# Screening of yeast strain that can efficiently utilize extracellular proline during fermentation

M-14

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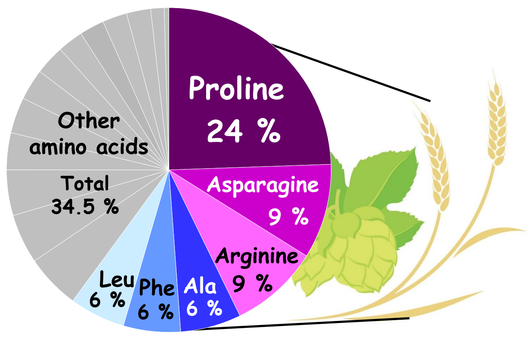
## 1. Abstract

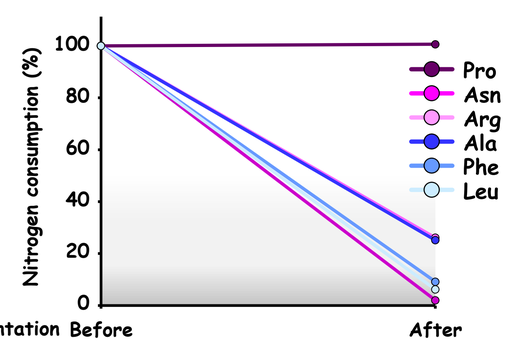
The yeast *Saccharomyces cerevisiae* plays important roles in the production of not only ethanol but also various metabolites involved in the aroma and taste of alcoholic beverages such as beer and wine. Thus, the quality of alcoholic beverages is strongly dependent on the metabolic characteristics of yeast, and nitrogen availability is one of the crucial factors in fermentation. While not a true amino acid but an imino acid, proline is the most abundant amino acid, and represents a potentially effective source of nitrogen. However, typical beer yeasts cannot assimilate proline during fermentation, leading to the high accumulation of proline in finished beer. An excess of residual proline results in risk of proline-polyphenol hazes, and a bitter and mild acidic taste. The purpose of this project is to determine whether beer- or wine-associated yeasts, including both *Saccharomyces* and non-*Saccharomyces*, could utilize proline, which could improve the fermentation and final quality of beer. We first developed and validated a colorimetric assay using the dye Isatin to quantify proline in liquid growth media. Using this protocol, we found that proline was not consumed in Synthetic Complete media inoculated with an ale strain of *S. cerevisiae*, while the media inoculated with a *Lachancea thermotolerans* strain that previously known to assimilate proline had no residual proline. After this method validation, 208 additional yeasts of beer or wine origin from Phaff Yeast Culture Collection (phaffcollection.ucdavis.edu) were subjected to the screening. These included commercial brewing and wine strains as well as research isolates. Twenty-six strains consumed more than 60% of the proline from the original media, while the ale strain was unable to consume proline. To determine whether yeasts that able to consume proline could produce ethanol in wort, seven of the 26 strains, all of species *S. cerevisiae*, were used to ferment 10°Plato wort produced from dry malt extract. With Gravity and ABV were recorded each day, we found two of these strains produced 3.62 % and 2.68 % (v/v) alcohol after 10 days fermentation. These results suggest that the two proline-utilizing strains may contribute to improvements in brewing. Our next approach will include scaled-up fermentation trials to explore how these yeasts reduce to the risk of negative proline effects such as proline-polyphenols hazes.

