Title

Characterization of pectinase-producing Saccharomyces cerevisiae UCDFST 09-448 and its effects on cull peach fermentations.

Authors

Gutierrez-Cano, Aaliyah

Jones, Bryce

Macario, Jordan

et al.

Publication Date

2024-01-09

DOI

10.1093/jimb/kuae037

Peer reviewed

Journal of Industrial Microbiology and Biotechnology, 2024, 51, kuae037
https://doi.org/10.1093/jimb/kuae037
Advance access publication date: 16 October 2024
Fermentation, Cell Culture and Bioengineering – Original Paper

Characterization of pectinase-producing *Saccharomyces cerevisiae* UCDFST 09-448 and its effects on cull peach fermentations

Aaliyah Raquel Gutierrez-Cano[1], Bryce Jones[1], Jordan Macario[1], Sofia Martin[1], Derrick Cardenas[1], Hannah Simpson[1], Kyria Boundy-Mills[2], Meredith Claire Edwards[1]

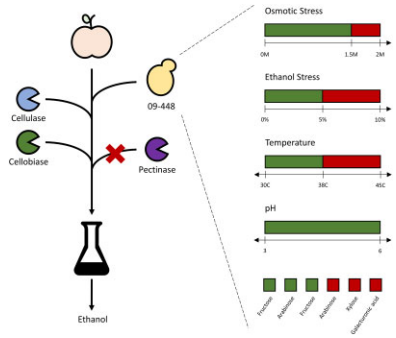
[1]Department of Biological Sciences, St. Edward's University, 3001 S Congress Ave, Austin, TX 78704, USA
[2]Phaff Yeast Culture Collection, Food Science and Technology, University of California Davis, One Shields Ave, Davis, CA 95616, USA
Correspondence should be addressed to: Meredith Claire Edwards at medward9@stedwards.edu

Abstract: Fermentation of pectin-rich biomass by *Saccharomyces cerevisiae* can produce bioethanol as a fuel replacement to combat carbon dioxide emissions from the combustion of fossil fuels. *Saccharomyces cerevisiae* UCDFST 09-448 produces its own pectinase enzymes potentially eliminating the need for commercial pectinases during fermentation. This research assessed growth, pectinase activity, and fermentative activity of *S. cerevisiae* UCDFST 09-448 and compared its performance to an industrial yeast strain, *S. cerevisiae* XR122N. *Saccharomyces cerevisiae* UCDFST 09-448's growth was inhibited by osmotic stress (xylose concentrations above 1 M), ethanol concentrations greater than 5% v/v, and temperatures outside of 30°C–37°C. However, *S. cerevisiae* UCDFST 09-448 was able to consistently grow in an industrial pH range (3–6). It was able to metabolize glucose, sucrose, and fructose but was unable to metabolize arabinose, xylose, and galacturonic acid. The pectinase enzyme produced by *S. cerevisiae* UCDFST 09-448 was active under typical fermentation conditions (35°C–37°C, pH 5.0). Regardless of *S. cerevisiae* UCDFST 09-448's limitations when compared to *S. cerevisiae* XR122N in 15% w/v peach fermentations, *S. cerevisiae* UCDFST 09-448 was still able to achieve maximum ethanol yields in the absence of commercial pectinases (44.7 ± 3.1 g/L). Under the same conditions, *S. cerevisiae* XR122N produced 39.5 ± 3.1 g/L ethanol. While *S. cerevisiae* UCDFST 09-448 may not currently be optimized for industrial fermentations, it is a step toward a consolidated bioprocessing approach to fermentation of pectin-rich biomass.

One-Sentence Summary: *Saccharomyces cerevisiae* UCDFST 09-448 demonstrates the potential to ferment pectin-rich biomass as part of a consolidated bioprocess, but is sensitive to industrial stressors.

Keywords: Pectin-rich biomass, Fermentation, Pectinase, *Saccharomyces cerevisiae*, Industrially related conditions

Graphical abstract



Introduction

Global warming has driven regional and seasonal temperature extremes, led to a reduction in snow cover and sea ice, more intense heavy rainfall, and changes in habitat ranges for plants and animals (Grimm et al., 2013). The amount of warming the Earth will experience depends on how much carbon dioxide and other greenhouse gasses are released into the atmosphere. In 2021, over 20% of carbon dioxide emissions in the United States were the result of petroleum-based transportation fuels (August 2024 Monthly Energy Review, 2024). Biofuels, in contrast, may contribute fewer greenhouse gasses into the atmosphere (Jeswani et al., 2020).

