Improving acid tolerance in Oenococcus oeni by Adaptive Evolution

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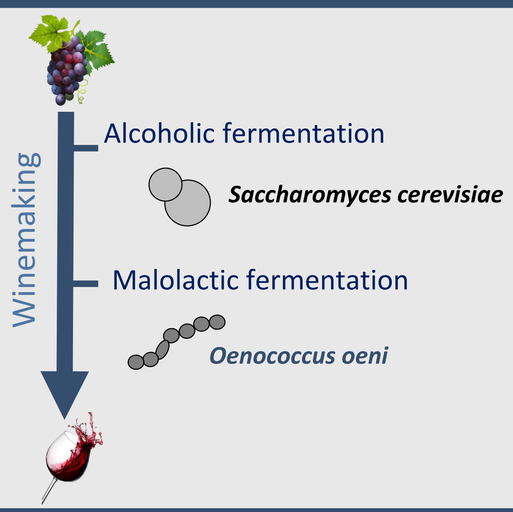
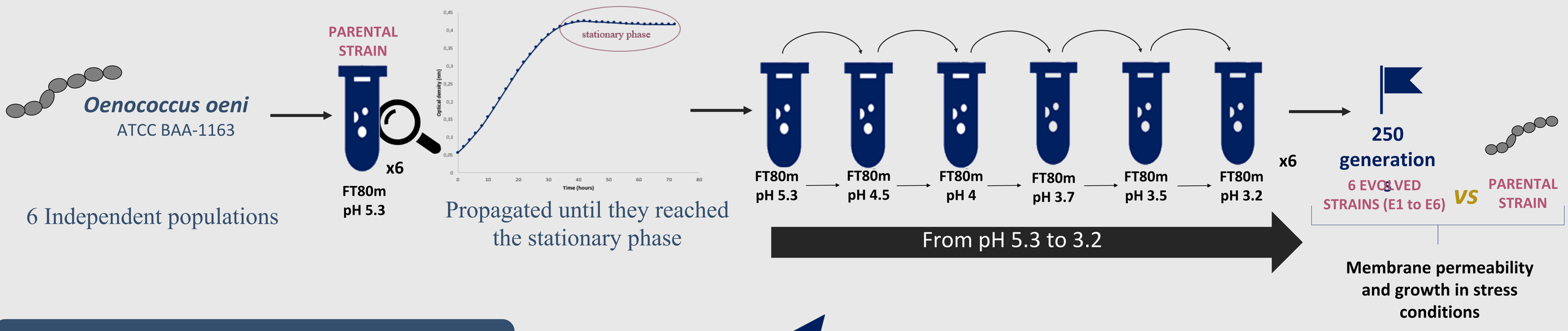
Background



Oenococcus oeni is a lactic acid bacterium (LAB) mainly responsible for the malolactic fermentation (MLF) in wine. MLF plays an important role in determining the final quality of wines[1]. Even though this LAB is naturally present in musts, wines and oenological environment, spontaneous MLF are usually unpredictable because of the stressful conditions and especially due to acidity[2]. The consequence of the mismanagement of this step might lead to the depreciation of wine quality.

To obtain a clone more tolerant to acidity, we undertaken to replicate *O. oeni* in a temporally varying environment (pH 5.3 to 3.2) to improve acid tolerance. To discriminate stress tolerance of evaluated populations *versus* parental strain an ethanol or acid stress were improved to both population.

