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# Review of *Lactobacillus* in the food industry and their culture media

## Revisión de Lactobacilos en la industria alimentaria y sus medios de cultivo

Óscar J. Sánchez\*; Pedro J. Barragán\*\*; Liliana Serna\*\*\*

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### ABSTRACT

Lactic acid bacteria (LAB) are currently of great importance given their increasing use in the improvement of human and animal health and nutrition. They exhibit complex nutritional requirements, which is the reason why their production costs are high. Research efforts are being made aimed at evaluating different substrates for their production as well as the production of valuable metabolites from them. The purpose of this paper is to expose the main research and development trends for LAB production for industrial purposes with emphasis on the culture media required for their growth. The web of Science databases as well as the Google Patent Search tool were used in order to gather and analyze the scientific and technical information published in the last twelve years relating to LAB and their culture media. The use of milk, industrial cheese whey, cane molasses, hydrolyzed starches, lignocellulosic materials, organic food waste and bovine blood plasma, among others, have been proposed for *Lactobacillus* cultivation with the purpose of reducing costs and increasing performance in their production. Research groups and centers have the responsibility of intensifying their efforts to offer highly efficient technological alternatives to the industry that allow the production and application of LAB as a growth factor for the food sector. Also, research in prebiotic ingredients or additives derived from LAB that allow the enhancement of the benefits to the consumer must be continued. In this regard, it is necessary to increase the international visibility of Colombian scientific production in this area.

**Key words:** Lactobacillus, patent search, probiotics, starter culture, submerged fermentation.

### RESUMEN

Las bacterias del ácido láctico (LAB) son actualmente de gran importancia dado su uso creciente en la mejora de la salud y nutrición humana y animal. Presentan requerimientos nutricionales complejos, por lo que sus costos de producción son altos. Se están realizando esfuerzos de investigación para evaluar diferentes sustratos para su producción, así como la producción de metabolitos valiosos a partir de ellos. El propósito de este documento es exponer las principales tendencias de investigación y desarrollo para la producción de LAB con fines industriales, con énfasis en los medios de cultivo necesarios para su crecimiento. Las bases de datos de Web of Science y la herramienta de búsqueda de patentes de Google se utilizaron para recopilar y analizar la información científica y técnica publicada en los últimos doce años relacionada con LAB y sus medios de cultivo. Se ha propuesto el uso de leche, suero de queso industrial, melaza de caña, almidones hidrolizados, materiales lignocelulósicos, desechos de alimentos orgánicos y plasma sanguíneo bovino, entre otros, para el cultivo de *Lactobacillus* con el fin de reducir costos y aumentar el rendimiento de su producción. Los grupos y centros de investigación tienen la responsabilidad de intensificar sus esfuerzos para ofrecer alternativas

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tecnológicas altamente eficientes a la industria que permitan la producción y aplicación de LAB como factor de crecimiento para el sector alimentario. Además, la investigación en ingredientes prebióticos o aditivos derivados de LAB que permite la mejora de los beneficios para el consumidor debe continuar. En este sentido, es necesario aumentar la visibilidad internacional de la producción científica colombiana en esta área.

**Palabras clave:** *Lactobacillus*, búsqueda de patentes, probióticos, cultivo iniciador, fermentación sumergida.

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## INTRODUCTION

At the beginning of the 20th century the concept of lactic acid bacteria (LAB) emerged as an independent group. Today this group shows a huge biotechnological potential, and it is present in a large number of fermentative food processes destined for human consumption such as dairy, vegetables, meat and bakery products, as well as in silage for animal food (Lee *et al.*, 2013; J. C. Ramírez *et al.*, 2011). These bacteria not only contribute to the development of the organoleptic, rheological and nutritional characteristics of foods, but also generate unfavorable environments for the development of pathogenic microorganisms such as *Bacillus*, *Pseudomonas*, *Listeria* and *Escherichia* among others, given their bio-conservative capacity to produce antimicrobial substances (Cizeikiene *et al.*, 2013; Topisirovic *et al.*, 2006). In addition to this important role in bio-conservation processes, some strains of lactic acid bacteria belonging to the genus *Lactobacillus* are beneficial to human and animal health, due to their potential in reducing enteric diseases in humans and farm animals, and the subsequent contamination of food products derived from them (Argyri *et al.*, 2013; Ávila *et al.*, 2010; L Axelsson, 1998). The purpose of this paper is to expose the main research and development trends in the production of lactic acid bacteria for industrial purposes with emphasis on the culture media required for their growth.

### Search procedure

This review is, focused on presenting the alternatives of culture media for the production of *Lactobacillus* in the last 12 years, the study was divided into two parts: A synthetic literature review on the generalities of LAB, and a systematic review on specific media for cultivation of *Lactobacillus*. With this purpose, the Web of Science databases, consisting of KCL (Korean Journal Database), Russian Science Citation Index, Scielo Citation Index, Springer and ScienceDirect, was used. The following search terms were used: 1) for the Title field: "*Lactobacillus*" with connector "AND" and in Topic field: "culture media"; 2) published between 2005 and 2016; 3) type of document: articles; 4) language (English, Portuguese and Spanish). Once the records were obtained, filters were used to focus on the ten research areas in

which this type of studies are done. In order to reduce the search size, the results were refined by using "Science and Technology" as research domain and even more specific research areas of this type of studies such as: "Food Science and Technology", "Applied Biotechnology and Microbiology", "Microbiology", "Applied Chemistry", and "Chemical Engineering".

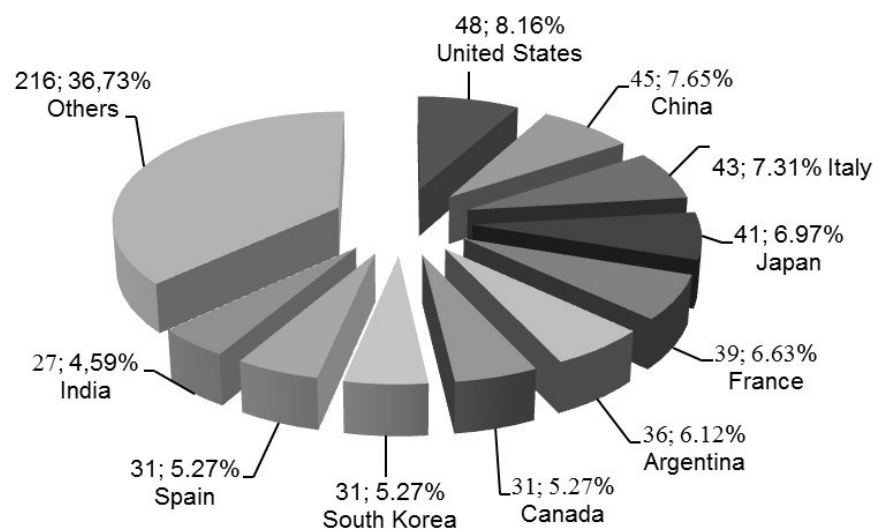
Using the Web of Science search engine, the internal analysis of articles obtained in the last twelve years (a reduced sample greater than 500 articles) was performed using the following criteria: 1) The ten most important research areas; 2) the thirteen authors registered as the most important in these research areas; 3) the record of publications in the same period of time; and 4) the ten most important countries in this type of study. From this reduced sample, about 60 articles were selected using the following exclusion criteria: 1) Reports that do not contribute to the objective of this review by not making explicit the culture media for LAB; 2) papers that do not report the type of microorganism, substrate or supplement, type of fermentation, fermentation conditions, and biomass production. The extracted information was compiled in an MS Excel spreadsheet, designed with the search criteria used. In this format, some works published prior to the time period covered by this review were also included given their relevance and pertinence. Moreover, patents reported between 2000 and 2016 on *Lactobacilli* were consulted through the Google Patent Search using the same search criteria indicated above.

