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REVIEW ARTICLE

Technological, functional and safety aspects of enterococci in fermented vegetable products: a mini-review

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Abstract Bacteria belonging to the genus *Enterococcus* spp.—a member of the lactic acid bacteria group—are natural inhabitants of various environments, including vegetable products. Although some strains show pathogenic determinants, overall these bacteria may have some pro-technological features. Some enterococci have been described as potential starter or protective cultures in the dairy industry, since they contribute to the organoleptic and quality characteristics of dairy products. Although several fermented vegetable products have a long history in human nutrition, studies regarding autochthonous enterococci and their application to fermented vegetable foods are much less numerous than those concerning dairy foods. In this review, after a general overview of enterococci, their presence and role in fermented vegetable foods (including table olives, sauerkraut, kimchi, tomato juice, French beans, caper berries and cereal-based products) will be covered.

Keywords *Enterococcus* · Fermented vegetable foods · Enterocin · Technological properties · Starter culture · Probiotic

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Abbreviations
E *Enterococcus*
L *Lactobacillus*

Introduction

The genus *Enterococcus* belongs to the large group of lactic acid bacteria that produce lactic acid by fermentation. On the one hand, enterococci are considered as indicators of fecal contamination (e.g., in water), or even as microorganisms carrying some pathogenic potential (Settanni and Corsetti 2007; Sabia et al. 2008). On the other hand, enterococci can play a positive role in various fermented products (El-Ghaisha et al. 2011). The contribution of enterococci to the organoleptic properties of fermented food products, and their common ability to produce bacteriocins (enterocins), may be of interest in food technology. These bacteria play a fundamental role in the ripening of dairy products, probably through proteolysis, lipolysis, exopolysaccharide production and citrate breakdown, thus conferring a unique taste and flavor to dairy foods (Manolopoulou et al. 2003; Yerlikaya and Akbulut 2011). Moreover, since some probiotic features have been reported for several strains of genus *Enterococcus* (Botes et al. 2008; Surono et al. 2011), such strains may be used to improve the microbial balance of the intestine or to prevent or treat gastroenteritis in humans and animals (Bhardwaj et al. 2008, 2010; Fortina et al. 2008). Recently, the use of enterococci as starter cultures or co-cultures (adjuncts) has increased considerably (Bhardwaj et al. 2010; Lee et al. 2008; Rodgers 2008). The majority of these studies have focused on the applicability of *Enterococcus* strains to food of animal origin (dairy and meat products) and, to a lesser extent, to vegetable products. Nowadays, there is an increasing trend towards consumption of vegetable products. Interest in fermented vegetable products is increasing since they are rich in antioxidants, vitamins, dietary fibres and minerals (Rodríguez et al. 2009). Among the various technological options, lactic acid fermentation may be considered as a simple and valuable technology for maintaining and/or improving the safety, nutritional, sensory and shelf-life properties of vegetables (Buckenhüskes 1997). Currently, lactic acid fermentation of vegetables has industrial significance for table olives and sauerkrauts. However, it has been shown that the safety, nutritional, sensory and shelf-life properties of several other vegetables (e.g., French beans, capers, cereals) may be improved through lactic acid fermentation.

The genus *Enterococcus* contains bacterial species associated with animals and plants. Species from mainly humans and domestic animals have been studied in some detail. Limited information is available on plant-associated species. To date, there have been several reviews on the functional aspects of enterococci in dairy foods (Bhardwaj et al. 2008; Chamba and Jamet 2008; Garcia de Fernando 2011; Ogier and Serror 2008). This review describes the most significant case studies of the application of enterococci to vegetable food products.

General characteristics of enterococci

Enterococci have a low (<50 mol %) guanine-plus-cytosine content in DNA, typical of related phylum genera *Clostridium* and *Bacillus* and, based on 16S rRNA analysis, are more closely related to the genera *Vagococcus*, *Tetragenococcus*, and *Carnobacterium* than *Streptococcus* and *Lactococcus*. Enterococci are Gram-positive, catalase-negative, non-spore-forming- bacteria and facultative anaerobic cocci that occur singly, in pair, or in chains (Foulquié Moreno et al. 2006). They grow typically in 6.5% NaCl, hydrolyze esculin in the presence of 40% bile, and have a broad pH range (Franz et al. 2003; Giraffa 2003). Enterococci are more thermophilic than other Gram-positive cocci, growing at both 10°C and 45°C, and surviving temperatures as high as 68°C for 30 min.

