

# Taking a walk on the wild side: food and beverage applications of wild yeasts

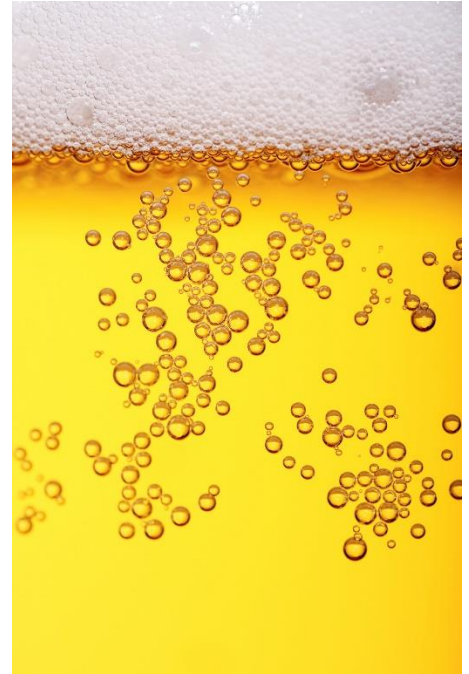
Brian Gibson

VTT Technical Research Centre of Finland Ltd.

21st November 2019

Gateways

Teagasc Food Research Centre, Fermoy, Cork



# VTT – beyond the obvious

VTT is one of the leading research, development and innovation organizations in Europe. We help our customers and society to grow and renew through applied research. The business sector and the entire society get the best benefit from VTT when we solve challenges that require world-class know-how together and translate them into business opportunities.

## Our vision

A brighter future is created through science-based innovations.

## Our mission

Customers and society grow and renew through applied research.

## Strategy

Impact through scientific and technological excellence.

Established in

**1942**

Owned by

Ministry of  
Economic  
Affairs and  
Employment

**268 M€**

Net turnover and  
other operating  
income (VTT  
Group 2018)

**2,177**

Total of personnel  
(VTT Group  
31.12.2018)

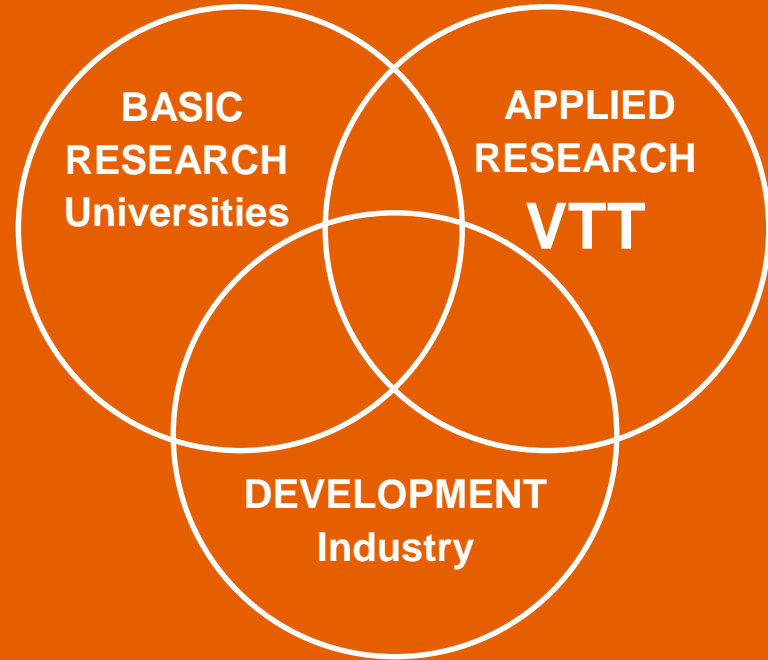
**31%**

Doctorates and  
Licentiatees  
(VTT Group  
2018)

**36%**

Net turnover  
from abroad  
(VTT Group  
2017)

# VTT's status as performer of R&D work



# VTT's business areas



- Sensing and integration
- Connectivity
- Data-driven solutions
- National Metrology Institute VTT MIKES
- Micronova manufacturing services

## KNOWLEDGE INTENSIVE PRODUCTS AND SERVICES



## SMART INDUSTRY AND ENERGY SYSTEMS



- Lifecycle solutions
- Nuclear safety
- Smart energy and transport solutions
- Digital engineering
- Business innovation foresight



- Industrial biotechnology and food solutions
- Biomass processing and products
- Sustainable energy and chemical technologies

## SOLUTIONS FOR NATURAL RESOURCES AND ENVIRONMENT



# The importance of yeast in fermented beverages

Influence of yeast not limited to alcohol!

Source of many beverage flavours

esters, higher alcohols, phenolics, organic acids

Removal of off-flavours

aldehydes, diacetyl, sugar

Antioxidant potential ( $\text{SO}_2 \uparrow$ ,  $\text{O}_2 \downarrow$ , metals  $\downarrow$ )

Secondary products/functions

carbonation, mouthfeel, toxin removal



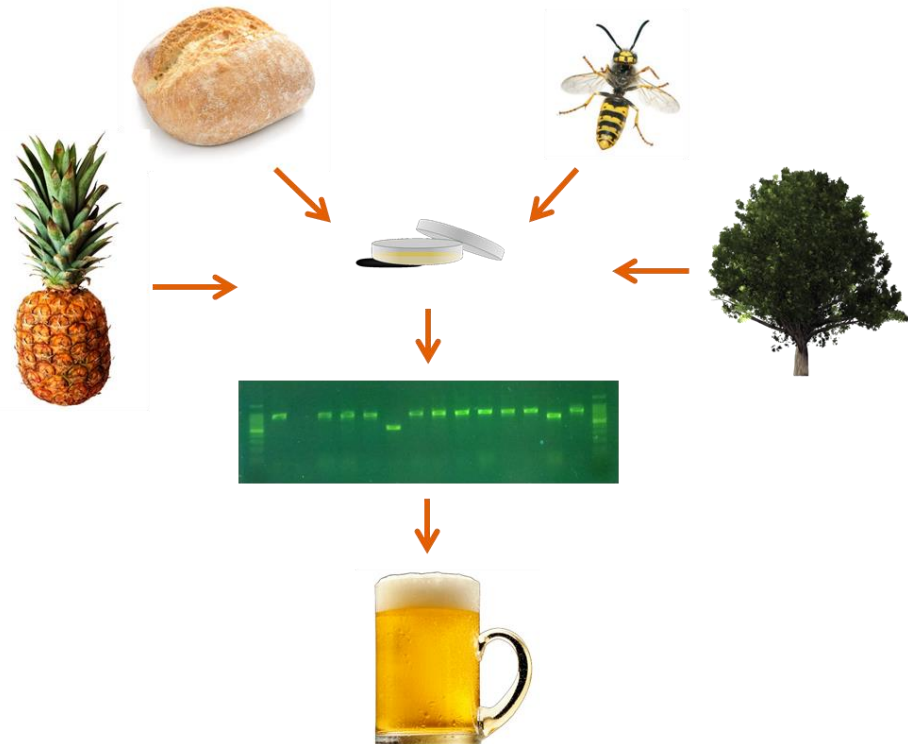
# VTT's brewing and baking yeast research

