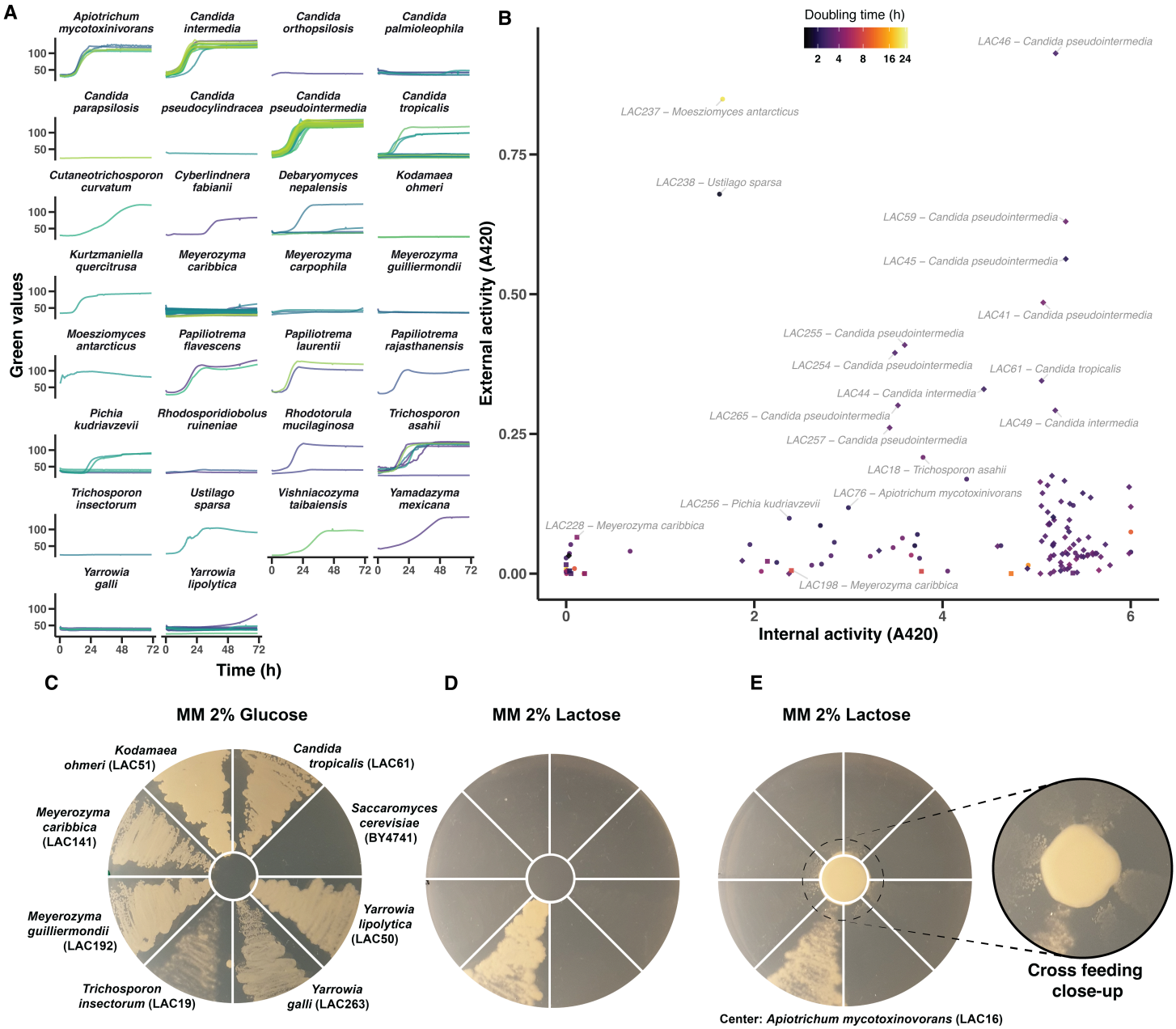

Figure 4

Yeasts growing in liquid and hydrolyzing lactose. A) Growth curves of yeast isolates while growing in microscale liquid MM 2% lactose medium. B) β-galactosidase activity measured in cells (x-axis) and supernatant (y-axis) after final time points in A. Point shapes are round for all genus except

Candida(diamonds) and *Meyerozyma* (squares). C) Strains streaked on MM glucose plates with negative control ScBY4741 in center. D) Strains in same layout as C, streaked on MM lactose plates with negative control ScBY4741 in center. E) Strains in same layout as C and D, streaked on MM lactose plates with *A. mycotoxinivorans* straked in center, close-up shows a zone of growth activation close to the center strain.

