Esterase activities of indigenous lactic acid bacteria from
Argentinean goats’ milk and cheeses

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Abstract

Esterase activities of indigenous lactic acid bacteria isolated from goats’ milk and cheese were investigated. All of the strains exhibited esterase activity on $\alpha$-naphthyl derivative of fatty acids of 2–6 carbon atoms. Lactobacillus fermentum ETC1 and L. bulgaricus ETC2 showed the highest specific activity on $\alpha$-naphthyl acetate; L. rhamnosus ETC14 presented the highest specific activity on $\alpha$-naphthyl butyrate and caproate. All enterococci strains presented the highest specific activities on $\alpha$-naphthyl propionate, butyrate and caproate, and the lowest specific activities on $\alpha$-naphthyl acetate. Pedicoccus pentosaceus ETC5 only had esterase activity on $\alpha$-naphthyl acetate. The electrophoretic zymogram showed for each strain an individual enzyme profile on $\alpha$-naphthyl acetate and revealed the presence of more than one esterase. L. plantarum ETC11 and Enterococcus faecium ETC9 showed four and five bands of esterase activity, respectively. The strains evaluated in this work showed different esterase activities, which were species and strain specific.

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Keywords: Lactic acid bacteria; Goats’ milk cheese; Esterase activity; Flavour compounds

1. Introduction

The semi hard goats’ milk cheeses from northwest Argentina are manufactured with raw milk. These cheeses possess a particular flavour due to the lipid fraction and the primary products of its degradation, e.g., free volatile fatty acids or esters. The enzymatic activities responsible for formation of these flavour compounds are esterases and lipases. Esterases from lactic acid bacteria, yeasts, and Pseudomonas may be involved in the development of fruity flavours in foods (Bardi, Dell’Oro, & Delfini, 1992; McKay, 1993), and pregastric lipase and esterases are essential for the development of typical flavour in Italian cheese (Fox & Stepaniak, 1993). Microbial esterases may improve quality or accelerate the maturation of cheeses, cured bacon, and fermented sausages (Lee & Lee, 1990).

Essentially nothing is known about the contribution of bacterial esterases to flavour formation in Cheddar, Dutch, and Swiss cheeses (Fox & Stepaniak, 1993).

Indigenous lactic acid bacteria isolated from goats’ milk and artisanal goats’ milk cheeses from the provinces of northwest Argentina were identified as lactobacilli (60%), enterococci (35%) and pediococci (5%). The species isolated were Lactobacillus plantarum (36 isolates), L. rhamnosus (15 isolates), L. delbrueckii subsp. bulgaricus (5 isolates), L. fermentum (5 isolates), Enterococcus faecium (36 isolates), and Pedicoccus pentosaceus (5 isolates) (Oliszewski, González, & Pérez Chaia, 2004).

Several authors have studied the free fatty acid profile of goats’ milk cheese and the changes on individual free fatty acids (FFA) during cheese ripening (Buffa, Guamis, Pavia, & Trujillo, 2001; Franco, Prieto, Bernardo, González

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E-mail address: rmedina@cerela.org.ar (R.B. Medina).
2. Materials and methods

2.1. Microorganisms and growth conditions

Lactic acid bacteria strains used in this study were: \textit{L. fermentum} ETC1, \textit{L. bulgaricus} ETC2, \textit{L. plantarum} ETC8, ETC11, ETC13, ETC15, ETC16, ETC19 and ETC20; \textit{L. rhamnosus} ETC14, ETC17 and ETC18, \textit{E. faecium} ETC3, ETC4, ETC6, ETC7, ETC9, ETC10, ETC12 and \textit{P. pentosaceus} ETC5 were isolated from goats' milk and cheeses (Oliszewski et al., 2004). Lactobacilli were grown in MRS broth (De Man, Rogosa, & Sharpe, 1960), enterococci and pediococci were grown in LAPTg broth (Raibaud, Caulet, Galpin, & Mocquot, 1961) for 16 h. The cultures were incubated at 30°C (\textit{Pediococcus}) or 37°C (\textit{Lactobacillus and Enterococcus}).

2.2. Preparation of cell free extracts

Cells were harvested after 16 h by centrifugation at 10,000 g for 10 min at 4°C, washed twice with 50 mM sodium phosphate buffer, pH 7.0, and resuspended at 50% (w/v) in the same buffer. The suspension was disrupted adding 1–2 g of glass beads (No. 31/14: diameter, 0.10–0.11 mm, B. Braun Biotech International, Germany) and using a cell disruptor (B. Braun Melsungen AG, Germany). Five mixing sequences (speed, 6.5/s) each of 1 min were successively applied under CO₂ atmosphere. Samples were cooled in ice for 1 min between each mixing sequence. Cellular debris was removed by centrifugation (20,000g for 30 min at 4°C) and the supernatant was used as a cell free extract (CFE).

