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ORIGINAL ARTICLE

Evaluation of the extent of spreading of virulence factors and antibiotic resistance in Enterococci isolated from fermented and unfermented foods

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Abstract The aim of this work was to evaluate the impact of fermentation processes on the incidence of virulence and antibiotic resistance determinants in *Enterococcus* strains from food origin (milk, fermented milk, cheese, fresh meat and fermented meat) with regards to potential pathogenicity. A total of 167 enterococcal strains were used in this study. Of those, 2 were reference strains, and 165 were isolated and identified by molecular methods and screened for virulence factors including *ace*, *agg*, *ccf*, *cpd*, *esp*, $efaA_{fm}$ and *gelE* as well as resistance against ten antibiotics. Enterococcal isolates were identified as *Enterococcus faecium* (54), *Enterococcus faecalis* (86), *Enterococcus casseliflavus* (10) and other *Enterococcus* spp. (15). Only 3 out of 88 fermented food isolates were free from virulence determinants while approximately 16 % of strains isolated from unfermented foods were free from virulence determinants. Unusually, the *Ace* gene was detected in *E. faecium*, especially in fermented foods. In contrast, antibiotic resistance in enterococci was not potentiated by the fermentation process. This study has revealed the important role that may be played by fermentation processes in virulence gene incidence and the potential of such processes to disseminate these traits throughout the enterococci food chain.

Keywords Safety evaluation · *Enterococcus* · Virulence factors · Antibiotic resistance

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Introduction

Enterococci are lactic acid bacteria (LAB) that form an important part of food microbiota, especially in those of animal origin such as fresh meat, fermented sausages and cheeses (Eaton and Gasson 2001). LAB have a predominant habitat in the gastrointestinal tract of humans and animals. Enterococci therefore have the ability to grow at between 10 °C and 45 °C, in the presence of 6.5 % sodium chloride, at a range of pH values (4.6–9.6) and to survive heating at 60 °C for 30 min (Foulquié-Moreno et al. 2006). These abilities explain their existence in various raw foods (such as milk and meat) and their multiplication in these materials during fermentation.

Moreover, several strains of the genus *Enterococcus* have also been used as probiotics, which have a positive impact on the gastrointestinal microbial balance or can be used in the treatment of gastroenteritis (Giraffa 2003; Ogier and Serror 2008). In addition, bacteriocinogenic potential has been proved by Santos et al. (2014). Some strains also play an important role in the production, ripening and aroma development of different types of cheeses (Giraffa 2003; Foulquié-Moreno et al. 2006; Xu and Kong 2013). Enterococci have also been found in many traditional and artisan fermented meat products in Mediterranean countries, and rely on the endogenous flora to retain their traditional organoleptic qualities. In contrast, their presence is not permitted in some types of cheese and processed meat products because they are considered a source of spoilage and, in some cases, a risk to human health (Franz et al. 1999). Some enterococcal strains are typical opportunistic pathogens, especially in nosocomial

infections, which are caused mainly by *Enterococcus faecalis* (80 %) and *Enterococcus faecium* (5–20 %), (Morrison et al. 1997; Martin et al. 2005).

Furthermore, antibiotic resistant enterococci have been found in food products, including meat and dairy products (Miranda et al. 2007; Gomes et al. 2008). The genetic determinants of antibiotic resistance in enterococci are generally located on conjugation plasmids or transposons (Clewell 1990). Likewise, the presence of virulence factors in enterococci and their transfer to other strains have been demonstrated (Eaton and Gasson 2001). Many putative virulence traits have been identified in enterococci, including *ace* (collagen adhesion), *agg* (aggregation substance), $efaA_{fm}$ (cell wall adhesin expressed in serum by *Enterococcus faecium*), *esp* (enterococcal surface protein), and *gelE* (gelatinase) (Shankar et al. 1999; Eaton and Gasson 2001; Klibi et al. 2015). Additionally, although sex pheromones are not considered as virulence factors per se, their role in enterococci may involve dissemination of virulence determinants and antibiotic resistance via pheromone-responsive conjunctive plasmids (Eaton and Gasson 2001; Valenzuela et al. 2008; Ben Belgacem et al. 2010).

