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Acetaldehyde metabolism by wine lactic acid bacteria

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Abstract

Acetaldehyde is a volatile flavor compound present in many fermented foods and is important in the production of red and white wines. Nine strains of the genera *Lactobacillus* and *Oenococcus* were able to metabolize acetaldehyde in a resting cell system, whereas two *Pediococcus* strains were not. Acetic acid and ethanol were produced from its degradation. A *Lactobacillus* and an *Oenococcus* were able to degrade SO_2-bound acetaldehyde, as well. A coincubation of resting cells of *Saccharomyces bayanus* Première Cuvée and *Oenococcus oeni* Lo111 showed that strain Lo111 metabolized acetaldehyde produced by the yeast. The ability of malolactic bacteria to degrade free and SO_2-bound acetaldehyde has implications for sensory and color qualities and the use of SO_2 in wine.

Keywords: Acetaldehyde; Malolactic fermentation; Lactic acid bacterium; Sulfur dioxide

1. Introduction

Malolactic fermentation (MLF) is a secondary fermentation in wine during which L-malic acid is degraded to L-lactic acid and carbon dioxide. MLF usually occurs after yeasts have completed the primary alcoholic fermentation and is important for the deacidification of high acid wines and for flavor modification [1]. MLF may occur spontaneously by lactic acid bacteria (LAB) naturally present in wine or may be induced by the addition of one or more strains of commercial wine LAB.

Acetaldehyde is one of the most important sensory carbonyl compounds formed during vinification and mainly originates from yeast metabolism during alcoholic fermentation [2]. Formation of acetaldehyde and its concentrations in several alcoholic beverages have been reviewed recently by Liu et al. [3]. Acetaldehyde is highly volatile and when present in excess imparts an undesirable green, grassy, apple-like aroma [4] which is usually masked by the addition of sulfur dioxide (SO_2) [5]. SO_2 is also used as an antimicrobial and antioxidant in wine and acetaldehyde-bound SO_2 is less effective in these roles [5,6]. Acetaldehyde further plays a role in the color development of red wines by promoting rapid polymerization between anthocyanins and catechins or tannins, forming stable polymeric pigments resistant to SO_2 bleaching [7,8].

Acetaldehyde consumption during MLF has been observed repeatedly [8,9]. Several studies demonstrated the inhibitory effect of acetaldehyde-bound SO_2 on LAB growth [10,11]. They suggested that the metabolism of the acetaldehyde moiety of SO_2-bound acetaldehyde by LAB led to release of free SO_2 and thus inhibited LAB growth. However, to date no definitive study of the impact of wine LAB on free and bound acetaldehyde in wine has been carried out [3]. More information is available about acetaldehyde metabolism in dairy LAB. Some dairy LAB (in particular *Leuconostoc mesenteroides* subsp. *cremoris*) are able to metabolize acetaldehyde, producing ethanol and acetic acid as final products [12,13]. At low levels of acetaldehyde (< 100 mg l^{-1}), growth of dairy LAB was stimulated while at high levels (> 100 mg l^{-1}) growth was inhibited [14]. It has been suggested that acetaldehyde is reduced to ethanol and thus acts as a hydrogen acceptor in the regeneration of NAD, necessary for sugar fermentation. This alternative NAD regeneration could lead to the production of extra ATP and thus increase growth of bacteria [14,15].

The aim of this research was to survey common malolactic wine LAB of the genera *Lactobacillus*, *Oenococcus* and *Pediococcus* for their ability to metabolize acetaldehyde. Because of its prevalence and importance in wine, the degradation of SO_2-bound acetaldehyde by selected

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LAB was investigated, as well. The ability of wine LAB to metabolize acetaldehyde produced by yeast during coincubation was also studied to investigate possible microbial interactions between yeast and wine LAB in wine produced by simultaneous alcoholic and MLFs.

2. Materials and methods

2.1. Microorganisms

LAB strains originally isolated from wine and wine yeast *Saccharomyces bayanus* 'Red Star' Première Cuvée (Universal Foods, Oakland, CA, USA) were from the Wine Microbiology Laboratory Culture Collection of the Institute of Molecular BioSciences, Massey University, Palmerston North, New Zealand. All wine LAB strains are heterofermentative with the exception of *Pediococcus damnosus* CUC-4, *Pediococcus* sp. 44.40 and *Lactobacillus delbrueckii* CUC-1 which are homofermentative.

