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Influence of nutrients and temperature on the production of fermentative aromas in winemaking conditions using a Box-Behnken design

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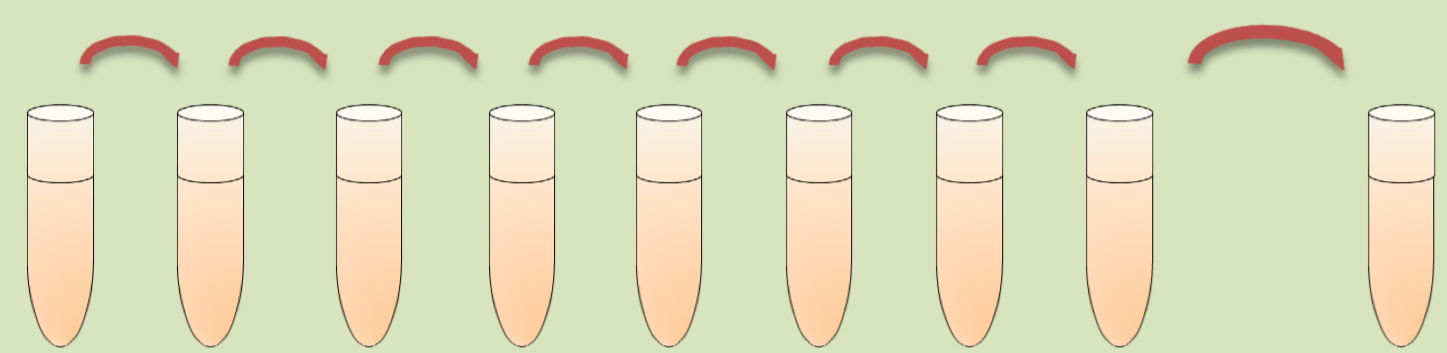
INTRODUCTION

Fermentative aromas (especially esters and higher alcohols) play an important role in the organoleptic profile of wines. In order to assess the effect of environmental factors on the production of fermentative aromas, we studied the production of these compounds by two *Saccharomyces cerevisiae* strains with contrasting aromatic profiles: Lalvin EC1118[®] and ECA5[™], an evolved strain with enhanced aroma production. We examined the combined effects of temperature, initial assimilable nitrogen and lipid contents on aroma production.

A Box-Behnken design was used to minimize the number of factor combinations required to evaluate the effects of these three factors. This plan allowed to identify the fermentation conditions (nitrogen and lipid contents and temperature) leading to the highest production of volatile compounds and to compare the two strains. A Box-Behnken design provides a response surface which is a valuable tool, because it can be used to determine the experimental-factor settings giving maximum (or minimum) response values.

EVOLUTIONARY ENGINEERING STRATEGY

Serial transfers on gluconate, a substrate poorly assimilated by *S. cerevisiae* and metabolized through the Pentose Phosphate Pathway (PPP) (Cadière *et al.*, 2011).