Previous works have indicated that the genetic adaptability and promiscuity of enterococci plays an important role in the development of problematic lineages (Franz et al. 2011). However, there is still a lack of information on the diffusion of antibiotic-resistant and virulent enterococci in fermented foods. Moreover, few data are available on the effect of the food environment on the occurrence of gene transfer in food microbiota and on the dynamics of antibiotic resistance diffusion during the fermentation process, such as during cheese and cured meat production.

Most previous studies have focused on presenting comparisons depending mainly on the source of strains. Furthermore, some studies on enterococci have been carried out using optimal environmental conditions, which allow the cells to grow and divide normally. The present research highlights the impact of the fermentation process through the actual prevalence of virulence and resistance determinants in fermented and unfermented foods. In this context, the aim of this work was to estimate the extent of diffusion of antibiotic resistance and virulence determinant within *Enterococcus* spp. in foods from different sources with regards to their potential pathogenicity, hence allowing a correct assessment of the spread of virulence within the *Enterococcus* genus.

Materials and methods

Bacterial strains and culture media

A total of 165 food samples was collected from local supermarkets, small factories and farms in the North of Spain in order to obtain the *Enterococci* isolates from both fermented and unfermented foods. A total of 167 enterococci and 4 non-enterococci strains was used in this study. Of these, 165 were isolated from various foodstuffs, 1 isolate from each food sample type (54 from fresh meat, 19 from fermented meat (chorizo), 23 from raw milk, 7 from fermented milk (Requeixón) and 62 from cheese (sheep and goat raw milk and mixed). In addition, two strains of *E. faecalis* (ATCC 27285, ATCC 19433) were used as reference strains. Finally, two *Lactococcus garvieae* (ATCC 43921 and KF012887G; GenBank accession number) and two *Vagococcus salmoninarum* (CECT 5810 and CECT 5811) strains were analysed and included as an out-group in the cluster analysis.

Enterococci isolates were collected from 25 g samples of each food, and were extracted aseptically and diluted in an appropriate volume (1/9 w/v) of 0.1 % peptone water (Merck, Darmstadt, Germany) and homogenised for 1 min in a masticator (AES, Combourg, France). After homogenisation, 1×10^{-1} to 1×10^{-4} dilutions of homogenates were surface plated on Chromocult Enterococci Agar plates (Merck). These plates were then incubated at 37 °C for 48 h. After the incubation period, two typical red, small and round presumptive LAB colonies from each sample were selected randomly. The frozen stored collection isolates were reactivated in brain-heart infusion (BHI) medium (Becton, Dickinson & Company, Le Pont de Claix, France) and subsequently surface plated on Chromocult Enterococci Agar and incubated at 37 °C for 24 h.

Isolate identification and phylogenetic analysis

All isolates were inoculated in BHI and incubated at 37 °C overnight. Afterwards, 1 mL broth was placed in a clean tube and centrifuged at 5160 *g* for 10 min. Bacterial DNA was subsequently extracted using a DNeasy Tissue Mini Kit (Qiagen, Valencia, CA) as described previously (Campos et al. 2006).

Enterococci isolated from dairy and meat samples were identified by amplification and sequencing of the 16S rRNA gene. Amplification of the 16S rRNA gene was performed with the universal primer pair p8FPL (5′-AGTTTGATCCTGGCTCAG-3′) and p806R (5′-GGACTACCAGGGTATCTAAT-3′) in a MyCycler Thermal Cycler (BioRad Laboratories, Hercules, CA) under the following conditions: (1) initial denaturing step at 94 °C/ 7 min; (2) 35 cycles of denaturation (94 °C/ 60 s), annealing (55 °C/ 60 s) and extension (72 °C/ 60 s); and (3) final extension at 72 °C/ 15 min (McCabe et al. 1999).

