Review

Revista Actualidades Biológicas

Actual. Biol. 45(119): 1-12, 2023 | DOI:10.17533/udea.acbi/v45n119a05

Characteristics of microorganisms used as probiotics and new probiotics

Características de microorganismos utilizados como probióticos tradicionales y nuevos probióticos

Claudia Karina Pacheco-Martínez[1], Gerardo Saucedo-Castañeda[1], Luis Víctor Rodríguez-Durán[2], María de Lourdes Pérez-Chabela[1*]

Abstract

Probiotics are live microorganisms that when properly administered confer a health benefit to the host. Lactic acid bacteria are among the main microorganisms recognized as probiotics. Not all lactic bacteria are considered probiotics as they must meet certain characteristics such as growth at a pH lower than 4.0, are able to exert control on pathogenic bacteria, can survive in the gastrointestinal tract, have tolerance to bile salts, are able to adhere to intestinal mucus and epithelial cells, have the ability to co-aggregate and self-aggregate, etc. Bacteria that possess these characteristics are called traditional probiotics. However, over the years other microorganisms with probiotic potential have been studied. Among the differences between traditional probiotics and new probiotics is their origin, as new probiotics are always isolated from the human gastrointestinal tract, which makes them difficult to cultivate because they are sensitive to oxygen. In contrast, traditional probiotics can be isolated from the gastrointestinal tract, but the main sources are from foods, fermented or not. An important characteristic of new probiotics is that benefits in the treatment of specific diseases are attributed to them. In this review, the main characteristics of traditional probiotics and new probiotics are reviewed.

Keywords: lactic acid bacteria, traditional probiotics, new probiotics

Resumen

Los probióticos son microorganismos vivos que al administrarse de forma adecuada confieren un beneficio a la salud del hospedero. Entre los principales microorganismos reconocidos como probióticos se encuentran las bacterias lácticas. No todas las bacterias lácticas son consideradas probióticas, estas deben reunir ciertas características como crecer a un pH menor a 4,0, ejercer control sobre bacterias patógenas, sobrevivir en el tracto gastrointestinal, tener tolerancia a sales biliares, presentar capacidad de adhesión al mucus intestinal y a células epiteliales, tener capacidad de co-agregarse y auto-agregarse, principalmente. Las bacterias que poseen estas características se les denomina probióticos tradicionales. Sin embargo, desde hace algunos años se han estudiado otros microorganismos con potencial probiótico. Entre las diferencias de los probióticos tradicionales y los nuevos probióticos se encuentra el origen de los mismos. Los nuevos probióticos tienen siempre que ser aislados del tracto gastrointestinal de seres humanos, lo que dificulta su cultivo pues son sensibles al oxígeno. En contraste, los probióticos tradicionales pueden ser aislados del tracto gastrointestinal, pero las fuentes principales son los alimentos, fermentados o no.

[1.] Departamento de Biotecnología, Universidad Autónoma Metropolitana Iztapalapa, México.

[2.] Unidad Académica Multidisciplinaria, Universidad Autónoma de Tamaulipas, México.

* Corresponding author: lpch@xanum.uam.mx

Received: September 2022; accepted: March 2023.

Una característica importante de los nuevos probióticos es que se les atribuyen beneficios en el tratamiento de enfermedades específicas. En esta revisión se muestran las principales características de los probióticos tradicionales y los nuevos probióticos

Palabras clave: bacterias lácticas, probióticos tradicionales, probióticos nuevos

INTRODUCTION

Traditional probiotics refer to a probiotic having viable and active cells. However, it is not enough that the cells are viable, they must exert health benefits to the consumer by lowering gut pH, producing vitamins, enzymes and antimicrobial compounds, balancing and rebuilding the intestinal microbiota after infections in the digestive tract, reducing the serum cholesterol level, regulating the immune system, exhibiting antioxidant activity, reducing poor lactose adsorption, and producing substances such as bacteriocins, hydrogen peroxide, butyric acid, lactic acid and acetic acid to mention a few (Zendeboodi et al., 2020). The term true probiotic is synonymous with traditional probiotic, considering all these properties (Guidelines for the Evaluation of Probiotics in Food, 2002). Castañeda-Guillot (2021) defined and compared the characteristics of traditional probiotics and new probiotics, describing mainly the use of traditional probiotics and their metabolites as binders, flavorings and preservatives for the food industry, which can be isolated from different substrates from the gastrointestinal tract of different animals from different ecosystems. Unlike traditional probiotics, new probiotics are characterized because their origin is primarily from the human gastrointestinal tract, and their use is mostly focused on the treatment of specific diseases, acting as biotherapeutics.

The objective of this review is to know the main characteristics of the microorganisms used as traditional probiotics and new probiotics.

Traditional probiotics

The word probiotic derives from the Greek pro (in favor) and biotic (life), and this term has undergone various changes over the years. The International Scientific Association for Probiotics and Prebiotics (ISAPP) defined them as "live microorganisms and their metabolites that when administered in an adequate manner and in adequate amounts provide a health benefit to the host" (Isolauri et al., 2001; Prats Capote, 2007). Finally, two more elements were added which state that probiotics have the capacity to stimulate bacterial growth or development, and to regulate their metabolic activity (Prieto, 2010). Among the main microorganisms used as probiotics are lactic acid bacteria and bifidobacteria, but some yeasts such as *Sacharomyces boulardii* have also been reported (Carnicé, 2006; Gómez, 2019). In this review we will focus on lactic acid bacteria (LAB) as probiotics.

Lactic acid bacteria

LAB have been used in food preservation for many years because they produce different compounds as a result of their metabolism, such as organic acids, hydrogen peroxide and bacteriocins. Likewise, their role as probiotics has also been studied (Agudelo et al., 2015; Gorbeña and Sáenz, 2008).

