



**HAL**  
open science

## Directed evolution of *oenococcus oeni* to improve acid-tolerance reveals fixed beneficial mutations in the citrate locus

Camille Eicher, Frédérique Julliat, Joana Coulon, Marion Favier, Hervé Alexandre, Stéphane Guyot, Critina Reguant, Cosette Grandvalet

### ► To cite this version:

Camille Eicher, Frédérique Julliat, Joana Coulon, Marion Favier, Hervé Alexandre, et al.. Directed evolution of *oenococcus oeni* to improve acid-tolerance reveals fixed beneficial mutations in the citrate locus. 27th International ICFMH Conférence FoodMicro, Jul 2022, Athènes, Greece. . hal-03773876

**HAL Id: hal-03773876**

**<https://institut-agro-dijon.hal.science/hal-03773876v1>**

Submitted on 9 Sep 2022

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



# DIRECTED EVOLUTION OF *OENOCOCCUS OENI* TO IMPROVE ACID-TOLERANCE REVEALS FIXED BENEFICIAL MUTATIONS IN THE CITRATE LOCUS

Camille Eicher<sup>1</sup>, Frédérique Julliat<sup>1</sup>, Joana Coulon<sup>2</sup>, Marion Favier<sup>2</sup>, Hervé Alexandre<sup>1</sup>, Stéphane Guyot<sup>1</sup>, Cristina Reguant<sup>3</sup>, Cosette Grandvalet<sup>1</sup>

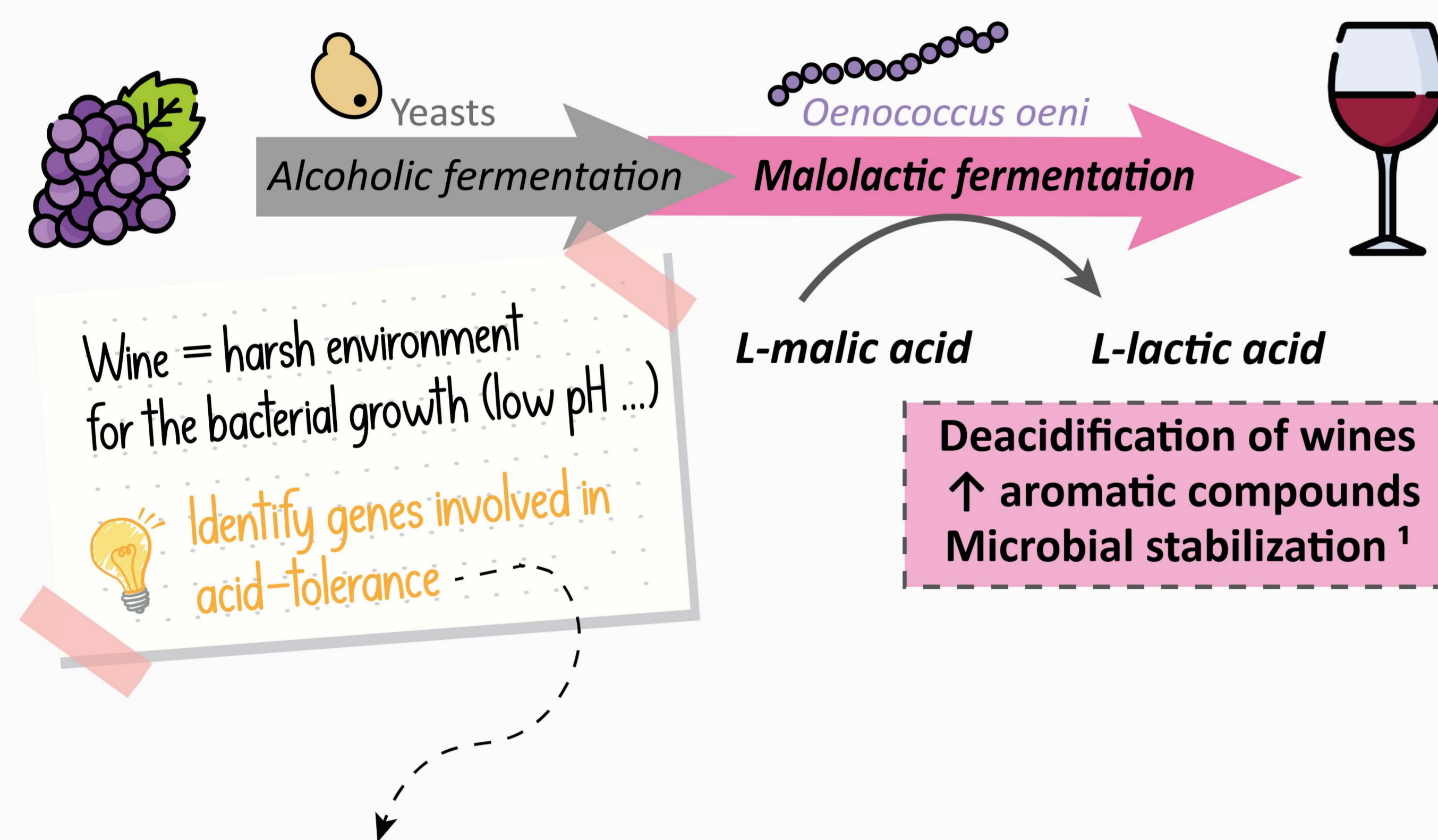
<sup>1</sup> UMR A. 02.102 Procédés Alimentaires et Microbiologiques, L'institut Agro Dijon, Université de Bourgogne Franche-Comté, Dijon, France

