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Original article

Influence of pH and temperature on the growth of Enterococcus faecium and Enterococcus faecalis

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Abstract – A total of 70 enterococcal strains isolated from traditional Italian raw milk cheeses were identified by API 20 Strep and Biolog GP. All the identifications were confirmed by species-specific PCR. Four species of genus Enterococcus were found: E. faecalis, E. faecium, E. durans and E. hirae. E. faecalis and E. faecium were the dominant enterococcal species isolated in artisanal cheeses. According to the most representative species and the origin of isolation, 21 strains (11 E. faecalis and 10 E. faecium) were selected for the conductimetric analysis. The influence of pH (5.0; 5.5; 6.0 and 6.5) and temperature (25 °C and 37 °C) on the metabolism and the development of enterococci was evaluated by determination of Generation Time (GT), Detection Time (DT) and the maximum conductance value (\(\Delta \mu S\)) of the curve during a period of 48 hours. Conductance trials showed that both species were able to adapt to adverse cultural conditions (low values of pH and temperature) during cheese-making and ripening. E. faecalis appeared to be less sensitive on the whole. In the worst conditions (pH 5.0; 5.5; 6.0 and 6.5) and temperature (25 °C and 37 °C) on the metabolism and the development of enterococci was evaluated by determination of Generation Time (GT), Detection Time (DT) and the maximum conductance value (\(\Delta \mu S\)) of the curve during a period of 48 hours. Conductance trials showed that both species were able to adapt to adverse cultural conditions (low values of pH and temperature) during cheese-making and ripening. E. faecalis appeared to be less sensitive on the whole. In the worst conditions (pH 5.0; 25 °C), after an adaptation phase (about 10 h), these microorganisms showed GT values almost double those at 37 °C. The same behavior can occur in cheese, where enterococci can reach numbers of up to \(10^5\)–\(10^8\) cfu·g\(^{-1}\) during ripening. This could explain the fact that enterococci (in particular, E. faecalis and E. faecium) represent the typical and important microflora in raw milk cheese.

E. faecalis / E. faecium / conductimetric analysis / pH / temperature / growth

Résumé – Influence du pH et de la température sur la croissance d’Enterococcus faecalis et Enterococcus faecium. Un total de 70 souches d’entérocoques, isolées de certains fromages italiens traditionnels, a été identifié à l’aide des galeries API 20 Strep et Biolog GP. Toutes les identifications ont été confirmées par PCR espèce-spécifique. Quatre espèces de ce genre ont été isolées : E. faecalis, E. faecium, E. durans et E. hirae. E. faecalis et E. faecium étaient les espèces dominantes isolées des fromages artisanaux. Un total de 21 souches (11 E. faecalis et 10 E. faecium) a été sélectionné pour les analyses de conductimétrie selon les espèces les plus représentées et selon l’origine de l’isolement. L’influence du pH (5,0 ; 5,5 ; 6,0 et 6,5) et de la température (25 °C et 37 °C) sur le métabolisme et sur le développement des entérocoques a été évaluée par détermination du temps de génération (GT), du temps de détection (DT) et de la valeur conductimétrique maximale (\(\Delta \mu S\)) de la courbe au cours d’une période de 48 h. Les mesures de conductance ont démontré que les deux espèces ont la capacité de s’adapter aux conditions de culture défavorables (valeurs faibles du pH

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et de la température) aussi bien pendant la fabrication du fromage que l’affinage. Les deux espèces ont un comportement différent : E. faecalis s’est avéré être moins sensible. Dans les plus mauvaises conditions (pH 5,0–25 °C), après la phase d’adaptation (environ 10 h), ces micro-organismes montrent une valeur du temps de génération deux fois plus élevée par rapport à celle relevée à 37 °C. Le même phénomène peut se produire dans le fromage où, pendant la maturation, les entérocoques peuvent atteindre 10⁵–10⁸ ufc·g⁻¹. Ceci pourrait expliquer le fait que les entérocoques (en particulier E. faecalis et E. faecium) représentent la flore typique dominante dans les fromages au lait cru.

**Enterococcus faecalis / Enterococcus faecium / conductimétrie / pH / température / croissance**

1. **INTRODUCTION**

Enterococci consist of ubiquitous microorganisms commonly found in dairy products and other foods. The ability to grow at 10 °C and 45 °C, to initiate growth in 6.5% NaCl broth at pH 9.6 and to survive at 60 °C for 30 min, are commonly used traits to segregate enterococci from other streptococci [11, 26].