### Search results on culture media for lactic acid bacteria

The initial search performed on *Lactobacillus* culture media, once the search criteria and the respective filters were applied, showed 594 records, which were analyzed for this review. From the records analyzed, 13.1% of the papers are authored by 13 researchers, who lead the studies on *Lactobacillus* in the last twelve years. These authors belong to research groups from Canada, Italy, South Africa, England, Poland, Japan, Argentina, Belgium, and the United States. Table 1 shows the 25 affiliation organizations of these authors. Taking into account the classification of research areas used by Web of Science, the articles found belong to the following

**Table 1.** Amount of publications of the 25 main organizations to which the research groups working on *Lactobacillus* belong between 2005-2016.

Organization (Country)	Records	Participation percentage
Institut National de Recherche Agronomique (INRA, France)	13	2.180
Consejo Nacional de Investigaciones Cientificas y Técnicas (CONICET, Argentina)	11	1.852
Consejo Superior de Investigaciones Cientificas (CSIC, Spain)	11	1.852
Universidade de São Paulo (Brazil)	11	1.852
Agriculture and Agri-Food Canada (Canada)	10	1.684
Istituto di Scienze di Alimentari (Italy)	9	1.515
Universidad Nacional de Tucumán (Argentina)	9	1.515
Universiti Putra Malaysia (Malaysia)	8	1.347
Islamid Azad University (Iran)	7	1.178
Jiangnan University (China)	7	1.178
Seoul National University (South Korea)	7	1.178
Technische Universität München (Germany)	7	1.178
Centro de Referencia de Lactobacilos (CERELAC, Argentina)	6	1.010
Instituto de Investigación de la Leche (Uruguay)	6	1.010
Tohoku University (Japan)	6	1.010
University of Manchester (United Kingdom)	6	1.010
Universidad de Vigo (Spain)	6	1.010
Università della Basilicata (Italy)	5	0.842
Universiteit Gent (Belgium)	5	0.842
University of Helsinki (Finland)	5	0.842
Université Laval (Canada)	5	0.842
Universidad Nacional del Litoral (Argentina)	5	0.842
Stellenbosch University (South Africa)	5	0.842
University of Tehran (Iran)	5	0.842
Wageningen University (Netherlands)	5	0.842



**Figure 1.** Distribution by country of the institutions to which the authors of articles on culture media for *Lactobacillus* belong in the period 2005-2016.

five research areas in descending order: Applied Microbiology and Biotechnology (196), Microbiology (176), Food Science and Technology (163), Molecular Biology and Biochemistry (31), Agriculture (28).

The ten countries with the highest participation in studies on culture media for *Lactobacillus* in the world in the last twelve years are shown in figure 1. It is noteworthy that only three records corresponding to Colombian researchers appear in Web of Science in the last twelve years, comprising a share of 0.5%. The amount of publications in the last twelve years was also consulted for this search in the selected databases and the results are illustrated in figure 2.

### Patent search

The literature reports an important amount of patents on new *Lactobacillus* strains, as well as processes for differentiating and quantifying lactic bacteria in some natural products, the composition and enrichment of media for their cultivation, and processes for producing, characterizing and applying products with probiotic properties obtained from *Lactobacillus*, among others. Table 2 presents the list of patents found in the last decade (from 2005 to August 2016 as a reference period for the analysis) regarding *Lactobacillus*.

It should be noted that the patents on *Lactobacillus* granted in the period from 2000 to August 2016, show a great interest in the identification of new strains based on their natural substrates (mainly dairy and meat products), as well as their potential use as preventive or therapeutic agents against pathologies related to the human

intestinal tract (Anonymous, 2005, 2016a; Bojrab, 2011; Connolly, 2008; Farmer, 2014). The companies filing patents on this topic aim their resources towards economic methods for production of *Lactobacilli* cultures at industrial level. Most patent documents refer to the use of whey (Yun *et al.*, 2015), corn starch (Li & Liu, 2014) and even whole milk (Anonymous, 2014a; Chen *et al.*, 2013). In other cases, the inventors resort to the design of synthetic media for the isolation of bioactive molecules, functional metabolites or production of LAB of interest (Elli *et al.*, 2002). For the enrichment of these media, the use of free bases, ribonucleosides, deoxyribonucleosides, casein hydrolysates, hydroxyproline, and glutamic acid was reported. Some patents introduced their inventions for preservatives, which enable the protection of the *Lactobacillus* culture, both in the subculture and during storage. It should be highlighted that, because of the recognized importance of probiotics, efforts are made to design growth media enriched with constituents such as sulfur amino acids, ovalbumin, bile powder, trehalose, and raffinose.

### Features of lactic acid bacteria

Lactic bacteria are chemo-organotrophic microorganisms. Their morphology appears as cocci or bacilli, commonly associated in short chains, are Gram positive, non-sporulated, and aerotolerant. They are negative in the oxidase, catalase and benzidine tests. They lack cytochromes and generally do not reduce nitrate to nitrite. These bacteria synthesize lactic acid as the main product of carbohydrate fermentation (Lars Axelsson, 2004; Bovo *et al.*, 2014; Stieglmeier *et al.*, 2009; Vázquez *et al.*, 2009; Waldir *et al.*, 2007).

Table 2. Patents on *Lactobacillus* in the period registered 2000-2016.

Patent number	Country	Description	Reference
EP 2316278	South Korea	Method for producing fermented edible plants or animals and foods containing	Choi et al. (2013)
US 8613964	United States	Method for making shelf-stable poi food products from dryland and wetland taro	Day and Boiser (2013)
CA 2598539	Canada	Lactic acid and <i>Bacillaceae</i> fertilizer and method of producing it	Blais (2013)
CN 103857297	China	Probiotic fruit drinks	Anonymous (2016b)
EP 0698347	Sweden	Food composition containing reuterin	Dobrogosz and Lindgren (2006)
US 8697055	United States	Probiotic based on lactic acid-producing bacteria	Farmer (2014)
CN 103224895	China	A novel strain of <i>Lactobacillus reuteri</i> for use by improving autoimmune diseases	Anonymous (2014c)
US 8748124	United States	Biodegradation process and composition	López et al. (2014)
CN 101919879	China	Method for preparing probiotics preparation by taking attapulgit as carrier	Anonymous (2012)
US 8815539	United States	Methods for producing melanin and inorganic fertilizer from fermentation leachates	Popa and Nealson (2014)
CN 103154235	China	Improvement of <i>Lactobacillus</i> strains immunomodulatory properties	Anonymous (2016a)
CN 102994644	China	Quantitative detection method and kit for <i>Lactobacillus plantarum</i> and its application	Anonymous (2014b)
US 7888062	United States	Process and composition for the manufacture of a microbial-based product	Garner and Flint (2011)
CN 103550258	China	<i>Lactobacillus</i> strains for regulation of the immune response	Anonymous (2015a)
US7344867	United States	Selection and use of lactic acid bacteria for reducing inflammation in mammals	Connolly (2008)
US 7901925	United States	Strain of <i>Lactobacillus delbrueckii</i> ssp. <i>bulgaricus</i> and compositions	Bojrab (2011)
CN103275921	China	Method for improving cholate tolerance of <i>Lactobacillus</i>	Anonymous (2015b)
US 6340585	United States	Synthetic medium for cultivation of <i>Lactobacillus</i> and <i>Bifidobacteria</i>	Elli et al. (2002)
CA 2534013	Canada	<i>Lactobacillus</i> as a preventive or therapeutic agent against infections	Anonymous (2005)
US 7901925	United States	Strain of <i>L. delbrueckii</i> ssp. <i>bulgaricus</i> and its composition	Bojrab (2011)
CN 102952772	China	Enrichment of culture medium for <i>L. bulgaricus</i> and method of preparation	Chen et al. (2013)
CN 103305435	China	Method for preparation of <i>L. plantarum</i> culture	Anonymous (2014a)
CN 104212743	China	Culture medium and method for high density culture of <i>L. amylolyticus</i>	Li and Liu (2014)
CN 104531596	China	Improved culture medium with whey proteins and their applications	Yun et al. (2015)
CN 104560799	China	Method of active bacterial preparation of bacteriocins by <i>L. plantarum</i> ssp. <i>plantarum</i>	Hongxing (2015) Zhang-LL
US 9265270	United States	<i>Lactobacillus</i> culture and method for producing it	Hoshi et al. (2016)