<sup>2</sup> BioLaffort, Flourens, France

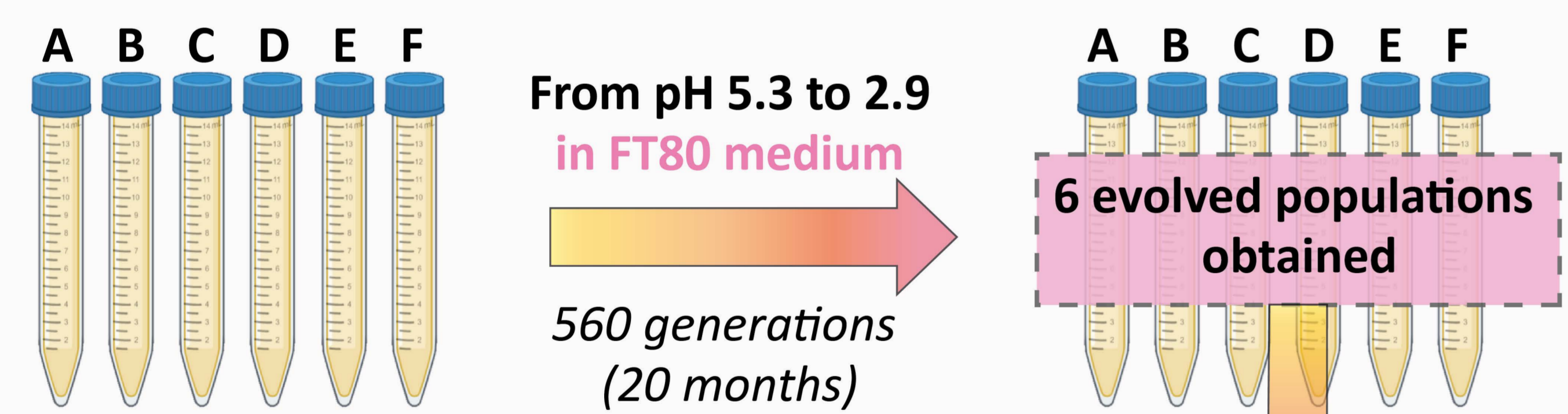
<sup>3</sup> Universitat Rovira i Virgili, Departament de Bioquímica i Biotecnologia, Facultat d'Enologia, Tarragona, Catalonia, Spain

## EXPERIMENTAL EVOLUTION PROCESS

*Oenococcus oeni* is a Lactic Acid Bacteria responsible for :