2.3. Esterase activity

The esterase activity of CFE was determined on \textit{α}-naphthyl derivatives of fatty acids of 2–10 carbon atoms as substrates. Esterase activity was present in the cell free extracts of all the strains assayed (Table 1). Among \textit{Lactobacillus} strains, \textit{L. fermentum} ETC1 showed the highest specific activity (32.47 ± 0.50 U/mg) on \textit{α}-NA acetate and this strain also hydrolyzed \textit{α}-NA butyrate, propionate and caproate. \textit{L. bulgaricus} ETC2 showed high specific activity on \textit{α}-NA acetate and low specific activity on \textit{α}-NA propionate and caproate. No esterase activity was observed in this strain on \textit{α}-NA butyrate. \textit{Lactobacillus casei} ETC19 had esterase activity on \textit{α}-NA acetate, propionate, butyrate and caproate. \textit{L. plantarum} strains ETC17 and ETC20 showed the highest specific activity on \textit{α}-NA butyrate and caproate, and \textit{L. plantarum} ETC11 showed the highest specific activity on \textit{α}-NA acetate. Of all lactobacilli studied,
**Table 1** Specific esterase activity* in cell free extract of lactic acid bacteria isolated from goat’s milk and artisanal cheese of northwest Argentine

<table>
<thead>
<tr>
<th>Source of enzyme</th>
<th>Substrate α-naphthyl derivative</th>
<th>Acetate</th>
<th>Propionate</th>
<th>Butyrate</th>
<th>Caproate</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. fermentum</em> ETC1</td>
<td></td>
<td>32.47 ± 0.50^A</td>
<td>22.97 ± 1.23^A</td>
<td>27.87 ± 2.34^A</td>
<td>14.93 ± 0.12^A</td>
</tr>
<tr>
<td><em>P. pentosaceus</em> ETC2</td>
<td></td>
<td>30.45 ± 0.89^B</td>
<td>3.65 ± 0.89^B</td>
<td>n.d.</td>
<td>0.58 ± 0.12^B</td>
</tr>
<tr>
<td><em>L. casei</em> ETC19</td>
<td></td>
<td>11.80 ± 0.23^F</td>
<td>3.80 ± 0.77^B</td>
<td>7.82 ± 0.48^E</td>
<td>4.25 ± 0.34^E</td>
</tr>
<tr>
<td><em>L. plantarum</em> ETC8</td>
<td></td>
<td>18.23 ± 0.35^C</td>
<td>7.23 ± 0.15^C</td>
<td>1.07 ± 0.23^B</td>
<td>1.41 ± 0.45^C</td>
</tr>
<tr>
<td><em>L. plantarum</em> ETC11</td>
<td></td>
<td>26.11 ± 1.05^D</td>
<td>16.11 ± 0.98^D</td>
<td>2.83 ± 0.34^E</td>
<td>2.86 ± 0.23^D</td>
</tr>
<tr>
<td><em>L. plantarum</em> ETC13</td>
<td></td>
<td>15.04 ± 0.45^CE</td>
<td>5.04 ± 0.67^DEH</td>
<td>1.60 ± 0.26^D</td>
<td>1.01 ± 0.43^C</td>
</tr>
<tr>
<td><em>L. plantarum</em> ETC15</td>
<td></td>
<td>16.0 ± 0.76^C</td>
<td>6.0 ± 0.59^B</td>
<td>0.91 ± 0.34^B</td>
<td>1.18 ± 0.68^C</td>
</tr>
<tr>
<td><em>L. plantarum</em> ETC16</td>
<td></td>
<td>13.93 ± 0.45^EF</td>
<td>3.73 ± 0.12^B</td>
<td>2.79 ± 0.66^C</td>
<td>1.96 ± 0.12^C</td>
</tr>
<tr>
<td><em>L. plantarum</em> ETC20</td>
<td></td>
<td>9.49 ± 0.12^F</td>
<td>7.49 ± 0.14^F</td>
<td>15.11 ± 0.09^F</td>
<td>11.85 ± 0.16^F</td>
</tr>
<tr>
<td><em>L. rhamnosus</em> ETC17</td>
<td></td>
<td>22.80 ± 0.83^H</td>
<td>12.80 ± 0.24^G</td>
<td>15.99 ± 0.25^H</td>
<td>14.90 ± 0.14^A</td>
</tr>
<tr>
<td><em>L. rhamnosus</em> ETC14</td>
<td></td>
<td>6.80 ± 0.08^G</td>
<td>16.80 ± 0.15^G</td>
<td>22.56 ± 0.23^G</td>
<td>19.84 ± 0.35^A</td>
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<tr>
<td><em>L. rhamnosus</em> ETC18</td>
<td></td>
<td>24.66 ± 0.52^D</td>
<td>4.66 ± 0.08^H</td>
<td>2.26 ± 0.14^C</td>
<td>1.25 ± 0.21^C</td>
</tr>
<tr>
<td><em>E. faecium</em> ETC3</td>
<td></td>
<td>6.33 ± 0.12^G</td>
<td>36.56 ± 1.34^I</td>
<td>31.58 ± 0.91^I</td>
<td>32.61 ± 0.83^I</td>
</tr>
<tr>
<td><em>E. faecium</em> ETC4</td>
<td></td>
<td>12.51 ± 0.22^I</td>
<td>23.81 ± 1.10^A</td>
<td>3.74 ± 0.14^F</td>
<td>20.00 ± 0.35^H</td>
</tr>
<tr>
<td><em>E. faecium</em> ETC6</td>
<td></td>
<td>4.71 ± 0.21^GJ</td>
<td>35.91 ± 1.23^D</td>
<td>39.11 ± 1.13^L</td>
<td>32.39 ± 1.32^K</td>
</tr>
<tr>
<td><em>E. faecium</em> ETC7</td>
<td></td>
<td>3.47 ± 0.08^J</td>
<td>30.72 ± 1.17^I</td>
<td>4.77 ± 0.09^M</td>
<td>30.61 ± 1.13^K</td>
</tr>
<tr>
<td><em>E. faecium</em> ETC9</td>
<td></td>
<td>11.23 ± 0.21^I</td>
<td>21.97 ± 0.97^A</td>
<td>5.84 ± 0.12^M</td>
<td>21.30 ± 1.03^H</td>
</tr>
<tr>
<td><em>E. faecium</em> ETC10</td>
<td></td>
<td>7.22 ± 0.23^G</td>
<td>17.43 ± 0.45^D</td>
<td>2.66 ± 0.07^C</td>
<td>17.97 ± 0.14^A</td>
</tr>
<tr>
<td><em>E. faecium</em> ETC12</td>
<td></td>
<td>13.65 ± 0.34</td>
<td>28.20 ± 0.67^L</td>
<td>n.d.</td>
<td>25.51 ± 0.25^M</td>
</tr>
<tr>
<td><em>P. pentosaceus</em> ETC5</td>
<td></td>
<td>10.54 ± 0.21^F</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