A revision of the genus *Enterococcus* spp., in order to accommodate the species *Streptococcus faecium* and *Streptococcus faecalis* was proposed. Indeed, studies based on DNA–DNA hybridization and sequencing of the gene coding for 16S rRNA had indicated that those two species were quite distinct from other streptococci. *E. faecalis* and *E. faecium* are natural members of the digestive microbiota in humans, varying in abundance (10^2–10^8 per gram of digestive content) among individuals and along the gastro-intestinal tract (Ogier and Serror 2008). Currently, there are 38 recognized species within the genus *Enterococcus* (http://www.bacterio.cict.fr/e/enterococcus.html). These species were *E. faecalis*, *E. faecium*, *E. durans*, *E. cassaliflavus*, *E. avium*, *E. hirae*, *E. columbae*, *E. gallinarum*, *E. sulfureus*, *E. pallens*, *E. raffinosus*, *E. saccharolyticus*, *E. mundtii*, *E. villorum*, *E. flavescens*, *E. asini*, *E. dispar*, *E. pseudoavium*, *E. ratti*, *E. saccharominimus*, *E. canintestini*, *E. canis*, *E. cecorum*, *E. devriesi*, *E. malodoratus*, *E. aquimarinus*, *E. termitis*, *E. camelliae*, *E. phoeniculicola E. gilvus*, *E. haemoperoxidus*, *E. hermanniensis*, *E. moraviensis*, *E. porcinus*, *E. solitarius*, *E. villorum*, *E. viikkiensis* and *E. thailandicus*.

Enterococci are ubiquitous, and nowadays are considered to be an essential part of many fermented foods (e.g., cheeses, sausages). In fact, some *Enterococcus* have achieved "generally recognized as safe" (GRAS) status. The beneficial properties of enterococci for consumers result from their specific biochemical traits.

Role and application of enterococci in fermented vegetable technology

Table olives

The olive phylloplane, and in particular the fruit surface, is a suitable habitat for the survival of microbial populations, especially the lactic acid bacteria used in lactic acid fermentation of table olives (Nychas et al. 2002). Enterococci were isolated from Spanish-style green olive fermentations (De Castro et al. 2002; Randazzo et al. 2010), and *E. faecalis* and *E. faecium* were found to be frequent contaminants (Foulquié Moreno et al. 2006). Other species like *E. casseliflavus* and *E. italicus* were found as a component of the autochthonous microbiota during the first days of table olive fermentation started by a probiotic strain of *Lactobacillus paracasei* (De Bellis et al. 2010). Table 1 summarizes the *Enterococcus* species isolated from fermented vegetable products. Concerning the viable cells of enterococci during olive fermentation, Kacem and Karam (2006) showed that cell density of enterococci tended to increase during fermentation from ca. 3.0 log CFU/g (day 15) to ca. 5.0 log CFU/g (day 90). However, lower values of enterococci cell density were found in the final product by other authors (Ben Omar et al. 2004; Pereira et al. 2008). Varying levels of enterococci in different olive fermentation result from olive type, extent of olive contamination and survival in the fermented-olive environment (seasonal temperature).

Sauerkraut

Sauerkraut prepared from shredded cabbage (Brassica) by natural or controlled lactic acid fermentation is a popular

Table 1 *Enterococcus* species associated with vegetable fermented products

Fermented vegetable products	*Enterococcus* species	References
Spanish-style green olive	*E. faecium* 6T1a *E.* sp.	Floriano et al. 1998
Aloreña table olive: naturally-fermented olives (Spain)	*E.* sp.	Abriouel et al. 2011
Black olives	*E. faecium*	Franz et al. 1996
Algerian fermented olives	*E. faecalis*	Kacem et al. 2004
Sicilian fermented green olives	*E. faecium*	Randazzo et al. 2004
Western Algerian green olives	*E. faecalis*, *E. faecium*, *E* durans	Kacem and Karam 2006
Italy olive phylloplane Italy olive brine	*E. faecium*	Lavermicocca et al. 1998
Olive phylloplane and olive brine	*E. faecium*	Lavermicocca et al. 2002
Spanish-style green olives	*E. casseliflavus*, *E. italicus*	De Bellis et al. 2010
Fresh and fermented cabbage (Argentine)	*E. hirae*	Pérez Borla et al. 2010
Cabbage (Brazil)	*E. faecalis*, *E. faecium*	Riboldi et al. 2009; Hui et al. 2004
Kimchi	*E. faecalis, E. faecium*	Lee et al. 1992; Shin et al. 1996
Tomato fruits	*E. faecium /E. faecalis*	Di Cagno et al. 2009; Sajur et al. 2007
French beans	*E. faecalis*	Di Cagno et al. 2008
Fermented caper berries	*E.* sp., *E. faecium*, *E. casseliflavus*; *E. faecalis*, *E. avium*	Pérez Pulido et al. 2005a, b; 2006
Fermented tea leaves	*E. camelliae*	Sukontasing et al. 2007
Portugal sourdough	*E. cassliflavus, E. durans, E. faecium*	Rocha and Malcata 1999
Sorghum sourdough Sudan	*E. faecalis*	Hamad et al. 1997
Sorghum Sudan kisra (flat bread)	*E. faecium*	Sulma et al. 1991
Tunisian wheat dough	*E. faecalis*	M'hir et al. 2007
Mexican pozol (maize dough fermentation)	*E. saccharolyticus*	Ben Omar and Ampe 2000
French wheat sourdough	*E. hirae*	Robert et al. 2009
German wheat sourdough	*E. faecium*	Kitahara et al. 2005
Tarhana: Turkish cereal food (a wheat flour-yoghurt mixture)	*E. faecium*	Sengen et al. 2009
Hussuwa (African fermented sorghum)	*E. faecium*	Yousif et al. 2005
Doenjang (Korean fermented soybean paste)	*E. faecium*	Kim et al. 2009
Japan fermented soybean paste	*E. faecium*	Onda et al. 2002
Chungkukjang (fermented soybean food)	*E. faecium*	Yoon et al. 2008
African locust been seeds	*E. faecium*, *E. hirae*	Ouaba et al. 2010
Tempeh (Malaysian soybean)	*E. faecium*	Moreno et al. 2002
Dochi fermented black beans	*E. faecium*	Chen et al. 2006
Bushera (alcoholic beverage: sorghum and millet)	*E. faecium*, *E. mundtii*	Muyunja et al. 2003
Tofu	*E. hermanniensis*, *E. casseliflavus, E. durans* *E. faecium /E. faecalis, E. mundtii/E. avium*	Chao et al. 2008; Lee et al. 1999
Locust been seeds fermentation (African Soumbala)	*E. faecium*, *E. hirae*	Ouaba et al. 2010
Malaysian Tempe	*E. faecium*	Moreno et al. 2002
Dochi (fermented black beans)	*E. faecium*	Chen et al. 2006
Idli (fermentation rice and black gram)	*E. faecalis*, *E. faecium*	Blandino et al. 2003 ;Rob Nout 2009 ; Vijayendra et al. 2010
Wheat mature sourdough and non conventional flour samples	*E. casseliflavus*, *E. durans*, *E. faecium*, *E. faecalis*, *E. mundtii*	Corsetti et al. 2007b