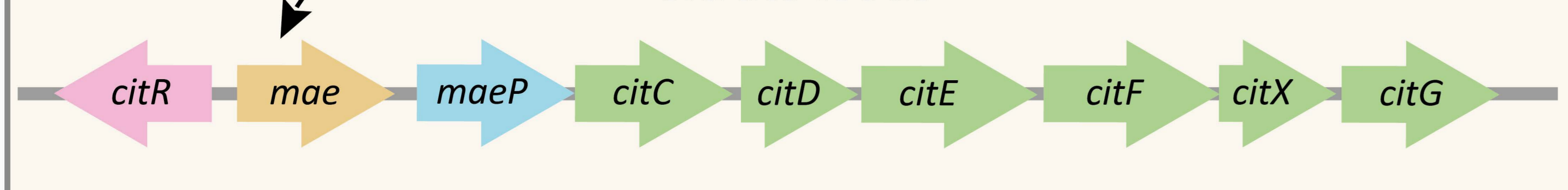
The **directed evolution approach**<sup>2</sup> was used on the *O. oeni* ATCC BAA-1163 laboratory strain to identify target genes :



What are the consequences of the acquisition of mutations in the *cit* locus during the evolution process?

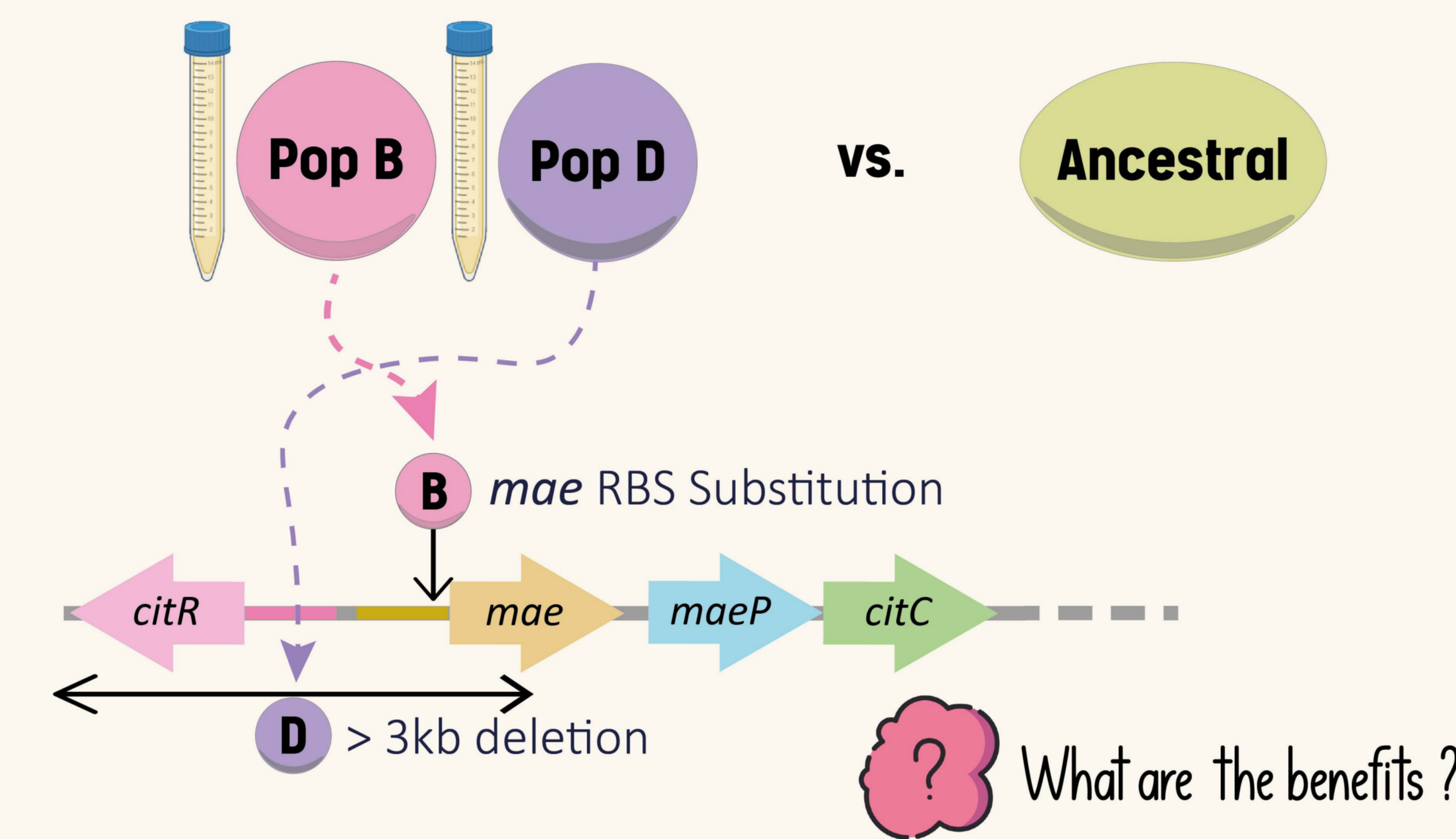
Fixed mutations affecting 5 genes in all the populations including the *mae* gene (citrate locus)

