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TRENDS IN TECHNOLOGICAL USE OF PROBIOTICS: A REVIEW

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ABSTRACT

Probiotics stimulate the growth of beneficial microorganisms to compete with the pathogenic microflora of the digestive system, and if consumed in certain quantities confer healthy effects on the host, being the lactic acid bacteria (LAB) referred as the main group of probiotics. The research of probiotic bacteria receives great interest related to the benefits of its consumption, it has approaches in areas such as the food industry, being considered as functional foods, which are already part of modern thinking based on prevention and/or alternatives to diminish the risk associated with chronic diseases. In human health, they stimulate the protective functions of the digestive system because they are biotherapeutic and bioprotective. As for animal production systems, they improve growth, increase the efficiency of digestion, and enrich the quality of production in the livestock sector. Probiotics have many applications, so it is important to know and study in new applications and benefits for consumers, producers, and animal species.

Keywords: probiotics, probiotic potential, food industry, human health, animal health.

INTRODUCTION

Probiotics are known as effective microorganisms in improving the intestinal flora to ensure health and well-being. The term "probiotics" originally refers to a group of living microorganisms that have significant influences on other cellular organisms and ensure well-being through the enhancement of microbial flora (Hill *et al.*, 2014; Salminen & Van Loveren, 2012). Bacterial genera such as *Lactobacillus* and *Bifidobacterium* are the most widely used, but the yeast *Saccharomyces cerevisiae*, in addition to some species of *Escherichia coli* and *Bacillus*, are also used as probiotics. However, other genera (*Pediococcus*, *Propionibacterium*, *Oenococcus*, *Bacillus*, *Faecalibacterium* and *Enterococcus*) are under study to verify their possible probiotic potential, assessing their growth and / or activity in the body (Miquel *et al.*, 2013; Park & Kim, 2014).

Since ancient times, it has been known of the beneficial effects of the consumption of probiotics due to the intake of fermented dairy products. A century ago, Eli Metchnikoff, Russian scientist and Nobel laureate in Physiology and Medicine in 1908, observed that the consumption of yogurt and fermented milk products in Bulgarian farmers, offered health benefits that enhance their longevity (Mackowiak, 2013). At present, the applications of probiotics are being directed to various areas, such as the enrichment of a great variety of foods, applications to treat some health problems and the improvement of animal health; starting from the isolation of new strains with probiotic potential.

Therefore, the objective of this document is to show the most recent scientific evidence of the use of probiotics, as well as the new applications that have emerged in the food industry, human health, feed, and animal health.

PROBIOTIC APPLICATIONS

Food industry

Many products of food industry have been incorporated with probiotics because can be considered as functional products. Cáceres & Gotteland (2010), mentioned that probiotics can be recognized as functional components that are used to give a beneficial property or added value to the food that contains it. The food products with probiotics are into the category of functional foods, since they provide benefits for the health of the consumer beyond the nutrients of the food (Hasler *et al.*, 2004). The feasibility of probiotics in food depends on technological factors such as resistance to low pH, acidity, tolerance to bile salts and different temperature ranges (Parvez *et al.*, 2006; Ren *et al.*, 2014), which should be monitored during processing and storage.

Lactic acid bacteria (LAB) are an important group of probiotic bacteria that have long been used in the manufacture of many fermented dairy products (Lourens-Hattingh & Viljoen, 2001). The *Lactobacillus* and *Bifidobacterium* strains are the genera commonly added to food, in the same way there are some microorganisms that do not belong to the LAB group, but they

have probiotic properties, such as *Enterococcus* spp., *Bacillus* spp., *Escherichia coli* strains and *Saccharomyces boulardii* (non-pathogenic yeast), which are commonly sold as food supplements or as additives (Kerry et al., 2018; Villanueva, 2015), some of which are shown in Table 1.

The use of different microorganisms for fermentation processes is a traditional biopreservation method for food manufacturing, which can be considered a simple, relatively inexpensive and valuable biotechnological tool to maintain or improve safety, properties sensory and the useful life of these products (Di-Cagno & Coda, 2014). *Streptococcus thermophilus* and *Lactobacillus bulgaricus* strains are specifically found in most dairy products and in the manufacture of yogurt; although some investigations have reformulated the product to include live strains of *Lactobacillus acidophilus* and species of *Bifidobacterium* (known as AB cultures), which increases the nutritional value (Lourens-Hattingh & Viljoen, 2001). In another study, Maurad & Meriem (2008), carried out the characterization of probiotic strains of *Lactobacillus plantarum* isolated from traditional butter made from camel milk, to incorporate these strains as starter cultures in the development of new products. On the other hand, LABs have been applied in fresh and matured cheeses in order to avoid the proliferation of pathogens such as *Listeria monocytogenes*, *Clostridium* and *Staphylococcus aureus* (Ozkaya, 2007), in addition to producing carbon dioxide for the formation of the eyes in some cheeses.

