

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

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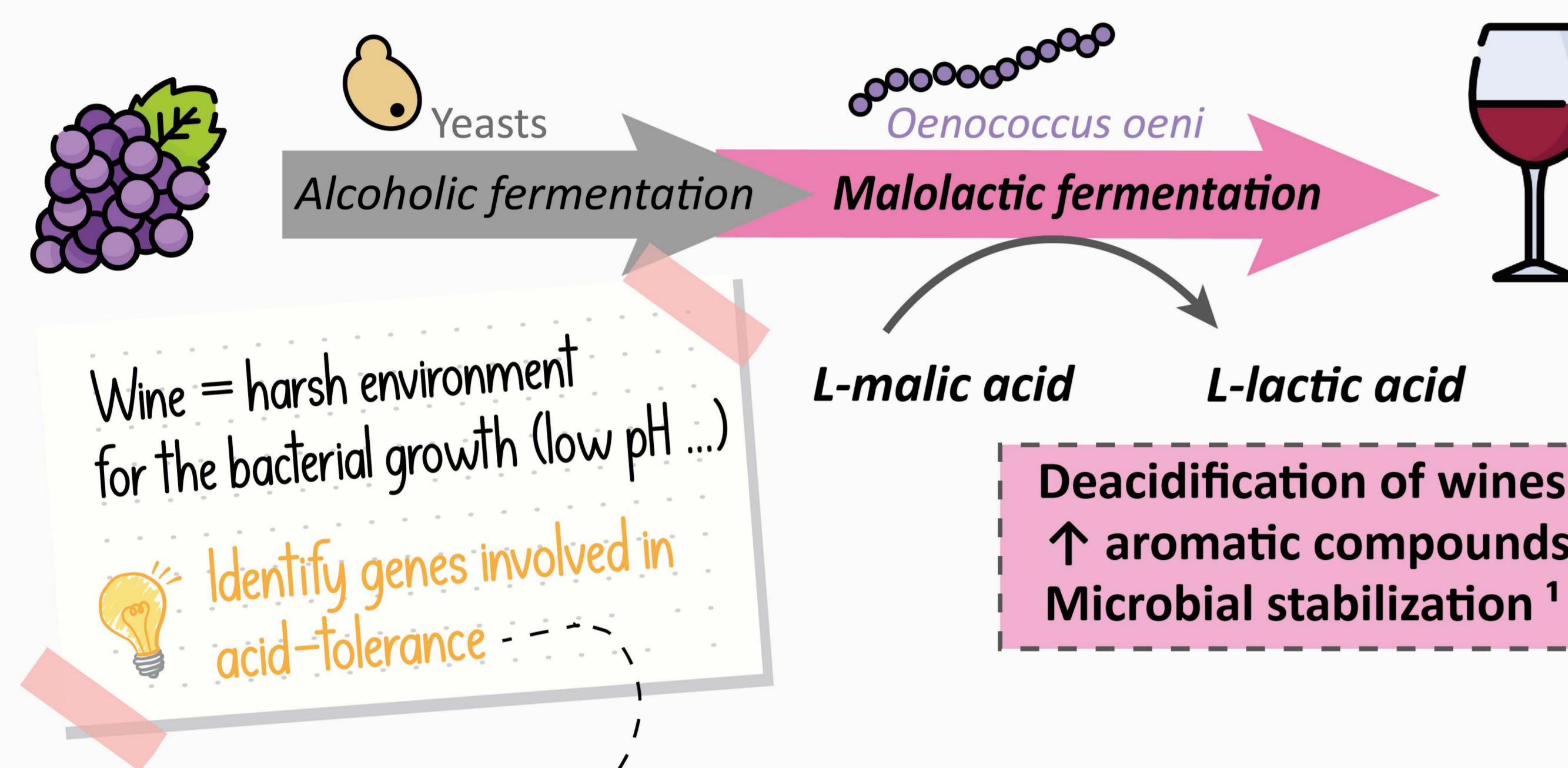
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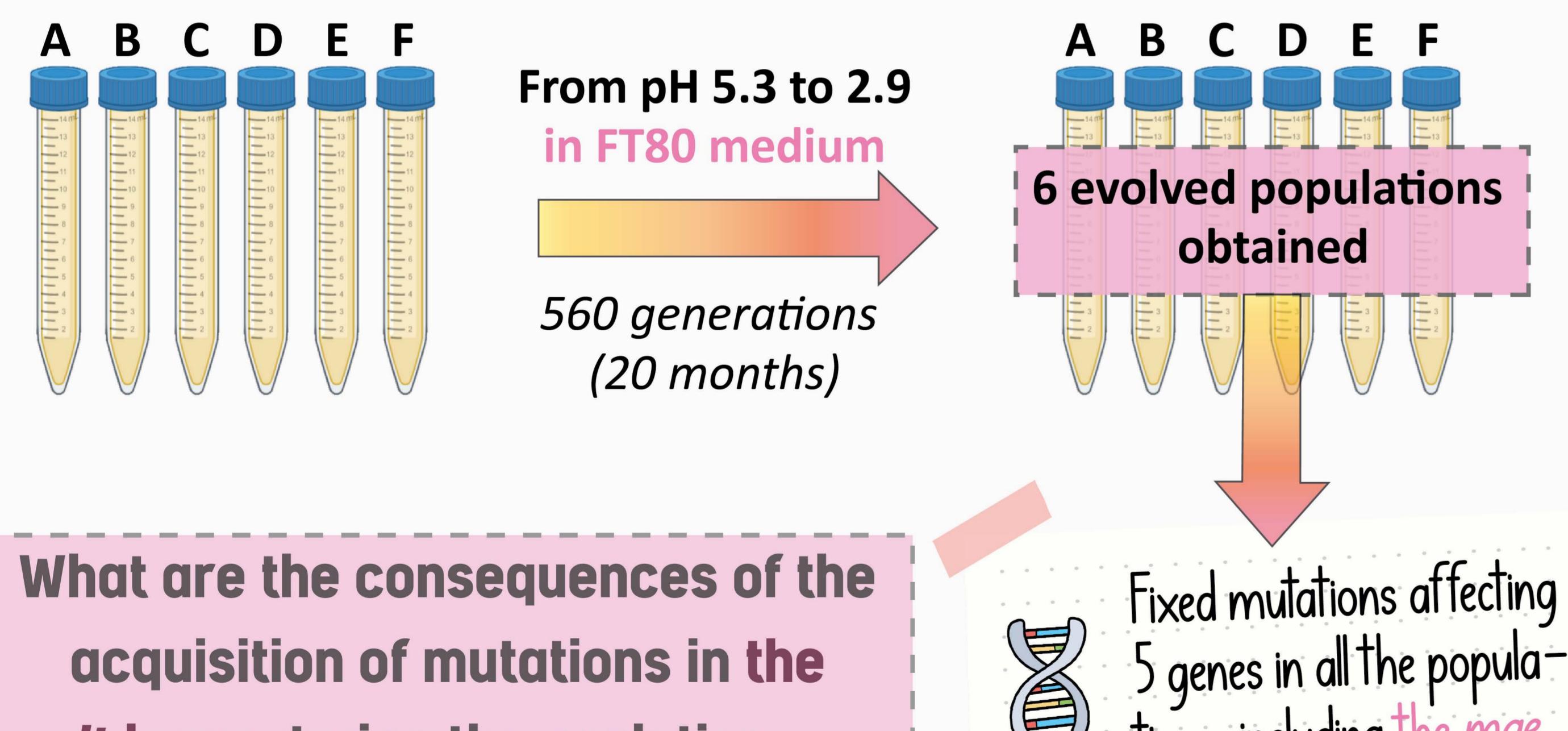
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EXPERIMENTAL EVOLUTION PROCESS

