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Characterization, health benefits and applications of fruits and vegetable probiotics

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ABSTRACT

Probiotics are live microorganisms that confer health benefits to human when administered in adequate amount. They are mainly lactic acid bacteria (LAB) of the genera *Lactobacillus* and *Bifidobacterium*, although the probiotics potential of other bacteria and yeast are not excluded. Modulation of the immune system, anti-inflammatory effect, improved intestinal functioning, inhibition of pathogens and treatment of diseases such as traveler's diarrhea are among probiotics benefits. While several health ailments such as inflammatory bowel, diabetes, hepatic, neurological, respiratory and cardiovascular diseases and some cancer like colorectal cancer are associated with probiotics imbalances. Probiotics are mainly isolated from the human microbiota pool, feces, breast milk and fermented products. Also, fermented fruits and vegetable described as prebiotics are among sources of probiotics. They are rich in functional antioxidants that synergistically provides health benefits to human. In the present work, fruits and vegetable probiotics, isolation, characterization, health benefits and their application are reviewed.

Caracterización, beneficios para la salud y aplicaciones de probióticos de frutas y verduras

RESUMEN

Los probióticos son microorganismos vivos que brindan beneficios a la salud humana cuando son administrados en la cantidad adecuada. Se trata principalmente bacterias del ácido láctico (LAB) de los géneros *Lactobacillus* y *Bifidobacterium*, aunque no se excluye el potencial probiótico de otras bacterias y levaduras. Entre los beneficios ofrecidos por los probióticos pueden mencionarse: modulación del sistema inmunitario, efecto antiinflamatorio, mejor funcionamiento intestinal, inhibición de patógenos y tratamiento de enfermedades, por ejemplo, la diarrea del viajero. Por otra parte, diversas enfermedades, como intestino inflamatorio, diabetes, enfermedades hepáticas, neurológicas, respiratorias y cardiovasculares y algunos tipos de cáncer como el cáncer colorrectal, están asociadas con desequilibrios en los probióticos. Los mismos se aíslan principalmente del conjunto de microbiota humana, heces, leche materna y productos fermentados. Además, entre las fuentes de probióticos se encuentran las frutas y verduras fermentadas descritas como prebióticos. Son ricos en antioxidantes funcionales que proporcionan beneficios para la salud humana de manera sinérgica. En el presente trabajo se revisan los probióticos de frutas y verduras, y se estudia su aislamiento, su caracterización, los beneficios para la salud y su aplicación.

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Probióticos; Prebióticos; Frutas; Verduras; *Lactobacillus*; *Bifidobacterium*

1. Introduction

Probiotics are live microorganisms that confers health benefit to human when administered in adequate amount, generally by improving or restoring the gut microflora (FAO/WHO, 2006; Lebeer et al., 2018). They include a viable mono or mixed culture of bacteria that beneficially improves the properties of indigenous gut microbiota when applied to human (FAO/WHO, 2006). Gut microbiota refers to microorganisms that colonize the entire human digestive system. Most of the probiotics belong to lactic acid bacteria (LAB) of the genera *Lactobacillus* and *Bifidobacterium* (Doğan, Tekiner, & Demirkesen Biçak, 2019). Others include *Bacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, *Enterococcus* and *Streptococcus* strains (Cutting, 2011; García-Ruiz et al., 2014; Le & Yang, 2018). They are mainly isolated from the human gut microbiota pool, feces, breast milk and fermented products (Fontana, Bermudez-Brito, Plaza-Díaz, Muñoz-Quezada, & Gil, 2013; Syngai

et al., 2016; Tarrah et al., 2019). Probiotics strains have to be autochthonous of the environment where they will be a part once ingested. Other microbes may also have probiotics potential including some yeast strains of *Saccharomyces boulardii*, *S. cerevisiae*, *S. bayanus*, *S. florentinus*, *S. pastorianus*, *S. sake*, *S. unisporus* and *Schizosaccharomyces pombe* and bacteria such as *Oenococcus oeni* (Pennacchia, Blaiotta, Pepe, & Villani, 2008; Tamang, Shin, Jung, & Chae, 2016). Probiotics effects can be strain specific, but may have more than one health benefits depending on their delivery method, host response or interaction with other microbes (Reid, 2016).

Probiotics competitively inhibit growth and proliferation of pathogens such as *Escherichia coli*, *Bacillus cereus*, *Listeria monocytogenes*, *Staphylococcus aureus* and *Clostridia* spp. in the gastric intestinal tract (GIT) (Floch, 2018; Ghasemian, Eslami, Shafiei, Najafipour, & Rajabi, 2018; Panghal et al.,

2018). The antimicrobial activity of probiotics occurs through: (1) reduced pH in the lumen due to production of acetic and lactic acids, (2) bacteriocins and reutericyclin accumulation and (3) compounds blocking bacterial adhesion to the epithelial cells and consequently reducing pathogen toxins production (Coman et al., 2014; Tejero-Sariñena, Barlow, Costabile, Gibson, & Rowland, 2012). Also, probiotics are involved in immune modulation, regulation of intestinal health, improved lactose digestion and maintaining bone health (Amorim, Piccoli, & Duarte, 2018; Collins, Kim, McCabe, & Weaver, 2017). They make functional components such as antioxidants and anti-hypertensive γ -aminobutyric acid (GABA) from fruits and vegetable (FV) accessible to human (Su et al., 2015). Whereas, imbalances in the composition and function of the intestinal microbiota have been associated with diseases, including inflammatory bowel disorders, obesity, diarrhea, *diabetes mellitus* type II, colorectal cancer as well as hepatic, neurological, respiratory and cardiovascular diseases (Shokryazdan, Faseleh Jahromi, Liang, & Ho, 2017). Exogenous and endogenous factors including genetic features, immune responses, diet, infection, antibiotics and other drugs use have been reported to influence gut microbiota (Collins et al., 2017; Schmidt, Raes, & Bork, 2018). Henceforth, the microbiota balances need to be improved and sustained.