preserved vegetable consumed widely in many European countries. Like most vegetable fermentations, sauerkraut fermentation is spontaneous and relies on a very small population of lactic acid bacteria, which are naturally present on fresh vegetables, for preservation. It is known that a succession of various lactic acid bacteria species and their metabolic activities are responsible for the quality and safety of these products. The process is characterized by an initial heterofermentative stage, followed by a homofermentative stage. The fermentation is started by *Leuconostoc* *mesenteroides*. *E. faecalis* and *Pediococcus cerevisiae* may also contribute to the fermentation (Hui et al. 2004), but only a limited number of studies considered the role of *Enterococcus* in cabbage fermentation.

A large percentage distribution (65%) of *E. faecalis* and *E. faecium* isolated from cabbage in Porto Alegre in Brasil was found (Riboldi et al. 2009). *E. hirae* was isolated from fresh and fermented cabbage, and its ability to produce extracellular proteases was also evaluated (Pérez Borla et al. 2010). As a non-pathogenic caseinolytic strain, *E. hirae* may be potentially useful for technological applications.

Kimchi

Kimchi is a traditional Korean food fermented from a variety of vegetables (Hui et al. 2004). It is also gaining popularity as a functional food. The magazine *Health* mentioned kimchi in its list of the top five "World's Healthiest Foods" (http://eating.health.com/2008/02/01/worlds-healthiest-foods-kimchi-korea/#). The beneficial effects of kimchi on human health may be derived from nutrients and functional components such as vitamins, minerals, fibers and phytochemicals, from the biological compounds present either in kimchi ingredients such as garlic, ginger, red pepper powder, the end-products of the lactic acid fermentation and the lactic acid bacteria involved, or a combination of these. Korean cabbage and radish are the major vegetables, minor ingredients include garlic, red pepper, green onion, ginger and salt (Lee 1997). Kimchi is produced traditionally only during the winter, because of problems with both fermentation and preservation. The preservation problem was solved, in part, by refrigerators. However, the fermentation remained difficult to control due to the unique characteristics of the fermenting microbiota (Settani and Corsetti 2008). The population dynamics of lactic acid bacteria in kimchi is influenced greatly by temperature, which in turn can greatly influence the organoleptic and storage properties of the products (Cho et al. 2006). *E. faecalis* and *E. faecium* have been isolated from kimchi by several authors (Lee et al. 1992; Shin et al. 1996). Lee (1997) reported that *E. faecium* DU0216, DU030, DU037, DU0253, DU0255 and DU0267 isolated from kimchi have a broad spectrum of bacteriocin activities.

Tomato juice

Recently, *E. faecium/ faecalis* POM3 was isolated from tomato fruits (Di Cagno et al. 2009). To the best of our knowledge, this is the only study to have considered the use of selected autochthonous lactic acid bacteria for the fermentation of tomato juices. *L. plantarum*, *L. brevis*, *Weisella cibaria*, *Pediococcus pentosaceus* and *E. faecium/ faecalis* POM3 were used as the single autochthonous starter. The use of autochthonous and selected lactic acid bacteria strains to ferment tomato juices maintained agreeable texture, nutritional and sensory properties. All autochtonous strains grew well in tomato juice without nutriment supplementation and pH adjustment. After storage (until 40 days at 4°C), tomato juice fermented with *E. faecium/faecalis* POM3, *L. brevis* POM2 and *L. plantarum* POM1 and POM5 had the highest values of total antioxidant activity. *Enterococcus* strains were also isolated from tomato surfaces by Sajur et al. (2007).