2.2. Culture conditions and resting cell experiments

Resting cell experiments were performed according to Mira de Orduña et al. [16] with modifications. Bacterial cells were grown in 500 ml of a complex medium (TJAG [17], without addition of arginine) at 30°C to the late-exponential phase and harvested by centrifugation at $5000 \times g$ for 10 min at 15°C. Yeast cells were grown in 500 ml YM broth (Difco, Detroit, MI, USA). The cells were washed twice with buffer (7.5 g tartaric acid, 1 g $MgSO_4 \cdot 7H_2O$ and 0.25 g $MnSO_2 \cdot 4H_2O$ per litre deionized water, adjusted to pH 4.2 with 5 M NaOH). Cell pellets were then resuspended in appropriate amounts (5–25 ml) of the same buffer adjusted to pH 3.6 to give cell suspensions with biomass concentrations of 3–6 mg l^{-1} dry weight and were pipetted into small glass vials. The glass vials were placed in a waterbath (30°C) and stirred gently using magnetic stirrers. To start experiments, acetaldehyde (free or SO_2-bound) was added to cell suspensions to give concentrations of approximately 50 mg l^{-1}. SO_2-bound acetaldehyde was prepared by adding potassium metabisulfite in excess to an acetaldehyde solution. SO_2 binds strongly to acetaldehyde and free SO_2 was removed by lowering the pH of the buffer to 1.5 with HCl (5 M) and purging with air until the absence of free SO_2 in the solution was confirmed by analysis (see below). For the coincubation of yeast and wine LAB, 1:1 mixtures of either yeast suspension and water or yeast and wine LAB suspensions adjusted to 2 g l^{-1} glucose were used. Samples were taken periodically during incubation, centrifuged (5 min at $10\,000 \times g$) and stored frozen (−18°C) for subsequent analysis.

2.3. Analysis

The dry weight of cells in resting cell experiments was determined by pipetting 1.5 ml of culture into a preweighed micro-centrifuge tube. The supernatant was removed after centrifugation ($10\,000 \times g$ for 10 min) and the tube containing the cell pellet dried overnight in a temperature-controlled oven at 100°C. The difference in weight after cooling was corrected for weight loss of the tubes by subjecting empty tubes to the same procedure. Acetaldehyde, ethanol and acetic acid concentrations were determined using enzymatic test kits from Roche Molecular Biochemicals, New Zealand. SO_2 concentration (free and total) was measured iodometrically by the Ripper procedure [18].

3. Results

Eleven strains of wine LAB (seven commercially available) were surveyed for their ability to degrade free acetaldehyde. Results of this survey are shown in Table 1. All strains, except *P. damnosus* CUC-4 and *Pediococcus* sp. 44.40, were able to utilize acetaldehyde.

Degradation of acetaldehyde led to production of ethanol in all strains. To consider a possible loss of acetaldehyde or ethanol during the incubation by evaporation, uninoculated controls containing acetaldehyde or ethanol

Table 1
Acetaldehyde degradation and ethanol production by resting cells of wine LAB in tartrate buffer (pH 3.6) at 30°C

Bacteria	Strain	Carbohydrate fermentation	Acetaldehyde degradation	Ethanol production
O. oeni	MCW[a]	heterofermentative	+	+
O. oeni	VFO[a]	heterofermentative	+	+
O. oeni	EQ54[a]	heterofermentative	+	+
O. oeni	ML34	heterofermentative	+	+
O. oeni	2001[a]	heterofermentative	+	+
O. oeni	Lo111[a]	heterofermentative	+	+
L. hilgardii	MHP[a]	heterofermentative	+	+
L. delbrueckii	CUC-1	homofermentative	+	+
L. buchneri	CUC-3	heterofermentative	+	+
P. damnosus	CUC-4	homofermentative	−	−
Pediococcus sp.	44.40[a]	homofermentative	−	−

[a]Commercially available strain.

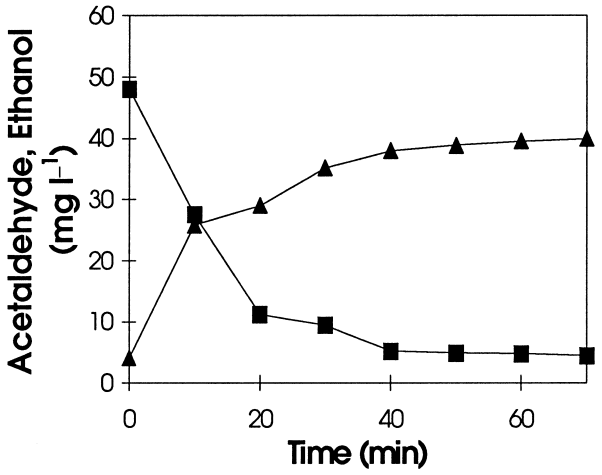

Fig. 1. Degradation of acetaldehyde and production of ethanol by resting cells of *O. oeni* VFO in tartrate buffer (pH 3.6) at 30°C and 50 mg l^{-1} initial acetaldehyde. Acetaldehyde (-■-), ethanol (-▲-).

