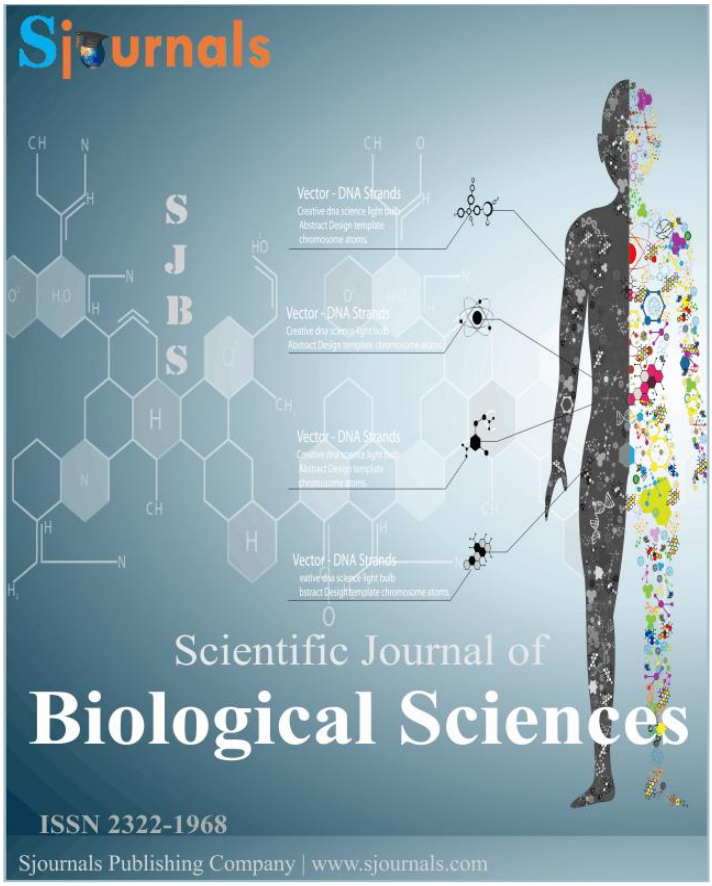

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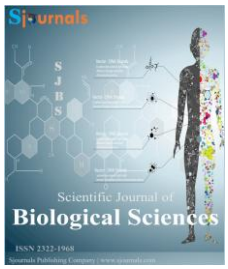

Review article

The genus *Enterococcus spp.*, a perspective from a focus on health to its biotechnological application

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ABSTRACT

The enterococci are a bacteria group that can be found in several environments, such as water, plants, animals and gastrointestinal tract in humans and animals. These microorganisms have entered into the spotlight for aspects related to health like: a bigger incidence in hospitable and community diseases, as the main causal agent; a phenomenon of resistance, in isolated food strains and clinical cases, to different antimicrobials, including those used on a regular basis in clinical therapies, and a spreading and transference capacity to other microorganisms. However, this group has also contributed to the well-being of human health, since it has been used around the globe as a biological indicator of water and food quality in order to both protect and look the health out against transmission risks of several pathogen agents; and finally, some species of this genre have been reported as factors involved in the production and preservation of foods, starting in the synthesis of several chemical compounds, favoring the consumer's health. Due to the aforementioned, this present document is focused on showing a general vision of the genre *Enterococcus spp*. and its duality in human health; i.e. its harmful side, as a causal agent of diseases; and its beneficial side, in the control and sanitary surveillance in

water and foods to protect the human health against biological risks, and its contribution to the development, foods preservation and effects on the consumer's health.

1. Introduction

The microorganisms are considered as one of the very first life forms on earth, being capable of living in any environment, and since its appearance they have been carried out important functions in the climatic, geological, biochemical and biological evolution aspects. Since they are present in any environment, foods have been colonized by microbial complex populations, named 'unwanted', which are responsible for diseases and foods disorders; the 'wanted', which ferment foods and make them functional, like the probiotics; and finally the 'diners', which doesn't either generate complications or are useful in any aspect (Diaz-Ruiz and Wacher, 2011). Into this context, the enterococci are found; they present a health duality, it means they are considered as pathogens for being responsible of several diseases in the human being, besides being labeled as microbiological indicators in the matters of control and surveillance of drinking water; on the other hand, it has been reported, by several researches carried out around the globe focused on the isolation and characterization of strains of this type, that some of this microorganisms can be used in human health, production and preservation of foods. Due to the aforementioned, this present document is focused on showing a general vision of the genre *Enterococcus spp*. and its duality in human health; i.e. its harmful side, as a causal agent of diseases; and its beneficial side, in the control and sanitary surveillance in water and foods to protect the human health against biological risks, and its contribution to the development, foods preservation and effects on the consumer's health.

2. Enterococci and their negative side to health

Enterococci is a group of microorganisms which are spherical or ovoid cells of 0.6-2 x 0.6-2.5 µm, gathered in short chains or positive Gram pairs, no spore producers, facultative anaerobes, chemotrophic, negative catalase but dismutase superoxide and peroxidase synthesizers, ferment a big amount of carbohydrates, including lactose and glucose and thus producing lactic acid, nogas producers, have a G+C content in their DNA <40% mol., present a growing temperature range from 10°C a 45°C, being the optimal at 37°C, can grow in 9.6 pH, 6.5% of NaCl concentrations and at 40% of bile, besides surviving to heating periods of 60°C for 30 minutes (Charles et al., 2007; Hikmate et al., 2008; Diaz-Perez et al., 2010).

This type of microorganisms can be found in several environments, like water, plants, foods, inside the gastrointestinal tract, genitourinary tract, and animals and human beings' saliva, being the *Enterococcus faecalis* and *Enterococcus faecium* the most abundant species inside the intestinal human tract and the most frequent isolated in animals, clinical cases and foods of animal origin (Charles et al., 2007; Hikmate et al., 2008; Caraffini et al., 2009; Diaz-Perez et al., 2010; Canton et al., 2103; Sparo et al., 2013). It's important to mention the amount of these microorganisms will depend on some factors like the geographic zone, the diet, and the gut flora (Charles et al., 2007; Hikmate et al., 2008; Caraffini et al., 2009; Diaz-Perez et al., 2010).

