Microbiología

Artículo Original

Response of microencapsulated *Lactobacillus casei* to in-vitro conditions that simulate the gastrointestinal environment and inhibitory potential on *Staphylococcus aureus*

Respuesta de *Lactobacillus casei* microencapsulado a condiciones in vitro que simulan el ambiente gastrointestinal y potencial inhibitorio sobre *Staphylococcus aureus*

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Fecha de recibido: 26 de octubre de 2023 - **Fecha de aceptado:** 21 de abril de 2024
ISSN: 0121-0319 | **eISSN:** 1794-5240

Abstract

Introduction: from a microbiological point of view, *Staphylococcus aureus* is one of the main contaminants causing foodborne illnesses, with symptoms such as nausea, vomiting, diarrhea, abdominal cramps, joint or back pain and fatigue. Bacterial resistance of pathogenic bacteria has recently been found to be a public health problem. An alternative is the use of microencapsulated probiotics for the inhibition of pathogenic microorganisms such as *Lactobacillus* casei. **Objective:** evaluate microencapsulated *Lactobacillus casei* ATCC 393® under *in-vitro* conditions simulating the gastrointestinal environment and the inhibitory potential on *Staphylococcus aureus* ATCC BAA 1708®. **Materials and methods:** reconstitution, seeding and adjustment of the inoculum; antibiogram of the two bacterial strains; fermentation kinetics of *Lactobacillus casei*; identification of peptides, amino acids and lactic acid of the supernatant; resistance of *Lactobacillus casei* to different temperatures (37 °C and 45 °C); microencapsulation of *Lactobacillus casei*; study, characterization and exposure to simulated gastrointestinal conditions of the microencapsulate after 90 days of storage and production of Exopolysaccharides. **Results:** the results indicate inhibitory action of the *Lactobacillus casei* strain against pathogenic bacteria; exponential phase at 15 hours (MRS culture medium) and 18 hours (PRO culture medium); results of the microencapsulation study and analysis: viability 100 %; efficiency 84,64 %; humidity 4,0 %; solubility 99,8 %; wettability 2 min with 22 seconds; water activity 0,617 and particle size between 2,10 µm and 5,28 µm. **Conclusion:** it was concluded that the microencapsulated *Lactobacillus casei* showed inhibitory properties against the pathogenic strain.

Keywords: Foodborne Diseases. Lactobacillales. *Lactobacillus casei*. Prebiotics. Probiotics. *Staphylococcus aureus.*

¿Cómo citar este artículo? Cerón-Córdoba JF, Bolaños-Bolaños JC, Jurado-Gámez H. Response of microencapsulated *Lactobacillus casei* to in-vitro conditions that simulate the gastrointestinal environment and inhibitory potential on *Sthaphylococcus aureus*. MÉD.UIS. 2024;37(2): 9-22. DOI: https://doi.org/10.18273/revmed.v37n2-2024001

Resumen

Introducción: desde un punto de vista microbiológico, *Staphylococcus aureus* es uno de los principales contaminantes causante de enfermedades de trasmisión alimentaria, con síntomas como náuseas, vómitos, diarrea, calambres abdominales, dolor articular o de espalda y fatiga. Recientemente, se ha encontrado resistencia bacteriana de bacterias patógenas siendo un problema de salud pública. Una alternativa es la utilización de probióticos microencapsulados para la inhibición de microorganismos patógenos como *Lactobacillus casei*. **Objetivo:** evaluar *Lactobacillus casei* ATCC 393® microencapsulada bajo condiciones in vitro que simulan el ambiente gastrointestinal y potencial inhibitorio sobre *Staphylococcus aureus* ATCC BAA 1708®. **Materiales y métodos:** reconstitución, siembra y ajuste de inóculo; antibiograma de las dos cepas bacterianas; cinética de fermentación de *Lactobacillus casei*; identificación de péptidos, aminoácidos y ácido láctico de sobrenadante; resistencia de *Lactobacillus casei* a diferentes temperaturas (37 °C y 45 °C); microencapsulación de *Lactobacillus casei*; estudio, caracterización y exposición a condiciones gastrointestinales simuladas del microencapsulado después de 90 días de almacenamiento y producción de Exopolisacáridos. **Resultados:** los resultados indican acción inhibitoria de la cepa *Lactobacillus casei* frente a la bacteria patógena; fase exponencial a las 15 horas (medio de cultivo MRS) y a las 18 horas (medio de cultivo PRO); resultados del estudio y análisis del microencapsulado: viabilidad 100 %; eficiencia 84,64 %; humedad 4,0 %; solubilidad 99,8 %; humectabilidad 2 min con 22 segundos; actividad de agua 0,617 y tamaño de partícula entre 2,10 µm y 5,28 µm. **Conclusión**: se concluyó que *Lactobacillus casei* microencapsulado presentó propiedades inhibitorias frente a la cepa patógena.

