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Biochemical characterisation of the esterase activities of wine lactic acid bacteria

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Abstract Esters are an important group of volatile compounds that can contribute to wine flavour. Wine lactic acid bacteria (LAB) have been shown to produce esterases capable of hydrolysing ester substrates. This study aims to characterise the esterase activities of nine LAB strains under important wine conditions, namely, acidic conditions, low temperature (to 10°C) and in the presence of ethanol (2–18% v/v). Esterase substrate specificity was also examined using seven different ester substrates. The bacteria were generally found to have a broad pH activity range, with the majority of strains showing maximum activity close to pH 6.0. Exceptions included an *Oenococcus oeni* strain that retained most activity even down to a pH of 4.0. Most strains exhibited highest activity across the range 30–40°C. Increasing ethanol concentration stimulated activity in some of the strains. In particular, *O. oeni* showed an increase in activity up to a maximum ethanol concentration of around 16%. Generally, strains were found to have greater activity towards short-chained esters (C2–C8) compared to long-chained esters (C10–C18). Even though the optimal physicochemical conditions for enzyme activity differed from those found in wine, these findings are of potential importance to oenology because significant activities remained under wine-like conditions.

Keywords Esters · *Oenococcus* · *Lactobacillus* · *Pediococcus* · Hydrolysis

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Introduction

Wine is produced by the enzyme-driven fermentation of grape juice. In addition to those enzymes produced by yeast during alcoholic fermentation, a range of additional enzyme activities of various sources can influence both the quality and the style of the final wine. Lactic acid bacteria (LAB) represent one source of such enzymes. Three genera of LAB are associated with grape juice and wine, namely, *Lactobacillus*, *Pediococcus* and, perhaps most importantly, *Oenococcus*, of which there is only one species, *Oenococcus oeni* (Fugelsang and Edwards 2007). The primary function of LAB in winemaking is to conduct the malolactic fermentation (MLF), which involves the decarboxylation of L-malic acid to L-lactic acid. In addition, LAB have further impacts on wine composition (Liu 2002), yet these activities are far from being characterised to the extent of the malolactic enzyme.

LAB associated with grape juice and wine have been shown to produce a range of enzymatic activities that could potentially be important in wine production, including those capable of hydrolysing ester substrates (e.g. Davis et al. 1988; Matthews et al. 2004, 2006). All strains of LAB examined were found to have activity towards three different ester substrates, although a genus and strain dependence was observed, with *O. oeni* generally having higher esterase activity than the lactobacilli and pediococci (Matthews et al. 2006). Potential substrates for such esterases in the wine environment are qualitatively one of the most important groups of volatile compounds in determining wine flavour (Ferreira et al. 1998; Lilly et al. 2006). Such esters can be generally categorised as either ethyl esters of fatty acids, acetate esters of higher alcohols or esters of organic acids. Esters of organic acids are the predominant group in wine, followed by the acetate esters

and ethyl esters of fatty acids (Etievant 1991). However, some individual ethyl esters of fatty acids and acetate esters are also important given their low sensory threshold (Simpson and Miller 1984; Aznar et al. 2001; Ferreira et al. 2002).

Minor amounts of some esters are derived from the grape berry, but the principle source of esters in wine is from their production by yeast during alcoholic fermentation. Ethanol is a key substrate in the formation of many esters thus their biosynthesis is, at least to some extent, concurrent with that of ethanol during alcoholic fermentation (Rapp and Mandery 1986; Herraiz and Ough 1993; Plata et al. 2003). The quantities and types of esters produced during fermentation are dependent upon the yeast strain conducting the fermentation (Soles et al. 1982; Antonelli et al. 1999; Rojas et al. 2003), as well as the physiochemical conditions including temperature, pH and nitrogen levels of the juice or must (Lambrechts and Pretorius 2000). Ethyl acetate is the most important ester found in wine, contributing a desirable fruity aroma at low concentrations. At higher concentrations (>200 mg/L), an undesirable 'solvent' character arises, which is detrimental to both wine aroma and quality (Dittrich 1983; Margalit 1997). Ethyl acetate along with other esters, such as methylbutyl acetate, can therefore contribute to 'ester taint' (Sponholz et al. 1982; Sponholz 1993). Therefore, selective production or degradation of esters could be valuable in enhancing wine composition and overcoming taints in wines. To achieve such selectivity, a greater understanding of the esterase profile of LAB is required.

Biochemical characterisations of LAB esterases, including those of *Lactobacillus* (El Soda et al. 1986a; Khalid et al. 1990; Castillo et al. 1999) and *Pediococcus* (Østdal et al. 1996) strains, have been published. Much of this work, however, has been carried out in the dairy industry, where esterases contribute to the flavour development of some foods, in particular, cheeses (Holland et al. 2005). While esters are generally accepted as being important volatile compounds in wine, parallel work has not been conducted in the wine context, despite the fact that physicochemical conditions are generally harsher. For example, wine pH ranges from 2.8 to 4.0, with low pH being important for reducing the risk of microbial spoilage and promoting red wine colour. Temperatures range from less than 10°C (for example, during juice settling) to 25°C (for example, during alcoholic fermentation). Also, the concentration of ethanol, a potential inhibitor of enzyme function, is particularly important after alcoholic fermentation, which coincides with the time when many wines undergo MLF (Liu 2002). LAB esterases must therefore be able to function under these conditions if they are going to impact on wine esters. In this study, we investigated the influence of the above critical oenological parameters on the esterase activity of nine LAB strains.