Figure 5

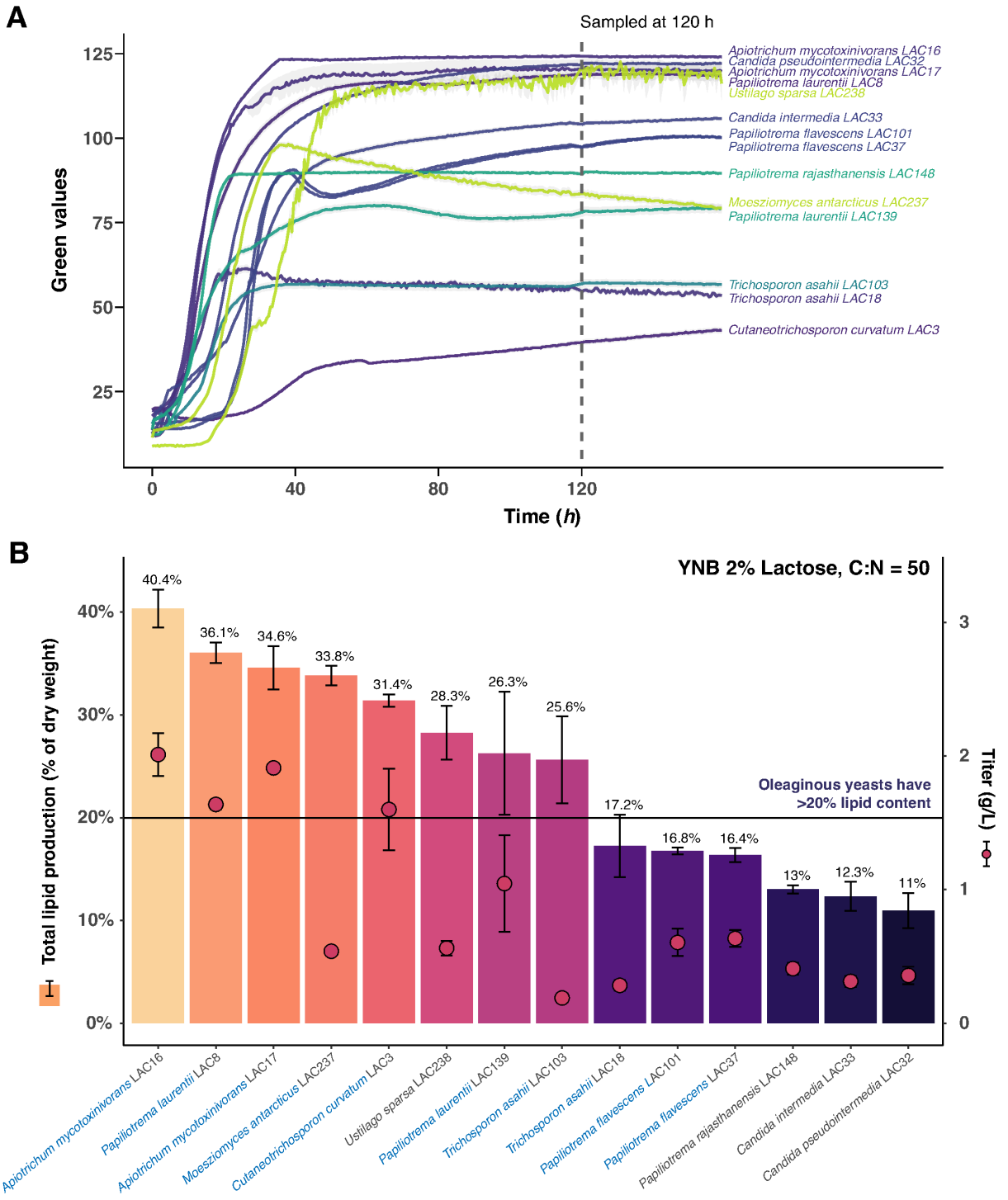

Figure 5

Yeasts converting lactose into lipids Growth and lipid production in 16 selected yeast isolates growing in a carbon-rich, nitrogen-poor medium (C/N = 50) that maximizes lipid production. A) Growth during microscale liquid cultivation. Each population size measure is an average of six replicates; fields indicate standard error of the mean B) Total lipid yield in stationary phase cells, after 120 h of growth in liquid medium in shake-flasks. Lipid content is expressed as percent of yeast dry weight (left y-axis) and lipid weight per liter of culture (right y-axis). Data shown represents an average of three independent cultures (*n*=3). Error bars = standard deviation. All tested strains except *C. intermedia* and *C. pseudointermedia* belong to the *Basidiomycota* phyla.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementaryfigures.pdf
- TableS1.tsv
- TableS2.tsv