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# IDENTIFICATION OF ENTEROCOCCI ISOLATED FROM COW'S MILK CHEESE: COMPARISON OF THE CLASSICAL METHODS AND THE API 20 STREP SYSTEM

(TECHNICAL NOTE)

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A comparison of the results obtained using the classical methods with those of the API 20 Strep system was carried out in identifying 24 enterococci strains isolated from San Simón cow's milk cheese, a traditional Spanish variety. The results of both identification systems coincided exactly in 9 strains (37.5% of the strains studied). In one strain the results obtained using the classical methods did not coincide with those using the API 20 Strep method. 3 strains (12.5%) could not be identified using the API 20 Strep system. However, 11 strains (45%), that remained doubtful between both species *E. faecalis* and *E. faecium* on the basis of the classical methods, were identified using the API 20 Strep system. The API 20 Strep system does not include some biochemical tests of importance in identifying of foodborne enterococci and could not identify the atypical strains of *Enterococcus*. Moreover, this system is adapted to the identification of enterococci of clinical origin and their database does not include some species common in foods. However, it could have an application in combination with the classical methods in order to carry out a reasonably rapid and reliable identification of enterococci related to cheese.

**Keywords**: Enterococci, cow's milk cheese, classical identification methods, API 20 Strep system

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## Introduction

The study of Enterococci population in cheeses has a double (both sanitary and technological) interest. The hygienic and sanitary interest derives from the fact that enterococci come from human and monogastric domestic animal gastrointestinal ducts where they carry out a probiotic action [1]. Their presence in food has been used as indicator of faecal contamination and related to the presence of enteric pathogens [2, 3]. Some enterococci could be responsible for the production of enterotoxins and biogenic amines [4, 5], and have important health hazards. The ability of this microbial group to survive under adverse environmental conditions does not always mean that its presence is the result of deficient hygienic conditions during the making and ripening of cheeses [6, 7, 8]. It is also sufficiently heat resistant so as to resist pasteurization and even higher degrees [9, 10], which means that its presence in pasteurized cheeses does not always indicate a postpasteurization contamination or an insufficient heat treatment.

The technological interest of this group is based on the fact that its members have an acidifying capacity and can have, to a greater or lesser extent, proteolytic and lipolytic activities (some strains of the species *E. faecalis* var. *liquefaciens* can become highly proteolytic), contributing to the development of the organoleptic characteristics of cheeses. They have also been related to alterations and defects in various foods, including cheese [11, 12, 13].

Several researchers studied the repercussion of the use of enterococci as a starter culture in cheese [14, 15, 16, 17, 18]. In general, one can arrive at the conclusion that they improve the quality of cheeses, increasing their aroma and flavour, and approximating their organoleptic characteristics to those of the artisanal cheeses. Either way, before the use of a *Enterococcus* strain as a starter culture it is essential to select it according to its technological characteristics and harmlessness [19].

Because of the health and technological repercussions which derive from the presence of enterococci in cheeses, their identification is a necessary and habitual task both in industries dedicated to cheese making and in research laboratories related to cheese and other fermented milk products.

The classical methods of enterococci identification are, of course, the most reliable, but rapid identification methods could be of interest. Simplified commercial systems have been designed and standardized for the identification of enterococci of clinical origin and they could also have application in the identification of enterococci isolated from foods.

The aim of this work is to evaluate the usefulness of the API 20 Strep system (bioMérieux S.A., Montalieu Vercieu, France) (identification system for streptococci)

in the identification of enterococci isolated from cow's milk cheese by the comparison of the results obtained with those obtained using the classical identification methods.

## Materials and methods

### *Enterococci strains*

Twenty-four enterococci strains isolated from plates of kanamycin aesculin azide (KAA) agar (Oxoid, Ltd., Basingstoke, U.K.) incubated for 18 to 24 h at 37ºC [20], throughout the manufacturing and ripening of San Simón cheese, an artisanal variety made from raw cow's milk in the NW of Spain, were used. The isolates were purified by four subcultures alternately in TSB (Tryptone Soya Broth) (Oxoid) and TSA (Tryptone Soya Agar) (Oxoid), and then maintained in TSB+20% glycerol at -30ºC.

### *Identification using classical methods*

Enterococci were identified according to the methods and criteria of Collins et al. [21, 22], Devriese et al. [23], Farrow et al. [24], Mundt [25] and Schleifer and Kilpper-Bälz [26].