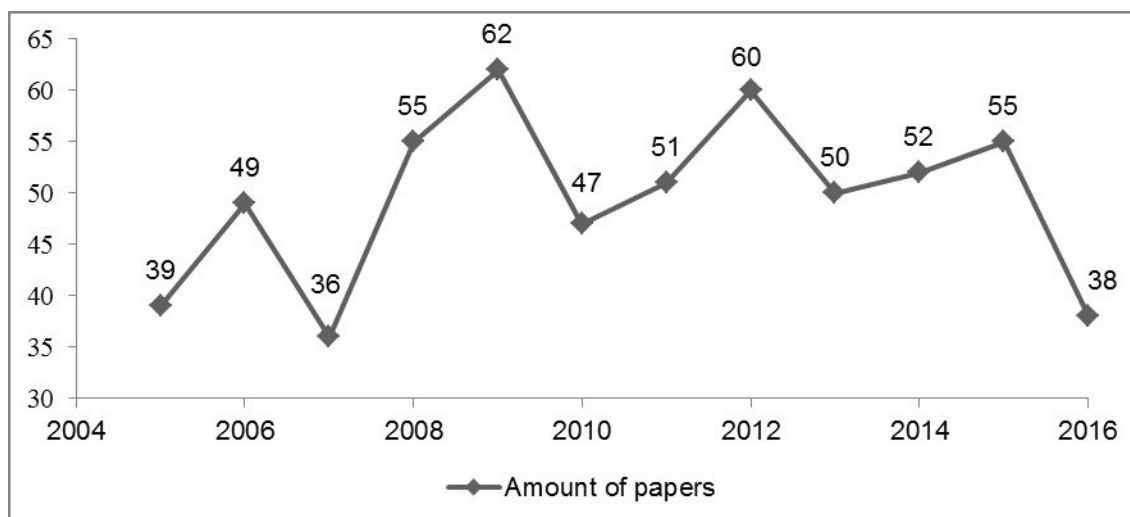


Figure 2. Amount of papers published on culture media for *Lactobacillus* in the period 2005-2016.

The LAB exhibit two metabolic pathways for fermentation of monosaccharides: Homolactic fermentation or glycolysis (pathway Embden-Meyerhof-Parnas) and heterolactic fermentation of the 6-phosphoglucanate (6FG) or pentoses pathway. Consequently, LAB have been classified as obligatory homofermentative, facultative heterofermentative, and obligatory heterofermentative microorganisms (Lars Axelsson, 2004; Gänzle, 2015). The acidolactic bacteria shows slight proteolytic activity due to the proteases and peptidases attached to the cell wall or released by them, as well as weak lipolytic activity due to the action of intracellular lipases.

The culture media and main fermentation conditions for production of the main *Lactobacillus* species used for production of probiotics, starter cultures, and bacteriocins, are shown in table 3 (organized alphabetically by species name followed by the year of publication). Among the most individually studied *Lactobacillus* or a mixture of them, isolated from natural substrates, the following species should be highlighted for their probiotic and bio-preservation activities: *L. acidophilus*, *L. casei*, *L. delbrueckii*, *L. plantarum*, *L. helveticus*, *L. rhamnosus*, and *Bifidobacterium bifidum*.

### Culture media for lactic acid bacteria

A culture medium must contain the nutrients and growth factors required by the microorganism and be free of contaminating microorganisms (Garcia *et al.*, 2010). These requirements are characteristic of each species. LAB show complex nutritional requirements such as amino acids, peptides, derivatives of nucleic acids, vitamins, salts, fatty acids or esters of fatty acids, and fermentable carbohydrates (Parra, 2010). These requirements are usually met when the culture medium contains ferment-

able carbohydrates, peptone meat, and yeast extract. In general, for *Lactobacillus* it is desirable for the nitrogen to be organic in the form of amino acids or peptides. In this sense, glutamic acid, isoleucine and valine are considered as growth factors and must be present in the culture medium (González *et al.*, 2008). Many species require the presence of manganese, acetate and esters of oleic acid, especially Tween 80 (Berbegal *et al.*, 2015).

According to table 3 and figure 4, it can be observed that most studies on the production of *Lactobacillus* biomass still make use of whey and sugar cane molasses as source of carbon and energy. In recent years, the use of fruit juices, as well as of by-products and agro-industrial waste has gained importance for media formulation. The above corroborates the research trend in the search for feedstocks of high availability and lower cost with the purpose of reducing the sale price of the final products derived from the *Lactobacilli* biomass.

In Latin America, different research efforts have been reported aimed at the use of native products for LAB growth given the great availability of biological resources and economies with a high weight of the agriculture and agro-industry in these countries. For example, the growth kinetics of *Lactobacilli* with probiotic potential in the intestinal tract are being studied in Mexico using the agro-industrial residue derived from the fermentation of agave honey, a pre-Hispanic drink called *pulque* in that country (Yépez *et al.*, 2013).

In Colombia, the use of wastewater sludge from sugar cane processing as well as the sugar cane molasses has been proposed as feedstocks given the importance of the sugar industries including the industry for production



**Table 3.** Culture media and fermentation conditions for *Lactobacillus* production.

$\mu_{max}$ : maximum specific speed of growth; OD: optical density; X: concentration of cellular biomass;  $Y_{p/s}$ : product yield from substrate;  $Y_{x/s}$ : biomass yield from substrate; YE: yeast extract; WP: whey permeate.