The abundant source of probiotics can be accessed by spontaneous fermentation of FV in exclusive uncontrollable environmental conditions (Xu, Luo, Bao, Liao, & Wu, 2018). Traditionally fermented dairy products are considered as the main vehicle for probiotics in different regions across the world. This is why several species of LAB, *Lactobacillus* and *Bifidobacterium* were isolated from fermented milk (Fontana et al., 2013; Mishra, Behera, Kar, & Ray, 2018). However, recently consumers awareness on health and nutrition has increased demand and consumption of functional foods (Salveti & O'Toole, 2017). The trend has created a need for formulation of non-dairy probiotics food from FV. Evidently, dairy products have high cholesterol, lactose and animal proteins that may limit consumption to some population groups (Panghal et al., 2018). Today probiotics products comes in different formulations including fruit juices, fermented vegetables, impregnated FV, infant formulas, condiments, sweeteners, pizza crust and dietary supplements (Champagne, Gomes Da Cruz, & Daga, 2018; Chen et al., 2014; Meybodi, Mortazavian, Sohrabvandi, Cruz, & Mohammadi, 2017). Moreover, exploring the potential of

FV prebiotics, probiotics yoghurt are formulated with FV extracts, soluble fibers, inulin and commercial FOS and XOS (Fazilah, Ariff, Khayat, Rios-Solis, & Halim, 2018; Sah, Vasiljevic, McKechnie, & Donkor, 2016; Zhang et al., 2019).

FV potential for probiotics has been studied and reported to be the next category of food to serve as probiotics carrier and a therapeutic microbiome target (Panghal et al., 2018; Septembre-Malaterre, Remize, & Poucheret, 2018). Therefore, this review presents research works on FV probiotics, their isolation and characterization, health benefits, application and related safety issues.

2. Probiotics and prebiotics selection

The selection of probiotics strain from the ecosystems (raw or fermented FV) implies several functional properties that are primarily *in vitro* investigated and proved in animal studies: they must be of human original and a normal inhabitant of the intestine; able to survive in the GIT and resistant to salivary enzymes, gastric acid, pancreatic juice and bile salts; able to colonize and adhere to the GIT epithelium; able to modulate human immune response, decrease incidences of diarrhea and improve the GIT health; maintain the mucosa integrity; are non-pathogenic and non-toxic, produce toxins binding substances and have detoxification activity (Markowiak, Śliżewska, Markowiak, & Śliżewska, 2017). Furthermore, probiotics must be capable of hydrolyzing constituents from FV that cannot be utilized by the host such as the fructans and galactans; fructooligosaccharides (FOS), mannanoligosaccharides (MOS), xylooligosaccharides (XOS), inulin and some antinutritional factors such as tannins (Gibson et al., 2017; Mohanty, Misra, Mohapatra, & Sahu, 2018). They must produce beneficial compounds such as vitamins, antioxidants and antimicrobial substances such as organic acids (lactic and acetic acids), hydrogen peroxide, bacteriocins, aldehydes, acetoin, carbon dioxide, reuterin, reutericyclin, phenolic acids, peptides, short chain fatty acids and competitively exclude pathogens, as presented in Figure 1 (Derrien & van Hylckama Vlieg, 2015).

FV are good source of vitamins, minerals, sugars and other phytonutrients and thus ideal for the microbial growth (Panghal et al., 2018). They are good prebiotics, possess intrinsic properties such as buffering capacity, FOS, inulin, fiber and antinutritional factors (tannins, phenols, phytates as examples) and provide substrates for proliferation of intestinal microbiota

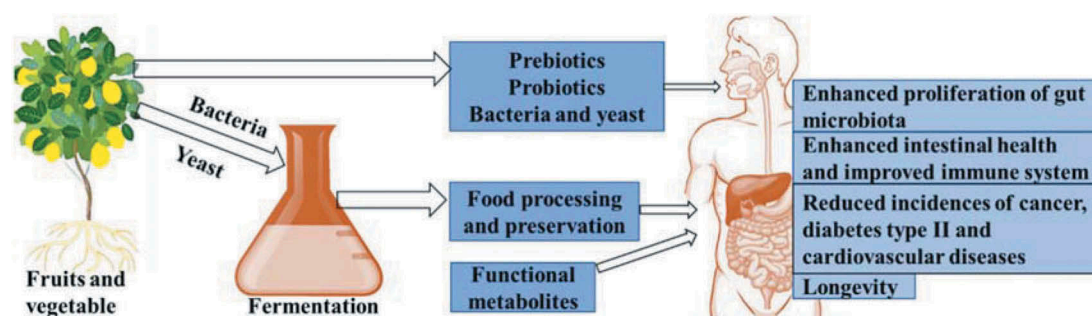


Figure 1. Prebiotics, probiotics and gut microbiota relationship in maintaining human health. Fruits and vegetable can provide prebiotics-probiotics benefits to human through direct consumption of fresh, fermented products and/or functional metabolites in synbiotics food.

Figura 1. Relación entre prebióticos, probióticos y microbiota intestinal para mantener la salud humana. Las frutas y verduras pueden proporcionar beneficios prebióticos-probióticos al ser humano a través del consumo directo de productos frescos fermentados y/o de metabolitos funcionales en los alimentos simbióticos.

(Septembre-Malaterre et al., 2018). Prebiotics are food components that are able to reach the colon undigested and not hydrolyzed in the upper digestive tract, stimulate the growth of microbiota and offers healthy composition for intestinal microflora (Di Gioia & Biavati, 2018). Recently, prebiotics are referred as substrates that are selectively utilized by host microorganisms conferring a health benefit (Gibson et al., 2017). Most prebiotics are non-digestible carbohydrates such as fibers and oligosaccharides, fructans and galactans (Gibson et al., 2017; Hill et al., 2014). In summary, prebiotics should have the following properties: they are neither hydrolyzed nor absorbed and/or can be partially digested in the upper intestine, reaching the colon undigested. They are fermented by intestinal microorganisms and represent selective substrates for one of the beneficial bacteria and must be able to selectively stimulate the growth, metabolism and/or activity of intestinal microflora associated with health, immune or wellbeing (Gibson et al., 2017). Of interest, a review by Schmidt et al. (2018) showed that dietary nutrient contributes only as low as one digit percent variation in microbiota population. The concept describes features of selective utilization by GIT microbiota that prebiotics are differentiated from other undigested nutrients such as vitamins and minerals.