# Table 1

<table>
<thead>
<tr>
<th>Source of enzyme</th>
<th>Substrate α-naphthyl derivative</th>
<th>Acetate</th>
<th>Propionate</th>
<th>Butyrate</th>
<th>Caproate</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. rhamnosus</em> ETC14</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Values in the same column with different superscript letters differ significantly (*P* < 0.05).

n.d.: not detected.

*a U/mg of protein. Mean ± SD.

L. *rhamnosus* ETC14 showed the highest specific activity on α-NA butyrate and caproate.

All enterococci strains showed the highest specific activities on α-NA propionate, butyrate and caproate, and the lowest specific activities on α-NA acetate. *E. faecium* strains, ETC3 and ETC6 showed highest activity with a lowest specific activities on α-NA propionate. ETC12 showed the highest specific activity on α-NA acetate. *L. rhamnosus* strains, ETC3 and ETC6 showed highest activity on α-NA propionate, butyrate and caproate than lactobacilli strains. Sarantinopoulos et al. (2001) reported that *E. faecium* were more esterolytic than *Enterococcus faecalis* and *Enterococcus durans*.

Of all the strains evaluated in this work, the enterococci showed the highest activities on α-NA derivatives of fatty acids of 3, 4 and 6 carbon atoms as substrates. Sarantinopoulos et al. (2001) reported that *E. faecium* were more esterolytic than *Enterococcus faecalis* and *Enterococcus durans*.

Pedicoccus *pentosaceus* ETC5 only had esterase activity on α-NA acetate (10.54 U/mg). Vafopoulou-Mastrojannaki et al. (1994) reported for *P. pentosaceus* isolated from traditional Greek cheeses a low esterase activity using 4-nitrophenyl butyrate as substrate.

None of the strains hydrolyzed α-naphthyl derivatives of fatty acids of 8 or 10 carbon atoms. Preferential or exclusive hydrolysis of esters containing C2–C6 of α-NA fatty acid derivatives has been reported for other lactic acid bacteria (Gobbetti, Fox, & Stepaniak, 1996; Katz et al., 2002).

Esterase activity was not observed with the extracellular fractions of the strains assayed. Several authors (El Soda, Abd El Wahab, Ezzat, Desazeaud, & Ismail, 1986; Katz et al., 2002) have reported that esterase activity is primarily intracellular.

Significant differences (*p < 0.05*) were observed with respect to specific esterase activity among studied strains. These strains would improve the organoleptic characteristics of goats’ milk cheese.

