



# Newly-created hybrid lager yeast strains (*S. cerevisiae* x *S. eubayanus*) outperform both parents during brewery fermentation

25.05.2015 – 35<sup>th</sup> Congress EBC Porto

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Brian Gibson

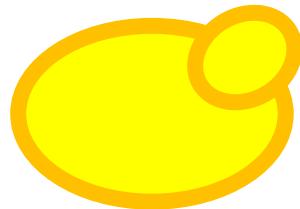
# Background

- Pale lager is the most popular beer style worldwide
- Clean flavour profile
  - Lack of yeast-derived aroma compounds

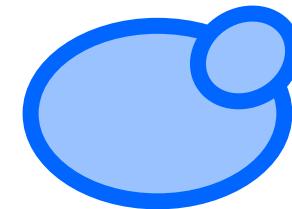


# Background

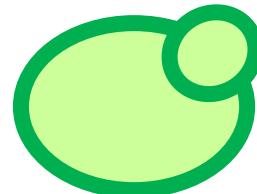
- *S. pastorianus* is a natural hybrid between *S. cerevisiae* and *S. eubayanus*:



*Saccharomyces cerevisiae*  
(cold-sensitive, good fermentation)



*Saccharomyces eubayanus*  
(cold-tolerant, poor fermentation)



*Saccharomyces pastorianus*  
(Good fermentation performance at low temperature, lager brewing)

# Background

- Poor diversity among the traditional lager yeasts
- Belong to one of two distinct lineages:
  - Saaz
  - Frohberg



Group 1  
(Saaz)

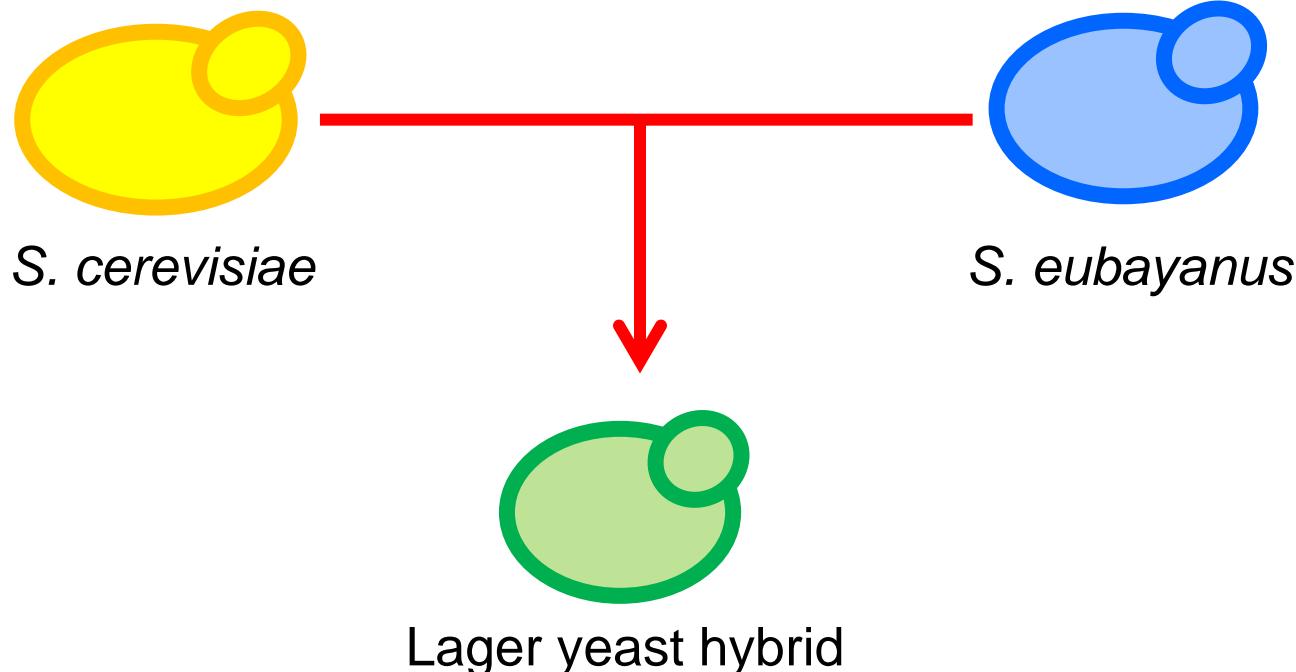


Group 2  
(Frohberg)

- Weak fermentation
- Very cold-tolerant
- *S. eubayanus* genome dominates
- Strong fermentation
- Cold-tolerant
- *S. cerevisiae* genome dominates

# Background

- Making new lager yeast hybrids by mating *S. eubayanus* with selected *S. cerevisiae* parents?



# Aim

- Generate artificial lager yeast hybrids by mating *S. eubayanus* with selected *S. cerevisiae* parents
- Assess fermentation performance of hybrids
  - Low temperature fermentation
  - Sugar utilization
  - Production of aroma compounds
  - Flocculation