Prior to sequencing, PCR products were purified using a ExoSAP-IT kit (GE Healthcare, Uppsala, Sweden). Direct sequencing was performed with a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA). The same primers used for PCR were also employed to

sequence both strands of the PCR products. Sequencing reactions were analysed using an automatic sequencing system (ABI 3730XL DNA Analyser, Applied Biosystems) with the POP-7 system and reviewed carefully using Chromas software (Griffith University, Queensland, Australia). Nucleotide sequences were aligned using CLUSTAL X software (Larkin et al. 2007), and phylogenetic analysis was conducted with MEGA software (Kumar et al. 2008), using the neighbour-joining (NJ) method (Saitou and Nei 1987) with 1000 bootstrap replicates to construct distance-based trees. All 16S rRNA sequences obtained were deposited in the GenBank database of the NCBI as reference data.

Random amplified polymorphic DNA–polymerase chain reaction analysis

All isolates were genotyped by random amplified polymorphic DNA (RAPD)-PCR. Reactions were carried out in a total volume of 25 μL using 200 ng template DNA, 12.5 μL Master mix (BioMix, Bioline, London, UK) and 14 pmol random primers M13R2 and CC1 (Table 1). The amplification program consisted of 5 min at 94 °C and 40 cycles of denaturation at 94 °C for 30 s (for M13R2) or for 1 min (for CC1), annealing for 1 min at 38 °C (for M13R2) or 33 °C (for CC1) and elongation at 72 °C (1.5 min for M13R2 and 2 min for CC1), followed by a final elongation at 72 °C for 5 min (primer M13R2) or 1 min (primers CC1).

To evaluate the reproducibility of the RAPD-PCR analysis, the molecular weight of each PCR product was determined in comparison with a DNA ladder using software Image Lab, version 4.1 (Bio-Rad). RAPD profiles of both primers were combined and calculation of the similarity of PCR fingerprinting profiles was based on the presence or absence of each DNA band in each molecular weight range. Molecular weight was recorded as ones or zeros for positive and negative reactions, respectively, and a cluster dendrogram was carried out with PASW statistics 18 (SPSS, Chicago, IL).

Determination of virulence factors

Screening of the virulence determinants *ace*, *agg*, *ccf*, *cpd*, $efaA_{fm}$, *esp* and *gelE* was performed by PCR amplification using the primers detailed in Table 1 following previously described procedures (McCabe et al. 1999; Shankar et al. 1999; Eaton and Gasson 2001; Dupre et al. 2003; Reviriego et al. 2005).

Antibiotic susceptibility testing

All *Enterococcus* spp. isolates were subjected to antimicrobial susceptibility testing using the disc diffusion method recommend by the Clinical and Laboratory Standards Institute (CLSI 2012) on Mueller-Hinton agar plates (BioMérieux, Marcy-l'Etoile, France). The antimicrobial discs used included ampicillin (Am, 10 μg), chloramphenicol ©, 30 μg), clindamycin (Cc, 2 μg), ciprofloxin (Cip, 5 μg), erythromycin (E, 15 μg), neomycin (N, 30 μg), penicillin (P, 10 μg), rifampicin (Ra, 30 μg), tetracycline (Te, 30 μg) and vancomycin (Va, 30 μg). All antibiotic discs were obtained from BioMérieux. The antibiotic resistance breakpoints used were the interpretative criteria for *Enterococcus* spp. as recommended by CLSI (2008). *E. faecalis* ATCC 29212 and *Staphylococcus aureus* ATCC 29213 were used as quality controls.

Table 1 Polymerase chain reaction (PCR) primers used for the detection of virulence factors and random amplified polymorphic DNA (RAPD)-PCR analysis

Gene	Responsible for	Sequence	Product size (bp)	Reference
Ace	Adhesion of collagen	AAAGTAGAATTAGATCCACAC TCTATCACATTCGGTTGCG	320	Dupre et al. (2003)
Agg	Cell aggregation and conjugation	AAGAAAAAGAAGTAGACCAAC AAACGGCAAGACAAGTAAATA	1553	Eaton and Gasson (2001)
Ccf	Sex- pheromone	GGGAATTGAGTAGTGAAGAAG AGCCGCTAAAATCGGTAAAAT	543	Reviriego et al. (2005)
Cpd	Sex- pheromone	TGGTGGGTTATTTTTCAATTC TACGGCTCTGGCTTACTA	782	Reviriego et al. (2005)
$efaA_{fm}$	Antigen of bacteria endocarditis	AACAGATCCGCATGAATA CATTTCATCATCTGATAGTA	735	Eaton and Gasson (2001)
Esp	Immune evasion	TTACCAAGATGGTTCTGTAGGCAC CCAAGTATACTTAGCATCTTTTGG	913	Shankar et al. (1999)
*gel*E	Hydrolysis of gelatin, collagen, haemoglobin	ACCCCGTATCATTGGTTT ACGCATTGCTTTTCCATC	419	Eaton and Gasson (2001)
M13R2	Genotypic characterization	GGAAACAGCTATGACCATGA	Random primer	Martín et al. (2005)
CC1	Genotypic characterization	AGCAGCGTGG	Random primer	Cocconcelli et al. (1995)

