

New frontiers of wine biotechnology: a perspective on the CRISPR technology in yeasts

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The CRISPR/Cas9 technology is gaining significant attention because of its biotechnological applications and its potential to boost innovation. It is considered an efficient, cheap and easy-to-use tool for genome editing that allows the simultaneous modification of all the alleles of a target gene. The wine industry could particularly gain an advantage from this engineering system; indeed, molecular studies would help understanding the microbial contribution of *Saccharomyces* and non-*Saccharomyces* yeast species and several bacteria to wine quality and safety. Moreover, the use of the CRISPR/Cas9 approach on wine selected yeast starters, widely used in winemaking but still poorly characterized from a genetic point of view due to their polyploid nature, can represent a further *plus* in oenology. In this work, a strategy to modify wine yeasts with the CRISPR/Cas9 system is outlined. Two commercial strains of *S. cerevisiae* (EC1118, AWRI796) have been genetically engineered in the arginine degradation pathway (*CANI* and *CARI* genes) to generate yeasts characterised by a reduced urea release in order to decrease the ethyl-carbamate production. Ethyl-carbamate is known as a potential carcinogenic compound, and it is found in alcoholic beverages in significant amounts. Results demonstrate that CRISPR/Cas9 system can be successfully established in *S. cerevisiae* wine yeasts and that, in the conditions under study, the editing of the *CANI* (Vigentini et al., 2017) and *CARI* genes leads to a significant reduction of urea production in laboratory synthetic and natural wines.

References:

Vigentini, I., Gebbia, M., Belotti, A., Foschino, R., Roth F.P. (2017) CRISPR/Cas9 system as a valuable genome editing tool for wine yeasts with application to decrease urea production. *Frontiers in Microbiology*, 8, 1225-1234. DOI: 10.3389/fmicb.2017.01225