French beans

Enterococcus faecalis (eight strains) was isolated from French beans (Di Cagno et al. 2008). Based on the rate of growth and acidification, these strains were not selected as starters for the fermentation of the carrots, French beans, or marrows. In fact, the main criteria for selecting starters to be used for vegetable fermentation are : (1) the rate of growth; (2) the rate and total production of acids which, in turn, affects the changes of pH; and (3) the environmental adaptation/tolerance (Gardner et al. 2001).

Caper berries

Caper berries are the fruits of *Capparis* species (*Capparis spinosa* L.)—a Mediterranean shrub cultivated for its buds and fruits. The fruits are collected during the months of June and/or July and immersed in tap water, where a spontaneous lactic acid fermentation takes place for approximately 5–7 days in a temperature range of 23 to 43°C. After this, fermented capers are placed in brine and distributed for consumption as seasoning. Manufacture of fermented capers is usually carried out in small production units or at home (Abriouel et al. 2008b). It has been reported that lactic acid bacteria are the dominant microorganisms during small scale industrial caper berry fermentation, causing a decrease in pH to below 4.0 in the final product (Pérez Pulido et al. 2005a, b). Among lactic acid bacteria found in fermented caper berries, *L. plantarum* was the species isolated most frequently. Out of 75 isolates of lactic acid bacteria, 2 were allotted to the species *E. faecium* (Pérez Pulido et al. 2005a, b). Besides this species, fermented caper berries have also been shown to harbor *E. faecalis*, *E. avium* and *E. casseliflavus* (Péréz Pulido et al. 2006). The cell density of enterococci in the final product varied from 2.3 to 5.3 log CFU/g, depending on the sample analyzed.

Cereal-based fermented food and beverages

Cereal fermentation is one of the oldest biotechnological processes, dating back to ancient Egypt, where both bread and beer were produced through metabolic activity of yeasts and lactic acid bacteria. Sourdough may be used as a leavening/flavoring agent for bread and other baked goods. Most lactic acid bacteria isolated from sourdough belong to the genus *Lactobacillus*, but species of *Enterococcus* are found occasionally in sourdough processes (see for review De Vuyst et al. 2009). Monitoring of 30 Tunisian wheat sourdough samples showed that ca. 35% of samples harbored enterococci at a cell density ranging from 2.0 to 6.0 log CFU/g (M'hir et al. 2007). Enterococci were also usually found as contaminant microorganisms in cereal kernels and flour (Corsetti et al. 2007b; De Vuyst and Neysens 2005). They may also be present during the early stages of sourdough production (Corsetti et al. 2007a; Van der Meulen et al. 2007) and may even dominate some sourdoughs (Rocha and Malcata 1999). *E. faecium* and *E. hirae* have been isolated from German (Kitahara et al. 2005) and French (Robert et al. 2009) wheat sourdoughs, respectively.

Selected strains of *Enterococcus* spp. may be used as starter cultures for sourdough. It was shown that they are able to grow during sourdough fermentation and to acidify the dough, thus possibly inhibiting undesired microorganisms, such as moulds and rope bacteria (Coppola et al. 1998; Corsetti et al. 2007a). Selected enterococci may play an important role for proteolysis during sourdough fermentation. Compared to other doughs started with *L. brevis* or *L. plantarum*, a sourdough fermented with *E. faecium* C-1 showed the highest concentration of free amino acids (Collar et al. 1992). Generation of free amino acids can positively influence the growth of yeasts during dough fermentation and the development of flavor compounds (Gobbetti et al. 2005). Furthermore, hydrolysis of gluten may influence the rheology of the dough and the texture of the finished baked goods, and has some repercussions on tolerance of celiac individuals towards food containing gluten (see Gobbetti et al. 2007 for a review). Prolammins (e.g., gliadin in wheat) and glutelins (e.g., glutenins in wheat) contained in wheat, rye, barley and oats are responsible for celiac disease—an inflammatory disorder of the small intestine (Gobbetti et al 2007). The proteolytic activity of *E. faecium* A86 against wheat gluten was demonstrated in vitro using Gluten Maltose Broth (Pepe et al. 2002). A strain of *E. faecalis* isolated from fermented sausage reduced the amounts of gluten macropolymer after 5 h of sourdough fermentation (Wieser et al. 2008). After having screened the proteolytic activity in Gliadin Glucose Broth, three selected strains (G32, ND3, HM3C) of *E. faecalis*, previously isolated from Tunisian sourdoughs, were used singly to ferment (24 h, 30°C) a liquid dough, causing a decrease of the gliadin concentration from 45 to 18 g/kg, as determined through sandwich ELISA test (M'hir et al. 2008). In a further study, a combination of these strains (initial cell density of each strains: 9.0 log CFU/ml) was used in association with fungal proteases during a long (48 h) fermentation of a wheat-based liquid dough, leading to a decrease of the gluten concentration of more than 98% (M'hir et al. 2009). However the residual gluten in the fermented dough after water removal was of 1.106 mg/kg, which is higher than the threshold level (0.02 mg/kg) indicated by the Codex Alimentarius Commissions of WHO and FAO for gluten-free foods (Gallagher et al. 2004).