Statistical analysis

The different species isolated from fermented (fermented milk, cheese and fermented meat) or unfermented (milk and meat) foods as well as the prevalence of virulence factors and antimicrobial resistance were compared using the X^2 test. Differences were considered significant when the *P*-value was lower than 0.05. All statistical analyses were carried out using Statgraphics version 5.0.1. (SAS Institute, Cary, NC).

Results

Identification and phylogenetic analysis

Enterococci isolates were identified as *E. faecium* (54), *E. faecalis* (86 isolates), *Enterococcus casseliflavus* (10 isolates), *E. gilvus* (4 isolates), *E. malodoratus* (4 isolates), *E. thailandicus* (5 isolates) and *E. durans* (2 isolate). The distribution of enterococci species isolated from unfermented and fermented foods was significantly different ($P=0.003$). *E. faecalis* was the most common species isolated from fermented foods (62 isolates out a total of 88), whereas *E. faecium* was the most common species isolated from unfermented foods (34 isolates out of a total of 77) (Table 2).

A phylogenetic tree was constructed showing the phylogenetic relationships (Supplementary Fig. S1). Within the cluster of all studied *Enterococcus* spp. strains, five species groups were found. Moreover, enterococci isolates were also discriminated genetically by RAPD-PCR analysis with two different primers: M13R2 and CC1. A total of 165 different RAPD fingerprint profiles was identified (Fig. 1). The number of different genotypes demonstrated the different origin of the isolates and also a good microbial diversity, which is important to ensure the significance of this study. A total of 23 RAPD clusters was defined at similarity 50 % and most genotypes of the same species were clustered together.

Virulence factors

The results indicate that 150 out of 165 enterococci isolates carryied virulence factors. As can be seen in Table 2, the $efaA_{fm}$ gene was significantly more frequently found in strains isolated from unfermented foods than from fermented foods. Meanwhile, the *ace*, *agg* and *esp* genes were significantly more frequent in enterococci isolated from fermented foods than in those isolated from unfermented foods. The *ccf* and *cpd* genes were the most widespread virulence determinants and were found in most of the studied strains (64 % and 53 %, respectively; Table 2).

None of *E. faecalis* isolates were free from virulence factors, whereas *E. gilvus* isolates were negative for all tested virulence factors. $efaA_{fm}$ was present in 93 % of *E. faecium* isolates while *ccf* and *ace* showed the highest incidence in *E. faecalis* (90 % and 83 %, respectively). In the case of *E. faecalis*, *ccf* and *ace* were the most commonly found virulence factors (>80 % of isolates), whereas $efaA_{fm}$ was found in only 14 % of isolates.

On the other hand, the presence of multiple virulence factors was detected more frequently in *E. faecalis* than in *E. faecium* ($P<0.001$). With the exception of one isolate, all *E. faecalis* isolates harboured at least two of the tested virulence determinants, whereas 20 % of *E. faecium* isolates showed the presence of only one virulence factor.

A comparison of enterococci isolated and unfermented or fermented foods revealed that the presence of multiple virulence factors was detected more frequently in strains isolated from fermented foods ($P<0.001$) (Fig. 2). Likewise, 96 % fermented foods isolates harboured at least two of the tested virulence determinants, whereas 18 % of strains isolated from unfermented foods showed the presence of only one virulence factor. Additionally, only one enterococcal strain isolated from unfermented food was positive for all tested virulence determinants while four (5 %) fermented food strains were positive.