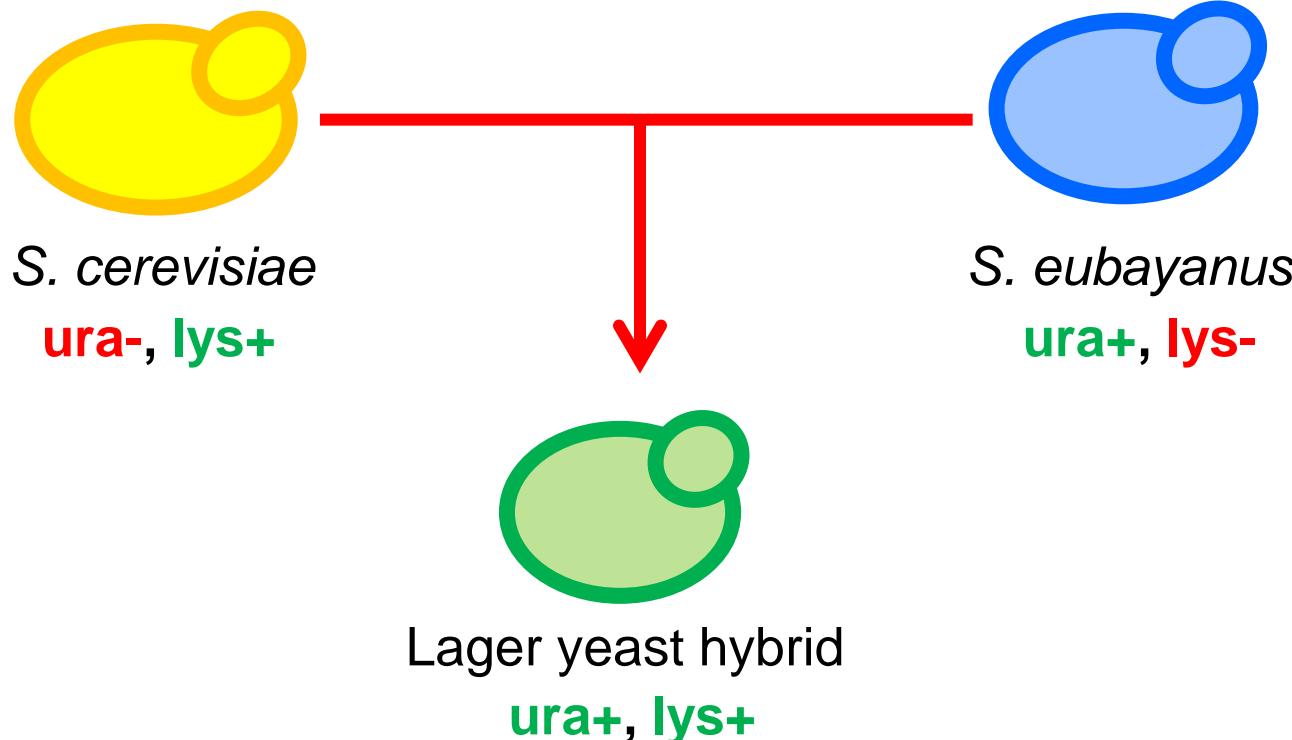
# Parent strains

- *Saccharomyces cerevisiae* VTT-A81062
  - Origin: Brewery in the United Kingdom
  - Strongly flocculent
  - Maltotriose utilization

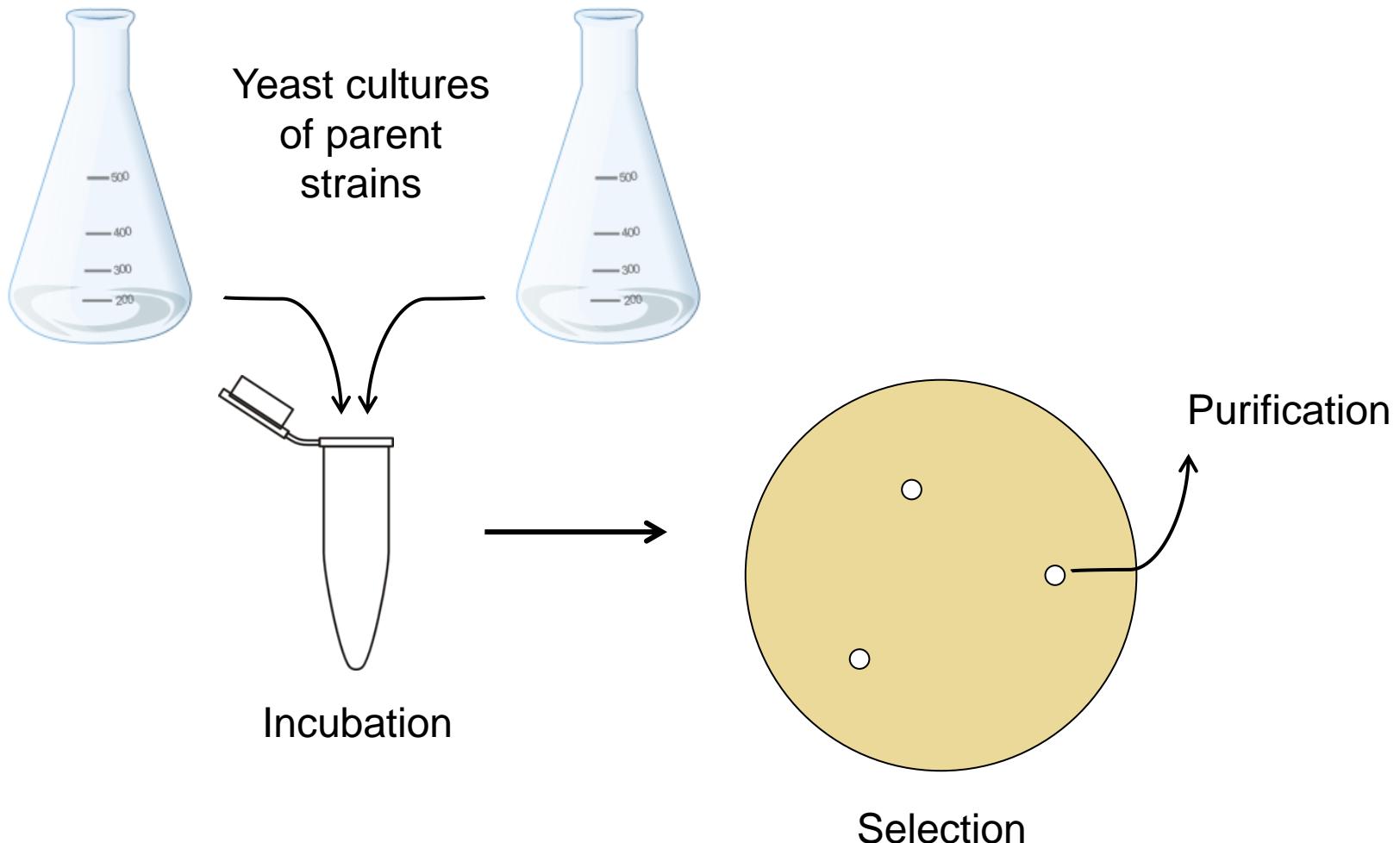
- *Saccharomyces eubayanus* VTT-C12902 (CBS12357)
  - Type strain
  - No maltotriose utilization
  - Good cold tolerance

# Selection of hybrids

- **Auxotrophic markers** (*ura-*, *lys-*, *leu-*, *ade-*, etc.)
  - Inability to grow with  $\text{NH}_4^+$  as sole nitrogen source