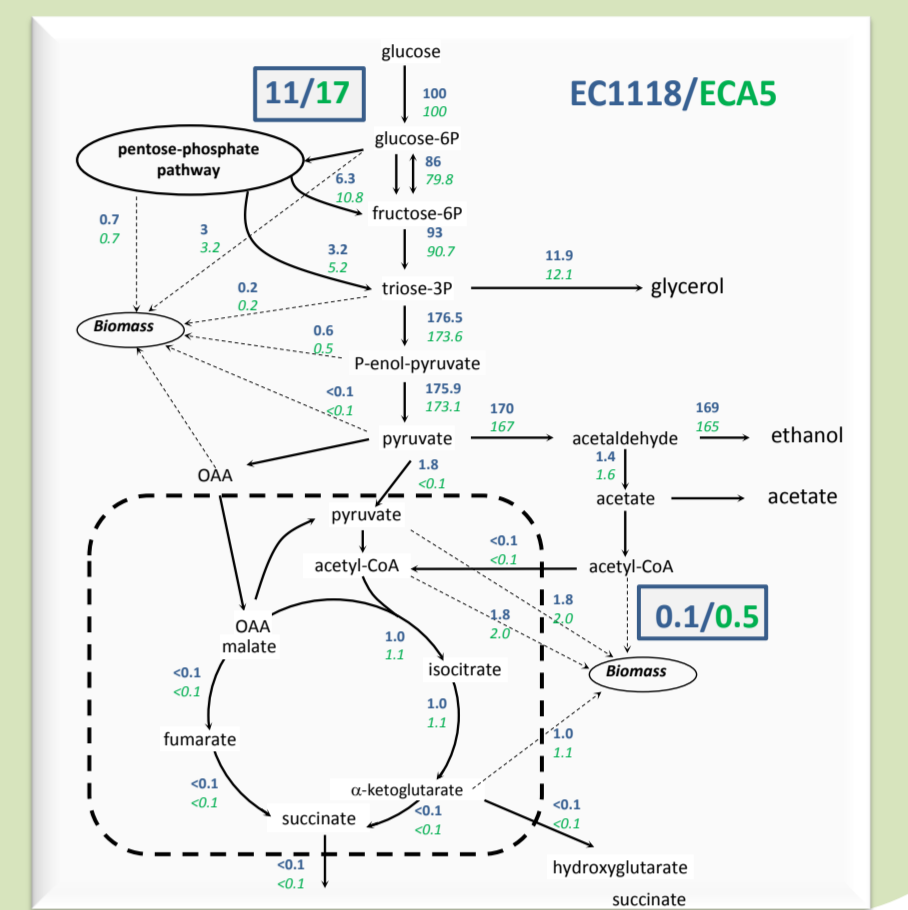


- Serial transfer on gluconate pH 6 during 240 generations
- 2 independently propagated cultures

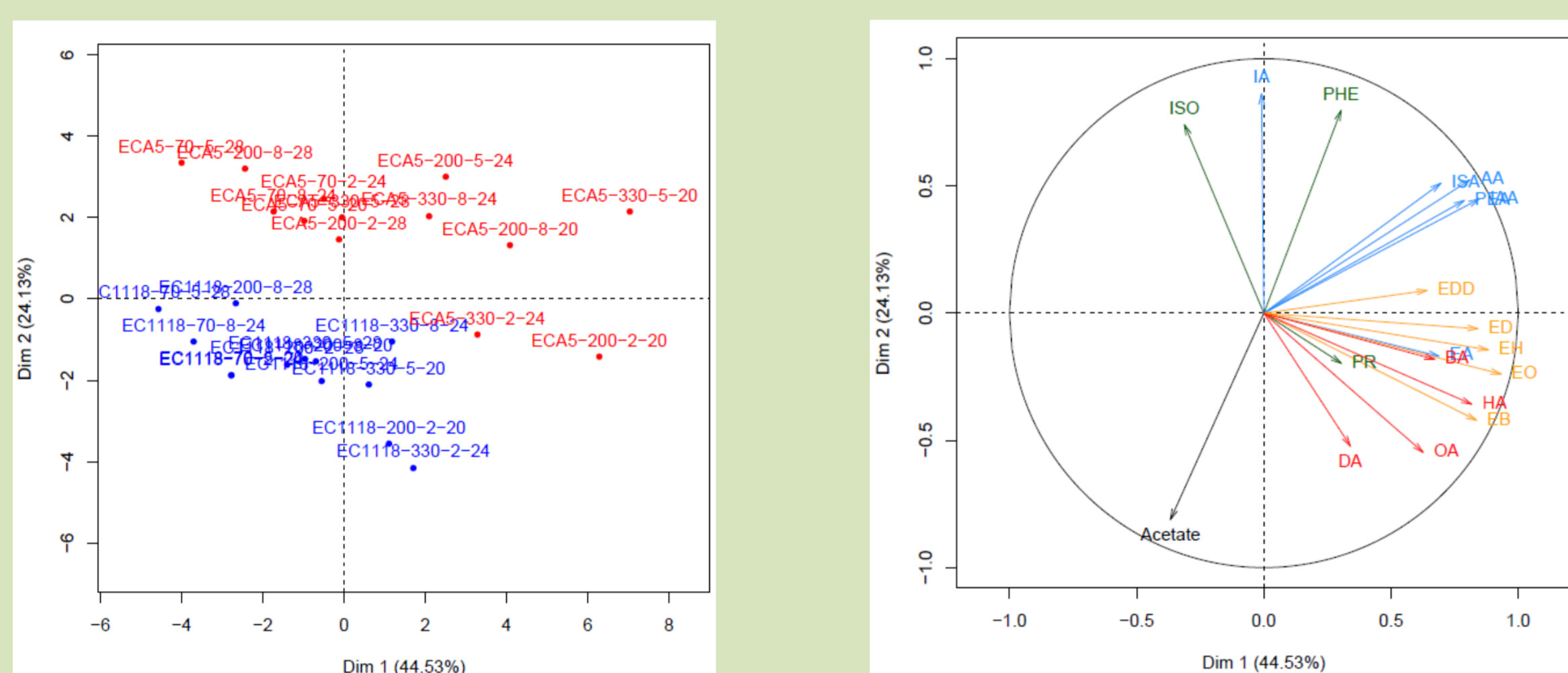
ECA5[™]: an evolved strain, obtained after 70 generations, showed a better assimilation of gluconate compared to the parental strain (Lalvin EC1118[®])