- **Industry-relevant phenotypes and their underlying mechanisms**
  - Flavour (diacetyl, aldehydes, esters and higher alcohols, sulphur volatiles)
  - Fermentation efficiency (sugar use, esp. maltotriose)
  - Stress tolerance/temperature tolerance
- **Strain development for industry (fermented beverages, baking)**
  - High-throughput screening
  - Evolutionary engineering
  - Bioprospecting for biotech applications
  - Hybridization



# Bioprospecting for brewers

- The attempt to discover living organisms, or biological processes, that have medical, agricultural, or industrial value
- Allows creation of novel, speciality beers
  - Wild lager (*S. eubayanus*)
  - Sour beer (*Lachancea thermotolerans*)
  - Low-alcohol beer (*Saccharomyces ludwigii*)
- Yeast can be sourced from:
  - Nature
  - Spontaneous fermentations





# Saccharomyces and domestication

- Eight species in genus
- *S. cerevisiae* most associated with human activities
  - but also found in nature
- Others species rarely contribute to human activities...



wolfblas.com

Winemaking



Dough leavening



Coffee fermentation



Gavin Smith

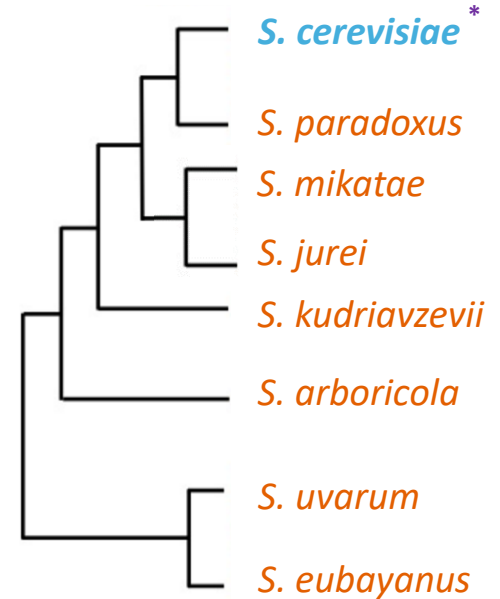
Brewing



Cocoa bean  
fermentation

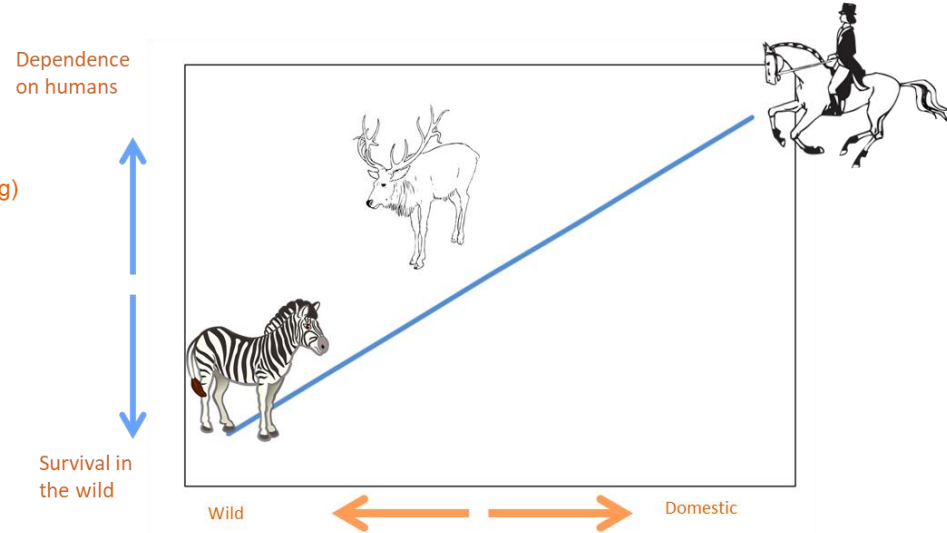
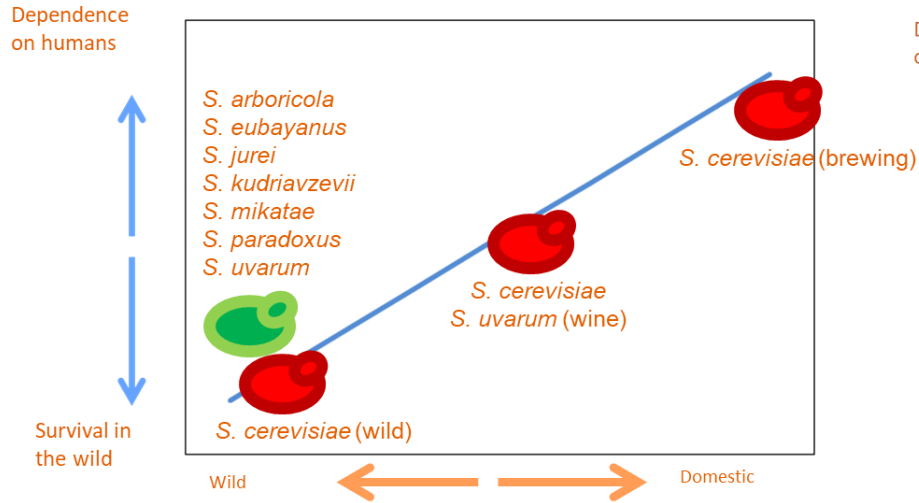


Wild





# Saccharomyces and domestication



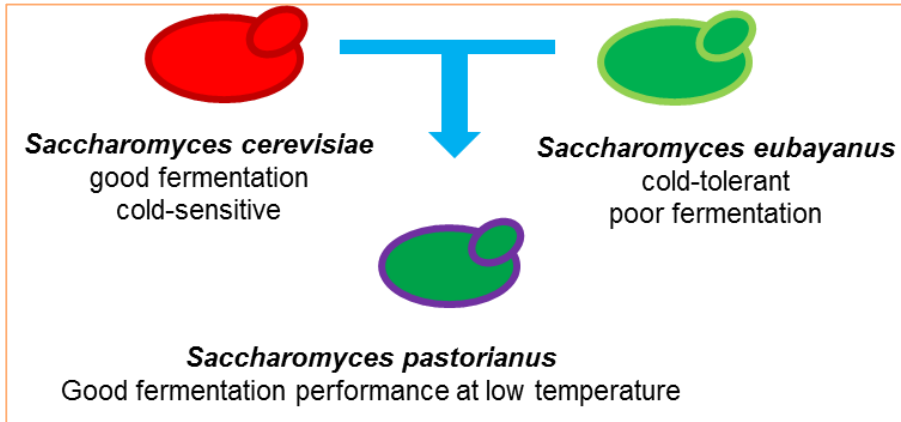
- Wild yeast rarely found in fermentation environments, at least not in pure form...

