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

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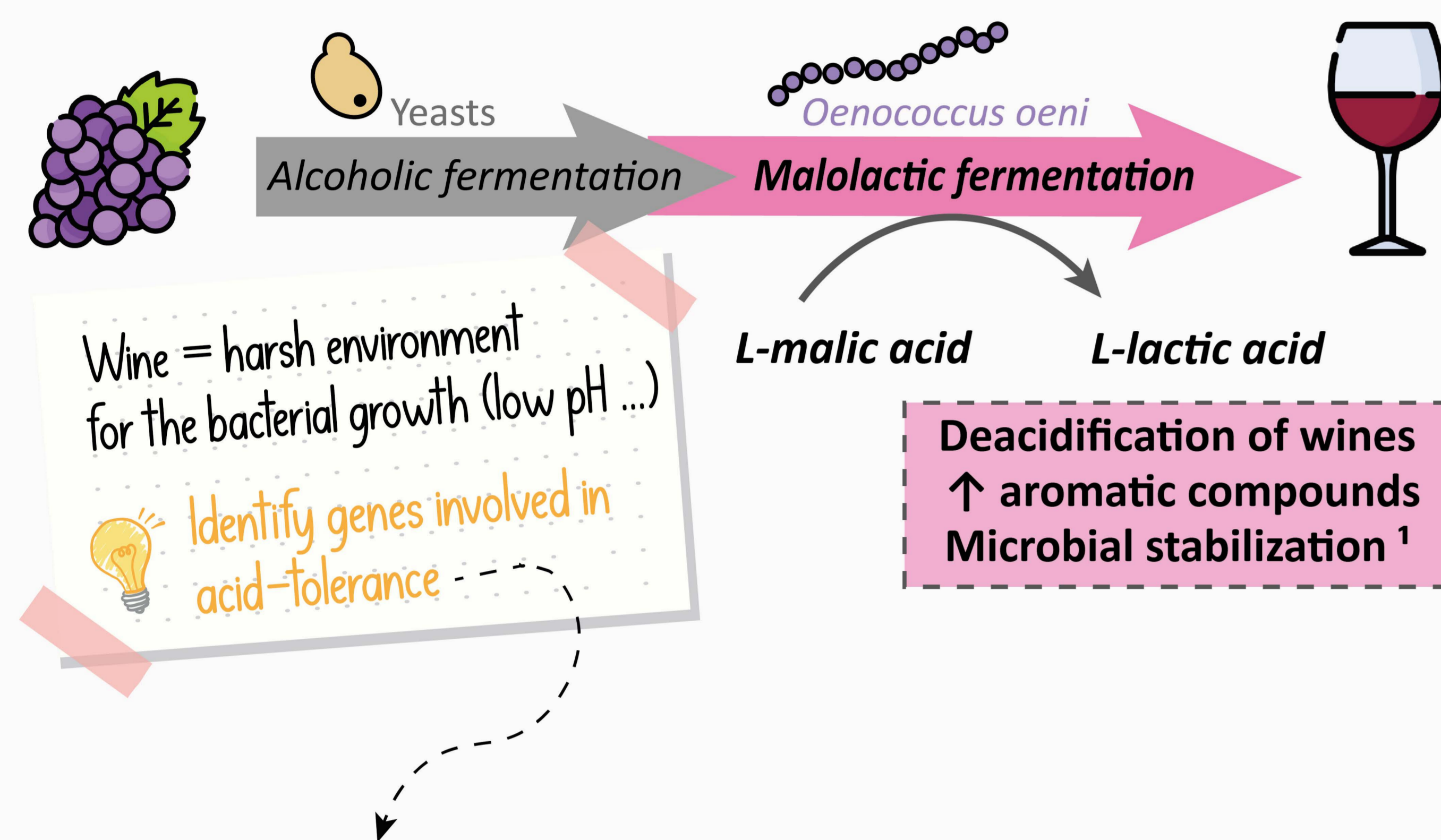
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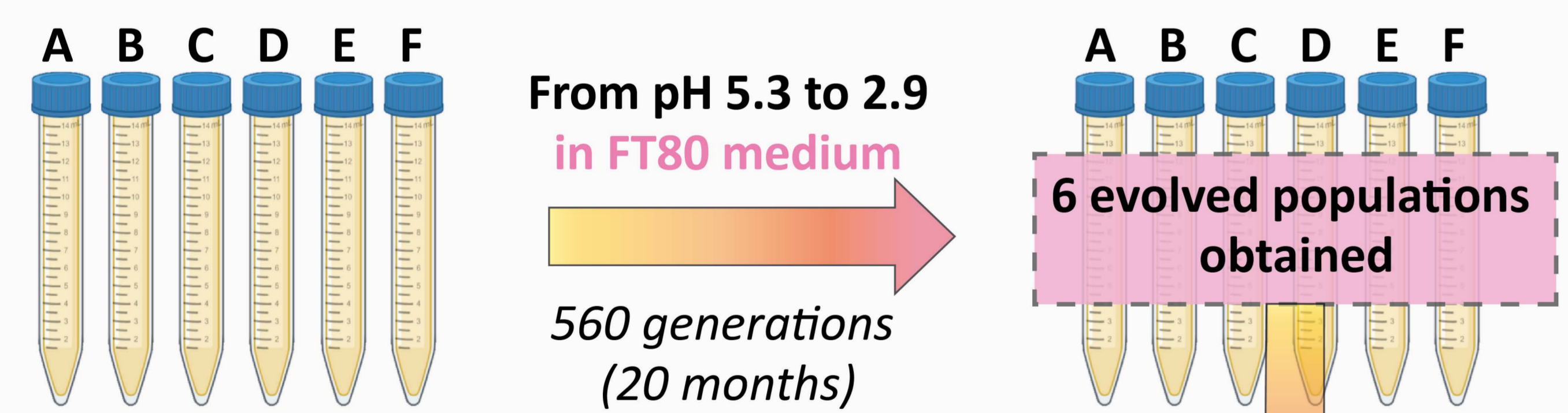
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## EXPERIMENTAL EVOLUTION PROCESS