Palabras clave: Enfermedades transmitidas por alimentos. Lactobacillales. *Lactobacillus* casei. Prebióticos. Probióticos. *Staphylococcus aureus*.

Introduction

Food safety issues arise from food contamination, which can stem from various sources found in the environment, such as water, dust, soil, insects, and feces from mammals, birds, and reptiles[1]. These contaminants have the potential to impact agricultural production, food processing, and food preparation processes, giving rise to Foodborne Diseases (FBD), it is important to note that all types of food, including vegetables, fruits, meat, milk and its products, sausages, fish, and ready-to-eat foods, can serve as potential vehicles for the transmission of FBD[2].

The World Health Organization (WHO) reports that 420 000 people die annually from FDB, which is caused mainly by Gram-negative bacteria and Gram-positive bacteria[3]. By 2020 in Europe, there were 3166 outbreaks of FDB, causing 22 010 illnesses, 1838 hospitalizations and 48 deaths, in the United States for the same time period there were 299 outbreaks, causing 5987 illnesses, 641 hospitalizations and fourteen deaths[4]. By 2020 in Colombia, 64,5 % (312/483) of the FBD outbreaks were sampled and 35,2 % (110/312) were identified by etiological agent, mainly *Salmonella* spp, *Escherichia coli, Staphylococcus aureus*, fecal coliforms, and total coliforms, and the symptoms presented in outbreaks of bacterial etiology were diarrhea (92,9 %), cramps (59,5 %), nausea and emesis (45,2 %), abdominal pain (16,7 %), headache (16,7 %), bloody diarrhea (14,3 %), fever (9,5 %), among others (10 %)[5].

A FBD outbreak is identified when there are two or more cases with similar symptoms and is caused by the consumption of a common food[6]. Once contaminated food with FDB is ingested, elderly people, children, and pregnant women experience more severe gastrointestinal and clinical symptoms such as nausea, vomiting, diarrhea, abdominal cramps, joint pain or back pain, and fatigue[7]. It is important to mention that biological factors related to FDB exist as saprophytic or ubiquitous microorganisms, in general, the bacteria involved are *S. aureus, E. coli*, *Salmonella* and *Listeria monocytogenes*[8]. Authors mention the evolutionary development of these microorganisms better adapted to higher stress scenarios, one of the most important is Antimicrobial Resistance (AMR), especially *S. aureus* resistant to metacilin (SARM) resistant to beta-lactam antibiotics and only sensitive to fifth generation cephalosporins[9].

S. aureus FBD is characterized by being an important cause of systemic infections, being the microorganism with the highest morbidity and mortality, a growing public health problem[10]. In Colombia, *S. aureus* is one of the main bacteria causing intramammary infections in dairy cattle, in these productions there is close contact between milkers, cattle and the zoonotic potential of *S. aureus* that represents a FBD[11]. In addition, about 40 % of raw milk is still produced under conditions of low safety, which can spread this pathogen in the general population[12].

The Lactic Acid Bacteria (LAB) strains *Pediococcus pentosaceus, Enterococcus faecium*, *Leuconostoc mesenteroides* subsp. *mesenteroides* and *Lactobacillus casei*, were classified as having high inhibitory capacity against *E. coli, L. monocytogenes, S. aureus.* and *P. pentosaceus*[13]. Research shows that strains of *Lactobacillus* genus, such as *L. casei,* release peptides with high radical-binding and antimutagenic activity, which have demonstrated good response in animal feeding and efficacy in treatments for *Helicobacter pylori* infections and inhibition of pathogenic bacteria such as *L. monocytogenes, E. coli* O157:H7, *Salmonella* spp., *S. aureus,* and SARM in in-vitro tests[14, 15]. *L. casei* is a well-researched species due to its commercial, industrial, and health potential. It is commonly used to ferment dairy products, resulting in foods with enhanced taste and texture. Additionally, it has been found to produce numerous bioactive metabolites that can provide health benefits to the consumer[15, 16]. *Lactobacillus* bacteria offer a potential solution to address AMR, recognized as probiotics by the FAO and WHO, these small living organisms, when administered in adequate concentrations, promote intestinal health and host development through fermentation by-products, pH regulation, organic acids (lactic and acetic), EPS, and bacteriocins, which are polypeptide substances[17, 18].