Some of the FV probiotics products are already available in the market, including fermented as well as unfermented FV, organic probiotics drinks and dried fruits enriched with microorganisms (Panghal et al., 2018). FV prebiotics includes aqueous extract of blueberry, China green tea, oolong tea, black and selenium-containing tea, dragon fruits, banana, mulberry leaves and fruit and asparagus among others (Choque Delgado & Tamashiro, 2018; Mohanty et al., 2018; Sun et al., 2018). Some commercial prebiotics are derived or formulated with fiber, inulin and oligofructose, the FOS and galactooligosaccharides (GOS). Whereas, technologies such microencapsulation, immobilization and vacuum impregnation can be used to incorporate probiotics into commercial prebiotics matrices (Mishra et al., 2018).

Combination of prebiotics and probiotics of FV makes it possible to formulate a synbiotics food (Markowiak et al., 2017). Consumption of synbiotics food is more potent to the human health than consumption of probiotics or prebiotics only (McFarland & Goh, 2018). Probiotics and prebiotics components in a single food improve probiotics viability and survival during storage and passage through the GIT (McFarland & Goh, 2018). It allows an efficient implantation of probiotics in the colon due to stimulating effect on the growth and activity of the microorganisms (Sánchez et al., 2017). Some of the prebiotics are able to modify the metabolic profile of probiotics, by supplying selective

compounds and branched short chain fatty acids (Fernando et al., 2018; Sánchez et al., 2017). Moreover, prebiotics may provide direct benefits to human health through cholesterol removal, lowering blood sugar, prevention of obesity, relieving constipation and inhibition of pathogens and carcinogens (Mohanty et al., 2018; Zhang et al., 2015). At the same time, they support intestinal and immune system functioning and improves calcium and magnesium absorption (Krupa-Kozak et al., 2017; Mohanty et al., 2018). Generally, fermented FV as prebiotics require few ingredients and minimal processing steps even though some contains only few dominant microbial taxa with remarkably complex population dynamics (Marco et al., 2017).

3. Probiotics isolation and identification

Researches have explored the use of LAB of *Lactobacillus* and *Bifidobacterium* and yeast strains isolated from FV and their addition effect to food systems, aimed at improving quality and functional properties. The main criterion for probiotics isolation is to survive under extreme pH of gastric juice and bile salts in the intestine to reach the target sites in human GIT (Figure 2). Probiotics need to be delivered to the desired sites in an active and viable form for their effectiveness and efficacy. The viability and activity have been reported as probiotics prerequisite for achieving health benefits. The recommended probiotics concentration in food to confer the desired health benefit is at least 10^8 to 10^9 colony forming unit per milliliter (CFU)/ml to ensure a minimum of 10^6 to 10^7 CFU/ml in the colon (Fernando et al., 2018; Shori, 2016, 2017). However, even non-viable culture or heat treated probiotics are proved to have functional properties, bacteriocin accumulation and immune system stimulation effects (Champagne et al., 2018; Lee et al., 2017). For instance, strains of *Oenococcus oeni*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Streptococcus salivarius* subsp. *thermophilus* and yeast (*Saccharomyces boulardii*) are qualified potential probiotics (Amorim et al., 2018; Foligné et al., 2010). Considering that probiotics are strain-specific and sometimes mixture of strains, culturing techniques are important to produce useful probiotics. Also, isolation and characterization of probiotics from FV need further development.

3.1. Isolation

Isolation is the first requirement for probiotics characterization, health claim submission and marketing. Determination of the genus, species and strain provides useful information for the

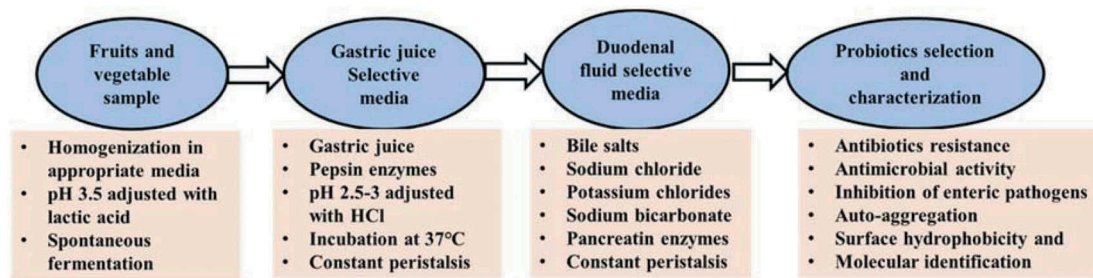


Figure 2. Fruits and vegetable-based probiotics isolation and identification schematic presentation using gastrointestinal tract conditions.

Figura 2. Presentación esquemática del aislamiento e identificación de probióticos a base de frutas y verduras utilizando condiciones similares a las del tracto gastrointestinal.

physiological and metabolic properties of probiotics. Several protocols have been developed for isolation of probiotics from FV so that a probiotics microorganism can resist gastric conditions of pH 2.5–3 and pepsin enzymes, bile salts, pancreatin enzymes and stimulated GIT peristalsis and adapt colon environment (Amorim et al., 2018; Lee et al., 2016). Also, the isolates are qualified for antibiotics resistance, antimicrobial activity and inhibition of enteric pathogens, auto aggregation, cell surface hydrophobicity in animal model and *in vitro* studies. Moreover, for safety reasons, probiotics isolates are tested for production of toxic compounds and hemolytic activity. Various types and forms of fermented FV products are commercially available, including Chinese *hum-coy*, *inziansang*, *sufu*, *pao-cai*, *jiang-gua* and *yan-taozih*; Sauerkraut, cucumber pickles and olives in Europe; Turkish *hardaliye*; Korean *kimchi*; Thai *paksian-don*; Malaysian *tempoyak*; Japanese *nazawana-zuke*; Philippine *puto*; *camuoi* in Vietnam; *kalpi* in Nepal and India; Ethiopian *enjera*; Sudanese *kisra* and; Brazilian cashew apple juice (Panghal et al., 2018; Urbonaviciene, Viskelis, Bartkiene, Juodeikiene, & Vidmantiene, 2015). Different strains of LAB: *Lactobacillus*, *Bifidobacterium* and yeast have been isolated, characterized and preserved for use as culture in commercial food formulations.