Antibiotic susceptibility

A very different prevalence of antibiotic resistance against the antibiotics tested was detected among *Enterococcus* isolates (Table 2). None of the isolates were resistant to Am or Va except two isolates that were identified as *E. faecalis*. Furthermore, *E. faecalis* strains were mostly resistant to N (85 %) and Cc (74 %) while fewer than 10 % of isolates were resistant to C (7 %), Ra (7 %) and Va (2 %). In contrast, *E. faecium* strains were mostly resistant to Cc (67 %) and N (57 %). However, the difference in these patterns of resistance between *E. faecium* and *E. faecalis* was not significant ($P>0.05$).

In keeping with the origin of the enterococci, it was found that strains isolated from fermented foods were more frequently resistant to E and C than those isolated from unfermented foods. Meanwhile, higher resistance rates against Cc, Cip and P were found in isolates obtained from unfermented foods, and no differences were detected for Am, Ra, Te, N and Va (Table 2). The antimicrobials for which higher rates of resistance were obtained in strains isolated from fermented foods were Cc (75 %) and N (72 %), while in strains isolated from unfermented foods the most common antimicrobial resistances found were to Cc (83 %), N (68 %) and P (45 %). Moreover, strains isolated from unfermented food had a higher incidence of multiple antibiotic resistance than those from fermented foods ($P=0.026$; Fig. 2).

Table 2 Incidence of virulence factors and resistance to different antibiotics for *Enterococcus* spp. isolated from fermented and unfermented food. Values in the same columns with different lower case letters are significantly different

	Fermented foods				Unfermented foods				Total/ %
Species (number of isolates)	*E. faecium* (20)	*E. faecalis* (62)	Other spp. (6)	Total/ % 88/ (100 %)	*E. faecium* (34)	*E. faecalis* (24)	Other spp. (19)	Total/ % 77/ (100 %)	(165)/ 100 %
Virulence factors*									
Ace	9	49	1	59/ (67 %) a	1	21	1	23/ (29 %) b	82/ (49 %)
Agg	4	31	2	37/ (42 %) a	0	14	2	16/ (21 %) b	53/ (32 %)
Ccf	9	52	1	62/ (70 %) a	19	23	2	44/ (57 %) a	106/ (64 %)
Cpd	6	46	1	53/ (60 %) a	13	21	1	35/ 45 %) a	88/ (53 %)
$efaAm_{fm}$	16	8	3	27/ (31 %) b	34	4	3	41/ (53 %) a	68/ (41 %)
Esp	3	33	0	36/ (41 %) a	0	12	1	13/ (17 %) b	49/ (30 %)
GelE	11	27	3	41/ (47 %) a	12	19	4	35/ (45 %) a	76/ (36 %)
Antibiotics**									
Am	0	0	0	0/ (0 %)	0	0	0	0/ (0 %)	0/ (0 %)
C	2	6	0	8/ (9 %) a	0	0	1	1/ (1 %) b	9/ (5 %)
Cc	10	44	6	60/ (68 %) b	26	20	18	64/ (83 %) a	124/ (75 %)
Cip	13	3	1	17/ (19 %) b	15	7	8	30/ (39 %) a	43/ (26 %)
E	9	15	0	24/ (27 %) a	3	10	1	14/ (18 %) a	38/ (23 %)
N	10	53	5	68/ (77 %) a	21	20	11	52/ (68 %) a	120/ (72 %)
Ra	5	5	0	10/ (11 %) a	6	1	7	14/ (18 %) a	24/ (15 %)
P	3	7	1	11/ (13 %) b	21	4	10	35/ (45 %) a	46/ (28 %)
Te	4	26	5	35/ (40 %) a	11	20	1	32/ (41 %) a	67/ (40 %)
Va	0	1	0	1/ (1 %) a	0	1	0	1/ (1 %) a	2/ (1 %)

* Virulence factors: *ace* collagen adhesin, *agg* aggregation substance, *ccf & cpd* sex pheromone-encoding genes, $efaA_{fm}$ cell wall adhesins expressed by *Enterococcus faecium*, *esp* enterococcal surface protein, *gelE* gelatinase

** Antibiotics: *Am* ampicillin, *C* chloramphenicol, *Cc* clindamycin, *Cip* ciprofloxin, *E* erythromycin, *N* neomycin, *P* penicillin, *Ra* rifampicin, *Te* tetracycline, *Va* vancomycin