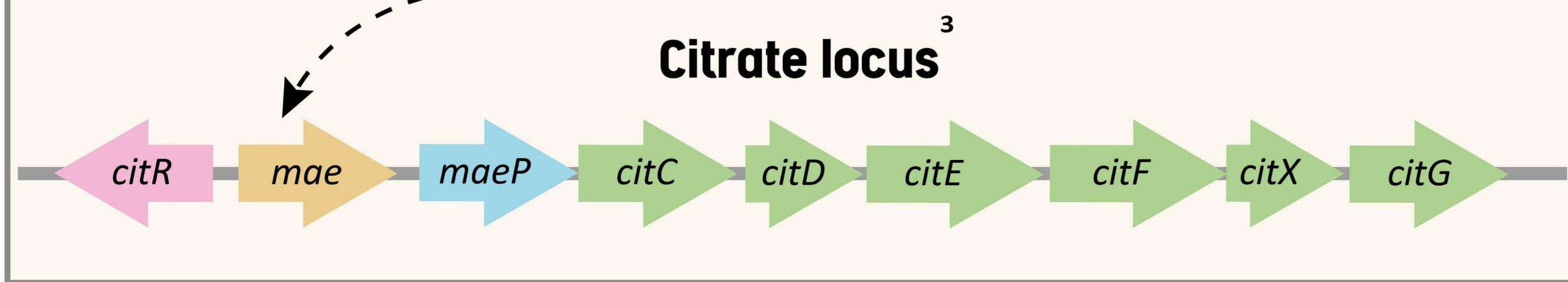
Oenococcus oeni is a Lactic Acid Bacteria responsible for :



