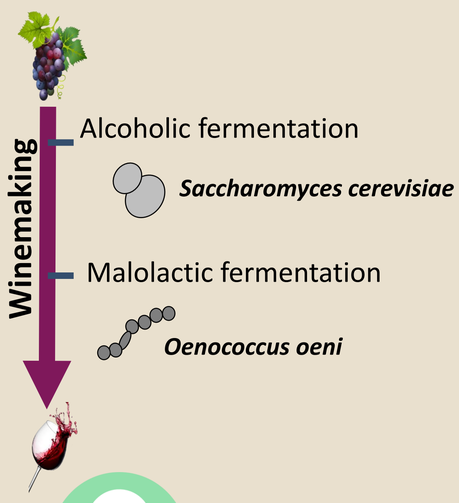
Adaptive evolution to improve acid tolerance in *Oenococcus oeni*

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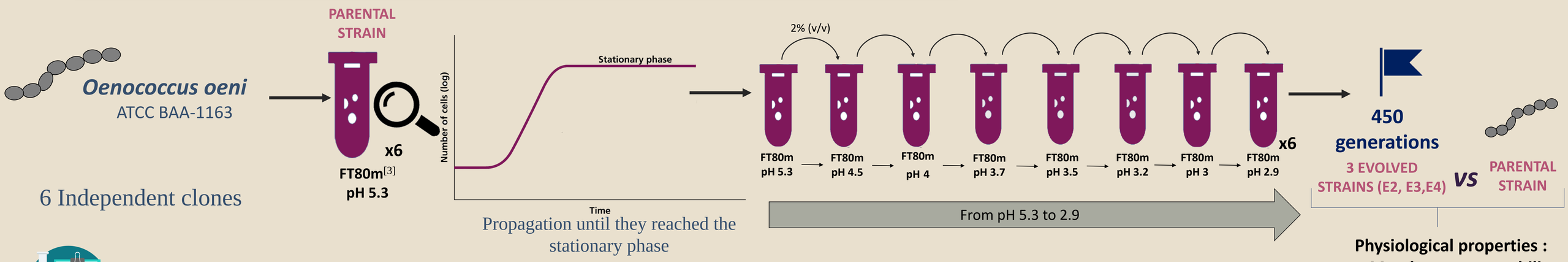
Background



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.

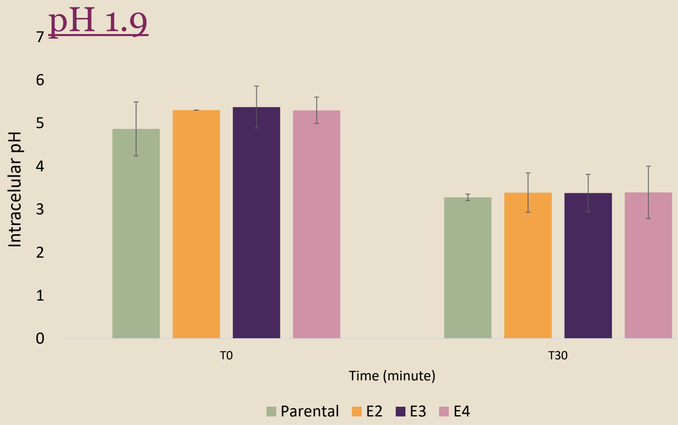
To obtain a clone more tolerant to acidity, we undertook a replication of *O. oeni* until 450 generations in a temporally varying environment (pH 5.3 to 2.9) to improve acid tolerance. To discriminate stress tolerance of evolved populations *versus* parental strain an acid stress was performed to both population.

Strategy involved



Parental *versus* evolved strains to extreme acidity challenge

1. Intracellular pH in growth conditions or during acid stress at pH 1.9

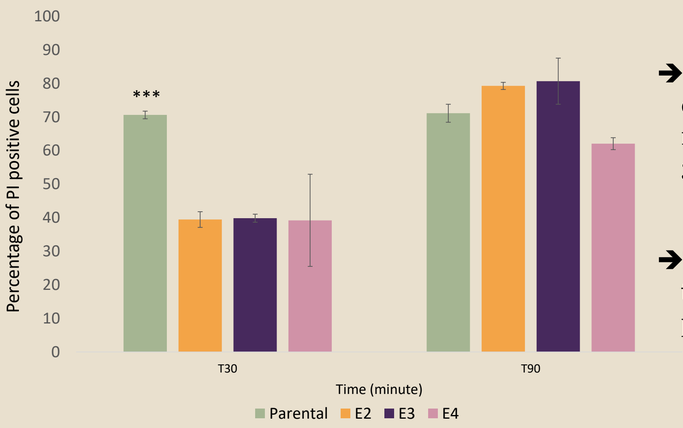


➔ Evolved strains maintain their intracellular pH even when they grow at pH 2.9

➔ Either parental nor evolved strain are able to regulate their intracellular pH during an acid stress at pH 1.9

Intracellular pH. Parental strain or evolved strains were gown respectively at pH 5.3 or 2.9 until mid-exponential growth phase. Intracellular pH was measured using CFDA-SE as probe. A first measurement has been made and then cultures were transferred into acidified FT80m (pH 1.9) and another measurement has been made after 90 min.