Until recent years, this microbial genre was segregated from the Lancefield's group D *Streptococcus* due to taxonomic and genomic studies (DNA-DNA and DNA-RNA hybridization), which demonstrated it was necessary to consider them as different genres (Charles et al., 2007; Diaz-Perez et al., 2010). Thus, the *Enterococcus spp.* genre presents around 33 different species: *E. faecalis, E. haemoperoxidus, E. moraviensis, E. silesiacus, E. termitis, E. caccae E. faecium, E. durans, E. hirae, E. mundtti,E. villorum, E. canis, E. ratti, E. asini, E. phoeniculicola, E. canintestini E. avium, E. pseudoavium, E. malodoratus, E. raffinosus, E. gilvus, E. pallens, E. hermanniensis, E. devriesei, E. gallinarum, E. casseliflavus E. cecorum E. cecorum, E. columbae E. saccharolyticus, E. sulfureus, E. aquimarinus, E. dispar, E. italicus,* from which the *E. faecalis* & *E. faecium* species are the most frequent causatives of human clinical cases (90%); *E. gallinarum* is found in birds, while the pigmented species like *E. casseliflavus, mundtii* and *E. sulfureus* are found in the vegetables surface, as epiphytes bacteria. For a proper identification of

both genre and species, several phenotypic tests have been created and developed inside a microbiological lab, which can be seen in table 1 (Hikmate et al., 2008; Diaz-Perez et al., 2010).

Table 1
Biochemical identification tests of different species from the *Enterococcus spp*genre (Herve et al., 2004; Diaz et al., 2010).

Microorganism	CA	Gram (Chain or pair)	AR	SA	TR	HA	PYR	MO	NaCl 6.5%	BE	MA
E. faecium	-	+	+	+	+	+	+	-	+	+	+
E. faecalis	-	+	-	+	+	+	+	-	+	+	+
E. casseliflavus	-	+	+	+	+	+	+	+	+	+	+
E. gallinarum	-	+	+	+	+	+	+	+	+	+	+
E. mundtii	-	+	+	+	+	+	+	-	+	+	+

CA: catalase Ar: Arabinose SA: sacarose TR: Trehalose HA: HydrolizateArginine PYR: (Pyrridonyl ß-naphthylamide) MO: Mobility BE: Bileesculin MA: Mannitol

This microbial group has been consider as emerging opportunists pathogens in the human being (mostly in elder people and immunocompromised), causing different diseases like endocarditis, bacteremia, urinary tract infections, neonatal infections, central nervous system, intra-abdominal and pelvic. Besides, in recent years, the incidence of infections and complications generated in hospitals and progressive strains isolation with a resistance to multiple antimicrobials has been acquired a bigger importance than in the past years (Charles et al., 2007; Hikmate et al., 2008; Caraffini et al., 2009; Diaz-Perez et al., 2010).

Although the members of *Enterococcus spp*. genre present a low level of pathogenicity in a normal host, it can react in an opportunist fashion in the risk groups (elder people and immunocompromised) generating some infections through virulence codified factors found inside the genes, generally caused by strains of *Enterococcus faecalis* (Silva et al., 2013). Among the virulence factors present in these enterobacteria are: surface proteins involved in adherence like the aggregation substance (*asaI* & *agg*), the collagen adhesion (*acel*), surface's protein (*esp*), endocarditis antigen A (*efa*A), adhesive fimbriae (Ebp fimbriae or endocarditis pilis and biofilms), polysaccharide capsules, cytolysin (*cylA*), gelatinase (*gelE*), hyaluronidase (*hyl*), DNA and superoxide ions (Caraffini et al., 2009; Diaz-Perez et al., 2010; Hikmate et al., 2008; Díaz Pérez et al., 2013; Silva et al., 2013).

The *Enterococcus spp*. infections in the human being are generally attributed to the endogenous gut flora. However, it can also be acquired by consuming foods and water, which are transmissions ways and putting the health at risk; besides, not only the microbial development can lead to deterioration, with its natural consequent economic problem if the amounts are big enough, but also can lead to a significant reduction in the nutritional quality (Díaz-Perez et al., 2010).

3. The phenomenon of resistance to antimicrobials

Until recent years, these microorganisms were labeled as 'non-invasive' and with a low pathogenic level when colonizing the digestive apparatus of human beings and animals; but the *Enterococcus spp*. genre started to present a tendency of being associated with different infectious scenarios and a difficulty for an appropriate treatment, mainly in the clinic area, to the point of being among the top places of hospitable pathogens around the world (Canton and Ruiz, 2013; Medell et al., 2014; Cercenado, 2016), being the biggest affected group those patients located in intensive care units, immunosuppressed, those with intravascular catheters, and those under multiple antibiotic plans (Rincon et al., 2014; Cercenado, 2016).

It has been reported the *Enterococcus spp*. genre presents several features that correlate its high incidence in hospitable infections with its difficulty of an appropriate treatment. Those features are: A high adaptation due to its genetic variability and intrinsic resistance (Cephalosporins or aminoglycosides) and acquired resistance to the action of some antimicrobials codified in some genes located in plasmids, transposons or in spontaneous mutations. Per example, with the β-lactams the resistance is due to the hyper-production or alterations in PBP5; meanwhile, for the glycopeptides like the vancomycin, the acquired resistance is due to the gain of *van*A, *van*B, *van*D, *van*E, *van*G, *van*L, *van*M y *van*N operons (This last microbial is used frequently in the treatment of infections produced by the aforementioned microorganisms). Likewise, we have to consider this genre as 'cosmopolitan',

since it's capable of growing in adverse conditions and with a great capacity of spreading and transferring resistance genes to other microbial strains; thus, the enterococci are labeled as a real worry and a big challenge in the public health affairs (Charles et al., 2007; Hikmate et al., 2008; Diaz-Perez et al., 2010; Silva et al., 2013; Rincon et al., 2014; Medell et al., 2014; Cercenado, 2016). The big incidence of antimicrobial resistance, and the knowledge of the infections caused by enterococci occurred in hospitals and the community have driven diverse health organisms around the world to develop a permanent surveillance of resistance in different microorganisms, specifically the positive Gram like the enterococci, with the purpose of detecting risks and predicting tendencies in the genes resistance acquisition (Cercenado, 2016; FDA, 2016).