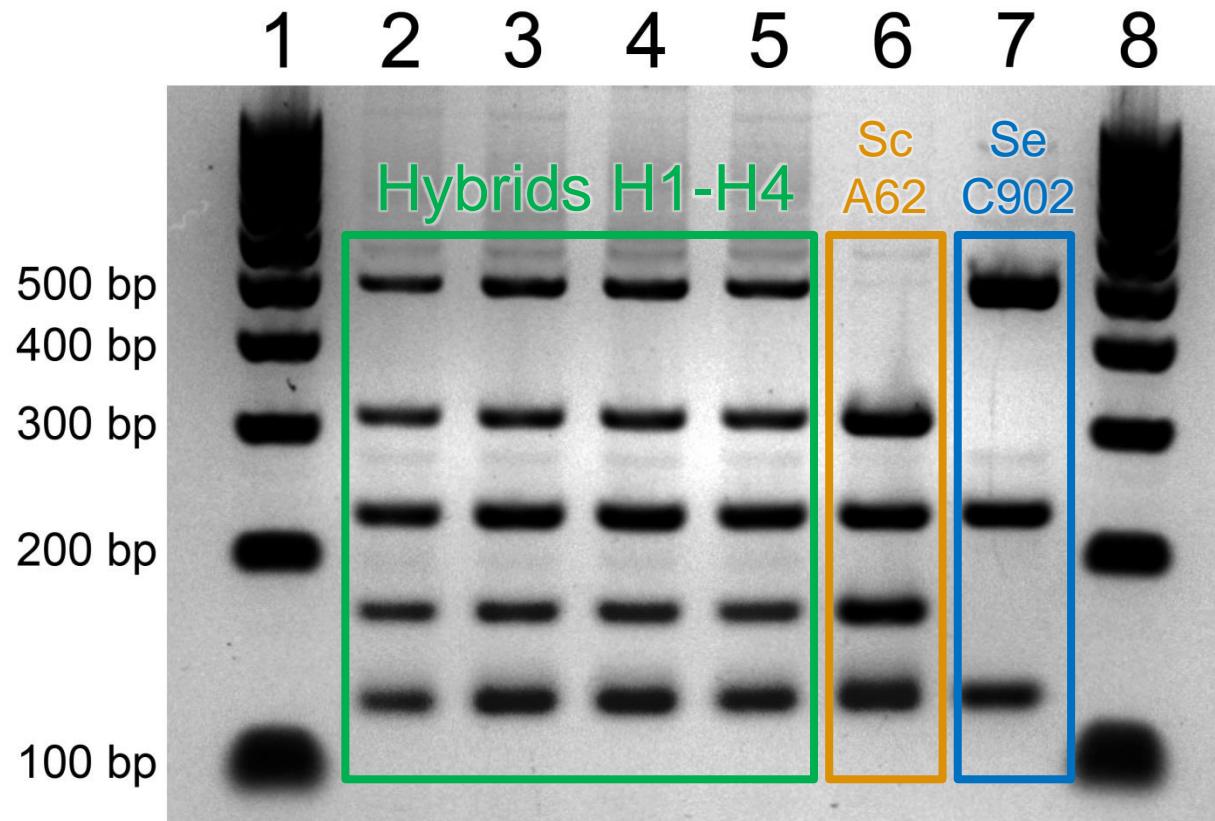


# Hybrid generation – mass mating



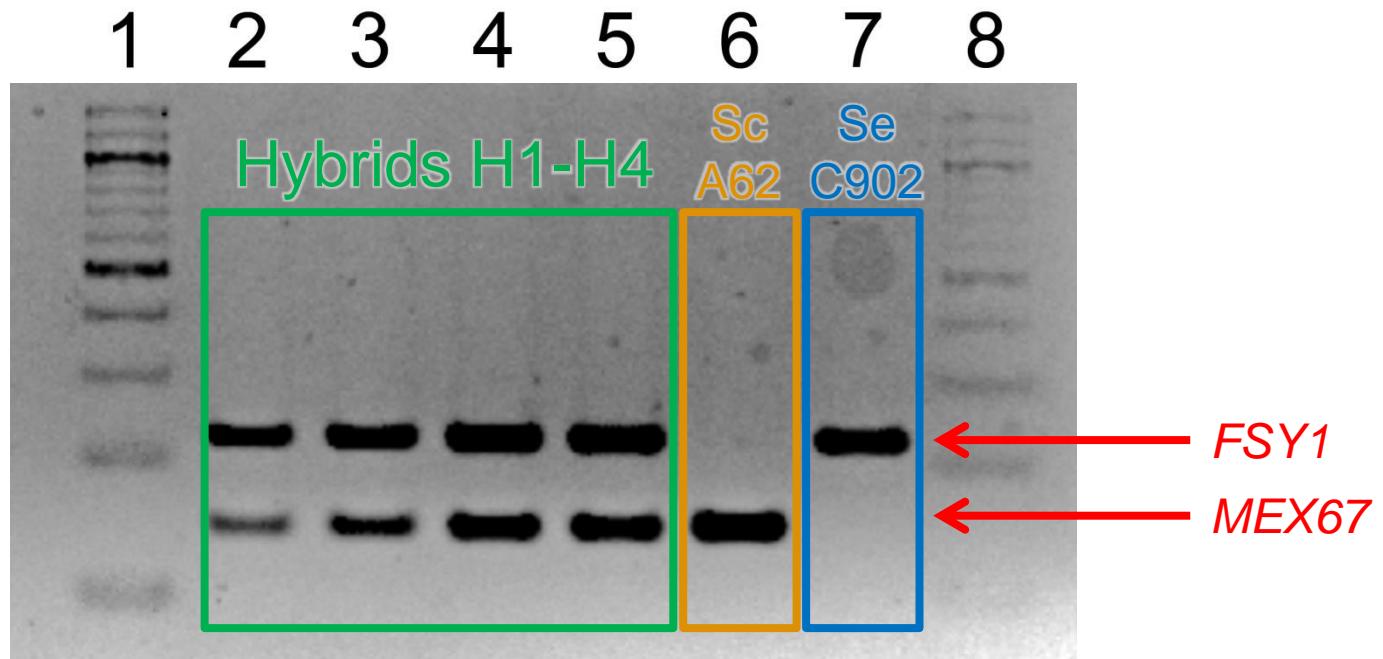
# Confirmation of hybrid status

- ITS-PCR and RFLP:



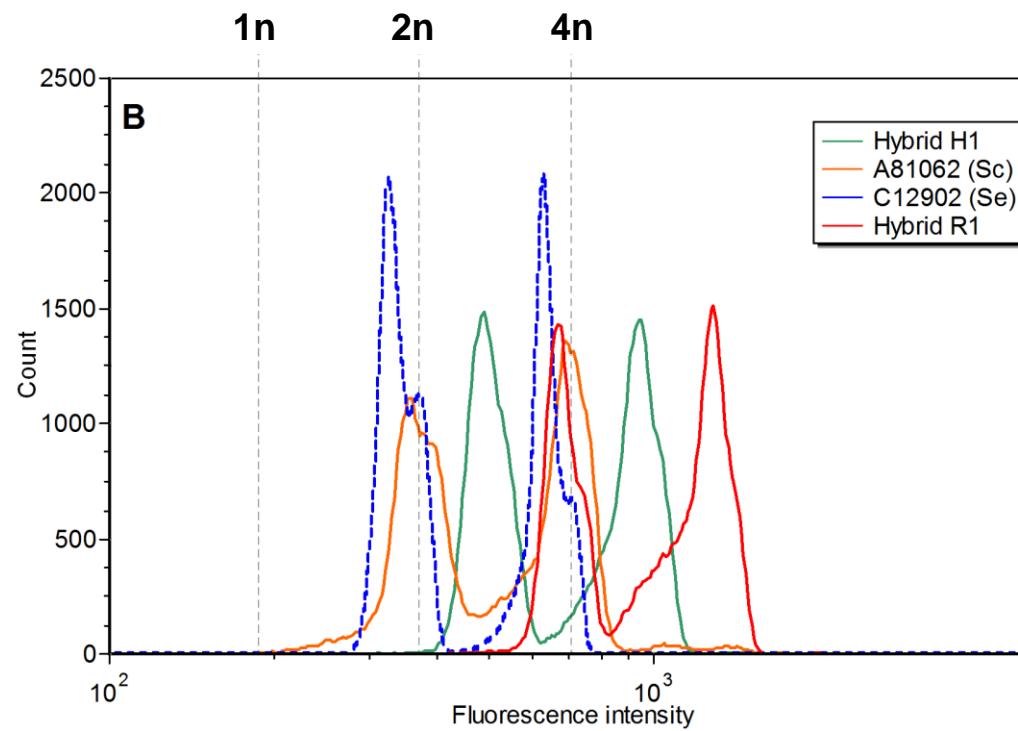
# Confirmation of hybrid status

- Amplification with *FSY1*- and *MEX67*-specific primers:



# DNA content of hybrids

- Estimated with SYTOX Green-staining and flow cytometry.

