The concentration of glucose and fructose influences the production of volatile phenols by wine Lactic Acid Bacteria

ISA S. SILVA*, FRANCISCO M. CAMPOS, TIM HOGG and J. ANTÓNIO COUTO
Escola Superior de Biotecnologia, U. Católica Portuguesa, R. Dr. A. Bernardino de Almeida, 4200-072 PORTO, PORTUGAL
*isfranco@esb.ucp.pt

CATÓLICA
UNIVERSIDADE CATÓLICA PORTUGUESA
ESCOLA SUPERIOR DE BIOTECNOLOGIA

ABSTRACT

Volatile phenols have characteristic aromas which, above a certain concentration threshold, have a negative effect on the overall aroma of wine. *Dekkera/Brettanomyces* are recognized as the main producer organisms, although certain strains of Lactic Acid Bacteria (LAB) are also capable of producing volatile phenols. In this work, we studied the influence of the concentration of glucose on the ability of LAB to produce volatile phenols in culture medium supplemented with *p*-coumaric acid. The capacity of *Lactobacillus plantarum* NCFB 1752 and *Lactobacillus collinoides* ESB 99 to produce 4-ethylphenol (4EP) was found to be significantly higher at 3 and 5 gl^{-1} of glucose than at 20 gl-1. The results suggest that the vinylphenol reductase activity of these bacteria is enhanced in the presence of low concentrations of glucose, which may be associated with the cells necessity to regenerate NAD$^+$, presumably the co-enzyme formed from the reduction of 4-vinylphenol (4VP) into 4EP. When fructose was added to the medium, the reduction step of 4VP was restrained, thus favouring the outcome of 4VP, for *L. plantarum* NCFB 1752. This effect might be related with the reduction of fructose into mannitol, accompanied by the regeneration of NAD$^+$. It was also found that aerobiose, in comparison to anaerobiose, notably favours the production of 4VP, suggesting that O_2 is used as electron acceptor allowing the regeneration of NAD+. This study shows that the ratio 4VP/4EP is considerably affected by certain wine factors.

INTRODUCTION

➢ Phenolic compounds are abundant in wine, originating from the grape material, yeast metabolism and wood barrels. These compounds include phenolic acids that are the precursors of volatile phenols (4-vinylphenol, 4-vinylguaiacol, 4-ethylphenol and 4-ethylguaiacol). Although they are often regarded as negative, imparting off-flavours to red wines described as "animal", "horse sweat" or "medicinal", some winemakers have considered that, at low concentrations, volatile phenols can contribute positively to the complexity of the bouquet of wines (Fugelsang, 1997).

➢ The conversion of *p*-coumaric acid in 4-ethylphenol involves the sequential activity of two enzymes: the first is the cinnamate decarboxylase which decarboxylates the hydroxycinnamic acid into the 4-vinylphenol (4VP), and the second is the vinylphenol reductase which reduces the latter in 4-ethylphenol (4EP) (Heresztyn, 1986) (Figure 1).

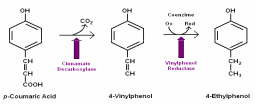

FIGURE 1: Biosynthesis of 4-ethylphenol from *p*-coumaric acid.

➢ The yeasts from the genera Dekkera/Brettanomyces are considered to be the main organisms responsible for the production of volatile phenols from hydroxycinnamic acids (Heresztyn, 1986; Chatonnet et al., 1995, 1997).

➢ There are, however, some works that report the ability of wine lactic acid bacteria (LAB) to produce volatile phenols. Cavin et al (1993) tested several strains of LAB isolated from wine for their ability to metabolize *p*-coumaric and ferulic acids, in culture medium. They found that *Lactobacillus* and *Pediococcus* were able to metabolize the phenolic acids with the production of the corresponding ethylphenols. Chatonnet et al (1995), compared the ability of LAB to synthesize volatile phenols with that of *Dekkera/Brettanomyces*. *L. plantarum* was capable of producing significant quantities of ethylphenols, particularly, 4-ethylphenol, however the concentrations released were very small when compared to those formed by *Dekkera/Brettanomyces* species. Recently, Couto et al (2006) tested the ability of twenty different species of LAB to produce volatile phenols, in culture medium. They have shown that 37% of the strains studied were capable of producing volatile phenols from *p*-coumaric acid.

➢ **This work aims to study the influence of certain wine factors on the synthesis of volatile phenols by LAB and on the ratio vinylphenols/ethylphenols produced.**

MATERIALS AND METHODS

TABLE 3 - List of strains used in this study.