Enterococci were found also in other cereal-based fermented food or beverages. *E. hirae*, *E. faecium* and *E. mundtii*, and *E. faecalis* were isolated from spelt (Van der Meulen et al. 2007) and sorghum (Hamad et al. 1997) sourdoughs, respectively. Enterococci were detected through culture-independent methods in *Pozol*, a Mexican maize-based spontaneously fermented beverage (Ben Omar and Ampe 2000). Enterococci are present during the first stages of fermentation of such products, whereas during further stages they are replaced by other species of lactic acid bacteria (belonging mainly to the genus *Lactobacillus*), more adapted to that kind of environment (Ben Omar and Ampe 2000; Van der Meulen et al. 2007). Another sorghum-based, semi-solid, dough-like food, named *Hussuwa* and popular in Sudan, was shown to harbor different strains of *E. faecium* (Yousif et al. 2005). These bacteria showed an important number (about 10%) of lactic acid bacteria isolated from this product as reported by Yousif et al. (2005). *E. faecium* strains, described in this study, exhibited a functional role in *Hussuwa* fermentation by bacteriocin production against *Listeria innocua*, *Listeria monocytogenes*, and *Staphylococcus aureus*. In addition, they produce other antimicrobial compounds such as hydrogen peroxide. They were also able to ferment indigestible oligosaccharides (raffinose and stachyose), leading to an improvement in the nutritionally quality of this sorghum product. In fact, removal of these sugars is considered a beneficial trait of bacteria associated with fermentation of vegetable foods containing these sugars.

Enterococcus faecium was isolated from *Tarhana*, a traditional Turkish fermented food prepared by mixing wheat flour and spontaneously fermented yogurt (Sengen et al. 2009). *E. faecalis* along with *Leuconostoc mesenteroides* are the main lactic acid bacteria involved in the fermentation of Idli, a mixture of rice and black gram used in several traditional foods in Southeast Asian countries. These lactic acid bacteria are responsible for acid production, leavening of the batter and flavor formation (Nout 2009). Two strains of *E. faecium* were isolated from *idli* batter samples, and showed anti-listerial properties, due to the synthesis of a heat-stable bacteriocin (Vijayendra et al. 2010). *Boza* is a low-alcoholic beverage coming from Turkey originally, but very common in the Balkan Peninsula, produced through fermentation of barley, oats, millet, maize, wheat or rice. Lactic acid bacteria present naturally in *boza* may thus contribute to the increase of the microbiological safety of this product and to control over food spoilage bacteria

(Todorov 2010). It was reported that a bacteriocin synthesized by *E. faecium* ST62BZ isolated from *boza* was active against a number of food spoilage and potentially pathogenic bacteria, including Gram-negative bacteria, such as *Pseudomonas* spp., *Escherichia coli* and *Klebsiella pneumoniae* (Todorov 2010). *Enterococcus faecium*, and *E. mundtii* have been isolated from *Bushera*, an alcoholic beverage prepared from sorghum and millet in Uganda (Muyunja et al. 2003).

Soybean- and other legume-based food

During recent decades, soybean-based food has captured the attention of researchers and consumers by virtue of its high nutritional value and health-promoting properties, especially against hyperlipidemic and atherogenic conditions. Miso-paste ("Soy-cheese")—a traditional Japanese fermented food—is a soybean-paste used for preparing soups and other foods. It is made by grinding a mixture of cooked soybeans and steamed rice, to which salt is then added before spontaneous fermentation (Ebine 1990). Although the most important lactic acid bacteria involved in fermentation of Miso are halophilic *Tetragenococcus halophila*, strains of halo-tolerant *E. faecalis* dominate the early stages of the fermentation process, playing roles in acid production and in the maintenance of the bright color of Miso-paste, among others (Onda et al. 2002). Enterococci isolated from Miso-paste inhibit the growth of undesirable bacteria by producing bacteriocin without inhibiting the growth of *T. halophila*. Onda et al. 2002) suggest that isolated enterococci could be useful as food biopreservative in Miso-paste products. Very similar to Miso, *Doenjang* is a fermented soybean paste that is used as a seasoning in Korea. *E. faecium* was found among the predominant species of lactic acid bacteria in Doenjang, as determined through culture-independent methods (Kim et al. 2009).

Chungkukjang—a Korean traditional fermented soybean food—harbored enterococci up to 6.0 log CFU/g, *E. faecium* being the predominant enterococcal species (Yoon et al. 2008). Seven strains of *E. faecium* isolated from *chungkukjang* showed potentially probiotic features (ability to survive at low pH, bile resistance, and bile salt hydrolase activity), were sensitive to vancomycin, and had neither hemolytic activity nor other virulence determinants. Furthermore, two (S2C10 and S2C11) out of seven strains inhibited the growth of *Listeria monocytogenes* in vitro and could therefore be used as starters or protective cultures in different soybean-based fermented food (Yoon et al. 2008). Two bacteriocinogenic *E. faecium* strains active against various Gram-positive bacteria (*Listeria monocytogenes* included) were isolated from Malaysian tempeh, a soybean-based fermented food (Moreno et al. 2002).