LAB are a diverse group of Gram-positive microorganisms (cocci or bacilli), with a length (depending on the genus) of 0.5 - 0.8 µm (Carr et al., 2002; Ibrahim and Raman, 2021), that are catalase negative (although in some cases some pseudo catalase can be found), and oxidase and benzidine negative (Vázquez et al., 2009). They are facultative anaerobes, do not form spores, lack cytochromes, are not toxigenic or pathogenic, and are tolerant to acidity, although some can grow at a pH of 9.6. They also grow at different temperatures and can be classified as mesophilic (optimum growth temperature of 20 to 25 °C) and thermophilic (temperature of 40 to 45 °C) (Parra Huertas, 2010). Some have the ability to be aero-tolerant and grow in the presence of oxygen (McKee and McKee, 2016). LAB synthesize ATP through carbohydrate fermentation, and are divided into two large homofermentative and heterofermentative groups based on their biochemical characteristics. Among the metabolites produced by LAB are lactic acid, as well as other acids in smaller proportions such as propionic, acetic, citric, succinic, and formic acids, in addition to hydrogen peroxide, diacetyl, acetaldehyde, reuterin, and bacteriocins (Heredia et al., 2017). Some LAB produces carboxylic acids (Kaiting, 2021).

Criteria for considering a probiotic strain

Among the main criteria for considering a LAB strain to be probiotic are growth at a pH below 4.0, exert control over pathogenic bacteria, survive in the gastrointestinal tract, tolerate bile salts, an ability to adhere to intestinal mucus and epithelial cells, and display co-aggregation and self-aggregation (Ramírez-Chavarín et al., 2013), in addition to not having antibiotic resistance genes, immunostimulation without proinflammatory effect, displaying resistance to phages, and having antimutagenic and anticarcinogenic properties (Rondon et al., 2015).

Beneficial effect of probiotics

Probiotics are responsible for mediating a wide variety of health problems, as they are involved in regulating intestinal homeostasis, suppressing pro-carcinogenic enzymatic activities, interfering with the ability of pathogenic bacteria to infect the intestinal mucosa, improving nutrient bioavailability, reducing symptoms of lactose intolerance, positively influencing the urogenital flora, reducing infections in the gastrointestinal tract, and improving the regulation of intestinal motility (Cunningham et al., 2021; Veiga et al., 2020). Probiotics establish competition for both nutrients and for adherence sites of digestive tract cells with other (pathogenic) bacteria and stimulate the local and systemic immune system by activating macrophages and elevating immunoglobulin concentrations (Prats Capote, 2007).

Mechanisms of action involved in probiotics

The mechanisms of action that give probiotics the ability to provide these benefits to humans are still largely unknown, but may involve modification of intestinal pH (Rondon et al., 2015), antagonism of pathogens through the production of antimicrobial compounds, competition for binding sites and receptors, competition for available nutrients and growth factors by stimulating immunomodulatory cells, increased erythrocyte regeneration, and production of short-chain fatty acids and regulation of gastrointestinal disorder (Cunningham et al., 2021; figure 1).

Patents on traditional probiotics

There are several patents on the use of traditional probiotics (table 1).

New Probiotics

New probiotics are also known as biotherapeutic products that contain live microorganisms, such as bacteria and yeast, and are applicable to the prevention, treatment or cure of human diseases. New probiotics are not considered vaccines, as they are microorganisms isolated mainly from the human intestinal microbiota. To be considered as new probiotics, certain characteristics must be met that are more stringent than those of a traditional probiotic, as they represent a part of the human intestinal microbiome that can be cultivated outside the intestine and offer physiological functions that are not obtained directly by bifidobacteria or *lactobacillus*, such as the production of butyrate, propionate and other bioactive compounds (Kirmiz et al., 2020).

New probiotics can target specific health problems and may have beneficial effects in the treatment of diseases such as hypercholesterolemia, hypertension, non-alcoholic fatty liver disease, diabetes, and obesity. They produce metabolites beneficial to host health such as short-chain fatty acids, acetate, propionate and succinate. The effects of some novel probiotics and some diseases can be treated by such an approach (figure 2); key species provide protection against pathogenic bacteria (Kumari et al., 2021).

Characteristics of new probiotics

For a probiotic to be considered new, it must meet certain characteristics, which are listed in table 2.

Classification of new probiotics

Among the new probiotics we can mention immunobiotics, psychobiotics, oncobiotics, pharmabiotics, paraprobiotics, postbiotics, probioceuticals and pseudoprobiotics (Martin and Langella, 2019). The characteristics of each of these are described below.

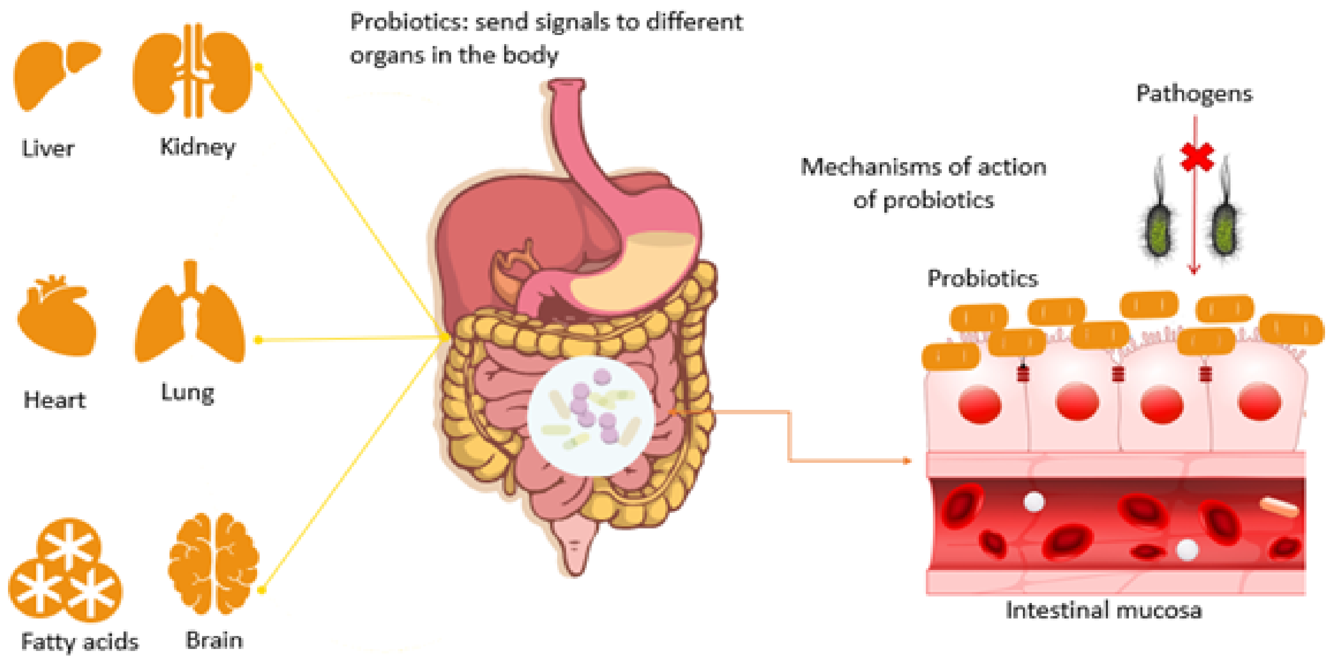

Figure 1. Mechanism of action of traditional probiotics (Adapted from Plaza-Diaz et al., 2019).

