TECHNOLOGY FOR BUSINESS

Non-conventional yeast as a new tool for beer flavour modification

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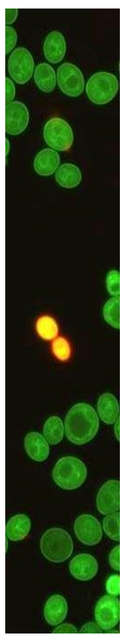
Background

Wild yeast invariably have a negative impact on brewery processes and beer quality[1]. However, the non-*Saccharomyces* yeast are a diverse group and harbour a variety of phenotypes that could be usefully employed in an industrial brewery. Such properties could be co-opted to help in the production of speciality beers, e.g. low alcohol or low calorie beer. In this study the potential of non-conventional yeast for flavour modification of beer was assessed. Flavour profiles of 13 diverse yeast species were determined after small-scale fermentations. Yeast with interesting flavour profiles and lacking Pad1 activity were selected for further trials, including 40L-scale co-culture wort fermentations and sensory analysis of the resultant beers. It is contended that the use of wild yeast, particularly those with limited ability to grow in brewer's wort, is a viable way to control beer flavour profile.

Methodology

Screening. For small-scale wort fermentations, 13 disparate non-*Saccharomyces* yeast[2] and three reference *Saccharomyces* species (Table 1) were pitched into 65ml of 15°P wort at 3g fresh yeast l^{-1}. Fermentations were conducted in 100ml shake flasks under anaerobic conditions at 20°C with shaking (100rpm) for 90h. Beers produced were tested for the presence of 4-vinylguaiacol (by HPLC) and a range of higher alcohols, acetate esters and ethyl esters (by GC-FID).

Table 1. Non-*Saccharomyces* yeast and reference yeast characterized in the investigation



Strain	VTT code*	Origin
Non-*Saccharomyces* yeast		
Kazachstania servazzi	C-00386^T	Soil, Finland
Naumovia dairenensis	C-95235	Salad, Finland
Zygotorulaspora florentinus	C-94199^T	Grape must, Italy
Zygotorulaspora mrakki	C-94202^T	Silage, Italy
Torulaspora delbrueckii	C-05716^T	Unknown
Lachancea fermentati	C-09854^T	Kvass, NE Asia
Kluyveromyces lactis	C-01448	Cheese, Finland
Kluyveromyces marxianus	C-75007^T	Cheese, Finland
Debaryomyces hansenii	C-00382^T	Unknown
Eromothecium coryli	C-05758	Unknown
Scheffersomyces stipitus	C-07806^T	Insect larva, France
Candida membranifaciens	C-96251	Fibre pulp, Finland
Hanseniaspora uvarum	C-99330	Malting, Finland
Reference strains		
Saccharomyces cerevisiae	A-60	Ale yeast, Finland
Saccharomyces pastorianus	W34/70	Lager yeast, Germany
Saccharomyces eubayanus	C-902	*Cytarria* gall, Patagonia

*http://culturecollection.vtt.fi/m/html

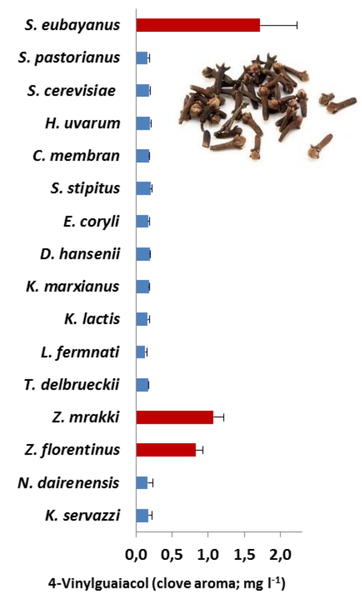


Figure 1. Phenolic off flavour in 'beers' produced by non-*Saccharomyces* wild yeast and reference yeast. Red bars indicate concentrations above the typical flavour threshold.

Pilot fermentations. Three different yeasts, *S. cerevisiae* (control), *K. servazzi* and *N. dairenensis*) were pitched into separate 30L-scale, 12°P fermentations at a rate of 1g fresh yeast l^{-1}. After 24 hours at a fermentation temperature of 20°C, a further 3g l^{-1} of *S. cerevisiae* was added to each fermentation. Fermentations continued till no further change in attenuation was observed. After removal of sedimented yeast, the beers were matured for 10 days, filtered, carbonated and bottled. Bottled beers were subjected to chemical and sensorial analysis.