Discussion

This study applied molecular methods based on 16S rRNA gene sequencing and RAPD fingerprint profiles to determine the hierarchical clustering of genomic profiles for species identification and illustrate the microbial biodiversity of *Enterococcus* species isolated from foodstuffs. Furthermore, the two primers (M13R2 and CC1) used in the RAPD-PCR assay increased the discrimination potential within enterococci species. Thus the results obtained from RAPD-PCR profiles (Fig. 1) were more discriminative in detecting intra-specific differences than those from phylogenetic analysis based on 16S rRNA sequences alone. RAPD-PCR profiles have proved an efficient molecular method for characterization inter-strain variation (Martin et al. 2005). Nevertheless, this method does not have the discrimination potential to distinguish strains according to their food of origin. Enterococci, especially *E. faecium* and *E. faecalis*, have long been present in numerous fermented and unfermented foods (Franz et al. 2001). Despite several studies over the last decade concerning virulence determinants in clinical and food isolates, little is known about the environmental cues that regulate their expression (Carlos et al. 2010). Previous works (Valenzuela et al. 2008; Cariolato et al. 2008; Semedo-lemsaddek et al. 2013) have reported that the gene encoding the collagen-binding protein gene *ace*, which is involved in attachment to extracellular matrix proteins, is usually found in *E. faecalis* strains. The results obtained in the present work have shown that the *ace* gene was detected in both *E. faecium* and *E. faecalis*. It is interesting to note that the *ace* gene, which has often been described only in *E. faecalis*, appeared within *E. faecium* strains only from fermented foods with the exception of one isolate from milk. This poses a question about the role of the fermentation process in the presence of these strains. Likewise, *agg* was also detected in *E. faecalis* and *E. faecium* (Cariolato et al. 2008; Barbosa et al. 2010; Ozmen et al. 2010). However, in all cases in the present study, *E. faecium* strains containing the *agg* gene were isolated from fermented foods. The possibly negative aspect of the presence of enterococci in fermented foods is their ability to frequently transfer risk factors. Hirt et al. (2002) reported that the frequency of transfer was influenced strongly by environmental conditions. In particular, a study by Cocconcelli et al. (2003) showed that mobile genetic elements carrying virulence