Of each isolate the following characteristics were studied: Gram-staining, catalase, gas production from glucose according to the Gibson and Abd-El-Malek technique [27], growth at 10ºC, 40ºC, 45ºC, growth at 4% and 6.5% NaCl, growth at pH 9.6, survival at 60ºC for 30 min, growth in bile aesculin agar (Oxoid), growth in KF agar (Oxoid) [28], and triphenyltetrazolium chloride (TTC) reduction.

The identification of *Enterococcus* strains at species level was carried out on the basis of growth in 0.1% methylene blue milk, ammonia from L-arginine production, reduction of 0.04% potassium tellurite, growth in KF agar, growth at 45ºC and 50ºC, fermentation of carbohydrates (L-arabinose, arbutin, melezitose, melibiose, sorbitol, sorbose, rhamnose, starch and sucrose) in bromocresol purple 1% broth base [29], and gelatin hydrolysis ability in gelatin nutritive agar [29] and in Frazier's gelatin agar [30].

### *Identification using the API 20 Strep system*

The API 20 Strep strips were used following the manufacturer's instructions [31]. The API 20 Strep strip consists of 20 plastic microtubes containing dehydrated substrates for the demonstration of enzymatic activities or the fermentation of sugars.

The microtubes of enzymatic tests are inoculated and rehydrated with a dense suspension of cells. The microtubes of fermentation tests are inoculated with the strain suspension in an enriched medium which reconstitutes the sugar substrates.

Strips, after inoculation, were incubated at 35–37ºC and the test results were observed after 4 and 24 hours. The metabolic end products produced during the incubation period are either visualized through spontaneous colored reactions or by the addition of reagents. Fermentation of carbohydrates is detected by a shift in the pH indicator.

Based on the test results, a seven-digit profile number was constructed as outlined in the instructions and the identification was made according to this seven-digit profile number. The database of the Analytical Profile Index of the API 20 Strep method [31] includes 32 species of streptococci (although only five of these are of *Enterococcus* genus).

## Results and discussion

Homofermentative, Gram-positive, catalase-negative, facultatively anaerobic cocci, which were capable of growing at 10ºC, 37ºC, 40ºC and 45ºC, in 4% and 6.5% NaCl, at pH 9.6, which survive after heating at 60ºC for 30 min, hydrolize aesculin in the presence of 40% bile, and in KF agar form red or pink colonies normally with a yelow halo, were considered belonging to the genus *Enterococcus*.

Using the classical identification methods, of the 24 *Enterococcus* isolates, 5 were identified as *Enterococcus faecalis* (3 as *E. faecalis* var. *faecalis* and 2 as *E. faecalis* var. *liquefaciens*), 5 as *E. faecium*, 13 as *E.* inter *faecalis-faecium*, because by paying attention to the criteria used to differenciate *Enterococcus faecalis* from *E. faecium* (above all the fermentation of L-arabinose, arbutin, melezitose, melibiose, sorbitol and sorbose, as well as 0.04% potassium tellurite and 0.01% TTC reduction) these strains showed intermediate characteristics between *E. faecalis* and *E. faecium*, and, finally, 1 strain that only fermented starch and sucrose was identified as *E. durans*. The cultural and biochemical characteristics of these 24 isolates are shown in Table I.

Using the API 20 Strep system, of the previously mentioned 24 isolates 12 were identified as *Enterococcus faecalis* 2, 9 as *E. faecium* 2, although with the possibility of identification as *E. casseliflavus*, and 3 could not be identified using the API 20 Strep strips. Table II shows the comparison of the identification results using the classical methods and using the API 20 Strep system.

Of the 5 strains identified as *Enterococcus faecalis* (2 as *E. faecalis* var. *liquefaciens* and 3 as *E. faecalis* var. *faecalis*) using the classical methods 4 were identified as *E. faecalis* 2 and 1 could not be identified using the API 20 Strep system,

because of the lack of leucine arylamidase activity. API 20 Strep system does not permit the identification of the biovarities of *E. faecalis* because the gelatin liquefaction test was not included among the tests.

The five strains identified as *Enterococcus faecium* using the classical methods were identified with high probability as *E. faecium* 2 using the API 20 Strep system, although with the possibility of identification as *E. casseliflavus*.

*E. faecium* is well identified by the API 20 Strep system. However, when the strains have an atypical biochemical profile, the API 20 Strep system can be less successful in identification, mainly when they are sorbitol-positive, β-galactosidase-negative and leucine arylamidase-negative strains in the API galleries [32]. In our case, the five strains identified as *E. faecium* using the classical methods were sorbitol-negative, β-galactosidase-positive and leucine arylamidase-positive in the API strips.