3.2. Identification and characterization

The species level identification is achieved by combination of techniques that includes biochemical information (growth in different media, carbon source, nitrate as well as Gram, catalase, urease and reductase tests), genotype (sequencing of the 16s rRNA gene) and DNA fingerprinting (Repetitive element palindromic PCR, Random Amplified Polymorphic DNA, Restriction Fragment Length Polymorphism (RFLP), PCR-RFLP and Pulse-Field Gel Electrophoresis (PFGE)) (de Melo Pereira, de Oliveira Coelho, Júnior, Thomaz-Soccol, & Soccol, 2018; Lefevre et al., 2017; Mianzhi & Shah, 2017). Some of the probiotics from traditional fermented FV products have been confirmed by various studies (Table 1). However, even with the advanced sequencing technologies some microbes such as *Archaea* could pass unnoticed (Sánchez et al., 2017). The effective probiotics product can only be regarded as safe and useful after the microbiota involved are properly identified, characterized and qualified for human use. It can be summarized that the dominant probiotics in naturally fermented FV are of the genera *Lactobacillus* (Table 1).

4. Health benefits of probiotics

There is a broad association between microbiome and human health status, though no claimed causality. It has been reported that overall microbiome shift, imbalance or reduced diversity are associated and/or predispose individuals to diseases and chronic conditions including inflammatory bowel diseases, cancer, irritable bowel syndrome, allergy, asthma, diabetes, obesity, cardiovascular diseases, neurological diseases and gastrointestinal disorders (Cremon, Barbaro, Ventura, & Barbara, 2018). The imbalances being caused by host genetics and environmental factor such as mode of a neonate delivery, breast-feeding history, diet, infections and use of antibiotics and other drugs (Cenit et al., 2015; Lange, Buerger, Stallmach, & Bruns, 2016; Leiva-Gea et al., 2018). Moreover, lifestyle factors such as smoking, alcohol consumption habit and physical exercises were

reported to modulates gut microbiota by changing not only its composition but also functionality (Schmidt et al., 2018; Tao et al., 2019).

Animal models, *in vitro* and human clinical studies have shown effectiveness and potential health benefits of probiotics (Collins et al., 2017). Probiotics have been reported to restore GIT microbiota and provide various health benefits (Table 2). Probiotics can synthesis certain vitamins, notably B group vitamins including biotin, cobalamin, pantothenic acid, pyridoxine, nicotinic acid, thiamine, riboflavin and folate and vitamin K so as to nourish the host (Kapasob, Kerdchoechuen, Laohakunjit, & Somboonpanyakul, 2018; Rowland et al., 2018). Additionally, probiotics have been associated with improved nutrient bioavailability, lowered blood cholesterol, alleviated lactose intolerance symptoms and reduced incidences of cardiovascular diseases, obesity, *diabetes mellitus* type II, production of anticancer and anti-mutagenic substances (Lebeer et al., 2018; Shokryazdan et al., 2017). Though, production of immunological, anticancer, bioactive and functional compounds are rarely characterized as strain specific (Hill et al., 2014). For instance, anticancer effect can be achieved by multiple mechanisms: production of compounds with anticarcinogenic activity, reduction of the activity of enzymes involved in the carcinogen formation, binding and degradation of mutagens, reduction of nephrotoxic and immunosuppressive mycotoxins, inhibition of tumor cell proliferation and induced cancer cells apoptosis. Likewise, immunomodulation effect might occur through enhanced production of anti-inflammatory cytokines, immunoglobulins and/or phagocytosis of pathogens (Azad, Sarker, & Wan, 2018; Maldonado Galdeano, Cazorla, Lemme Dumit, Vélez, & Perdigón, 2019).

5. Applications of probiotics

5.1. Probiotics as starter culture in fruits and vegetable products

The potentials of probiotics in fermented FV have been studied and led to the development of starter culture (Xu et al., 2018). Studies have shown successful use of commercial probiotics culture in FV (Bujna, Farkas, Tran, Dam, & Nguyen, 2017). Use of probiotics starter culture for most of the formulations has been a preferred technological approach to minimize variations and to ensure uniformity of the product (Peñas, Martínez-Villaluenga, & Frias, 2017). Some of the probiotics products available in the market contains different formulations of *Lactobacillus acidophilus*, *L. casei*, *L. bulgaricus*, *L. fermentum*, *L. lactis*, *L. plantarum*, *L. reuteri*, *L. brevis*, *L. salivarius*, *L. paracasei*, *L. rhamnosus*, *Bifidobacterium longum*, *B. bifidum*, *B. infantis*, *B. breve*, *B. animalis*, *B. lactis*, *Streptococcus thermophilus*, *Streptococcus* spp., *Enterococcus faecium* and *Saccharomyces cerevisiae* (Dias et al., 2018; Lugli et al., 2019; Morovic, Hibberd, Zabel, Barrangou, & Stahl, 2016). However, *Lactobacillus*, *Bifidobacterium* and *Saccharomyces* spp. are dominant probiotics in most of commercial products. Due to increased consumer interest in probiotics, products containing a mixed or single culture of live microorganisms are delivered in different formulations within the food matrixes in forms of juice, liquids, suspension, pills, capsules, powder, granules, sachets, chewable bar and so on (Kolaček et al., 2017). Apple, pineapple, cashew apple, orange, banana, black current, cantaloupe, grape, passion fruit, raspberry, mango and blueberry

Table 1. Traditional and emerging fermented fruits and vegetable products and their corresponding probiotics.**Tabla 1.** Frutas y verduras fermentadas tradicionales y emergentes y sus probióticos correspondientes.