Materials and methods

Bacterial strains, preculturing conditions and preparation of biomass

In total, nine strains of LAB, three lactobacilli, three pediococci and three oenococci, were used in this study (Table 1). Some of the bacteria were isolated from freeze-dried LAB starter cultures for use in commercial wine-making. Two *Lactobacillus* strains, Lac34 and Lac35, and *Pediococcus* strain Ped42 were purchased from the Australian Starter Culture Research Centre, while *Pediococcus* strain Ped18 was isolated from a commercial olive product. Bacteria were stored and cultured as described previously (Matthews et al. 2006). Briefly, all isolates were held on cryopreservative-treated beads (Protect, Technical Service Consultants) at −80°C until required. In preparation for experiments, bacteria were precultured in de Mann Rogosa Sharp medium, which was supplemented with 20% (v/v) preservative-free apple juice and adjusted to pH 5.0 prior to autoclaving (MRS + AJ). When required for experiments, the precultures of bacteria were used to inoculate 5 mL volumes of fresh MRS + AJ broth to yield an optical density at 600 nm (OD_{600}) of approximately 1.0 (*O. oeni*) or 0.3 (lactobacilli and pediococci) and incubated at 26°C for 24 h (lactobacilli and pediococci) or 40 h (*O. oeni*).

All of the esterase assays conducted in this study consisted of liquid suspensions of whole bacterial cells. The number of bacterial cells required to produce an OD_{600} of 0.5 in the final assay was determined spectrophotometrically. The required volume of cells was harvested and washed once in 0.85%

Table 1 Details of the LAB strains used in the biochemical characterisation study

Strain	Genus/species[a]	Origin
Lac26	*Lactobacillus* sp.	Lallemand nuovi Ceppi Oo1
Lac34	*Lactobacillus hilgardii* CSCC-5489	SCRC
Lac35	*Lactobacillus buchneri* CSCC-5400	SCRC
Ped11	*Pediococcus* sp.	Lalvin MCW
Ped18	*Pediococcus* sp.	Olives
Ped42	*Pediococcus pentosaceus* CSCC-2304	SCRC
O.oeni8	*Oenococcus oeni*	CHR Hansen Viniflora oenos
O.oeni9	*Oenococcus oeni*	CHR Hansen Viniflora oenos
O.oeni12	*Oenococcus oeni*	Lalvin 3X 1Step

SCRC purchased from the Australian Starter Culture Research Centre collection

[a] Species listed where it is known

(w/v) NaCl solution by centrifugation (5,000×*g*, 5 min) to remove the culturing media before being resuspended in an appropriate volume of 0.1 M McIlvaine buffer (Dawson et al. 1986) and aliquoted into the reaction tubes. Dry cell weights were determined using 10-mL culture samples adjusted to an OD_{600} of 0.5.

Determination of esterase activity

Esterase assays were conducted using *p*-nitrophenyl (*p*NP) octanoate substrate with the exception of the substrate-specificity trials, in which seven different *p*NP-linked ester substrates were used, as described below. Reaction mixtures (final volume 1 mL) were prepared as detailed in each section below and contained bacterial biomass to yield an optical density at OD_{600} of 0.5. Reactions were incubated at 37°C for 2 h, with the exception of the temperature optima experiments (see below). Following incubation, cells were pelleted by centrifugation (13,000×*g*, 7 min), the supernatant (900 μL) was transferred to a 48-well microplate (Costar No 3548, Corning) and the liberated *p*-nitrophenol was quantified immediately by measuring the absorbance at 410 nm using a microplate spectrophotometer (μQuant, Bio-Tek Instruments). The results were then corrected for non-enzymatic degradation of the ester substrate using a cell-free control of the same reaction mixture that was treated in the same manner. All assays and controls were performed in triplicate and activities were quantified by comparing with *p*-nitrophenol standards.

Influence of pH, temperature or ethanol on esterase activity

The influence of pH on esterase activity was determined by varying the pH of the reaction mixture across the range pH 3.0 to 8.0. The buffers used were 0.1 M McIlvaine buffer (pH 3.0 to 7.5) and Na_2HPO_4/NaH_2PO_4 buffer (pH 8.0) (Dawson et al. 1986). Briefly, 1-mL reactions were prepared by combining 860 μL of the appropriate buffer, 100 μL of the required cell suspension in the same buffer and 40 μL of a 25-mM stock *p*NP-octanoate solution in ethanol, to yield a final substrate concentration of 1 mM. Following incubation and centrifugation (13,000×*g*, 7 min), 900 μL of each supernatant was transferred to a fresh 48-well microplate. For alkalinisation, 300 μL of 0.5 M NaOH was then added to the pH-3.0 to -4.0 samples and 100 μL of 0.5 M NaOH was added to the pH-4.5 and -5.5 samples prior to absorbance measurements being taken as described above. Results were quantified by comparison with standard *p*-nitrophenol solutions prepared under the same conditions.