This research focuses on bioethanol from second-generation wastes, indicated in previous studies to have a lower global

Received: August 23, 2024. **Accepted:** October 15, 2024.

warming potential than first-generation biofuels or fossil fuels (Jeswani et al., 2020). In addition, the use of food waste does not require any land-use change. Overall, the ethanol produced from plant biomass has the potential to cut greenhouse gas emissions by up to 86%, making it a promising replacement for gasoline (Wang et al., 2007).

Pectin-rich biomass is generated as waste products from industrial processing of fruits and vegetables like apples, lemons, limes, peaches, nectarines, grapes, and almonds, of which 7.75 million metric tons were produced in the United States in 2023 alone (2023/2024 Almonds Production, n.d.; 2023/2024 Apples Production, n.d.; 2023/2024 Lemons & Limes Production, n.d.; 2023/2024 Peaches & Nectarines Production, n.d.; 2023/2024 Table Grapes, n.d.). Pectin-rich biomass is not currently used as a source for biofuel because, like other second-generation biomass, its cell wall structure is more complex than that of corn kernels (Robak & Balcerek, 2018). This requires that the biomass undergo pretreatment and the addition of commercial enzymes to digest cellulose, hemicellulose, and pectin. The addition of commercial enzymes increases the cost of ethanol production compared to first-generation biomass, where pretreatment is unnecessary (Robak & Balcerek, 2018).

Consolidated bioprocessing, where a single organism produces saccharification enzymes as well as ferments the free sugars, is the preferred platform for commercial ethanol production as it would remove the need for commercial enzymes. *Saccharomyces cerevisiae* UCDFST 09-448 from the Phaff Yeast Culture Collection is of particular interest for pectin-rich biomass fermentation as it produces pectinase. It was originally isolated from Sicilian-style fermented olives that were spoiled by its excessive production of pectinase (Golomb et al., 2013).

Generally, the performance of *S. cerevisiae* strains during fermentation is compromised by increased osmotic pressures, temperatures, acidic conditions, and ethanol accumulation. *Saccharomyces cerevisiae* UCDFST 09-448's potential as an ethanologen was assessed through growth analysis under industrially relevant conditions (0–2 M xylose, 30°C–45°C, pH 3–6, and 0%–20% v/v ethanol) and on various carbohydrates (sucrose, glucose, fructose, arabinose, xylose, and galacturonic acid). Its pectinase activity was also quantified in industrial conditions, and 15% dw/v cull peach fermentations were performed with and without commercial pectinases and compared to an industrial yeast strain, *S. cerevisiae* XR122N.

Methods

Saccharomyces cerevisiae UCDFST 09-448 Growth Phenotyping Assays

Saccharomyces cerevisiae UCDFST 09-448 was obtained from the Phaff Yeast Culture Collection, University of California Davis (https://phaffcollection.ucdavis.edu). Single colonies were inoculated into 5 mL of tryptic soy broth (TSB; tryptone 17 g/L, soy peptone 3 g/L, K_2HPO_4 2.5 g/L, NaCl 5 g/L) containing 2% w/v glucose and incubated at 37°C with shaking at 225 rpm, in alignment with typical fermentation conditions (Doran-Peterson et al., 2009). Overnight cultures were inoculated at a 600 nm optical density of 0.01 into a final volume of 200 μl stressor media in a 96-well plate and incubated at 37°C without shaking in a BioTek Synergy H1 multimode plate reader (Santa Clara, CA). Stressor media contained TSB supplemented with 2% w/v glucose and one of the following stressors: ethanol (0%, 5%, 10%, 15%, and 20% v/v), acidity (pH 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, and 6.0 using 3 M HCl), osmotic pressures (0.0, 0.5, 1.0, 1.5, and 2.0 M xylose), or no additional stressor but varying temperatures (30°C, 34°C, 37°C, 40°C, 43°C, and 45°C). Aside from temperature control, the stressors were not monitored or maintained during the course of the experiment. Growth was monitored spectrophotometrically at 600 nm every 15 min for up to 36 hr. All conditions were tested in triplicate.

Similarly, *S. cerevisiae* UCDFST 09-448's capability to grow on carbon sources associated with the plant cell wall (sucrose, glucose, fructose, arabinose, xylose, and galacturonic acid) was tested by replacing the 2% w/v glucose in the TSB media with 2% w/v of the respective sugar.

Saccharomyces cerevisiae UCDFST 09-448 Pectinase Assay

Polygalacturonic acid (PGA) plates were made as described previously (Starr et al., 1977) using 0.5% synthetic PGA. PGA plates were spotted with 10 μL of overnight culture grown in yeast malt extract broth (glucose 10 g/L, peptone 5 g/L, yeast extract 3 g/L, malt extract 3 g/L, pH 5.0) supplemented with 1.5% w/v citrus pectin. The pH of the PGA plates was adjusted to 7.0, 5.5, and 5.0 by adding 5 M NaOH to the plate medium. *Escherichia coli* LY40A (Edwards et al., 2011) and *E. coli* LY40A containing pWEB (Boland et al., 2010) were used as negative and positive controls, respectively. The plates were incubated at 30°C or 37°C overnight. PGA activity was detected as visual zones of clearing around the spot after flooding the PGA plates with 2 M HCl (Starr et al., 1977).