An example that has driven the scientists to take surveillance actions is that, inside the clinical area, the isolation, resistant to vancomycin enterococci strains are the causes of 25% of the nosocomial infections in America, making it one of the top three most isolated pathogens (Silva et al., 2013). Besides, these microorganisms are considered causal agents of diseases through the foods, mainly due to the consumption of wrongly processed meat and dairy products; for such reason, the Food and Drugs Administration (FDA) and the Center for Diseases Control and Prevention (CDC) have developed the National Antimicrobial Resistance Surveillance System (NARSS) for different microorganisms, including food pathogens (*Campylobacter, Salmonella, Escherichia coli, V. cholerae* and *Enterococcus*), in order to document the level, spreading, tendencies, risk factors, reservoirs, and to generate policies and action guidelines for a containment of this phenomenon; also, adapting enterococci as an indicator organism for tracking of microbial resistance of the positive Gram group caused by the antimicrobial use (FDA, 2016).

On the other hand, in Europe, in countries like Spain the incidence of nosocomial diseases produced by positive Gram microorganisms goes from 31.8% to 35%, making it an important problem when handling hospitalized patients. Also, according to the European Center for Diseases Control and Prevention (ECDC) and the Prevalence Study of Nosocomial Infections in Spain (EPINE), it is shown that, in conducted studies to the *Enterococcus spp*. genre, it is located in the third place among the pathogens causes of hospitable diseases, with a percentage around 9.38-9.6, pointing out its multi-resistant feature to the microbial all across Europe, being the 10.2% of the isolated strains resistant to vancomycin (Canton and Ruiz, 2013; Cercenado, 2016). This situation has been taken as a warning prior to a growing problem, in such fashion, several recommendations have been made to try to mitigate the infections for multi-resistant enterococci inside hospitals; some of them are: the correct hands hygiene of the sanitary staff, proper contact precautions, rational use of antibiotics, an establishment of an active surveillance program, appropriate environmental cleanse, and education to the sanitary staff to guarantee the permanence of these actions (Canton and Ruiz, 2013).

Taking the isolation of microbial resistant strains phenomenon to the antimicrobial in foods, from several years ago, some researchers around the world have witnessed the presence of enterococci with this ability, which they consider as a relevant focus of attention in health and foods affairs due to phenomenon like: spreading, a growth in the resistance to antimicrobial, and possible infections in human beings via the foods; such is the case of Novais et al. (2005), that carried out a research in poultry, obtaining strains of enterococci resistant to vancomycin in 48% of the cases, gentamicin 34%, streptomycin 32%, or kanamycin 30%; and a co-resistance to tetracycline, erythromycin, ciprofloxacin and quinupristin/dalfopristinin the most of the isolated strains. Meanwhile, in more recent years, another study was conducted in the livestock industry, more specifically in farms of Tunisia; Klibi et al. (2015) conducted a study in animals used as a source of food like poultry, cattle and sheep by analyzing feces samples; the results: a report of isolation of *Enterococcus faecium* and *Enterococcus hirae* strains resistant to erythromycin, tetracycline aminoglycosides and ciprofloxacin.

It also indicated that the animals destined for human consumption can become an important spreading factor throughout the food chain of virulent bacteria, and also the fact of its resistance to the action of different antimicrobial that can provoke a risk in the health of human beings. And finally, in the dairy products industry, Pesavento et al. (2014) reported the isolation of *Enterococcus faecalis* & *Enterococcus faecium*in products like cheese, ready-to-serve salads, ham and raw meat which were resistant to several microbial such as gentamicin, tetracycline, vancomycin, teicoplanin, linezolid and amoxicillin in combination with clavulanic acid; thus, they concluded the enterococci must be considered potential pathogens and genes reservoirs that codify the resistance against some antibiotic which can be transferred to other microorganisms, making vital and necessary the surveillance activities to regulate its incidence and resistance in the foods industry to find potential harmful strains that might become a risk for the population and ensure an effective treatment in infections.

4. Enterococci and their role in favor of health

The *Enterococcus spp*. genre is considered as indicator microorganisms inside the sanitary quality and food safety, sediments, water destined for human consumption, agriculture and recreation (Charles et al., 2007; Díaz-Perez et al., 2010; Díaz Pérez et al., 2013; Boehm et al., 2014). They are also considered a sanitary indicator for marine water, this due to resist the waters' conditions, including its temperature, and presenting a major relation with gastrointestinal, respiratory and dermatological diseases (Rodriguez, 2011; Boehm et al., 2014). For example, the *E. faecalis* has a role of being an indicator of contamination for human-origin feces; the *E. bovis* & *E. equinus* are indicators of contamination for hot-blooded animals, and finally, the *E. faecium* and other species are indicators of contamination for other sources (Díaz-Perez et al., 2010; Díaz Pérez et al., 2013).

Around the globe there have been established a series of limits for the presence of these microorganisms in a fashion of parameters of the microbial quality of marine waters or brackish in order to not put the human health at risk, being the limit of 40 Colonies Forming Units (CFU)/100 mL, in accordance with the World Health Organization (WHO), for the marine water. In America, the criteria for recreational marine water, in accordance with the Environmental Protection Agency (EPA) for enterococci, has to be less than 104 CFU/ mL (sample's standard) and 35 CFU/100 mL (average geometrical standard) (Boehm et al., 2014). In Central America countries, like Mexico, the norm NMX-AA-120-SCFI-2006 establishes the requirements and specifications for sustainability of quality in beaches, with a permissible limit going from 0 to 100 enterococci NMP/100 mL in recreational beaches, employing the procedure of multiple tubes. Meanwhile, in the European Union, the levels for WC water vary from 100-400 CFU/mL, depending on if the beach is either marine or fresh, and if it is qualified as excellent or enough (Boehm et al., 2014). Likewise, for the water destined for human consumption, the enterococci are not allowed in 100 mL for water coming from the sink, and no enterococci are allowed in 250 mL of bottled water (BOE, 2011; Boehm et al., 2014).

5. The analysis in the microbiological laboratory

Due to its high incidence and persistence in hospitalized patients, the microbial resistance phenomenon and the fact they are considered as indicators of foods and water contamination, several techniques have been developed for its quantification, isolation and identification of enterococci inside the lab, that include different types of conventional cropping ways that employ selective agents such as sodium azide, sodium chloride, thallium acetate, potassium tellurite, potassium thiocyanate, crystal violet, 2,3,5-triphenyltetrazolium chloride (TTC) and antibiotics; likewise, several chromogenic and / or fluorogenic ways have been developed, being the fluorogenic based on the detection of specific enzymatic activities like the β-D-glucoside (Díaz et al., 2013; Diaz et al., 2014).