Two strains of *E. faecium* were raffinose positive both in the classical tests and in the API 20 Strep strips after 4 hours of incubation, while after 24 hours of incubation the five strains of *E. faecium* were raffinose positive in the API strips. The acid production from raffinose in *E. faecium* strains is a variable character, although generally the *E. faecium* strains from mammals are raffinose-negative. Thus, raffinose fermentation can pose problems in identifying these strains using the API 20 Strep system, their adscription to other species such as *E. casseliflavus* or *E. gallinarum* is also probable thus other conventional tests are necessary [32]. In fact, even though these 5 strains were identified, with a high probability, as *E. faecium* 2, with the API 20 Strep system, the numerical profile obtained also shows the possibility that they could be strains of the species *E. casseliflavus*.

Of 13 strains identified as *E.* inter *faecalis-faecium* using the classical methods, seven were identified as *E. faecalis* 2 and four as *E. faecium* 2 (with the possibility of identification as *E. casseliflavus*) using the API 20 Strep system. The remaining two could not be differentiated in a reliable way.

The seven strains of *E.* inter *faecalis-faecium* identified as *E. faecalis* 2 by the API 20 Strep system were identified with regard to their characteristics of β-Galactosidase-negativity and β-Glucuronidase-negativity, the negative fermentation of arabinose and positive fermentation of sorbitol. In these strains L-arabinose fermentation was positive or weakly positive in the classical tests, which makes the relationship to *E. faecalis* difficult following the classical identification methods. The acidification of L-arabinose is difficult to interpret in uncovered phenol red broths [32] and possibly also in bromocresol purple broths (base medium used for test of the carbohydrates fermentation in this study). These strains showed the same numerical profile in the API 20 Strep system as the strains identified as *E. faecalis* using the classical tests, since the API 20 Strep strips do not include the fermentation test of

certain carbohydrates for those which differ in the strains identified as *E. faecalis* and as *E.* inter *faecalis-faecium* using the classical method. These strains were submitted to gelatin liquefaction test, resulting in one strain positive and six negative strains.

**Table I**

*Biochemical and cultural characteristics of the 24* Enterococcus *strains isolated from San Simón cow's milk cheese*

| | *E. faecalis* | *E. faecalis* var. *liquefaciens* | *E. faecalis* var. *faecalis* | *E. faecium* | *E.* inter *faecalis–faecium* | *E. durans* |
|---|---|---|---|---|---|---|
| Number of isolates | 5 | 2 | 3 | 5 | 13 | 1 |
| Growth at 45ºC | 5 | 2 | 3 | 5 | 13 | 1 |
| Growth at 50ºC | 0 | 0 | 0 | 0 | 0 | 0 |
| Growth at pH 9.6 | 5 | 2 | 3 | 5 | 13 (1 w) | 1 |
| Growth in 6.5% NaCl | 5 | 2 | 3 | 5 (1 w) | 13 | 1 |
| Growth in 0.1% methylene blue | 5 | 2 | 3 | 5 | 13 | 1 |
| Survival after 60ºC / 30 min. | 5 (1 w) | 2 | 3 (1 w) | 5 (1 w) | 12 | 1 w |
| Growth in 0.04% K-tellurite | 5 | 2 | 3 | 5 (2 w) | 13 (1 w) | 1 |
| Fermentation | | | | | | |
| L-Arabinose | 0 | 0 | 0 | 5 | 13 (1 w) | 0 |
| Arbutin | 5 | 2 | 3 | 5 | 13 | 0 |
| Melezitose | 2 | 2 | 0 | 1 w | 13 | 0 |
| Melibiose | 0 | 0 | 0 | 5 | 13 (4 w) | 0 |
| Sorbitol | 4 (1 w) | 2 (1 w) | 2 | 0 | 10 (4 w) | 0 |
| Sorbose | 5 (1 w) | 2 (1 w) | 3 | 0 | 9 (2 w) | 0 |
| Rhamnose | 5 (1 w) | 2 | 3 (1 w) | 1 w | 12 (2 w) | 0 |
| Raffinose | 2 w | 2 w | 0 | 2 (1 w) | 10 (8 w) | 0 |
| Starch | 5 | 2 | 3 | 5 | 13 | 1 |
| Sucrose | 4 | 2 | 2 | 5 | 10 | 1 |
| Growth in KF agar | 5[a] (3 w) | 2[a] (1 w) | 3[a] (2 w) | 5[a] (1 w) | 13[a] | 1[a] |
| Ammonia from | 5 | 2 | 3 | 5 | 13 | 1 |

arginine

[a]All strains reduce TTC and form red colonies with yellow halo.
The numbers included in the table correspond to the number of positive strains in each test.
w = weak reaction