Microorganism	Substrate	Supplements	Fermentation conditions	Biomass production	Type of fermentation	References
<i>Bifidobacterium bifidum</i>	Medium 1: Sugar cane molasses 32 g/L	YE: 5.0 g/L	Anaerobic conditions	4.5 g/L-2.8 g/L	Discontinuous	Vargas et al. (2004)
	Medium 2: UHT wastewater 45 g/L			3.8 g/L-3.4 g/L		
<i>L. acidophilus</i>	Medium 3: Sugar cane molasses 32 g/L	YE: 5.0 g/L Casein peptone: 10 g/L	37°C, 48 h, 100 rpm, 5-10% oxygen	5.5 g/L	Discontinuous	Vargas et al. (2004)
<i>L. acidophilus</i> ACC	Milk	YE: 0.3, 0.5 and 1%	37 °C, 100 rpm, controlled pH 6.5	CFU/ mL: $7.94 \times 10^7$ $6.30 \times 10^9$ $X_{max}$ : 1.2-3.5 g/L $\mu_{max}$ : 0.36-0.92 h <sup>-1</sup>	Discontinuous	Avonts et al. (2004)
<i>L. acidophilus</i> IBB 01	Milk	YE: 0.3, 0.5 and 1%	37 °C, 100 rpm, controlled pH 6.5	CFU/ mL: $7.94 \times 10^7$ $6.30 \times 10^9$ $X_{max}$ : 1.2-3.5 g/L $\mu_{max}$ : 0.36-0.92 h <sup>-1</sup>	Discontinuous	Avonts et al. (2004)
<i>Lactobacillus bulgaricus</i> RR	Medium: glucose (20g/L), Tween 80 (1.0 g/L), ammonium citrate (2 g/L), sodium acetate (5 g/L), MgSO <sub>4</sub> 7H <sub>2</sub> O (0.1 g/L), MnSO <sub>4</sub> (0.05 g/L), K <sub>2</sub> HPO <sub>4</sub> (2 g/L)	Yeast nitrogenous base (5 g/L) and Bacto casitone (10 g/L)	42°C, 12 h, pH 6.56	$1.28 \times 10^8$ CFU/mL	Discontinuous	Kimmel and Roberts (1998)
<i>L. casei</i> Imunitas	Milk	YE: 0.3, 0.5 and 1%	37 °C, 100 rpm, controlled pH 6.5	CFU/ mL: $7.94 \times 10^7$ $6.30 \times 10^9$ $X_{max}$ : 1.2-3.5 g/L $\mu_{max}$ : 0.36-0.92 h <sup>-1</sup>	Discontinuous	Avonts et al. (2004)
<i>L. casei</i> YIT 9029	Milk	YE: 0.3, 0.5 and 1%	37 °C, 100 rpm, controlled pH 6.5	CFU/ mL: $7.94 \times 10^7$ $6.30 \times 10^9$ $X_{max}$ : 1.2-3.5 g/L $\mu_{max}$ : 0.36-0.92 h <sup>-1</sup>	Discontinuous	Avonts et al. (2004)
<i>L. casei</i>	Sugar cane molasses 32 g/L	YE: 5.0 g/L	37°C, 48 h, 100 rpm, 5-10% oxygen	5.5 g/L	Discontinuous	Vargas et al. (2004)
<i>L. casei</i> NRRL 1445	Juice of Aloe vera variety barbariensis: 25, 50, 75 and 100%		30 °C, 150 rpm, initial pH of medium: 6,5	<i>L. casei</i> : $1 \times 10^{11}$ CFU/mL	Discontinuous	González et al. (2008)
<i>L. casei</i> Shirota	Sugar cane juice		37°C, 24 h	$3.98 \times 10^7$ CFU/mL	Discontinuous	Magaña et al. (2008)
	Coconut milk			$1.58 \times 10^8$ CFU/mL		
	Orange juice			$1.58 \times 10^8$ CFU/mL		
	Whey			$3.16 \times 10^9$ CFU/mL		
<i>L. casei</i>	WP from goat milk		Batch regime: 37 °C, pH 5.5, effective volume 2 L, 300 rpm, inoculum concn. 0.5-1.0 g/L, concn., initial substrate concn. 33-50 g/L, 20 h	Productivity: 0.067 0.46 g biomass/(L×h)	Discontinuous	Aguirre et al. (2009)
			Fed-batch regime: 1 L supernatant replaced by 1 L fresh culture medium at 14 h	X: 2.5-3.5 g/L		
				$Y_{x/s}$ : 0.0990-0.16 g biomass/g substrate)		
				$5.17 \times 10^9$ CFU/mL		
				Fed-batch: $2.43 \times 10^{10}$ CFU/mL	Fed batch	
<i>L. casei</i>	WP from goat milk		Batch regime: 37 °C, pH 5.5, effective volume 2 L, 300 rpm, inoculum concn. 0.5-1.0 g/L, concn., initial substrate concn. 33-50 g/L, 20 h	Productivity: 0.067 0.46 g biomass/(L×h)	Discontinuous	Aguirre et al. (2009)
			Fed-batch regime: 1 L supernatant replaced by 1 L fresh culture medium at 14 h	X: 2.5-3.5 g/L		
				$Y_{x/s}$ : 0.0990-0.16 g biomass/g substrate)		
				$5.17 \times 10^9$ CFU/mL		
				Fed-batch: $2.43 \times 10^{10}$ CFU/mL	Fed batch	



**Table 3.** Culture media and fermentation conditions for *Lactobacillus* production.

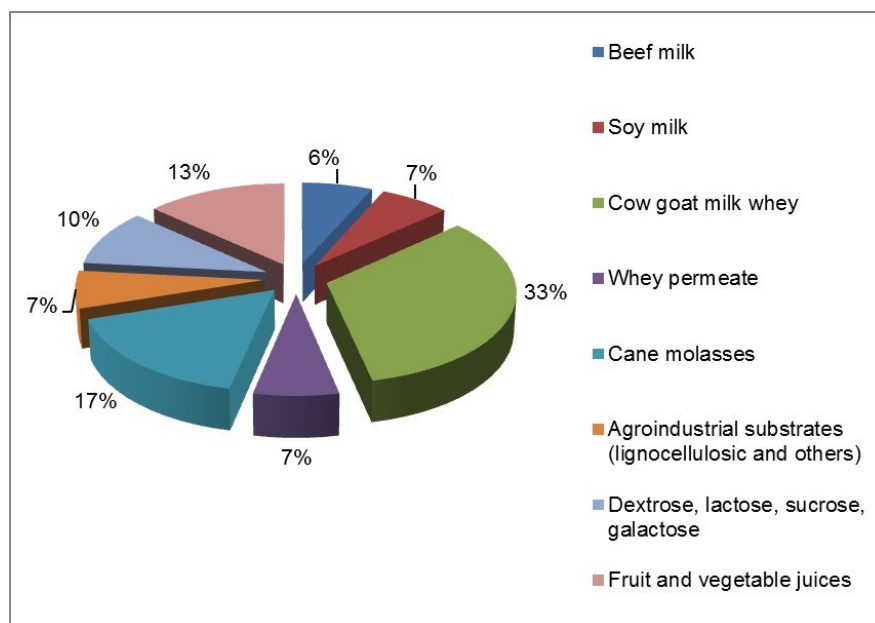
$\mu_{max}$ : maximum specific speed of growth; OD: optical density; X: concentration of cellular biomass;  $Y_{p/s}$ : product yield from substrate;  $Y_{x/s}$ : biomass yield from substrate; YE: yeast extract; WP: whey permeate.