The directed evolution approach² was used on the *O. oeni* ATCC BAA-1163 laboratory strain to identify target genes :

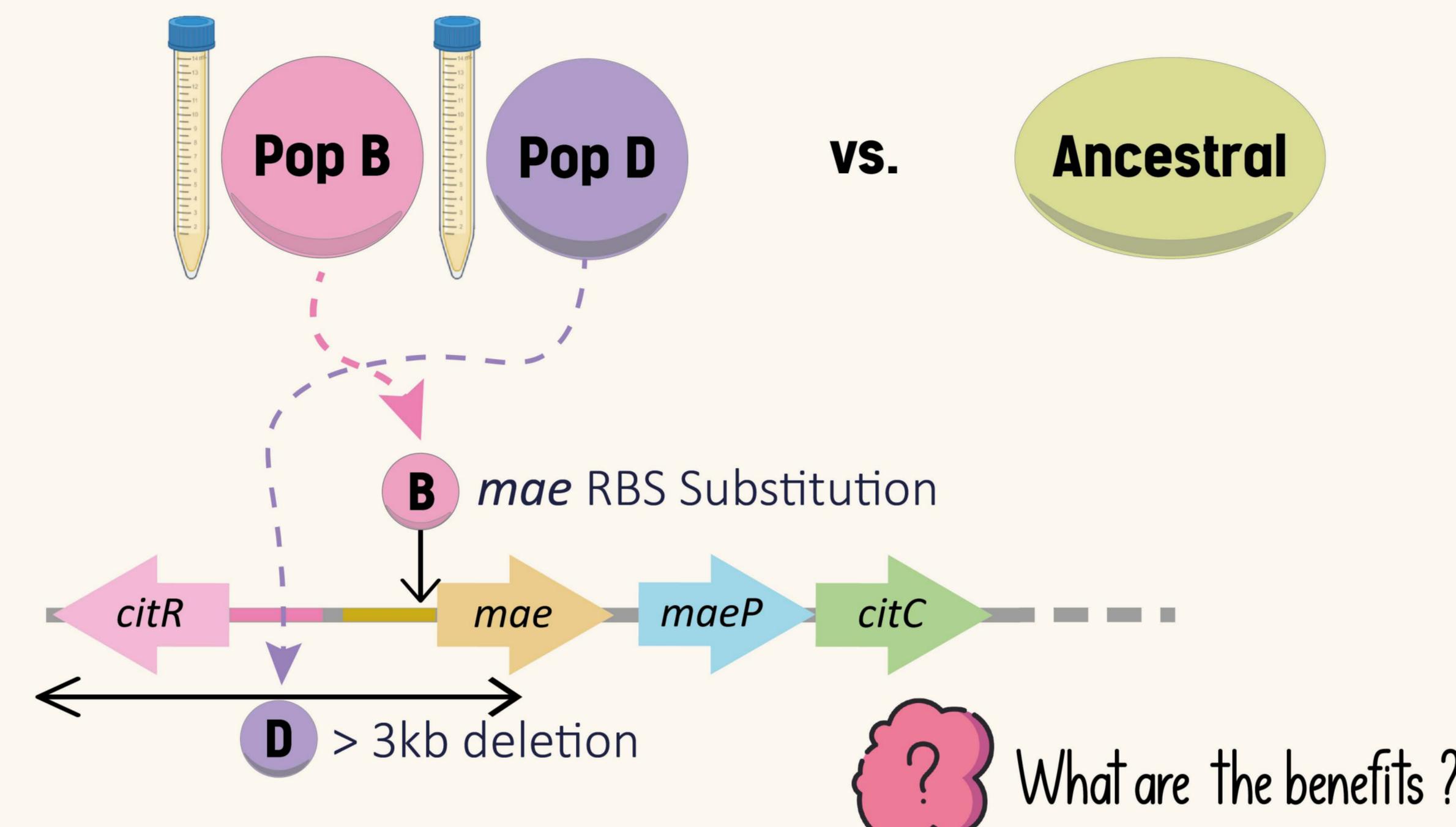


What are the consequences of