Enterococci are widely distributed in nature. They are found in such diverse habitats as the gastrointestinal tract, oral cavity and upper genital tract of man and other mammals, and also in birds, reptiles, insects, plants, soil and water [28].

The significance of their presence in food, particularly in dairy products, has been a source of discussion for a long time. Their possible use as fecal contamination indicators and their contribution to the development of flavor are controversial topics.

The ubiquitous nature of enterococci and their ability to survive in unfavorable conditions almost guarantee the presence of this group in many types of foods, particularly in dairy products.

Enterococci are the predominant microflora in a large number of typical Mediterranean cheeses with the European Protected Designation of Origin. They produce acetaldehyde and diacetyl, and consequently they contribute to the organoleptic characteristics of cheese and are considered to be an important part of the microflora [2, 24, 39].

Some enterococci of food origin also produce bacteriocins that exert activity against Listeria and Salmonella [3, 23, 27].

Moreover, enterococci are used as probiotics to improve the microbial equilibrium of the intestine, or as treatments for gastroenteritis in humans and animals. However, recent studies have pointed out that E. faecium and E. faecalis might be a potential recipient of vancomycin resistance genes, and consequently, the FAO/WHO have recommended that E. faecium should not be considered as probiotics for human use. Furthermore, enterococci have become recognized as serious nosocomial pathogens causing bacteraemia, endocarditis and infections of the urinary tract [14].

This is in part explained by the resistance of some of these bacteria to most of the antibiotics that are currently in use. Resistance is acquired by gene transfer systems, such as conjugative or nonconjugative plasmids or transposons. The virulence of enterococci is not well understood, but adhesins, haemolysin, hyaluronidase, aggregation substance and gelatinase are potential virulence factors. It also appears that food could be a source of vancomycin-resistant enterococci [14, 16, 25].

Impedimetric methods have been developed to analyze a range of foods and pharmaceuticals. These methods are based on the principle that as bacteria grow and metabolize, uncharged or weakly charged substrates are transformed into charged end products. The accumulation of these products leads to increased conductance of the medium and capacitance at the electrode-medium interface [29].

Measurement of the rate of growth permits investigation into the effects of inhibitors, sensitivity to some parameters such as pH, temperature and salt concentration, in a much shorter time than conventional techniques.

Impedance measurement, defined as the resistance to flow of an alternating current through a conducting material, can be used to monitor the growth of LAB [10] and to
Growth of *E. faecium* and *E. faecalis* 183
determine the shelf life and bacteriological
growth of raw materials of different food
products [6, 15, 29, 30, 32].

Conductance is considered a valid method
for evaluating the growth and acidity of
LAB in milk [4, 22, 31, 36].

Changes in the conductance of the
medium, which is the reciprocal of resist-
ance, have been successfully employed for
the detection of several microbial species,
including *E. faecalis* and *E. faecium* [5, 20].

The aim of this study was to characterize
and investigate the effects of different cul-
tural conditions (pH and temperature),
present in cheese durin g different cheese-
making processes and different ripening
periods, on the metabolism and growth
rates of enterococci, using conductance
measurements that allowed us to accurately
evaluate the growth of these species (Tab. I).

### 2. MATERIALS AND METHODS

#### 2.1. Enumeration, isolation
and identification of enterococci

Different Italian cheeses produced in
northern Italy from raw milk (Valtellina
Casera, Bitto, Scimudin, pasta filata cheese,
Formai de Mut, goat cheese, Fontina,
Semuda and hard cheese) were analyzed in
order to verify the presence of enterococci.

For the enumeration, a 5-g sample of
each kind of cheese was placed in 45 mL of
dipotassium hydrogenphosphate solution
[21] and homogenized with a Stomacher for
approximately 1 min.

A selective medium for detection and
isolation of enterococci (Kanamycin Aes-
culin Azide agar, Sharlau Microbiology,
Spain) was used. After 24 h of incubation
at 37 °C, typical colonies of presumptive
enterococci (surrounded by a black halo)
were randomly picked from the plates.