Strains
L. plantarum NCFB 1752
L. collinoides ESB 99
P. pentosaceus NCFB 990
ESB – Escola Superior de Biotecnologia Collection, Porto, Portugal NCFB – National Collection of Food Bacteria, Reading, England

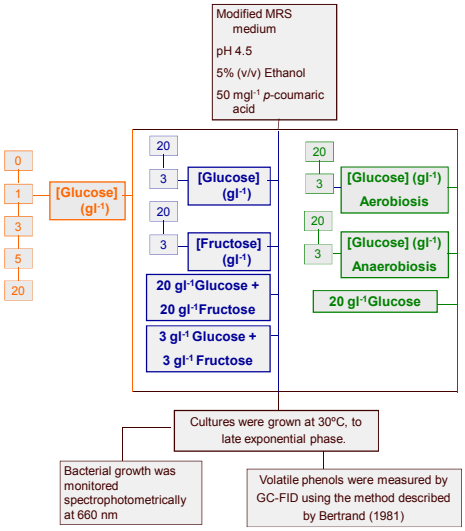

FIGURE 2 – Schematic representation of the methodology.

RESULTS

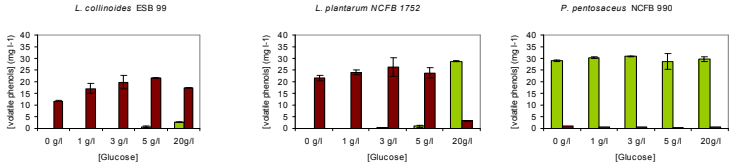

FIGURE 3 – Effect of glucose concentration on the production of 4-vinylphenol (Green bars) and 4-ethylphenol (Bordeaux bars) by *L. collinoides* ESB 99, *L. plantarum* NCFB 1752 and *P. pentosaceus* NCFB 990. Results are the average values of two experiments (with standard deviation).

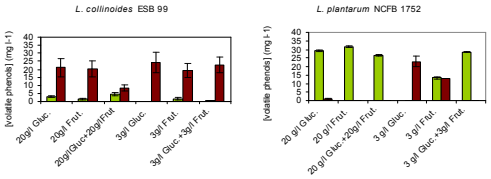

FIGURE 4 – Effect of fructose concentration on the production of 4-vinylphenol (Green bars) and 4-ethylphenol (Bordeaux bars) by *L. collinoides* ESB 99, *L. plantarum* NCFB 1752. Results are the average values of two experiments (with standard deviation).

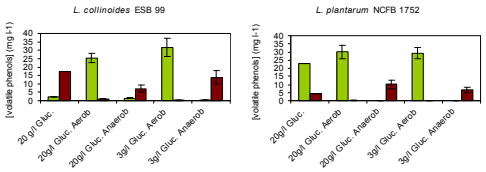

FIGURE 5 – Effect of oxygen on the production of 4-vinylphenol (Green bars) and 4-ethylphenol (Bordeaux bars) by *L. collinoides* ESB 99, *L. plantarum* NCFB 1752. Results are the average values of three experiments (with standard deviation).

RESULTS / CONCLUSIONS

▪ The concentration of glucose in media strongly influences the behaviour of *L. collinoides* ESB 99 and *L. plantarum* NCFB 1752. *L. plantarum* NCFB 1752 produces mainly 4VP in the presence of 20 gl^{-1} of glucose, while at 5 gl^{-1} and lower concentrations, 4EP is mainly or solely produced. The capacity of *L. collinoides* ESB 99 to produce 4EP is also higher at 3 and 5 gl^{-1} of glucose than at 20 gl^{-1}. The results suggest that the vinylphenol reductase activity of these bacteria could be enhanced in the presence of low concentrations of glucose, which may be associated with the cells necessity, in these conditions, to regenerate NAD$^+$, presumably the co-enzyme formed from the reduction of 4VP. The production of volatile phenols by *P. pentosaceus* NCFB 990 was not affected by the variation of glucose (Figure 3). Low levels of glucose (0-5 gl^{-1}) have strongly inhibited the growth of all strains (results not shown), however they were still able to produce significant amounts of volatile phenols.

▪ The addition of fructose to the medium, apparently has no effect on the reduction of 4VP into 4EP by *L. collinoides* ESB 99. In the case of *L. plantarum* NCFB 1752 the outcome of 4VP is favoured in the presence of fructose or when it is added to the medium along with glucose, being remarkable at lower concentrations. This effect might be related with the reduction of fructose into mannitol, accompanied by the regeneration of NAD$^+$. The growth of both strains was inhibited in the presence of fructose (results not shown).

▪ In the presence of oxygen the lactobacilli only produced 4VP suggesting that O_2 is used as electron acceptor allowing the regeneration of NAD$^+$. In anaerobiosis, the concentration of volatile phenols produced is lower, however the reduction of 4VP into 4EP is favoured.

▪ This study highlights the capacity of wine lactic acid bacteria to produce volatile phenols in sensorially significant amounts and that the ratio 4VP/4EP is considerably affected by certain wine factors which could be related to the intracellular NAD$^+$/NADH balance.

▪ Further research is required to elucidate the real impact of the metabolic activity of lactic acid bacteria on the levels of volatile phenols found in wines.

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