Saccharomyces cerevisiae UCDFST 09-448 culture supernatant was screened for pectate lyase activity and polygalacturonase activity over 72 hr. *Saccharomyces cerevisiae* UCDFST 09-448 was grown in YPD broth (peptone 20 g/L, yeast extract 10 g/L, 40% w/v glucose 50 mL/L) with or without 1% w/v synthetic PGA supplementation. *Saccharomyces cerevisiae* XR122N (North American Bioproducts Corp., now part of Lallemand Biofuels and Distilled Spirits) was used as a negative control grown under the same conditions with 1% w/v synthetic PGA supplementation. Growth was monitored spectrophotometrically at 600 nm. Supernatant was collected via centrifugation at 19280 × *g* for 5 min and tested for reducing sugars, glucose, galacturonic acid, pectate lyase, and polygalacturonase activity. Reducing sugars were measured using the dinitrosalicylic acid method (Miller, 1959). Glucose and galacturonic acid were measured as described in the "sample analysis" section.

Pectate lyase activity was monitored as previously described (Edwards et al., 2011), with the noted changes. Supernatant and substrate (50 mM sodium acetate buffer [pH 5.0], 1.5 mM $CaCl_2$, 0.24% w/v synthetic PGA) were equilibrated to 35°C and then rapidly mixed. The formation of 4,5-unsaturated products was monitored at 235 nm every 5 s for 3 min with a linear rate of reaction for at least 30 s. Units were μmol products formed per min per mL.

Polygalacturonase activity was measured using a colorimetric method (Kashyap et al., 2000). Briefly, 200 μL supernatant and 200 μL substrate (50 mM sodium acetate buffer [pH 5.0], 1% w/v synthetic PGA) were incubated for 3 hr at 35°C. The mixture was boiled with 400 μL dinitrosalicylic acid for 15 min and diluted to 5 mL. Absorption of reducing sugars (galacturonic acid) was measured at 530 nm. Polygalacturonase units (PGU) were μmol products formed per minute per mL. Protein concentration in the supernatant was measured using the Bradford method (Bradford, 1976). Final enzyme activity was reported as specific activity (PGU/mg protein).