## 2. Introduction

### Amino acids composition in wort




### Amino acids consumption



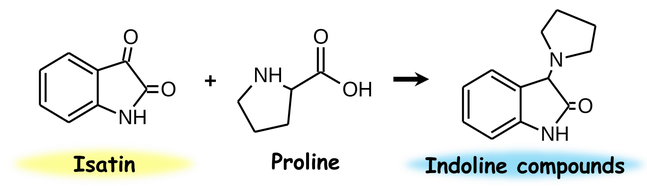

Matjaž Deželak *et al.*, *J. Inst. Brew.*, 2014,

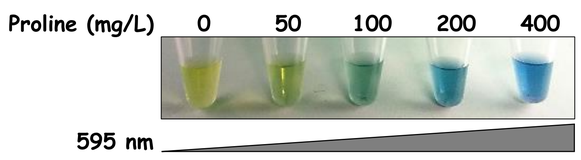
The yeast *Saccharomyces cerevisiae* plays important roles in the production of ethanol and in dictating the aroma and taste of alcoholic beverages. The quality of alcoholic beverages is thus strongly dependent on the metabolic characteristics of yeast cells. Nitrogen availability is a crucial factor in fermentation. Proline is the predominant amino acid in grape must and wort, and represents a potentially significant and effective source of nitrogen. However, yeast cells cannot utilize proline during wine and beer fermentation, leading to the high accumulation of proline in wines and beers and resulting in a bitter and low acid taste. Hence, a yeast strain that better utilizes proline would be promising for overcoming nutrient-related fermentation problems, and thereby improving the fermentation ability and quality of wine and beer.

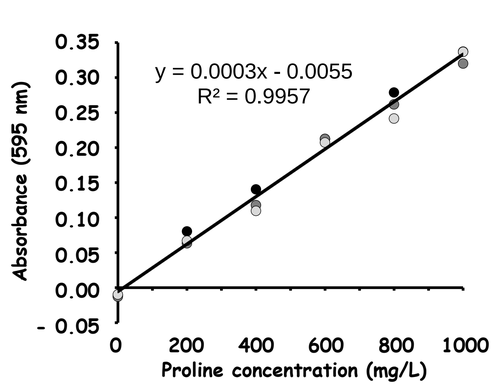
## 3. Development of a rapid method to measure residual proline

We first developed a rapid assay method with the dye Isatin with 96-well plate. To validate the method, residual proline was determined from cultured media with a typical ale yeast *S. cerevisiae* UCDFST 96-12, or with *Lachancea thermotolerans* UCDFST 04-833 that was previously known to assimilate proline.

### Determination of residual proline by Isatin and 96 well Plate

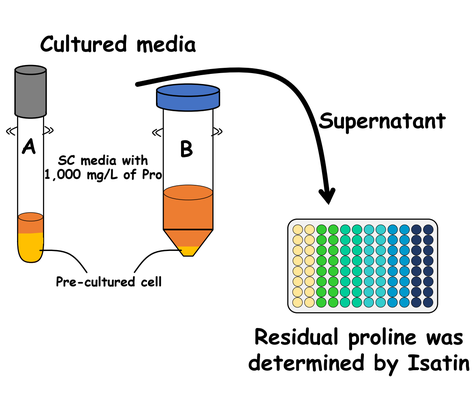


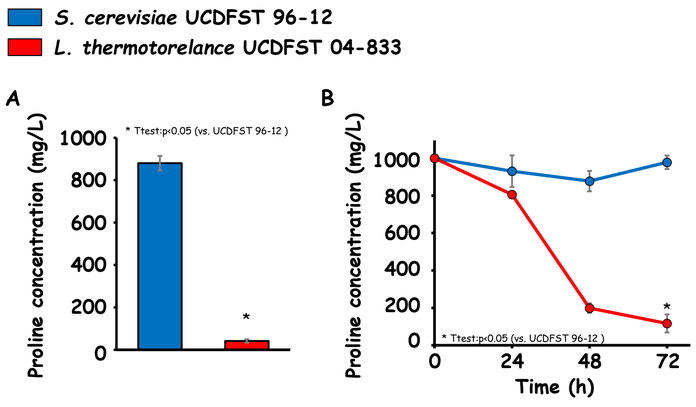




Isatin specifically reacts with proline and forms Indoline compounds. This reaction changes the yellow color of Isatin to blue. The standard curve showed a high degree of linearity over the working range of 0–1,000 mg/L, with a correlation coefficient of 0.9957.