After purification, the ability to initiate
growth in 6.5% NaCl broth and in broth at
pH 9.6 at 10 °C and 45 °C, and survival at
60 °C for 30 min were verified. The ente-
rococci were cultured in MRS broth (Shar-
lau Microbiology, Spain) with incubation at
37 °C for 16 h. Identification was per-
fomed by using API 20 STREP galleries
(bioMérieux, Marcy l’Étoile, France) and
Biolog GP MicroPlate (Biolog, USA)
according to the instructions of the manu-
facturer. Since *E. faecium* and *E. faecalis*
are the dominant enterococcal species, their
identification was also performed by separate
PCR reactions using either primer pair E

<table>
<thead>
<tr>
<th>Ripening (d)</th>
<th>Enterococcus sp. (cfu·g⁻¹)</th>
<th><em>E. faecalis</em></th>
<th><em>E. faecium</em></th>
<th><em>E. durans</em></th>
<th><em>E. hirae</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Scimudin</td>
<td>20</td>
<td>10⁶–10⁷</td>
<td>8 (3*)</td>
<td>3 (3*)</td>
<td></td>
</tr>
<tr>
<td>Goat cheese</td>
<td>20</td>
<td>10⁵–10⁷</td>
<td>2 (1*)</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Formai de Mut</td>
<td>45</td>
<td>10⁵–10⁷</td>
<td>4</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Caciocavallo</td>
<td>60</td>
<td>10⁵–10⁸</td>
<td>1</td>
<td>1 (1*)</td>
<td>1</td>
</tr>
<tr>
<td>Ragusano</td>
<td>60</td>
<td>10⁵–10⁸</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Fontina</td>
<td>60</td>
<td>10⁶–10⁷</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Semuda</td>
<td>60</td>
<td>10⁶–10⁷</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Valtellina</td>
<td>70</td>
<td>10⁵–10⁷</td>
<td>8 (4*)</td>
<td>1 (1*)</td>
<td></td>
</tr>
<tr>
<td>Casera</td>
<td>70</td>
<td>10⁵–10⁷</td>
<td>5 (3*)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bitto</td>
<td>70</td>
<td>10⁹–10⁷</td>
<td>13 (3*)</td>
<td>7 (2*)</td>
<td>5 (3*)</td>
</tr>
<tr>
<td>Hard cheese</td>
<td>70</td>
<td>10⁹–10⁷</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Strains selected for the conductimetric analysis.
Enterococci were grown overnight in MRS broth, then dilutions of broth culture (1:10, 1:50, 1:100) in reconstituted non-fat dry milk containing Bromo Cresol Purple (0.025 g·L⁻¹) were made and incubated at 37 °C.

The highest dilution showing clotted milk and yellow color was selected as the inoculum for conductance trials.

### 2.2.2. Milk inoculation

The pH of the reconstituted non-fat dry milk was adjusted with 1N HCl to 5.0, 5.5, 6.0 and 6.5 after sterilization.

Milk was inoculated into triple Malthus cells at a rate of 1% (v/v) using the inoculum prepared as in Section 2.2.1. Six decimal dilutions for each pH and each temperature tested were made.

All the cells were incubated simultaneously in two water-baths at 25 and 37 °C for 48 h.

### 2.3. Experimental design

Conductimetric analyses were performed on 11 E. faecalis and 10 E. faecium strains.

The influence of four different levels of pH (5.0, 5.5, 6.0 and 6.5) and two levels of temperature (25 °C and 37 °C) on the metabolism and the development of enterococci was evaluated by the determination of the Generation Time (GT), the maximum conductimetric values of the curve during a period of 48 h (ΔµS max) and the Detection Time (DT). Overnight cultures were diluted and then inoculated into triple Malthus cells by preparing 6 decimal dilutions for each pH and temperature combination tested. The cells were incubated simultaneously in water-bath incubators at 25 and 37 °C for 48 h.

### 2.3.1. Growth parameters

The Detection Time (DT), the maximum conductimetric value (ΔµS max) and the Generation Time (GT) were determined for each strain subjected to each combination of tested parameters (Fig. 1).
The Generation Time was determined according to Firstenberg-Eden and Eden [16] by recording the delay in DT for 6 serial dilutions of the initial samples. The maximum conductimetric value of the curve was used to estimate the enterococcal metabolic activity during a period of 48 h.

The DT is the time elapsed between the start of a test and the detection of an accelerating impedance signal by the instrument. The DT is a function of numerous parameters, including initial concentration of microorganisms, lag phase, generation time and metabolic activity [16, 32]. The Malthus computer program automatically records the DT; however, in some cases it is necessary to correct values by directly reading them from the curves.