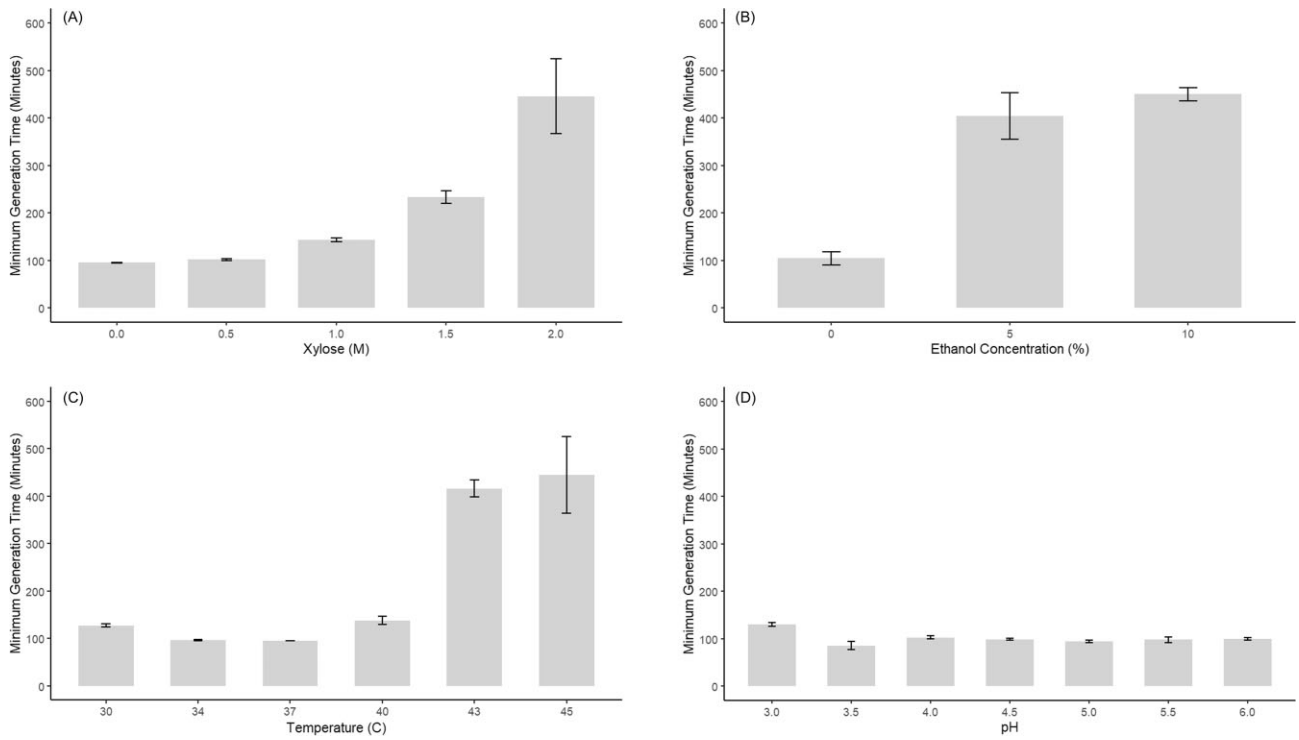


Fig. 1. Average generation times of *Saccharomyces cerevisiae* UCDFST 09-448 when exposed to increasing (A) osmotic pressures (B) ethanol concentrations, (C) temperatures, and (D) acidic conditions. The error bars represent the standard deviation from the mean, $n = 3$.

15% dw/v Cull Peach Fermentation

Fermentations were conducted essentially as described previously (Doran-Peterson et al., 2009; Edwards et al., 2014), with the basic conditions noted below. Cull peach (15% dw/v) fermentations were conducted in 500 mL bioreactors at a final volume of 200 mL and a final concentration of 1 × TSB. At least three biological replicates were completed for each fermentation. Cull peach dw was determined using a Denver Instrument IR 35 moisture analyzer (Denver, CO). Cull peach (30 g dw) and any additional water needed were autoclaved at 121°C and 1 atm for 20 min. After autoclaving, the pH was adjusted to 5.0 using 10 M KOH and 5 M HCl, and commercial enzymes were added (cellulase [Novozymes 5013, Novozymes [now called Novonesis], Franklin, NC] measured in filter paper units [FPU]/g dw biomass, pectinase [Pectinex P2736, Novozymes] from *Aspergillus niger* measured in PGU/g dw biomass, and cellobiase [Novozymes 188; Novozymes] measured in cellobiase units [CBU]/g dw biomass). *Saccharomyces cerevisiae* XR122N fermentations were inoculated with 2 g/L lyophilized active dry yeast (approximately 2.0×10^7 cells/mL). *Saccharomyces cerevisiae* UCDFST 09-448 fermentations were inoculated with 2.0×10^7 cells/mL from TSB supplemented with 5% w/v glucose and grown overnight at 35°C without shaking.

After inoculation, the fermentations were placed in water baths at 37°C and mixed with magnetic stir bars. The pH was maintained at 5.0 throughout the fermentation. Samples were taken at the time of inoculation and at 24, 48, and 72 hr. Samples were centrifuged at 19280 × *g* for 7 min. The supernatant was collected and centrifuged again in a 0.22 μm filter (Corning Inc., Corning, NY) and stored at −20°C for further analysis.

Fermentation Sample Analysis

Ethanol concentrations were measured using gas chromatography (GC) performed on a Shimadzu (Columbia, MD) GC-8A instrument as previously described (Doran-Peterson et al., 2009). Sugar analysis was conducted using high-performance liquid chromatography with refractive index detection (HPLC-RID). Chromatographic separation was achieved on a Shimadzu Prominence LC-20AT liquid chromatographic system (Shimadzu Scientific Instruments, Columbia, MD) using a Bio-Rad (Hercules, CA) Aminex HPX-87H 300 mm by 7.8 mm column with a Bio-Rad Cation H guard column. H_2SO_4 (5 mM) was used as the mobile phase to perform an isocratic run at 0.6 mL/min with the column temperature at 60°C.

Results and Discussion

Saccharomyces cerevisiae UCDFST 09-448 Growth Phenotyping Assays

Phenotypic analysis of *S. cerevisiae* using a plate reader is a quantitative and high-throughput technique for assessing the sensitivity of yeast strains to stressors. When performed without agitation, cells form a uniform and reproducible lawn at the bottom of the well that can be measured to assess growth (Hung et al., 2018). The small volume (200 μl) precludes the ability to monitor or maintain stressors (like ethanol and pH), but nevertheless is commonly used to evaluate yeast physiology (Toussaint & Conconi, 2006; Olsen et al., 2010; Hung et al., 2018; Monteiro de Oliveira et al., 2021).