Esterase activity was studied at six temperatures across the range of 10 to 60°C in dry heating blocks or a PCR thermocycler. Accuracy of temperature settings was confirmed with a thermometer immediately prior to initiation of the experiment. Assays were carried out as described for the pH optima study, but for all samples, 0.1 M McIlvaine buffer of pH 5.0 was used. Reaction mixtures containing only buffer and substrate were equilibrated at the appropriate temperature for 10 min before the bacterial cell suspension was added to initiate the reaction. Following incubation, all samples were centrifuged, transferred into 48-well microplates and alkalinised as above before absorbances were measured.

The effect of the presence of ethanol on esterase activity was studied at nine ethanol concentrations ranging from 2 to 18% (v/v). Reaction mixtures were prepared as described for the temperature optima experiments, but different volumes of ethanol were added from 20 μL (2% v/v) up to 180 μL (18% v/v) and the volume of buffer was adjusted accordingly to maintain a final reaction volume of 1 mL. Reactions were incubated for 2 h at 37°C and treated post-incubation as described for the temperature optima samples.

Determination of substrate specificity

To determine the specificity of individual LAB strains for different ester substrates, seven *p*NP-linked esters of varying carbon-chain length were used: *p*NP-acetate (Sigma), -butyrate (Sigma), -octanoate (Jomar), -decanoate (Sigma), -dodecanoate (Fluka), -tetradecanoate (Fluka) and -octadecanoate (Fluka). The method of Pencreac'h and Baratti (1996) was used with some modifications. Stock solutions (25 mM) of each substrate were prepared in ethanol (*p*NP-acetate, -butyrate, -octanoate) or propan-2-ol (*p*NP-decanoate, -dodecanoate, -tetradecanoate, -octadecanoate) and stored at −20°C until required. Reaction mixtures (final volume 1 mL) contained the following: 755 μL 0.1 M McIlvaine buffer (pH 5.0), 5 μL Triton X-100, 40 μL of stock substrate solution (to yield a final substrate concentration of 1 mM), 100 μL of 1% (w/v) gum arabic (Sigma) solution, and 100 μL of cell suspension in buffer. All samples were vortexed vigorously for 1 min and incubated at 37°C for 2 h with agitation (100 rpm) on a platform shaker. Following incubation, all samples were centrifuged and treated as described for the temperature optima experiments.

Statistical analysis

Analysis of variance of the data was conducted using Microsoft Excel. Significant differences between the means ($p=0.05$) were determined using the Multiple Range Test and Studentised Range Q values.

Results

Influence of pH on esterase activity

The effect of pH on the esterase activity of the nine LAB strains towards *p*NP-octanoate is shown in Fig. 1. Overall,

the results suggest that the influence of pH varies between LAB strains, with different patterns of activity being observed for each of the strains studied. Compared to the other strains, the *O. oeni* appeared least influenced by pH, particularly across the range of pH 4.0 to 6.5. For O.oeni12, esterase activity was highest between pH 6.0 and 6.5 (average of 48 units of activity) and there was no significant difference in activity at pH 4.5 compared to pH 6.5. More than 40% of maximal activity (Ave.=21 units) was retained at pH 3.0 for this strain. Similarly, O.oeni9 showed no significant difference in activity at pH 6.5 compared to that seen at pH 4.0. Of the three *O. oeni* strains examined, O.oeni8 had the greatest activity of all strains (Ave.=62 units); however, this activity was also most strongly inhibited by low pH.

All of the lactobacilli showed maximum esterase activity close to pH 6.5, and all three strains showed a rapid decrease in activity at pH above optimum. Thus, for Lac34, of the order of 80% of the activity seen at pH 7.0 was lost at pH 7.5. Lac34 also showed a marked decrease in esterase activity at pH values below 5.0, and minimal activity was observed at pH 3.0. Of the pediococci, Ped42 had the maximum esterase activity at pH 4.5 to 5.0, values which were lower than the optimum pH values for Ped18 (6.0 to 6.5) and Ped11 (6.0). Ped18 was the only strain of the pediococci examined to retain at least some esterase activity at pH 3.0.

Influence of temperature optima on esterase activity

Maximum esterase activity was observed between 30 and 40°C for all three of the *O. oeni* strains (Fig. 2). For both O.oeni8 and O.oeni9, no significant difference in esterase activity was observed between 30 and 40°C, but O.oeni12 had significantly higher activity at 40°C. Similar trends were obtained for the lactobacilli, albeit at lower activities. Significantly higher activity was observed at 40°C than at 30°C for both Lac26