In the specialized literature there are different methodologies, normal and standard, used around the world for enterococci enumeration in drinking water, environmental water, waste water or recreational water, based on techniques of multiple tubes, membrane filtration, or chromogenic substratum, like the international norm ISO 7899-1:1998, Water quality-Detection and enumeration of intestinal enterococci in surface and waste water, ISO 7899-2:2000 Water quality Detection and enumeration of intestinal enterococci , the Standard test method for Enterococci in wáter using enterolert-ASTM D6503-99 (2009)-, the Method 1600: Enterococci in Water by Membrane Filtration Using membrane-Enterococcus Indoxyl-β-D-Glucoside Agar(mEI) reported in 2006 by the environmental protection agency (EPA) in America and the official Mexican norm NOM-210-SSA1-2014, being the membrane filtration method the most suggested for the enumeration of enterococci in diverse types of water samples and large volumes, besides being faster, in the obtaining of results, than the method of multiple tubes (Diaz et al., 2014). Likewise, several modern methods have been developed based on molecular biology, like the Quantitate PCR (QPCR), which analysis target and identification is the rRNA operon (Boehm et al., 2014).

In the fig. 1 it is shown the method for isolation and visualization of enterococci colonies in water samples subjected to a filtration process of a determined volume of water, through a membrane filter with a pore size (0.45µm) (ISO 7899-2:2000). The membrane is placed in a selective environment with sodium azide, which inhibits Gram negative bacteria, facultative aerobic and anaerobic, which obtain energy from the cytochromes due to its compound inhibits the hydrogen transportation through the cytochrome system, it blocks the cytochrome molecule's iron in the ferric state, and so blocking the final transference from the electron to the molecular oxygen (Diaz et al., 2013). The 2,3,5-triphenyltetrazolium chloride is a colorless compound that, once it is metabolized by

intestinal enterococci, is reduced to red-colored formazan. The typical colonies of these bacteria present an elevation of color red, brown or pink; afterwards, the confirmation takes place by transferring the membrane to an Agar BilisEsculinAzide, where these intestinal microorganisms hydrolyze the esculin, producing the 6,7-dihydroxycoumarin, which combined with the Fe3+ ions, they form a colorful compound going from brown to black that spreads in the agar, doing later the colonies' enumeration with typical morphology (ISO 7899-2:2000). Optionally, biochemical confirmation tests can be carried out, like the case of Diaz et al. (2014), evaluation and comparison of alternate chromogenic enumeration tests, or the ones reported by Herve et al. (2004), in which it was used an analysis for genre and species identification at clinical level that included many biochemical tests, such as: catalase, oxidase, mobility, telluride tolerance, growth in ram's blood agar, hemolysis, arginine hydrolysis, growth in broth with 6.5% of NaCl, growth in presence of 40% of bile, growth at 45°C, growth at pH 9.6%, esculine hydrolysis, Voges-Proskaüer pyruvate fermentation, arabinose, mannitol, sorbitol, lactose and sorbose, besides the PYR test. Once the isolation and identification of the strain are made, in view of the growing problem of the incidence of antimicrobial resistance by members of the genre *Enterococcus spp.*, a study can be carried out.

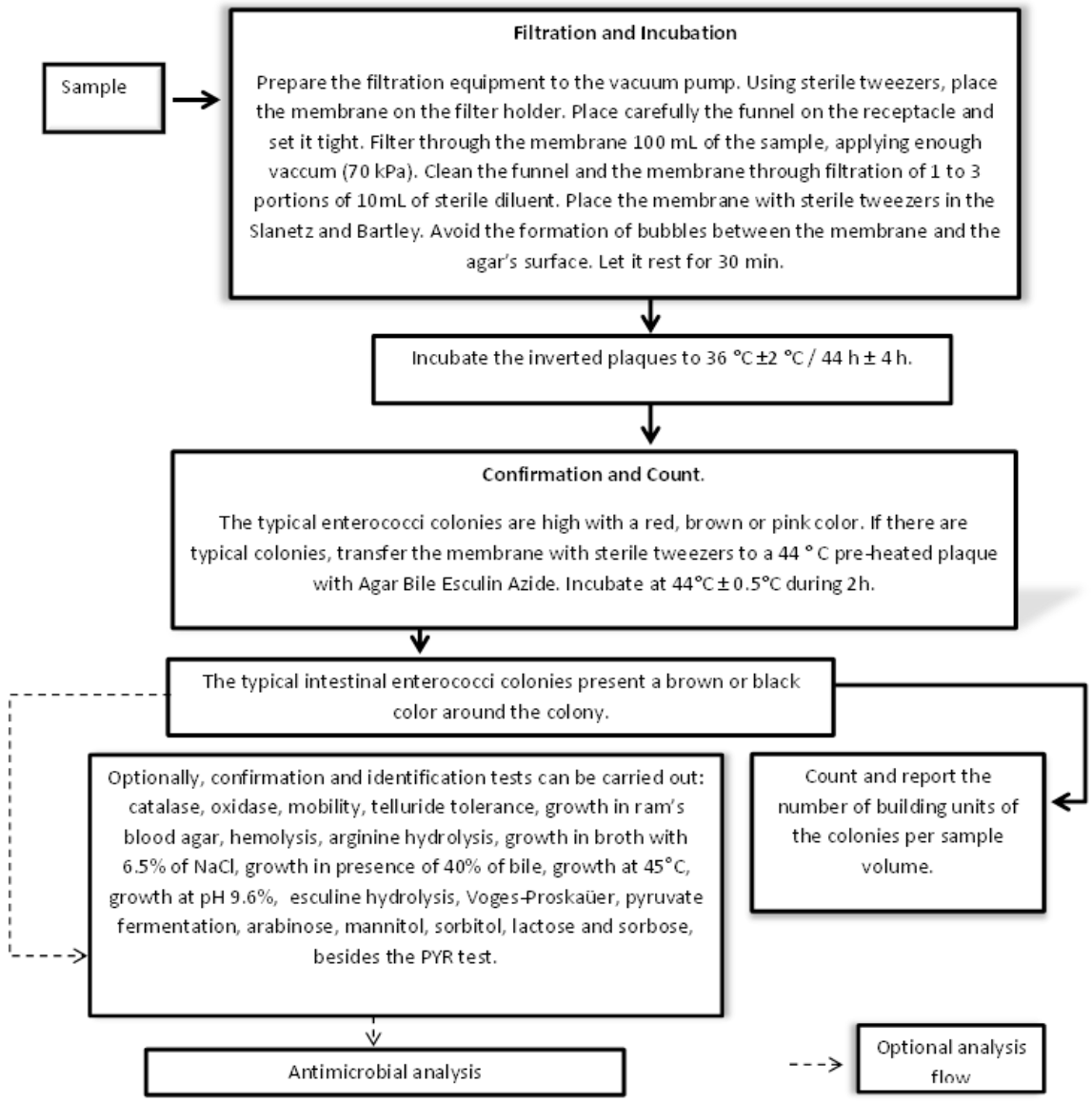

Fig. 1. Analysis flow diagram for detection and numeration of *Enterococcus spp*., in water (ISO 7899-2:2000; Herve et al., 2007; Díaz et al., 2014; NOM-210-SSA1-2014).