# Hybrids and fermentation

## ■ Natural hybrids in ale, cider and wine fermentations

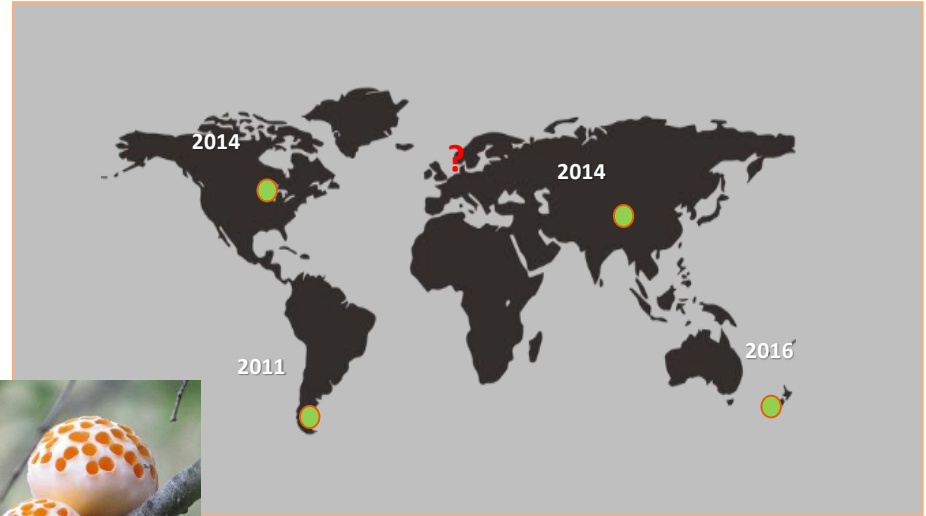
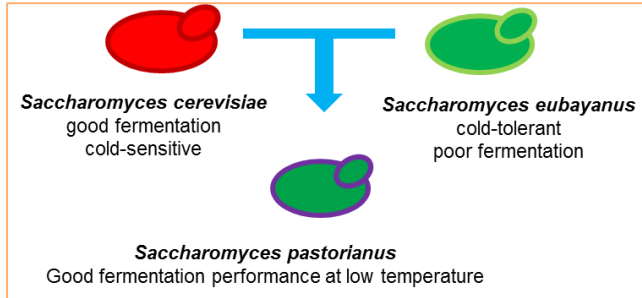
- *S. cerevisiae* x *S. uvarum*
- *S. cerevisiae* x *S. kudriavzevii*
- *S. cerevisiae* x *S. uvarum* x *S. kudriavzevii*

## ■ Natural lager yeast hybrids



# *S. eubayanus* discovery

- A recent discovery
  - Diego libkind, Bariloche, 2011
  - From southern beech
  - Apparent world-wide distribution
  - Not Europe...yet
  - Parent strain of lager yeast



- Brewing potential?

# *S. eubayanus* as a brewing yeast

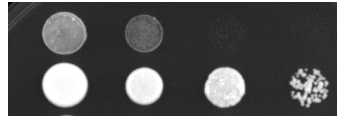
- *S. eubayanus* suitable for lager brewing

- But with certain limitations

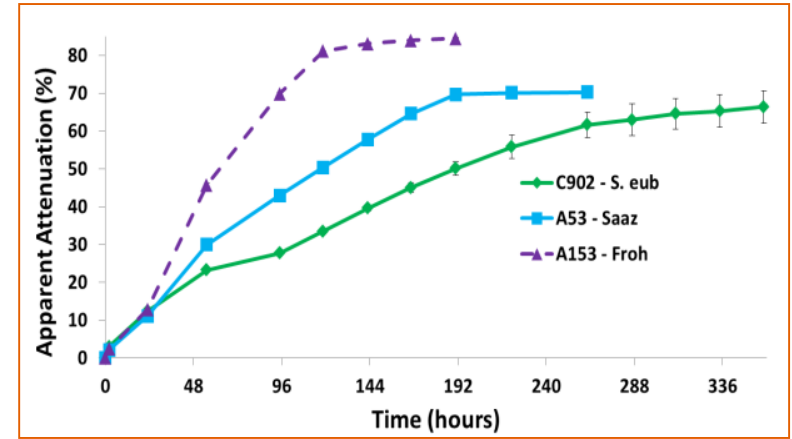
- Low yield ✗
- Phenolic flavour (4-VG) ✗/✓
- Poor flocculation ✗
- Aromatic ✓
- Cold tolerant ✓

*S. cerevisiae*

*S. eubayanus*



8°C, 10 days



- One of the rare industrial applications of wild yeast



## Yeast

Yeast 2013; 30: 255–266.  
Published online in Wiley Online Library  
(wileyonlinelibrary.com) DOI: 10.1002/yea.2960

## Research Article

### Comparative physiology and fermentation performance of Saaz and Frohberg lager yeast strains and the parental species *Saccharomyces eubayanus*

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Discovery  
2011

Characterization  
2013

Commercialization  
2016

# Other *Saccharomyces* species?

- Eight species in genus
- We know surprisingly little about most *Saccharomyces* yeasts
  - Few comparative studies
- Have potential applications been overlooked?