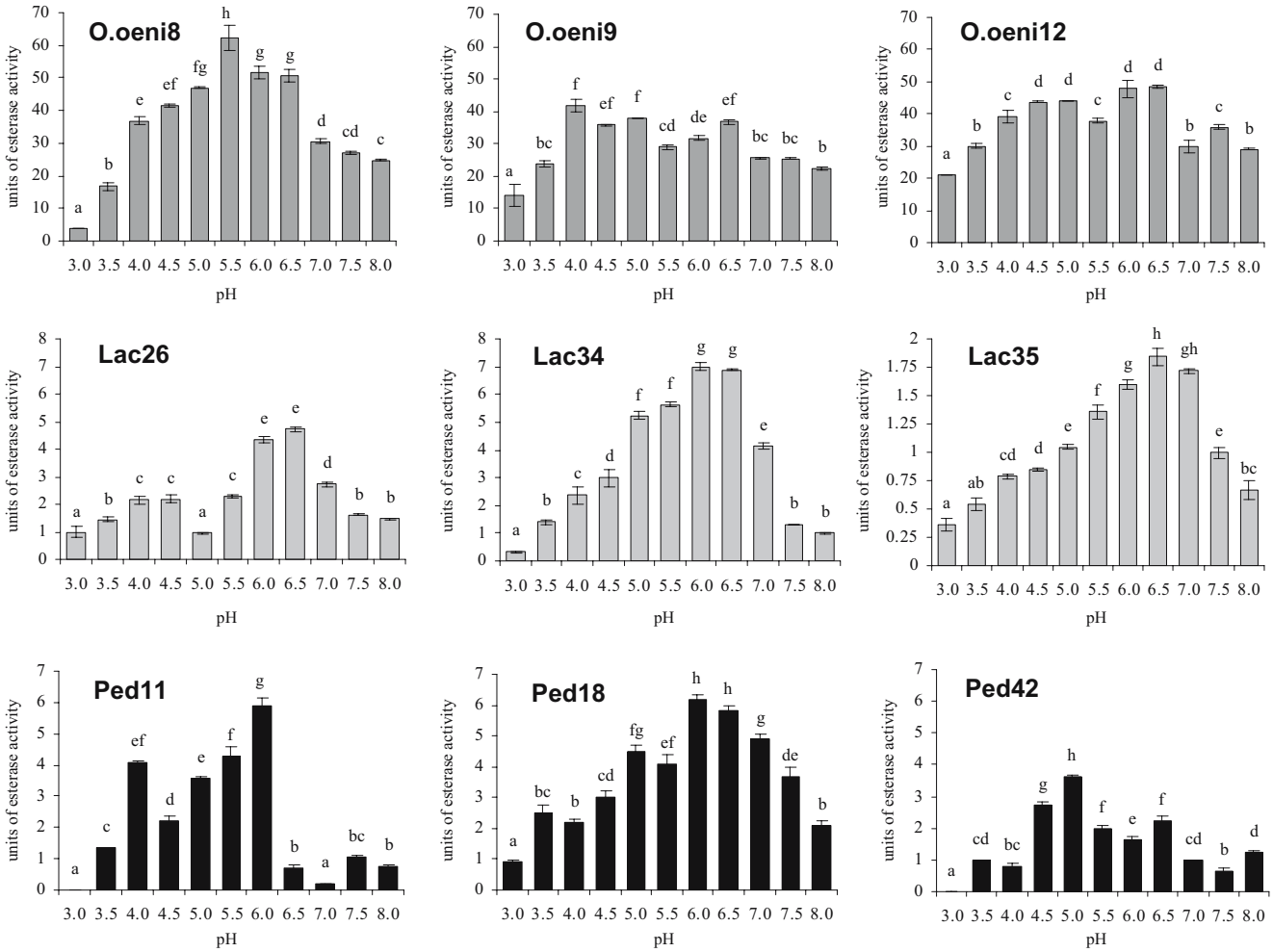

Fig. 1 Influence of pH on esterase activity of LAB strains. Units of esterase activity are defined as micromoles of *p*-nitrophenol liberated per minute per microgram of dry cell weight. Values are the mean of triplicates ± standard deviation. Within each panel columns with different letters are significant at the 5% level according to the multiple range test. NB: The scales differ across the genera and Lac35 has a different scale to Lac26 and Lac34

and Lac34. Interestingly, esterase activity diminished quickly above 40°C for all three lactobacilli strains and was similarly reduced at 10°C. By comparison, activities associated with two of the pediococci (Ped11 and Ped18) had lower temperature optima than other strains, with significantly higher activities at 20°C than at 40°C. At 10°C, Ped18 maintained 64% of the maximum activity observed at 30°C, a fact that could be important in wine production where such lower temperatures are common.

The influence of ethanol on esterase activity

Ethanol concentration is an important consideration during winemaking because MLF is often conducted after the completion of alcoholic fermentation, when ethanol concentrations of wine can exceed 13% (v/v). For this reason, the influence of ethanol on the ability of LAB strains to hydrolyse esters was investigated. Ethanol was not inhibitory to the esterase activity of all of the strains (Fig. 3). In fact, for all oenococci, ethanol was found to progressively increase the detected activity up to concentrations of the order of 16% (v/v). The influence of ethanol on the lactobacilli was generally inhibitory, with Lac26 showing the most dramatic reductions commencing from ethanol concentrations as low as 4%. Lac35 showed similar results, retaining as little as 30% of the activity found at 2% ethanol when exposed to an ethanol concentration of 18%. For Ped18, there was no significant difference in esterase activity at 2% compared to 16%, with intermediate concentrations yielding activities of up to approximately 30% higher.