6. The *Enterococcus spp*. Genre and its function in production and preservation of foods

Beyond the fact the Enterococci have been involved in the deterioration of processed foods, they work as indicators of sanitary quality and pathogens causing several diseases (Charles et al., 2007; Ghrairi et al., 2008; Díaz-Perez et al., 2010; Díaz-Pérez et al., 2013; Boehm et al., 2014). To some species of the genre, they have been given some beneficial properties in human health and in the production of foods, mainly the dairy products and the 'traditional' products (Ghrairi et al., 2008). The enterococci are considered into a group called 'Lactic Acid Bacteria' (LAB) (Parra-Huertas, 2010). The LAB are a group of bacteria of great importance to the food industry, focused on the production of several products such as yogurt, cheeses, pickles, sausages, silage, wine, beer, and other due to their ability to ferment sugars, producing diverse metabolites, preserving the foods by avoiding the growth of pollutant microorganisms or pathogens, besides contributing to the nutritional and sensorial features, like texture, taste, smell and aroma of the foods (Dolz, 2008; Parra-Huertas, 2010; Ramirez et al., 2011; Vallejo et al., 2014).

The LAB are cosmopolitan microorganisms since they have been isolated from different foods, soils, plants and animals (Ramirez et al., 2011; Londoño et al., 2015). Their ability to preserve foods is due to the production of antimicrobial metabolites with a non-specific activity like the lactic acid, acetic acid, hydrogen peroxide, diacetyl and ethanol, in addition to ribosomal synthesis peptides called bacteriocins that act specifically against cell membranes of bacteria related phylogenetically, and inhibiting their growth (Londoño et al., 2015; Vallejo et al., 2014).

Inside the microbial genres that form the LAB are: *Aerococcus, Alloinococcus, Enterococcus, Vagococcus, Weisella, Carnobacterium, Globicatella, Oenococcus, Lactococcus, Pediococcus, Bifidobacterium, Streptococcus, Leuconostoc,* and *Lactobacillus*, being the last one the most known. The LAB are classified according to some different characteristics: A.Fermentation products, either homo-fermentative (They only produce lactic acid through the biochemical route Embden-Meyerhoff-Parnas) or hetero-fermentative (They produce sugars through the glycolytic route Embden-Meyerhoff; the pentoses route produces tagatose 6-phosphate; or Leloir route, which produces compounds like lactic acid, ethanol, CO_2 and others). B. Growth temperature (mesophylls and thermophiles). C. Configuration of the produced lactic acid. D. Growth ability in large concentrations of salt and tolerance to alkaline or acid conditions (pH from 3.2 to 9.6) (Parra-Huertas, 2010; Ramirez et al., 2011; Londoño et al., 2015).

Some genres belonging to the LAB are considered as probiotics, which definition is all the living microorganisms, or a mix of so, which improve their host's health once they are ingested in suitable quantities. Among the benefits they bring are: a reduction in the incidence of diarrheas and their severity, antimicrobials by production of compounds (H_2O_2 bacteriocins, organic acids, among others), prevention of intestinal pathogens colonization, (block in adherence and a nutrients competence), reduction of cancer, lactose intolerance, cholesterol reduction, prevention of allergic reactions, and a stimulation of the immune system (Amores et al., 2004; Taranto et al., 2005; Cáceres and Gotteland, 2010; Guarner et al., 2011; Ramirez et al., 2011; Ortiz et al., 2014).

In table 2 is shown some bacteria and yeasts considered probiotic, being the most generally used those belonging to the *Lactobacillus spp., y Bifidobacterium spp.* genres, which have been isolated from feces of healthy individuals. It is important to mention that the process of selection of microorganisms, to be considered as probiotics, involves different characteristics like the pH resistance to the stomach's acid, digestive enzymes and intestine bile salts, the adherence to the mucus or intestinal epithelial cells, features that favors their survival and permanence in the digestive tube, production of antimicrobial substances, and have the ability to increase the immune functions and metabolic activities in the host (Sanz et al., 2003; Amores et al., 2004; Castro and De Rovetto, 2006; Cáceres and Gotteland, 2010; Guarner et al., 2011; Ramirez et al., 2011; Ortiz et al., 2014).

It is important to mention that, despite the benefits to the consumer's health the probiotics bring, there has been very sporadic reports of an association of systematic infections with probiotics consumption, which have been taken place in persons with specific health conditions, as immune-depressed, and under medical treatment (Sanz et al., 2003). Strains from the *Enterococcus spp.* genre are said to be considered as risky, since it has been reported they are the cause of nosocomial diseases and of possessing a large resistance to the antimicrobials (Sanz et al., 2003; Silva et al., 2013; Rincon et al., 2014; Medell et al., 2014). Therefore, to consider the species of *Enterococcus spp.* genre, or others, as safe, probiotics, or with its equivalent potential in the production of foods, it becomes imperative not only to limit, but also to deepen in researches of characterization and functionality, as

well as to secure them with a special focus on: antibiotics resistance, check the absence of transferable virulent, resistance genes, harmful metabolic activities, epidemiological studies of possible adverse effects in consumers, determination in the production of toxins and hemolytic capacity, and absence of infection in immune-depressed animals (Sanz et al., 2003; Ghrairi et al., 2008).

Table 2
Lactic acid bacteria, fungi and yeasts considered probiotics (Sanz et al., 2003; Amores et al., 2004; Dolz, 2008; Cáceres and Gotteland, 2010; Guarner et al., 2011; Ramirez et al., 2011).