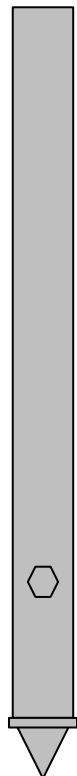


# Growth at various temperatures

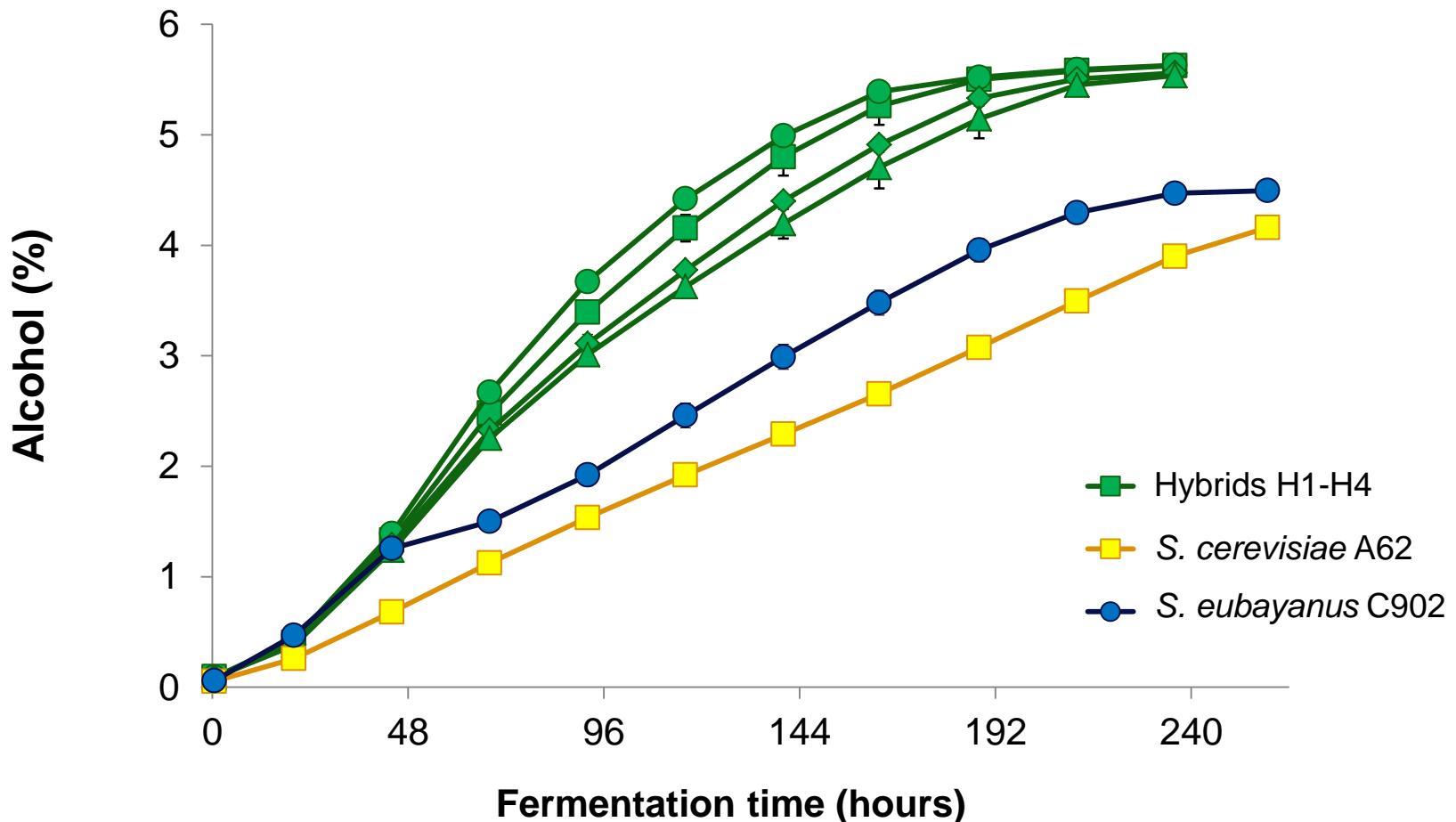
Strain	8 °C	25 °C	37 °C
<i>S. cerevisiae</i> A62	Red	Green	Green
<i>S. eubayanus</i> C902	Green	Green	Red
Hybrid H1	Yellow-green	Green	Yellow-green
Hybrid H2	Yellow-green	Green	Yellow-green
Hybrid H3	Yellow-green	Green	Yellow-green
Hybrid H4	Yellow-green	Green	Yellow-green

# Fermentation

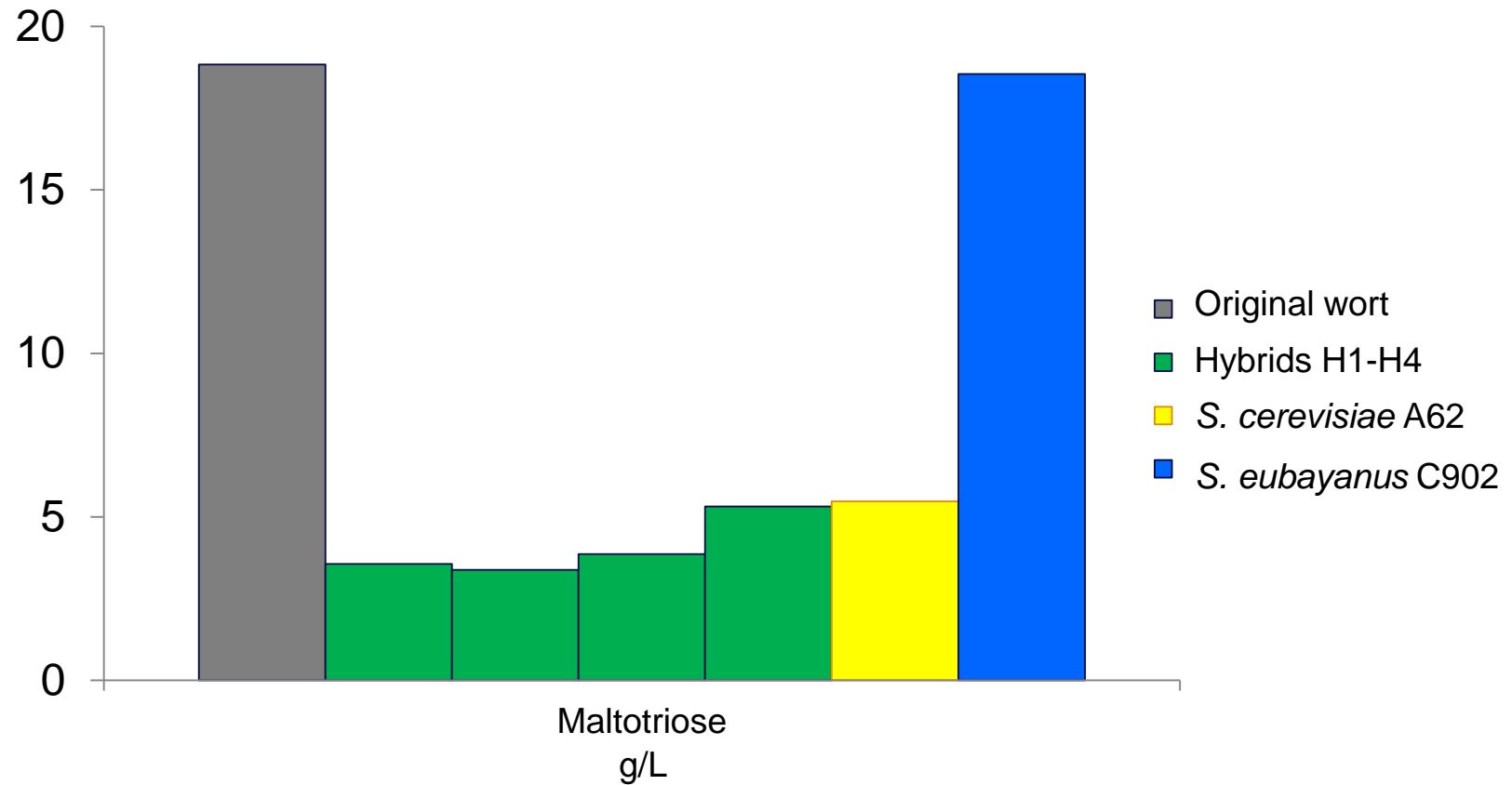
- The fermentation performance of 4 hybrids was compared in 2L all-malt wort fermentations at 12 °P and 12 °C.



