Fermentation ability of bottom fermenting yeast exhibiting defective entry into the quiescent state

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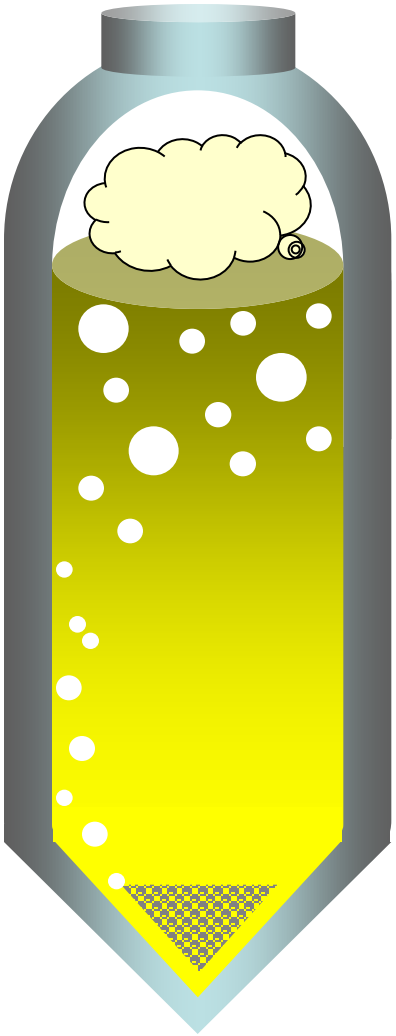
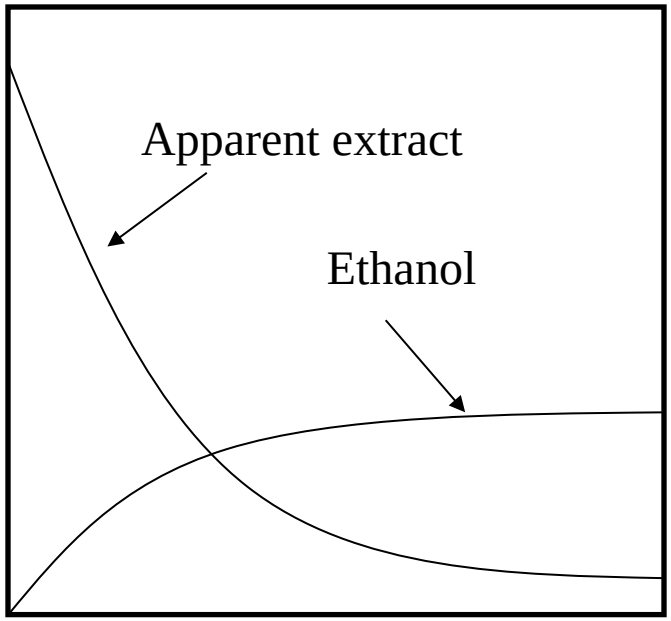
Outline

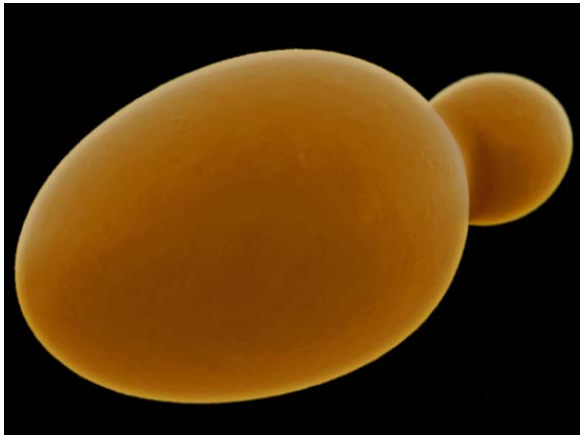
- **Introduction**
- **Research objective**
- **Methods**
- **Results**
- **Conclusion**