Microorganism	Substrate	Supplements	Fermentation conditions	Biomass production	Type of fermentation	References
<i>L. casei</i> ATCC 393	Uchuva pulp and glucose solution (14% p/p)		37 °C, 72 h, microaerophilic conditions	$5.40 \pm 2.36 \times 10^8$ to $7.34 \pm 7.88 \times 10^8$ CFU/mL	Discontinuous	Cortés R (2009)
<i>L. casei</i>	Deproteinized whey	Glucose (0 % and 5%)	Initial pH=6.05, 93 h	$5.9 \times 10^{10}$ CFU/mL	Discontinuous	Escobar et al. (2010)
<i>L. casei</i> var. <i>rhamnosus</i>	Whey	35-70 g/L lactose	37°C, pH 5.5, 300 rpm, volume 2 L, inoculum concn. 0.5-1.0 g/L	$Y_{x/s} = 1.5819$ g/g $Y_{p/s} = 0.5684$ g/g $X: 5$ g/L	Discontinuous	Alvarez et al. (2010)
<i>L. casei</i> IMAU60214 (isolated from commercial dairy products)	Wastewater from corn nixtamalization		37°C, 48 h	By absorbance (650 nm): 0.740	Discontinuous	G. Ramírez et al. (2013)
<i>Lactobacillus delbrueckii</i> ssp	MRS		42°C, 12 h, pH 6.56	$1.62 \times 10^8$ CFU/mL	Discontinuous	Kimmel and Roberts (1998)
<i>L. gasserii</i> K7	Milk	YE: 0.3, 0.5 and 1%	37 °C, 100 rpm, controlled pH 6.5	CFU/ mL: $7.94 \times 10^7$ - $6.30 \times 10^9$ $X_{max}: 1.2-3.5$ g/L $\mu_{max}: 0.36-0.92$ h <sup>-1</sup>	Discontinuous	Avonts et al. (2004)
<i>L. helveticus</i>	Whey permeate (48g/mL of lactose)	YE: 20 g/mL, casein peptone: 5 g/mL	42 °C, controlled pH 5.9, 25 h	$\mu_{max} = 0.477$ h <sup>-1</sup> $X = 4.0$ g/L	Discontinuous	Amrane (2005)
<i>L. helveticus</i> IMAU70129 (isolated from commercial dairy products)	Wastewater from corn nixtamalization		37°C, 48 h	By absorbance (650 nm): 0.735	Discontinuous	G. Ramírez et al. (2013)
<i>L. johnsonii</i> La1	Milk	YE: 0.3, 0.5 and 1%	37 °C, 100 rpm, controlled pH 6.5	CFU/ mL: $7.94 \times 10^7$ - $6.30 \times 10^9$ $X_{max}: 1.2-3.5$ g/L $\mu_{max}: 0.36-0.92$ h <sup>-1</sup>	Discontinuous	Avonts et al. (2004)
<i>L. jonhsonii</i>	Sugar cane juice Coconut milk Orange juice Whey		37°C, 24 h	$3.98 \times 10^7$ CFU/mL $1.58 \times 10^8$ CFU/mL $3.16 \times 10^9$ CFU/mL $1.58 \times 10^8$ CFU/mL	Discontinuous	Magaña et al. (2008)
<i>L. paracasei</i> HA9-2 (isolated from humans)	Media 1 and 4: Whey with 70 g/L carbohydrates Media 2 and 3: Whey	Medium 2: $MgSO_4 \cdot 7H_2O$ (0.3 g/L), $MnSO_4 \cdot 4H_2O$ (0.03 g/L) Medium 3: $MgSO_4 \cdot 7H_2O$ (0.3 g/L), $MnSO_4 \cdot 4H_2O$ (0.03 g/L) and YE (0.5%). Medium 4: 0.5% and 1.0% acacia gum	Concn. inoculum: $1.0 \times 10^8$ CFU/mL, pH: 5.0, 5.5 and 6.0	$1.5$ to $3.53 \times 10^9$ CFU/mL	Discontinuous	Vázquez et al. (2009)
<i>L. plantarum</i> ATCC 8014	Media 1 and 2: Sugar cane molasses 3%	Medium 1: 0.5% YE, ammonium citrate and sodium acetate Medium 2: 0.5% YE, sodium acetate, ammonium phosphate and ammonium citrate, 0.1% Tween 80 and 0.005% $MnSO_4$	35 °C, 24 h, 150 rpm, 0.7 vvm	Productivity of medium 1: 0.0507 g/(L×h) Productivity of medium 2: 0.0768 g/(L×h)	Discontinuous	Villavicencio et al. (1999)
<i>L. plantarum</i>	Medium 1: Sugar cane molasses 32 g/L Medium 2: UHT wastewater 45 g/L Medium 3: Sugar cane molasses 32 g/L	YE: 5.0 g/L YE: 5.0 g/L Casein peptone: 10 g/L	<i>Lactobacillus</i> spp.: 37°C, 48 h, 100 rpm, 5-10% oxygen	5.5 g/L	Discontinuous	Vargas et al. (2004)
<i>L. plantarum</i> (NCIMB 11718) and	Juice of <i>Aloe vera</i> variety <i>barbariensis</i> : 25, 50, 75 and 100%		30 °C, 150 rpm, initial pH of medium: 6,5	$1 \times 10^9$ CFU/mL	Discontinuous	González et al. (2008)

**Table 3.** Culture media and fermentation conditions for *Lactobacillus* production.

$\mu_{max}$ : maximum specific speed of growth; OD: optical density;  $X$ : concentration of cellular biomass;  $Y_{P/S}$ : product yield from substrate;  $Y_{X/S}$ : biomass yield from substrate; YE: yeast extract; WP: whey permeate.

Microorganism	Substrate	Supplements	Fermentation conditions	Biomass production	Type of fermentation	References
<i>L. plantarum</i> LPBM10 (isolated from fermented cabbage)	Uchuva pulp and glucose solution (14% p/p)		37 °C, 72 h, microaerophilic conditions	1.64±1.57×10 <sup>9</sup> to 3.23±3.35×10 <sup>9</sup> CFU/mL	Discontinuous	Cortés R (2009)
<i>L. plantarum</i> WS417	Sterile molasses (5%, 10%, 20%, 25% and 30%)		25°C, 30°C and 35°C, 24 h, pH 5.2, 100 rpm	43×10 <sup>8</sup> CFU/mL	Discontinuous	Ossa et al. (2010)
<i>L. plantarum</i> 1 H1 and <i>L. plantarum</i> 1 H2 (isolated from pig large intestine)	Medium 1: MRS	Medium 2: 14 g/L soy milk, 30 g/L wheat bran	32°C, 24 h, without pH control	MRS: 1.0×10 <sup>8</sup> CFU/mL <i>L. plantarum</i> 1 H1 and 4.0×10 <sup>12</sup> CFU/mL <i>L. plantarum</i> 1 H2	Discontinuous	Jurado et al. (2013)
	Medium 2: 20 g/L sucrose, 130 g/L whey	Medium 3: 12 g/L soy milk, 10 g/L wheat bran		Medium 2: Not shown		
	Medium 3: 15 g/L sucrose, 120 g/L whey	Medium 4: 15 g/L soy milk, 15 g/L wheat bran		Medium 3: 1.0×10 <sup>13</sup> CFU/mL <i>L. plantarum</i> 1 H1		
	Medium 4: 10 g/L sucrose, 150 g/L whey			Medium 4: 4.0×10 <sup>14</sup> CFU/mL <i>L. plantarum</i> 1 H2		
<i>L. plantarum</i> CIDCA 83114 (isolated from Kefir grains)	Medium 1: WP with 80% lactose, 6% ash, 3% protein	Galacto-oligosaccharides	37°C, 18 h	Medium 1: 4.75×10 <sup>8</sup> ±7.07×10 <sup>7</sup> CFU/mL, $\mu_{max}$ = 0.110 h <sup>-1</sup>	Discontinuous	Ayelén et al. (2016)
	Medium 2: WP			Medium 2: 4.0×10 <sup>8</sup> CFU/mL		
				$\mu_{max}$ = 0.160 h <sup>-1</sup>		
<i>L. reuteri</i>	Medium 1: Sugar cane molasses 32 g/L	YE: 5.0 g/L	37°C, 48 h, 100 rpm, 5-10% oxygen	4.5-5.5 g/L	Discontinuous	Vargas et al. (2004)
	Medium 2: UHT wastewater 45 g/L					
	Medium 3: Sugar cane molasses 32 g/L	YE: 5.0 g/L				
		YE: 5.0 g/L				
		Casein peptone: 10 g/L				
<i>L. reuteri</i> DSPV002 of porcine origin (ileum)	Whey	<5% YE and 0.003% MnSO <sub>4</sub>	37°C, 18 h		Discontinuous	Fusari et al. (2014)
		5% YE and 0.003 % MnSO <sub>4</sub>				
		<5% YE and 0.003% MnSO <sub>4</sub>				
	Whey	5% YE and 0.003 % MnSO <sub>4</sub>				
<i>L. rhamnosus</i> GG	Milk	YE: 0.3, 0.5 and 1%	37 °C, 100 rpm, controlled pH 6.5	CFU/ mL: 7.94×10 <sup>7</sup> , 6.30×10 <sup>9</sup> $X_{max}$ : 1.2-3.5 g/L $\mu_{max}$ : 0.36-0.92 h <sup>-1</sup>	Discontinuous	Avonts et al. (2004)
<i>L. rhamnosus</i> ATCC 7469	Medium 1: Glucose 50 g/L and YE 60 g/L	KH <sub>2</sub> PO <sub>4</sub> 2.7 g/L; MgSO <sub>4</sub> ·7H <sub>2</sub> O 0.2 g/L; MnSO <sub>4</sub> ·H <sub>2</sub> O 0.05 g/L; Tween-80 1 mL/L; vitamins in solution (g/L): 12.8 pyridoxine HCl; pantothenic acid 1.0, niacin 1.0, riboflavin 1.0 and folic acid 1.0.	37 °C, constant pH 6.9, stirred-tank fermenter, N <sub>2</sub> injection, environment without O <sub>2</sub>	Max cell viability.: 1.3×10 <sup>10</sup> CFU/mL (from 5.86 to 7.61 g/L)	Continuous	Ling et al. (2006)
	Medium 2: Glucose 40 g/L and YE 60 g/L.		Dilution rates: from 0.05 to 0.4 h <sup>-1</sup> ; constant volume 600 mL	$Y_{X/S}$ from 2.9 to 3.7×10 <sup>11</sup> CFU/g glucose (from 0.15 to 0.22 g/g glucose)		
<i>L. rhamnosus</i>	Apple juice concentrate	YE; tryptone; wheat; soy; YE and wheat; YE and soy	Initial pH 6.0, 2L fermenter, 500 rpm, 4.3 L/min air	Medium without supplements: OD <sub>max</sub> =0.21, $\mu_{max}$ =0.27 h <sup>-1</sup> Max. biomass concn. for medium with YE and wheat: OD <sub>max</sub> =0.51, $\mu_{max}$ =0.39 h <sup>-1</sup>	Discontinuous	Champagne and Gardner (2008)
				Medium without supplements: OD <sub>max</sub> =0.21, $\mu_{max}$ =0.13 h <sup>-1</sup> Max. biomass concn. for medium with YE and wheat: OD <sub>max</sub>		
	Molasses	YE; tryptone; wheat; soy; YE and wheat; YE and soy molasses		=0.51, $\mu_{max}$ =0.47 h <sup>-1</sup>		
<i>L. rhamnosus</i> GG	Wastewater from corn nixtamalization		37°C, 48 h	By absorbance (650 nm): 0.770	Discontinuous	G. Ramírez et al. (2013)
<i>Lactobacillus</i> sp. (isolated from pulque)	Medium 1: Whole UHT milk with 7% lactose	Medium 2: 100% salts of MRS medium	23°C, 29°C and 37°C, pH 6.4, without agitation, with 2 and 5% inoculum, 34 h	35 to 42 g/L	Discontinuous	Yépez et al. (2013)
	Medium 2: Whole UHT milk with 7% lactose	Medium 4: 100% salts of MRS Medium 5: 100% salts of MRS medium and 0.25% YE				
	Media 3-6: whey with 7% lactose	Medium 6: 100% salts of MRS medium and 5 g/L YE				