Saccharomyces cerevisiae UCDFST 09-448 was negatively affected by all stressors tested except pH. The minimum generation time of *S. cerevisiae* UCDFST 09-448 increased with increasing osmotic pressures (Fig. 1A) and ethanol concentrations (Fig. 1B). Growth was inhibited above 1 M xylose, and no growth was observed above 10% w/v ethanol. *Saccharomyces cerevisiae* UCDFST 09-448 grew most rapidly at 34°C–37°C, and no growth was observed above 43°C (Fig. 1C). However, *S. cerevisiae* UCDFST 09-448 grew at a relatively consistent rate at pH values between 3 and 6 (Fig. 1D).

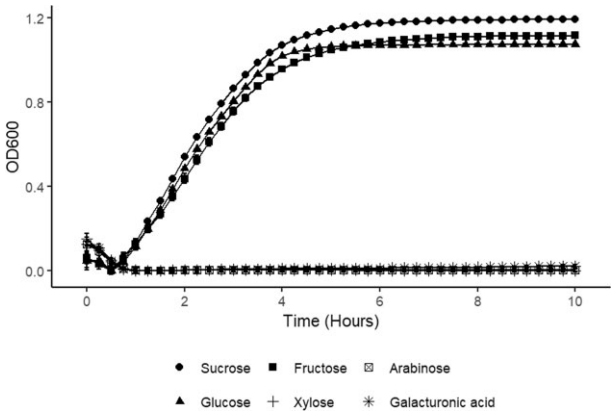


Fig. 2. Average growth curves of *Saccharomyces cerevisiae* UCDFST 09-448 when grown in TSB containing 2% w/v sucrose, glucose, fructose, arabinose, xylose, or galacturonic acid. The error bars represent the standard deviation from the mean, $n = 3$.

The ability of *S. cerevisiae* UCDFST 09-448 to metabolize sugars commonly found in pectin-rich biomass was also examined. During fermentation, enzymes including cellulases, hemicellulases, and pectinases are added to release monomeric sugars from the biomass. Common sugars found in pectin-rich cell walls include sucrose, glucose, fructose, xylose, arabinose, and galacturonic acid. Compared to all other carbon sources tested, *S. cerevisiae* UCDFST 09-448 grew most rapidly when sucrose was available as a sole carbon source. Like most *S. cerevisiae* strains, it was capable of metabolizing six-carbon sugars, but could not metabolize five-carbon sugars or galacturonic acid (Fig. 2).

Saccharomyces cerevisiae UCDFST 09-448 Pectinase Activity

Pectinase activity was observed on plates with a pH of 5.0 incubated at 37°C, indicating that *S. cerevisiae* UCDFST 09-448 produced pectinase under these conditions. No pectinase activity was observed on PGA plates at pH 7.0, 5.5, or those incubated at 30°C at pH 5.0, 5.5, or 7.0. This is in contrast to previous reports that *S. cerevisiae* UCDFST 09-448 has pectinase activity at room temperature using an agarose diffusion assay (Golomb et al., 2013).

To further characterize the pectinase activity, the supernatants of *S. cerevisiae* UCDFST 09-448 grown with and without additional PGA were tested for polygalacturonase and pectate lyase activity over 72 hr. *Saccharomyces cerevisiae* XR122N was used as a negative control. The growth of *S. cerevisiae* UCDFST 09-448 and *S. cerevisiae* XR122N were comparable (Fig. 3A). The degradation of PGA in the growth medium was observed over time through the measurement of reducing sugar equivalents (Fig. 3B). *Saccharomyces cerevisiae* UCDFST 09-448 without any PGA had the lowest amount of reducing sugars, followed by *S. cerevisiae* XR122N. *Saccharomyces cerevisiae* UCDFST 09-448 with PGA had higher concentrations of reducing equivalents, indicating that *S. cerevisiae* UCDFST 09-448 was degrading the PGA present in the growth medium. Residual glucose did not contribute to the concentration of the reducing equivalents as glucose was not detected in the media using HPLC after 24 hr (data not shown).

Pectate lyase activity was not observed (data not shown). However, there was significant polygalacturonase activity in *S. cerevisiae* UCDFST 09-448 cultures grown with and without PGA. Supernatant from *S. cerevisiae* UCDFST 09-448 cultures grown with PGA reached maximum activity (138.8 ± 15.2 PGU/mg protein) within 48 hr of inoculation (Fig. 3C). No galacturonic acid was detected in the media at any time (data not shown).