Table 1. Some patents on the use of traditional probiotics (Boletín Tecnológico: Alimentos funcionales con probióticos, bancos de patentes SIC, 2014)

Patent Number	Year	Title	Applicants
EP 2878204	2019	Pectin-containing sour milk drink and method for its production	Nakano Masatoshi; Nihei Daichi; Kobayashi Yukiko; Rolin Claus; Ushiyama Soko y Mamiya Hiroyuki
MAT-MX-2001688	2018	Enterogermina	Sanofi-Aventis de México S.A. de C.V.
EP 1884566	2018	Fermented substance with lactic acid bacteria and fermented dairy food product containing it	Ogasawara Nobuhiroc; Ishii Mayumic; Yoshikawa Masakic; Kudo Tatsuyukic; Akahoshi Ryoishic; Matsui Akihisac; Mizusawa, Susumuc
EP 2548948	2018	New lactobacillus classified as *Lactobacillus plantarum* and its use	Iino Tohru; Masuoka Norie; Ishikawa Fumiyasu; Yoshimura Koichi y Hayashida Eiji
EP 2471544	2016	*Lactobacillus casei* for reducing the risk of developing cancer	Toi Masakazu y Ohashi Yasuo
ES 2255730	2006	Topical use of probiotic bacillus spores to prevent or control microbial infections	Ganeden Biotech Inc
ES2243697	2005	Combination of probiotics	Mayra-Makinen Annika; Suomalainen Tarja; Vaarala Outi
MXPA01007144	2002	Use of *Lactobacillus salivarius*	Collins John Kevin; Entpr Irlanda haciendo negocio
ES2164299	2002	Pet food containing probiotics	Cavadini Christof; Ballevre Olivier; Gaier Walter; Nestle
ES2176338	2002	Probiotic Compositions	Brown Ian; Mcnaught Kenneth J; Ganly Robert N; Conway Patricia Lynne; Evans Anthony John; Topping David Lloyd

Immunobiotics

Defined as probiotic microorganisms with favorable immunological action for the organism. Immunobiotics increase the humoral immune response and promote better functioning of the intestinal barrier, stimulate resistance to pathogenic organisms and decrease the hypersensitivity

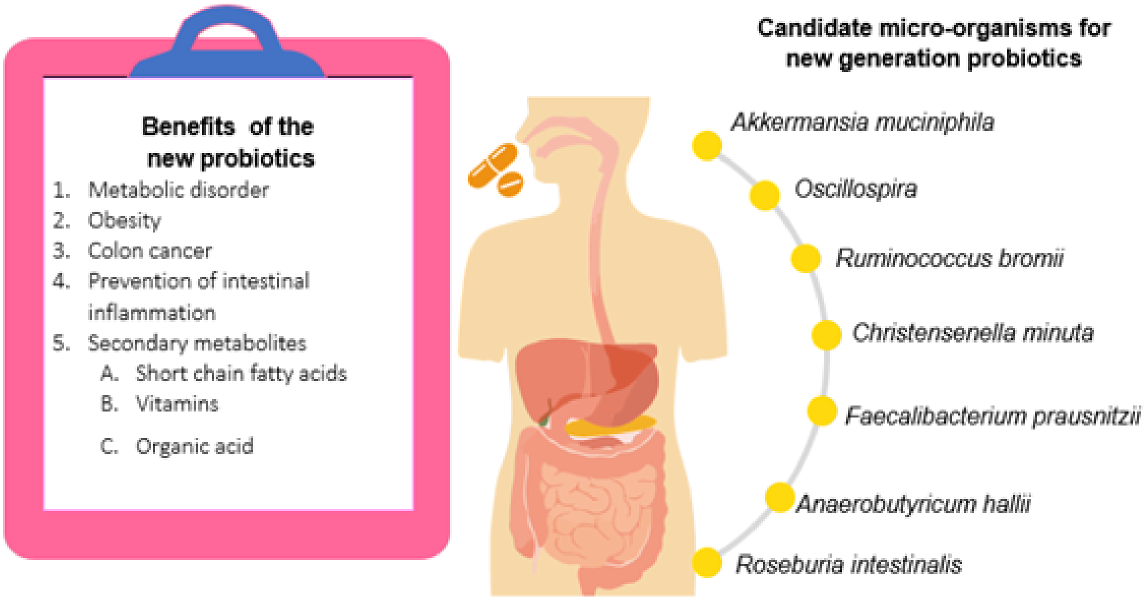

Figure 2. Beneficial effect of new probiotics (adapted from Kumari et al., 2021).

Table 2. Comparative table between traditional and new probiotics (Modified from Castañeda-Guillot, 2019; Castañeda-Guillot, 2021; Martin and Langella, 2019; Tzu-Lung et al., 2019)

Traditional probiotics	New probiotics
Isolated from variable sources, from human intestine and fermented foods, to plants and other ecosystems.	Isolated mainly from the indigenous human intestinal microbiome.
Used for different processes, in medical treatments, and in the production of fermented and enriched foods.	Focused on the treatment of specific and systemic diseases, regulation of inflammation, metabolic diseases, obesity, help in the production of vitamins of the host, increase the immune system, a single strain can help in different diseases.
Used as additives, binders, flavorings, food preservatives, starter cultures for fermented products, and as treatments for some specific diseases.	Development of specific drugs, production of secondary metabolites such as vitamins. They are divided into immunobiotics, psychobiotics, oncobiotics and pharmabiotics. Functional components such as paraprobiotics, postbiotics, probioceuticals or probiotaceuticals are in development.

reaction (Aviña et al., 2006). The activity on non-specific immune modulation allows immunobiotics to increase the host immune response and facilitate the elimination of pathogenic germs in the intestine by releasing proinflammatory cytokines such as Tumor Necrosis Factor-alpha (TNF-α). It has been reported that immunobiotics can stimulate macrophages and increase phagocytosis by early activation of the inflammatory response prior to antibody production (Isolauri et al., 2001; Molina et al., 2016).

