

ABSTRACT

Some wine microorganisms can produce volatile phenols (4-vinylphenol and 4-ethylphenol) from wine phenolic (p-coumaric and ferulic) acids. Volatile phenols have characteristic aromas which, certainly above a certain concentration threshold, have a negative effect on the overall aroma of a wine. Previous works showed that some strains of Lactic Acid Bacteria (LAB) can produce low concentrations of volatile phenols in near-wine conditions. In this work, thirty five strains of LAB (19 species) were screened for their ability to produce volatile phenols from the corresponding phenolic acids. Cells were cultivated in liquid media supplemented with p-coumaric or ferulic acid at 500 mg L⁻¹. The concentration of volatile phenols in the growth media was analysed by GC-FID. The results indicate that 13 strains (37%) were able to produce volatile phenols from p-coumaric acid, although only 3 (9%) produced 4-ethylphenol as the final product. Seven strains (20%) were able to convert ferulic acid to 4-vinylguaicol but none produced 4-ethylguaicol. Seven (of 8) strains of *Pediococcus* and 6 (of 25) strains of *Lactobacillus* were able to produce volatile phenols from phenolic acids in sensorially significant amounts. The two *Oenococcus oeni* strains studied did not produce volatile phenols. Strains which were found to produce volatile phenols were used in subsequent studies at lower phenolic acid concentrations. Experiments with added 5 mg L⁻¹ of p-coumaric acid showed that some strains can still produce relatively high (up to 800 µg L⁻¹) concentrations of 4-ethylphenol.

INTRODUCTION

Volatile phenols are aromatic compounds that affect, in a negative way, the wine quality giving "animal", "leather", "horse sweat" odors to the wine. The presence of these compounds in wine is nowadays of great concern among wine producers being considered a key point in the control of wine quality.

The precursors of the volatile phenols are natural constituents of grape juice and wine – the hydroxycinnamic acids p-coumaric and ferulic acids. The transformation involves a sequence of two enzymatic activities. In the first, a carboxylase decarboxylates the hydroxycinnamic acid in the corresponding vinyl derivative (4-vinylphenol from p-coumaric acid or 4-vinylguaicol from ferulic acid). In the second reaction a reductase converts the vinyl in the corresponding ethyl compound (4-ethylphenol or 4-ethylguaicol) (FIGURE 1).

Which microorganisms are involved in the metabolism of the hydroxycinnamic acids has been under discussion. The ability of *Brethanomyces* to produce volatile phenols has been well demonstrated in the literature (Heresztyn, 1986, Chatonnet *et al.*, 1995, Edlin *et al.*, 1995) being probably the main organism involved.

Other yeasts and bacteria have, however, been shown to be able to metabolize hydroxycinnamic acids (Edlin *et al.*, 1995). Concerning bacteria, Cavin *et al.* (1993) have shown that p-coumaric and ferulic acids were decarboxylated by *Lactobacillus brevis*, *Lact. plantarum* and *Pediococcus*. Chatonnet *et al.* (1995) reported that some strains of *Lact. brevis* and *Ped. pentosaceus* were capable of decarboxylating p-coumaric acid to form 4-vinylphenol as actively as *S. cerevisiae*. *Oenococcus oeni* synthesized very small quantities of 4-vinylphenol. Only *Lact. plantarum* was capable of producing ethylphenols, but in low concentrations when compared to *Brethanomyces*.

Thus, it can be seen in the literature, and also from preliminary assays in our laboratory, that certain LAB strains decarboxylate phenolic acids into vinylphenol; the capacity then to produce ethylphenols being much rarer.

Aside from standard media favorable to their development, the ability of LAB to produce volatile phenols in wine was never studied in depth. Further research is needed concerning the role of wine microorganisms (other than *Brethanomyces*), and possible interactions between them, in the production of volatile phenols.