Introduction

The fermentation process affects the character of beer products







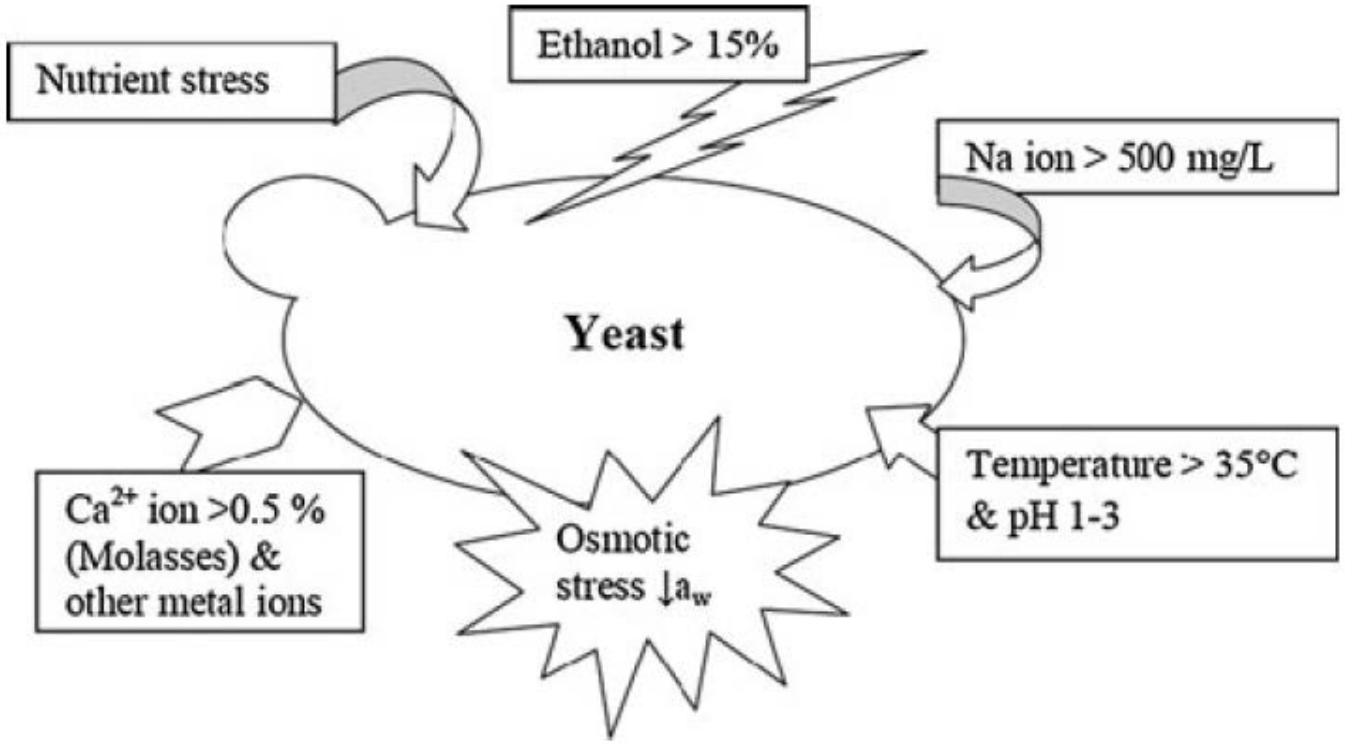
Ethanol, CO_2, etc…

The factors involved in the fermentation rate of brewing yeast

Factors

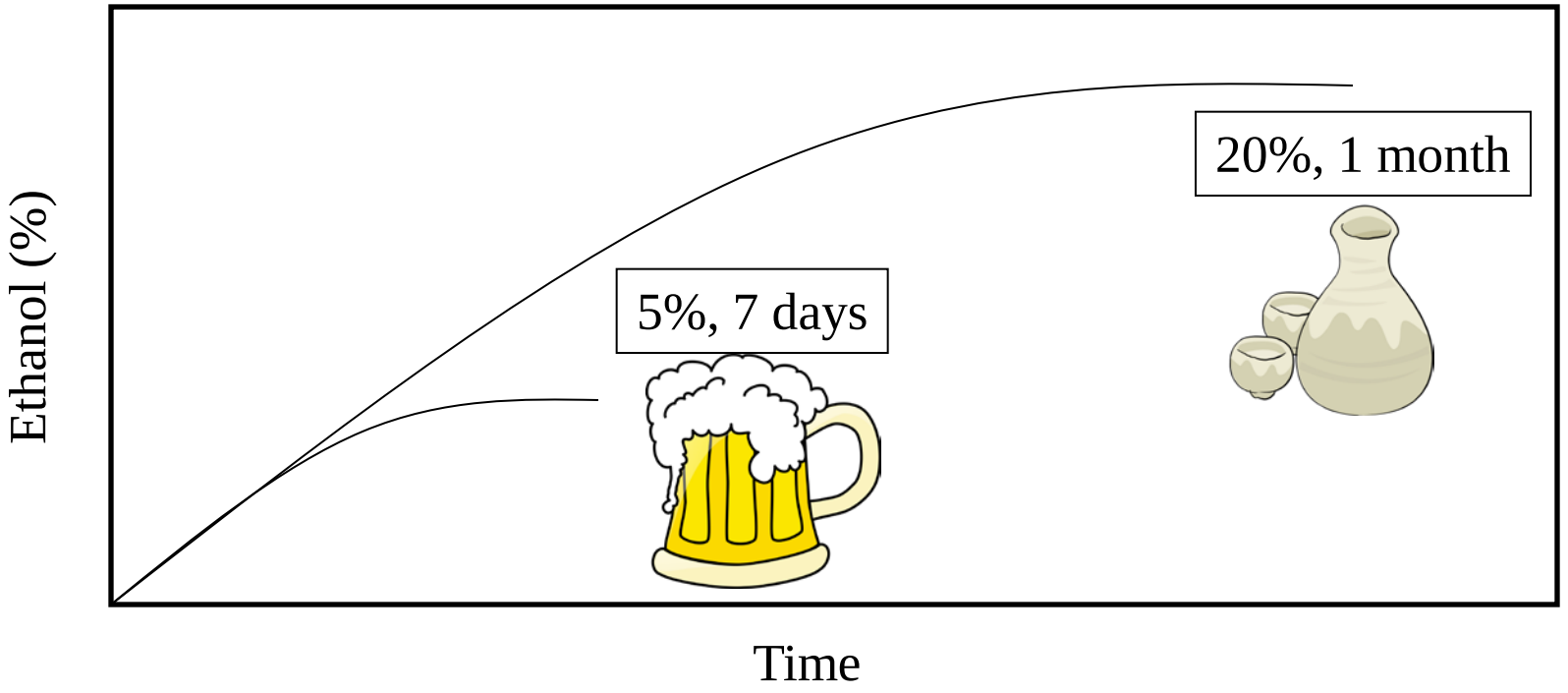
- **Fermentation conditions**
- **Wort compositions**
- **Characteristics of brewing yeast strains**