In the meat industry, LABs have been used for some years as starter cultures to

suppress the growth of pathogenic microorganisms such as *Staphylococcus aureus* and in a variety of fermented meat products because of they provide a more appropriate environment (without heat treatment) for the viability of microorganisms. The use of probiotic microorganisms in other types of meat products, such as cooked meat sausages and raw meat, remain scarce mainly due to the processing required for these products, such as heating, the use of additives (sodium chloride, nitrite sodium, among others) and conditions of consumption. Probiotics increase the quality of pork meat production, improving the water retention capacity and decreasing its hardness (Ceslovas et al., 2005). Rubio et al. (2014), isolated probiotic strains (*L. casei* / *paracasei* CTC1677, *L. casei* / *paracasei* CTC1678 and *L. rhamnosus* CTC1679) from infant feces, to test their ability to grow in a fermented sausage environment, which indicates their suitability as probiotic starter cultures in these types of products. Currently, the meat industry is focusing on the production of meat products with new potential benefits such as bioprotection for human health, the most widely used being the genus *Lactobacillus* and *Bifidobacterium*, however, its use in cooked products presents significant problems in terms of maintaining the viability during the processing and storage periods until the moment of consumption to provide health benefits to consumers (Pasqualin-Cavalheiro et al., 2015). In alcoholic beverages, LABs play a significant role in the production of wines, due to the malolactic fermentation, which is an important secondary reaction that occurs in some wines after alcoholic

fermentations by yeasts (Arribas & Polo, 2008; Jenn-Shou *et al.*, 2008). The feasibility of including probiotics in plant matrices for fruit and vegetable products has recently been investigated (Bernal *et al.*, 2017), using strains of *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus plantarum*, *Lactobacillus rhamnosus* and *Bifidobacterium lactis* (Shori, 2016). For bakery products, it has been proposed to add *Bacillus subtilis*, which is a sporulated bacterium that can survive thermal shocks, UV rays, adverse environments that present an acidic pH, low water activity and high sugar content; some studies have shown that its addition improves bread texture (Almada-Érix *et al.*, 2021; Setlow, 2006). Another topic of interest has been to search, identify and characterize a large number of bacteriocins, substances produced by LABs, which constitute a heterogeneous group of ribosomal synthesis peptides or proteins that present a great variety of physicochemical characteristics and antimicrobial action spectra against Gram positive bacteria. The mode of action of these peptides can be bactericidal or bacteriostatic (Cintas *et al.*, 2001). Recent studies have discovered regulatory links between competition and antimicrobial peptide production in multiple species of streptococci, which reveals a widely distributed strategy among streptococci to develop their adaptive ability through their bacteriocin activity (Wang & Dawid, 2018). The antimicrobial activity of bacteriocins has represented great benefits for the food industry due to their use as pure biological preservatives, which have replaced chemical preservatives (González-Martínez *et al.*, 2003). Its function as a preservative

substance is due to the production of bacteriocins that exert antibacterial action that contribute to the prevention of decomposition (Campos, 2002); such is the case of nisin, from *Lactococcus lactis* subsp. *lactis*, which has been studied for its biopreservative effect in fruits and vegetables. For example, it has shown effectiveness in controlling the bacterium *Listeria monocytogenes* in cut lettuce and broccoli (Bari *et al.*, 2005; Oliveira *et al.*, 2015). On the other hand, Bagde & Vigneshwara (2019), showed that the bacterium *Enterococcus faecium* isolated from *Vigna mungo* produced a bacteriocin that inhibits gram negative and positive bacteria and is heat stable (100 ° C for 30 min).

Human health

More than 1000 microbial species live in the human intestine, which mainly modify the internal environment of the host, since these probiotic microorganisms affect the intestinal ecosystem by stimulating the immune mechanisms of the mucosa (OMGE, 2008), improving the protective functions of the digestive system. So, they can play an important role in the health of the host as biotherapeutics and bioprotectors, preventing enteric and gastrointestinal infections. Studies have associated pathologies such as inflammatory bowel disease (Scaldaferri *et al.*, 2013), obesity (Jenzsch *et al.*, 2009), colon cancer and polyposis (Scanlan *et al.*, 2008), and atopic disorders (Penders *et al.*, 2007) with alterations in the gut microbiome. The effect of probiotics in the prevention and treatment of dysentery caused by *Clostridium difficile* has been extensively studied, since the use of probiotics restores the microflora,

allowing to alleviate the signs and symptoms of infection. The administration of *Lactobacillus rhamnosus* has shown evidence of a positive effect in the prevention of primary infections caused by *C. difficile* (Goldstein et al., 2017). The administration of probiotics to treat gastrointestinal inflammatory disorders has been proposed by many research groups, since they are capable of occupying niches that compete with pathogens in the gastrointestinal tract (GIT) (Hugh, 2016).