Psychobiotics

Defined as a special class of probiotics that when ingested exert mental health benefits through an interaction with the gut microbiota, stimulating the production of short-chain fatty acids, enteroendocrine hormones, anti-inflammatory cytokines and neurotransmitters such as gamma amino butyric acid, serotonin, acetylcholine, catecholamines and norepinephrine, which helps regulate mood, cognitive, memory and learning functions (Sharma and Shukla, 2016; Zendeboodi et al., 2020). An ability to modulate the expression of neurochemical receptors such as endocannabinoids are also attributed to psychobiotics. Table 3 lists the most commonly used psychobiotics in neurological conditions.

Oncobiotics

Oncobiotics are probiotics that enhance the response to cancer immunotherapy, helping the immune system to eliminate tumor cells through cytotoxicity stimulated by biological agents aimed at preventing apoptosis or lymphocyte inhibition (Zitvogel et al., 2016). There are studies that suggest that oncobiotic probiotics play an important role in the immune response after chemotherapy and radiotherapy treatment, thus helping the host to avoid future relapses (Gopalakrishnan et al., 2018).

Pharmabiotics

Pharmabiotics are probiotics that can function as pharmacological active ingredients with preventive and curative potential. Pharmabiotics are used for the adjunctive treatment of various diseases such as acute diarrhea and irritable bowel syndrome. Studies have determined that specific pharmabiotics can improve many lower gastrointestinal tract symptoms in adults (Belkaid and Hand, 2014; Navarro et al., 2021).

Paraprobiotics or phantom probiotics

Taverniti and Guglielmetti (2011) described paraprobiotics as inactive, dead, and non-viable cells, or cell fractions, from intact or broken probiotics that provide health benefits to the recipient. Other authors have shown that cells from probiotics, even when dead or inactivated, continue to have a beneficial effect on the host, such as decreasing the inflammatory response, as well as preventing the adhesion of pathogenic bacteria (Zendeboodi et al., 2020). They are also attributed with attenuation of colitis, stimulation of the intestinal immune system, anti-adhesion capacity against several pathogens in CaCo-2 experimental models (Zhang et al., 2005; Nataraj et al., 2020). Some paraprobiotics produce compounds such as teichoic acid, peptidoglycan-derived muropeptides, exopolysaccharides and biosurfactants bound to their cell wall (Nataraj et al., 2020).

Postbiotics

Defined as a complex mixture of molecules that are part of the products or by-products derived from probiotic metabolism and must be cell-free (Aguilar-Toalá et al., 2018; Salminen et al., 2021). Postbiotic molecules have a known chemical formula, prolonged storage stability, as well as an ability to trigger various treatment mechanisms for diseases such as inflammation, adhesion of pathogens to the gastrointestinal tract, obesity, coronary artery disease, cancer, and oxidative stress (Aguilar-Toalá et al., 2018). Some examples of postbiotic molecules are enzymes, peptides, proteins, exopolysaccharides, organic acids, serine protease inhibitors (serpin), short-chain fatty acids, and cell wall components such as teichoic acid, peptidoglycan, hydrogen peroxide, reuterin, diacetyl, bacteriocins, phenol, amino acids, benzoic acid, phenylactic acid, and vitamins (Jastrząb et al., 2021). There is scientific evidence attributing different antimicrobial, antioxidant and immunomodulatory functions to postbiotics, properties that could positively affect the homeostasis of the gut microbiota, as well as signaling pathways and metabolic pathways of the host, thus having effect on specific physiological, immunological, neuronal, hormonal, biological, regulatory and metabolic reactions (Sharma and Shukla, 2016; Salminen et al., 2021).

Table 3. Most commonly used psychobiotics in neurological conditions (Adapted Misra and Mohanty, 2019; Nataraj et al., 2020)

Neurological condition	Psychobiotic strains
Anxiety	*Lactobacillus fermentum* NS9, *L. casei Shirota*, *L. rhamnosus* JB-1, *L. helveticus* ROO52, *B. breve* 1205, *B. infantis*, *B. longum* 1714, *B. longum* NCC3001, *B. longum* R0175
Depression	*L. acidophilus*, *L. acidophilus* W37, *L. brevis* W63, *L. casei*, *L. casei Shirota*, *L. casei* W56, *L. gasseri* OLL2809, *L. helveticus* NS8, *L. lactis* S19, *L. lactis* W58, *B. infantis*, *B. bifidum*, *B. bifidum* S23, *B. lactis* W52, *B. longum* R0175
Stress	*L. casei Shirota*, *L. helveticus*, *L. helveticus* R0052, *L. plantarum* PS128, *L. rhamnosus*, *B. infantis*, *B. longum* R0175

Probioceuticals

Probioceuticals are biologically active substances derived from the metabolism of probiotics that are attributed with health benefits, as well as the treatment and prevention of diseases. An example of probioceutical compounds are exopolysaccharides which are long-chain polymers of sugars with antioxidant and immunomodulatory properties (Kumar et al., 2020; Adebayo et al., 2018).

Pseudoprobiotics

Pseudoprobiotics are viable, but not active, probiotic cells that were inactivated by environmental factors such as high or low temperatures, extreme pH, lack of nutrients, low water activity, so they have low or no growth rates. Based on these characteristics they cannot be classified as traditional or true probiotics since they need to be viable and active cells. The advantages that these pseudoprobiotics have is that they can function in an inactive manner as paraprobiotics or be activated and used as traditional probiotics, with the advantages that both classifications give them (Zendeboodi et al., 2020; Blinkova et al., 2014).

Candidate microorganisms for new probiotics

Among the species that have been reported as new probiotics are *Akkermansia miciniphila* (Castañeda-Guillot, 2019), *Ruminococcus bromii* (Kumari et al., 2021), *Roseburia intestinalis* (Kasahara et al., 2018), *Anaerobutyricum hallii* (Kumari et al., 2021), *Faecalibacterium prausnitzii* (Castañeda-Guillot, 2019; Kumari et al., 2021), *Oscillospira* (Yang et al., 2021) and *Christensenella minuta* (Castañeda-Guillot, 2019). The main characteristic of these microorganisms is that they are isolated from the human gastrointestinal tract. Therefore, they must be resistant to a low pH in the intestinal tract, have no resistance to antibiotics and exercise pathogen control, although they may be sensitive to oxygen.