The fermentation rate declines under the high gravity brewing conditions



cf) J Ind Microbiol Biotechnol (2011) 38:1133–1144

Sake yeasts produce more than 20% ethanol



Japanese sake

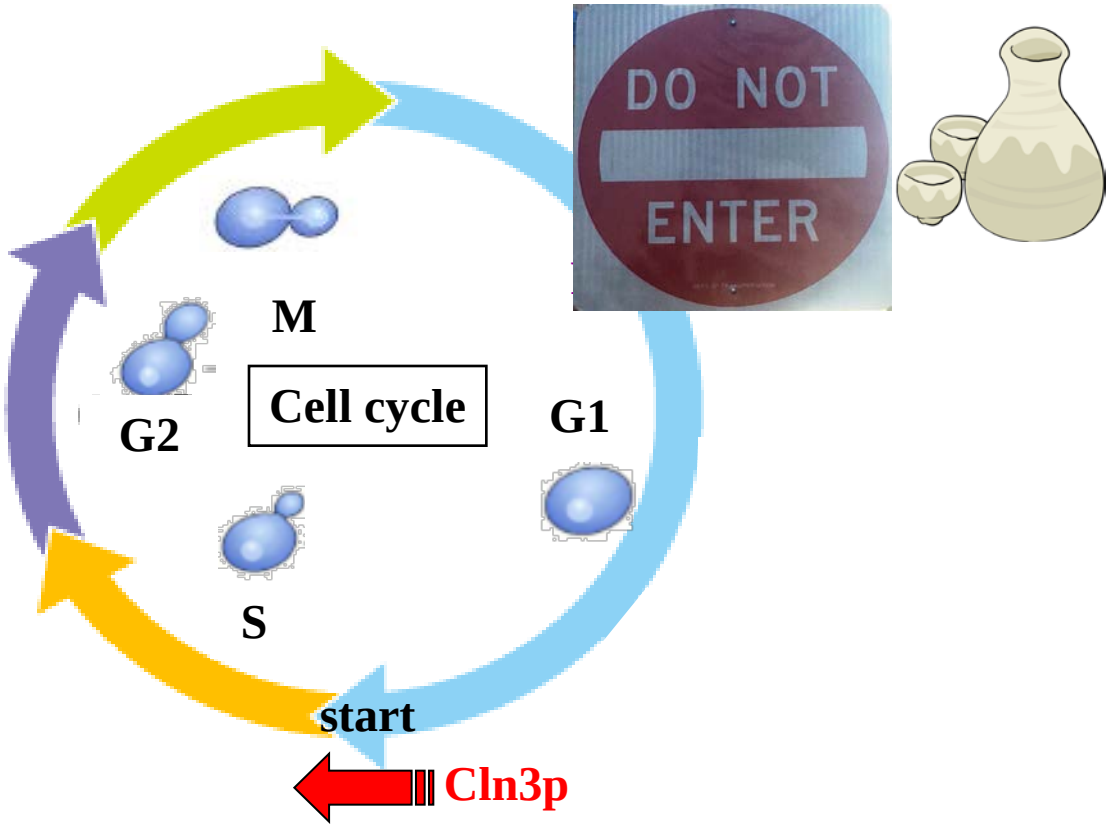
- Traditional alcoholic drink in Japan
- Ethanol content being more than 20%
- Steamed rice as a starting material for fermentation.
- Saccharification of rice starch by *Aspergillus oryzae*
- Fermentation by sake yeasts (*Saccharomyces cerevisiae*)

Sake yeasts are workaholic

Defective transition into the G0 phase

Rim15p : Loss of function by mutation

Cln3p : Gene expression is increased



cf.) WATANABE Daisuke et al. *Appl. Environ. Microbiol.* **78**: 4008-4016 (2012)
WATANABE Daisuke et al. *J. Biosci. Bioeng.* **112**: 577-582 (2011).

Research objective

Investigation of the relationship between the fermentation rates and cell cycle transitions in bottom fermenting yeast.

Methods

Scheme of analysis

Construction of bottom fermenting yeasts which exhibit the defective entry into the quiescent state.

Evaluation of the constructed strains

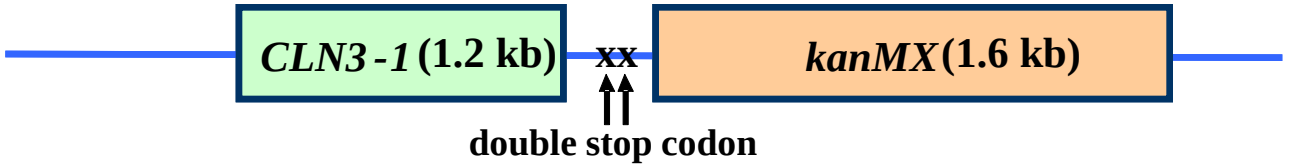
- **Fermentation rate**
- **Cell cycle**

Construction of the strains

Wild type strain

Saccharomyces pastorianus Weihenstephan 34/70 (W34/70)

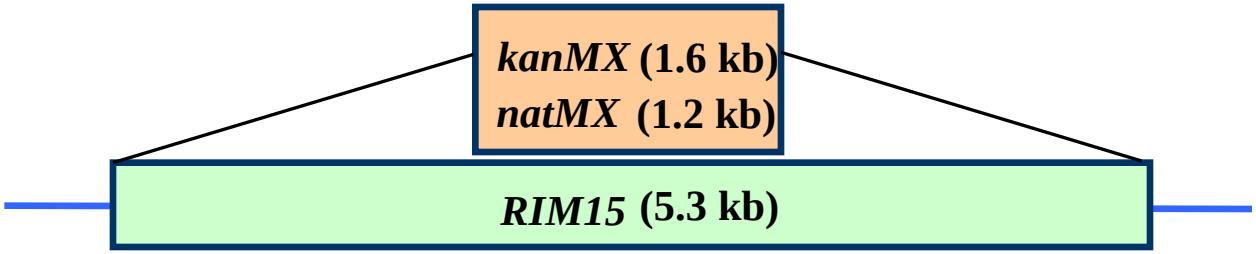
W34/70 *S. cerevisiae* type *CLN3-1* mutant (*CLN3-1*)