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



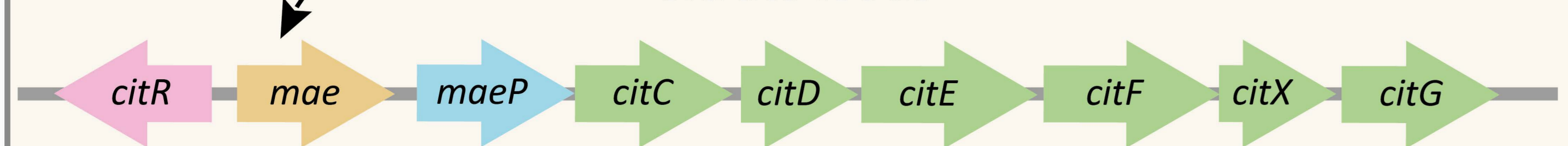
The **directed evolution approach**<sup>2</sup> was used on the *O. oeni* ATCC BAA-1163 laboratory strain to identify target genes :



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

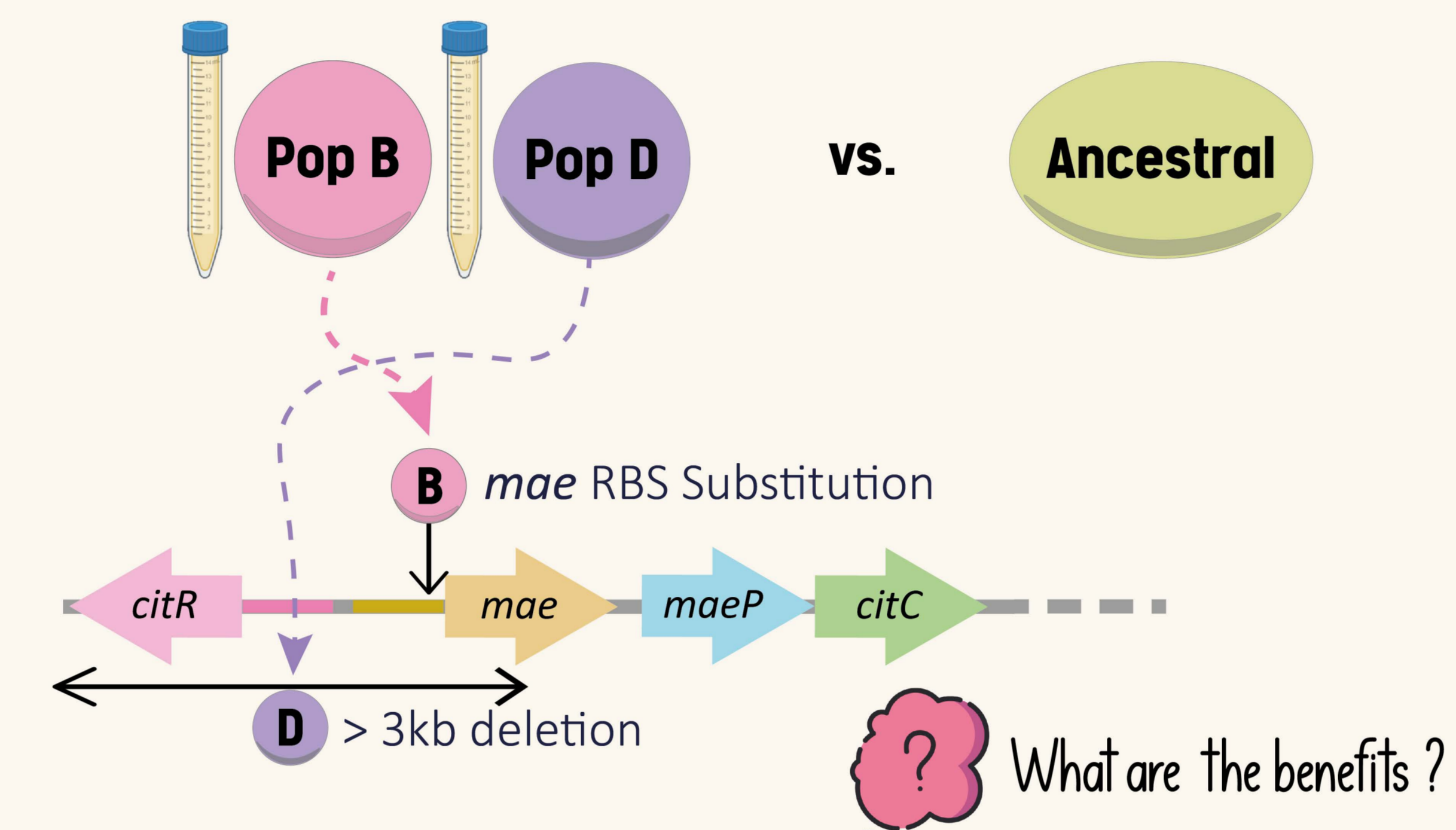
Fixed mutations affecting 5 genes in all the populations including the *mae* gene (citrate locus)