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Winemaking



Dough leavening



Coffee fermentation



Gavin Smith

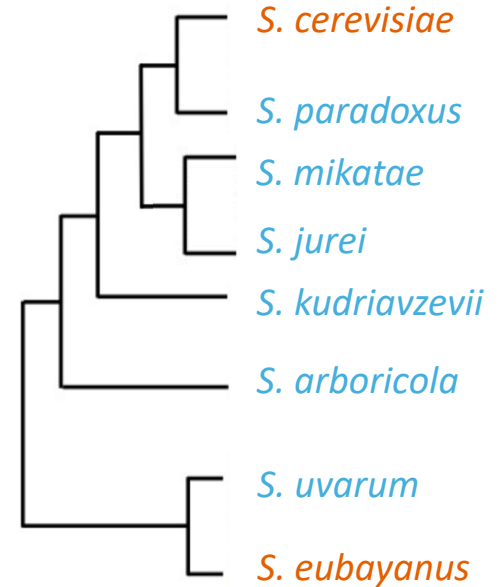
Brewing



Cacao bean  
fermentation

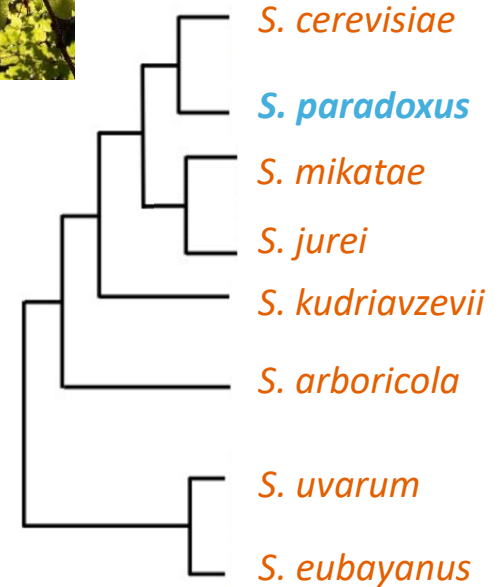


Wild



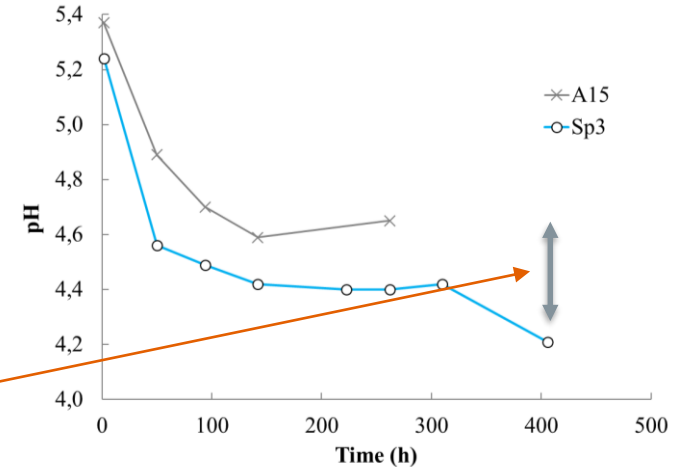
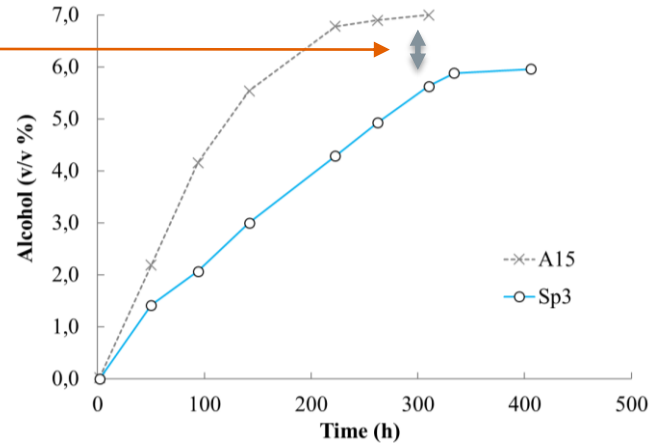
# *S. paradoxus*

- Closest relative to *S. cerevisiae*
- Associated with oak trees
- Fully wild, never associated with human activities
- Used for brewing of Sinebrychoff's 1819 beer (24.9.19)
  - Strain isolated and characterized at VTT



# *S. paradoxus* fermentation characteristics at 15°C (Lager beer)

No maltotriose usage  
-> deviation!

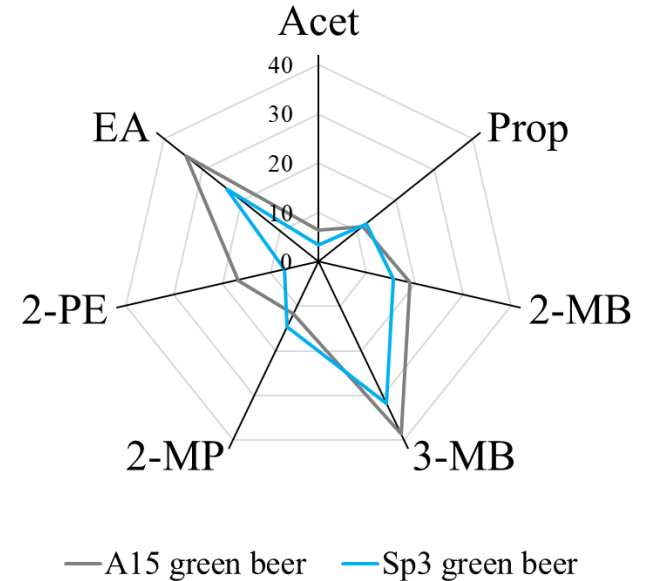


*S. paradoxus* has greater acidifying power (acetic acid)

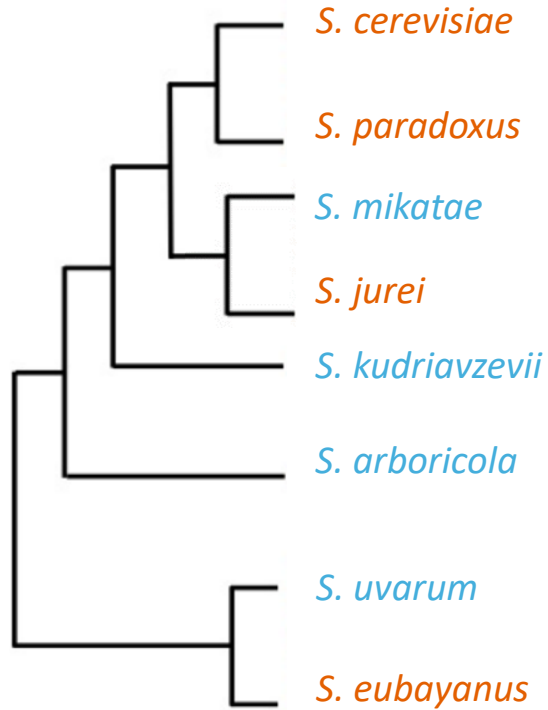


# *S. paradoxus* aroma production at 15°C (Lager beer)

- Volatile aromas similar or lower than reference
- Notes by professional sensory panel:
  - Spicy phenolic notes
  - Absence of off-flavours
  - Sulphuric, fatty acid notes only in A15 reference beer
  - *S. paradoxus* beer perceived as more fruity, estery, hoppy, acidic

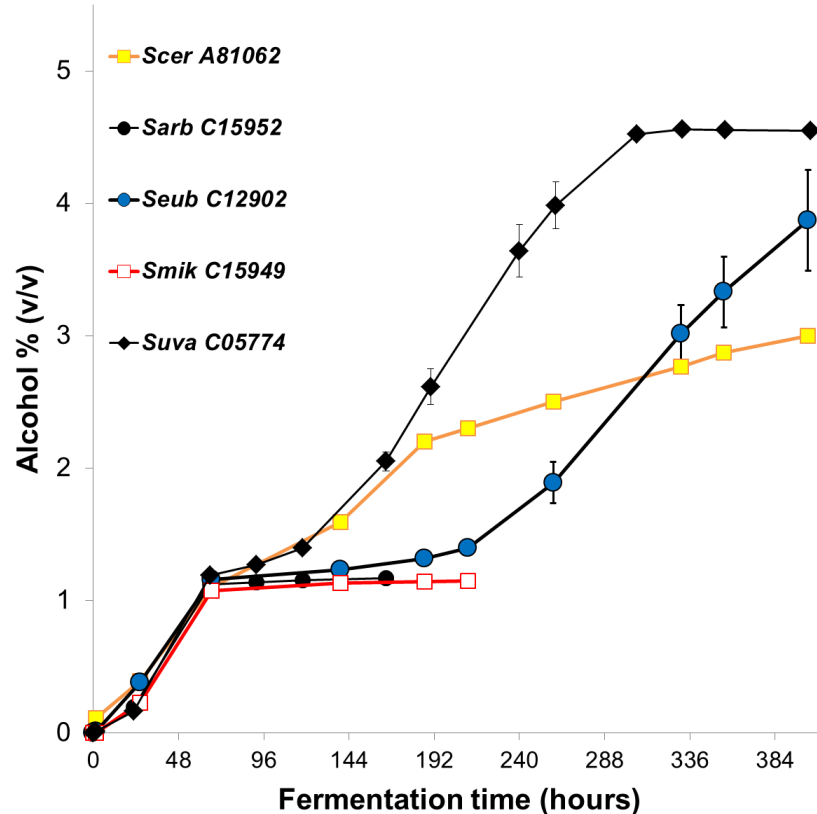
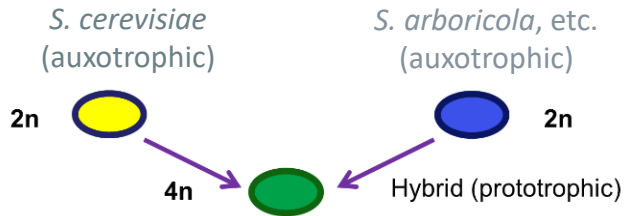