### Validation of the method by using media cultured with proline-utilizing strain

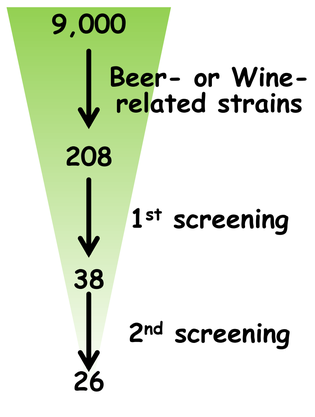



Proline remained in the media cultured with *S. cerevisiae* UCDFST 96-12 (Wyeast #1056 American Ale), while *L. thermotorelans* UCDFST 04-833 significantly consumed proline (Fig. A). The changes in proline level was also observed in the scaled-up condition (Fig. B). Taken together, we concluded that the rapid assay method was reliable in culture media.

## 4. Outline of screening with Phaff Yeast Culture Collection

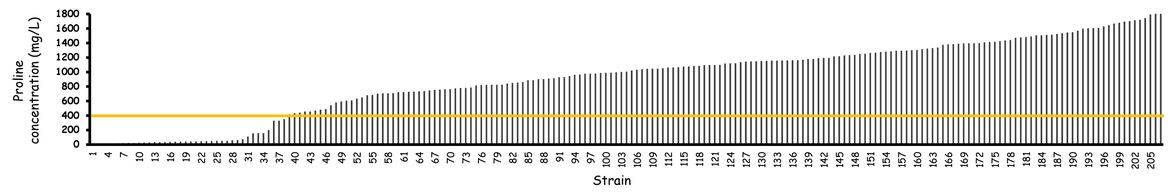

We conducted screenings with the Phaff Yeast Culture Collection, the 4th largest public wild yeast collection in the world with over 1,000 yeast species and over 9,000 strains. From the collection, 26/208 beer- or wine-related strains of *S. cerevisiae* and other species were selected from the 1st and 2nd screening.




## 5. Results of the 1st and 2nd screening

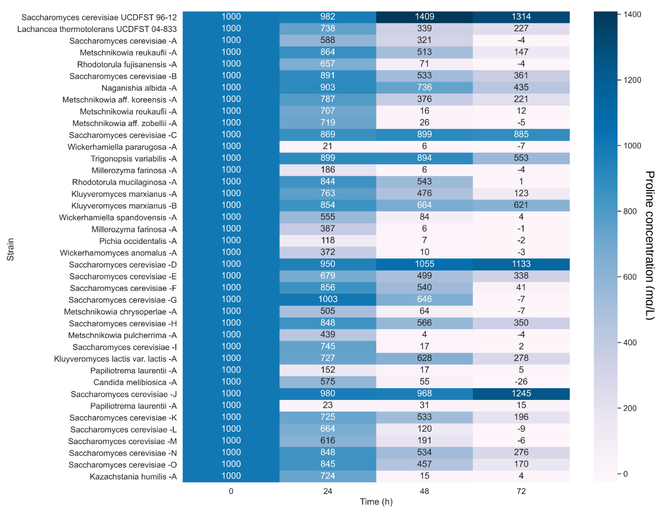
Residual proline was measured after two days growth in SC media that initially contained 1,000 mg/L proline. Strains exhibiting less than 400 mg/L of residual proline after 2 days growth were selected and subjected to the 2nd screening, in which proline consumption was assayed daily for three days.

### 1st screening




38 strains showed less than 400 mg/L of residual proline.