Products	Main ingredients	Probiotics	References
Burong mustala, Inziangsang, Suan-tsai, Pak-Gard-Dong and Dakguadong	Mustard leaves	<i>Lactobacillus brevis</i> , <i>L. plantarum</i> , <i>Pediococcus cerevisiae</i> , <i>Pediococcus pentosaceus</i> and <i>Tetragenococcus halophilus</i>	(Panghal et al., 2018)
Broccoli juice	Broccoli	<i>Leuconostoc mesenteroides</i> , <i>Weissella</i> spp., <i>Enterococcus gallinarum</i> and <i>Lactococcus</i> spp.	(Xu et al., 2018)
Cherries juice	Cherries	<i>Pediococcus pentosaceus</i> and <i>Enterococcus gallinarum</i>	(Xu et al., 2018)
Cucumbers	Cucumbers, vinegar, salt	<i>Pediococcus</i> spp., <i>Lactobacillus</i> spp. and <i>Leuconostoc</i> spp.	(Zieliński, Surma, & Zielińska, 2017)
Dhamuoi Hardaliye	Cabbage and other vegetables Red grape juice, black mustard seeds	<i>Lactobacillus plantarum</i> , <i>Leuconostoc mesenteroides</i> <i>Lactobacillus paracasei</i> , <i>L. brevis</i> , <i>L. casei</i> subsp. <i>caseipseudopiantarum</i> , <i>L. pontis</i> , <i>L. acetotolerans</i> , <i>L. sanfranciscensis</i>	(Panghal et al., 2018)
Jiang-gua	Cucumbers, salt, sugar, vinegar and soy sauce	<i>Leuconostoc lactis</i> , <i>Leuconostoc mesenteroides</i> , <i>Lactobacillus pentosus</i> , <i>Lactobacillus plantarum</i> , <i>Lactobacillus paraplantarum</i> , <i>Lactococcus lactis</i> subsp. <i>lactis</i> , <i>Weissella hellenica</i> , <i>Weissella cibaria</i>	(Chen et al., 2012)
Kimchi	Cabbage, radish, spices (garlic, ginger, pepper and onion)	<i>Lactobacillus plantarum</i> , <i>L. brevis</i> , <i>L. curvatus</i> , <i>L. sakei</i> , <i>L. maltaromicus</i> , <i>L. bavaricus</i> , <i>Weissella confusa</i> , <i>Weissella kimchi</i> , <i>Weissella koreensis</i> , <i>Leuconostoc mesenteroides</i> , <i>Leuconostoc kimchi</i> , <i>Leuconostoc cireum</i> , <i>Leuconostoc gasicomitatum</i> and <i>Leuconostoc pseudomesenteroides</i>	(Le & Yang, 2018)
Pao-cai	Cabbage, celery, cucumber, and radish, ginger, salt, sugar, hot red pepper	<i>Lactobacillus pentosus</i> , <i>L. plantarum</i> , <i>L. brevis</i> , <i>L. lactis</i> , <i>L. fermentum</i> and <i>Leuconostoc mesenteroides</i>	(Swain et al., 2014)
Pickles	Varieties of fruits and vegetable-cucumbers, mangoes: usually in 10–15% salt NaCl, brine	<i>Lactobacillus casei</i> , <i>L. plantarum</i> , <i>L. rhamnosus</i> , <i>L. Pentosus</i> , <i>L. acidophilus</i> , <i>Pediococcus pentosaceus</i> and <i>P. acidilactic</i>	(Cao et al., 2016; Fan, Breidt, Price, & Pérez-Díaz, 2017)
Radish juice Salgam	Radish Black carrots, turnip, bulgur flour, sourdough, yeast, salt and water	<i>Weissella confusa</i> , <i>Enterococcus durans</i> and <i>Bacillus coagulans</i> <i>Lactobacillus plantarum</i> , <i>L. paracasei</i> , <i>L. fermentum</i> , <i>L. brevis</i> , <i>L. pentosus</i> , <i>L. buchneri</i> , <i>L. helveticus</i> , <i>L. acidophilus</i> , <i>Lactococcus</i> spp., <i>Pediococcus</i> spp., <i>Leuconostoc</i> spp. and <i>Saccharomyces</i> spp.	(Xu et al., 2018) (Coskun, 2017; Ekinici et al., 2016)
Sauerkraut	Cabbage, salt	<i>Leuconostoc mesenteroides</i> , <i>Lactobacillus brevis</i> and <i>L. plantarum</i>	(Peñas et al., 2017)
Tursu	Cucumbers, cabbage, green tomatoes, green peppers and other vegetables	<i>Lactobacillus plantarum</i> , <i>L. brevis</i> , <i>Leuconostoc mesenteroides</i> and <i>Pediococcus pentosaceus</i>	(Çetin, 2011)
Wine (red)	Grapes	<i>Oenococcus oeni</i> , <i>Pediococcus parvulus</i> , <i>P. pentosaceus</i> , <i>Lactobacillus casei</i> and <i>L. plantarum</i>	(García-Ruiz et al., 2014)
Yan-taozih	Peaches, salt, sugar and pickled plums	<i>Leuconostoc mesenteroides</i> , <i>Lactococcus lactis</i> subsp. <i>lactis</i> , <i>L. brevis</i> , <i>Weissella cibaria</i> , <i>W. paramesenteroides</i> , <i>W. minor</i> and <i>Enterococcus faecalis</i>	(Chen et al., 2013)

juices are some of the successful fruit juices used for probiotics delivery (Fernandes Pereira & Rodrigues, 2018).

Conventionally, not all LAB strains from FV can guarantee the same performance during processing. The main criteria used to select them can be categorized as follows:

- (1) Pro-technological: origin, pathogenicity, toxicity, antibiotics resistance, synthesis of antimicrobial compounds, growth rate and viable cell count, acidification rate, tolerance to low pH and bile salts (Vasiljevic & Shah, 2008).
- (2) Processing technological: the strain must be genetically stable and survive food processing conditions such as fermentation, oxidative stress and variation in temperatures and pH, phage resistance, viable and stable during storage (Vasiljevic & Shah, 2008).
- (3) Sensory aspects: synthesis of aroma compounds, change in taste induced by lactic acid accumulation, and other flavor active compounds from proteolytic and lipolytic probiotics enzymes.
- (4) Nutrition and functional components: synthesis of biogenic compounds, increased antioxidants activity, hydrolyze exo-polysaccharides and tolerant to phenolics, whereas such compounds are abundant in FV (Jiménez-Gómez, García-Fraile, Flores-Félix, & Rivas, 2018; Rowland et al., 2018).