15% dw/v Cull Peach Fermentation

Previous work has shown that pectinase was required for maximum ethanol production from cull peaches, but the entire suite of commercial pectinases could be replaced by a single pectate lyase, PelB (Edwards et al., 2014). However, it might prove advantageous to have the fermenting organism also produce the pectinase, resulting in a type of consolidated bioprocessing. Fermentations of pectin-rich biomass (15% dw/v cull peaches) performed with *S. cerevisiae* UCDFST 09-448 produced ethanol at comparable levels to *S. cerevisiae* XR122N when both received a full complement of commercial cellulases, cellobiase, and pectinases (1.88 FPU/g dw, 6.25 CBU/g dw, and 7.5 PGU/g dw, respectively). When pectinase was removed from the fermentation, ethanol production from *S. cerevisiae* XR122N dropped from 45.9 ± 5.5 g/L to 39.5 ± 3.1 g/L. However, the ethanol yield from *S. cerevisiae* UCDFST 09-448 was not affected by the removal of pectinase, and the percent of maximum theoretical ethanol yield remained above 95%. The ethanol concentration with pectinase, cellulase, and cellobiase in *S. cerevisiae* UCDFST 09-448 fermentations was 45 ± 0.8 g/L, when pectinase was removed, the ethanol concentration was 44.7 ± 3.1 g/L. Therefore, the native pectinase expressed by *S. cerevisiae* UCDFST 09-448 was as effective as an entire suite of commercial pectinase enzymes (Fig. 4).

Conclusion

Saccharomyces cerevisiae UCDFST 09-448 produces a polygalacturonase enzyme that is active at typical fermentation conditions (pH 5.0 and 35°C–37°C) and can be used to degrade PGA. Although

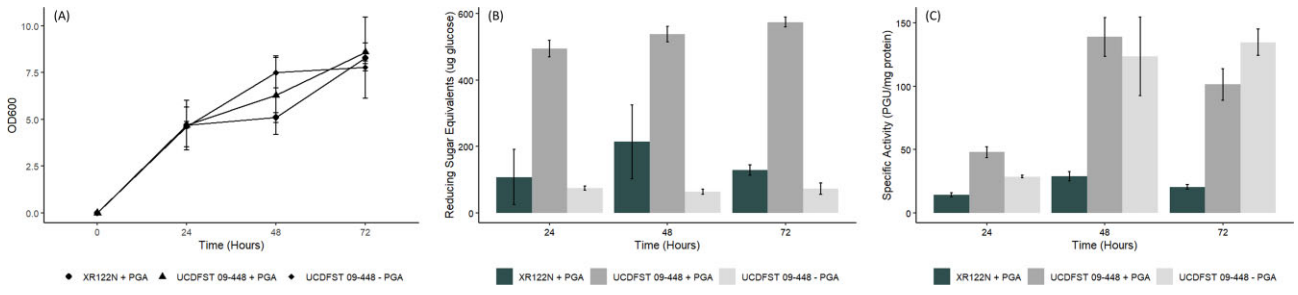


Fig. 3. Growth of *Saccharomyces cerevisiae* strains (A), XR122N with PGA (●), UCDFST 09-448 with PGA (▲) and UCDFST 09-448 without PGA (◆). Reducing sugar equivalents in growth media supernatant over time (B) from XR122N with PGA, UCDFST 09-448 with PGA, and UCDFST 09-448 without PGA and the specific activity (PGU/mg protein) in growth media supernatant (C) from XR122N with PGA, UCDFST 09-448 with PGA, and UCDFST 09-448 without PGA.

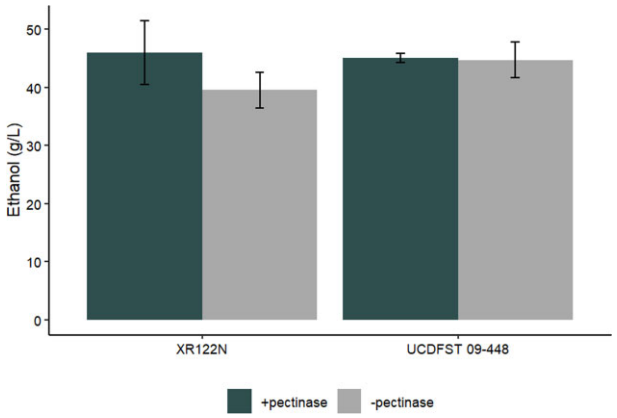


Fig. 4. Fermentation of 15% dw/v cull peach with *Saccharomyces cerevisiae* XR122N and an enzyme loading of 1.88 FPU/g dw, 6.25 CBU/g dw, and 7.5 PGU/g dw (dark gray); *S. cerevisiae* XR122N and enzyme loading of 1.88 FPU/g dw, 6.25 CBU/g dw, and 0 PGU/g dw (light gray); *S . cerevisiae* UCDFST 09-448 and an enzyme loading of 1.88 FPU/g dw, 6.25 CBU/g dw, and 7.5 PGU/g dw (dark gray); and *S. cerevisiae* UCDFST 09-448 and enzyme loading of 1.88 FPU/g dw, 6.25 CBU/g dw, and 0 PGU/g dw (light gray).