Bifidobacteriumbifidum	*Saccharomycesboulardii*
Bifidobacteriumanimalis	*Aspergillus oryzae*
Bifidobacteriuminfantis	*Candidapintolopesli*
Bifidobacteriumlactis	*Lactobacillusparacasei*
Bifidobacteriumlongum	*Lactobacillusjohnsonii*
Lactococcuslactis spp., lactis	*Lactobacillus casei*
Lactococcuslactis spp. cremoris	*Lactobacillus rhamnosus*
Lactococcusdiacetylactis	*Lactobacillus bulgaricus*
Enterococcus faecalis	*Lactobacillus plantarum*
Enterococcus faecium	*Lactobacillusacidophilus*
Bacillussubtilis	*Lactobacillusreuteri*
Bacilluscoagulans	*Lactobacillushelveticus*
Saccharomycescerevisiae	*Streptococcussalivarius spp. thermophilus*

7. Bacteriocins

Due to the increasing demand of fresh and chemical, conservative free and innocuous foods, a special interest has emerged for studying and using antimicrobial peptides labeled as bacterocins, which present activity against food pathogens (*L. monocytogenes, Staphylococcus aureus, Bacillus cereus*, and vegetative cells or spores of *C. botulinum*), and they are generated by lactic acid bacteria, where factors such as the carbon source, nitrogen, vitamins, pH, temperature, or NaCl influences its synthesis. Bacteriocins are being considered as potential agents in the bio-preservation process in the foods industry, which means, achieve the extension of the average life and foods safety through the controlled use of microorganisms, or the incorporation of metabolites with an antimicrobial activity (Charles et al., 2007; Rojas and Vargas, 2008; Bautista et al., 2010; Vallejo et al., 2014). The application of these compounds in foods can be through the inoculation of the food with the lactic bacteria which will produce the bacteriocin, its use as a food ingredient from a previous fermented environment with a producer strain and/or the addition of the purified/semi-purified bacteriocins (Cardoso, 2010; Mondragon et al., 2013).

Bacteriocinsare a heterogenic group of synthetized peptides in the microbial ribosome as a defense mechanism (Aguado et al., 2010; Rojas and Vargas, 2008; Vallejo et al., 2014). These compounds can be in an extended or globular shape, and can show different molecular weights, generally stable when in heat and inactivated by the action of proteases, which make them being considered as innocuous and safe for the consumer at being inactivated by intestinal enzymes (Bautista et al., 2010; Rojas and Vargas, 2008; Vallejo et al., 2014; Gonzalez et al., 2015).

The classification of these compounds of protein nature is divided into various classes, according to their genetic and biochemical features: Class I. lantibiotics, unstable in heat, poli-cyclical peptides (<5 KDa) with amino acids modified post-translationally, like Lanthionine, β-methyl-lanthionine and dihydroalanine. This class is subdivided into the Ia class: it consist of elongated peptides, cationic, amphipatic with a molecular mass between 2-4 KDa, being the nisin the most studied and used bacteriocin in foods preservation, besides being recognized as safe according to the Food Drugs Administration (FDA), and Ib class: peptides with globular features, hydrophobic, their molecular mass is between 2-3 KDa and can have a negative charge. Class II Small (<10 KDa) no-lantibiotics stables in heat, lineal, and no post-translated modified. Class IIa: Active peptides against Listeriae, present a consensual sequence in the region N-terminal TGNGVXC (Tyr-Gly-Asn-Gly-Val-Xaa-Cys). Class IIb: Builders of complexes for the formation of pores that consist of two different peptides. Class IIc: Small peptides, thermos-stables, no modified and going through guide peptides. Class III Big molecules (>10 KDa) and unstable in heat. Class

IV. Complex bacteriocins, characterized by being peptides with a protein part, and one or more lipid fractions or indispensable carbohydrates for their biological activity, and finally Class V. Bacteriocins of circular structure and unmodified postraduccion (Gonzalez et al., 2003; Rojas and Vargas, 2008; Monroy et al., 2009; Mondragon et al., 2013). In table 3, there are shown some bacteriocins' producers and their classification.

Table 3
Lactic acid bacteria producers of bacteriocins (Gonzalez et al., 2003; Lopez et al., 2008; Monroy et al., 2009).

Microorganism	Bacteriocin	Bacteriocins classification
Lactococcuslactissubsplactis	Nisin	I
Pediococcusacidilactici y Lactobacillus plantarum WHE92	Pediocin PA-1	IIa
Lactobacillus sake 706	Sakakina A	IIa
Lactobacillus curvatus LTH1174	Curvacin A	IIa
Leuconostocmesenteroides	Mesentericine Y105	IIa
L. plantarum CTC305	Plantaractin A	IIb
Enterococcus faecalis	Enterocin AS-48	IIc

8. The bacteriocins' action mode

Bacteriocins' action mode is determined by several factors involving the composition of the cytoplasmic membrane of the target cell, structure and expression of a protein in function of immunity, and the chemical composition of the environment. In the positive Gram microorganisms is at membrane's cell level, due to electrostatic interactions, the formation of pores in the bacterial membrane, giving space to a permeability phenomenon, so that the cell starts losing ions and essential metabolites for its survival, giving place to cell death. This action can also take place at wall cell level by synthesis inhibition and the DNA biosynthesis inhibition, as a secondary mechanism of these peptides (Gonzalez et al., 2003; Lopez et al., 2008; Monroy et al., 2009; Mondragón et al., 2013).

9. Bacteriocins produced by enterococci

Inside the LAB group, *Enterococcus spp.* genre has shown multiple bacteriocins producer species, which have been named enterocins (Charles et al., 2007; Vallejo et al., 2014). Producer strains have been isolated from foods in a large rate, which has allowed to open a safety antecedent about these microorganisms and their metabolites of protein nature in the potential use on food systems (Bautista et al., 2010). However, some species are related to diverse diseases, being the *E. faecalis* and *E. faecium* species frequently related to human pathologies, meanwhile the rest of them are rare causal agents (Vallejo et al., 2014). Inside the food matrix, where the producer strains grows and IIa class enterocins are produced, degradative enzymes are produced that metabolize compounds such as citrate, and contribute to develop typical flavors and aromas helping to generate the sensory characteristics of foods (Vallejo et al., 2014). In dairy products and fermented meat, bacteriocins generation by enterococci can possess the function of colonizing and becoming the controlling compound of the microbiota of these products, taking into account, besides, some tolerance factors in temperature and salinity conditions which contribute to the aforementioned goal (Charles et al., 2007).