The main objective of this work

is to evaluate the ability of wine lactic acid bacteria to produce volatile phenols, i.e. to evaluate the cinnamate decarboxylase and reductase activity of these organisms and the ratio vinylphenols / ethylphenols produced

References

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PRODUCTION OF VOLATILE PHENOLS BY LACTIC ACID BACTERIA

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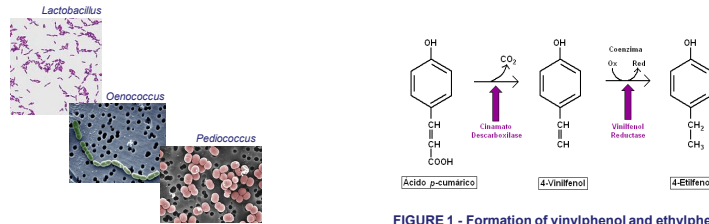


FIGURE 1 - Formation of vinylphenol and ethylphenol from p-coumaric acid

TABLE 1 - Production of volatile phenols by lactic acid bacteria, in MRS/Tomato Juice broth (50:50) (pH 4.5, 5% v/v ethanol) supplemented with 500 mg/L of p-coumaric acid. The % conversion values represent molar conversions (moles of product/moles of substrate in media).

Strain	Conversion		Conversion	
	(RVPI) (ppm)	(% molar)	(4EPI) (ppm)	(% molar)
<i>L. brevis</i> 118	1.84	11%	364.07	103%
<i>L. brevis</i> 6908	39.38	11%	364.07	103%
<i>L. buchneri</i>	0.76	0%	0.11	0%
<i>L. casei</i> 119	3.18	1%	0.01	0%
<i>L. casei</i> 13087	0.48	0%	0.06	0%
<i>L. casei</i> 29	0.63	0%	0.01	0%
<i>L. collinoides</i> 37	382.00	104%	6.62	2%
<i>L. collinoides</i> 99	0.13	0%	375.16	105%
<i>L. confusus</i>	3.49	1%	0.00	0%
<i>L. curvulus</i>	0.63	0%	0.01	0%
<i>L. fructivorans</i> 122	0.32	0%	0.01	0%
<i>L. fructivorans</i> 92	0.21	0%	0.05	0%
<i>L. hilgardii</i> 17	0.68	0%	0.03	0%
<i>L. hilgardii</i> 19	0.67	0%	0.01	0%
<i>L. hilgardii</i> 34	0.67	0%	0.01	0%
<i>L. hilgardii</i> 35	0.41	0%	0.01	0%
<i>L. hilgardii</i> 37	0.42	0%	0.01	0%
<i>L. hilgardii</i> 41	0.63	0%	0.03	0%
<i>L. hilgardii</i> 5	2.39	1%	0.00	0%
<i>L. hilgardii</i> 9	0.48	0%	0.04	0%
<i>L. kefir</i>	0.87	0%	0.01	0%
<i>L. mali</i>	358.56	103%	2.58	1%
<i>L. plantarum</i>	290.19	80%	20.97	6%
<i>L. sakei</i>	382.66	110%	0.37	0%
<i>L. vitisaezensis</i>	304.63	87%	0.62	0%
<i>Le. mesenteroides</i>	0.76	0%	0.10	0%
<i>O. oeni</i> 132	0.77	0%	0.02	0%
<i>O. oeni</i> VIF	1.24	0%	0.00	0%
<i>P. acidilactici</i>	399.25	119%	0.85	0%
<i>P. damnosus</i>	88.52	25%	0.17	0%
<i>P. parvulus</i>	0.71	0%	0.04	0%
<i>P. pentosaceus</i>	299.08	86%	0.59	0%
<i>Pediococcus</i> 185	264.68	75%	0.70	0%
<i>Pediococcus</i> 222	230.34	68%	0.55	0%
<i>Pediococcus</i> X	293.91	84%	0.48	0%

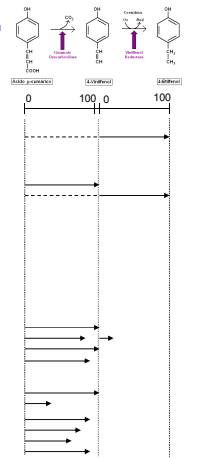


FIGURE 2 - representation of the extension of conversion of p-coumaric acid into 4-vinylphenol and/or 4-ethylphenol.