Currently, probiotics are widely used in the manufacture of fermented dairy products, fruit, meat, sausages, fish, freeze-dried and functional foods. It is also increasing as an alternative for lactose-intolerant people and in vegetarian diets[19]. However, the application of probiotics is limited since it must face several adverse conditions (environmental, gastrointestinal, and industrial processes) that affect viability and survival[20].

Therefore, the microencapsulation of probiotics is used in biotechnology and refers to cover substances or elements for protection against temperature, pH, enzyme activity and controlled release of viable probiotic cells[17]. The characteristics of the microencapsulation may vary according to the microencapsulating material or matrix and matrices recognized as prebiotics such as maltodextrin and inulin that are generally used[20, 21].

Maltodextrin is chosen as a microencapsulating agent due to its solubility in water, low viscosity, and clear solution. Additionally, it is easily digested. This allows microencapsulated probiotics to be released and influenced by the gastrointestinal system during digestion. On the other hand, inulin, a fructooligosaccharide with slightly branched structure, is composed of fructose units linked by β-(2-1) bonds[21]. Inulin, partially soluble in water, resists human digestion due to its glycosidic bonds. Nevertheless, it supports intestinal microorganism growth. Consequently, inulin acts as a colonic release biopolymer, maintaining its integrity during passage through the upper digestive tract and releasing bioactive compounds in the colon through enzymatic and fermentative processes[20, 21].

Intestinal cell cultures are valuable tools for assessing the potential of probiotic bacteria and prebiotics in terms of their ability to adhere to intestinal cells, modulate immune function, and promote intestinal health[22].

Lactic Acid Bacteria (LAB) microencapsulation involves creating a protective barrier to enhance probiotic activity[20]. The objective of the study was to evaluate microencapsulated *Lactobacillus casei* ATCC 393® under *in-vitro* conditions simulating the gastrointestinal environment and its inhibitory potential on *Staphylococcus aureus* ATCC BAA 1708®.

Materials and methods

This research was carried out in the PROBIOTEC-FORAPIS Research Group Laboratory located in the teaching laboratory block and in the specialized laboratories of the University of Nariño in the city of Pasto, located in the department of Nariño, Colombia, for year 2022.

The *L. casei* and *S. aureus* strains were used for the study. The strains were reconstituted, sown, and inoculated[23]. The strains were purchased from the distributor MDM cientifica.

Test inhibition L. casei against S.aureus and antibiogram

To evaluate the inhibition of *L. casei* against *S. aureus* different methods were used, including impregnated agar discs, method pads with supernatant, diffusion in plastic cylinder with supernatant, and diffusion in double-layer plastic cylinder with supernatant. These methods were conducted under various conditions such as pH 6, filtered (F), unfiltered (UF), heat exposure at a temperature of 80°C for a duration

of 10 minutes, both with and without thermal processing[24, 25]. The antibiotic resistance profiles of the *L. casei* and *S. aureus* strains were determined with specific antibiotics[26]. Test performed prior to microencapsulation.

Fermentation Kinetic:

Fermentation kinetics were studied using MRS culture medium and PRO medium for the growth of *L. casei*. Colony Forming Units (CFU/mL)[27], sugars consumed (mg/L)[28], protein production (mg/L)[29], pH, and lactic acid percentage.

The specific rate of growth was estimated with equation 1, The cell duplication time (dt) was calculated by equation 2, the generation time (*g*) was calculated using equation 3, the growth rate (K) was calculated using equation 4, units are expressed in generations/hour (K), and the maximum harvest was calculated by equation 5.

$$y = (mx + b) \quad (1)$$

$$dt = \left(\frac{Ln2}{vmax}\right) \quad (2)$$

$$g = \frac{0{,}693}{\mu} \quad (3)$$

$$K = \frac{1}{g} \quad (4)$$

$$M = Mt - M0 \quad (5)$$

Peptide identification, lactic acid analysis, an amino acid profiling

Peptide identification, lactic acid analysis, and amino acid profiling were conducted using HPLC. The peptide profile was determined by HPLC, while lactic acid was measured from the filtered supernatant. Amino acid profiling was carried out for *L. casei* and *S. aureus* grown in MRS and BHI broth, respectively. *L. casei* growth at 37 °C and 45 °C: *L. casei* growth was analyzed under two different temperatures[30].