**Table II**

*Comparison of the identification results in the 24* Enterococcus *strains using the classical methods and the API 20 Strep system*

| Strain | Classical methods | API 20 Strep system | Strain | Classical methods | API 20 Strep system |
|---|---|---|---|---|---|
| 1 | *E. faecalis var. liquefaciens* | *E. faecalis* 2 | 13 | *E. faecium* | *E. faecium 2** |
| 2 | *E. faecalis* var. *faecalis* | *E. faecalis 2* | 14 | *E. faecium* | *E. faecium 2**** |
| 3 | *E. faecium* | *E. faecium 2**** | 15 | *E.* inter *faecalis-faecium* | N.I. |
| 4 | *E.* inter *faecalis-faecium* | *E. faecium 2**** | 16 | *E. faecium* | *E. faecium 2**** |
| 5 | *E.* inter *faecalis-faecium* | *E. faecalis* 2 | 17 | *E.* inter *faecalis-faecium* | *E. faecalis* 2 |
| 6 | *E. faecalis* var. *liquefaciens* | *E. faecalis* 2 | 18 | *E.* inter *faecalis-faecium* | *E. faecalis* 2 |
| 7 | *E.* inter *faecalis-faecium* | N.I. | 19 | *E. durans* | *E. faecalis 2* |
| 8 | *E.* inter *faecalis-faecium* | *E. faecium* 2* | 20 | *E.* inter *faecalis-faecium* | *E. faecalis 2* |
| 9 | *E.* inter *faecalis-faecium* | *E. faecalis* 2 | 21 | *E. faecalis* var. *faecalis* | N.I. |
| 10 | *E. faecium* | *E. faecium 2** | 22 | *E. faecalis* var. *faecalis* | *E. faecalis* 2 |
| 11 | *E.* inter *faecalis-faecium* | *E. faecalis* 2 | 23 | *E.* inter *faecalis-faecium* | *E. faecium* 2* |
| 12 | *E.* inter *faecalis-faecium* | *E. faecalis* 2 | 24 | *E.* inter *faecalis-faecium* | *E. faecium* 2* |

* Possibility of identification as E. casseliflavus
N.I.: Not identified

The four strains of *E.* inter *faecalis-faecium* identified as *E. faecium* 2 with the API 20 Strep system showed in the API strips the same profile as the strains that had been identified as *E. faecium* using the classical methods, because some carbohydrates such as melezitose or melibiose (whose fermentation made the identification as one concrete species confusing using the classical methods) are not included in the API 20 Strep data base.

Finally, one strain identified as *E. durans* using the classical identification methods was identified as *E. faecalis* 2 using the API 20 Strep system. This strain presents in the API strips an identical profile as the strains identified as *E. faecalis* 2. However, this strain does not have a typical profile, as it does not ferment almost any

sugar in the classical tests (Table I), it only ferments sucrose and starch, although in API 20 Strep galleries it is mannitol and sorbitol positive. On the basis of the principal characteristic of not fermenting most sugar, this strain remained doubtful between *E. faecalis* 2 and *E. durans*.

Atypical strains of *E. durans* can ferment some carbohydrates. API 20 Strep system easily identifies the atypical *E. durans* strains (melibiose-positive and/or sucrose-positive) [32], but the mannitol positive and/or sorbitol positive strains were not identified as *E. durans*, independent of the fact that other sugars are not fermented.

The incapacity to ferment both mannitol and sorbitol allows for the differentiation of *E. durans*. However, atypical strains of *E. durans* could ferment mannitol [21, 24, 33]. On the other hand, the sorbitol fermentation is always negative. In the classical test this strain does not ferment this carbohydrate.

So as to correctly identify this strain, further investigation is necessary since the identification of atypical melibiose-negative and sucrose-positive *E. durans* strains (characteristics of this strain) can also pose other problems. API 20 Strep system recognizes as *E. durans* the atypical melibiose positive and sucrose negative *E. durans* strains, as well as the sucrose positive strains, whatever the fermentation of the melibiose is [32], since it does not include these tests. *E. durans* is normally negative in these tests, but the atypical strains of *E. durans* could be melibiose positive and/or sucrose positive, characteristics of *E. hirae*, in the same way that some *E. hirae* strains could not ferment these carbohydrates [34]. Other identification systems such as the Rapid ID 32 Strep identify as *E. durans* the melibiose negative and sucrose positive strains and as *E. hirae* the melibiose and sucrose positive strains. Therefore, the differentiation of *E. hirae* from *E. durans* is complex.