# Other species?



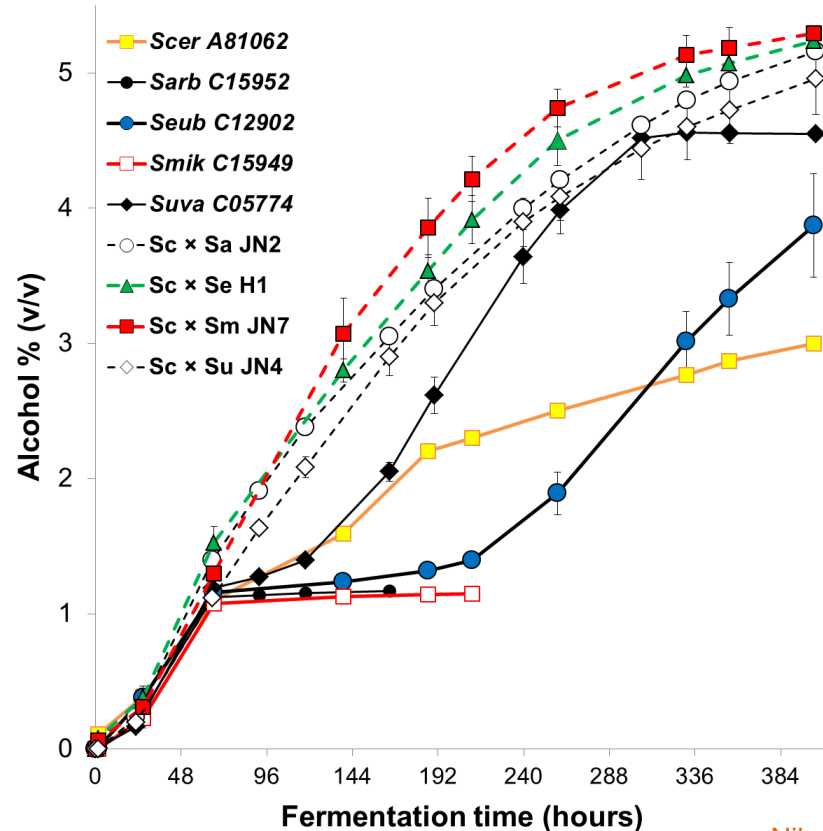
# Other wild yeast for lager brewing (12°C)

- All species vary in performance
- Due to:
  - Temperature tolerance (*S. cerevisiae*)
  - Sugar use (esp. *S. mikatae*, *S. arboricola*)
- Performance after hybridization?
- Rare mating performed with
  - *S. arboricola*
  - *S. eubayanus*
  - *S. mikatae*
  - *S. uvarum*



# 'Alternative' hybrids for lager brewing

- Fermentation similar or better than *S. cerevisiae* x *S. eubayanus*
- Lag phase (a wild feature) absent
- *S. eubayanus* **not** essential for low-temperature fermentation
- Other cold-tolerant species equally good
  - cf *S. mikatae*
- Some strain development may be needed for wild yeast

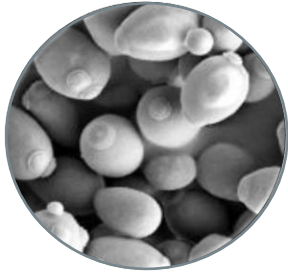


## Other uses for wild *Saccharomyces* yeasts?

- Frozen-dough technology allows fresh bread to be available throughout the day.
- Baker's yeast sensitive to freezing leading to poor proofing.
- Genus includes many cold tolerant species that can potentially tolerate freezing.