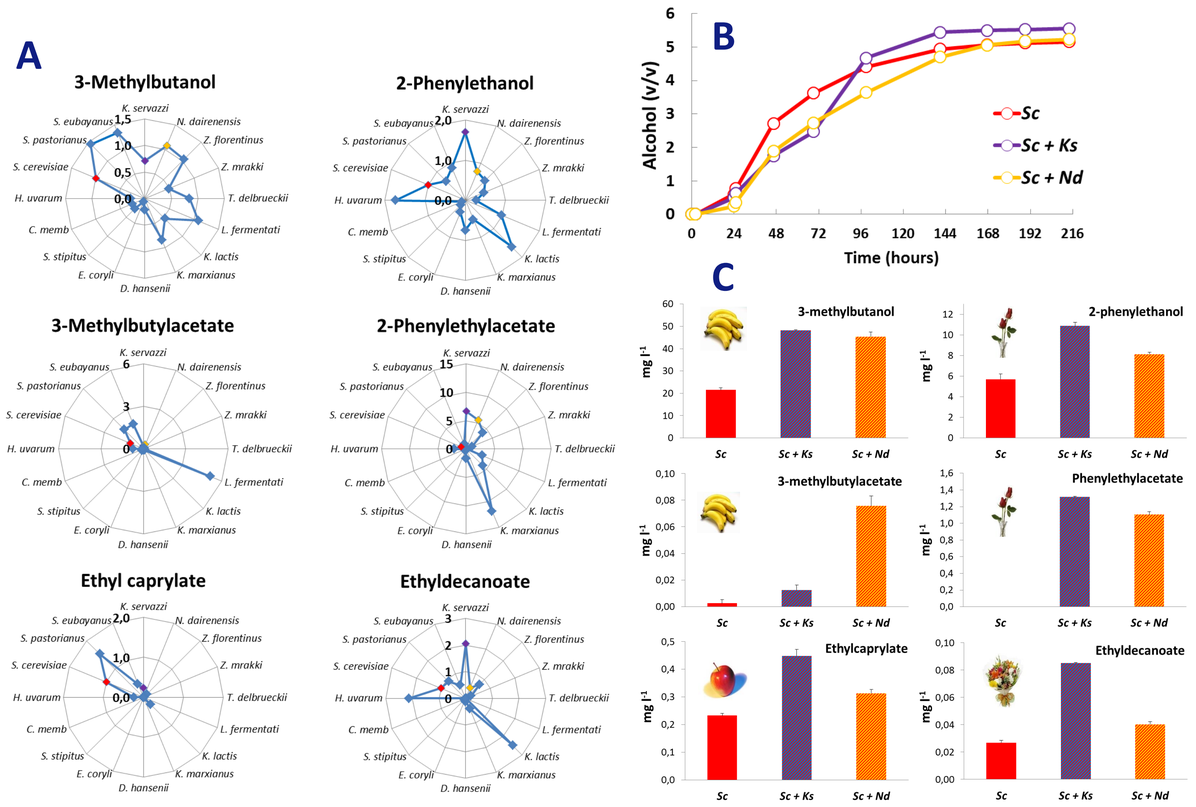


Figure 2. **A.** Flavour profile of worts fermented with wild yeast strains relative to a control strain *S. cerevisiae* ale strain A60 (♦). *K. servazzi* (♦) and *N. dairenensis* (♦) were chosen for further study based on their flavour profiles. **B.** Alcohol evolution during 15°P wort fermentations co-cultures of *S. cerevisiae* with *K. servazzi* or *N. dairenensis*. **C.** Concentrations of key flavours in finished beers adjusted to 5% ABV.

Results

- A screen of 13 'wild' yeast (Table 1) revealed a range of phenotypes from fermentation of 15°P wort (Fig. 1 & 2A).
- Two species, *K. servazzi* and *N. dairenensis* were chosen for further investigation based on their flavour profiles (Fig. 2A) and suitability for fermentation (Fig. 2B).
- Sequential co-culture fermentations at pilot scale showed that wild yeast could be effectively used to modify final flavour profiles without detriment to fermentation rate or beer quality.
- Fermentation with *K. servazzi* resulted in beers with higher levels of 3-methylbutanol, phenylethanol and phenylethyl acetate as well as ethyl decanoate and caprylate (Fig. 2C).
- *N. dairenensis* produced beers with relatively high levels of of 3-methylbutanol, 3-methylbutylacetate and phenylethyl acetate (Fig. 2C).
- A team of 11 trained tasters evaluated the beers and detected no off-flavours. The beers produced via wild yeast co-culture more deemed to have a more fruity character than the control.

Conclusions

- **Non-*Saccharomyces* yeast have potential for effective and controlled modification of beer flavour profile**
- **Utilization of non-adapted species like *K. servazzi* and *N. dairenensis* ensures that cell number in wort is controlled and the brewing yeast remains dominant**
- **Careful screening ensures that off-flavours are avoided and fermentation remains unimpeded**

References

1. Campbell, I. (1996) Wild yeasts in brewing and distilling. In:Brewing Microbiology, 2nd Ed. F.G. Priest & Campbell. Chapman and Hall. London.
2. Hagman, A., et al. (2013) Yeast "make-accumulate-consume" life strategy evolved as a multi-step process that predates the whole genome duplication. PLOS One. E68734

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