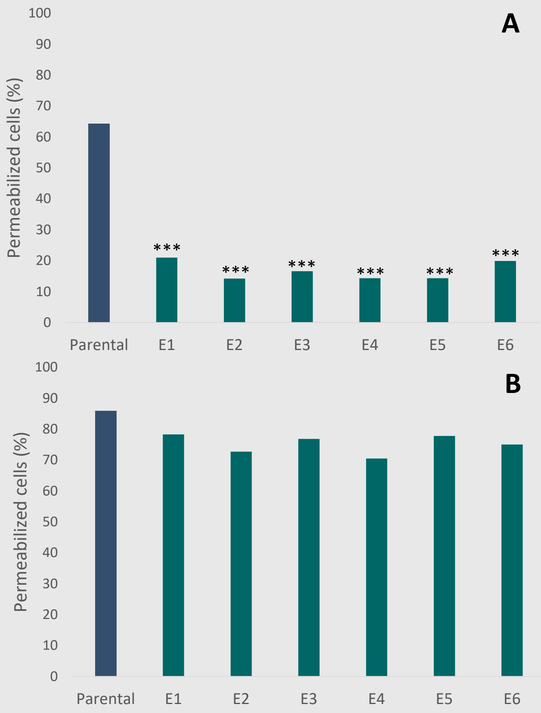
Strategy



First Results

250 generations

Modification of membrane fluidity of evoluated populations during stress



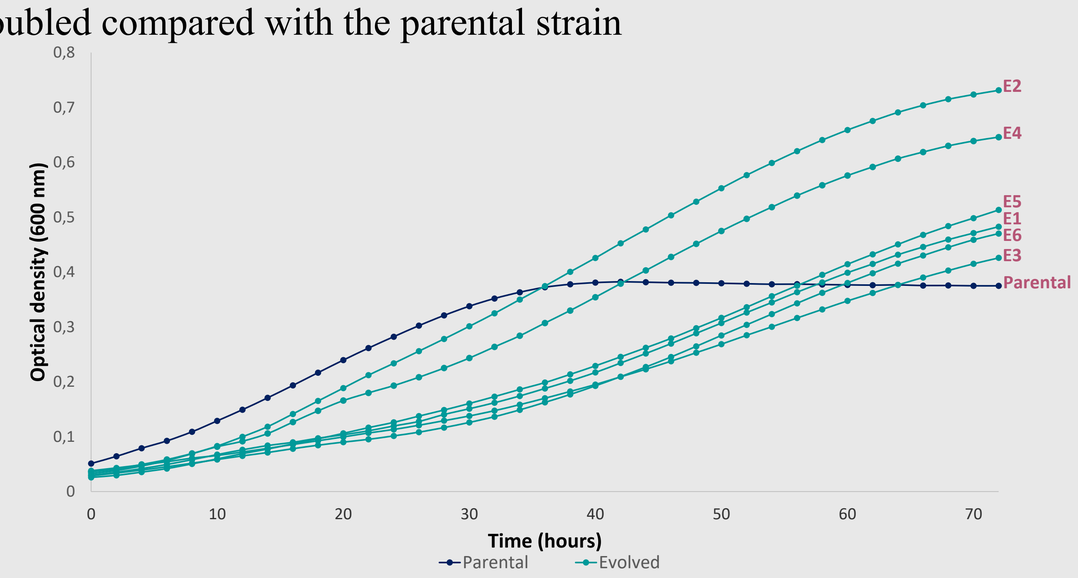
The membrane permeability was measured on the evolved and parental strains during ethanol or acid stress. Evolved strains maintained better membrane integrity during acid stress than the parental strain.

Nervertheless, no significant difference between parental and evolved strains during ethanol stress

Membrane permeability during stress conditions. Cells were cultivated at 28 °C in FT80m medium (pH 5.3 for parental strain and 3.2 for evolved strain) until mid-exponential growth phase (OD_{600nm}= 0.8 for parental strain and OD_{600nm}= 0.3 for evolved strain). Cells were transferred into (A) medium supplemented by 8% ethanol (v/v) or into (B) medium at pH 2.4 and 90 min after transfer membrane permeability was measured by flow cytometry using propidium iodide as probe.

Growth of evoluated populations in ethanol stress condition

The growth of evolved and parental strains was monitored in ethanol supplemented medium. Biomass of evolved strains increase in medium containing ethanol and especially for two clones where biomass has doubled compared with the parental strain



Growth follow up of evolved and parental strains in ethanol supplemented medium. Cells were cultivated at 28°C in FT80m medium (pH 5.3 for parental strain and 3.2 for evolved strain) until mid-exponential growth phase (OD_{600nm}=0.8 for parental strain and OD_{600nm}=0.3 for evolved strain) and inoculated in FT80m medium at pH 5.3 supplemented by 8% (v/v) ethanol.

Conclusions & Perspectives

- **Two clones appears discriminant** by increase biomass in ethanol supplemented medium compared with the parental strain.
- An adaptation based on improve acidity tolerance enabled **ethanol tolerance for two clones**.
- Further works will focus on **the origin of this phenotype** by an exploration of the genome and transcriptome.
- It would be necessary to test evolved clones in oenological condition to **perform MLF**.

References

[1] Bartowsky, E.J. (2005). *Oenococcus oeni* and malolactic fermentation – moving into the molecular arena. Aust. J. Grape Wine Res. *11*, 174–187.
[2] Bauer, R., and Dicks, L.M.T. (2004). Control of malolactic fermentation in wine. A review. Afr J Enol Vitic *25*, 74–88.