the acquisition of mutations in the cit locus during the evolution process?

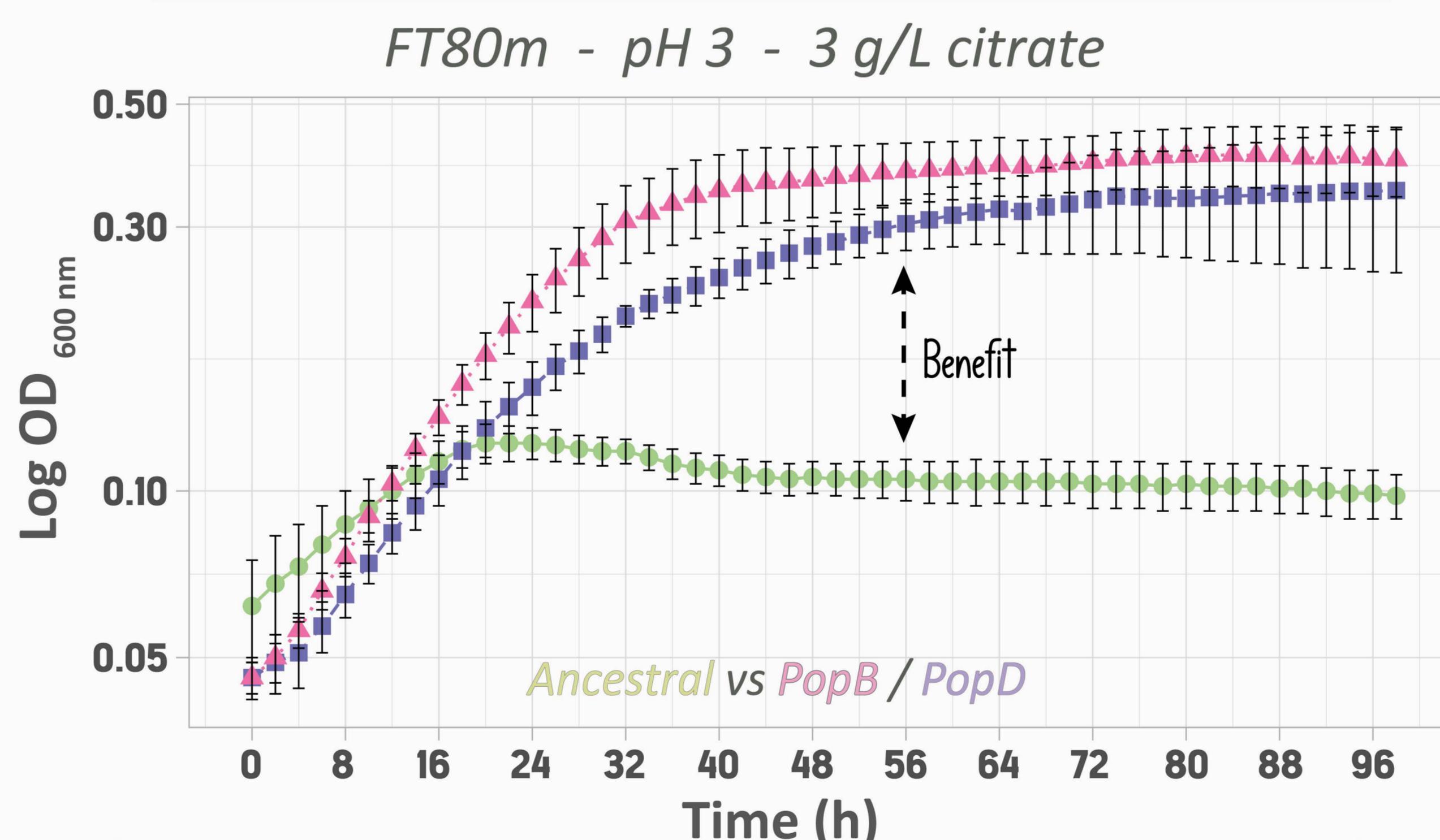


IMPACT OF THE MUTATIONS

Focus on the evolved populations B and D :