Inflammatory bowel diseases are chronic diseases that affect the GIT, the main forms of which are represented by Crohn's disease (CD) and ulcerative colitis (UC) (Cosnes et al., 2011; Scaldaferri et al., 2013). In 2005, Bousvaros et al. conducted a randomized, double-blind, placebo-controlled trial to establish the efficacy of *Lactobacillus* GG as an adjunct to standard therapy in maintaining remission in 75 patients with Crohn's disease. Similarly, Sood et al. (2009), conducted a multicenter, randomized, double-blind, placebo-controlled study of a high-potency probiotic to verify the use of a probiotic preparation (VSL # 3: *Bifidobacterium breve*, *B. longum*, *B. infantis*, *Lactobacillus acidophilus*, *L. plantarum*, *L. paracasei*, *L. bulgaricus* and *Streptococcus thermophilus*), inducing remission in patients with moderately active to mild ulcerative colitis.

According to Qin et al. (2010), the human intestinal tract has a diverse and complex microbiota that interferes with human health, since certain elements that make up the intestinal microflora can produce carcinogenic substances such as nitroreductase, azoreductase and β -glucuronidase. However, there is also

data that indicates that probiotic microorganisms have the ability to exert an anticarcinogenic effect in humans, which relates them to a potential for the prevention and treatment of cancer, one of the main diseases responsible for morbidity and death in the world population (Stewart & Wild, 2014). Among neoplasms, colorectal cancer is one of the leading causes of death in men and women, and there is evidence that it may be due to an imbalance of the intestinal microflora (Uccello et al., 2012). LeBlanc et al. (2010), demonstrated the cytotoxic and antiproliferation activity *in vitro* against the colorectal cancer cell lines Caco-2 and HRT-18, of two probiotic strains of *Lactobacillus acidophilus* LA102 and *Lactobacillus casei* LC232. *In vitro* studies have shown that the probiotic strains *Lactobacillus fermentum* NCIMB-5221 and 8829 have great potential in suppressing colorectal cancer cells and promote normal epithelial cell growth, through the production of short chain fatty acids (SCFA) (Kich et al., 2016). Mojibi et al. (2019), tested an extraction of exopolysaccharide from *L. paracasei* and *L. brevis* on HT-29 cancer cells, where they observed that they prevented the proliferation of these cells, thereby demonstrating that probiotics have the ability to stimulate the immune system towards the destruction of carcinogenic enzymes. Currently, progress in the medical field is mainly related to the development of genetically modified probiotic bacteria strains, which can produce and discharge immunomodulators such as interleukin-10, trefoil factors (compact proteins) co-expressed with mucins in the GIT or lipoteichoic acid (a main component of the cell wall of Gram-positive bacteria),

which can stimulate the host's immune system, in the restoration of protective commensal bacteria (Shahverdi, 2016; Patel *et al.*, 2015). In urogenital health, it is known that the dominant microflora in a healthy human vagina is made up of a variety of *Lactobacillus* species, which play an essential role in protecting against genital infections, so a microbial imbalance can cause a quantitative change and qualitative of the microflora, dominated by anaerobic bacteria such as *Gardnerella vaginalis*, *Bacteroides*, *Prevotella* and *Mobiluncus* species (Petricevic *et al.*, 2014). Lactobacilli have been shown to produce biosurfactants and collagen-binding proteins that inhibit the adhesion of the pathogen to cells, making it less receptive to pathogens (Waigankar & Patel, 2011). Currently, the only clinically proven strains that have a beneficial effect are *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* (Reid & Bruce, 2006); when administered intravaginally once a week, or twice a day orally, they reduce recurrences of urinary tract infection (UTI) and restore a normal vaginal flora dominated by lactobacilli, promoting the colonization of beneficial microbiota by supporting the general health of the vagina (Vujic *et al.*, 2013).