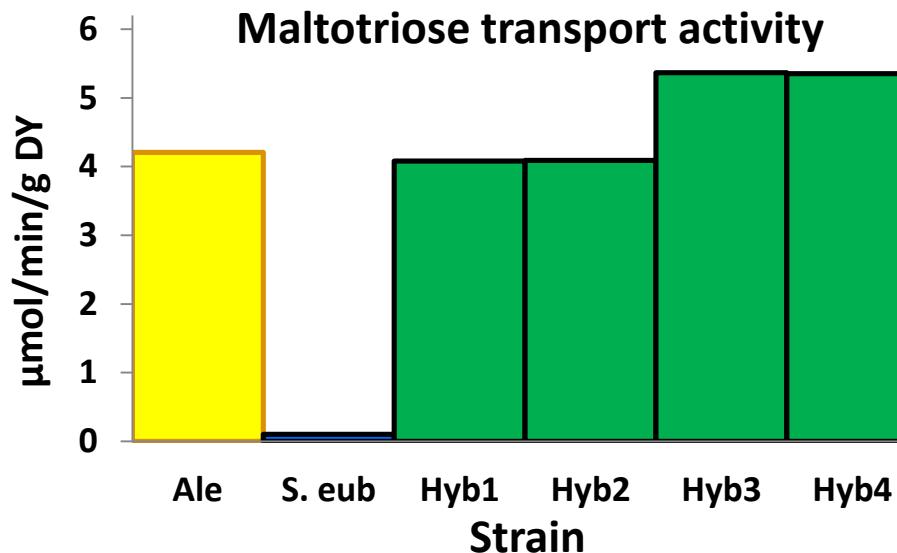
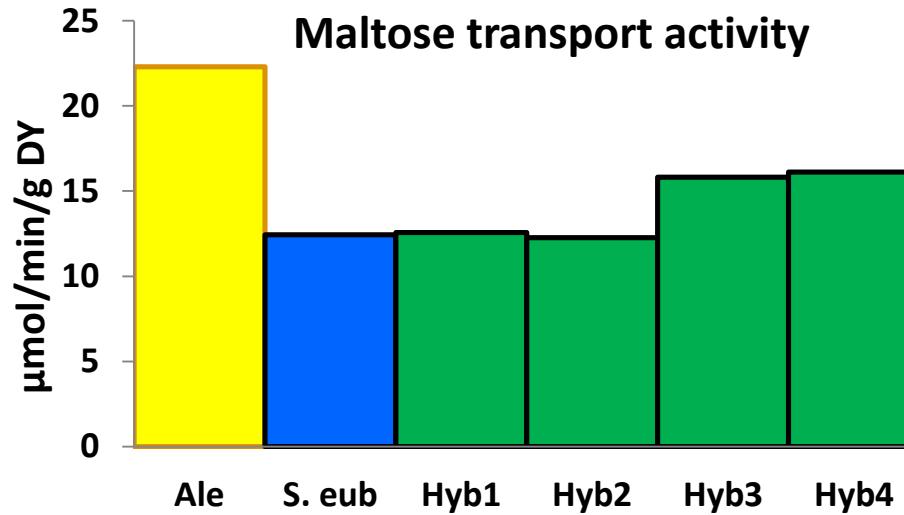
# Fermentation performance (12 °C, 12 °P)