S. cerevisiae UCDFST 09-448 itself cannot yet withstand industrial fermentation conditions (specifically osmotic pressure over 1 M xylose, ethanol concentrations over 5% v/v, and temperatures over 37°C), we have determined that *S. cerevisiae* UCDFST 09-448 fermentations can produce maximum ethanol production even when no commercial pectinase is added. This is an improvement from *S. cerevisiae* XR122N, which requires the addition of heterologous pectinases to achieve maximum ethanol production, which increases the cost of fermentation. Like all known wild-type strains of *S. cerevisiae*, *S. cerevisiae* UCDFST 09-448 cannot metabolize galacturonic acid or five-carbon sugars, but strains have been genetically engineered in the past to utilize xylose, arabinose, and galacturonic acid (Moysés et al., 2016; Ye et al., 2019; Jeong et al., 2020). The full genome of *S. cerevisiae* UCDFST 09-448 was published in 2018 (Peter et al., 2018) and could support future experiments to produce stress tolerant *S. cerevisiae* UCDFST 09-448 mutants, and engineering of five-carbon and galacturonic acid metabolism pathways could make progress toward a consolidated bioprocessing strain for bioethanol production from pectin-rich biomass.

Acknowledgments

We thank Dr. Joy Doran-Peterson at the University of Georgia for support with the peach fermentations and data analysis and Duke Lane III from Lane Southern Orchards for providing cull peaches.

Funding

This work was supported in part by a Research Opportunity Award through the St. Edward's Institute for Interdisciplinary Science (i4) funded through a National Science Foundation award under [grant no. 1832282] and in part by the Ronald E. McNair Post-Baccalaureate Achievement Program [Award # P217A220167] to A.G.C.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability

The data underlying this article will be shared on reasonable request to the corresponding author.

References

2023/2024 Almonds Production. (n.d.). USDA Foreign Agricultural Service. Accessed June 12, 2024. https://fas.usda.gov/data/production/commodity/0577400

2023/2024 Apples Production. (n.d.). USDA Foreign Agricultural Service. Accessed April 11, 2024. https://fas.usda.gov/data/production/commodity/0574000

2023/2024 Lemons & Limes Production. (n.d.). USDA Foreign Agricultural Service. Accessed April 11, 2024. https://fas.usda.gov/data/production/commodity/0572120

2023/2024 Peaches & Nectarines Production. (n.d.). USDA Foreign Agricultural Service. Accessed April 11, 2024. https://fas.usda.gov/data/production/commodity/0579309

2023/2024 Table Grapes. (n.d.). USDA Foreign Agricultural Service. Accessed June 12, 2024. https://fas.usda.gov/data/production/commodity/0577400

August 2024 Monthly Energy Review (No. DOE/EIA-0035(2024/8)). (2024). U.S. Energy Information Administration. Accessed September 26, 2024. http://www.eia.gov/totalenergy/data/monthly

Boland, W. E., Henriksen, E. D., & Doran-Peterson, J. (2010). Characterization of two *Paenibacillus amylolyticus* strain 27C64 pectate lyases with activity on highly methylated pectin. *Applied and Environmental Microbiology*, *76*(17), 6006–6009. https://doi.org/10.1128/AEM.00043-10

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*(1-2), 248–254. https://doi.org/10.1006/abio.1976.9999

Doran-Peterson, J., Jangid, A., Brandon, S. K., DeCrescenzo-Henriksen, E., Dien, B., & Ingram, L. O. (2009). Simultaneous saccharification and fermentation and partial saccharification and co-fermentation of lignocellulosic biomass for ethanol production. *Methods in Molecular Biology*, *581*, 263–280. https://doi.org/10.1007/978-1-60761-214-8_17

Edwards, M. C., Henriksen, E. D., Yomano, L. P., Gardner, B. C., Sharma, L. N., Ingram, L. O., & Doran Peterson, J. (2011). Addition of genes for cellobiase and pectinolytic activity in *Escherichia coli* for fuel ethanol production from pectin-rich lignocellulosic biomass. *Applied and Environmental Microbiology*, *77*(15), 5184–5191. https://doi.org/10.1128/AEM.05700-11

Edwards, M. C., Williams, T., Pattathil, S., Hahn, M. G., & Doran-Peterson, J. (2014). Replacing a suite of commercial pectinases with a single enzyme, pectate lyase B, in *Saccharomyces cerevisiae* fermentations of cull peaches. *Journal of Industrial Microbiology & Biotechnology*, *41*(4), 679–686. https://doi.org/10.1007/s10295-013-1394-z

Golomb, B. L., Morales, V., Jung, A., Yau, B., Boundy-Mills, K. L., & Marco, M. L. (2013). Effects of pectinolytic yeast on the microbial composition and spoilage of olive fermentations. *Food Microbiology*, 33(1), 97–106. https://doi.org/10.1016/j.fm.2012.09.004