The enterocins present a great diversity, functionality and distribution among the isolation of different sources, either foods or clinical. This diversity can be a result of their capacity to tolerate adverse conditions, cosmopolitan distribution, ability for reception and transference of genetic material of enterococci and it has given room to researchers like Charles et al. (2007) to suggest a way to classify the bacteriocins of this microbial genre, which consists of three classes: Class I enterocins (lantibioticenterocins), Class II enterocins (small, nonlantibiotic peptides), Class III enterocins (cyclic enterocins) and Class IV enterocins (large proteins). Class II can be subdivided into other three classes: II 1. Enterocins of the pediocin family, II 2. Synthetized enterocins without a leader peptide, and II 3. Other linear nonpediocin-like enterocins.

Table 4
Classification of bacteriocins "enterocins" produced by different species of enterococci (Charles et al., 2007).

Class	Bacteriocin example	Producer microorganism	Opposite biologic activity
Class I. Lantibioticenterocins	Cytolysin	*E. faecalis*	Eukaryotic cells (erythrocytes) and Gram-positive bacteria
Class II enterocins			
II.1. Enterocins of the pediocin family	Enterocin A	*E. faecium* CTC492, T136 and P21	*L. monocytogenes*
	Enterocin P	*E. faecium* P13, AA13, G16 and L50	*B. cereus* *C. botulinum* *C. perfringens* *S. aureus*
	Mundticin	*E. mundtii* ATO6	*L. monocytogenes C. botulinum*
II.2. Enterocins synthesized without a leader peptide	Enterocin L50A	*E. faecium* L50 and 6T1a	*L. monocytogenes B. cereus*
	Enterocin EJ97	*E. faecalis* EJ97	*Bacillus spp, Listeria spp. S. aureus*
II.3. Other linear nonpediocin-like enterocins	Enterocin B	*E. faecium* CTC492, T136, WHE 81 and BFE 900	*L. monocytogenes, S. aureus* *C. perfringens*
Class III. Cyclic antibacterial peptides	Enterocin AS-48	*E. faecalis* S-48	Gram +
Class IV. Large proteins	Enterolysin A	*E. faecalis* LMG 2333 and DPC5280	*Bacillus spp. Listeria spp. Staphylococcus spp.*

Several studies have been conducted around the world with a special focus on the search and isolation starting from different sources of *Enterococcus spp.* strains, enterocins producers, and the biologic effect of these compounds of protein nature that are still accumulating and giving weight as an evidence of that positive duality of the potential usage of bacteriocins in production and preservation of foods. Such is the case of Cardoso et al. (2010), who focused on a research of capacity to generate bacteriocins by *Enterococcus faecalis* DBFIQ E24, isolated from raw milk produced in the region of Santa Fe, Argentina, reporting the synthesis of a protein substance which shows antimicrobial activity against positive Gram strains (*Listeria monocytogenes, Bacillus cereus y Bacillus subtilis)* and negative Gram strains (*Pseudomonas sp., Escherichia coli, Vibrio cholerae*O1 Tor serotype Inaba and *Salmonella Enteritidis*), concluding that the substance presents a technological potential to be used as a natural foods preserver.

Bautista et al. (2010), conducted a study of the antimicrobial effect in vitro of bacteriocins produced by *E. faecium* MXVK29, isolated from Mexican sausage at different concentrations (3.33, 6.66 and 13.32 Arbitrary Units/mL), and its combination with each of the following compounds: potassium sorbate at concentrations of 100, 200 & 400 µg/mL, sodium benzoate at concentrations of 100, 200 & 400 µg/mL, and sodium nitrate at 5, 100 & 200 µg/mL in adulterant microorganisms and pathogens in foods, reporting the microbial synergetic effect of the bacteriocin with the different conservative compounds against microorganisms like *B. thermosphacta, E. coli* and *L. Innocua*. On the other hand, Vallejo et al. (2014), reported the production of a bacteriocin by *Enterococcus mundtii* Tw56, isolated from the intestinal system of the *Odontesthes spp*., which presented a high antimicrobial activity against *Listeria innocua*; besides, it showed that factors like nitrogen and carbon sources, physicochemical parameters like pH, NaCl, and temperature, affect the generation of biomass and the antimicrobial compounds. Finally, González et al. (2015), analyzed the antimicrobial activity of extracellular compounds of protein nature-

enterocins-produced by autochthonous, wild strains of *Enterococcus faecalis*, isolated from foods of the Zulia state, Venezuela, indicating that the aforementioned compounds are stable at pH variations and temperature with restricted biological activity against the spectrum of positive Gram bacteria. The aforementioned cases match at the end of the investigations that, strains and generated enterocins have a potential use as bio conservatives of foods, being required deeper studies in the areas concerning the food safety and the genomic characterization related to the virulence and resistance to antimicrobials (Cardoso, 2010; Bautista et al., 2010; Vallejo et al., 2014; Gonzalez et al., 2015).

10. Not only antimicrobials like the bacteriocins… but also other bioactive enterococci compounds

Studies carried out in isolation, metabolism, and functionality of strains coming from foods, mainly dairy products, have reported that different genres of lactic acid bacteria, due to their proteolytic systems, are producers of flavor amino acids precursors, besides providing sensorial abilities like texture and an increasing nutritional value and bioactive peptides with anti-hypertensive activity (inhibitors of the converting enzyme of angiotensin), antithrombotic and antioxidants (Miguel et al., 2005; Domínguez et al., 2014; Pieniz et al., 2015). *Enterococcus spp*. strains belong to the lactic acid bacteria and are capable of producing inhibitor peptides of the angiotensin convertor enzyme and antioxidants (Miguel et al., 2005; Gutiez et al., 2013; Pianiz et al., 2015). The angiotensin convertor enzyme is part of the renin-angiotensin system, an important element in hemodynamic regulation, and also in liquid and electrolytes. It is a dicarbopeptidase produced by different tissues and the central nervous system, proximal tubules epithelium, vascular endothelium, lung, pulmonary epithelium and its activity in maintaining blood pressure. This enzyme catalyzes the conversion from angiotensin I to angiotensin II, being this last a potent vasoconstrictor which can increase the peripheral vascular endurance and, as a consequence, increase the arterial tension (Fernández and Gallego, 1995; Garcia and Toruncha, 1997). Thus, the angiotensin convertor enzyme inhibitors (ACEI), IECA in Spanish, have been set as meds in the treatment of arterial hypertension, which is consider a common disorder and a progressive disorder in the population's health. If it is not treated properly, it presents large death rates (Garcia and Toruncha, 1997; Fernández and Gallego, 1995; Miguel et al., 2005; Domínguez et al., 2014).