Microencapsulation by spray drying:

L. casei microencapsulation by spray drying, study, and characterization. A 500 mL solution with a concentration of 10 % w/v was prepared for *L. casei*. The process utilized a Spray Dryer Bilon 6000s, operating at an input temperature of 170 °C and an output temperature of 67 °C for a 4-hour cycle. The resulting microencapsulated product was sterilized, stored in metallized Ziploc bags, and kept at room temperature (19+2 °C)[31, 32]. To evaluate the microencapsulated material and the encapsulant binary matrix, some aspects were used as stability criteria: viability, efficiency, humidity, water activity, solubility, wettability, morphology, and particle size[31, 32].

The microencapsulated material was evaluated after 90 days of storage. Viability percentage (equation 6)[32], and efficiency[33], were determined using specific equations 6 and equation 7.

$$\%Viability = \left(\frac{N}{N_0}\right) x100 \quad (6)$$

$$\%Efficiency = \left(\frac{A - B}{A}\right) x100 \quad (7)$$

Humidity was measured using a moisture analyzer, and water activity was determined with Hygrolab Rotronic team (Nürnberg, Germany). Solubility was evaluated by measuring the remaining solids after centrifugation and equation 8. Wettability was determined using the static wetting method[32].

$$\%Solubility = \left(\frac{mi - mf}{mi}\right) x100 \quad (8)$$

Simulated gastrointestinal

Microencapsulated *L. casei* was also subjected to simulated gastrointestinal conditions to evaluate its behavior[34, 35]. The process involved exposure to lysozyme activity, pepsin, NaCl, HCl, pancreatin, bile, pH (2,5-6,8) and NaOH. Bacterial viability was assessed, and plaque counts were performed[27]. Exopolysaccharide production was examined at different temperatures and time periods (28±2 °C / 7 days; 35±2 °C / 48 hours and 42±2 °C / 24 hours), the presence of EPS was determined by the appearance of mucoid colonies and confirmed through alcohol mixing[36, 37].

Intestinal adhesion assays will be conducted to assess the adhesion of *L. casei* and *S. aureus* using the mucin from stomach porcine-Type III medium (Sigma-Aldrich)[38]. The process will involve activation of bacterial strains, harvesting and purification of

cells (9000 rpm for 10 min at 4 °C), dilution of biomass with 1 mL of sterile saline solution, and evaluation of cell adhesion (80 µL of prepared sample and anaerobic incubation for 24 hours at 37 °C).

After incubation, non-adherent bacteria will be removed, samples will be stained using the Giemsa stain[39] and they will be observed under the microscope to analyze adhesion to mucin.

Statistical design: A descriptive evaluation of the results was carried out to determine their mean and standard error. Each variable had 5 replicates for its determination.

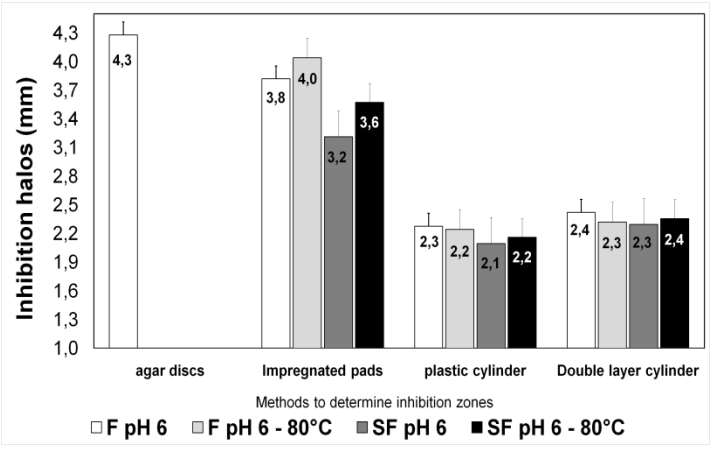
Ethical considerations

As an in-vitro study, it did not undergo ethical committee approval.