2.4. Statistical treatment of the data

The data were subjected to general linear model (GLM) analysis of the Statistical Analysis System [35]. All means shown in this paper are least square means (LSM). Statistical significance was accepted if the probability value was less than or equal to 0.05.

3. RESULTS AND DISCUSSION

3.1. Isolation and identification

Enterococci were recovered from all the fully ripened cheeses at levels ranging from $10^5$ to $10^8$ cfu·g$^{-1}$, thus confirming them to be an important part of the microflora of many Italian cheeses, especially for those produced from raw milk (Tab. I). A total of 70 isolates were identified as belonging to *E. faecalis*, *E. faecium*, *E. durans* and *E. hirae*. The identifications performed by API 20 Strep, Biolog GP and species-specific PCR were always in agreement, except for *E. faecium*: for this species Biolog identification differed from API and species-specific PCR. *E. durans* and *E. hirae* were identified by phenotypical methods.
Among the isolated strains, *E. faecalis* and *E. faecium* were the dominating species present, as reported by many authors for different kinds of cheese [19, 34].

According to the most representative species and the origin of isolation (Tab. I), 21 strains (11 *E. faecalis* and 10 *E. faecium*) were selected for the conductimetric analysis.

### 3.2. Influence of pH and temperature on enterococcal growth

#### 3.2.1. Generation Time (GT)

GT mean values related to the 21 tested strains of enterococci were highly correlated to both pH and, in particular, temperature (*P* < 0.001): GT values increased when pH decreased, and they ranged from 0.85 to 1.21 h (*P* < 0.001) at 25 °C while they varied from 0.44 to 0.67 h at 37 °C (*P* < 0.001).

At the same pH, when the temperature decreased the growth of all the enterococcal strains was greatly slowed down; in fact, GT values were almost double those at 25 °C than at 37 °C (Fig. 2, Tab. II).

In fact, at 37 °C a GT average emerges, which is about half that at 25 °C in the same conditions of acidity (Fig. 2, Tab. II).

Both species showed similar behavior at optimal temperature (37 °C), but *E. faecium* was more sensitive than *E. faecalis* at 25 °C and with increasing acidity: the maximum value of GT for this species was recorded at 25 °C and pH 5.0 (1.50 h), while in the same conditions *E. faecalis* duplicated in 0.93 h.

The development of *E. faecium* was influenced by temperature in the same way as by pH (*P* < 0.001); in fact, the GT values were bigger at 25 °C than at 37 °C, and differed in a remarkable way with increases in medium acidity. In addition, this species was particularly sensitive at pH 5.0; in fact, at both temperatures, at this pH there was a considerable increase in GT (Tab. II).

For *E. faecalis* at 25 °C there were no particular differences regarding the GT when acidity varies, while at 37 °C we had a bigger increase in this parameter with a decrease in the pH.

Thus, it seems that the development of the strains belonging to the species *E. faecalis* is above all influenced by variations in temperature (*P* < 0.001), rather than by pH, which is significant only at 37 °C (*P* < 0.002) (Tab. II).

#### 3.2.2. Metabolic activity (∆µS max)

Although grown in acid media, the same strains have high ∆µS max values; consequently, we can assume that the max values of ∆µS are an index of the metabolic activity of strains in acid media as well.

Observing the mean of ∆µS max values of the 21 tested strains, we can deduce that the metabolic activity of enterococci is also influenced by pH and temperature (*P* < 0.0001) (Fig. 3); in fact, the maximum values of ∆µS for both temperatures decreased with increasing acidity in a similar way (Tab. II), and at 37 °C they were always higher than at 25 °C.

Conductimetric trials pointed out that the metabolic curves took a longer time to reach the ∆µS max at 25 °C than at 37 °C; moreover, the count of the cfu·mL⁻¹ carried out at the plateau of the curves for each strain at different pH indicated that the varying ∆µS max values are not due to decreased microbial development. We can suppose that the different activity is not bound to a minor microbial development nor to a different reaction of the medium at different pH, but rather to minor metabolic activity at decreasing pH, even if the strains show the same number of cells.

Metabolic activity of *E. faecalis* was less influenced than *E. faecium* by temperature: at 25 °C the ∆µS max was 335 vs. 301 (*P* < 0.05), while at 37 °C it was 394 vs. 424 (*P* = NS) (Fig. 3).