Regarding other legume-based foods, *E. faecium* was isolated both from *dochi*, a traditional Taiwan food consisting in fermented black beans (Chen et al. 2006), and *Soumbala*, a traditional African food obtained from alkaline fermentation of locust been seeds (Ouaba et al. 2010). *Enterococcus hirae* was also isolated from Soumbala (Ouaba et al. 2010).

Application of enterococci in food fermentation

Starter for vegetable food preservation

Starter cultures are microorganisms (previously isolated from the original food product) that are added intentionally to raw material at high numbers to create a desired outcome in the final product, most often through their metabolic activities. They cause a rapid pH decrease in the raw material through the production of lactic acid as the main catabolic product. In addition, production of bacteriocin leads to microbial stability in the final products of fermentation (El-Ghaisha et al. 2011). Enterococci have the ability to produce bacteriocins called enterocins. Various bacteriocin-producing enterococci have been isolated from plant matrices (Corsetti et al. 2008; Settani and Corsetti 2008). Enterocins are small, ribosomally synthesized, extracellularly released, antibacterial peptides produced mainly by *E. faecalis* and *E. faecium* strains (Leroy et al. 2003). Enterocins usually belong to class II bacteriocins, i.e., they are small and heat stable. They have stability over a wide range of pH values, and a general compatibility with starter lactic acid bacteria species (Foulquié Moreno et al. 2006). Overall, the enterocins display inhibitory activity towards spoilage or food-borne pathogens such as *Bacillus* spp., *Listeria* spp. and *Clostridium* spp (Bayoub et al. 2011; Javed et al. 2011). Activity towards Gram-negative bacteria such as *Escherichia coli*, and *Vibrio cholerae* has also been shown (Javed et al. 2011; Khan et al. 2010; Vijayendra et al. 2010). Enterocins, like most bacteriocins, have the cytoplasmic membrane as their primary target. They form pores in the cell membrane, thereby depleting the transmembrane potential and/or the pH gradient, resulting in the leakage of indispensable intracellular molecules (Cleveland et al. 2001).

Franz et al. (1996) approached the selection of potential starter cultures, focusing on the screening of table olive-associated lactic acid bacteria for bacteriocin production. Among the bacteria studied in this work, *E. faecium* BFE 900 was one the most interesting bacteriocin-producing strains. This strain produced a chromosomally bacteriocin termed enterocin 900, which was antagonistic towards *Clostridium butyricum* and *Listeria monocytogenes*. Inhibition of *Listeria monocytogenes* is of special interest, due to its pathogenicity and ability to grow at refrigeration temperatures (Lavermicocca et al. 1998). It has been thought that the ability of enterococci to inhibit *Listeria* spp. was due to the close phylogentic relationship of enterococci and listeria (Javed et al. 2011).

Selected enterococci can be used successfully as a starter for fermentation of table olives (De Castro et al. 2002). It was shown that inoculation of table olives with *E. casseliflavus* cc45 on the 1st day and with *Lactobacillus pentosus* CECT 5138 on the 2nd day caused quicker brine acidification and a greater consumption of carbohydrates with respect to other combinations. This protocol of fermentation was successful probably because of the tolerance of *E. casseliflavus* to the initial high pH characterizing the first steps of the Spanish style olive fermentation technology, which starts with an alkaline treatment to decrease the bitter taste of the olives (De Castro et al. 2002).

Manufacture of table olives relies on the microbiota naturally present on the fruit surface, on water (type and quantity), and fermentation plants. This can frequently explain the variations in the flavour of the product, as well as economic losses due to microbial spoilage. In this respect, the use of selected starter cultures (for example, strains producing antimicrobial substances) would facilitate elaboration of a more uniform product and at the same time decrease the risk of spoilage (Rubia Soria et al. 2006). Furthermore, enterococci not only produce bacteriocin to inhibit the growth of pathogenic and degradative bacteria (Foulquié Moreno et al. 2006), but also induce bacteriocin production in *L. plantarum* by co-culturing (Maldonado et al. 2003, 2004). Recently, Ruiz Barba et al. (2010) showed that *E. faecium* BFE 6T1a-20 induces bacteriocin production in *L. plantarum* NC8 (predominant in olive fermentation) by co-cultivation in olive fermentation.

Currently, the only bacteriocin that can be used as a food additive is nisin. Therefore, the use of bacteriocinogenic strains in food could represent an effective and applicable tool for enhancing food safety and shelf-life. As described in the two preceding paragraphs, many reports have considered the use of bacteriocin-producing enterococci as protective starters in different food models. Table 2 shows the application of some enterocins for biopreservation of various vegetable foods.