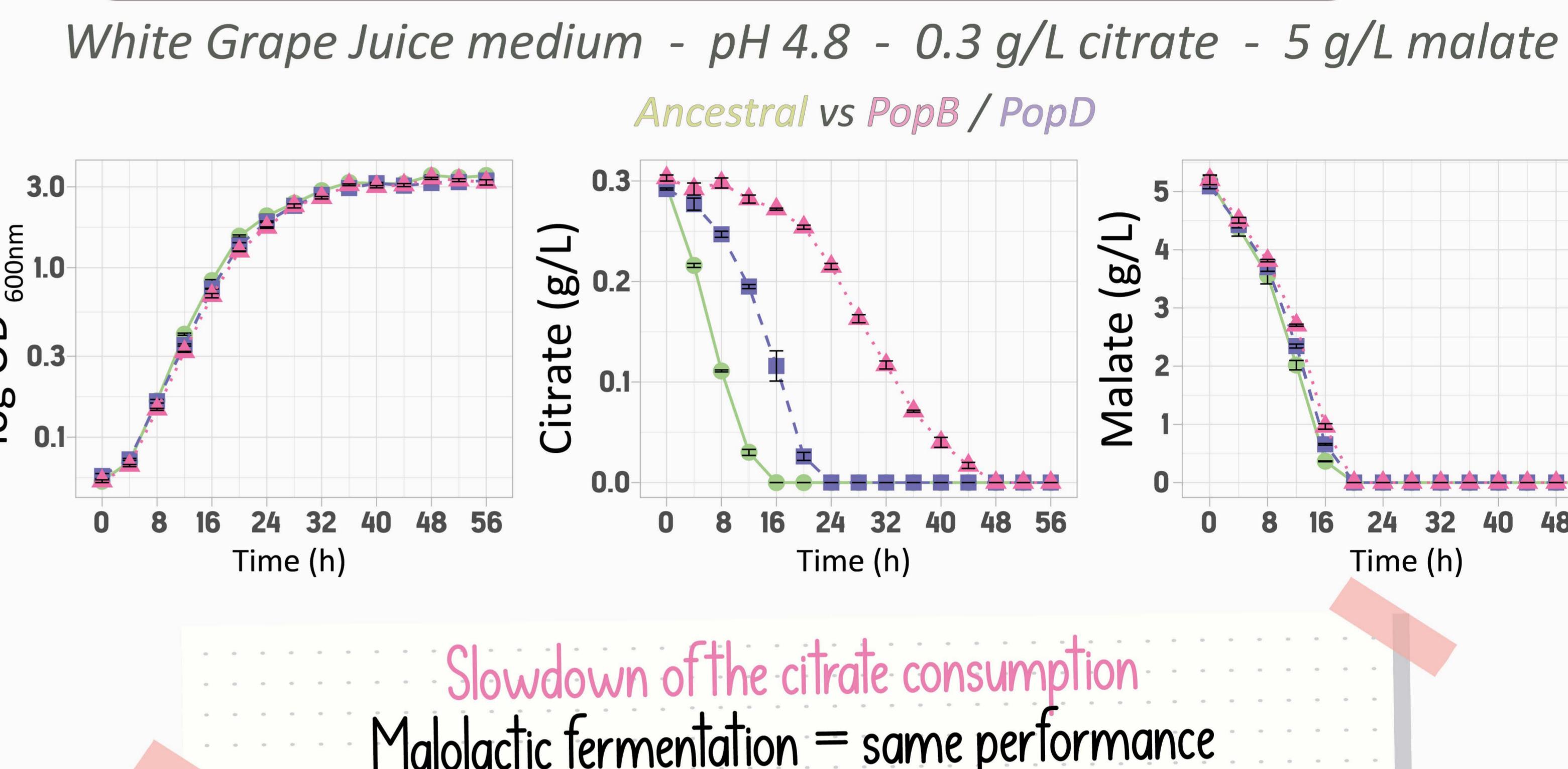
1 The growth in acidic medium supplemented with citrate