Substrate specificity of esterases

Overall, the strains studied tended to show greater hydrolytic activity against the short-chained ester substrates (i.e. ≤C8) (Fig. 4). An exception was Ped18, which had equal or higher activity towards some of the longer esters. The remaining pediococci failed to hydrolyse any substrates from C12 and longer. All three *O. oeni* strains showed similar substrate specificity profiles with highest activity

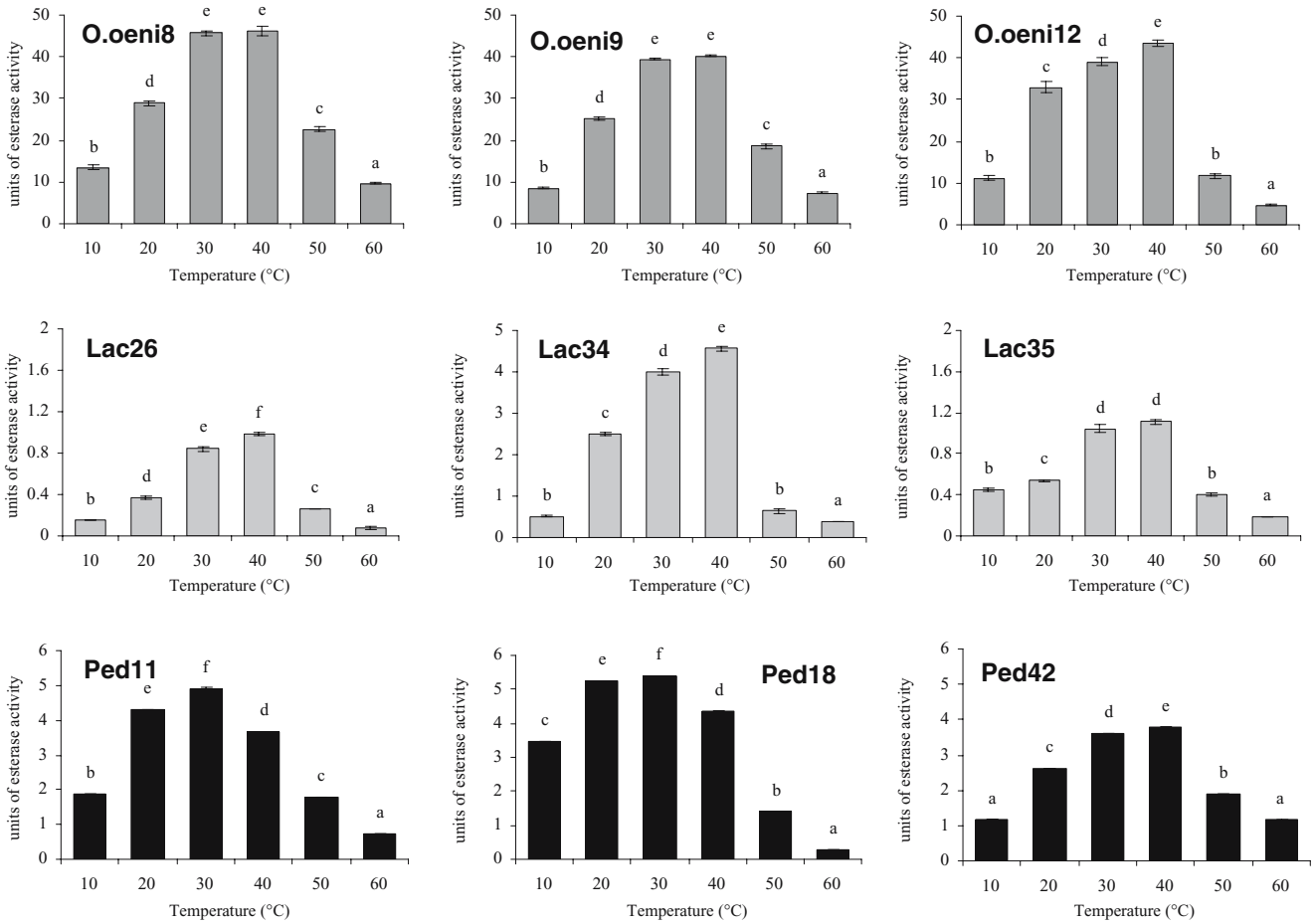

Fig. 2 Influence of temperature on esterase activity of LAB strains. Units of esterase activity are defined as micromoles of *p*-nitrophenol liberated per minute per microgram of dry cell weight. Values are the mean of triplicates ± standard deviation. Within each panel columns with different letters are significant at the 5% level according to the multiple range test. NB: The scales differ across the genera and Lac34 has a different scale to Lac26 and Lac35