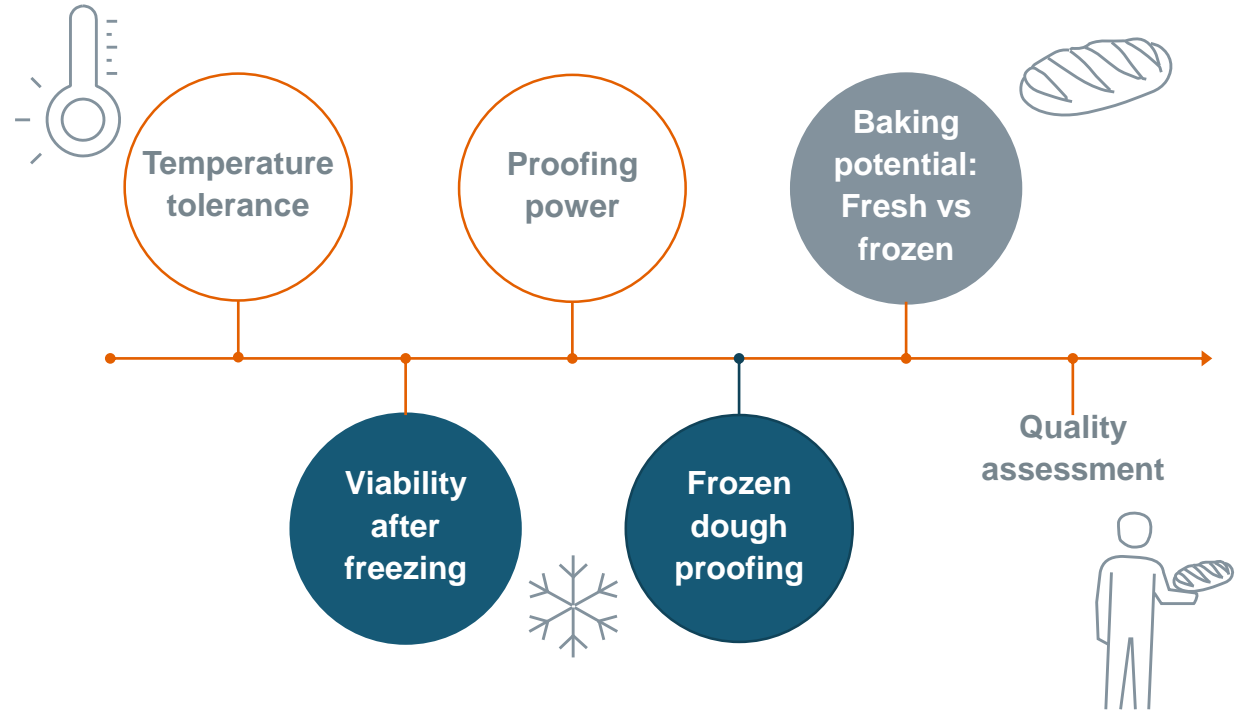


# Screening for cold/freeze resistance and baking potential

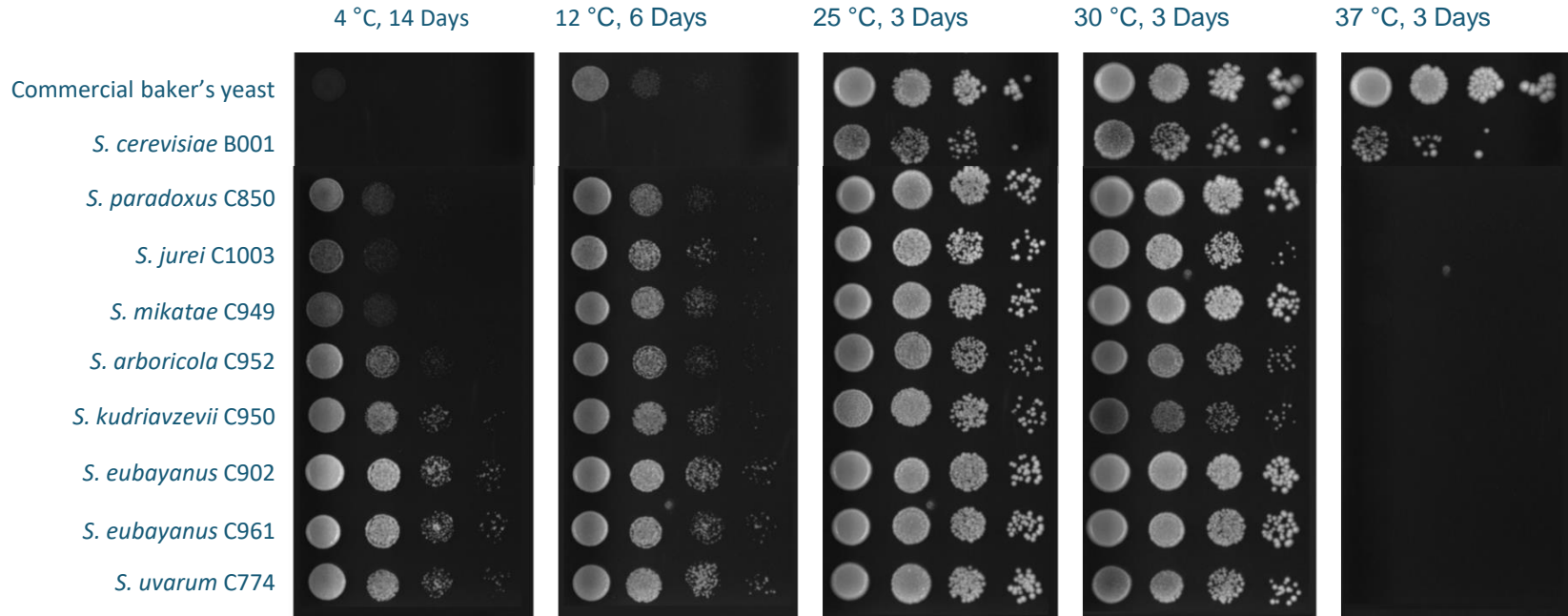


*Saccharomyces* spp.  
(Type Strains)

*S. cerevisiae*  
*S. paradoxus*  
*S. jurei*  
*S. mikatae*  
*S. arboricola*  
*S. kudriavzevii*  
*S. eubayanus*  
*S. uvarum*



# Temperature tolerance of *Saccharomyces* species

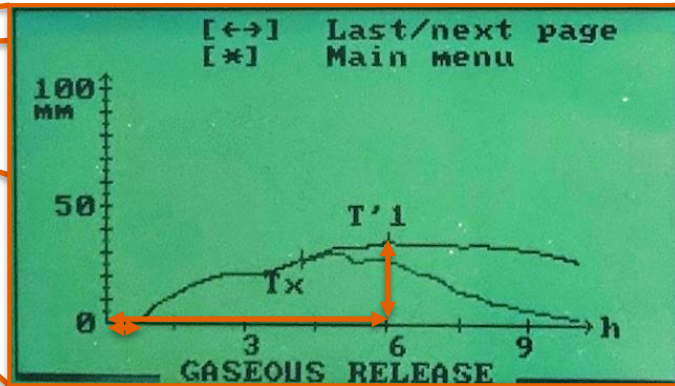
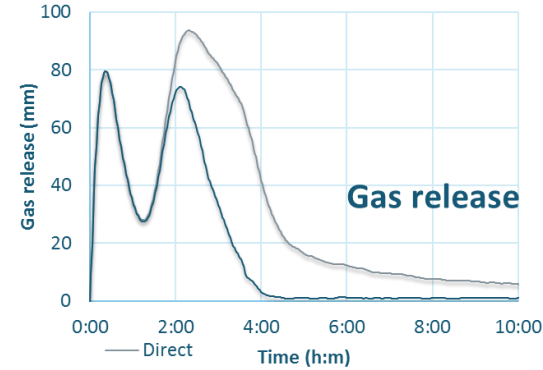
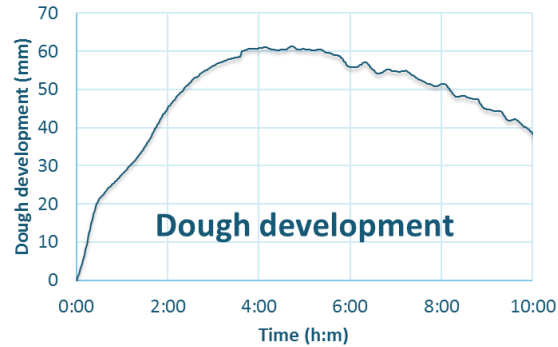


- *S. cerevisiae* strains capable of 37°C growth
- *S. eubayanus* particularly cold-tolerant



# Monitoring of proofing (fermentation) power

Rheofermentometer follows dough development by measuring the gas release and variation in volume.