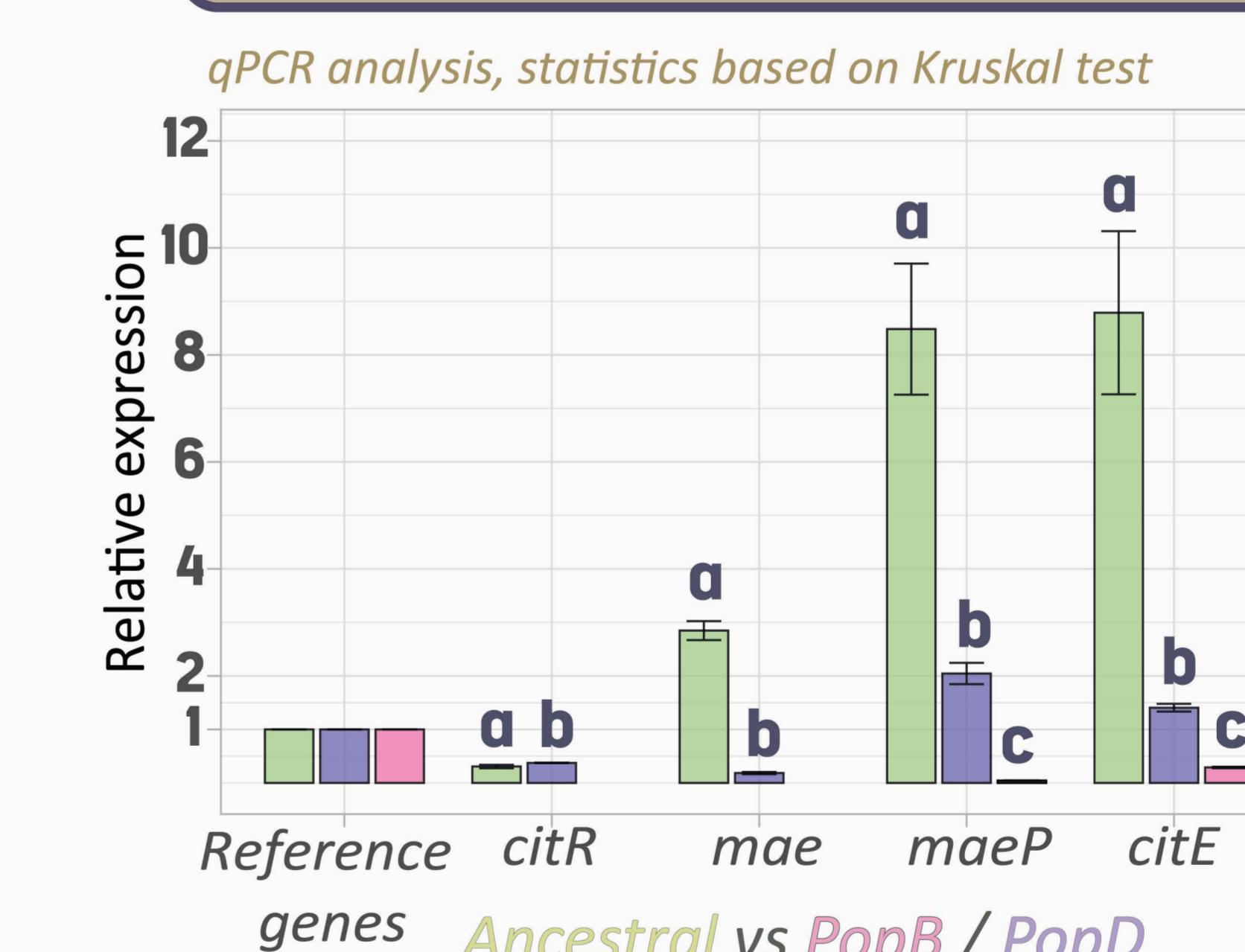
Evolved populations = Better acid-tolerance

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

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



3 Focus on the cit locus expression



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.

¹ 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)

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

³ 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>