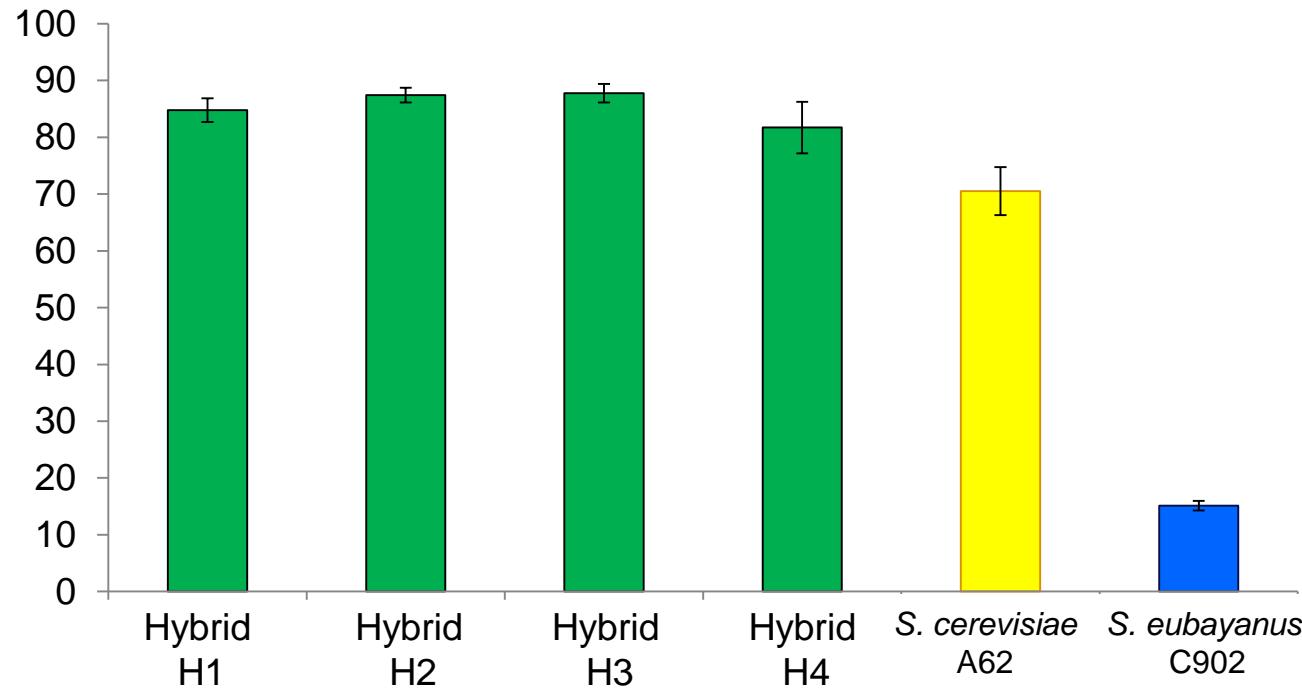
# Maltotriose utilization



# Maltotriose transport

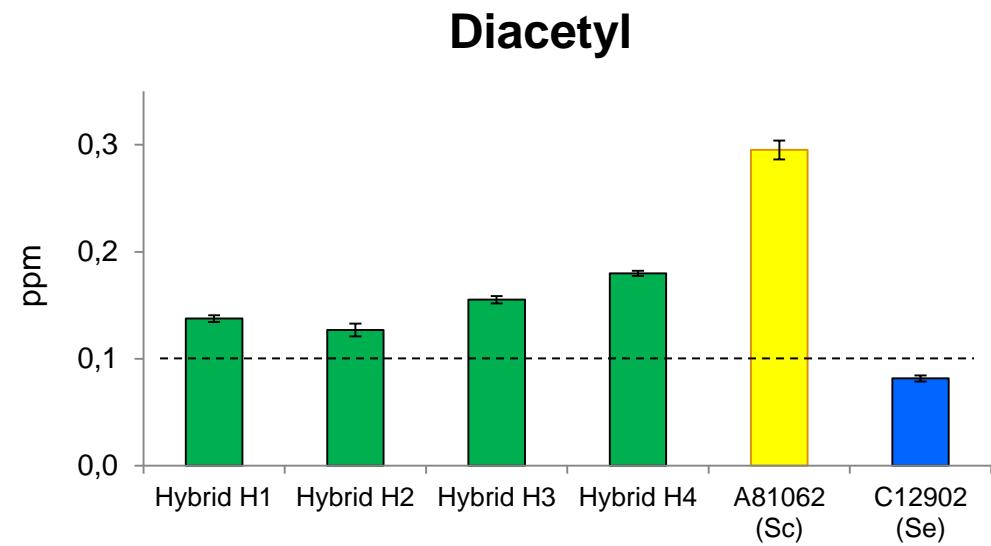


# Flocculation potential



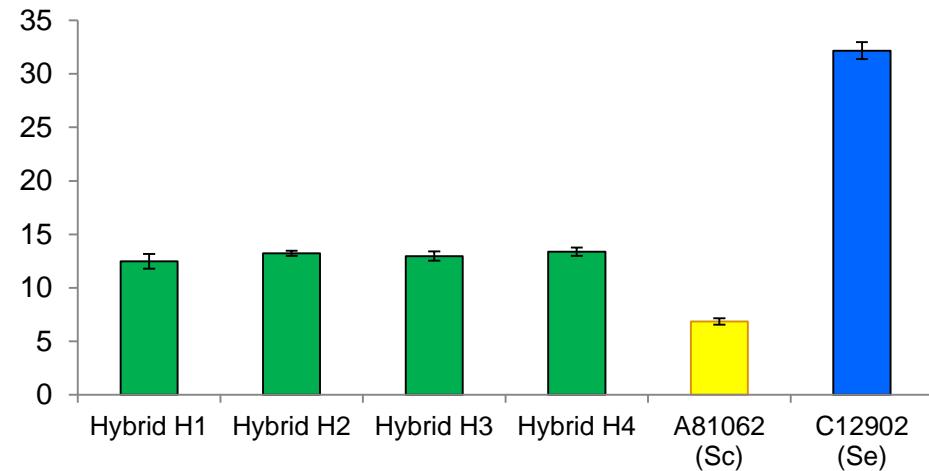
# Vicinal diketones

- Diacetyl is unwanted in lager beers
- Concentrations of diacetyl in beers fermented with hybrid strains in between those of the parent strains