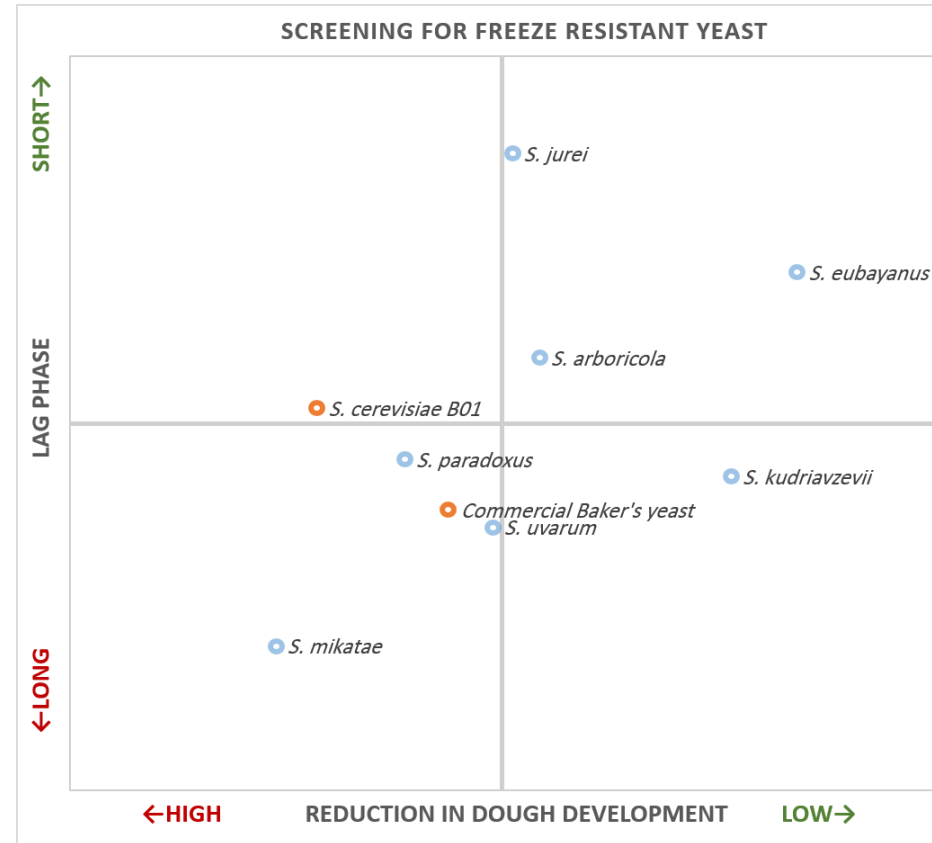
Lag phase

Maximum gas release rate

Time to maximum gas release rate

# Effect of freezing on dough development

- Baker's yeast strains strongly affected by freezing
- *S. arboricola*, *S. eubayanus* and *S. jurei* maintain good proofing capacity with shorter lag phase

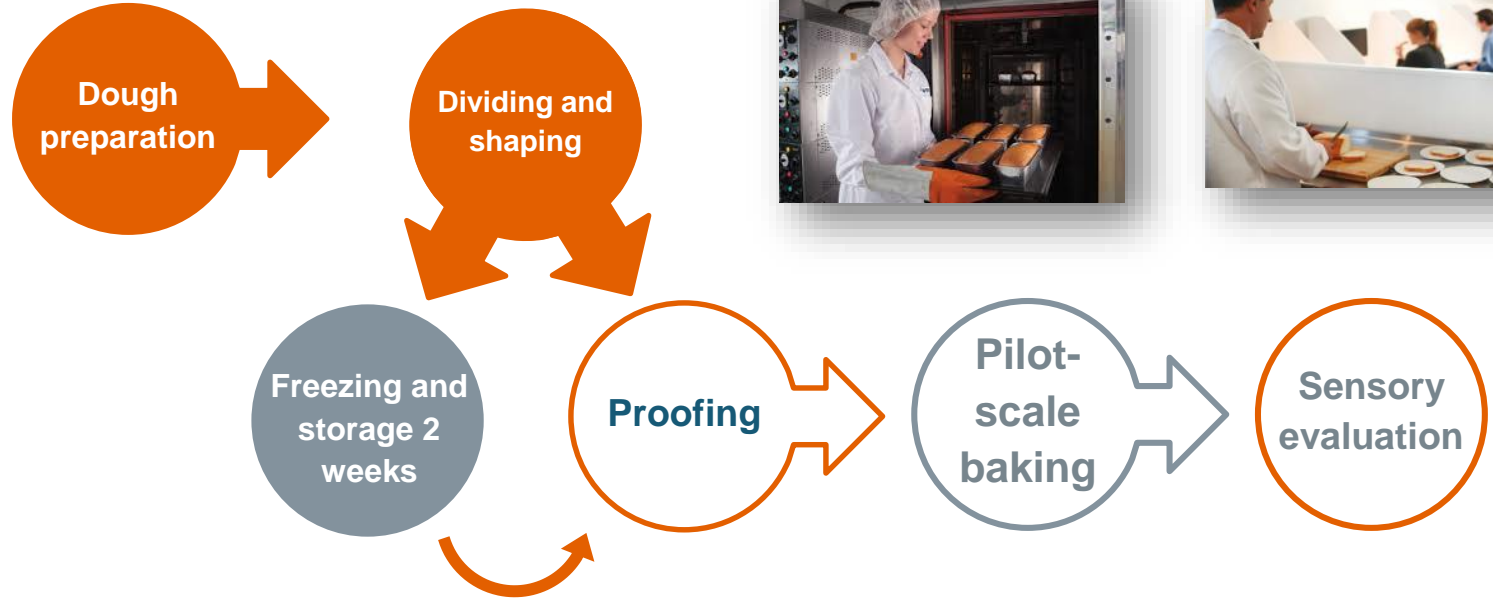


# Best performing strains selected for baking trials

*S. cerevisiae* (Baking strain)

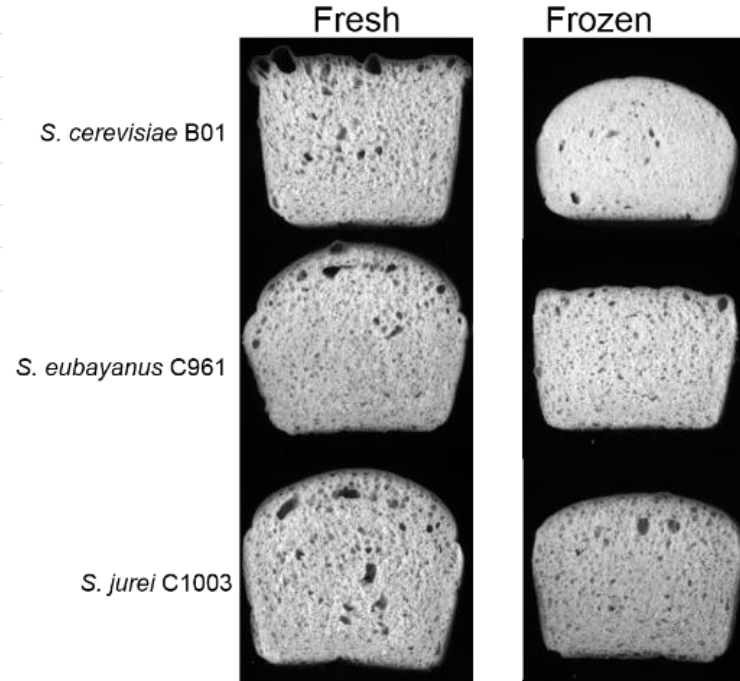
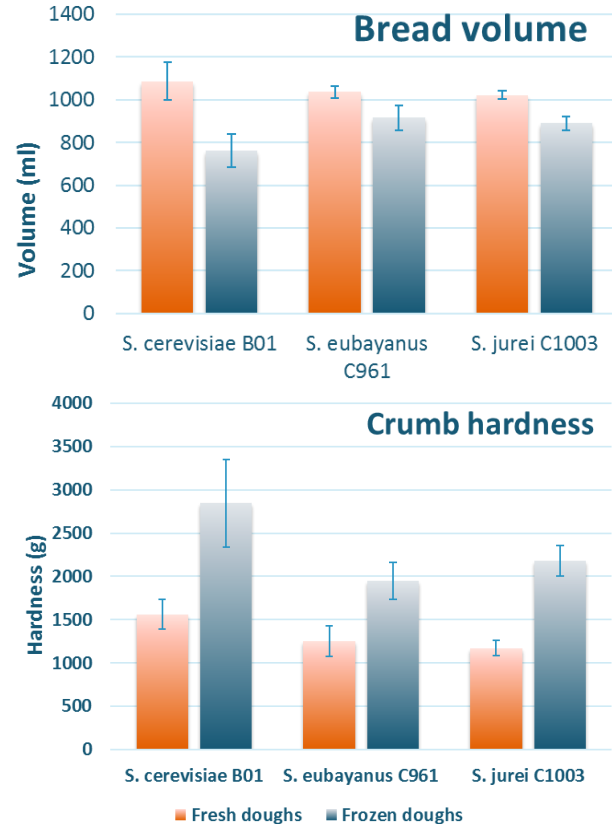
*S. jurei*