TABLE 2 - Production of volatile phenols by lactic acid bacteria, in MRS/Tomato Juice broth (50:50) (pH 4.5, 5% v/v ethanol) supplemented with 500 mg/L of ferulic acid. The % conversion values represent molar conversions (moles of product/moles of substrate in media)

Strain	Conversion		Conversion	
	(RVGI) (ppm)	(% molar)	(4EGI) (ppm)	(% molar)
<i>L. brevis</i> 118	2.06	3%	0.02	0%
<i>L. brevis</i> 6908	9.52	1%	0.00	0%
<i>L. buchneri</i>	2.42	1%	0.00	0%
<i>L. casei</i> 119	1.84	1%	0.02	0%
<i>L. casei</i> 13087	2.30	1%	0.00	0%
<i>L. casei</i> 29	2.32	1%	0.00	0%
<i>L. collinoides</i> 37	0.00	0%	0.00	0%
<i>L. collinoides</i> 99	75.82	21%	0.43	0%
<i>L. confusus</i>	0.38	0%	0.00	0%
<i>L. curvulus</i>	1.68	0%	0.00	0%
<i>L. fructivorans</i> 122	2.58	1%	0.01	0%
<i>L. fructivorans</i> 92	0.39	0%	0.02	0%
<i>L. hilgardii</i> 17	3.00	1%	0.02	0%
<i>L. hilgardii</i> 19	2.61	1%	0.01	0%
<i>L. hilgardii</i> 34	2.05	1%	0.01	0%
<i>L. hilgardii</i> 35	2.73	1%	0.02	0%
<i>L. hilgardii</i> 37	2.35	1%	0.01	0%
<i>L. hilgardii</i> 41	2.71	1%	0.02	0%
<i>L. hilgardii</i> 5	2.57	1%	0.00	0%
<i>L. hilgardii</i> 9	2.83	1%	0.01	0%
<i>L. kefir</i>	2.84	1%	0.02	0%
<i>L. mali</i>	0.00	0%	0.00	0%
<i>L. plantarum</i>	0.00	0%	0.00	0%
<i>L. sakei</i>	4.81	1%	0.00	0%
<i>L. vitisaezensis</i>	10.60	3%	0.00	0%
<i>Le. mesenteroides</i>	1.70	0%	0.01	0%
<i>O. oeni</i> 132	1.83	0%	0.01	0%
<i>O. oeni</i> VIF	1.68	0%	0.00	0%
<i>P. acidilactici</i>	17.58	0%	0.00	0%
<i>P. damnosus</i>	3.07	1%	0.02	0%
<i>P. parvulus</i>	1.79	0%	0.00	0%
<i>P. pentosaceus</i>	29.01	8%	0.20	0%
<i>Pediococcus</i> 185	12.41	3%	0.37	0%
<i>Pediococcus</i> 222	64.68	18%	0.56	0%
<i>Pediococcus</i> X	64.83	18%	0.15	0%

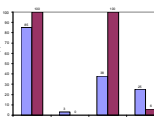


FIGURE 4 - % conversion values of p-coumaric acid (at 50 ppm: purple bars; at 500 ppm: bordeaux bars) into 4-ethylphenol.

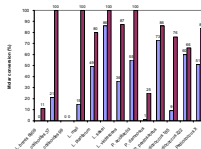


FIGURE 3 - % conversion values of p-coumaric acid (at 50 ppm: purple bars; at 500 ppm: bordeaux bars) into 4-vinylphenol.

CONCLUSIONS

It is shown that certain lactic acid bacteria are able to produce volatile phenols:

- Thirteen strains (37%) were able to produce volatile phenols from p-coumaric acid, although only three (9%) produced 4-ethylphenol (TABLE 1).

- Seven (out of 8) strains of *Pediococcus* and six (out of 25) strains of *Lactobacillus* were able to produce volatile phenols from p-coumaric acid in sensorially significant amounts.

- The two *Oenococcus oeni* strains studied did not produce volatile phenols.

- Strains which were found to produce volatile phenols were used in subsequent studies at lower phenolic acid concentrations. Experiments with added 50 or 5 mg L⁻¹ of p-coumaric acid showed that some strains can still produce relatively high concentrations of 4-ethylphenol (data not shown).

- The % conversion is dependent on the concentration of the substrate. Except for *Lact. plantarum*, higher % conversion values were obtained with 500 ppm of p-coumaric acid than with 50 (FIGURES 3 and 4).

- The capacity to produce volatile phenols from ferulic acid is much lower (TABLE 2) than from p-coumaric acid. Seven strains (20%) were able to convert ferulic acid to 4-vinylguaicol, at low conversion rates, but none produced 4-ethylguaicol.

Future steps

- To study the behaviour of volatile phenols LAB producing strains in real wine conditions

- To study the factors which may influence the production of volatile phenols

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