The environmental adaptation of the targeted starter is a prerequisite, which affect all the other potential metabolic features (Tripathi & Giri, 2014). Buffering capacity, pH, concentration of fermentable carbohydrates, species diversity and presence of inhibitory compounds affect the growth and acidification of the probiotics (Shori, 2016). In some foods, even minor alteration to species diversity or numbers can result in inconsistent fermentation, varied metabolites composition and significantly different product in terms of acidity and texture (Marco et al., 2017). Thus, it is worth maintaining ecological environment for improving and maintaining the functional properties and organoleptic quality of probiotics product containing commercial culture.

5.2. Biocontrol, preservation and antagonistic probiotics

Traditionally, LAB have been used in FV processing and preservation to prevent them from spoilage and pathogens proliferation (Swain, Anandharaj, Ray, & Parveen Rani, 2014). Spontaneous FV fermentation produce several beneficial compounds which deserves interest as tools to improve the quality and microbial safety of fermented foods. Some of the probiotics isolate have shown antagonistic towards plant pathogens. Several species of LAB were reported to

Table 2. Example of some probiotics and reported specific benefits to human health.**Tabla 2.** Ejemplos de algunos probióticos y beneficios específicos reportados para la salud humana.

Probiotics	Reported specific benefits/antagonistic activity	Reference
<i>Lactobacillus acidophilus</i>	Antimicrobial activity against <i>Clostridium jejuni</i> and <i>Listeria monocytogenes</i>	(Campana, van Hemert, & Baffone, 2017)
<i>Lactobacillus casei</i>	Antimicrobial activity against <i>Clostridium sakazakii</i> , <i>Clostridium jejuni</i> and <i>Listeria monocytogenes</i>	(Campana et al., 2017)
<i>Lactobacillus paracasei</i>	Anticancer activity, <i>E. coli</i> and <i>Listeria innocua</i> inhibition effects	(Tarrach et al., 2019)
<i>Lactobacillus plantarum</i>	Antimicrobial activity against <i>Streptococcus enteritidis</i> , <i>Clostridium sakazakii</i> , <i>Clostridium jejuni</i> , <i>Listeria monocytogenes</i> , <i>E. coli</i>	(Campana et al., 2017)
<i>Lactobacillus lactis</i>	Antimicrobial activity against <i>Streptococcus enteritidis</i> , <i>Clostridium sakazakii</i> , <i>Clostridium jejuni</i> , <i>Listeria monocytogenes</i> and <i>E. coli</i>	(Campana et al., 2017)
<i>Lactobacillus rhamnosus</i>	Antimicrobial activity against <i>Streptococcus enteritidis</i> , <i>Clostridium sakazakii</i> , <i>Clostridium jejuni</i> , <i>Listeria monocytogenes</i> , <i>E. coli</i> and <i>Clostridium difficile</i>	(Campana et al., 2017; Valdés-Varela, Gueimonde, & Ruas-Madiedo, 2018)
<i>Saccharomyces boulardii</i>	Treatment of antibiotics associated diarrhea as well as by <i>Clostridium difficile</i> (traveler's disease)	(Pennacchia et al., 2008; Valdés-Varela et al., 2018)
<i>Weissella cibaria</i>	Anti-inflammatory effect, antimicrobial activity against <i>Listeria monocytogenes</i> , <i>E. coli</i> and <i>Salmonella</i> spp.	(Le & Yang, 2018)
<i>Weissella koreensis</i>	Anti-obesity, antimicrobial activity against <i>Listeria monocytogenes</i> , <i>E. coli</i> and <i>Salmonella</i> spp.	(Le & Yang, 2018)
<i>Lactobacillus buchneri</i>	Antimicrobial against <i>Candida albicans</i>	(Shokryazdan et al., 2017)
<i>Bifidobacterium animalis</i>	Anti-rotaviral, treatment against antibiotics uses and <i>Clostridium difficile</i> related diarrhea. Also improves immune functioning in human	(Miller et al., 2017; Vasiljevic & Shah, 2008)
<i>Bifidobacterium bifidum</i>	Antimicrobial activity against <i>Clostridium sakazakii</i> , <i>Clostridium jejuni</i> , <i>Listeria monocytogenes</i> and <i>E. coli</i>	(Campana et al., 2017)
<i>Lactobacillus acidophilus</i> and <i>Bifidobacterium</i> spp.	Inhibit growth of tumor cell, produce anti-carcinogens and reduces the chances for cancer	(Vasiljevic & Shah, 2008)
<i>Lactobacillus reuteri</i>	Improves immune system	(Indrio et al., 2017)
LAB	Control of blood cholesterol-Hypocholesterolemia-effect and hyperlipidemia, Immune modulation, Effective against <i>Salmonella enterica</i> ver. <i>Typhimurium</i> , Rota viral infections and <i>Clostridium difficile</i> diarrhea.	(Miller et al., 2017; Valdés-Varela et al., 2018; Wang et al., 2014)
<i>Lactobacillus salivarius</i>	Antimicrobial activity against <i>Listeria monocytogenes</i> , <i>Streptococcus enteritidis</i> , <i>S. mutans</i> , <i>Candida albicans</i> , <i>Clostridium sakazakii</i> and <i>C. jejuni</i>	(Campana et al., 2017; Krzyściak et al., 2017)
<i>Lactobacillus helveticus</i> and <i>Saccharomyces cerevisiae</i>	Reduction of hypertension effects	(Hendijani & Akbari, 2018)
<i>Oenococcus oeni</i>	Immune modulation and anti-inflammatory effects	(Foligné et al., 2010)
<i>Lactobacillus acidophilus</i> and <i>Bifidobacterium longum</i>	Associated with reduced incidences of colorectal cancer	(Parvez, Malik, Ah Kang, & Kim, 2006)
<i>Bacillus subtilis</i>	Stimulates the immune system, reduce frequency of infections, has antagonistic effect on <i>H. pylori</i> infection, also has protective effect on other LAB during food processing, storage and in the GIT	(Lefevre et al., 2017; Lefevre et al., 2015; Pinchuk et al., 2001; Yahav, Berkovich, Ostrov, Reifen, & Shemesh, 2018)
<i>Bacillus clausii</i>	Anti- <i>Helicobacter pylori</i> and reduce side effects related to <i>H. pylori</i> antibiotics therapy, treatment of acute diarrhea in children and immunomodulating activity	(Ianiro et al., 2018; Nista et al., 2004; Sudha, Jayanthi, Pandey, & Verma, 2019)
<i>Bacillus licheniformis</i>	Prevents insulin resistance, has antibiotics effect against <i>H. pylori</i> , reduce the effect of antibiotics use in treatment of diarrhea and can detoxify aflatoxin B1 up to 94.7% in food matrixes.	(Elshaghabee, Rokana, Gulhane, Sharma, & Panwar, 2017; Raksha Rao, Vipin, Hariprasad, Anu Appaiah, & Venkateswaran, 2017)