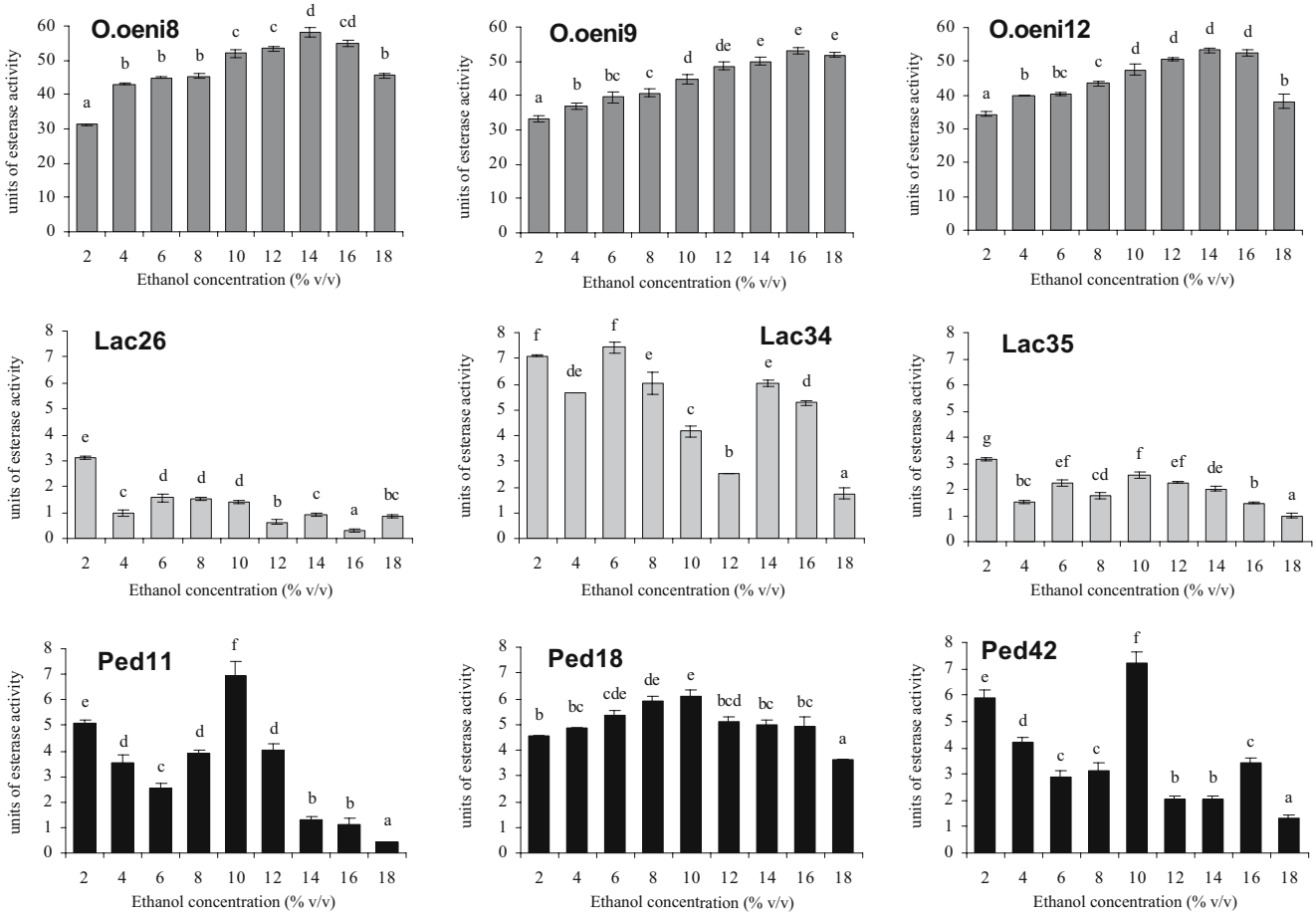

Fig. 3 Influence of ethanol on esterase activity of LAB strains. Units of esterase activity are defined as micromoles of *p*-nitrophenol liberated per minute per microgram of dry cell weight. Values are the mean of triplicates ± standard deviation. Within each panel columns with different letters are significant at the 5% level according to the multiple range test. NB: The scales differ across the genera

towards the four-carbon *p*NP-butyrate, but with high activity also towards *p*NP-acetate and *p*NP-octanoate. Similarly, all three strains had minimal activity towards the 14- and 18-carbon substrates. Amongst the lactobacilli, strong specificity towards the shortest substrate (*p*NP-acetate) was observed and all three strains showed minimal or no hydrolysis of the longer chained ester substrates.

Discussion

Esters are important contributors to the aroma profile of wine, and changes in their concentration have the potential to influence wine quality. The exploitation of esterases to modulate the ester composition of wine could either utilise purified enzyme preparations or selected microbial cultures bearing specific esterase activities. LAB and *O. oeni* in particular make good candidates for the latter approach because these organisms are routinely used to effect the MLF. Before such a notion can be tested, a fuller characterisation of the esterolytic capabilities of LAB is necessary. The work that is reported in this paper provides some of these data. In this study, the activities of nine LAB strains from the genera of *Lactobacillus*, *Pediococcus* and *Oenococcus* were characterised in relation to parameters of importance in the winemaking environment. The results of this investigation indicate that LAB possess esterase activities that could potentially alter the ester profile of wine.