It appears that the API 20 Strep system is not appropriate to be applied successfully with other enterococci species (*E. mundtii*, *E. hirae*, *E. casseliflavus* or *E. malodoratus*) since API Strep galleries do not contain the necessary tests to differenciate most of these enterococci. Additional tests are necessary but these are often not included in the galleries.

A number of studies on the API 20 Strep method showed that the majority of *E. faecalis*, *E. faecium*, *E. avium* and *E. durans* strains isolated of a clinical origin are correctly identified [35]. However, because this system was developed prior to the recent taxonomy changes, some identifications may be in error, especially for species other than *E. faecalis* and for “enterococcus-like” strains. This system needs to be reevaluated with these new species definitions.

Certain decisive tests in the identification of the strains as the genus *Enterococcus* are needed since the API 20 Strep system includes in the same data base

streptococci belonging to different Lancefield serological groups. Thus, a same numerical profile could correspond, for example, to enterococci and to lactococci.

However, the API 20 Strep system could have an application in combination with the classical methods so as to carry out a reasonably rapid (most of these test systems provide an identification in approximately 4 h) and reliable identification of enterococci related to cheese.

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## References

1. Vanos,V.: Importance of streptococci group D in fermented dairy products, as indicators of quality assurance in comparison with coliforms. Bull Int Dairy Federation nº **264**, 22–25 (1991)
2. Godfree,A.F., Kay,D., Wyer,M.D.: Faecal streptococci as indicators of faecal contamination in water. J Appl Microbiol, Symposium Supplement **83**, 110S–119S (1997)
3. Laukovà,A., Juris,P.: Distribution and characterization of *Enterococcus* species in municipal sewages. Microbiol **89**, 73–80 (1997)
4. Joosten,H.M.L.J., Núñez,M.: Prevention of histamine formation in cheese by bacteriocin-producing lactic acid bacteria. Appl Environ Microbiol **62**, 1178–1181 (1996)
5. Tham,W.: Histamine formation by enterococci isolated from home-made goat cheeses. Int J Food Microbiol **7**, 103–108 (1988)
6. Gatti,M., Borio,P., Fornasari,E., Neviani,E.: Enterococci in Italian cheeses. Latte **18**, 392–397 (1993)
7. Mucchetti,G., Neviani,E., Todesco,R., Lodi,R.: Ruolo degli enterococchi nei formaggi italiani. II. Attività caseinolitica e lipolitica. Latte **7**, 821–831 (1982)
8. Neviani,E., Mucchetti,G., Contarini,G., Carini,S.: Ruolo degli enterococchi nei formaggi italiani. I. Loro presenza in formaggi di monte e impiego in un innesto selezionato. Latte **7**, 722–728 (1982)
9. Otogalli,G., Rondinini,G., Conti,D.: Coliformi, streptococchi fecali e test di sedimentazione in alcuni formaggi freschi. Ann Microbiol Enzimol **29**, 41–48 (1979)
10. Wessels,D., Jooste,P.J., Mostert,J.F.: Technologically important characteristics of *Enterococcus* isolates from milk and dairy products. Int J Food Microbiol **10**, 349–352 (1990)
11. Kurmann,J.A., Schilt,P.: Behaviour of gas-producing streptococci in Emmental cheese. Schweiz Milch Forsch **99**, 57–58 (1973)
12. Nath,K.R., Kostak,B.J.: Etiology of white spot defect in Swiss cheese made from pasteurized milk. J Food Prot **49**, 718–723 (1986)
13. Ritter,W.: The problem of the biochemical cause of the late fermentation in Emmental cheese. Some suggestions for further study. Deutsche Molkereiztg **97**, 680–684 (1976)