**Figure 3.** Type of substrate and percentage of use in culture media for *Lactobacillus* in the last fifteen years.

of non-centrifugal sugar or solid brown sugar (called *panela* in that country) (Ossa *et al.*, 2010). Likewise, the use of lignocellulosic materials for LAB production with probiotic functionality by submerged fermentation, such as wheat bran, has been reported (Jurado *et al.*, 2013). Obtained *Lactobacillus* may be used to produce probiotics, which can colonize the intestine when consumed orally. This would open new possibilities in the diversification of the feedstocks basis for industrial fermentation processes. Other researchers have proposed the use of cereals like barley and malt for the development of lactic acid bacteria with probiotic potential such as *L. plantarum* NCIMB 8826 and *L. acidophilus* NCIMB 8821 (Rathore *et al.*, 2012). Complex or enriched liquid media are currently being used supplemented with some components that increase the production costs of lactic cultures. Particularly, as can be seen in this review, the nitrogen sources used such as yeast extract, tryptic casein peptone, and meat extract have a high cost in industrial fermentations. Table 3 shows the most widely used substrates in the last twelve years, supplements added to the media, conditions of fermentation, type of fermentation, and data on production of biomass obtained. Table 3 does not report the average biomass yields since these data are scarcely published in the available literature, being risky to make any comparison about the performance of synthetic media related to those based on agro-industrial waste. Considering the final viable biomass concentration (in CFU/mL), the medium based on whey and sucrose as carbon sources, and soy milk and wheat bran as nitrogen sources employed by Jurado *et*

*al.* (2013) allowed reaching the highest concentration of *L. plantarum*. In the case of *L. casei*, the best medium was almost exclusively based on a by-product (or waste) from the dairy industry (Escobar *et al.*, 2010): the cheese whey (see table 3). These outcomes suggest that the utilization of waste materials for media formulation is an attractive way to enhance the performance of LAB cultivation, enabling, at the same time, the decrease in the feedstock costs during production of probiotic microorganisms and starter cultures. In this sense, the evaluation of lignocellulosic hydrolyzates for LAB fermentation could be a promising research trend in the near future. In general, the range of cell concentrations (in CFU/mL) for main LAB presented in table 3 is as follows: *L. acidophilus*  $7.94 \times 10^7 - 6.30 \times 10^9$ , *L. casei*  $7.94 \times 10^7 - 6.30 \times 10^9$ , *L. plantarum*  $4.0 \times 10^8 - 4.0 \times 10^{14}$  and *L. rhamnosus*  $7.94 \times 10^7 - 3.7 \times 10^{11}$ . It should be noted that the papers reviewed for this work reported the data on biomass concentrations with different units (CFU/mL, g/L, Optical Density); this difficulties the comparison among the different media used in those papers.

#### Culture conditions for *Lactobacillus*

The temperature for the cultivation of mesophilic and thermophilic LAB found in this study ranges from 23 to 37°C and 42°C, respectively, with incubation times from 18 to 93 hours and a pH from 5.2 to 6.9. The discontinuous cultivation was the type of fermentation used in almost all the studies reviewed (table 3), which does not allow a more precise evaluation of other alternative regimes that are more efficient like the continuous culture.

The highest production of viable cells ( $4.0 \times 10^{14}$  CFU/mL) mentioned above, corresponded to a batch system for production of two *L. plantarum* strains. More research efforts are required at the bench and pilot levels in continuous and discontinuous fermentations in order to assess the efficiency of alternative cultivation regimes for *Lactobacillus* production.

### Applications of LAB

Lactic acid bacteria are increasingly used in the improvement of human and animal health and nutrition (Senz *et al.*, 2015; Süle *et al.*, 2014). It has been known for several decades that LAB have an inhibitory potential in food against the development of altering microorganisms and pathogens, which is useful for prolonging shelf-life and increasing the hygienic quality of foods (Concha-Meyer *et al.*, 2011; Forney *et al.*, 2004; Siamansouri *et al.*, 2013). Therefore, LAB are widely investigated as natural biological agents because of their antimicrobial potential and as a response to the demand for safe, fresh or minimally processed foods, without preservatives and with a longer shelf-life (Serna & Enríquez, 2013). In general, from the industrial viewpoint, LAB are being applied in the production of antimicrobial substances, sugar polymers, sweeteners, aromatic compounds, vitamins, and are also used as probiotics (Aro & Rojas, 2013; Leroy & De Vuyst, 2004).

The term probiotic was redefined in 2002 by the United Nations Food and Agriculture Organization (FAO) and the World Health Organization (WHO) as a "living microorganism that ingested in the right amounts confers a healthy benefit to the host" (Colombo *et al.*, 2014; Miranda *et al.*, 2013). Fermented dairy products are the most used foods to carry probiotic bacteria. The probiotic strains predominating in these dairy derivatives are *L. acidophilus*, *L. casei*, and *B. lactis* (Geng & Boyer, 2014; Tabasco *et al.*, 2014). These bacteria have been used in recent decades as probiotic supplements in functional food and animal nutrition (Senz *et al.*, 2015). Also, a limited amount of LAB species are used as a starter culture in the meat industry; the main cultures are *L. sakei*, *L. curvatus*, *L. plantarum*, *Pediococcus pentosaceus*, and *P. acidilactic*.

### CONCLUSIONS

Most papers on *Lactobacillus* production are focused on the production of lactic acid and, in some cases, on evaluating the growth of *Lactobacillus* of industrial importance, mainly for human and animal health, and food. Currently, the best production system for LAB corresponds to the submerged fermentation in a batch regime at mesophilic conditions using whole whey or

whey permeate supplemented with nitrogenous materials like yeast extract, soy milk or wheat bran.