Patents for new probiotics

Table 4 shows some of the patents for new probiotics.

Studies on probiotics

Studies using traditional probiotics

Several authors have studied the role of probiotic LAB. Ramírez-Chavarín et al. (2013) evaluated the probiotic potential of ten thermotolerant LAB strains, which were studied for tolerance to low pH and bile salts, co-aggregation and self-aggregation, adhesion to epithelial cells, concluding that the thermotolerant LAB studied are promising for their probiotic potential. Sanchez and Tromps (2014) characterized 72 LAB strains by growth at different pH, temperature and tolerance to high sodium chloride concentrations, selecting 14 strains as probiotic candidates which were tested for hydrophobicity, self-aggregation and microbial antagonism. The results showed that strains such as *Lactobacillus* spp., *Leuconostoc* spp., *Lactococcus* spp., *Streptococcus* spp. and *Pediococcus* spp. have microbial antagonism to *Bacillus cereus* and *Staphylococcus aureus* at an inhibition rate of 63.6%, to *Salmonella typhimurium* at 75.7% and to *Pseudomonas aeruginosa* at 72.7%. Jurado and Fajardo (2017) evaluated the susceptibility of two strains of lactic acid bacteria to different antibiotics, the inhibition effect of *Lactobacillus gasseri* and its supernatant on *Staphylococcus epidermidis*, the growth of the lactic acid strain at different pH and different temperatures, resistance to bile salts and bovine bile, established fermentation kinetics and determined the count of viable microorganisms for each condition. They concluded that both strains have resistance to the antibiotics gentamicin and dicloxacillin, with *L. gasseri* showing growth inhibition of *S. epidermidis*, reaching the maximum growth peak at 12 h, at pH 4.2, and a lactic acid percentage of 1.26. Zachary et al. (2020) evaluated the mechanisms of adhesion to intestinal mucus of different probiotic strains, for which they immobilized the mucus on the surface of the plate and tested the use of different cell concentrations, finding that surface proteins and cellular components influence mucoadhesion and were heterologously expressed or altered in *Lactococcus lactis* and *Escherichia coli*, concluding that adhesion to mucus depends on the type of strain, as well as on the concentration of cells.

Studies with new probiotics

In recent years, studies on new probiotics have been developed. Molina et al. (2016) evaluated the use of the immunobiotic *Lactobacillus rhamnosus* and its influence on the immunological alterations that occur naturally in aging mice, demonstrating that this strain is capable of improving the

Table 4. Patents of new probiotics (Adapted from Eligo Bioscience Announces Successful Outcome in US Patent Interference against SNIPR Biome on CRISPR-Cas antimicrobials, 2021; SNIPRBIOME A CRISPR COMPANY, 2022)

Patent number	Year	Title	Applicants
EP/V027743/1	2021	Next-generation probiotics: the development of oral microbe-based formulations for microbiome-altering applications	Institute of Clinical Sciences, University of Birmingham
WO2014124226	2021	Eligobiotics	Eligo Bioscience; Rockefeller University
US 10,953,090 B2	2021	Selectively altering microbiota for immune modulation	Jasper Clube, London; Christian Grondahl, Copenhagen; Morten Sommer, Copenhagen; SNIPR Technologies Limited, London (GB)
US 10,920,222 Cl	2021	Treating and preventing microbial infections	Morten Sommer; Virginia Martinez; Eric Van Der Helm; Jakob Krause Haaber; Ana Dc Santiago Torio; Christian Grøndahl; Jasper Clube, SNIPR BIOME APS
EP 3 291 679 B1	2021	Altering microbial populations and modifying microbiota	SNIPR Technologies Limited
US 9, 701, 964, B2	2017	Altering microbial populations and modifying microbiota	Jasper Clube; Morten Sommer; Christian Grondahl; Erick Van Der Helm; Rubén Vázquez Uribe

phagocytic activity of macrophages. Therefore, they concluded that *L. rhamnosus* may have application as an immunomodulator given its positive influence on aging and help in reinforcing intestinal and systemic immunity.

O'Toole et al. (2017) sought a sustainable route to the delivery of novel probiotics from a pharmaceutical perspective, where this relatively new concept overlaps with live biotherapeutic products. Through the development of improved cultivation methodologies, accessibility to genome and metagenome sequencing will allow these probiotics to be targeted.

Nishida et al. (2017) analyzed the relationship that the parapsychobiotic *Lactobacillus gasseri* has with stress and sleep quality. A group of 21 men and eleven women were orally administered the parapsychobiotic for 5 weeks, finding that sleep quality can improve with the administration of *L. gasseri*, particularly in men, shortening sleep latency and increasing the sleep duration. A decrease in the growth of strains such as *Bacteroides vulgatus* was observed, responsible for intestinal inflammation, suggesting that the *L. gasseri* strain is a good ally against stress and its side effects, as well as improving sleep.

Salva and Alvarez (2017) studied the role of microbiota and immunobiotics in granulopoiesis (the process of renewal of granulocytic cells circulating in the blood that are part of the body's defense system), which occurs in the bone marrow of immunocompromised hosts. The authors determined that dietary supplementation with immunobiotics is an interesting alternative to improve granulopoiesis in stationary and emergency states, improving the respiratory innate immune response and resistance against respiratory pathogens in immunocompromised hosts.

Chang et al. (2019) conducted a review of existing information on novel probiotics and how they influence most diseases such as chronic intestinal inflammation, colitis, obesity, metabolic syndromes, diabetes mellitus, liver disease, cardiovascular diseases, cancer, neurodegenerative diseases, using new strains such as *Prevotella copri*, *Christensenella minuta*, *Parabacteroides goldsteinii*, *Akkermansia muciniphila*, *Bacteroides thetaiotaomicron*, *Faecalibacterium prausnitzii* and *Bacteroides fragilis*.

Kumar et al. (2020) studied the probioceutical produced by *Pediococcus acidilactis*, in its exopolysaccharide form and its influence on human health. They analyzed the *Pediococcus acidilactis* genome and identified ten genes responsible for the production of exopolysaccharides, which are linear homopolysaccharides (α-glucans) with few α-(1→3) branches, which have antioxidant activity, reducing power, and anticancer activity, suggesting this probioceutical can be used as an antioxidant and anticancer agent.