Otherwise *E. faecium* and *E. faecalis* showed a similar behavior for pH: when the acidity of the medium decreased, ∆µS max increased for both species without significant differences.

Considering all the different conditions of temperature and pH, *E. faecium* showed a wider range of metabolic activity (from 188 ∆µS at 25 °C and pH 5.0 to 530 ∆µS at 37 °C and pH 6.5) than *E. faecalis* (from...
Growth of *E. faecium* and *E. faecalis* 187 ∆µS at 25 °C and pH 5.0 to 484 ∆µS at 37 °C and pH 6.5) (Tab. II).

3.2.3. Detection Time (DT)

Even the DT of cells, the expression of time necessary for the adaptation to different culture conditions was very correlated with species, pH and temperature. DT values were higher when temperature decreased and acidity increased (Fig. 4).

The ratio between DT values related to ideal conditions (pH 6.5, 37 °C) and the worst ones (pH 5.0, 25 °C) is almost 1:10 (Tab. II).

Also in this case *E. faecium* was sensitive to medium acidity and temperature; in fact, a shift in the DT for growth was detected with respect to the control curve (37 °C and pH 6.5). At 37 °C, it adapted better, with values ranging from 1.3 h to 4.6 h; at 25 °C from 3.5 h to 9.9 h.

For *E. faecalis* DT values at 37 °C were not significantly different when acidity increased to 5.5 but at pH 5.0 they doubled with respect to other pH levels.
Table II. Least square means and significance of temperature and pH on Generation Time (GT), Metabolic Activity (MAX) and Detection Time (DT) of *Enterococcus* sp. (21 strains), *E. faecalis* (11 strains) and *E. faecium* (10 strains).

<table>
<thead>
<tr>
<th>T (°C)</th>
<th>pH</th>
<th>5.0</th>
<th>5.5</th>
<th>6.0</th>
<th>6.5</th>
<th>SE</th>
<th>5.0 vs. 5.5</th>
<th>5.0 vs. 6.0</th>
<th>5.0 vs. 6.5</th>
<th>5.5 vs. 6.0</th>
<th>5.5 vs. 6.5</th>
<th>6.0 vs. 6.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>GT (h)</td>
<td><em>Enterococcus</em> sp.</td>
<td>25</td>
<td>1.21</td>
<td>1.05</td>
<td>0.94</td>
<td>0.85</td>
<td>0.1</td>
<td>*****</td>
<td>NS **</td>
<td>*** NS</td>
<td>*** NS</td>
<td>NS **</td>
</tr>
<tr>
<td></td>
<td></td>
<td>37</td>
<td>0.67</td>
<td>0.58</td>
<td>0.46</td>
<td>0.44</td>
<td>0.1</td>
<td>** NS</td>
<td>NS ***</td>
<td>** NS</td>
<td>** NS</td>
<td>NS NS</td>
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<tr>
<td></td>
<td><em>E. faecalis</em></td>
<td>25</td>
<td>0.93</td>
<td>0.91</td>
<td>0.87</td>
<td>0.83</td>
<td>0.1</td>
<td>NS NS</td>
<td>NS NS</td>
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<tr>
<td></td>
<td></td>
<td>37</td>
<td>0.69</td>
<td>0.61</td>
<td>0.48</td>
<td>0.41</td>
<td>0.1</td>
<td>NS NS</td>
<td>NS **</td>
<td>NS NS</td>
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<tr>
<td></td>
<td><em>E. faecium</em></td>
<td>25</td>
<td>1.50</td>
<td>1.18</td>
<td>1.02</td>
<td>0.86</td>
<td>0.1</td>
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<td>37</td>
<td>0.66</td>
<td>0.55</td>
<td>0.44</td>
<td>0.46</td>
<td>0.1</td>
<td>NS NS</td>
<td>NS NS</td>
<td>NS NS</td>
<td>NS NS</td>
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</tr>
<tr>
<td>MAX (ΔμS)</td>
<td><em>Enterococcus</em> sp.</td>
<td>25</td>
<td>211</td>
<td>311</td>
<td>345</td>
<td>406</td>
<td>18</td>
<td>*** ***</td>
<td>*** ***</td>
<td>*** ***</td>
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<td></td>
<td></td>
<td>37</td>
<td>293</td>
<td>379</td>
<td>457</td>
<td>507</td>
<td>17</td>
<td>*** ***</td>
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<tr>
<td></td>
<td><em>E. faecalis</em></td>
<td>25</td>
<td>235</td>
<td>345</td>
<td>326</td>
<td>434</td>
<td>29</td>
<td>** NS</td>
<td>** NS</td>
<td>** NS</td>
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<tr>
<td></td>
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<td>* NS</td>
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</tr>
<tr>
<td></td>
<td><em>E. faecium</em></td>
<td>25</td>
<td>188</td>
<td>278</td>
<td>363</td>
<td>377</td>
<td>22</td>
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<td></td>
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<td>37</td>
<td>306</td>
<td>405</td>
<td>455</td>
<td>530</td>
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<td>** ***</td>
<td>** ***</td>
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<tr>
<td>DT (h)</td>
<td><em>Enterococcus</em> sp.</td>
<td>25</td>
<td>10.0</td>
<td>5.6</td>
<td>4.2</td>
<td>3.1</td>
<td>0.4</td>
<td>*** ***</td>
<td>*** ***</td>
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<td><em>E. faecalis</em></td>
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* P < 0.05; ** P < 0.01; *** P < 0.001;    NS: not significant;    SE: standard error.
Growth of E. faecium and E. faecalis