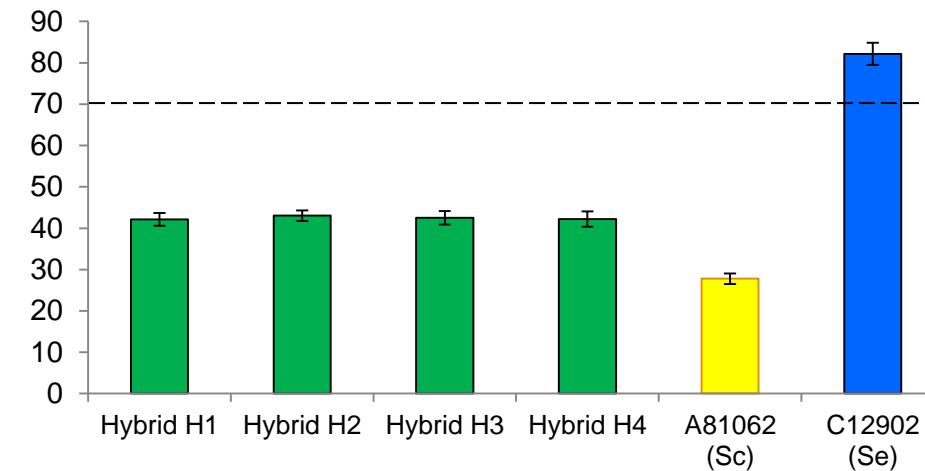


# Aroma compounds - higher alcohols

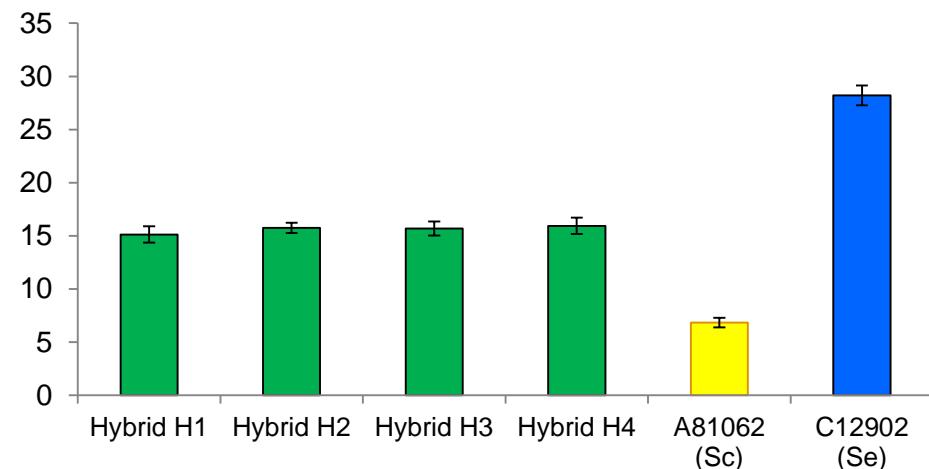
**2-Methylpropanol**



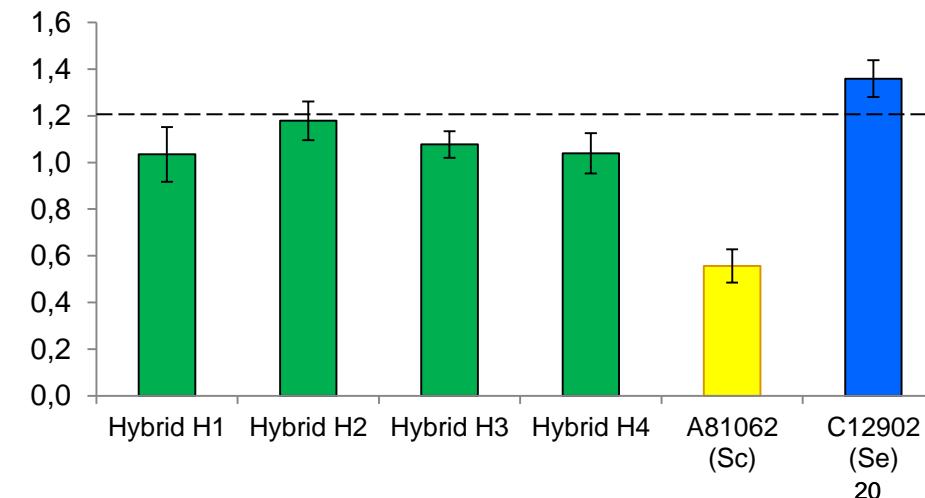
**3-Methylbutanol**



**2-Methylbutanol**

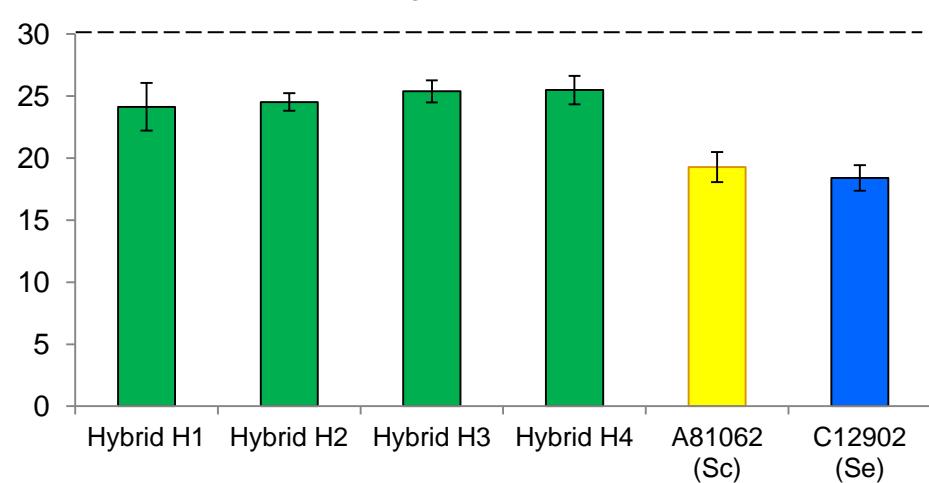


**3-Methylbutyl acetate**

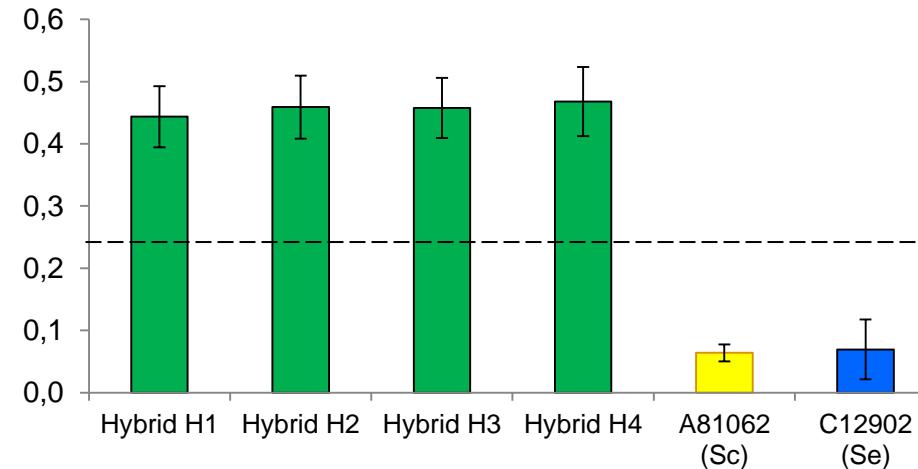


# Aroma compounds – ethyl esters

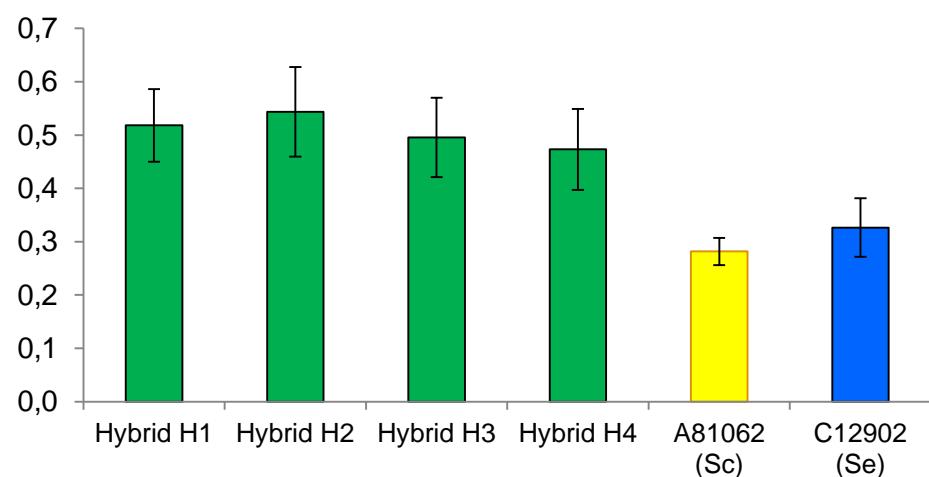
**Ethyl acetate**



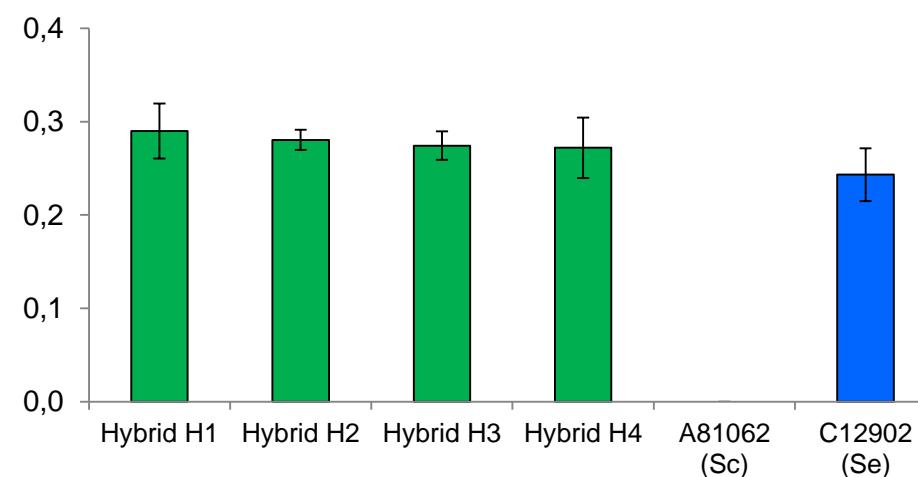
**Ethyl hexanoate**



**Ethyl octanoate**



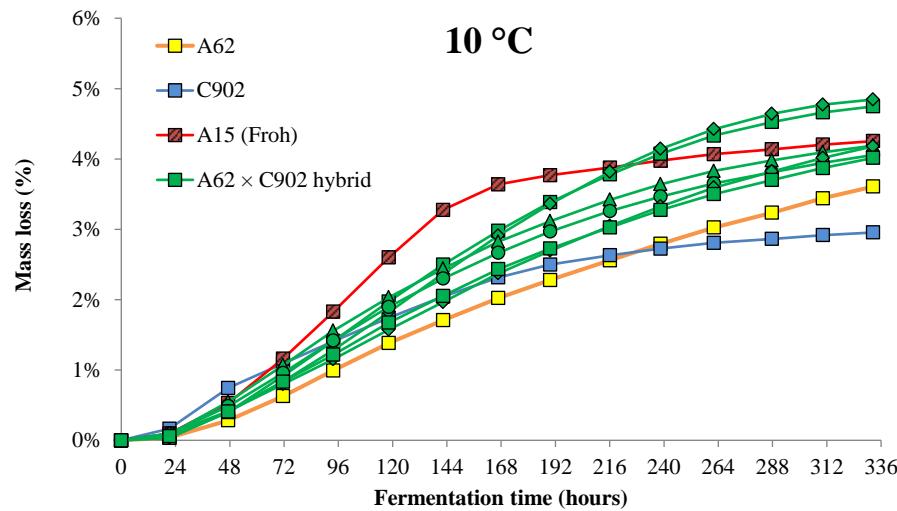
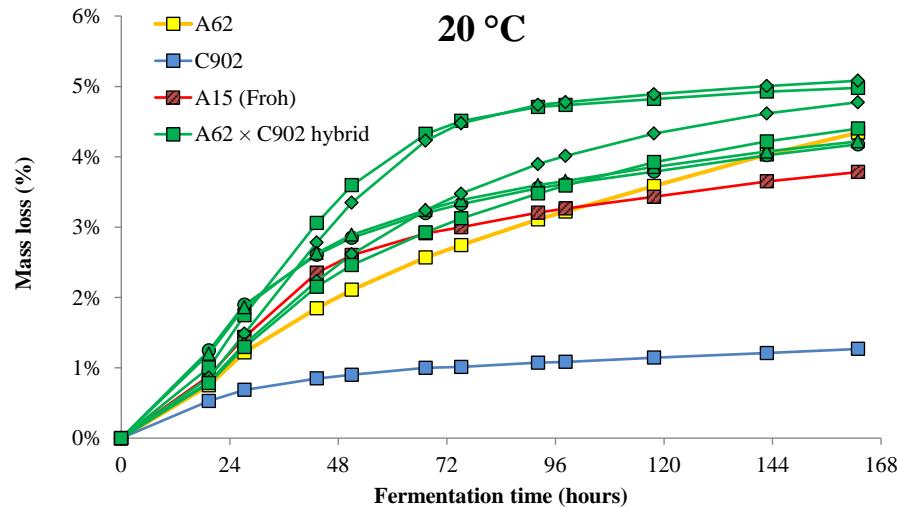
**Ethyl decanoate**



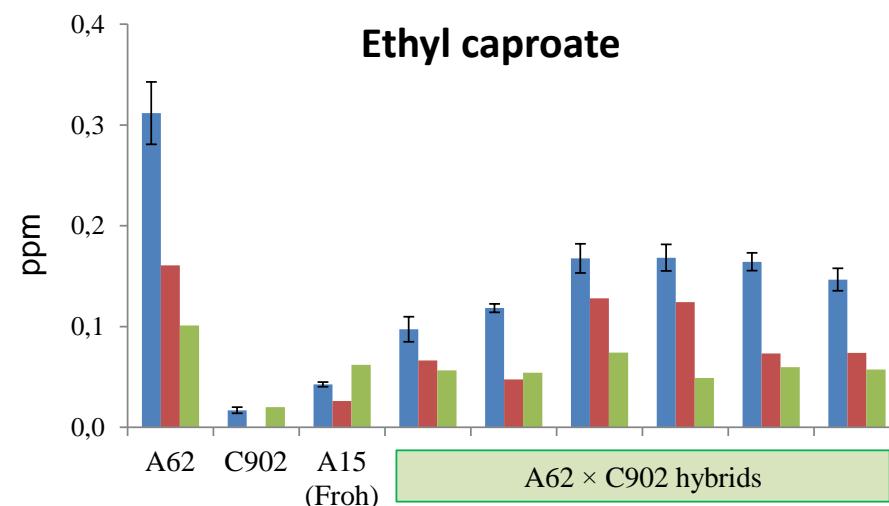
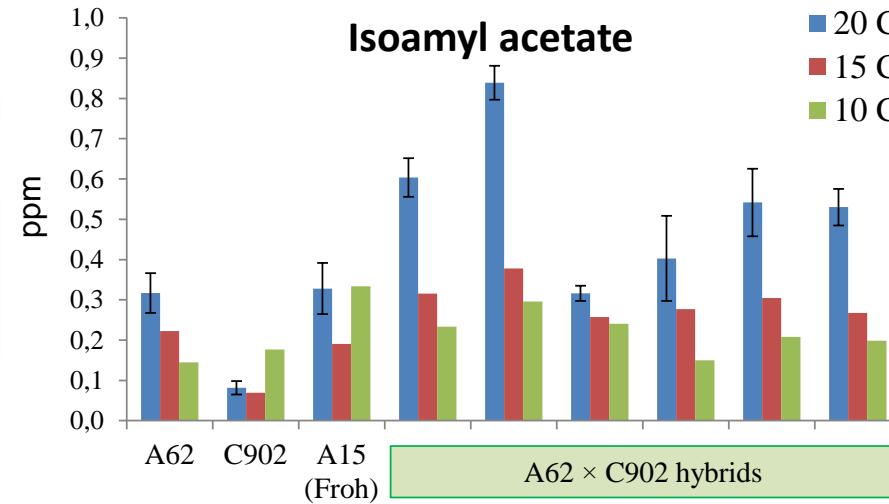
# Aroma compounds

- The beers fermented with the hybrid strains:
  - All had similar aroma profiles
  - Concentrations of higher alcohols and their esters in between those of the parent strains
  - Concentrations of ethyl esters higher than those of the parents strains

# Fermentation at various temperatures



# Aroma compounds at various temperatures



# Conclusions

- Successful generation of *de novo* lager yeast hybrids by interspecific hybridization
  - Non-GM
- Hybrid strains inherited beneficial properties from both parents, and showed apparent hybrid vigour:
  - Cryotolerance
  - Flocculation ability
  - Maltotriose utilization
  - Faster fermentation
  - Higher aroma production

# Acknowledgements

- Alfred Kordelin Foundation
- PBL Brewing Laboratory
- Academy of Finland
- FP7 Marie-Curie ITN YEASTCELL
  
- Sirpa Jylhä
- Annika Wilhelmson
  
- Aila Siltala
- Arvi Wilpolä
- Eero Mattila