### Citrate locus<sup>3</sup>

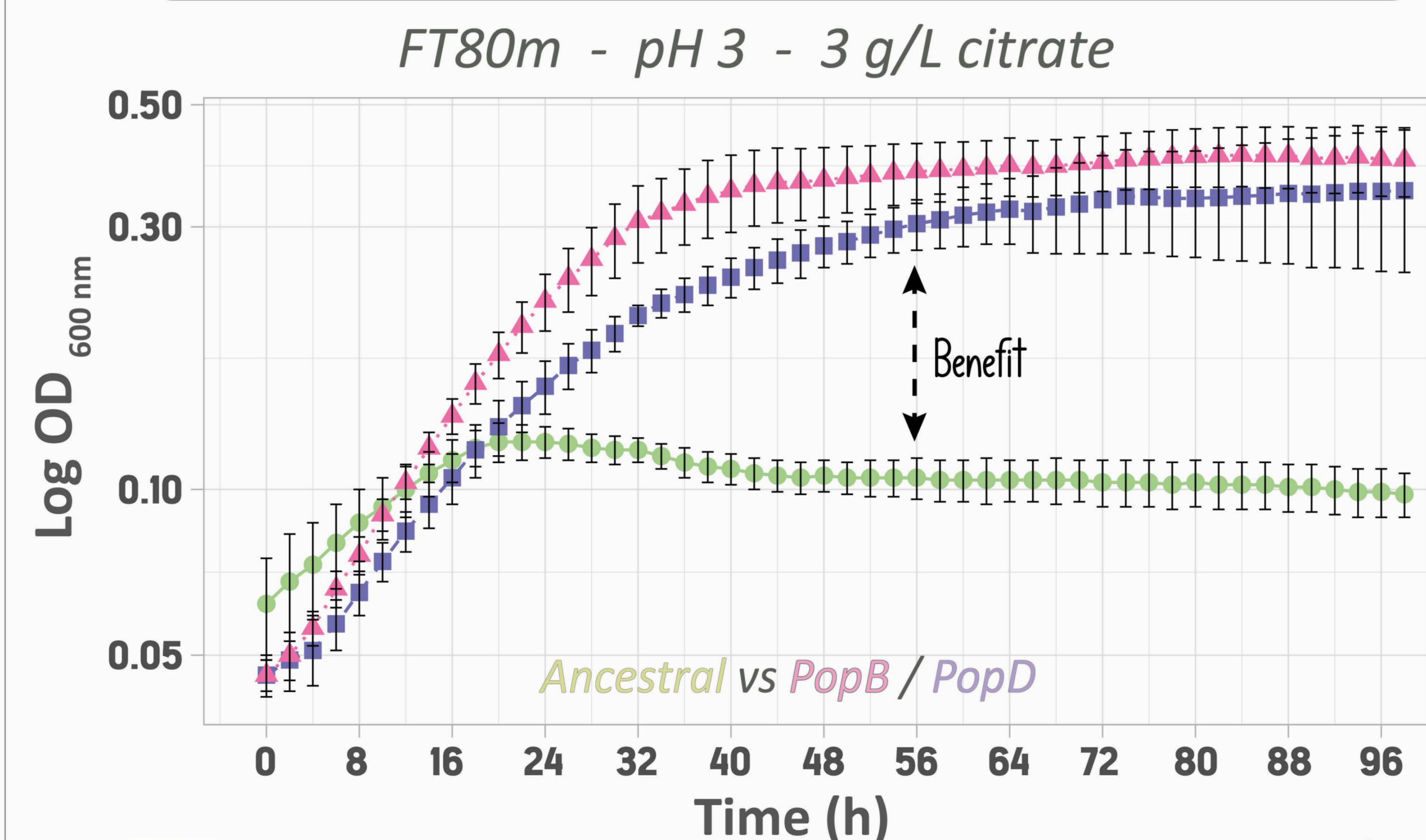


## IMPACT OF THE MUTATIONS

Focus on the evolved populations B and D :