CHARACTERIZATION OF THE EVOLVED STRAIN ECA5[™] DURING WINE FERMENTATION

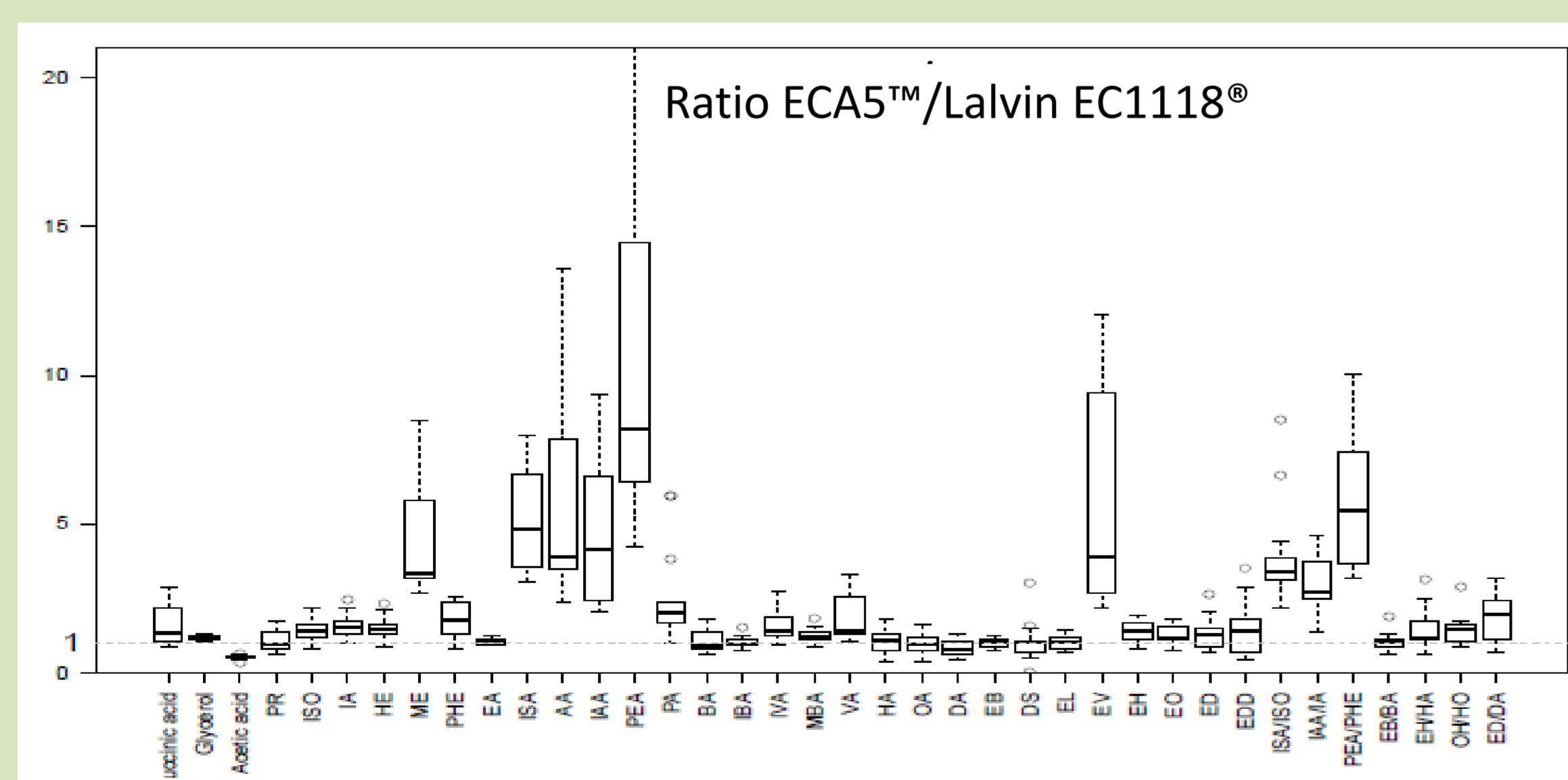
- Increased flux through the PPP (1.5 fold)
- Increased flux through lipid synthesis
- Lower production of acetate
- Enhanced production of fermentative aromas
- Higher fermentation rate (Cadière *et al.*, 2011)