Research efforts are being made all over the world to improve the production systems, as well as cultivation methods and media for LAB production. In the case of Colombia, research groups and centers have the responsibility of intensifying their efforts to offer highly efficient technological alternatives to the national industry, that allow the production and application of LAB as a growth factor for the food sector. In this regard, among other aspects, it is necessary to increase the international visibility of Colombian scientific production in this area, as it is evident from the results of this review. Thus, national researchers will contribute to supply the demand for high quality scientific knowledge on starter microorganisms for fermented foods such as meat, dairy and vegetables, among others; as well as on the culture media in which they are grown (including the use of agro-industrial wastes as raw materials). Furthermore, research in prebiotic ingredients or additives derived from LAB allowing the increase of the benefits to the consumer must be continued.

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### REFERENCES

- Aguirre, D., Ramirez, A., Aguilar, J., & Alvarez, M. (2009). Producción de proteína y biomasa probiótica de *Lactobacillus casei* liofilizadas a partir de suero de leche de cabra. *Revista Mexicana de Ingeniería Química*, 8(1), 67-76.
- Alvarez, M., Aguirre, E., Ramírez, A., & Rodríguez, A. (2010). Kinetic analysis and mathematical modeling of growth and lactic acid production of *Lactobacillus casei* var. *rhannosus* in milk whey. *Journal of Dairy Science*, 93(12), 5552-5560.
- Amrane, A. (2005). Analysis of the kinetics of growth and lactic acid production for *Lactobacillus helveticus* growing on supplemented whey permeate. *Journal of Chemical Technology and Biotechnology*, 80(3), 345-352.
- Anonymous. (2005). China Patent No. CN 1842588.
- Anonymous. (2012). China Patent No. CN 101919879.
- Anonymous. (2014a). China Patent No. CN 102747009.

- Anonymous. (2014b). China Patent No. CN 102994644 .
- Anonymous. (2014c). China Patent No. CN 103224895.
- Anonymous. (2015a). China Patent No. CN 103550258 .
- Anonymous. (2015b). China Patent No. CN 103275921 .
- Anonymous. (2016a). China Patent No. CN 103154235 .
- Anonymous. (2016b). China Patent No. CN 103857297 .
- Argyri, A. A., Zoumpopoulou, G., Karatzas, K., Tsakalidou, E., Nychas, G., Panagou, E., & Tassou, C. (2013). Selection of potential probiotic lactic acid bacteria from fermented olives by in vitro tests. *Food Microbiology*, 33(2), 282-291.
- Aro, J., & Rojas, E. (2013). Efecto de cultivos iniciadores en la proteólisis y su característica sensorial en salchichas fermentadas. *Revista de Investigaciones Altoandinas*, 15(01), 13-22.
- Ávila, J., Ávila, M., Tovar, B., Brizuela, M., Perazzo, Y., & Hernández, H. (2010). Probiotic Properties of Strains of *Lactobacillus* Genus extracted from intestinal tract of farm Animals. *Revista Científica, Facultad de Ciencias Veterinarias, Universidad del Zulia*, 20(2), 161-169.
- Avonts, L., Van Uytven, E., & De Vuyst, L. (2004). Cell growth and bacteriocin production of probiotic *Lactobacillus* strains in different media. *International Dairy Journal*, 14(11), 947-955.
- Axelsson, L. (1998). Lactic Acid Bacteria: Classification and Physiology. In: *Salminen S & von Wright A (Eds) Lactic acid bacteria. Microbiology and functional aspects*. (2<sup>nd</sup> ed., pp. 1-72). New York: Marcel Dekker.
- Axelsson, L. (2004). Lactic acid bacteria: Classification and physiology. In S. Salminen & A. von Wright (Eds.), *Lactic Acid Bacteria. Microbiological and Functional Aspects* (3rd ed ed., Vol. 139, pp. 1-66). New York: Marcel Dekker.
- Ayelén, H., Bruno, F., & Golowczyc, M. (2016). Whey permeate containing galacto-oligosaccharides as a medium for biomass production and spray drying of *Lactobacillus plantarum* CIDCA 83114. *LWT-Food Science and Technology*, 69, 185-190.
- Berbegal, C., Benavent-Gil, Y., Pardo, I., & Ferrer, S. (2015). A novel culture medium for *Oenococcus oeni* malolactic starter production. *Food Science and Technology*, 64(1), 25-31. doi: <http://dx.doi.org/10.1016/j.lwt.2015.05.020>.
- Blais, A. (2013). Canada Patent No. CA 2598539 .
- Bojrab, G. (2011). United States Patent No. US 7901925 B2.
- Bovo, F., Franco, L. T., Rosim, R. E., & Oliveira, C. A. (2014). Ability of a *Lactobacillus rhamnosus* strain cultured in milk whey based medium to bind aflatoxin B1. *Food Science and Technology (Campinas)*, 34, 566-570.
- Cizeikiene, D., Juodeikiene, G., Paskevicius, A., & Bartkiene, E. (2013). Antimicrobial activity of lactic acid bacteria against pathogenic and spoilage microorganism isolated from food and their control in wheat bread. *Food Control*, 31(2), 539-545.
- Colombo, M., Zimmermann de Oliveira, A. E., Fernandes de Carvalho, A., & Nero, L. A. (2014). Development of an alternative culture medium for the selective enumeration of *Lactobacillus casei* in fermented milk. *Food Microbiology*, 39, 89-95. doi: <http://dx.doi.org/10.1016/j.fm.2013.11.008>.
- Concha-Meyer, A., Schöbitz, R., Brito, C., & Fuentes, R. (2011). Lactic acid bacteria in an alginate film inhibit *Listeria monocytogenes* growth on smoked salmon. *Food Control*, 22(3), 485-489.
- Connolly, E. (2008). United States Patent No. US7344867 .
- Cortés R, M. (2009). Evaluación de la viabilidad de crecimiento de la cepa nativa *Lactobacillus plantarum* LPBM10 y la cepa comercial *Lactobacillus casei* ATCC 393 en pulpa de uchuva y en solución isotónica de glucosa. *Vitae*, 16(2), 210-217.
- Champagne, C. P., & Gardner, N. J. (2008). The growth and recovery of an exopolysaccharide producing *Lactobacillus rhamnosus* culture on growth media containing apple juice or molasses. *Journal of General and Applied Microbiology*, 54(4), 237-241.
- Chen, Li, Shu Guowei , & Wang Yong. (2013). United States Patent No. CN 102952772.
- Choi, J. C., Choi, J. H., & Choi, S. H. (2013). Korea Patent No. EP 2316278 .
- Day, P., & Boiser, P. (2013). United States Patent No. US 8613964 .
- Dobrogosz, W. J., & Lindgren, S. E. (2006). Sweden Patent No. EP 0698347 .
- Elli, M., Zink, R., Marchesini-Huber, B., & Reniero, R. (2002). United States Patent No. US 6340585.
- Escobar, L., Rojas, C., Giraldo, G., & Padilla, L. (2010). Evaluación del crecimiento de *Lactobacillus casei* y producción de ácido láctico usando como sustrato el suero de leche de vacuno. *Revista de Investigación Universidad del Quindío*, 20, 42-49.
- Farmer, S. (2014). United States Patent No. US 8697055.
- Forney, L. J., Zhou, X., & Brown, C. J. (2004). Molecular Microbial Ecology: land of the one-eyed king. *Current Opinion in Microbiology*, 7(3), 210-220.
- Fusari M., Blajman J., Astesana D., Berisbil A., Rossler E., Soto L., Zbrun M., . . . L., Frizzo. (2014). Evaluación del crecimiento de la cepa porcina probiótica *Lactobacillus reuteri* DSPV002C en medios de cultivos derivados de subproductos de la industria láctea. Artículo presentado en el VII Congreso de Producción Porcina del Mercosur, XII Congreso Nacional de Producción Porcina., Argentina.
- Gänzle, M. G. (2015). Lactic metabolism revisited: metabolism of lactic acid bacteria in food fermentations and food spoilage. *Current Opinion in*