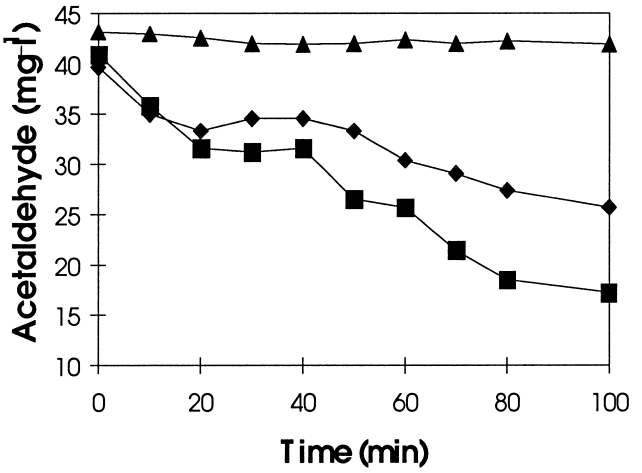

Fig. 3. Degradation of SO_2-bound acetaldehyde by resting cells of *L. buchneri* CUC-3 and *O. oeni* MCW in tartrate buffer (pH 3.6) at 30°C and 50 mg l^{-1} initial SO_2-bound acetaldehyde. *O. oeni* MCW (-◆-), *L. buchneri* CUC-3 (-■-), uninoculated control (-▲-).

at 50 mg l^{-1} were evaluated at the same time. In these control assays, no significant reduction of the substrates occurred. Controls containing only the cell suspensions and no substrates showed no increase in concentrations of acetaldehyde or ethanol during the course of the experiment.

Fig. 1 shows a typical example of acetaldehyde degradation and ethanol production by *Oenococcus oeni* VFO. Acetaldehyde degradation rates were found to be strain specific. On a molar basis, the total amount of ethanol produced during incubations did not fully account for the amount of acetaldehyde degraded by any of the strains. Molar recoveries ranged between 40 and 60%. Therefore, two strains (*Lactobacillus hilgardii* MHP and *L. delbrueckii* CUC-1) were also tested for the production of acetic acid in addition to ethanol from acetaldehyde degradation. Fig. 2 shows data from this experiment for strain MHP. Both strains MHP and CUC-1 produced acetic acid besides ethanol. The added total amounts of ethanol and acetic acid produced during acetaldehyde degradation accounted for about 75% of the acetaldehyde degraded in the case of *L. hilgardii* MHP and about 60% for *L. delbrueckii* CUC-1. It was not possible to recover the total amount of degraded acetaldehyde as ethanol or acetic acid.

Strains *L. buchneri* CUC-3 and *O. oeni* MCW were further tested for their ability to degrade SO_2-bound acetaldehyde (Fig. 3). Compared to the uninoculated control, both strains degraded significant amounts of SO_2-bound acetaldehyde (57% for strain CUC-3 and 40% for strain MCW). It was not possible to measure the release of free

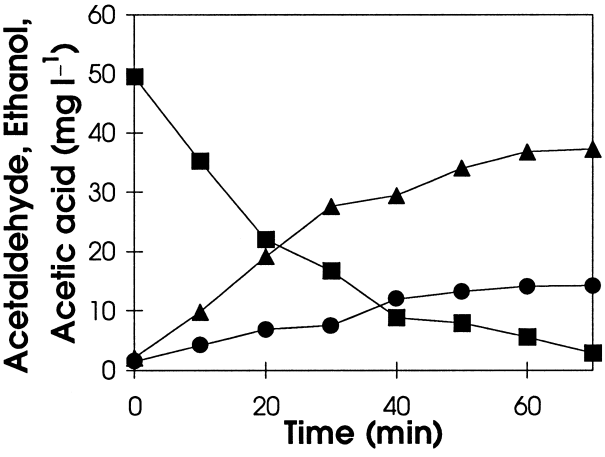

Fig. 2. Degradation of acetaldehyde and production of ethanol and acetic acid by resting cells of *L. hilgardii* MHP in tartrate buffer (pH 3.6) at 30°C and 50 mg l^{-1} initial acetaldehyde. Acetaldehyde (-■-), acetic acid (-●-), ethanol (-▲-).