Grimm, N. B., Chapin, F. S., III, Bierwagen, B., Gonzalez, P., Groffman, P. M., Luo, Y., Melton, F., Nadelhoffer, K., Pairis, A., Raymond, P. A., Schimel, J., & Williamson, C. E. (2013). The impacts of climate change on ecosystem structure and function. *Frontiers in Ecology and the Environment*, *11*(9), 474–482. https://doi.org/10.1890/120282

Hung, C.-W., Martínez-Márquez, J. Y., Javed, F. T., & Duncan, M. C. (2018). A simple and inexpensive quantitative technique for determining chemical sensitivity in *Saccharomyces cerevisiae*. *Scientific Reports*, *8*(1), 11919. https://doi.org/10.1038/s41598-018-30305-z

Jeong, D., Ye, S., Park, H., & Kim, S. R. (2020). Simultaneous fermentation of galacturonic acid and five-carbon sugars by engineered *Saccharomyces cerevisiae*. *Bioresource Technology*, 295, 122259. https://doi.org/10.1016/j.biortech.2019.122259

Jeswani, H. K., Chilvers, A., & Azapagic, A. (2020). Environmental sustainability of biofuels: A review. *Proceedings of the Royal Society A: Mathematical, Physical and Engineering Sciences*, *476*(2243), 20200351. https://doi.org/10.1098/rspa.2020.0351

Kashyap, D. R., Chandra, S., Kaul, A., & Tewari, R. (2000). Production, purification and characterization of pectinase from a *Bacillus* sp. DT7. *World Journal of Microbiology and Biotechnology*, *16*(3), 277–282. https://doi.org/10.1023/A:1008902107929

Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry*, *31*(3), 426–428. https://doi.org/10.1021/ac60147a030

Monteiro de Oliveira, P., Aborneva, D., Bonturi, N., & Lahtvee, P.-J. (2021). Screening and growth characterization of non-conventional yeasts in a hemicellulosic hydrolysate. *Frontiers in Bioengineering and Biotechnology*, 9, 659472. https://doi.org/10.3389/fbioe.2021.659472

Moysés, D. N., Reis, V. C. B., de Almeida, J. R. M., de Moraes, L. M. P., & Torres, F. A. G. (2016). Xylose fermentation by *Saccharomyces cerevisiae*: challenges and prospects. *International Journal of Molecular Sciences*, *17*(3), 207. https://doi.org/10.3390/ijms17030207

Olsen, B., Murakami, C. J., & Kaeberlein, M. (2010). YODA: software to facilitate high-throughput analysis of chronological life span, growth rate, and survival in budding yeast. *BMC Bioinformatics*, *11*(1), 141. https://doi.org/10.1186/1471-2105-11-141

Peter, J., De Chiara, M., Friedrich, A., Yue, J.-X., Pflieger, D., Bergström, A., Sigwalt, A., Barre, B., Freel, K., Llored, A., Cruaud, C., Labadie, K., Aury, J.-M., Istace, B., Lebrigand, K., Barbry, P., Engelen, S., Lemainque, A., Wincker, P., … Schacherer, J. (2018). Genome evolution across 1,011 *Saccharomyces cerevisiae* isolates. *Nature*, 556(7701), 339–344. https://doi.org/10.1038/s41586-018-0030-5

Robak, K., & Balcerek, M. (2018). Review of second generation bioethanol production from residual biomass. *Food Technology and Biotechnology*, 56(2), 174–187. https://doi.org/10.17113/ftb.56.02.18.5428

Starr, M. P., Chatterjee, A. K., Starr, P. B., & Buchanan, G. E. (1977). Enzymatic degradation of polygalacturonic acid by *Yersinia* and *Klebsiella* species in relation to clinical laboratory procedures. *Journal of Clinical Microbiology*, *6*(4), 379–386.

Toussaint, M., & Conconi, A. (2006). High-throughput and sensitive assay to measure yeast cell growth: A bench protocol for testing genotoxic agents. *Nature Protocols*, *1*(4), 1922–1928. https://doi.org/10.1038/nprot.2006.304

Wang, M., Wu, M., & Huo, H. (2007). Life-cycle energy and greenhouse gas emission impacts of different corn ethanol plant types. *Environmental Research Letters*, *2*(2), 024001. https://doi.org/10.1088/1748-9326/2/2/024001

Ye, S., Kim, J., & Kim, S. R. (2019). Metabolic engineering for improved fermentation of L-arabinose. *Journal of Microbiology and Biotechnology*, *29*(3), 339–346. https://doi.org/10.4014/jmb.1812.12015

Received: August 23, 2024. **Accepted:** October 15, 2024