14. Casalta,E., Zennaro,R.: Effect of specific starters on microbiological, biochemical and sensory characteristics of Venaco, a Corsican soft cheese. Sci Aliments **17**, 79–94 (1997)
15. Centeno,J.A., Menéndez,S., Hermida,M., Rodríguez Otero,J.L.: Effects of the addition of *Enterococcus faecalis* in Cebreiro cheese manufacture. Int J Food Microbiol **48**, 97–111 (1999)
16. Hegazi,F.Z.: Some properties of white pickled cheese made with *Streptococcus faecalis* subsp. *liquefaciens* as a starter. Nahrung **33**, 721–728 (1989)
17. Hegazi,F.Z., Abo-Elnaga,I.G.: Characteristics of white pickled cheese made by various starters and ripened without brine. Microbiol Aliment Nutr **9**, 331–334 (1991)
18. Tzanetakis,N., Vapoulou-Mastrojiannaki,A., Litopoulou-Tzanetaki,E.: The quality of white-brined cheese from goat's milk made with different starters. Food Microbiol **12**, 55–63 (1995)
19. Kalantzopoulos,G.: Enterococci in food fermentations. Functional and safety aspects, *in* F. Toldrá, D. Ramón and J.L. Navarro (ed.), Proceedings of the International Congress Improved Traditional Foods For The Next Century. Gráficas Barrasil (Valencia, Spain). 1999. pp. 57–59
20. Mossel,D.A.A., Bijker,P.G.H., Eelderink,I.: Streptokokken der Lancefield-gruppe D in Lebensmittel und Trinkwasser. Ihre Bedeutung, Erfassung und Bekämpfung. Arch Lebensmittelhyg **29**, 121–127 (1978)
21. Collins,M.D., Jones,D., Farrow,J.A.E., Kilpper-Bälz,R., Schleifer, K.H.: *Enterococcus avium* nom rev, comb nov; *E. casseliflavus* nom rev, comb nov; *E. gallinarum* comb nov; and *E. malodoratus* sp nov. Int J Syst Bacteriol **34**, 220–223 (1984)
22. Collins,M.D., Farrow,J.A.E., Jones,D.: *Enterococcus mundtii* sp nov. Int J Syst Bacteriol **36**, 8–12 (1986)
23. Devriese,L.A., Van de Kerckhove,A., Kilpper-Bälz,R., Schleifer,K.H.: Characterization and identification of *Enterococcus* species isolated from the intestines of animals. Int J Syst Bacteriol **37**, 257–259 (1987)
24. Farrow,J.A.E., Jones,D., Phillips,B.A., Collins,M.D.: Taxonomic studies on some group D streptococci. J Gen Microbiol **129**, 1423–1432 (1983)
25. Mundt,J.O.: Enterococci. *in* P.H.A. Sneath, N.S. Mair, M.E. Sharpe and J.G. Holtz (ed.), Bergey's Manual of Systematic Bacteriology. Vol II. Williams & Wilkins (Baltimore, U.S.A.). 1986. pp. 1063–1065
26. Schleifer,K.H., Kilpper-Bälz,R.: Molecular and chemotaxonomic approaches to the classification of Streptococci, Enterococci and Lactococci: a review. Syst Appl Microbiol **10**, 1–19 (1987)
27. Gibson,T., Abd-El-Malek,Y.: The formation of carbon dioxide by lactic acid bacteria and *Bacillus licheniformis* and a cultural method of detecting the process. J Dairy Res **14**, 35–44 (1945)
28. Kenner,B.A., Clark,H.F., Kabler,P.W.: Fecal streptococci. I. Cultivation and enumeration of streptococci in surface water. Appl Microbiol **9**, 15 (1961)
29. Harrigan,N.F., Mc Cance,M.E.: Métodos de laboratorio en microbiología de alimentos y productos lácteos. Ed. Academia. León, Spain. 1979
30. Smith,N.R., Gordon,R.E., Clark,F.E.: Aerobic sporeforming bacteria. US Dept Agric Monograph No 16. US Dept of Agriculture, Washington, 1952
31. API SYSTEM S.A.: API 20 Strep Identification system for streptococci. Analytical Profile Index. 3rd edition. Montalieu Vercieu, France. 1987
32. Devriese,L.A., Pot,B., Van Damme,L., Kersters,K., Haesebrouk,F.: Identification of *Enterococcus* species isolated from foods of animal origin. Int J Food Microbiol **26**, 187–197 (1995)

33. Centeno,J.A., Cepeda,A., Rodríguez Otero,J.L.: Identification and preliminary characterization of strains of enterococci and micrococci isolated from Arzúa raw cow's milk cheese. Nahrung **39**, 55–62 (1995)
34. Facklam,R.R., Collins,M.D.: Identification of *Enterococcus* species isolated from human infections by a conventional test scheme. J Clin Microbiol **27**, 731–734 (1989)
35. Murray,B.E.: The life and times of the *Enterococcus*. Clin Microbiol Rev **3**, 46–65 (1990)