### 3.2. Electrophoretic zymogram

The electrophoretic zymogram of strains studied is shown in Table 2. Based on electrophoretic mobility (*R* values), each strain gave an individual enzyme profile on α-NA acetate and butyrate. *L. fermentum* ETC1 presented three bands with esterase activity, E1, E2 and E3 on α-NA acetate. E3 band also showed activity on α-NA butyrate. *L. delbrueckii* subspp. *bulgaricus* ETC2 exhibited three bands with esterase activity, E1, E2 and E3 on α-NA acetate. *L. casei* ETC19 contained two bands, E1 and E2 with activity on α-NA acetate. Among *L. plantarum* strains, *L. plantarum* ETC13 and ETC15 showed only one band with esterase activity on α-NA butyrate. The strains ETC8 and ETC16 showed two bands only with activity on α-NA acetate. *L. plantarum* ETC11 showed four bands with esterase activity, E1, E3 and E4 bands had activity on α-NA butyrate. E2 band only had activity on α-NA acetate. This strain possessed a very complex intracellular esterolytic system, producing four enzymes that hydrolyze carboxyl ester linkages with different specificity.
L. rhamnosus strains C18 and C14 presented two bands with activity. Various authors (El Soda et al., 1986; Gobbetti, Fox, & Stepaniak, 1997; Katz et al., 2002; Lee & Lee, 1990) have observed that lactobacilli possess more than one intracellular esterase.

E. faecium ETC4 and ET12 possessed two active esterolytic bands, E. faecium ETC3, ETC6 and ETC10 possessed three, E. faecium ETC7 showed four bands and E. faecium ETC9 five active esterolytic bands. Sarantinopoulos et al. (2001) reported for E. faecium strains five active esterolytic bands with a maximum of two bands per strain.

In P. pentosacens only ETC5 possessed two bands with esterase activity on acetate. There is little information about the esterolytic system of the Pediococcus genus.

Our results show that esterases from lactic acid bacteria strains tested released preferentially short-chain fatty acids and that several strains possessed more than one intracellular esterase.

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Our results show that esterases from lactic acid bacteria strains tested released preferentially short-chain fatty acids and that several strains possessed more than one esterase. Multiple esterases were also found in other lactic acid bacteria (Gobbetti et al., 1997; Katz et al., 2002; Sarantinopoulos et al., 2001; Tsakalidou, Zoidou, & Kalantzopoulos, 1992).

Franco et al. (2003) quantified the major free fatty acids during ripening of traditional Babia-Laciana cheese. Despite the variation in free fatty acids concentrations and proportions, these cheeses contained high levels of C4–C10 free fatty acids, which contribute to cheese flavour.

4. Conclusion

Enterococcus and Lactobacillus were the major LAB found in goats’ milk and artisanal Argentine cheeses. These bacteria play a role in fat breakdown. Both genera of lactic acid bacteria tested in this study exhibited esterase activities that may release C2–C6 fatty acids during ripening and therefore, would actively contribute to flavour development in goats’ milk cheese. Short-chain fatty acids such as butanoic and hexanoic acids are potent flavour compounds at concentrations below 10 mg/ml (Brennand, Ha, & Lindsay, 1989; Fenster, Rankin, & Steele, 2003). Esterases have the capacity for both hydrolyzing and synthesizing esters by esterification of fatty acids and ethanol (Liu, Holland, & Crow, 2004). These esters, which are potent flavour compounds at less than 5 ppm, are important for development of the characteristic “fruity” type

<table>
<thead>
<tr>
<th>Strains</th>
<th>Intensity of esterase activity with substrate (Table 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bands</td>
<td>Specific activity esterase (α-Acetate)</td>
</tr>
<tr>
<td>E. faecium ETC3</td>
<td>E1 0.37 ++ –</td>
</tr>
<tr>
<td>E. faecium ETC4</td>
<td>E1 0.23 +++ –</td>
</tr>
<tr>
<td>E. faecium ETC6</td>
<td>E1 0.55 ++ –</td>
</tr>
<tr>
<td>E. faecium ETC7</td>
<td>E1 0.30 – +++</td>
</tr>
<tr>
<td>E. faecium ETC9</td>
<td>E1 0.36 – ++</td>
</tr>
<tr>
<td>E. faecium ETC10</td>
<td>E1 0.30 – ++</td>
</tr>
<tr>
<td>E. faecium ETC12</td>
<td>E1 0.30 – ++</td>
</tr>
</tbody>
</table>

Substrate used: α-naphthyl derivatives of acetate and butyrate.
Activity was expressed from W (weak) to ++++: from a very thin to very deep coloured band. (–) absence of activity.

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flavours such as ethyl butyrate and ethyl hexanoate (Liu et al., 2004).

The LAB strains evaluated in this work showed different esterase activities, which were species and strain specific. Wide variations in activity between strains highlight the need for selecting appropriate starters.

Acknowledgements

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