The first of the physicochemical parameters examined, pH, is monitored throughout the entire winemaking process. Low pH, commonly within the range 3.0 to 3.6, is sought in both grape juice and wine for a number of reasons; to optimise the colour of red wines (Somers 1971), to reduce the likelihood of growth by spoilage micro-organisms (du Toit and Pretorius 2000), and to increase the concentration of molecular sulphur dioxide (Margalit 1997). In this study, esterase activity was examined across the pH range 3.0 to 8.0. Under these conditions, most of the LAB strains were found to have the highest esterase activity when the pH was close to 6.0 (Fig. 1). Esterases with pH optima close to neutral have previously been reported in LAB, including *Lactococcus lactis* (Holland and Coolbear

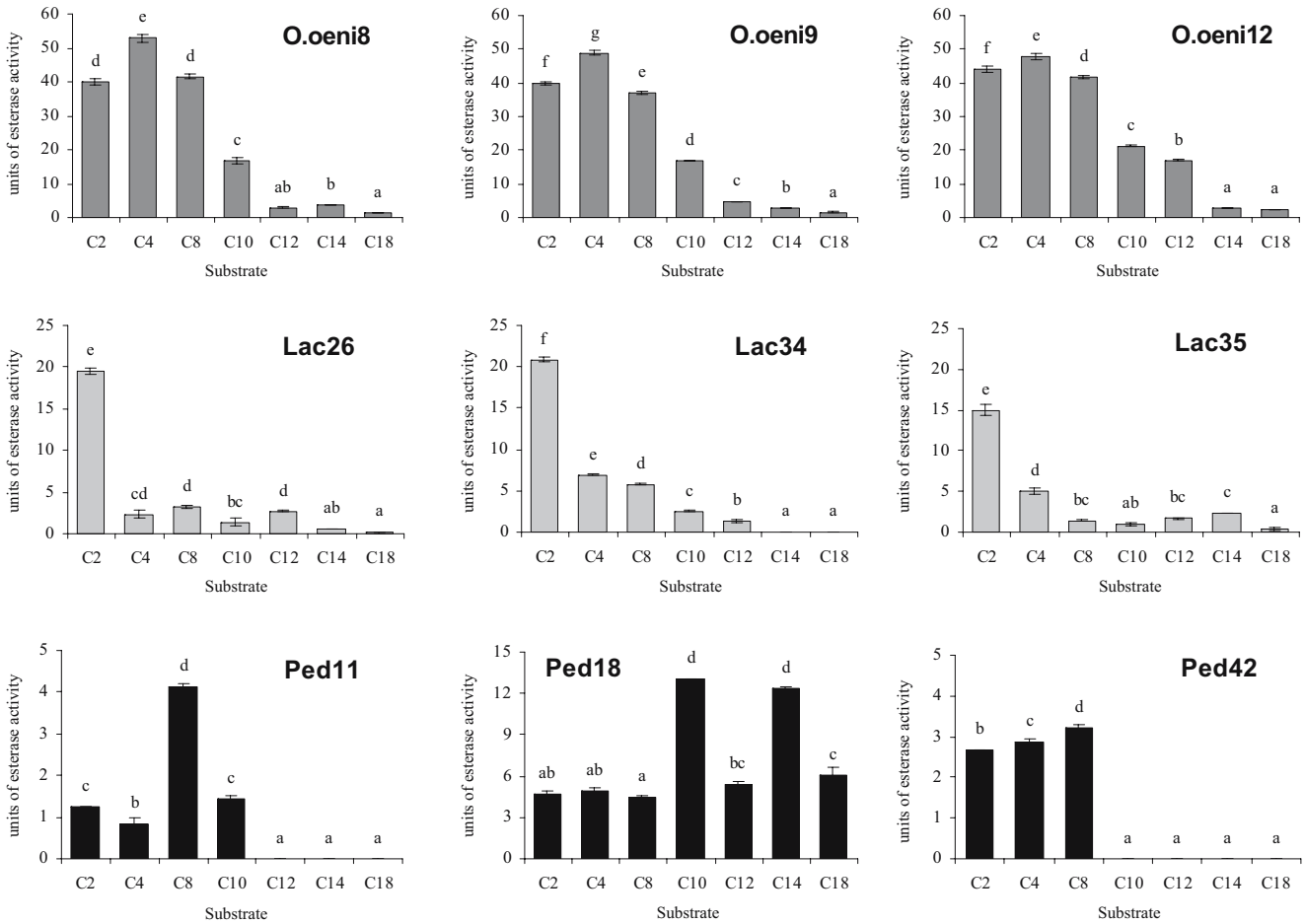

Fig. 4 Esterase activity of LAB strains towards substrates of different chain length. Substrates used were *p*NP-acetate (C2), -butyrate (C4), -octanoate (C8), -decanoate (C10), -dodecanoate (C12), -tetradecanoate (C14) and -octadecanoate (C18). Units of esterase activity are defined as micromoles of *p*-nitrophenol liberated per minute per microgram of dry cell weight. Values are the mean of triplicates ± standard deviation. Within each panel columns with different letters are significant at the 5% level according to the multiple range test. NB: The scales differ across the genera and the scale of Ped18 differs from Ped11 and Ped42

1996; Chich et al. 1997), *Lactobacillus casei* (Fenster et al. 2003) and *Lactobacillus helveticus* (Fenster et al. 2000). However, all of these results were obtained using purified enzyme preparations, rather than whole cell biomass, which was the case in this study. Despite having maximum activity close to pH 6.0, some of the strains, O.oeni9 in particular, showed activity over a broad pH range. Reports from studies conducted using dairy isolates of LAB have also described such ranges in pH optima (Chich et al. 1997; Castillo et al. 1999). In this report, some strains maintained significant esterase activity in the wine pH range. For example, both O.oeni12 and O.oeni9 retained some 50–60% of their maximum esterase activity at pH 3.5 compared to their optimum pH values.