*S. eubayanus*

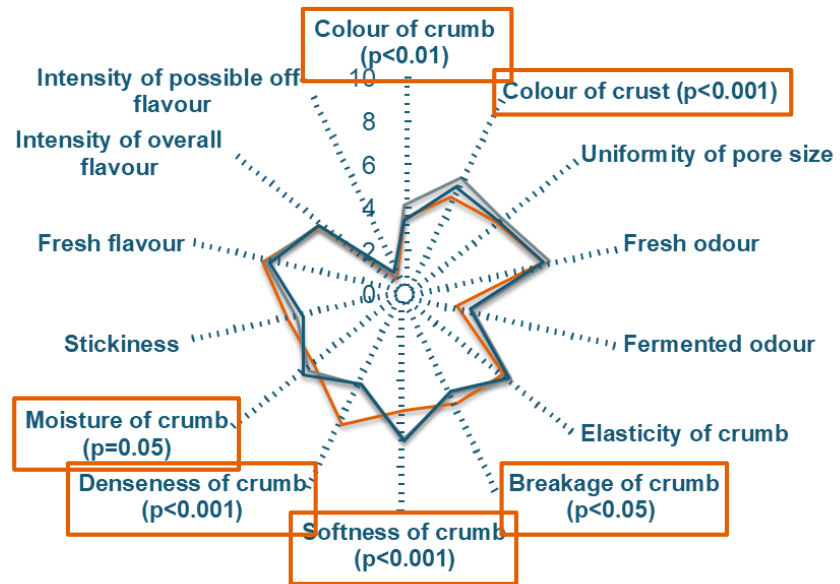
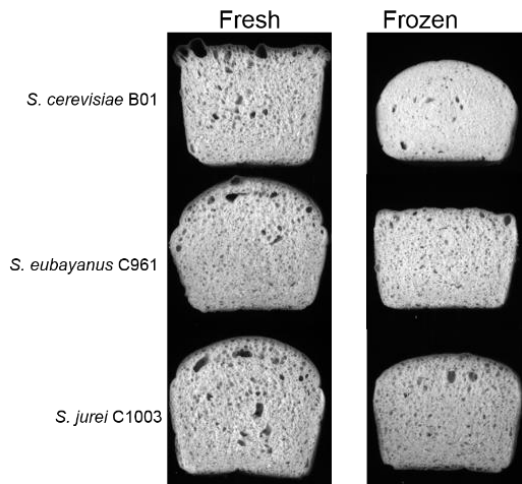


# Baking trials

- All strains capable of fermenting fresh dough
- *S. cerevisiae* was strongly affected by the freezing
  - smaller
  - denser



# Baking trials

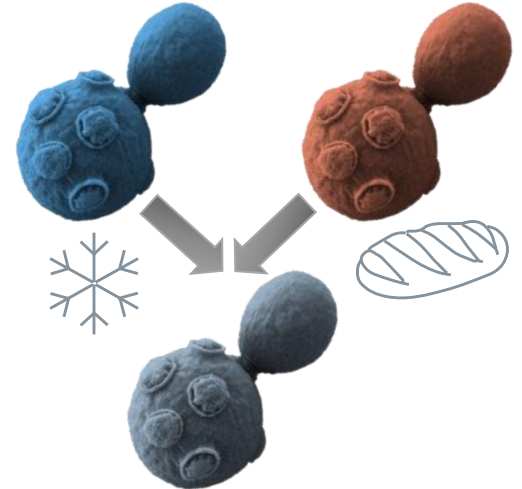


— *S. cerevisiae* B01 — *S. jurei* C1003 — *S. eubayanus* C961

- Differences in sensorial attributes are directly linked to good/bad proofing characteristics
- No off-flavour detected

# Non-conventional baking yeasts

- Cold tolerance of *S. eubayanus* and *S. jurei* confers good frozen-dough baking potential
  - No off-flavours are detected in these breads (equal to baking strains)
  - Considerable potential for industrial exploitation
- 
- Can yeast strains with good proofing capacity in fresh and frozen dough be generated by interspecific hybridization?



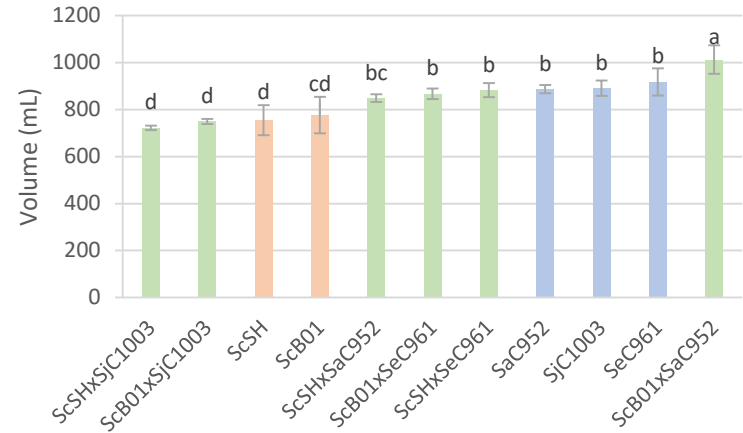
# Baking with hybrid yeast

Hybrids were created between two baker's yeast and the best performing non-cerevisiae strains:

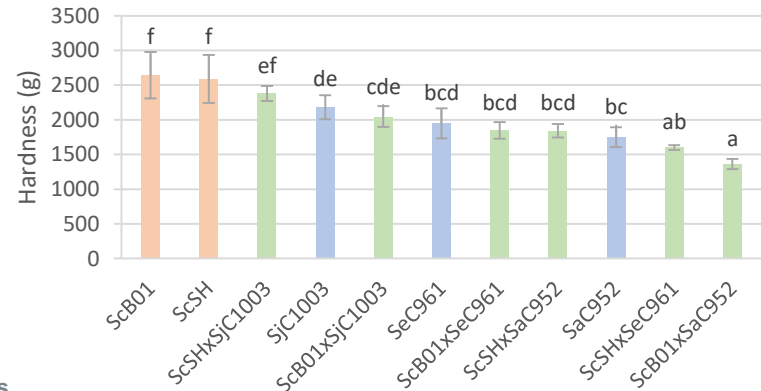
*S. cerevisiae* B01      *S. eubayanus*  
*S. cerevisiae* SH    x   *S. jurei*  
   *S. arboricola*

- Hybrids including *S. arboricola* and *S. eubayanus* had the best performance - larger and softer breads
- S. jurei* hybrids performed weakly, similar to the baker's yeast

Volume of breads - frozen dough



Hardness of breads - frozen dough





# Improvement of wild strains

- Potential of wild species overlooked with respect to industrial application
- Isolation and characterization is a first step
- Various non-GM strategies for further improvement



Screening and selection



Hybridization



Experimental domestication



# Acknowledgements



PBL Brewing  
Laboratory



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-  Eero Mattila
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-  Maximilian Michel (TUM)

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