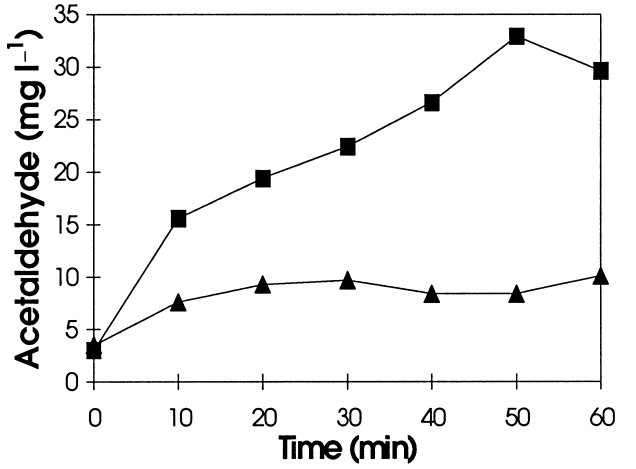

Fig. 4. Comparison of acetaldehyde degradation by resting cells of *S. bayanus* Première Cuvée and a mixture of resting cells of both *S. bayanus* Première Cuvée and *O. oeni* Lo111. Both assays were carried out in tartrate buffer (pH 3.6) at 30°C with 2 g l^{-1} initial glucose. *S. bayanus* Première Cuvée (-■-), *S. bayanus* Première Cuvée and *O. oeni* Lo111 (-▲-).

SO_2 from the degradation of SO_2-bound acetaldehyde because the SO_2 analysis method was not sensitive enough at the small volumes used here.

Fig. 4 shows the comparison of the incubation of wine yeast *S. bayanus* Première Cuvée with the coincubation of the same yeast and *O. oeni* Lo111. Both assays contained glucose as sole substrate. Whereas incubation of only the yeast led to significant production of acetaldehyde reaching a maximum of 33 mg l^{-1} after 50 min, the presence of malolactic strain Lo111 in the coincubation limited acetaldehyde formation to a maximum of 10 mg l^{-1}.

4. Discussion

Acetaldehyde is an important flavor compound in wine and plays a role in the color development of red wines. In this study, the degradation of free and SO_2-bound acetaldehyde by several wine LAB in a model wine buffer was investigated. Acetaldehyde degradation was independent from the sugar fermentation pathway – both heterofermentative and homofermentative strains were able to degrade acetaldehyde. However, two pediococci tested did not degrade acetaldehyde and the degradation rates calculated from oenococci and lactobacilli were strain dependent. This result has implications for the selection of wine LAB for conducting MLF. Depending on the wine style, it may be beneficial to use efficient acetaldehyde-degrading strains; e.g. in white wines with high acetaldehyde concentrations from alcoholic fermentation or to reduce the need to mask acetaldehyde with SO_2, which has health implications [19]. On the other hand, partial or complete acetaldehyde degradation may be undesirable; e.g. in red wine production for color development or to avoid masking of other flavor compounds (e.g. diacetyl) by free SO_2 released from degradation of SO_2-bound acetaldehyde [20].

Although it was not possible to recover the entire amount of acetaldehyde degraded as end products, two major catabolic products were identified as ethanol and acetic acid, confirming data from dairy LAB [21] for malolactic bacteria. The impact of both products on the chemical and sensory composition of a wine is believed to be limited, since the increase in ethanol and acetic acid from acetaldehyde degradation would be insignificant. This is because acetaldehyde levels found in wines that have not undergone MLF are small (50–80 mg l^{-1}) [22].

SO_2-bound acetaldehyde was degraded by lactobacilli and oenococci, though degradation rates were significantly lower in comparison with those calculated for free acetaldehyde. The slower degradation was probably the result of metabolic inhibition by the antimicrobial agent SO_2 released from SO_2-bound acetaldehyde during its degradation [10,11]. Since SO_2 binds very strongly to acetaldehyde, the latter can be regarded as an SO_2 reservoir in wine. The degradation of SO_2-bound acetaldehyde by SO_2-sensitive strains may therefore play a role in causing stuck or sluggish MLF. But release of free SO_2 from this reservoir will mean, as well, that less SO_2 will have to be added to fulfil its functions as an antimicrobial and antioxidant in wine.

During coincubation experiments with resting cells, acetaldehyde formed by wine yeast was degraded simultaneously by malolactic bacteria. This indicates that it may be possible to decrease or even avoid acetaldehyde formation in wine production by carrying out simultaneous alcoholic and MLFs. This technique also provides the possibility to produce a wine without the addition of SO_2 when a suitable combination of a high SO_2-producing yeast and a strong acetaldehyde-degrading LAB was used.

This work has shown the impact of malolactic bacteria on free and SO_2-bound acetaldehyde. Strain selection for conducting MLF is likely to be important regarding sensory and color qualities and the use of SO_2 in wines. Therefore, strain specific characteristics regarding acetaldehyde metabolism will be examined further in wine.

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