As stated earlier, MLF is often conducted following alcoholic fermentation (Liu 2002). At that time, wine storage temperatures are preferably kept below 20°C, which coincides with the optimum growth temperature of LAB in wine (Asmundson and Kelly 1989). As with pH, some LAB have previously been reported to have esterases with broad temperature optima (Castillo et al. 1999). In this study, maximum esterase activity was generally found at temperatures that were higher than those considered suitable for wine production and storage (Fig. 2). Activity was, however, observed across a wide temperature range and some strains retained significant activity at lower temperatures. All strains had more than 40% of their maximum activity at 20°C, and *O. oeni* retained more than 60% of maximal activity at 20°C. These results therefore again suggest that esterases of wine LAB could be active at the temperatures applied during MLF and wine storage.

A further consideration of the inoculation of wine with LAB post-alcoholic fermentation for initiation of the MLF is that ethanol will be present at a high concentration. The growth of some LAB is inhibited by ethanol concentrations as low as 4% in wine (Capucho and San Romão 1994). By contrast, the malolactic activity of some strains of *O. oeni* is unaffected by ethanol concentrations of up to 12%, and

partial activity is retained at 16% (Capucho and San Romão 1994). The results reported for some strains are comparable with observations for the malolactic enzyme, that is that esterases are not inhibited by ethanol at concentrations commonly found in wine (Fig. 3). In fact, esterolytic capabilities of the *O. oeni* strains were stimulated by increasing ethanol concentration, up to about 14%. Other reports from our group on the β-glucosidase activity of LAB isolates describe a similar enhancement of activity by ethanol (Grimaldi et al. 2000, 2005a, b). A possible explanation for this trend is related to influence of ethanol on the permeability of the cell membrane, and hence, the degree of substrate and enzyme interaction.

Based on the findings reported for the artificial substrate used here, it appears probable that LAB esterases will retain at least partial activity under wine-like conditions of pH, temperature and ethanol concentration. While further work with natural substrates is necessary, a preliminary investigation of substrate specificity was also conducted to more fully define the potential applications of specific strains. As shown in Fig. 4, a distinct specificity for short- and medium-chained esters was found, with the exception of Ped18. Similar observations have been reported in dairy LAB, including some species also associated with wine, such as *L. helveticus* (Fenster et al. 2000), *L. casei* (El Soda et al. 1986b; Castillo et al. 1999; Fenster et al. 2003; Choi et al. 2004), *Lactobacillus plantarum* (El Soda et al. 1986b; Macedo et al. 2003), *Lactobacillus fermentum* (El Soda et al. 1986b) and *Lactobacillus brevis* (El Soda et al. 1986b). Examples of such esters in wine include ethyl esters, namely, ethyl acetate and ethyl butyrate. Such esters can contribute a desirable, fruity aroma to wine at low concentrations (Lilly et al. 2000); thus, degradation of these aromatic compounds would not necessarily be advantageous to wine quality. However, a beneficial role of these esterases might arise out of their deliberate application to treat wines tainted with unacceptably high concentrations of these esters. Such concentrations may arise through the action of the inoculated yeast or spoilage organisms including *Pichia anomala*, *Kloeckera apiculata* and *Hanseniaspora uvarum* in grape juice or wine (du Toit and Pretorius 2000) or species of *Acetobacter* growing in wine (Drysdale and Fleet 1989). The removal of excess ethyl acetate, which, at higher concentrations (>200 mg/L; Peynaud 1984), confers an acescent character to wine, would be an advantage. Strain Lac26, shown in our study to have high activity toward the C2 ester substrate and minor activity toward the larger substrates might therefore be used to specifically target this problem compound in wine.

Conversely, a number of the LAB had significant activity towards some of the longer chained esters. Both ethyl dodecanoate and ethyl tetradecanoate are examples that have been identified in wine (Francioli et al. 1999). The aroma contribution of these larger compounds becomes less desirable, and descriptors such as ‘soaplike’ and ‘stearic’ predominate (Rapp and Mandery 1986). Thus, amelioration of wines higher in undesirable long-chained ethyl esters could also be achieved through treatment with selected LAB strains.

While the work presented here goes some way to characterising the activity of LAB esterases, their action in wine is still not fully elucidated. Wine is a complex combination of all of the factors discussed here, as well as others, such as sulphur dioxide, which has previously been shown to inhibit the malolactic activity of LAB (Carreté et al. 2002). The consequences of the combined influences of such individual parameters remains to be studied, particularly because outcomes from such challenges are not necessarily predicted by the response to the single parameters (Grimaldi et al. 2000, 2005a, b). The effectiveness of the detected esterases against natural substrates is currently under investigation, as is the question of whether esterase activities of wine LAB are capable of making significant and detectable changes to the flavour of wines. The sensory contribution of ester-degradation products including fatty acids, higher alcohols and organic acids also requires exploration. Nevertheless, the results reported here show great promise for the deliberate use of LAB to selectively modify wine ester composition and thereby help justify further investigation.

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