Profiling commercial and industrial yeast strains with Odin

Introduction

The single-celled yeast species *Saccharomyces cerevisiae* has been used for thousands of years to produce fermented foods and beverages. For much of this time, people took advantage of the natural diversity that was found in different isolates, and selected for desired properties. More recently, *S. cerevisiae* has been engineered to generate artificial diversity and even wider genetic variation, resulting in strains with characteristics beneficial for industrial production. Not surprisingly, this variability also yields noticeable phenotypic diversity. Screening or confirming for desired phenotypes and verifying that there are no unintended consequences from gene editing is important when selecting a production strain.

Figure 1: The Odin family are all-in-one systems for cellular characterization. Odin VIII has a capacity of 8 plates, and Odin L allows you to load and run up to 50 plates at a time. Both systems are ideal for monitoring growth curves, measuring cell respiration kinetics, and identifying unknown microbes.

Phenotype MicroArrays™ (PM) from Biolog, used in conjunction with the Odin™ family of instruments (Figure 1) enable phenotypes of any microbes to be screened against a large library of substrates and conditions. Odin L can incubate and read up to 50 plates at a time, while Odin VIII has an 8-plate capacity. Both systems can collect kinetic data in two ways: the Optical Density (OD) of each plate can be read to directly measure cell proliferation, or one can use a Biolog redox dye and measure the color change to determine the metabolic activity of the cells in response to the compound found in each well. Both measurements can be made in parallel, allowing the user to collect two orthogonal types of data from a single starting culture, over the desired time period.

There are 20 PM arrays designed to be used for microbial phenotyping. PM 1-8 contain metabolic substrates including 190 carbon sources, 95 nitrogen sources, 285 di- and tri-peptides, along with a variety of sulfur- and phosphate- containing compounds and other nutritional supplements. Each of these substrates was selected to probe various points along specific metabolic pathways, providing a comprehensive overview of the cells' metabolic profile. Additional PM panels were designed to test organisms' susceptibility to various osmotic and ionic effects, pH, and a wide array of chemical inhibitors.

To get started with PM technology, the user prepares a cell suspension in a minimal inoculating fluid devoid of most nutrients, and aliquots that cell suspension into each of the wells for the desired plates. The inoculating fluid can be supplemented with a redox dye to measure respiration, or plates can be run without dye to focus on growth and turbidity changes over the incubation period. Once plates have been inoculated, they are transferred to an Odin system for incubation, data acquisition, and analysis. For this study we utilized PM1 to probe differences between various strains of *S. cerevisiae* in their ability to utilize the 95 distinct carbon sources arrayed on the plate.

There are well-reported differences in the ability of various strains of *S. cerevisiae* to utilize a range of carbon sources. This application note shows how the Odin platform can be used to streamline the phenotypic characterization of several strains at once, and quickly identify specific compounds that highlight differences between the selected strains.

By profiling 4 commercially available strains of yeast that have been used for applications ranging from wine-making to human gut health to recombinant protein production, we show how this method can complement genomic or other data to identify preferred carbon sources for a given strain. This work can be easily extended to include other PM panels, to thoroughly characterize the functional properties of any strain, including responses to any metabolic substrates and susceptibility to stressors or growth inhibitors.

Methods

Phenotype Microarray Experiments

PM profiles were generated using the standard Biolog PM procedures for yeast. *Saccharomyces cerevisiae* strains BY4741, INVSc1, ICV-D47, and MYA967, were grown on Biolog Universal Yeast (BUY) solid media at 30 °C for 24 hours. Cell suspensions for each strain were then made to a transmittance of 62% T in 16 mL 1x Biolog Yeast PM inoculating fluid (IFY-0) using a Turbidimeter. Two fresh tubes with 11.75 mL IFY-0, supplemented with 0.25% Yeast Extract, were then inoculated with 0.25 mL of cells from the 62% T suspensions for each strain. 120 µL Biolog Dye Mix D was then added to one tube for each strain to complete the sample preparation. Eight PM1 plates were pre-warmed to room temperature and inoculated with each of the eight cell suspensions with 100 µL per well. PM plates were then transferred to Odin VIII for incubation at 30 °C for 24 hours with reads every 20 minutes at 590 and 740 nm. Kinetic metabolic and growth profiles were compared by plotting OD590 data for each strain alongside others for each well of PM1 for both dye and no-dye plates using the Curation function in Odin software..

Results

Saccharomyces cerevisiae has been used for millennia in the making of bread, beer, and wine, and a host of other products. *S. cerevisia*e has also served as a workhorse of science, allowing researchers to gain new insights into genetic regulation and cell division, among other topics. To better serve these various research and industrial niches, there exist many unique strains of yeast, including those that have been isolated from the environment, passed down through generations of brewers, or genetically modified to serve a specific purpose. Here we selected 4 such strains to serve as the basis for probing the metabolic variability among them. These include BY4741, one of the most widely used yeast for basic research in synthetic biology, and the progenitor of commercially available yeast knockout libraries; *S. cerevisiae var. boulardii* (MYA-796), a probiotic yeast with several documented differences relative to BY4741; INVSc1, a fast growing diploid strain engineered for expression of protein products; and ICV-D47, a commercial wine yeast available to the average consumer from online markets that may have interesting differences relative to lab strains.