- Food Science*, 2, 106-117. doi: <http://dx.doi.org/10.1016/j.cofs.2015.03.001>.
- Garner, M. R., & Flint, J. (2011). United States Patent No. US 7888062 .
- Geng, J., & Boyer, M. (2014). Advancing analytical microbiology in the dairy industry. *New Food*, 22. [www.newfoodmagazine.com/advent-calendar/advancing-analytical-microbiology](http://www.newfoodmagazine.com/advent-calendar/advancing-analytical-microbiology).
- González, B., Domínguez, R., & Alcocer, B. (2008). Aloe vera como sustrato para el crecimiento de *Lactobacillus plantarum* y *L. casei* CyTA - *Journal of Food*, 6(2), 152-157.
- Hongxing, Z. (2015). China Patent No. CN 104560799.
- Hoshi, R., Matsui, A., & Suzuki, T. (2016). United States Patent No. US 9265270.
- Jurado, G. H., Ramírez, C., & Aguirre, D. (2013). Cinética de fermentación de *Lactobacillus plantarum* en un medio de cultivo enriquecido como potencial probiótico. *Revista de la Facultad de Medicina Veterinaria y de Zootecnia*, 7(2), 37-53.
- Kimmel, S. A., & Roberts, R. F. (1998). Development of a growth médium suitable for exopolysaccharide production by *Lactobacillus delbrueckii* ssp. *bulgaricus* RR. *International Journal of Food Microbiology*, 40(1), 87-92.
- Lee, K., Kim, H.-J., & Lee, E.-J. (2013). Mixed cultures of Kimchi lactic acid bacteria show increased cell density and lactate productivity. *African Journal of Biotechnology*, 12(25).
- Leroy, F., & De Vuyst, L. (2004). Lactic acid bacteria as functional starter cultures for the food fermentation industry. *Trends in Food Science & Technology*, 15(2), 67-78. doi: <http://dx.doi.org/10.1016/j.tifs.2003.09.004>.
- Li, L., & Liu, L. (2014). China Patent No. CN 104212743.
- Ling, L. S., Mohamad, R., Rahim, R. A., Wan, H. Y., & Ariff, A. B. (2006). Improved production of live cells of *Lactobacillus rhamnosus* by continuous cultivation using glucose-yeast extract medium. *Journal of Microbiology Seoul*, 44(4), 439.
- López, J., Sánchez, D., & Rochin, K. (2014). United States Patent No. US 8748124 .
- Magaña, A., Jimenez, R., González, N., Chab, C., & Zetina, Z. (2008). Crecimiento de biomasa probiótica en sustratos naturales. Paper presented at the Semana de Divulgación y Video Científico 2008. <https://scholar.google.com/citations?bav=on.2,or&bvm=bv.127984354,d.dmo&biw=1138&bih=548&dpr=1.2&um=1&ie=UTF-8&lr&q=related:P0qK0CEZJvOzcM:scholar.google.com/>
- Miranda, R. O., Neto, G. G., de Freitas, R., Fernandes de Carvalho, A., & Nero, L. A. (2013). Enumeration of bifidobacteria using Petrifilm™ AC in pure cultures and in a fermented milk manufactured with a commercial culture of *Streptococcus thermophilus*. *Food Microbiology*, 28(8), 1509-1513. doi: <http://dx.doi.org/10.1016/j.fm.2011.07.002>.
- Ossa, J. A., Vanegas, M. C., & Badillo, Á. M. (2010). Evaluación de la melaza de caña como sustrato para el crecimiento de *Lactobacillus plantarum* Revista U.D.C.A Actualidad & Divulgación Científica, 13(1), 97-104.
- Parra, R. (2010). Bacterias acidolácticas: papel funcional en los alimentos *Biotecnología en el Sector Agropecuario y Agroindustrial*, 8(1), 93-105.
- Popa, R., & Nealsen, K. H. (2014). United States Patent No. US 8815539 .
- Ramírez, G., Reyes, M., & Cruz, G. (2013). Estudio del nejayote como medio de crecimiento de probióticos y producción de bacteriocinas. *Revista Mexicana de Ingeniería Química*, 12(3), 463-471.
- Ramírez, J. C., Ulloa, P. R., Velázquez, M. Y., González, J. A., & Romero, F. (2011). Bacterias lácticas: Importancia en alimentos y sus efectos en la salud. *Revista Fuente*, 2(7).
- Rathore, S., Salmerón, I., & Pandiella, S. (2012). Production of potentially probiotic beverages using single and mixed cereal substrates fermented with lactic acid bacteria cultures. *Food Microbiology*, 30(1), 239-244.
- Senz, M., Van, L., Bader, J., & Stahl, U. (2015). Control of cell morphology of probiotic *Lactobacillus acidophilus* for enhanced cell stability during industrial processing. *International Journal of Food Microbiology*, 192, 34-42. doi: <http://dx.doi.org/10.1016/j.ijfoodmicro.2014.09.015>.
- Serna, L., & Enríquez, C. E. (2013). Antimicrobial activity of *Weissella confusa* and its metabolites against *Escherichia coli* and *Klebsiella pneumoniae*. *Revista Colombiana de Biotecnología*, 15(2), 63-69.
- Siamansouri, M., Mozaffari, S., & Alikhani, F. E. (2013). Bacteriocins and lactic acid bacteria. *Journal of Biology*, 2(5), 227-234.
- Stieglmeier, M., Wirth, R., Kminek, G., & Moissl-Eichinger, C. (2009). Cultivation of anaerobic and facultatively anaerobic bacteria from spacecraft-associated clean rooms. *Applied and Environmental Microbiology*, 75(11), 3484-3491.
- Süle, J., Körösi, T., Hucker, A., & Varga, L. (2014). Evaluation of culture media for selective enumeration of bifidobacteria and lactic acid bacteria. *Brazilian Journal of Microbiology*, 45, 1023-1030.
- Tabasco, R., Fernández de Palencia, P., Fontecha, J., Peláez, C., & Requena, T. (2014). Competition mechanisms of lactic acid bacteria and bifidobacteria: Fermentative metabolism and colonization. *LWT-Food Science and Technology*, 55(2), 680-684. doi: <http://dx.doi.org/10.1016/j.lwt.2013.10.004>.
- Topisirovic, L., Kojic, M., Fira, D., Golic, N., Strahinic, I., & Lozo, J. (2006). Potential of lactic acid bacteria



- isolated from specific natural niches in food production and preservation. *International Journal of Food Microbiology*, 112(3), 230-235.
- Vargas, E. M., Gómez, C. J., Parra, M. E., & Romero, M. A. (2004). Producción de microorganismos probióticos como aditivo para alimentos concentrados para ganado vacuno (primera parte). *Revista de Ingeniería Universidad de los Andes*(19), 166-176.
- Vásquez, S. M., Suárez, H., & Zapata, S. (2009). Utilización de sustancias antimicrobianas producidas por bacterias ácido lácticas en la conservación de la carne. *Revista Chilena de Nutrición*, 36(1), 64-71.
- Villavicencio, A., Anna, S., Ernani, S., & Tôrres, R. C. (1999). Produção de *Lactobacillus plantarum* em Melaço de Cana-de-açúcar. *Brazilian Archives of Biology and Technology*, 42(2), 0-0.
- Waldir, E., Rychtera, M., Melzoch, K., Quillama, E., & Egoavil, E. (2007). Producción de ácido láctico por *Lactobacillus plantarum* L10 en cultivos batch y continuo. *Revista Peruana de Biología*, 14(2), 271-276.
- Yépez, B. C., Ballinas, A. M., Herrera, E. M. S., Lobato, A. C. Z., Hernández, I. C., Villanueva, A. L. A., & Brito, R. S. (2013). Formulación y optimización de un medio de cultivo económico para *Lactobacillus* con potencial probiótico aislado del pulque. *Revista de Investigación de la Universidad Simón Bolívar*(12), 133-144.
- Yun, Y., Xilin, H., & Chao, X. (2015). China Patent No. CN 104531596.