Repression of G1 cyclin Cln3p degradation

Promotion of G1/S transition

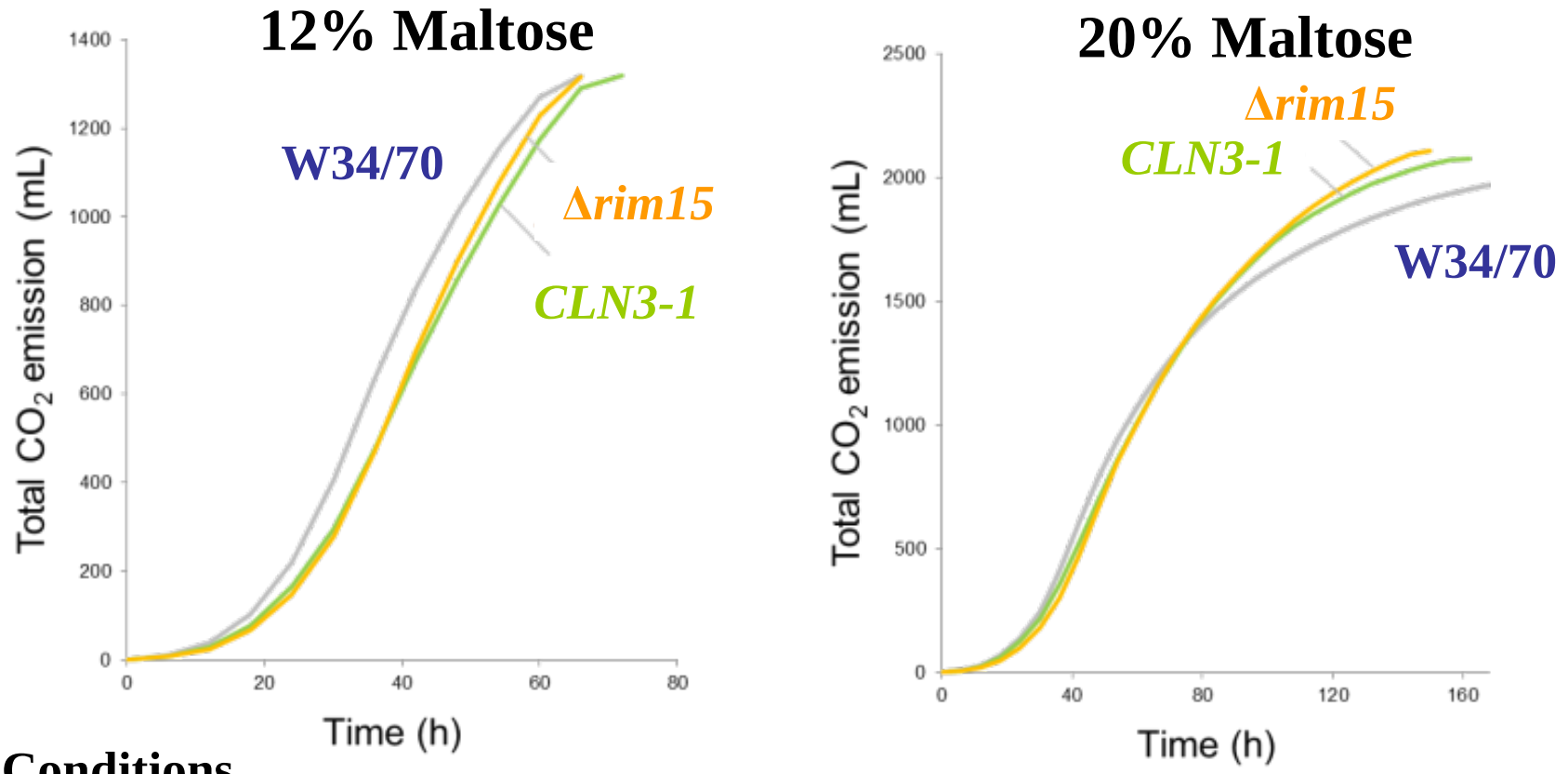
W34/70 *S. cerevisiae* type *RIM15* gene disruptant (Δ*rim15*)



Defective transition into the G0 phase

Results

The constructed strains exhibited higher fermentation rates in high-sugar medium (Monitoring by total CO_2 emissions)