### 2nd screening

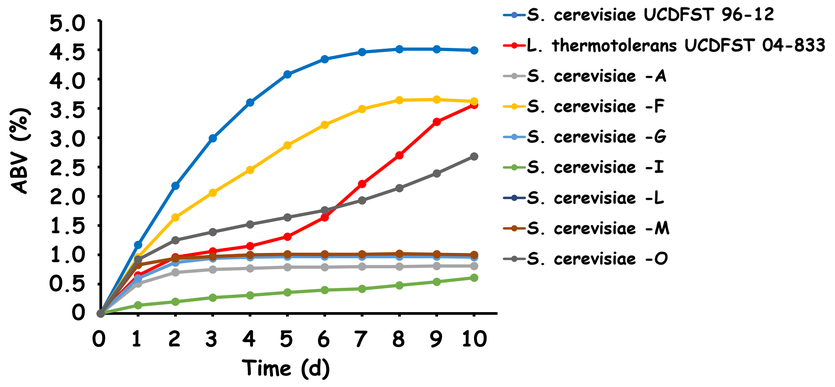



7 strains of *S. cerevisiae* (7/143; 4.9%) and 19 strains of non-*Saccharomyces* (19/65; 29.2%) consumed over 60% of the proline, suggesting that proline utilization is more common in non-*Saccharomyces* species than *Saccharomyces*.

Some *S. cerevisiae* strains were able to utilize proline, indicating that genetic differences may contribute to the ability to utilize proline.

## 6. Beer fermentation test with proline utilizing *S. cerevisiae*

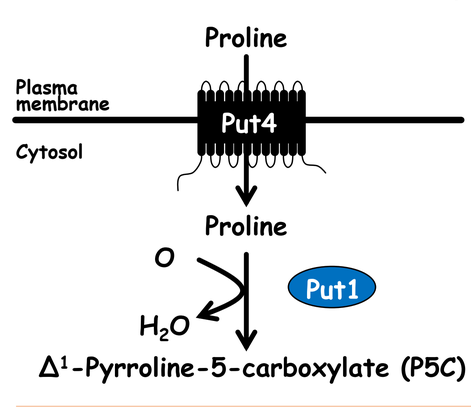
Seven strains of *S. cerevisiae* were selected from the 26 candidates. Selected strains were inoculated with wort for 10 days to determine alcohol production.

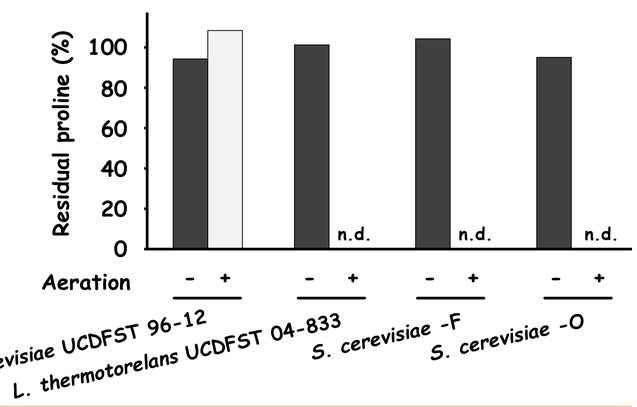



Commercial ale strain *S. cerevisiae* UCDFST 96-12 produced 4.50 % of alcohol as expected. *S. cerevisiae* –F and –O produced 3.62 % and 2.68 % alcohol after 10 days fermentation.

## 7. Proline consumption in wort with/without aeration

*Oxygen is an essential factor to reduce proline level, since proline is degraded by the proline oxidase Put1 right after incorporated into cells. We thus tested whether strains S. cerevisiae* –F and –O consume proline in the wort with or without aeration.




Proline was not consumed by any strains under anaerobic conditions. Under aerobic conditions, ale strain *S. cerevisiae* UCDFST 96-12 did not consume proline, but *S. cerevisiae* –F and –O as well as *L. thermotorelans* UCDFST 04-833 did. While these yeast strains were able to consume proline, the aerobic conditions required for this consumption are not compatible with brewing practices.

## 8. Summary

- A rapid and large-scale proline measurement method was established using Isatin and 96-well plate.
- 26 yeast strains strongly consumed extracellular proline under SC media.
- Two of *S. cerevisiae* produced low level of alcohol for 10 days fermentation and were able to utilize proline even under wort condition. Further process development is needed.

## 9. Acknowledgement

This study was partly supported by Project for International Collaborative Laboratories under the MEXT Program for Promoting the Enhancement of Research Universities.