Functional and probiotic starters

Recently, *E. avium* G-15 isolated from carrot leaf showed the ability to synthesize gamma-aminobutyric acid (GABA) from monosodium L-glutamate at a hyper conversion rate (Tamura et al. 2010). GABA, a four-carbon non-protein amino acid, is produced by animals and plants. Being an analog of glutamic acid, GABA has been reported to possess several physiological functions such as neurotransmission (Okuma et al. 1997), tranquilization (Komatsuzaki et al. 2005), antihypertension (Vemulapalli and Barletta 1984), prevention of diabetic condition (Hagiwara et al. 2004) and diuretic effects. Nowadays, several functional foods supplemented with GABA are produced to satisfy market demand. However, the direct addition of chemical GABA to food is regarded as unsafe. Tamura et al. (2010) reported that *E. avium* G-15 has a potential ability to produce GABA through fermentation. The functional potential of enterococci was exploited when used in combination with soybean with the aim of achieving a functional food. The strain *E. faecium* CRL183, in association with the flavor improving *L. helveticus* subsp. *jugurti* 416, was used as a starter for a fermented soymilk, which lowered total cholesterol and raised the level of HDL cholesterol in rabbits (Rossi et al. 2000) and men (Rossi et al. 2003). Since the fermentation process leads to a high loss of isoflavones (Rossi et al. 2004), in a further study, the same research group supplemented this food with isoflavones and tested its biological activity in vivo (Rossi et al. 2008). The resulting supplemented food, possessing sensory attributes not significantly different from a non-supplemented fermented soymilk, showed a lipid-lowering effect in hypercholesterolemic rats, relative to the animals that did not consume this product (Rossi et al. 2008). In another study, it was reported that, during fermentation of soymilk by a mixed starter culture, composed of *L. paraplantarum* KM and *E. faecium* 35, more than 90% of the soy isoflavones daidzin and genistin was converted to the corresponding aglycones (Chun et al. 2008). Since aglycones are more bioavailable than their glucosidic precursors, the use of that mixed starter culture for fermenting soymilk could be exploited to obtain a functional food targeted at reducing the risk of age-related and hormone-related diseases (cancer, menopausal symptoms, cardiovascular disease, osteoporosis, etcetera) (Omoni and Aluko 2005).

Some probiotic features have been reported for several strains of genus *Enterococcus* (Botes et al. 2008; Hyeran et al. 2002; Surono et al. 2011). A number of probiotics products currently on the market include some enterococci preparations. Examples of these are: Symbioflor 1 (SymbioPharm, Herborn, Germany), Cylactin® (Hoffmann-La Roche, Basel, Switzerland) and ECOFLOR (Watlters Health Care, Den Haag, The Netherlands) (Domann et al. 2007; Senok 2009).

Enterococci as pathogens are of increasing concern due to their intrinsic and acquired resistance to many antibiotics. Therefore, safety assessment with regards to virulence traits and antibiotic resistance is an essential phase in the selection of enterococci as potential probiotics. Probiotic assessment of *E. faecalis* CP58 isolated from human gut was evaluated recently (Nueno-Palop and Narbad 2011). Among 70 lactic acid bacteria isolated from the faeces of healthy humans, only 5 were found to resist bile salts and indicated survival in the simulated in vitro

Table 2 Examples of the application of enterocins for food preservation (adapted from Javed et al. 2011)

Enterocin	Strain	Application	Reference
Enterocin 900	*E. faecium* BFE 900	Inhibition of *Listeria monocytogenes*, *Clostridium butyricum*, *Clostridium perfringens*, *Lactobacillus sakei*, enterococci	Franz et al. 1996
Enterocin AS-48	*E. faecalis*	Inhibition of *Bacillus coagulans*: Applied in three vegetable canned foods	Lucas et al. 2006
		Tomato paste	Grande et al. 2006a
		Syrup from canned peaches	Molinos et al. 2008b
		Juices from canned pineapple; inhibition of toxicogenic psychotrophic strain *Bacillus cereus* in rice-based foods; inhibition of *B. cereus* and *Bacillus weihenstephanensis* inoculated on alfalfa, soybean sprouts and green asparagus; inhibition of *Bacillus licheniformis* in apple cider	Grande et al. 2006b
Enterocin AS-48 + additional treatment	*E. faecalis*	Reduced or inhibited growth of *Salmonella enterica*, *Escherichia coli* O157:H7, *Shigella* spp., *Enterobacter aerogenes*, *Yersinia enterolitica*, *Aeromonas hydrophila*, *Pseudomonas fluorescens*	Molinos et al. 2008b
Enterocin	*E. mundtii* ST4V	Prevent the growth of *L. sakei* DSM 20017 during boza storage (low-alcohol cereal-based beverage)	Todorov et al. 2009
Enterocin	*E. faecium*	Antimicrobial substances against *L. monocystogenes* strains	Ibarguren et al. 2010
Enterocin EJ97	*E. faecalis* EJ97	Prevention of food spoilage caused by *Bacillus macroides* and *Bacillus maroccanus*	Garcia et al. 2004
Bacteriocin-like inhibitory substances (BLIS)	*E. faecium*, *E. mundtii*	Inhibition of *Listeria innocua* 4202	Corsetti et al. 2008
Enterocin AS-48	*E. faecalis* A-48-32	Inactivation of exopolysaccahides and 3-hydroxypropionaldehyde (3-PHA) in apple juice and apple cider	Martínez Viedma et al. 2008
		Inhibition of *Geobacillus stearothermophilus* in canned food samples (corn and peas) and in coconut milk	Martínez Viedma et al. 2009
		Inhibition of *Listeria monocytogenes* in Russian type salad during storage for 1 week at 10°C	Molinos et al. 2009a
		Control of *Alicyclobacillus acidoterrestris* in fruit juices (orange and apple juice)	Grande et al. 2005
Enterocin L50 A and B	*E. faecium* L50	Inhibition of alcoholic and non-alcoholic beer-spoilage bacteria *L. brevis*, *Pediococcus pentosaceus*)	Basanta et al. 2008
Enterocin AS-48 + additional treatment	*E. faecalis*	Inhibition of *Salmonella enterica* in Russian salad	Molinos et al. 2009b
Enterocin EJ97	*E. faecalis* EJ97	Inhibition of *Geobacillus stearothermophilus* CECT and CECT43 and CECT49 in canned foods and drinks, canned corn, peas, coconut milk, and coconut juice	Martínez Viedma et al. 2010