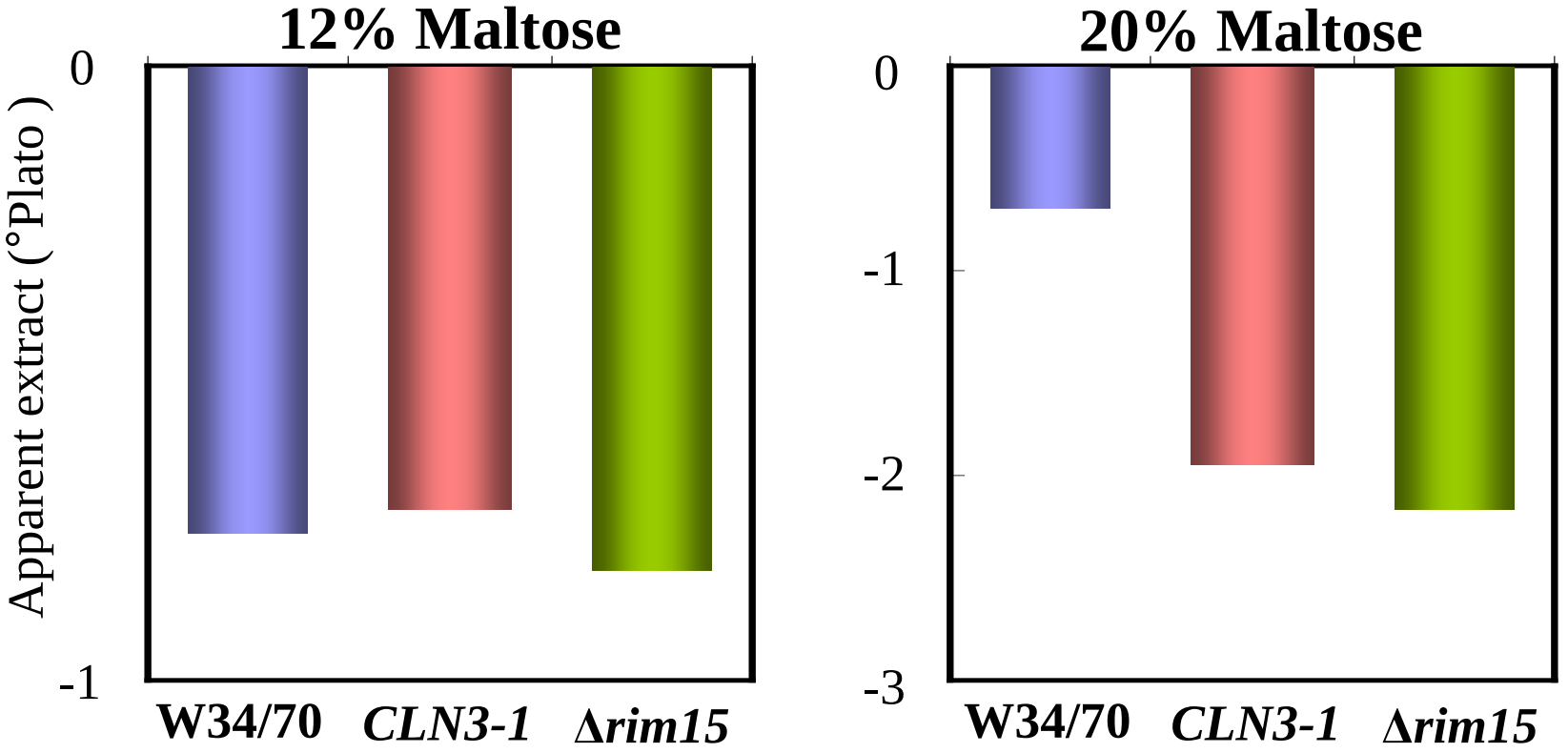
Conditions

Temp. 15 ºC **Synthetic medium**

Scale 50 mL **YNB (w/o a.a.) , 1% Yeast extract, 12% or 20% Maltose**

The constructed strains exhibited higher fermentation rates in high-sugar medium

(Apparent extract at the end of fermentation)



Conditions

Temp. 15 ºC **Synthetic medium**

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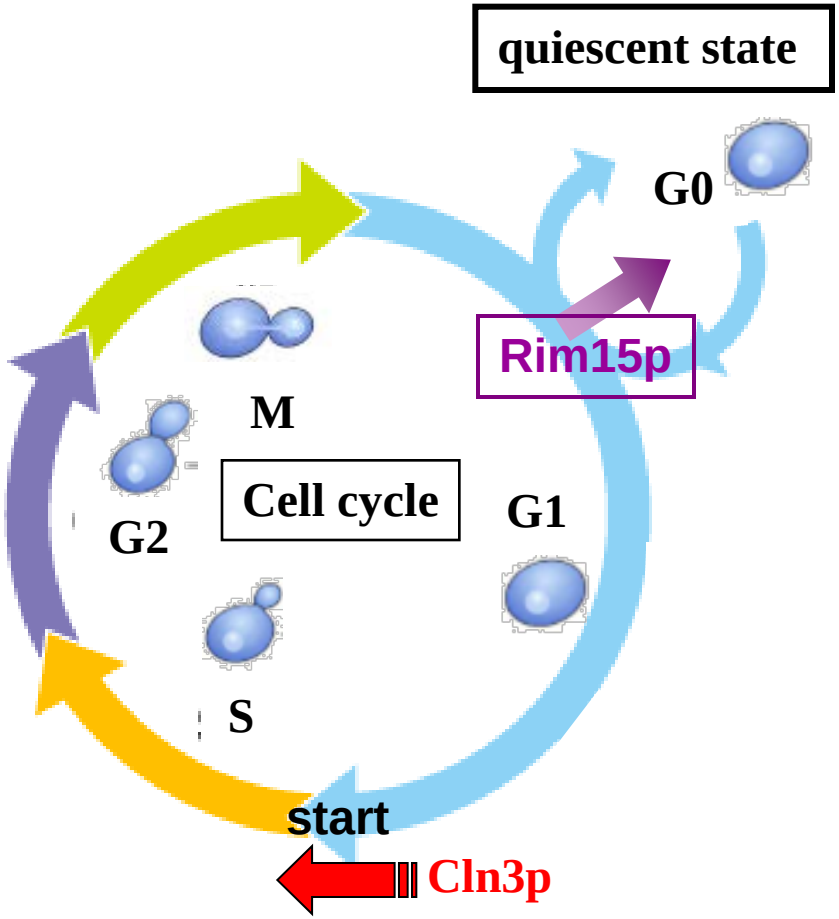
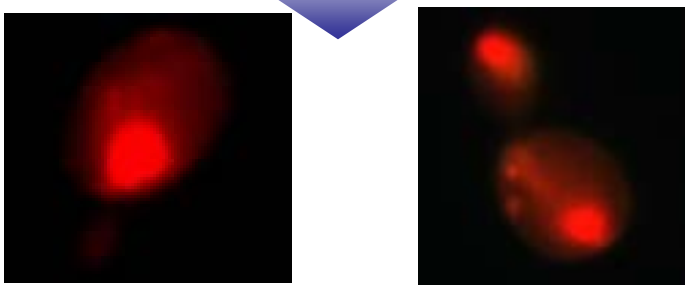
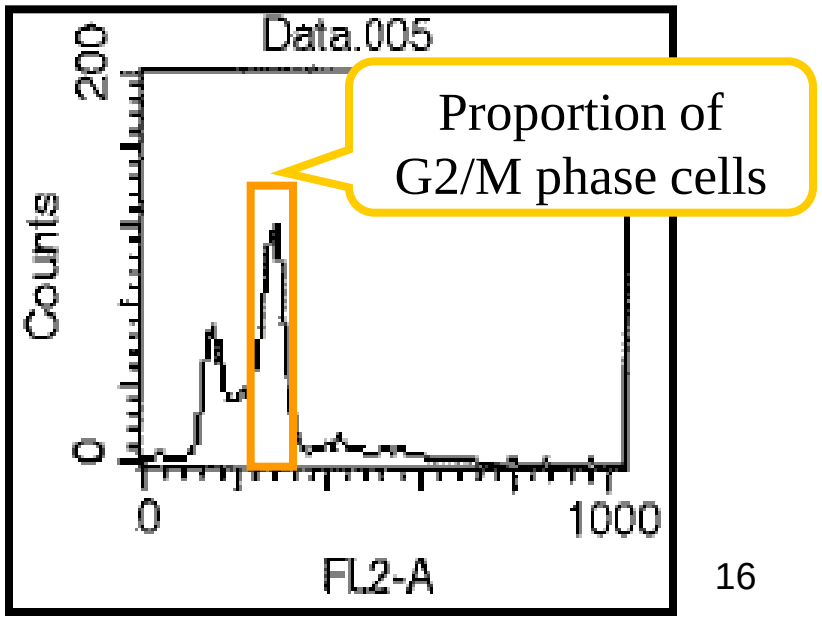
Cell cycle analysis