Results

The results obtained for the antibiogram in *L. casei* indicated resistance against ampicillin (AM 20 µg), florfenicol (FFC 30 µg), and penicillin (P 10 µg), sensitivity against ciprofloxacin (CIP 5 µg), gentamycin (CN 10 µg) and tetracycline (TE 30 µg).

The results obtained for the antibiogram in *S. aureus* express the strain resistant to cefquinone (CEQ 30 µg), penicillin (PEN 10 µg) and sensitive to amoxicillin (AMC 30 µg), gentamycin (CN 10 µg), doxycycline (D 30 µg), florfenicol (FFC 30 µg), and tetracycline (TE 30 µg). Summary of the inhibiting halos and the respective evaluation methods used under different conditions (See figure 1). The agar disc method was not done with the supernatant but specifically with the lactic strain.



a)

b.

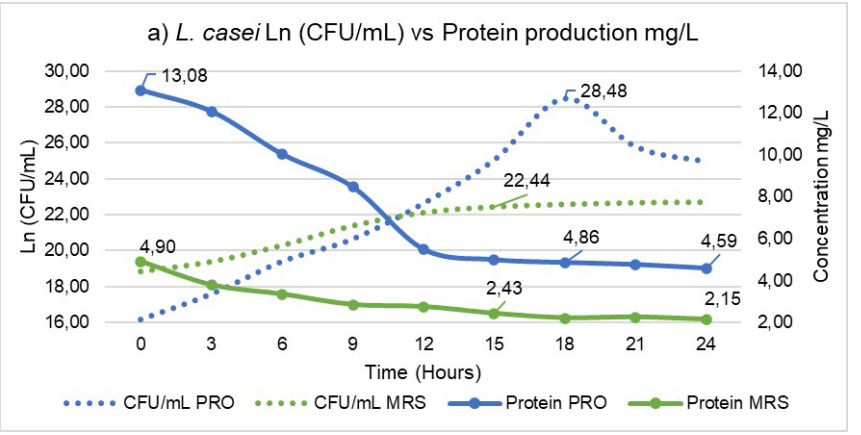
Figure 1. a) Inhibition halo in mm and methods for determining inhibition under different concentrations of L. casei on S. aureus. b) Inhibition halos of L. casei on S. aureus using the agar disk method. *F filtered. **SF Unfiltered.
Source: authors.

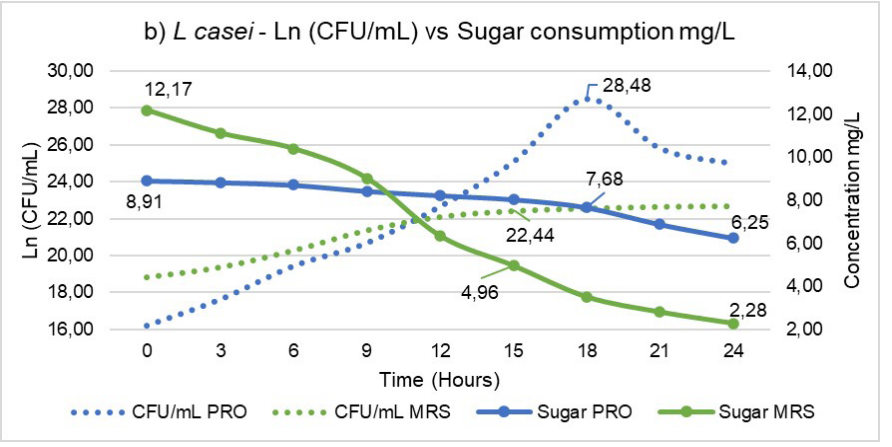
The results indicate that the probiotic strain's supernatant possesses antimicrobial activity under the tested conditions and methods. The agar disc method, with *L. casei*, demonstrated a stronger inhibitory effect against *S. aureus*.