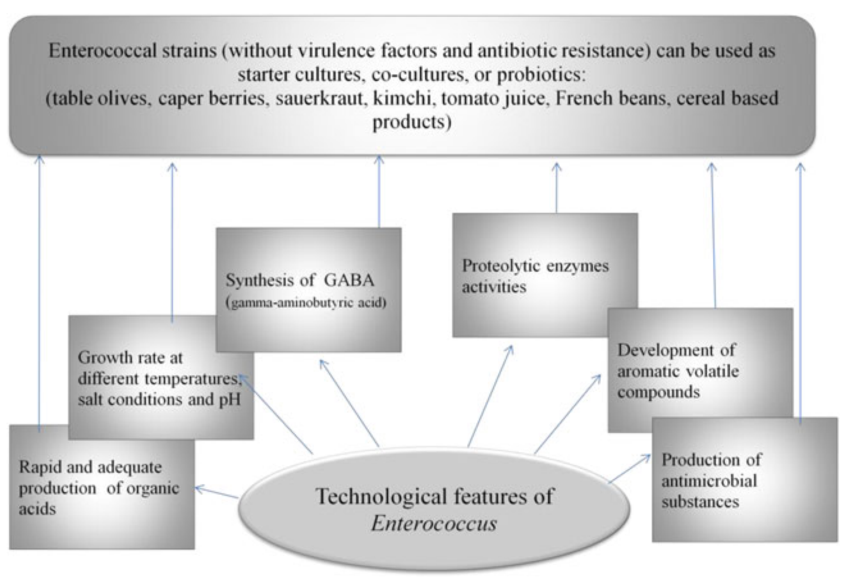

Fig. 1 Examples of functional application of enterococci in vegetable fermented foods

digestion assay that reproduces stomach and intestinal digestion, indicating their tolerance to gastric enzymes and low pH conditions. *E. faecalis* CP58 was the most adherent strain. Examination of the virulence determinants for this strain indicated that it was positive for *efaAfs* (cell wall adhesins), *gelE* (gelatinase), *agg* (aggregation substance), *cpd*, *cob*, *ccf* and *cad* (sex pheromones) (genes involved in the expression of virulence determinants) (Nueno-Palop and Narbad 2011).

In general, *E. faecalis* strains are known to contain multiple virulence determinants (Nueno-Palop and Narbad 2011). Lempiäinen et al. (2005) investigated the prevalence of virulence factors among human intestinal enterococcal isolates and concluded that commensal enterococcal strains differ from the clinical isolates and that the most frequent virulence factors present in the commensal enterococci were *cpd*, *agg* and *gelE*. Although many of the genes present in *E. faecalis* CP58 are classed as virulence genes, they are not necessary associated with pathogenesis, and in fact the expression of some of these genes that encode aggregation factors may be an advantageous trait that allows the bacteria to colonize the host tissue and display probiotic functionality (Domann et al. 2007). A recent study by Carlos et al. (2010) indicated that expression of individual virulence genes was environment- and strain-dependent. Concerning antibiotic susceptibility, it is found that enterococcal isolates of non-clinical origin display a lower incidence of antibiotic resistance compared to clinical isolates (Abriouel et al. 2008a; Garcia de Fernando 2011).

Conclusion

Enterococci are widespread in vegetable raw materials and are present in vegetable fermented food products. This review focuses on the importance of enterococci in vegetable products. Over the last decade, interest in the physiology and genetics of enterococci has increased greatly, reflecting the growing importance of these bacteria as starters. Enterococci possess large desirable properties, such as improvements in sensory characteristics, natural preservation and health-related benefits, that could increase the value of vegetable-based fermented foods. Enterocin production, proteolytic activity, synthesis of GABA, and production of aromatic volatile compounds are examples of the functional application of enterococci in vegetable fermented foods (Fig. 1). Bacteriocinogenic enterococci may help to control over undesired bacteria, such as *Listeria* and *Bacillus*. However, enterococci may possess virulence factors and resistance to antibiotics. These are strain-dependent characteristics. Virulence factors and antibiotic resistance of food-related enterococci could be transferred to human strains in the gastrointestinal tract. Thus, the food chain may play a role in the spread of antibiotic-resistant enterococci. Therefore, prior to their application to food as starters or protective cultures, enterococci should be screened carefully not only for the presence of desirable properties but also, and above all, for the absence of virulence factors and antibiotic resistance.

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