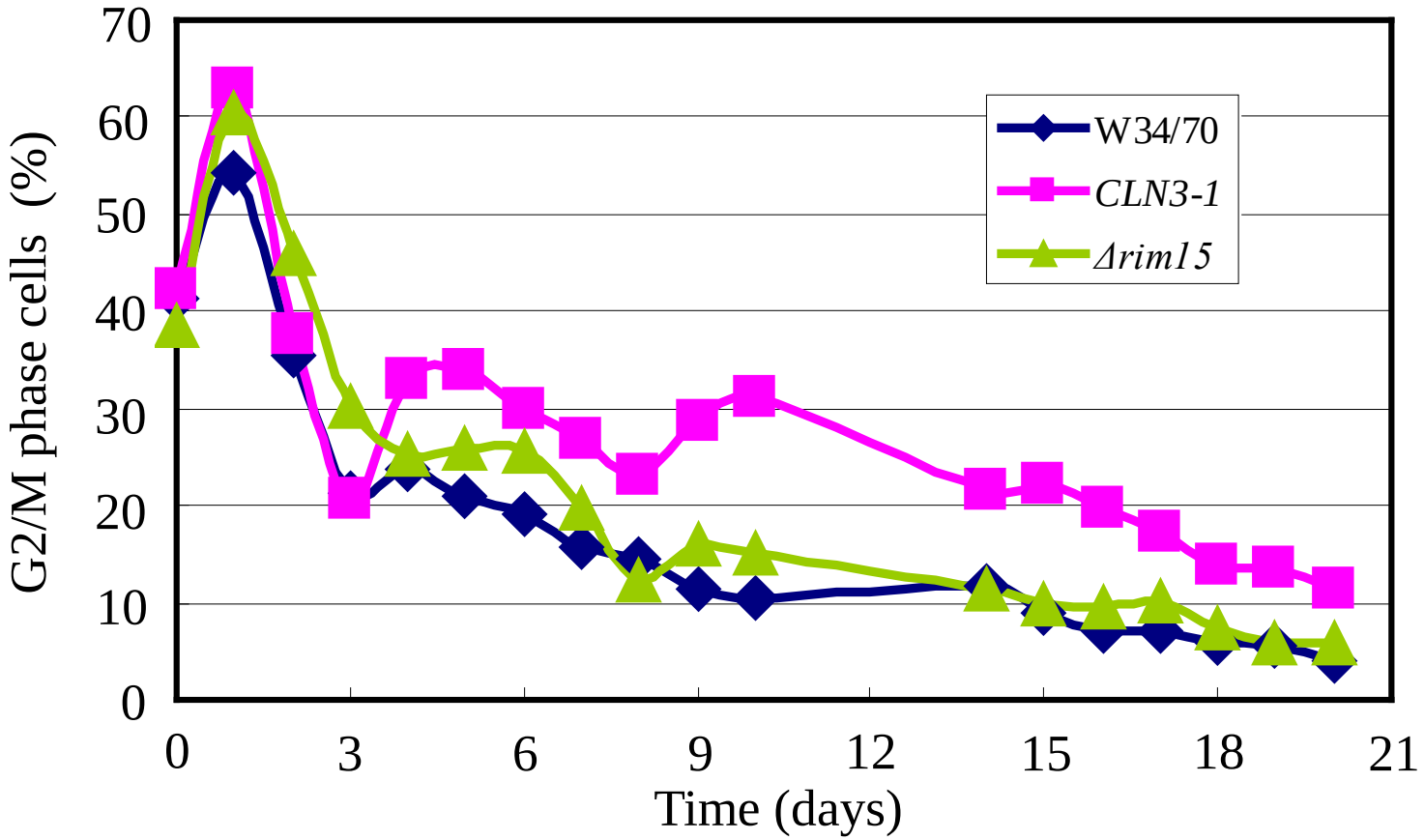


Analysis of cell cycle by flow cytometer.