### 1 The growth in acidic medium supplemented with citrate

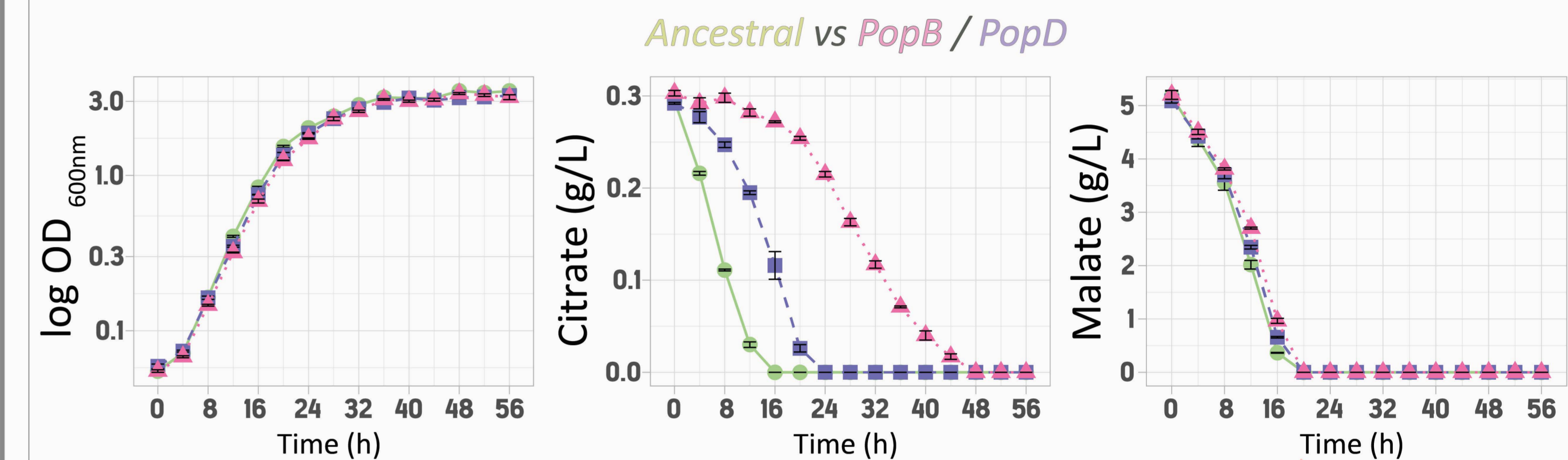


Evolved populations = **Better acid-tolerance** **BEST**

**PERSPECTIVE** : Could the evolved populations be good starters for the industry?

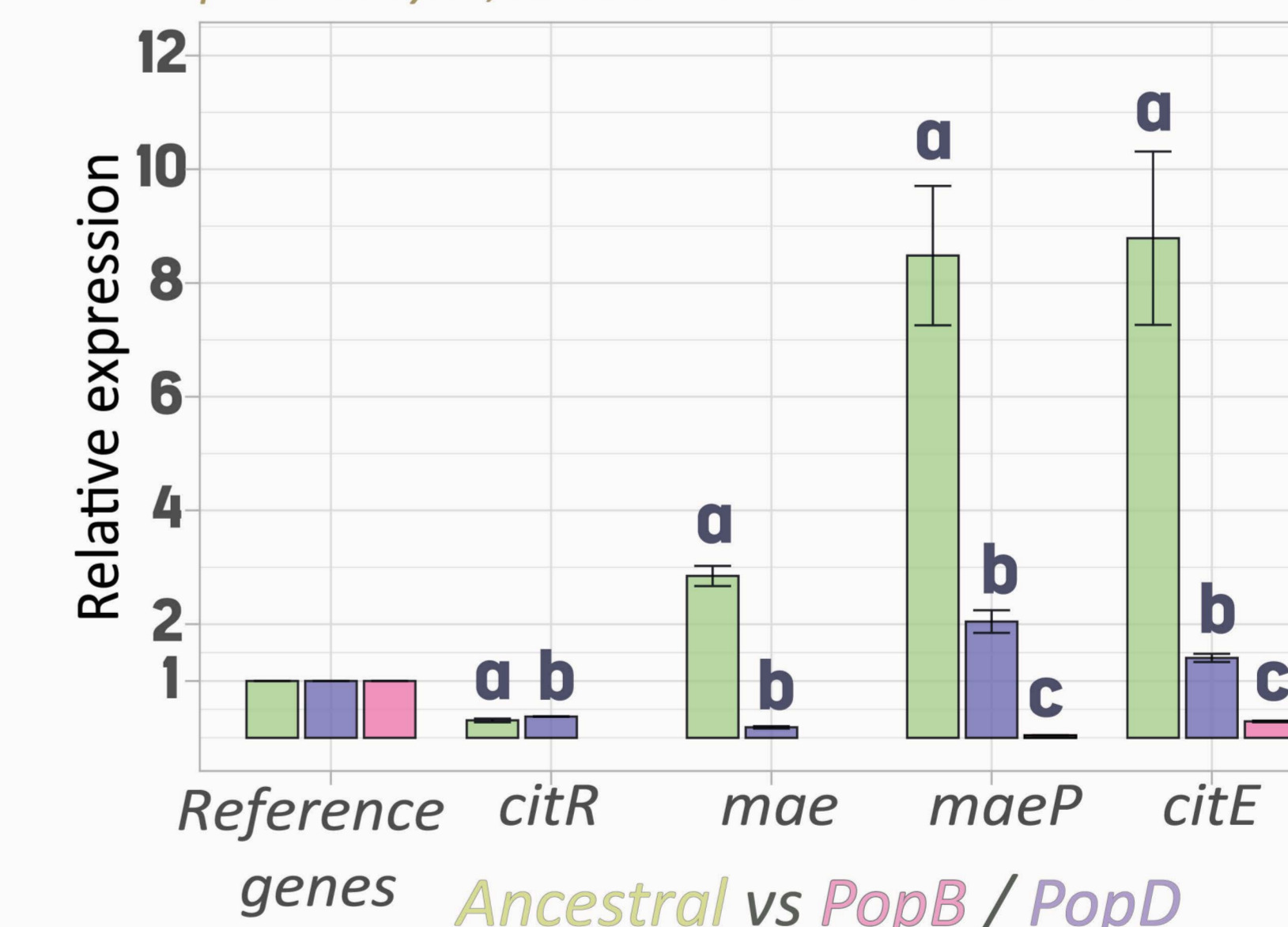
### 2 The organic acid consumption in "oenological-like" conditions

White Grape Juice medium - pH 4.8 - 0.3 g/L citrate - 5 g/L malate



### 3 Focus on the *cit* locus expression

qPCR analysis, statistics based on Kruskal test



Drastic down-regulation of the *cit* locus genes in the evolved populations

## CONCLUSION

The *mae* mutation, acquired during the experimental evolution process, results in a **severe down-regulation of the citrate locus** in the two evolved populations D and B. As a result, **the citrate consumption is reduced** which seems to confer a **better acid-tolerance** to the evolved populations **without affecting the malolactic fermentation efficiency**.

<sup>1</sup> Kunkee, R.E., 1991. Some roles of malic acid in the malolactic fermentation in wine making. FEMS Microbiol. Lett. 88, 55–71. [https://doi.org/10.1016/0378-1097\(91\)90696-8](https://doi.org/10.1016/0378-1097(91)90696-8)

<sup>2</sup> Julliat, F., 2020. Molecular mechanisms involved in the regulation of the stress response in *Oenococcus oeni* and experimental evolution (PhD)

<sup>3</sup> Olguín, N., Bordons, A., Reguant, C., 2009. Influence of ethanol and pH on the gene expression of the citrate pathway in *Oenococcus oeni*. Food Microbiol. 26, 197–203. <https://doi.org/10.1016/j.fm.2008.09.004>