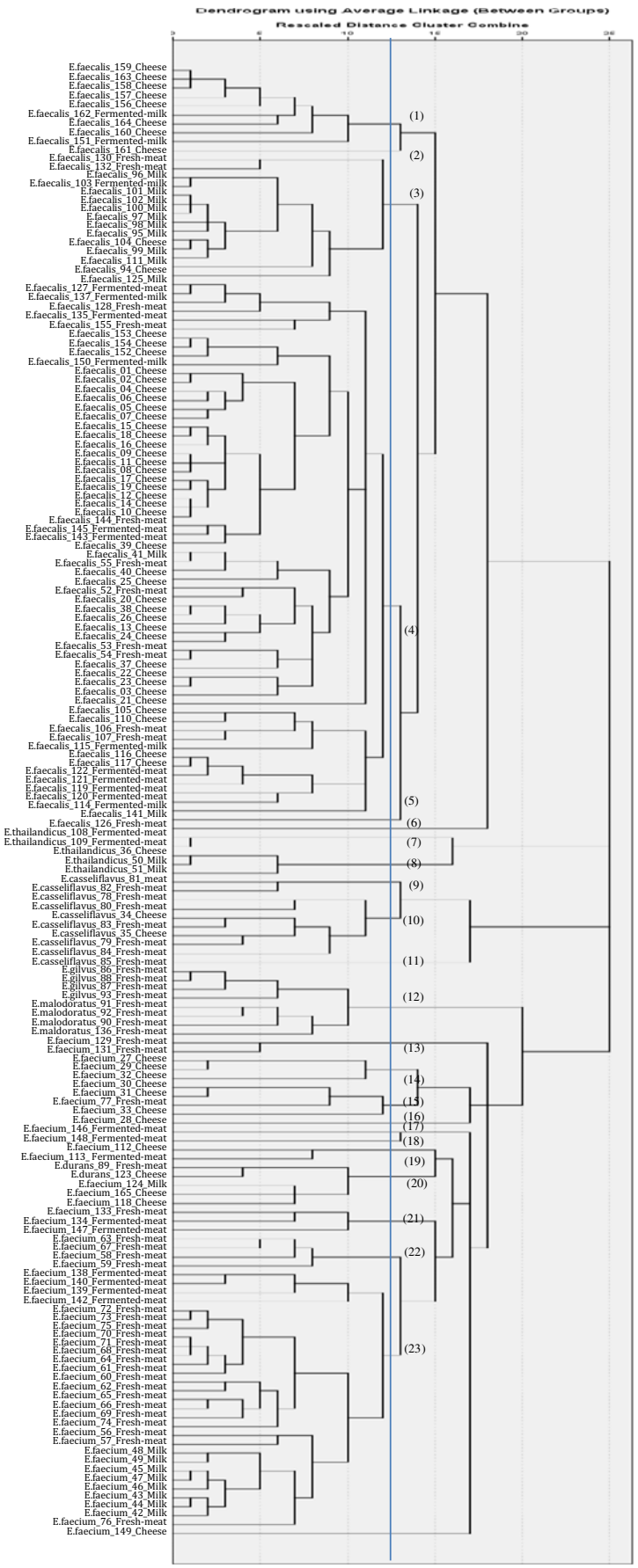

Fig. 1 Dendrogram generated from random amplified polymorphic DNA–polymerase chain reaction (RAPD-PCR) of 165 different profiles showing the relationships between related enterococcal bacterial species isolated from foodstuffs. "0" indicates maximum similarity. The *vertical line* marks cut-off value at 50 % arbitrarily chosen to define RAPD groups within the dendrogram

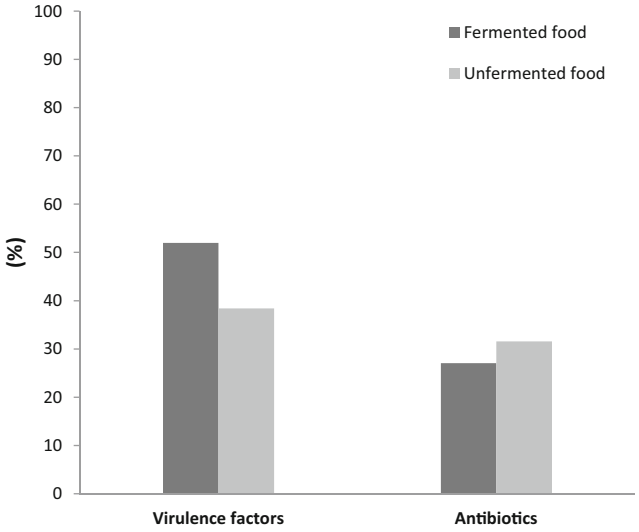

Fig. 2 Comparative analysis of virulence factors and antibiotic resistance as a rate over the total possible, among enterococci isolated from fermented foods and from non-fermented foods

determinants and antibiotic resistance can be transferred at high frequency in food-associated enterococci during cheese and sausage fermentation; however, no other study has proved this.

The *efaA*$_{fm}$ gene was found in various enterococci species isolated from both fermented and unfermented foods, and appeared in all *E. faecium* isolated from unfermented foods. It is possible to explain this distribution of the *efaA*$_{fm}$ gene given the nature of its association with *E. faecium* (Martin et al. 2005). Previous studies have detected the *efaA*$_{fm}$ gene in both *E. faecium* and *E. faecalis* isolated from fermented foods (Barbosa et al. 2010; Ozmen et al. 2010). As previously reported by Ozmen et al. (2010), a higher incidence of the *esp* gene was found in both *E. faecalis* and *E. faecium* isolated from naturally fermented food. These findings are in agreement with our results, which revealed the higher incidence of the *esp* gene in fermented food than in unfermented food. Notably, the *esp* gene was found within *E. faecium* strains from fermented food in this study, although it has been found only rarely in *E. faecium* by others (Valenzuela et al. 2008; Barbosa et al. 2010).