The constructed strains showed moderate inhibition of G0 entry

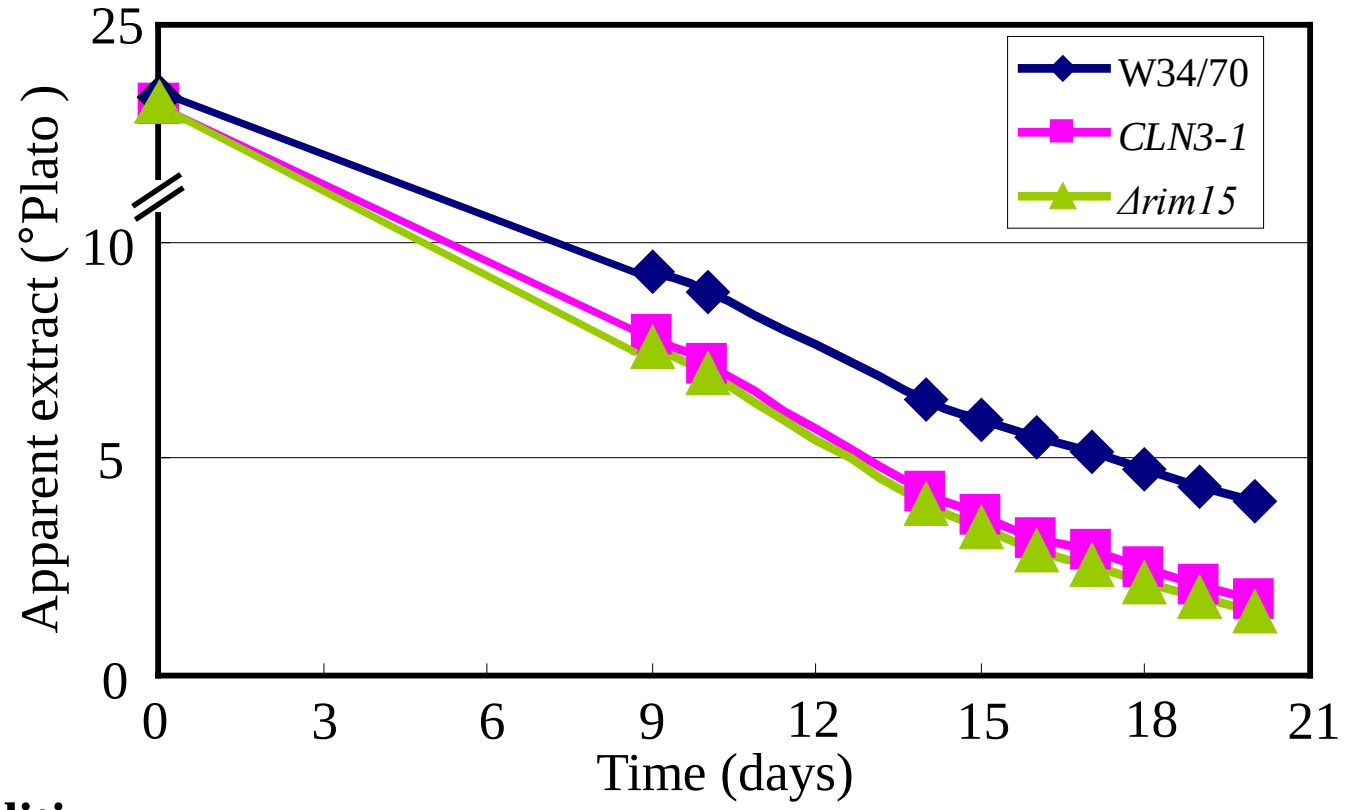


Conditions

Temp. 15 ºC **Synthetic medium**

Scale 1 L **・YNB (w/o a.a.) , 0.25% Yeast extract, 20% Maltose**

The constructed strains showed the increased fermentation rate

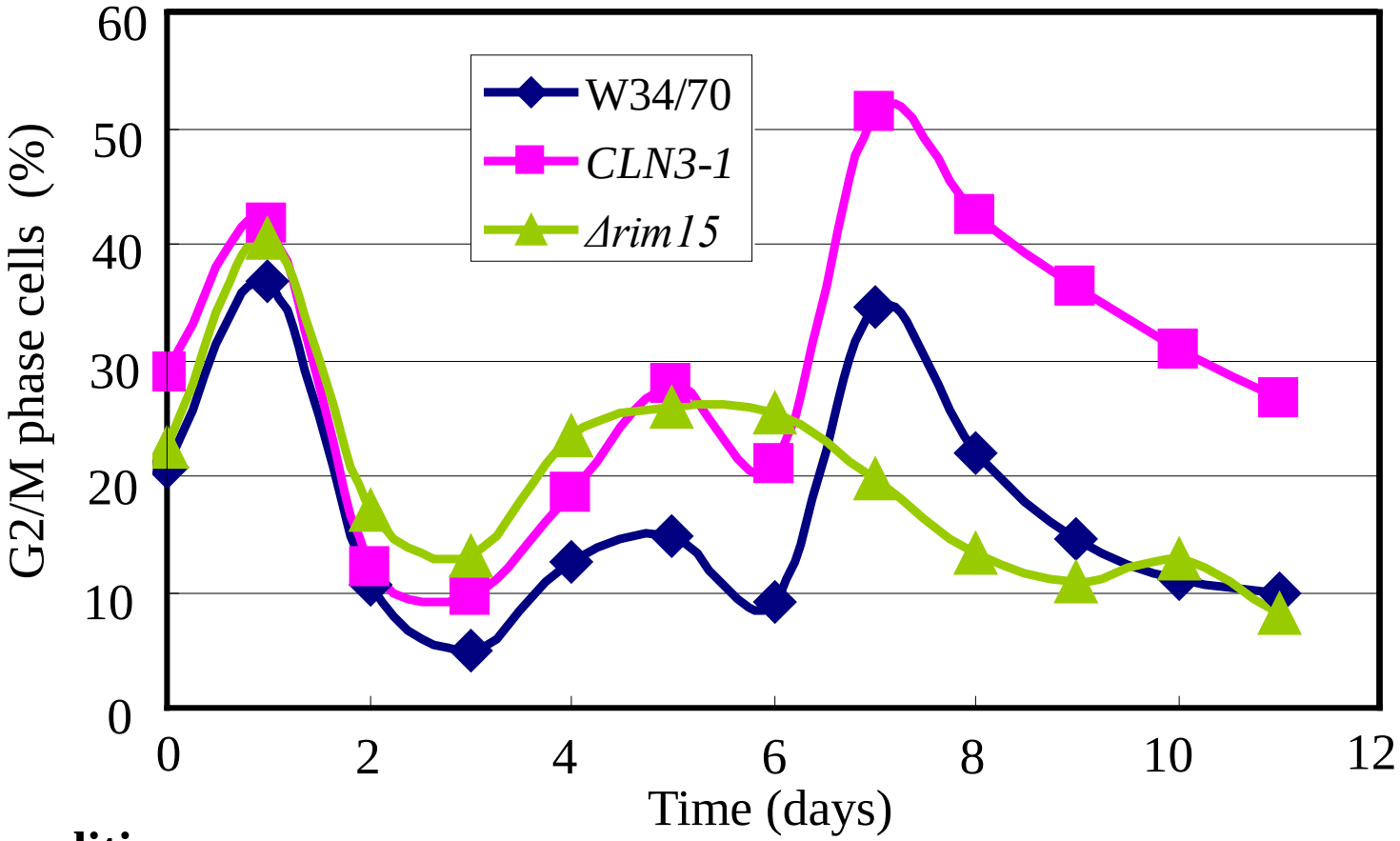


Conditions

Temp. 15 ºC **Synthetic medium**

Scale 1 L **・YNB (w/o a.a.) , 0.25% Yeast extract, 20% Maltose**

The constructed strains also showed moderate inhibition of G0 entry with high gravity wort

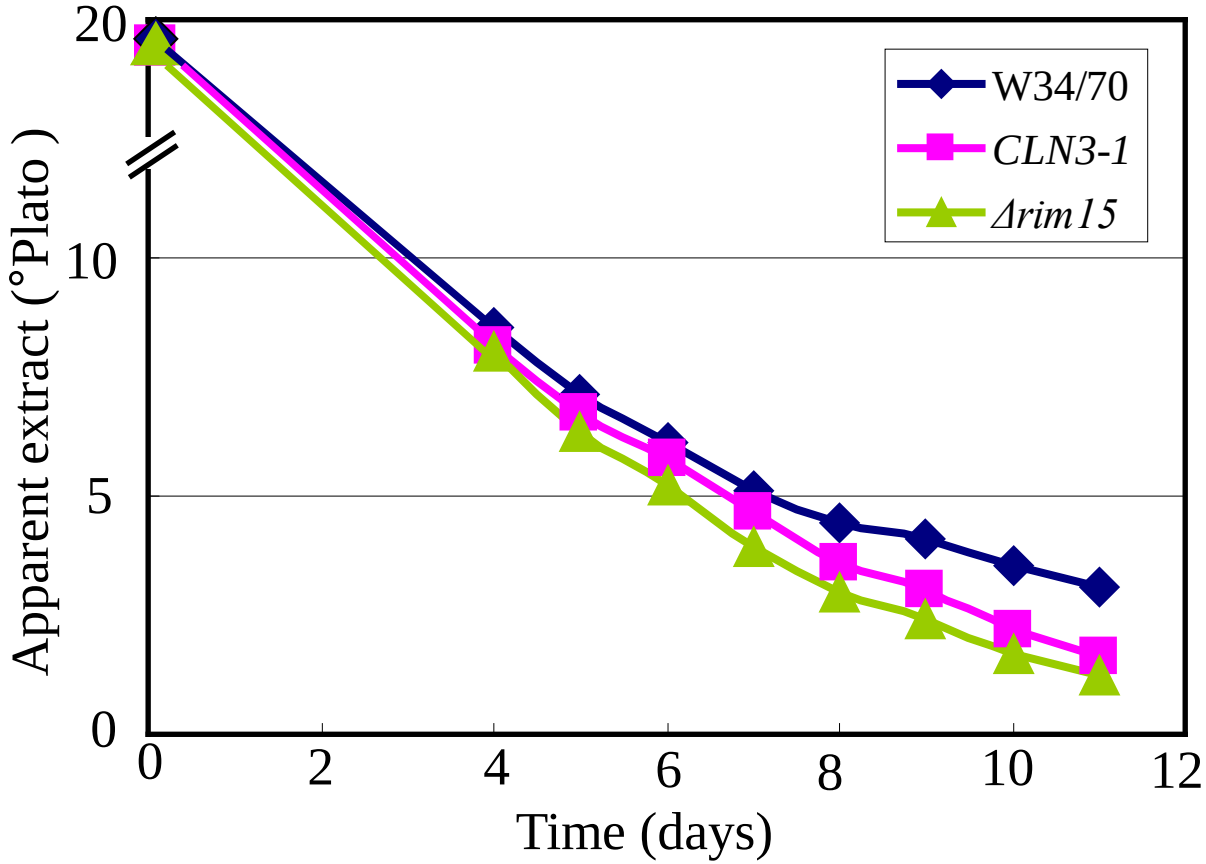


Conditions

Temp. 15 ºC **20 °Plato wort**

Scale 1 L **▪Normal wort supplemented with maltose**

The constructed strains also showed the increased fermentation rate with high gravity wort



Conditions

Temp. 15 ºC **20 °Plato wort**

Scale 1 L **▪Normal wort supplemented with maltose**

Summary

1. **We constructed two W34/70 variants (*CLN3-1*,Δ*rim15*) .**

2. ***CLN3-1* mutant and Δ*rim15* strain showed moderate inhibition of G0 entry compared with W34/70.**

3. **The fermentation rates of the constructed strains were increased both in synthetic medium and high gravity wort.**

Conclusion

A relationship was found between the fermentation rate and cell cycle transitions in bottom fermenting yeast.

We found a clue in the screening of yeast strains suited to high gravity brewing.

Thank you for your kind attention