Akter et al. (2020) suggested the use of paraprobiotics in the treatment for people with weak immune systems as live probiotics could do more harm than good regarding host health, highlighting their long shelf life, modulation of immune responses, biological response modification, anti-inflammatory and antiproliferative properties.

Different methods of extraction of paraprobiotics and postbiotics produced by bacilli have been studied, such as heat treatment, enzymatic treatments, solvent extraction, radiation, sonication, supercritical CO_2, chromatography, centrifugation, dialysis and lyophilization (Teame et al., 2020).

Moradi et al. (2020) analyzed the applications of postbiotics in food preservation, packaging and biofilm control, as well as their use as a biodegradant against chemical compounds in food (e.g., biogenic amines), concluding that further studies on food biosafety are needed to develop international standards and regulations on the use and applications of postbiotics.

Amirí et al. (2021) proposed the use of postbiotics to produce linoleic acid, exopolysaccharides, and bacteriocins using *Bifidobacterium lactis*, showing how the effect of yeast extract had a beneficial effect on the production yield of postbiotic metabolites.

Recent studies have determined that complementary probiotic therapy can reduce the duration and severity of intestinal diseases, since using the pharmabiotic *Lactobacillus acidophilus* LB can reduce intestinal pH and inhibit the growth of pathogenic organisms (Navarro et al., 2021).

Michels et al. (2022) investigated the molecular patterns of the immune response using *Bifidobacterium lactis*, *Lactobacillus casei*, *L. gasseri*, *L. paracasei* and *Streptococcus thermophilus*. Different doses of these strains on macrophage cell lines stimulated with polysaccharides was examined; the immune response was analyzed after application of these strains as paraprobiotics, concluding that the paraprobiotics had an effect on the production of interleukin in addition to inhibiting free radicals, increasing cell viability, making it likely that these paraprobiotics contribute to improve intestinal homeostasis, immunomodulation and host metabolism. The identification of microbial strains of intestinal origin for the development of new probiotics requires extensive knowledge about the cultivation of these microorganisms, because unlike traditional probiotics these strains are very sensitive to oxygen (De Filippis et al., 2022).

CONCLUSION

The beneficial role of traditional probiotics in human health is undeniable. With the discovery of new probiotics with functions different from traditional probiotics, a door is opened for the development of treatments for different diseases in a specific and systematic way. However, more applied research in humans is needed to determine their biosafety.

ACKNOWLEDGMENTS

Claudia Karina Pacheco Martínez thanks the Consejo Nacional de Ciencia y Tecnología for grant 807331 awarded for her doctoral studies. The English version was translated by Actualidades Biológicas Journal.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

Adebayo, T. B., Ishola, R., & Oyewunmi, T. (2018). Characterization, antioxidant and immunomodulatory potential on exopolysaccharide produced by wild type and mutant *Weissella confusa* strains. *Biotechnology reports*, *19*, 1–8. https://doi.org/10.1016/j.btre.2018.e00271

Aviña, F. J. A., Ángel, O. J., & Ramírez, C. P. J. (2006). Microorganismos probióticos y modulación inmunológica. *Médicas UIS*, *19*, 105–112. https://revistas.uis.edu.co/index.php/revistamedicasuis/article/view/2162

Aguilar–Toalá, J. E., García–Varela, R., García, S. H., Mata–Haro, V., González–Córdova, A. F., Vallejo–Córdova, B., & Hernández–Mendoza, A. (2018). Postbiotics: An evolving term within the functional foods field. *Trends* *in food science & technology*, *75*, 105–114. https://doi.org/10.1016/j.tifs.2018.03.009

Agudelo, L. N., Torres, T. M. M., Álvarez, L. C., & Vélez, A. M. L. (2015). Bacteriocinas producidas por bacterias ácido lácticas y su aplicación en la industria de alimentos. *Revista alimentos hoy*, *23*(35), 186–205. https://alimentoshoy.acta.org.co/index.php/hoy/article/view/356

Akter, S., Park, H. J., & Kil, J. H. (2020). Potential health–promoting benefits of paraprobiotics, inactivated probiotic cells. *Journal of microbiology and biotechnology, 30(*4), 477–481. https://doi.org/10.4014/jmb.1911.11019_

Amirí, S., Rezazadeh–Bari, M., Alizadeh–Khaledabad, M., Rezaei–Mokarram, R., & Sowti–Khiabani, M. (2021). Fermentation optimization for co–production of postbiotics by *bifidobacterium lactis* BB12 in cheese whey. *Waste and biomass valorization*, *12*, 5869–5884. https://doi.org/10.1007/s12649–021–01429–7

Belkaid, Y., & Hand, T. W. (2014). Role of the microbiota in immunity and inflammation. *Cell*, *157*(1), 121–141. https://doi.org/10.1016/j.cell.2014.03.011

Blinkova, L., Martirosyan, M. D., Pakhomov, Y., Dmitrieva, O., Vaughan, R., & Altshuler, M. (2014). Nonculturable forms of bacteria in lyophilized probiotic preparations. *Functional foods in health and disease*, *4*(2), 66–76. https://doi.org/10.31989/ffhd.v4i2.29

Boletín Tecnológico: Alimentos funcionales con probióticos, bancos de patentes SIC. (2014, noviembre). Recuperado de https://issuu.com/quioscosic/docs/alimentos_probioticos___28noviembre_

Castañeda–Guillot, C. (2019). Probióticos de nueva generación. *Belize journal of medicine*, *8*(2), 26–33.

Castañeda–Guillot, C. (2021). Nueva Bioterapéutica: Probióticos de próxima generación. *Revista cubana de pediatría, 93*(1), e1384.

Carnicé, T. R. (2006). Probióticos concepto y mecanismo de acción. *Anales de pediatría, 41*, 30–41.