RELATIVE PERFORMANCES OF ECA5[™] COMPARED TO LALVIN EC1118[®]



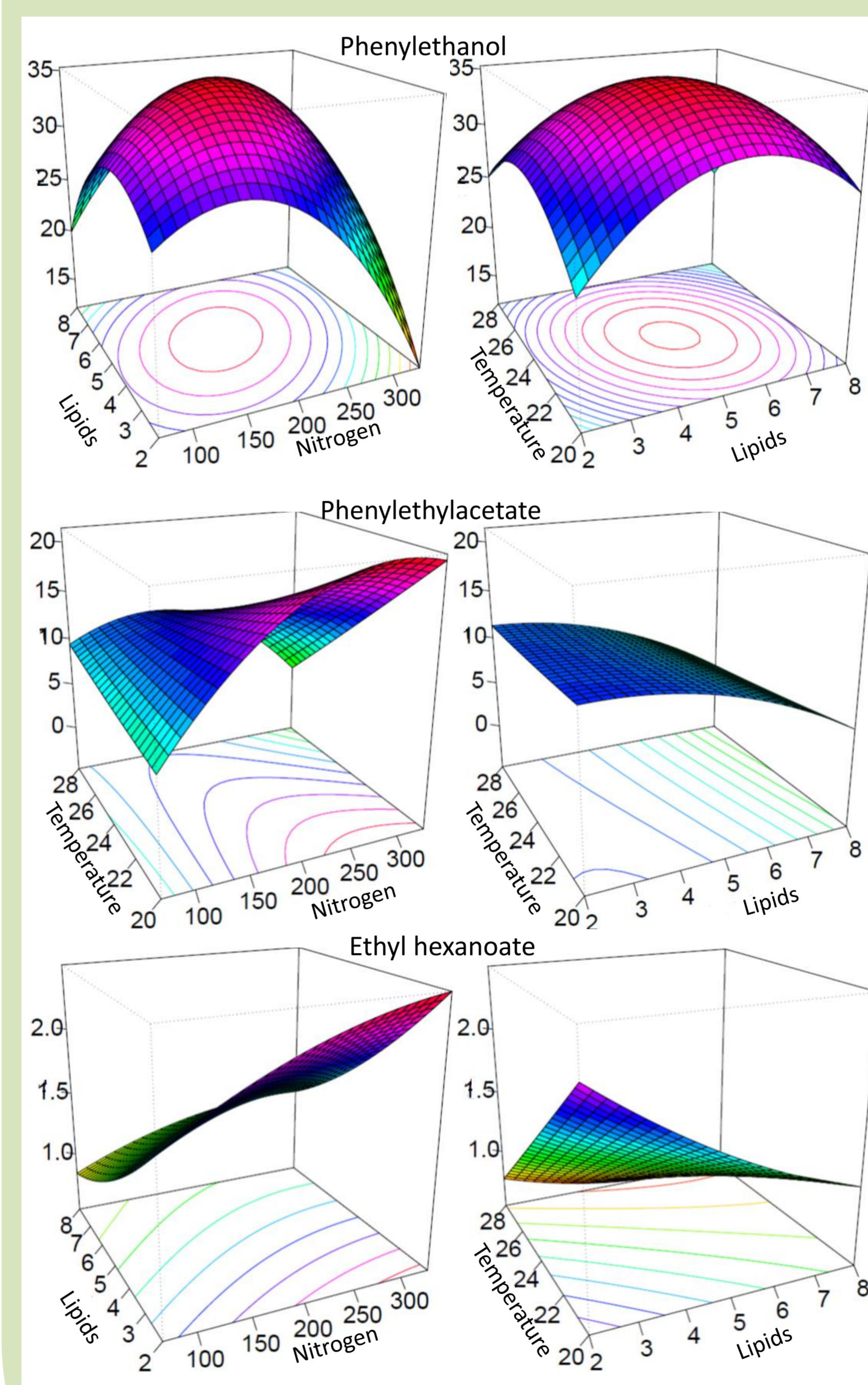
- Whatever the fermentation conditions : systematic separation of the 2 strains due to the acetate esters (in light blue), phenylethanol (PHE) and acetate
- PHE (marker of the PPP) involved in the discrimination of the strains
→ In agreement with the higher flux through the PPP in ECA5[™]
- PHE and acetate: markers of the 2 pathways involved in NADPH production
→ Negative correlation between these 2 compounds