Here we challenged these strains and their ability to respire and/or divide using a variety of single carbon substrates contained in PM1. This was achieved by growing the strains on PM1 with or without Biolog redox Dye Mix D which indicates energy production by turning purple after responding to NADH/NADPH production in the cell. Odin can quantify the respiration (dye reduction) and cell growth (no-dye) side by side in this way. PM1 contains 95 unique carbon substrates including simple sugars such as glucose and galactose as well as more complex or intermediate substrates such as L-Galactonic acid-γ-lactone, L-lyxose, and tricarballylic acid. The carbon source in each well of the PM1 plate serves as the sole carbon source in the growth medium, which enables us to draw conclusions about a strain's ability to grow using only that substrate. When grown on PM1, seven substrates were able to elicit log phase growth in at least 1 of the 4 yeast strains including: D-galactose, D-mannose, D-fructose, α-D-glucose, maltose, sucrose, and maltotriose (Figure 2).

The status of *Saccharomyces cerevisiae var. boulardii* (MYA-796) has been contested in the past as some have proposed it is a different species from cerevisiae entirely (Khatri et al. 2017). One of the assertions made in this case is that MYA-796

is unable to utilize galactose as a primary sugar source. Here, we were able to demonstrate this differentiating phenotype using PM1 where MYA-796 was the only strain unable to use D-galactose in well A6. Interestingly MYA-796 was also the only strain able to efficiently utilize sucrose in contrast to ICV-D47 which was able to grow modestly on this substrate, while BY4741 and INVSc1 were unable to grow at all (Figure 2). Maltose and maltotriose were also differentially utilized from one strain to the next with ICV-D47 being the sole strain to utilize both while MYA-796 could grow using maltose (Figure 2). All four yeast strains were able to utilize and proliferate in wells containing D-fructose and α-D-glucose (Figure 2). These results are reflected in both growth (no-dye) and metabolic activity via dye reduction.

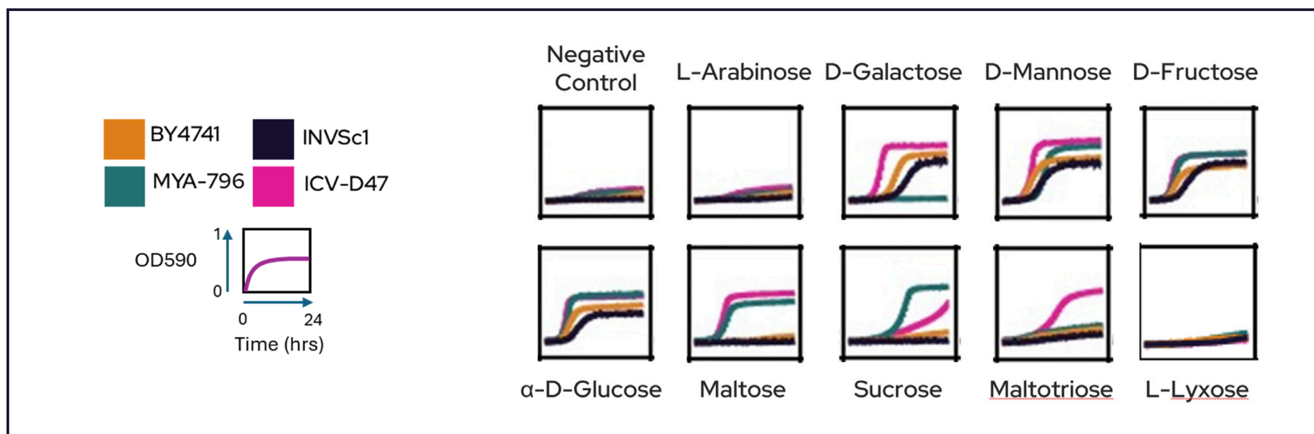

Figure 2: Kinetic growth curves for *Saccharomyces* strains on PM1 - OD590 measurements representing growth are indicated on the y-axis, and time (0-24 hours) is represented on the x-axis. The negative control (well A1 in the plate) as well as a selection of carbon sources are displayed here. D-galactose, D-mannose, D-fructose, α-D-glucose, maltose, sucrose, and maltotriose induced log phase growth in at least one strain. None of the strains were able to grow on L-arabinose or L-lyxose. MYA-796 (*S. cerevisiae var. boulardii*) was unable to utilize D-galactose which is one of the factors differentiating this strain.

Conclusion

Phenotypic screening of various strains of the same species can give significant insights into what metabolic pathways are conserved and which ones are more subject to intra-species variation. Here we demonstrated how Odin VIII, combined with Phenotype MicroArrays, provides a powerful toolkit for rapidly screening multiple strains for carbon source utilization. With one run of the instrument, we found that there are substrates whose utilization are conserved, as well as those which are differentially preferred by one strain or another, including a historically noted inability to metabolize galactose by *Saccharomyces cerevisiae var. boulardii*.

References

Khatri I, Tomar R, Ganesan K, Prasad GS, Subramanian S. Complete genome sequence and comparative genomics of the probiotic yeast *Saccharomyces boulardii*. Sci Rep. 2017 Mar 23;7(1):371. doi: 10.1038/s41598-017-00414-2. PMID: 28336969; PMCID: PMC5428479

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