Carr, F. J., Chill, D., & Maida, N. (2002). The lactic acid bacteria: A literature survey. *Critical reviews in microbiology*, *28*(4), 281–370. https://doi.org/10.1080/1040–840291046759

Cunningham, M., Azcarate, P. A. M., Barnard, A., Benoit, V., Grimaldi, R., Guyonnet, D., Holscher, D. H., Hunter, K., Manurung, S., Obis, D., Petrova, M. I., Steinert, R. E., Swanson, S. K., Sinderen, D. V., Vulevic, J., & Gibson, G. R. (2021). Shaping the future of probiotics and prebiotics. *Trends in microbiology*, *29*(8), 667–685. https://doi.org/10.1016/j.tim.2021.01.003

Chih–Jung, C., Tzu–Lung, L., Yu–Ling, T., Tsung–Ru, W., Wei–Fan, L., Chia–Chen, L., & Hsin–Chih L. (2019). Next generation probiotics in disease amelioration. *Journal of food and drug analysis*, *27*(3), 615–622. https://doi.org/10.1016/j.jfda.2018.12.011

De Filippis, F., Esposito, A., & Ercolin, D. (2022). Outlook on next–generation probiotics from the human gut. *Cellullar & molecular life sciences*, *79*, 76. https://doi.org/10.1007/s00018–021–04080–6

Eligo Bioscience Announces Successful Outcome in US Patent Interference against SNIPR Biome on CRISPR–Cas antimicrobials. (2021, diciembre 2). Recuperado de https://eligo.bio/successful–crispr–patent–interference/

Guidelines for the Evaluation of Probiotics in Food. (2002, Mayo 1). Recuperado de https://www.mhlw.go.jp/file/05–Shingikai–11121000–Iyakushokuhinkyoku Soumuka/0000197343.pdf

Gómez, L. A. (2019). Microbioma, salud, y enfermedad: probióticos, prebióticos, y simbióticos. *Biomédica: revista del Instituto Nacional de salud, 39*(4), 617–621.

Gorbeña, J. C. R., &. Sáenz, T. A. (2008). Bacterias acido lácticas: Biopreservante de los alimentos. *Biotempo*, *8*, 54–64. https://doi.org/10.31381/biotempo.v8i0.865

Gopalakrishnan, V., Helmink, B. A., Spencer, C. N., Ruben, A., & Wargo, A. J. (2018). The influence of the gut microbiome on cancer, immunity, and cancer immunotherapy. *Cancer cell*, *33*(4), 570–580. https://doi.org/10.1016/j.ccell.2018.03.015

Heredia, C. P. Y., Hernández, M. A., Gonzales, C. A. & Vallejo, C. B. (2017). Bacteriocinas de bacterias acido lácticas: mecanismos de acción y actividad antimicrobiana contra patógenos en quesos. *Interciencia*, *42*(6); 340–346.

Isolauri, E., Sütas, Y., Kankaanpää, P., Arvilommi, H., & Salminen, S. (2001). Probiotics: effects on immunity. *The american journal of clinical nutrition*, *73*(2), 444–450. https://doi.org/10.1093/ajcn/73.2.444s

Ibrahim, M., & Raman, K. (2021). Two–species community design of lactic acid bacteria for optimal production of lactate. *Computational and structural biotechnology journal, 19*, 6039–6049. https://doi.org/10.1016/j.csbj.2021.11.009

Jastrząb, R., Graczyk, D., & Siedlecki, P. (2021). Molecular and Cellular Mechanisms Influenced by Postbiotics. *International journal of molecular sciences*, *22*(24), 13475. https://doi.org/10.3390/ijms222413475

Jurado, G. H., & Fajardo, A. C. (2017). Determinación del efecto probiótico *in vitro* de *Lactobacillus gasseri* sobre una cepa de *Staphylococcus epidermis*. *Biosalud, 16*(2), 53–69. https://doi.org/10.17151/biosa.2017.16.2.6

Kaiting, J. (2021). Lactic acid bacteria antibacterial peptides: classification and current application. *E3S Web of conferences*, *271*, 03016. https://doi.org/10.1051/e3sconf/202127103016

Kirmiz, N., Galindo, K., Cruz, L. K., Luna, E., Rhoades, N., Podar, M., & Flores, G. E. (2020). Comparatives genomics guides elucidation of vitamin B12 biosynthesis in novel human– associated *Akkermansia* Strains.

Applied and environmental microbiology, *86*, 117–119. https://doi.org/10.1128/AEM.02117–19

Kumar, R., Bansal, P., Singh, J., & Dhanda, S. (2020). Purification, partial structural characterization and health benefits of exopolysaccharides from potential probiotic *Pediococcus acidilactici* NCDC 252. *Process biochemistry*, 99, 79–86. https://doi.org/10.1016/j.procbio.2020.08.028

Kumari, M., Singh, P., Nataraj, H. B., Kokkiligadda, A., Naithani, H., Ali, A. S., Behare, V. P., & Nagpal, R. (2021). Fostering next–generation probiotics in human gut by targeted dietary modulation: An emerging perspective. *Food research international, 150*(A), 110716. https://doi.org/10.1016/j.foodres.2021.110716

McKee, T., & McKee, J. R. (2016), *Bioquímica: Las bases moleculares de la vida*. Nueva York, USA, McGRAW–HILL.

Martin, R., & Langella, P. (2019). Emerging health concepts in the probiotics field: streamlining the definitions. *Frontiers in microbiology*, *10*, 1047. https://doi.org/10.3389/fmicb.2019.01047

Michels, M., Fernández, A. J. G., Lorenzo, V. A. P., Rosseto, M., Ramlov, F., Corneo, E., Feuser, P., Gelain, D., & Dal–Pizzol, F. (2022). Immunomodulatory effect of *Bifidobacterium, Lactobacillus*, and *Streptococcus* strains of paraprobiotics in lipopolysaccharide–stimulated inflammatory responses in RAW–264.*7 Macrophages. current microbiology*, *79*(1), 9. https://doi.org/10.1007/s00284–021–02708–1

Misra, S., & Mohanty, D. (2019). Psychobiotics: a new approach for treating mental illness? *Critical reviews in food science and nutrition*, *59*(8), 1230–1236. https://doi.org/10.1080/10408398.2017.1399860

Moradi, M., Kousheh, A. S., Almasi, H., Alizadeh, A., Guimarães, T. J., Yilmaz, N., & Lotfi, A. (2020). Postbiotics produced by lactic acid bacteria: the next frontier in food safety. *Comprehensive reviews in food science and food safety*, *19*, 3390–3415. https://doi.org/10.1111/1541–4337.12613

Molina, V., Médici, M., Villena, J., Font, G., & Taranto, M. P. (2016). Dietary supplementation with probiotic strain improves immune–health in aged mice. *Open journal of immunology, 6*, 73–78. https://doi.org/10.4236/oji.2016.63008.

Navarro, D., Camacho, C. N., Torres, J. B., & Alonzo, L. (2021). Terapias complementarias en diarrea aguda. *Archivos venezolanos de puericultura y pediatría, 84*(1), 62–71.