produce a wide array of antagonistic metabolites to FV pathogens as well as spoilage microbes (Choi, Patra, Kim, & Kang, 2018; Gajbhiye & Kapadnis, 2016). This is due to the synthesis of various primary as well as secondary metabolites including organic acids (acetic and lactic acids), carbon dioxide, ethanol, hydrogen peroxide, acetaldehyde, acetoin, antifungal compounds, free fatty acids, phenyl-lactic acids, hydroxyphenyl acids, bacteriocins (nisin), and antibiotics (reutericyclin), all of which may affect broad spectrum of fungi, bacteria, virus, and protozoa (Shokryazdan et al., 2017; Stanton, Ross, Fitzgerald, & Sinderen, 2005).

Recently, use of probiotics in FV preservation has increased as consumer demands for fresh taste and free from chemical preservatives produce. For fresh FV, use of probiotics and bacteriocins do not induce changes as compared to other processing technologies such as heat treatment (Barbosa, Mantovani, & Jain, 2017). Some bacteriocins such as nisin, enterocin, bovicin, bificin and pediocin have proven to be successful in FV preservation against spoilage and pathogenic microorganisms (Barbosa et al., 2017). Bacteriocins are produced *in situ* by probiotics culture incorporated on the surface of fruits or vegetables and/or on the

packaging materials (Karunaratne, 2018). With bioprocessing technology advancement, bacteriocins can be added in FV in the purified or semi-purified forms.

Various studies demonstrated successful inhibition or antagonistic effect of bacteriocin against pathogens in juice: enterocin AS-48 reduced viability of *Listeria monocytogenes*, *Bacillus cereus* and *Staphylococcus aureus*; combination of enterocin AS-48 and nisin inactivated the growth of *Alicyclobacillus acidoterrestris* a spoilage bacteria that survives pasteurization temperatures; combination of nisin, acetic acid and sodium lactate was effective against *Listeria monocytogenes*; and nisin-sodium lactate-potassium sorbate formulation effectively inhibited growth of different *Salmonella* spp. (Settanni & Corsetti, 2008). Moreover, enterocin AS-48 inhibited growth of *Pediococcus parvulus*, *Lactococcus collinoides* and *Lactobacillus diolivorans* strains in most of apple cider tested (Abriouel, Lucas, Ben, Valdivia, & Gálvez, 2010). Iglesias, Abadias, Anguera, Sabata, and Viñas (2017) reported that *Lactobacillus rhamnosus* successfully inhibited the growth of *Salmonella* spp. and *Listeria monocytogenes* in fresh cut pears fruit. Likewise, nisin producing *Lactococcus lactis* strain inoculated in the minimally processed sliced

apple enhanced safety of the product by inhibiting the growth of spoilage yeast and *Listeria monocytogenes* (Siroli et al., 2016).

5.3. Probiotics application in cosmetics

Today a large number of probiotics-based cosmetics are used in different forms and numerous products are available in the market. Cosmetics refer to substance or products intended to be rubbed, poured, sprinkled or sprayed on, introduced into, or otherwise applied to the human body or any part thereof for cleansing, beautifying, promoting attractiveness or altering appearance (Huang & Tang, 2015). Microbiome such as *Staphylococcus* spp. (*S. epidermis*), *Propionibacterium* spp. (*P. acnes*, *P. granulosum* and *P. avidum*) and *Corynebacterium* spp. (*C. simulans*, *C. tuberculostearium*, *C. tenuis*, *C. jeikeium* and *C. xerosis*) are dominant member in the human skin (Byrd, Belkaid, & Segre, 2018; Marini & Krutmann, 2012). Some of the skin pathogens such as *Staphylococcus aureus* are responsible for several skin infection, including impetigo, furuncles, subcutaneous abscesses, skin ulcers and several systemic infections when they get into the blood stream (Ingen-Housz-Oro, Del Giudice, & Chosidow, 2016; Olaniyi, Pozzi, Grimaldi, & Bagnoli, 2016).

It has been suggested that orally administered probiotics exerts effect on the skin via mechanisms initiated in the GIT, most likely due to immune responses such as modulation of specific T-cells and stimulating toll-like receptors (Kober & Bowe, 2015). Some of the products used in beauty industry with probiotics as bioactive ingredients include lotion, anti-aging serum, soap, wipe and aftershave for topical application and ingested probiotics drinks. The most common probiotics claimed on the label are *Lactobacillus* spp. and *Bifidobacterium* spp. (Cinque et al., 2017; Huang & Tang, 2015). *Lactobacillus* spp. probiotics were reported to be effective in managing skin inflammation when administered orally, but their effectiveness and efficacy in children varies. *Staphylococcus epidermis* a commensal skin bacterium is used for treatment of acne related to *P. acne* and other related skin infections (Ouwehand, Lahtinen, & Tiihonen, 2017). *Streptococcus salivarium* spp. *thermophilus* S244 an ingredient in cosmetic formulations has been reported to produce enzymes that reduce skin dryness, loss of tone and water, hence slowing the process of skin aging (Cinque et al., 2017). Animal and human studies had demonstrated that orally administered *Lactobacillus johnsonii* La1 reduces effects of ultra-violet radiation (UV) on immune suppression (Ouwehand et al., 2017).