- The production of acetate is twice lower with ECA5[™]
- Acetate esters and higher alcohols are systematically overproduced by ECA5[™], which is not the case for ethyl esters and acids
- Overproduction of acetate esters is greater than the higher alcohols
- The 2 strains are discriminated by the ratios acetate esters/higher alcohols

IMPACT OF TEMPERATURE, NITROGEN AND LIPIDS CONTENTS ON THE SYNTHESIS OF VOLATILE COMPOUNDS BY ECA5[™]

- For PHE, optimum of production given by the model: 180 mgN/L, 5 mg/L of lipids and 24°C
- For other higher alcohols, acetate and ethyl esters, no optimum given by the model



Impacts of nutrients and temperature

Higher alcohols :

- Favorable concentrations of **nitrogen**: between 100-200 mgN/L
- Temperature** and **lipids**: no significant effect

Acetate and ethyl esters :

- Temperature**: lower concentrations of esters at high temperature (combined effect of evaporation and lower production)
- Lipids**: Strong impact on the production of ethyl esters
→ Consistent with their biosynthetic pathways
Moderate effect on the synthesis of acetate esters
→ Requirement of acetyl-CoA
- Nitrogen**: increased production at high nitrogen content
→ Link between acetate esters and nitrogen consistent with their metabolic pathways (Ehrlich pathway)
→ Link between ethyl esters and nitrogen more surprising: contribution of acyl-CoA deriving from α -cetoacids ?

CONCLUSION

Using adaptive evolution on gluconate, we recently obtained an evolved strain with a higher flux through PPP and novel metabolic traits during wine fermentation, in particular reduced acetate and increased aroma formation (Cadière *et al.*, 2011, Cadière *et al.*, 2012). We show here that the evolved and parental strains are strongly discriminated by their production of higher alcohols and acetate esters, regardless the environmental conditions (lipids, nitrogen, temperature), while a difference on ethyl esters was not systematic. Moreover, we highlighted differences in the response of ECA5[™] and Lalvin EC1118[®] to environmental parameters (data not shown), which suggests changes in the metabolic and regulation network of the evolved strain. This study also revealed a differential impact of nutrients and temperature on the synthesis of aromas depending on the class of compounds: higher alcohols, acetate or ethyl esters.