2. Plasma membrane permeability after acid stress at pH 1.9

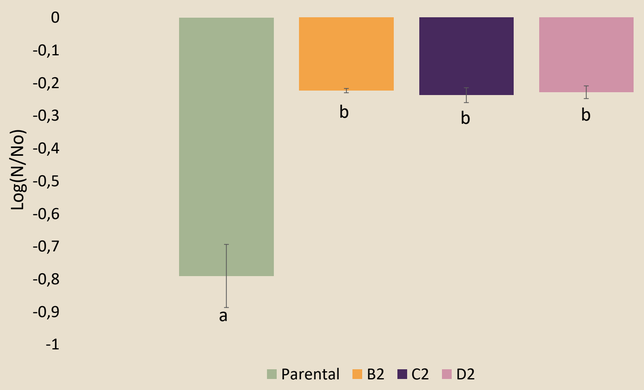


➔ Compared to parental strain, evolved strains hold their membrane integrity for at least 30min during an acid stress

➔This state seems to be temporary : all strains become permeable after 90 min

Percentages of permeable parental and evolved strains. Parental strain or evolved strains were gown respectively at pH 5.3 or 2.9 until mid-exponential growth phase. Cultures were transferred into acidified FT80m (pH 1.9). Plasma membrane permeability was performed using propidium iodide as a probe at 30min or 90min after acid stress at pH 1,9.

3. Lost of cultivability of evolved strains after acid stress at pH 1.9

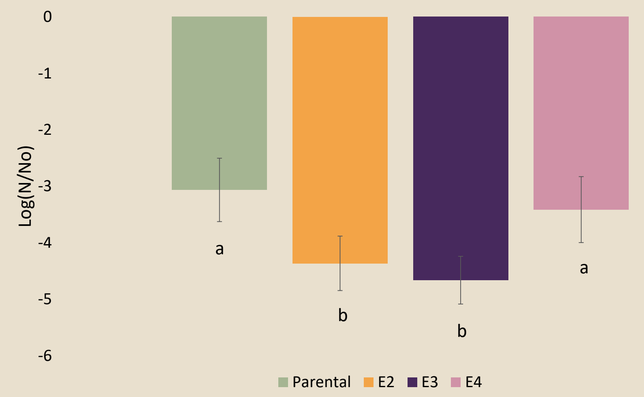


➔ Evolved strains resist better than parental strain at an acid stress at pH 1.9

➔ This resistance may be due to adaptation or acclimatization of cells

Cultivability following stress treatment. Parental strain or evolved strains were gown respectively at pH 5.3 or 2.9 until mid-exponential growth phase. Cultures were transferred into acidified FT80m (pH 1.9). Cultivability was estimated by plating on agar medium after 60min treatment.

3. Lost of cultivability of evolved strains after a recovery to initial pH 5.3



➔ Return to intial pH 5.3 disrupt avantage of evolved strains

➔ The resistance of evolved strains at pH of 1.9 does not seem to be due to adaptation but rather to a transitional acclimatization of cells

Cultivability following stress treatment. Parental strain and evolved strains were gown respectively at pH 5.3 until mid-exponential growth phase. Cultures were transferred into acidified FT80m (pH 1.9). Cultivability was estimated by plating on agar medium after 60 min treatment.

Conclusions and perspectives

- Evolved strains **maintain the same intracellular pH** in acidic conditions (pH 2.9) than the parental strain in optimal conditions (pH 5.3)
- Tolerance to acidity of evolved strains is a transitional state which could **optimize MLF performance** in oenological conditions
- Further works will focus on **genome sequencing and transcriptome (RNAseq)**.

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References

[1] Bartowsky, E.J. (2005). Oenococcus oeni and malolactic fermentation – moving into the molecular arena. Australian Journal of Grape and Wine Research *11*, 174–187.

[2] Bauer, R., and Dicks, L.M.T. (2004). Control of malolactic fermentation in wine. A review. S. Afr. J. Enol. Vitic *25*, 74–88.

[3] Cavin, J.F., Prevost, H., Lin, J., Schmitt, P., and Divies, C. (1989). Medium for Screening Leuconostoc oenos Strains Defective in Malolactic Fermentation. Appl Environ Microbiol *55*, 751–753.