Some probiotics contributes to increased antioxidants, exopolysaccharides, bioactive proteins, fermented lyases and enzymes activity in modulation of oxidative stress, protecting cells against induced damage by carcinogens and reactive oxygen species generated during body metabolism (Meng et al., 2018). Glutathione, glutathione reductase, s-transferases, glutathione peroxidases, super-peroxide dismutase and catalase are among beneficial probiotics antioxidant enzymes involved in the complex skin antiaging mechanism (Cinque et al., 2017). For instance, *Lactobacillus plantarum* K8 lyases administered orally in human resulted in improved forearm and face skin hydration (Ouwehand et al., 2017). Another skin protection enzyme, sphingomyelinase (SMase) produced by *Streptococcus thermophilus* delivered in exogenous probiotics formulations was reported to improve skin health and effective in treatment of dermatitis

and other skin ailments (Lew & Liong, 2013). Moreover, polyphenols and antioxidants from FV as prebiotics composition play roles in skin protection and antiaging mechanisms providing synergetic effect on probiotics (Kwon et al., 2019; Ouwehand et al., 2017).

6. Probiotics safety and regulation

Development and use of probiotics has no universal standard for safety evaluation and regulation and varies from country to country or regional wise (Kolaček et al., 2017; Pineiro & Stanton, 2007). Unlike commercial drugs, probiotics are mostly categorized as food or dietary supplements in the United State of America and Europe, natural health products in Canada, food for specific health use in Japan, thus, comply with significantly less stringent regulations (Kolaček et al., 2017). In China, the Chinese State Food and Drug Administration (SFDA) regulates and oversees functional food and nutraceuticals (Baldi & Arora, 2015; Foligné, Daniel, & Pot, 2013). Probiotics among functional food take a large market share in China influenced by traditional dietary culture and habits along with economic growth.

In the United States of America (U.S.A.), bacteria safe for human consumption are categorized as GRAS (generally recognized as safe) by the Food and Drug Administration (FDA) and such products are not subjected to close monitoring (Degnan, 2008). In Canada, Health Canada accepts 1×10^9 CFU per serving for nonspecific claims as probiotics, which might include the matrix of *Bifidobacterium* and *Lactobacillus* spp. in the formulated probiotics product (Hill et al., 2014).

The European Union, through the European Food Safety Authority (EFSA) proposed an introduction on the Qualified Presumption of Safety (QPS) that could be applicable to select groups of microorganisms for human direct consumption (Salveti & O'Toole, 2017). The QPS works on taxonomy, characterization, pathogenicity and end user information for conformity to qualify a probiotic. Also, EFSA is responsible for assessment of the probiotics health claim made on food that are submitted by different manufacturers (Salveti & O'Toole, 2017). The Italian ministry of health regulates the use of probiotics bacteria in food and set the allowable minimum number of viable cell in food per serving at 1×10^9 CFU per day (Hill et al., 2014).

In Japan, probiotics are regulated under Food for Specific Health Use (FOSHU), which permits labelling with health claims on food or ingredients that meet scientific evidence required for safety and efficacy (Amagase, 2008). The FOSHU products are approved by the Japanese Minister of Health, Labor and Welfare (Foligné et al., 2013). The claims are categorized as: special dietary uses, specific health application and food with nutrient function (Kumar et al., 2015). The health claims may cover aspects of cholesterol, triglycerides, blood pressure, blood sugar, bone minerals and dental health. Also, the assessment of novelty is based on the source and the traditional use of foods or food ingredients.

The FAO/WHO has recommended that the following information on the probiotic products should be labelled: genus, species, and strain designation, minimum viable number of each probiotics strain at the end of the shelf life, storage conditions and traceability information such as cooperate contact (Cremon et al., 2018). Although, regulatory standards on probiotics are not established on an international basis, there are no periodic screenings of the product's safety and quality.

While probiotics products are not strictly regulated, a review by Kolaček et al. (2017) presented some aspects of the quality assessment from the U.S.A., Europe, Asia, South Africa and Australia with concern on misinterpretation at the genus, species and strain level and mislabeling. Often, claimed number of viable cells per dose of many products are significantly lower than those on their labels (Chen et al., 2014, 2017). Among major concern are contamination and lower functional properties of probiotics which are influenced by processing, handling and food matrixes.

Some probiotics species might produce problematic effects such as transmission of antibiotics resistance gene to pathogens and production of biogenic amine (Zoumpopoulou et al., 2018). Therefore, the following information are recommended to be disclosed: isolation history or origin, taxonomic identification, absence of virulence, toxicity and chances of antibiotics resistance gene transfer to pathogenic bacteria. Notably, EFSA and GRS in 2007 and 2012, respectively introduced antimicrobial resistance as the safety concern on probiotics consumption due to horizontal gene transfer from beneficial to pathogenic bacteria in the GIT (de Melo Pereira et al., 2018).

7. Conclusions

Probiotics have shown remarkable health benefits to human in a way that their utilization may reduce the use of antibiotics for treatment of diseases such as traveler's diarrhea reducing the risks of antibiotics resistance and other health problems related to microbiota imbalances. Increase of consumer's demand for the non-dairy functional probiotics has promoted development of novel FV based probiotics product. Probiotics and their bacteriocin application to FV products as well as biocontrol use to plant pathogens and spoilage microbes provide a promising alternative to chemical additive to control food borne pathogens and spoilage microorganisms. Lastly, it is an urging need to establish international standards to regulate probiotics and prebiotics products and functional health claim on the label to ensure product effectiveness and safety.

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