At 25 °C they were higher and strictly dependent on the pH (from 2.8 h to 10.1 h). Both species were influenced in particular at pH 5.0 (Tab. II, Fig. 4).

4. CONCLUSION

Enterococci occur and grow in a variety of cheeses, especially in artisanal cheeses produced from raw milk in southern Europe (Italy, Spain, Portugal and Greece) [1, 2, 7, 17, 18, 33, 37, 38]. In our research we found, as remarked by other authors, that levels of enterococci in milks and cheese curds range from 10^2 to 10^4 cfu·mL^{-1} or g^{-1}, whereas in the fully ripened cheeses (from 20 to 70 days) they reach a level from 10^5 to 10^8 cfu·g^{-1} (numbers vary with cheese type and production season).

The dominance or persistence of enterococci in some cheeses during ripening can

Figure 3. Metabolic activity mean values (ΔμS max) of E. faecalis (11 strains), E. faecium (10 strains) and Enterococcus sp. (21 strains) at different temperatures and pH.
be attributed to their wide range of growth temperatures and their high tolerance to heat, salt and acidity [1, 12, 18, 34]. For this reason the enterococci, in particular *E. faecalis* and *E. faecium*, usually represent an important part of the bacterial flora of ripened cheeses [1, 2, 7, 17, 18, 33, 37, 38].

Conductance analysis allowed the evaluation of the influence of pH and temperature on the growth and metabolic activity of enterococci in milk and to measure their ability to adapt to conditions present during cheese-making and ripening (without antagonists). In particular, it was possible to evaluate that speed of duplication and adaptation to low temperatures and acidity for *E. faecium* was slower than *E. faecalis*. All this information can justify the different behavior of these species in different cheeses, as reported by many authors.

Enterococci produce typical flavor components such as acetaldehyde, acetoin and

**Figure 4.** DT mean values (h) of *E. faecalis* (11 strains), *E. faecium* (10 strains) and *Enterococcus* sp. (21 strains) at different temperatures and pH.
Growth of *E. faecium* and *E. faecalis* 191
diacetyl [7, 17, 34]. Some enterococci of
dairy origin have also been reported to pro-
duce bacteriocins (enterocins) inhibitory
against pathogenic bacteria (e.g. *Listeria
monocytogenes* and *Salmonella* sp.) [3, 23,
27]. The technological application of ente-
ocins, which have been shown to be pro-
duced during food manufacture, led to pro-
posing enterococci as adjunct or protective
cultures in cheeses [8]. Moreover, *E. faec-
cium* strain SF68 finds application in pro-
biotic preparations for humans [9].

However, the fact that enterococci have
recently assumed importance as nosocomial
corphens is not neglected. Enterococci are
low grade pathogens, but their intrinsic
resistance to many antibiotics and their
acquisition of resistance to the few antibi-
otics available for treatment in clinical ther-
apy, such as the glycopeptides, have led to
difficulties and searching for new drugs and
therapeutic options [17, 19].

Clearly, there is no consensus on the
acceptance of their presence in foodstuffs
and their role as primary non starter lactic
acid bacteria (NSLAB) is still a question
mark.

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