Other studies are investigating the ability of orally administered lactobacilli to colonize the vagina and / or reduce vaginal colonization, as well as *Candida* infection. Reid *et al.* (2003), administered *Lactobacillus rhamnosus* GR-1 and *Lactobacillus fermentum* RC-14 daily, orally to a group of women, while another group received placebo. The cultures of the vaginal smears studied showed a significant increase in vaginal lactobacilli four weeks after

administration, and a significant reduction in yeast in the women treated with lactobacilli, compared to those treated with placebo. Although properties required by probiotics have been identified, evidence for *in vivo* expression is scant.

Feed and animal health

The improvement of animal nutrition and health through the development and use of biotechnological products have been of great interest. Currently, probiotics or probiotic combinations are being studied to improve growth, increase the efficiency of digestion and enrich the quality of production in the livestock sector (swine, poultry, aquaculture, among others). Probiotic mixtures are made up of two or more of these microorganisms, isolated from the digestive tracts of healthy animals (Brizuela, 2011). Within animal feeding, it is worth mentioning the production of silage, which is a conservation method for most of the forages used in livestock feeding, where the native LAB present in the forage or added as a starter culture, convert sugars soluble in organic acids as a consequence of the decrease in pH, managing to inhibit the growth of bacteria and generating satisfactory sensory and nutritional characteristics for feeding ruminants (Ramírez *et al.*, 2011). Specific probiotic strains and their combinations may be useful in canine and feline nutrition, therapy, and care. Probiotic supplements have been successful in preventing and treating acute gastroenteritis, treating infections, and preventing allergies in companion animals. New challenges for probiotic applications include fighting diseases

such as obesity and overweight, urogenital tract infections, *Helicobacter* gastritis, and parasitic infections. In clinical trials, probiotics have been reported to enhance the growth of many domestic animals, including cows, newborn calves, piglets, and broilers (Grzeńskowiak *et al.*, 2015; Scharek *et al.*, 2007; Simon *et al.*, 2005). Alexopoulos *et al.* (2004), evaluated the efficacy of BioPlus2B, a probiotic that contains spores of *Bacillus licheniformis* and *B. subtilis*, on the health status of female pigs and their litters, observing a higher concentration of proteins and fat in milk in the lactation period and better general health of the piglets, since they presented less frequency of periods of diarrhea, weight gain and lower mortality rate. In addition, Pérez *et al.* (2012) and Rodríguez *et al.* (2015), conducted studies evaluating the effect of probiotic mixtures (*L. salivarius* C65 and *B. subtilis* E44), included in poultry diets; the results obtained showed an improvement in health and in the maintenance of the beneficial microflora, greater feed consumption, an increase in egg production for each kilogram of feed, improvements in the live weight of the birds and a decrease in mortality. The positive effects of probiotics are not only presented at the GIT level but were also reflected in results such as live weight gain and the improvement of the relationship between the food consumed by the animal and the weight it gains (Gil & Gil-Turnes, 2005). In aquaculture, probiotics are used in food as one of the strategies to reduce the consumption of antibiotics in intensive systems and mitigate impacts on the environment; in addition to being used to increase the immune response, growth, and survival in marine species, including shrimp and

mollusks. Waché *et al.* (2006), suggest that the use of probiotics can optimize the growth of fish cultures due to the beneficial effects that occur in digestive processes and to improve stress tolerance, since in fish, the intestine is the main organ for digestion and absorption of nutrients, as well as influence on the water and electrolyte balance, endocrine regulation of digestion, metabolites, and immunity (Ramírez *et al.*, 2016). Most probiotics used in aquaculture belong to the *Lactobacillus* and *Bacillus* genera, although other organisms such as photosynthetic bacteria and yeasts are also used (Gatlin *et al.*, 2006; Vázquez *et al.*, 2005; Yu *et al.*, 2008). Ramírez *et al.* (2016) evaluated the effect of microencapsulated probiotics included in the diet of fingerlings of red tilapia (*Oreochromis* sp.). The results showed that the addition of strains of *Bacillus megaterium*, *B. polymyxa* and *L. delbueckii*, caused a greater gain in weight, size, faster growth rate and in the use of the relationship between the food consumed and weight—what win. Enriching the diets for fish and shrimp with probiotics reflects benefits by improving the nutritional value of the diet, aids digestion with enzymatic input, and promotes growth factors (Zhou *et al.*, 2010).