Carried our studies around the world reveal that enterococci strains are capable of producing peptides with an antimicrobial activity and enzymes' inhibitors involved in regulation of arterial pressure, such is the case of the researches made by Miguel et al. (2005), where it was reported that *Enterococcus faecalis* strains CECT 5727, CECT 5728, CECT 5826 and CECT 5827 are producers of inhibitor peptides of the angiotensin convertor enzyme during the milk fermentation, Specifically, the CECT 5728 showed being a bioactive peptides producer (with a main peptic sequence consisting on Leucine-Histidine-Leucine-Proline-Leucine-Proline) in an animal model when fermenting milk with a calcium addition. Regazzo et al. (2010), mentions the angiotensin convertor enzyme activity is inhibited by fermented milks, either by *Enterococcus faecalis TH563* in a 69.43 ± 3.12% *or Lactobacillus delbrueckii subsp. Bulgaricus* (*L. delb. bulgaricus*) LA2 in a 60.86 ± 1.01%, which were isolated from Italian dairy products. Meanwhile, Gutiez et al. 2013 reported that isolated *Enterococcus faecalis* foods, environment and clinic strains are capable of producing angiotensin convertor enzymes, after their growth in bovine milk, besides showing an antimicrobial activity against *Pediococcus damnosus* CECT4797 and *Listeria ivanovii* CECT913.

Pianiz et al. (2014) reported the probiotic, antimicrobial, and antioxidant potential qualities of *Enterococcus durans* LAB18s isolated from cheese. It resists acid conditions, it has a survival inside simulated gastric juices, with and without bile salts, it is capable of growing and bio-accumulate selenium, it does not present hemolytic activity; besides, the crop supernatant and intracellular extract of *Enterococcus durans* showed antimicrobial activity against pathogen microorganisms and indicators of food safety, such as *L. monocytogenes, E. coli, P. aeruginosa, A. hydrophila*and *C. fimi*; meanwhile, the antioxidant activity of the crop supernatant and intracellular extract were analyzed with three different methods: 2,2 azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical cation method ABTS.+, scavenging ability on 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals and thiobarbituric acid reactive substances (TBARS). The results showed that *E. durans* LAB18s can be considered as a microorganism with a high antioxidant potential, being used in the decrease of the oxidative damage in foods and fodders. Finally the same author, Pieniz et al. (2015) reported the antioxidant and antimicrobial activity of crop supernatants and cells-free intracellular extracts from different *E. faecium* strains isolated from dairy products and meat, reporting that both bioproducts present antimicrobial activity (against *Listeria monocytogenes*) and also antioxidant activity, due to

their ability to 'hijack' free radicals through the radical ration ABTS•+ (2,2 azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) method. The supernatants showed the highest percentage of inhibition, between 59 and 92.5%; meanwhile, the extracts showed a minor antioxidant activity, between 0.6 and 6%, suggesting that this can be due to the used extraction method, and the fact this ability can be extracellular. In a common conclusion, all the researchers mentioned that the peptide producer enterococci strains with hypertensive, probiotic and antioxidant activity, isolated from foods, present a potential in biotechnological applications related to the elaboration and preservation of foods, and also in the consumers health, but being pending on deeper researches about their benefits and safety in the foods industry and the human health.

Other bioactive compounds produced by different microorganisms (Bacteria, fungi and yeasts) generally during their growth in hydrophobic substrates in an extracellular way or linked to cellular components and that have acquired relevance in the last years, due to multiple properties exhibited are the so-called biosurfactants (BS). These compounds of amphiphilic nature because they contain hydrophilic and hydrophilic groups exhibit an emulsifying and surface activity, besides stability to extreme conditions, structural diversity, biodegradability, low toxicity and different biological properties (antimicrobial, antiadhesive, immunological, and anticancer) being considered potential substitutes for surfactants of chemical origin as well as of biotechnological application in the environmental area, petroleum, food and pharmaceutical industry (Raiger and Lopez, 2009; Okoliegbe and Agarry, 2012; Becerra and Horna, 2016). It has been reported that different lactic acid bacteria are producers of BS and that these compounds can contribute to the prevention of infections in man, such is generally the case of species of the genus *Lactobacillus spp, Streptococcus spp., and Propionibacterium spp*., which are found in different environments including gastrointestinal tract of humans and animals (Hajfarajollah et al., 2014; Sharma et al., 2015; Deepansh et al., 2016). Otherwise, not only lactobacilli are producers of BS since it has been recently reported by researchers as Sharma et al. (2015) that strains of enterococci such as *Enterococcus faecium* MRTL9 isolated from traditional fermented foods is able to generate a glycolipidicbiosurfactant by growing in paraffin as a carbon source. The BS consists of hexadecanoic acid and xylose and shows to be heat stable and different pH values, it has antiadhesive properties against different pathogens (*L. monocytogenes, E. coli, B. cereus, P. aeruginosa* and *S. aureus*), activity of surface by reducing the surface tension of 72.0 to 40.2 mN m-1 and emulsifying activity in 64% against kerosene. Concluding therefore, that this BS can be potentially used as a cleaning material / coating of biomedical equipment, it also gives another perspective on antimicrobial alternatives coming from lactic acid bacteria like some enterococci in favor of the combat against colonization and microbial infections.

11. Final comments

Enterococci are a versatile microbial group with a notable spreading capacity and resistance to adverse conditions for its survival. This bacteria, through various global researches, has demonstrated a duality in matters of human health, going from the fact they are causal agents of hospitable diseases, to the phenomenon of resistance to microbial that difficult therapeutic actions. On the other hand, inside this duality, it is the fact that some species of the genre have been used to favor the human health as microbiologic indicators in the surveillance and sanitary control, alerting about the contamination and presence of pathogens in foods and water, thus avoiding risks for the human health. Likewise, it has been reported that different species of the genre, despite their pathogen backgrounds, intervene in production and preservation of foods through their growth and synthesis of some chemical compounds, antimicrobials, emulsifiers, antioxidants, and antihypertensive, which has given them the potential of being used in the foods industry in favor of human health. However, this last statement is still under debate, and it is not recommendable to take it lightly, since some conclusions point at the fact of making more detailed research studies related to the safety of these microorganisms, given their pathogen characteristics and multi-resistance to the antimicrobials.

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