Although previous studies have also shown variation in the spread and distribution of the *gelE* gene, there were no differences in the distribution of this gene according to the fermentation process. However, the presence of the *gelE* gene in an *Enterococcus* strain does not mean that the gene is functional, as various isolates carrying *gelE* have been shown not to produce gelatinase (Eaton and Gasson 2001). With respect to sex pheromone determinants, several studies are in agreement with our detection of sex pheromone determinants in *E. faecalis* and *E. faecium* (Valenzuela et al. 2009; Ben Belgacem et al. 2010; Ozmen et al. 2010). *Ccf* and *cpd* genes were identified in a large number of enterococci strains, particularly in those isolated from fermented foods, accounting for about 70 % and 60 % of strains, respectively. These large numbers of sex pheromone determinants, which involve dissemination of virulence determinants, might explain the high incidence of virulence determinants in fermented foods. In accordance with this, enterococcal strains isolated from fermented foods that carry multiple virulence traits have been reported (Canzek Mahjhenic et al. 2005).

The dissemination of antibiotic resistance among enterococci in fermented and unfermented foods was also investigated. Indeed, most enterococci isolates (83 %) showed resistance to multiple antibiotics. This frequent detection of antibiotic resistance among enterococci could be related to efficient mechanisms for transfer of resistance genes via conjugative plasmids and transposons (Franz et al. 1999; Clewell 1990). In agreement with the results of this study, enterococci resistant to several antibiotics ©, Cip, E, N, Ra and Te) as well as being sensitive to Am and P have been found in recent studies (Miranda et al. 2007; McGowan-Spicer et al. 2008; Valenzuela et al. 2009). In the present work, when considering the distribution of antibiotic resistance according to species, it was found that *E. faecalis* possessed a higher number of resistances than *E. faecium*. The result sof this study agree with the findings of Valenzuela et al. (2009).

Another major concern in the emergence of antibiotic resistant enterococci is sex pheromone responsive plasmids, which may carry one or more antibiotic resistance genes (Clewell 1990; Wirth 1994). In this context, the efficient transfer mechanisms of resistance genes via conjugative plasmids and transposons may explain the weak effect of the fermentation process on the transfer of resistance genes. Furthermore, Te resistance is one of the most commonly acquired forms of antibiotic resistance in *Enterococcus* food isolates (Peters et al. 2003), and Templer and Baumgartner (2007) observed a high incidence of Te resistance (68 %) among enterococci isolated from raw milk cheeses. This study found broadly similar results in both fermented and unfermented foods (40 % and 41 %, respectively). Moreover, we also can not overlook the role of legislative policies regarding the treatment of animals with antibiotics, which have an effect on the prevalence of genetic resistance. On the other hand, Va-resistant enterococci pose a major problem in the treatment of human clinical infections, as Va is used as a last resort treatment for multiple antibiotic resistant enterococci (Huycke et al. 1998). However, only two isolates from the studied enterococcal isolates were resistant to the clinically important antibiotic Va.

It should be pointed out that, throughout our results, and in contrast to the results gathered on the presence of virulence factors, enterococci resistance to antibiotics was not potentiated by the fermentation process. Indeed, we observed a higher incidence of multiple resistances to antimicrobials in unfermented enterococcal strains than in fermented

enterococcal strains. This might depend on the type of resistance (whether acquired or intrinsic), or be due to efficient transfer mechanisms of resistance genes and the role of legislative policies governing the use of antibiotics, which have an effect on the prevalence and distribution of the genetic determinants of resistance.

Conclusions

Meat and dairy products can play a clear role in the dissemination of virulence and resistance traits through the food chain of enterococci, especially for *E. faecalis*, which contains multiple virulence determinants and antibiotic resistances compared to *E. faecium*. In addition, virulence determinants were more prevalent within strains from fermented foods, while antibiotic resistance was not necessarily correlated with the fermentation process. Notably, our results have also shown that the *ace* gene is found in *E. faecium*, especially in strains from fermented foods. Therefore, strains to be used for biotechnological applications should be tested carefully for the presence of antibiotic and virulence determinants, and the evolution of virulence within the *Enterococcus* genus should be monitored precisely to reduce the incidence of nosocomial infection associated with enterococci.

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