The use of probiotic strains, native to fish, confers greater benefits than can be provided by commercial probiotics isolated from humans or other mammals. Jiménez-Rojas *et al.* (2012), reported that in angelfish (*Pterophyllum scalare*), an artificial diet with worms (*Enchytraeus buchholzi*) and probiotics provides greater weight gain and higher growth rates. Among the marine animals of commercial interest are mollusks, the

“mule leg” or pustular burlap (*Anadara tuberculosa*), belonging to the Arcidae family, an animal that lives among the root mangroves, a stressful environment. The difficult conditions in its habitat suggest that it encourages the development of its intestinal microbiota, which favors its survival in the mangrove swamp. Ortiz *et al.* (2015), isolated bacteria with probiotic potential from *Anadara tuberculosa* by conducting *in vitro* and *in vivo* tests in white shrimp (*Litopenaeus vannamei*) culture. The strains obtained had a beneficial effect on growth and immune response, for the control of bacterial and viral diseases. The improper and intense use of antibiotics to treat infections has led to the appearance and development of multi-resistant bacteria in different animal species (Defoirdt *et al.*, 2007; Santos & Ramos, 2018); Therefore, the isolation of

strains with probiotic potential from several healthy animals to be studied and used in the same species has become essential to achieve beneficial effects and thus combat infections and pathologies. Given this, Laurencio-Silva *et al.* (2017), evaluated the *in vitro* probiotic potential of 28 strains of *Lactobacillus* spp., isolated from the vagina of dairy cows for use as probiotics in females with urogenital disorders. The results concluded that the selected strains are candidates for probiotics, for their use in the prevention of said pathology in bovine females. In the selection of strains with a probiotic effect on the urogenital tract, it is important to verify the resistance capacity to these extreme conditions to consider that they can grow and colonizing the vaginal mucosa (Sánchez *et al.*, 2015; Zárate *et al.*, 2005).

Table 1. Probiotics and its commercial sources.

Type	Comercial Product
<i>Lactobacillus acidophilus</i> NCFM	Additive
<i>Bifidobacterium lactis</i> HN019 (DR10)	Additive
<i>Lactobacillus rhamnosus</i> HN001 (DR20)	Additive
<i>Saccharomyces cerevisiae boulardii</i>	Supplement (Florastor)
<i>Bifidobacterium infantis</i> 35,264	Supplement (Align)
<i>Lactobacillus fermentum</i> VRI003 (PCC)	Additive and supplement
<i>Lactobacillus rhamnosus</i> R0011	Additive and supplement
<i>Lactobacillus acidophilus</i> R0052	Medicine
<i>Lactobacillus acidophilus</i> LA5	Additive
<i>Lactobacillus paracasei</i> CRL 431	Additive and supplement
<i>Bifidobacterium lactis</i> Bb-12	Additive
<i>Lactobacillus casei</i> strain Shirota	Product (Yakult)
<i>Bifidobacterium breve</i> strain Yakult	Product (Yakult)
<i>Bifidobacterium animalis</i> DN173 010 (“ <i>Bifidobacterium regularis</i> ”)	Product (Activia)
<i>Lactobacillus casei</i> DN-114 001 (“ <i>L. casei</i> Immunitas”)	Supplement (DanActive)
<i>Lactobacillus reuteri</i> RC-14	Supplement (Fem-Dophilus)
<i>Lactobacillus rhamnosus</i> GR-1	Supplement
<i>Lactobacillus johnsonii</i> Lj-1	Supplement (LC1)
<i>Lactobacillus plantarum</i> 299 v	Supplement (Good Belly)
<i>Lactobacillus rhamnosus</i> 271	Additive
<i>Lactobacillus rhamnosus</i> GG (“LGG”)	Supplement (Culturelle, Danimals)
<i>Lactococcus lactis</i> L1A	Supplement

<i>Lactobacillus salivarius</i> UCC118	Supplement
<i>Bifidobacterium longum</i> BB536	Additive
<i>Bacillus coagulans</i> BC30	Supplement (Sustenex)
<i>Streptococcus oralis</i> KJ3	Medicine (ProBiora3)
<i>Streptococcus uberis</i> KJ2	Supplement (EvoraPlus)
<i>Streptococcus rattus</i> JH145	Supplement
<i>Lactobacillus rhamnosus</i> PBO1	Supplement (EcoVag)
<i>Lactobacillus gasseri</i> EB01	Supplement

Font: Adapted from Kerry *et al.*, 2018.

CONCLUSION

The use of probiotic systems for the creation or modification of products or processes applicable to the food industry, human health and animal production systems has shown favorable results in a significant number of studies. The development of foods that have a beneficial effect on health beyond basic nutrition is of great interest, and the food industry is trying to meet these demands by developing products with benefits for health. Several studies have shown the importance of probiotics in the treatment of gastrointestinal and urinary infections, and on some cancers in *in vitro* studies. Also, the development of various species to produce functional food products increases the benefits of their application of probiotics in food and health; however, it is still necessary to confirm the efficacy and safety of its large-scale implementation in *in vivo* systems and its possible impact on the environment.

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