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

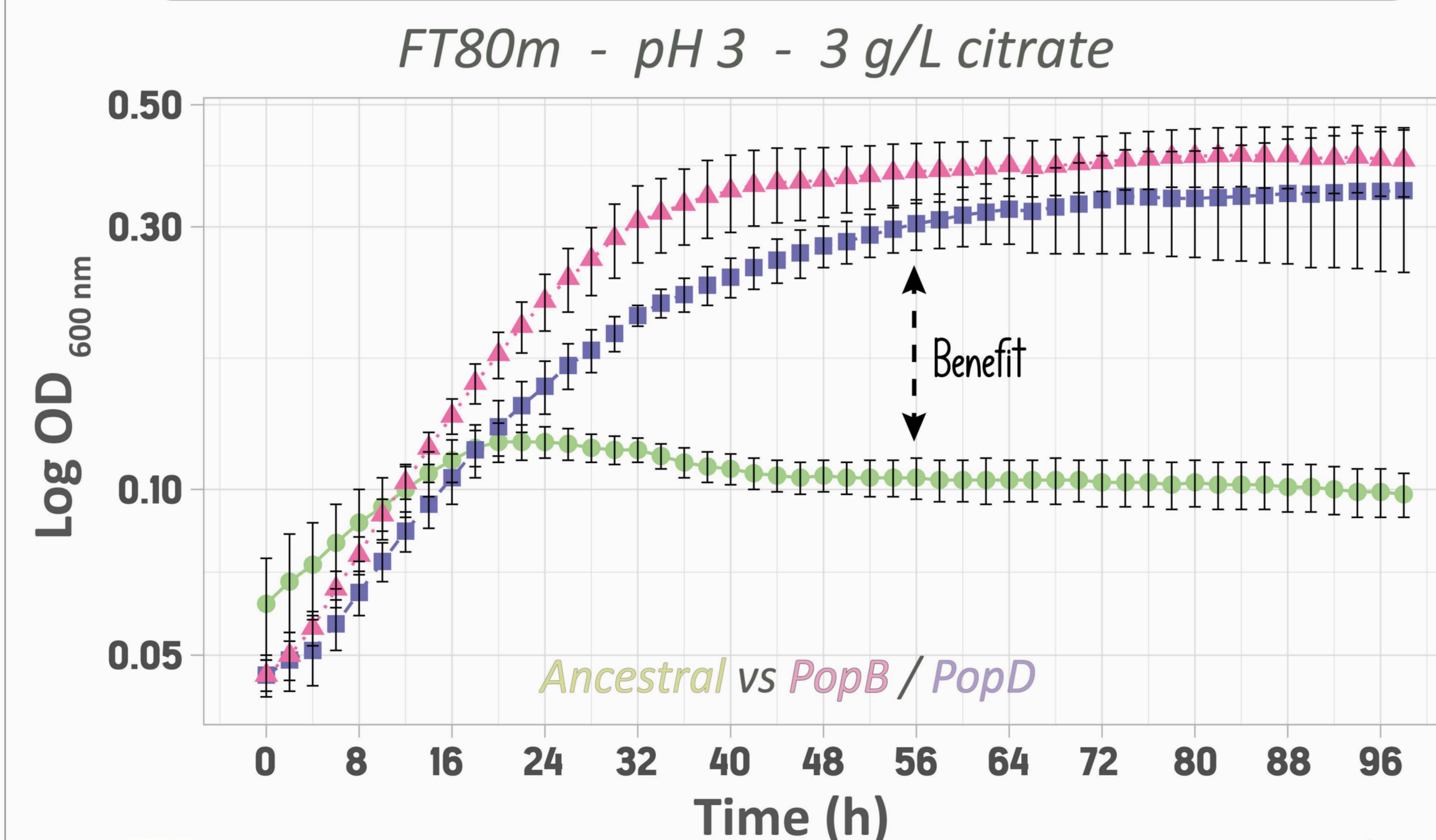


## IMPACT OF THE MUTATIONS

Focus on the evolved populations B and D :



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

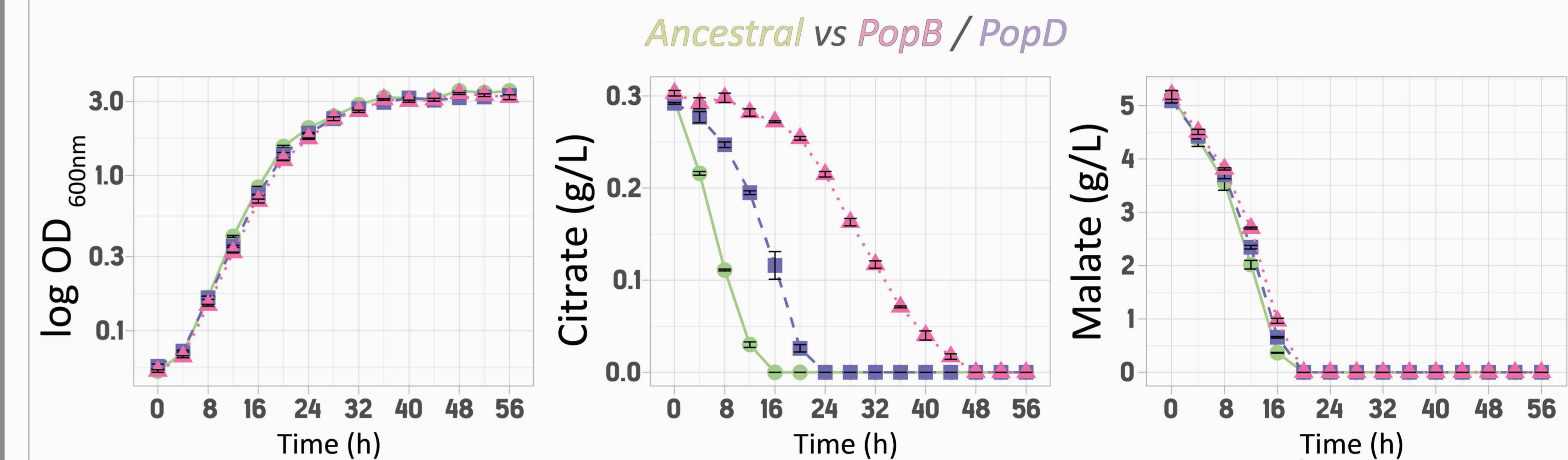


Evolved populations = **Better acid-tolerance**

**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



Slowdown of the citrate consumption  
Malolactic fermentation = same performance

### 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>