Nataraj, B. H., Ali, A. S., Behare, V. P., & Yadav, H. (2020). Postbioticsparabiotics: the new horizons in microbial biotherapy and functional foods. *Microbial cell factories, 19*, 168. https://doi.org/10.1186/s12934–020–01426–w

Nishida, K., Sawada, D., Kawai, T., Kuwano, Y., Fijiwara, S., & Rokutan, K. (2017). Para–psychobiotic *Lactobacillus gasseri* CP2305 ameliorates stress–related symptoms and sleep quality. *Journal of applied microbiology*, *123*(6), 1561–1570. https://doi.org/10.1111/jam.13594

O´Toole, W. P., Marchesi, R. J., & Hill, C. (2017). Next–generation probiotics: the spectrum from probiotics to live biotherapeutics. *Nature microbiology, 2*, 17057. https://doi.org/10.1038/nmicrobiol.2017.57

Parra Huertas, A. R. (2010). Review lactic acid bacteria: functional role in the foods. *Biotecnología en el sector agropecuario y agroindustrial*, *8*(1), 93–105.

Plaza–Diaz. J., Ruiz, O. J. F., Gil, C. M., & Gil, A. (2019). Mechanisms of action of probiotics. *Advances in nutrition*, *10*(1), 49–66. https://doi.org/10.1093/advances/nmy063

Prats Capote, A. (2007). Probióticos: una alternativa natural como promotores de la salud. *Revista CENIC. Ciencias biológicas*, *38*(1), 49–53.

Prieto, P. A. (2010). Aspectos moleculares de los prebióticos. *Revista de gastroenterología de México, 2*(75). 210–211.

Ramírez–Chavarín, M. L., Wacher, C., Eslava–Campos, C. A., & Pérez–Chabela, M. L. (2013). Probiotic potential of thermotolerant lactic acid bacteria strains isolated from cooked meat products. *International food research journal*, *20*(2), 991–1000.

Rondon, L., Añez, Z. M. R., Salvatierra, H. A., Meneses, B. R. T., & Heredia, R. M. T. (2015). Probióticos: generalidades. *Archivos venezolanos de puericultura y pediatría, 78*(4), 123–128.

Salva, S., & Álvarez, S. (2017). The role of microbiota and inmunobiotics in granulopoiesis of inmunocompromised hosts. *Frontiers in immunology, 8,* 507 https://doi.org/10.3389/fimmu.2017.00507

Salminen, S., Collado, M. C., Endo, A., Colin, C., Lebeer, S., Quigley, E. M. M., Sanders, E. M., Shamir, R., Swann, J. R., Szajewska, H., & Vinderola, G. (2021). The international scientific association of probiotics and prebiotics (ISAPP) consensus statement on the definition and scope of postbiotics. *Nature reviews: gastroenterology & hepatology, 18*, 649–667. https://doi.org/10.1038/s41575–021–00440–6

SNIPRBIOME A CRISPR COMPANY. (2022). Recuperado de https://www.sniprbiome.com/publications

Sánchez, L., & Tromps, J. (2014). Caracterización in vitro de bacterias ácido–laticas con potencial probiótico. *Revista salud animal*, *36*(2), 124–129.

Sharma, M., & Shukla, G. (2016). Metabiotics: one step ahead of probiotics; an insight into mechanisms involved in anticancerous effect in colorectal cancer. *Frontiers in microbiology*, *7*, 1940. https://doi.org/10.3389/fmicb.2016.01940

Taverniti, V., & Guglielmetti, S. (2011). The immunomodulatory properties of probiotic microorganisms beyond their viability (ghost probiotics: proposal of paraprobiotic concept). *Genes nutrition*, *6*, 261–274. https://doi.org/10.1007/s12263–011–0218–x

Teame, T., Wang, A., Xie, M., Zhang, Z., Yang, Y., Ding, Q., Gao, C., Olsen, E. R., Ran, C., & Zhou, Z. (2020). Paraprobiotics and postbiotics of probiotic *Lactobacilli*, their positive effects on the host and action mechanisms: a review. *Frontiers in nutrition, 7* 570344. https://doi.org/10.3389/fnut.2020.570344

Tzu–Lung, L., Ching–Chung, S., Wei–Fan, L., Chi–Meng, T., Hsin–Chih L., & Chia–Chen L. (2019). Investiture of next generation probiotics on amelioration of diseases – Strains do matter. *Medicine in microbiology*, *1–2*, 100002. https://doi.org/10.1016/j.medmic.2019.100002

Vázquez, M. S., Suarez, M. H., & Zapata, B. S. (2009). Utilización de sustancias antimicrobianas producidas por bacterias acido lácticas en la conservación de la carne. *Revista chilena de nutrición*, *36*(1), 64–71. https://doi.org/10.4067/S0717–75182009000100007

Veiga, P., Suez, J., Derrien, M., & Elinav, E. (2020). Moving from probiotics to precision probiotics. *Nature microbiology*, *5*, 878–808. https://doi.org/10.1038/s41564–020–0721–1.

Yang, J., Li, Y., Wen, Z., Liu, W., Meng, L., & Huang, H. (2021). Oscillospira – a candidate for the next–generation probiotics. *Gut microbes*, *13*(1), 1–18. https://doi.org/10.1080/19490976.2021.1987783

Zachary, J. S., Mays, T. C., & Nikhil, U. N. (2020). Quantifying and engineering mucus adhesion of probiotics. *American chemical society: synthetic biology*, *9*(2), 356–367. https://doi.org/10.1021/acssynbio.9b00356

Zitvogel, L., Ayyoub, M., Routy, B., & Kroemer, G. (2016). Microbiome and anticancer immunosurveillance. *Cell, 165*(2), 275–287. https://doi.org/10.1016/j.cell.2016.03.001

Zendeboodi, F., Khorshidian, N., Mortazavian, M. A., & Da Cruz, A. G. (2020). Probiotic: conceptualization from a new approach. *Food and science*, *32*, 103–123. https://doi.org/10.1016/j.cofs.2020.03.009

Zhang, L., Li, N., Caicedo, R., & Neu, J. (2005). Alive and dead *Lactobacillus rhamnosus* GG decrease tumor necrosis factor–α–induced interleukin– 8 production in Caco– 2 cell. *The journal of nutrition*, *135*(7), 1752–1756. https://doi.org/10.1093/jn/135.7.1752