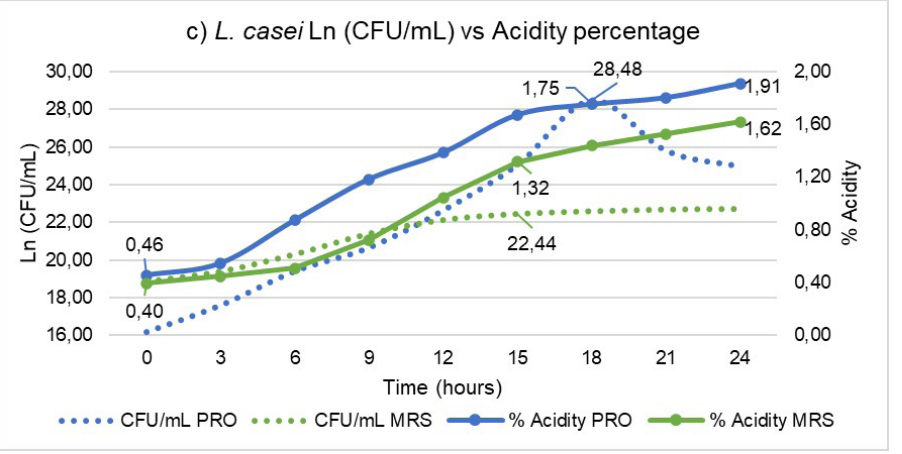
The data obtained for Ln CFU/mL of *L. casei* growth in fermentation kinetics indicate differences between culture media (PRO and MRS) ($p<0,05$) and differences between the times sampled for the PRO medium (9 times) ($p<0,05$). The MRS medium showed no differences between times ($p>9$).

For the MRS medium, the exponential phase is obtained at 15 hours with 22.8 Ln CFU/mL ($5,6x10^9$ CFU/mL); in the PRO medium, the exponential phase is observed at 18 hours with 28.48 Ln CFU/mL ($2,3x10^{12}$ CFU/mL). The results obtained from fermentation kinetics are described below: specific growth rate of 0,264 (MRS) and 0,698 (PRO); cell duplication time

2,63 hours (MRS) and 0,993 hours (PRO); number of generations per hour 0,381 (MRS) and 1,007 (PRO); maximum harvest 3,73 Ln CFU/mL (MRS) and 9,61 Ln CFU/mL (PRO), exponential protein production 2,43 mg/L (MRS) and 4,86 mg/L (PRO) (See figure 2a); consumption of sugars for the exponential phase 4,96 mg/L (MRS) and 7,68 mg/L (PRO) (See figure 2b); acidity 1,32 % (MRS) and 1,75 % (PRO) (See figure 2c); pH, 4,07 (MRS) and 3,86 (PRO), with a pH range between 5,84 and 3,81 (MRS) and 5,82 to 3,74 (PRO) (See figure 2d).







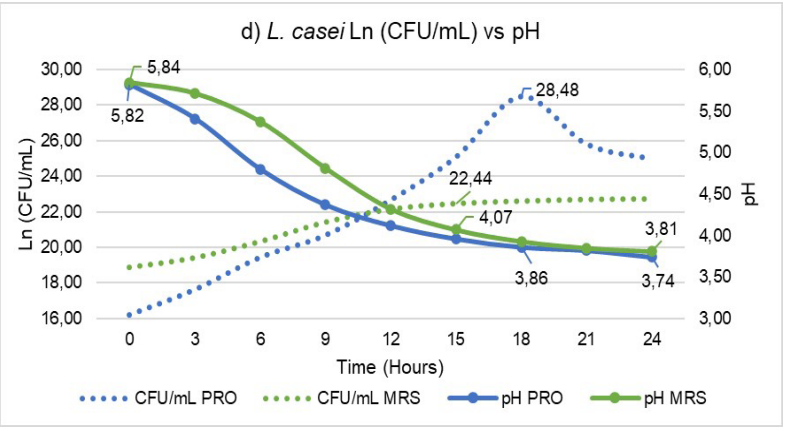


Figure 2. a) Determination of protein production of L. casei in PRO and MRS medium. b) Determination of sugar consumption by Lactobacillus casei in PRO and MRS media. c) Determination of acidity percentage of Lactobacillus casei in PRO and MRS medium. d) Determination of the pH of Lactobacillus casei in PRO and MRS medium.
CFU: Colony Forming Units
Source: authors.

Growth of *L. casei* at 37 °C and 45 °C, statistical analysis indicated differences between 37 °C and 45 °C temperatures (p<0,05). Bacterial growth was recorded for dilutions 10^{-9}, 10^{-10}, 10^{-11}, and 10^{-12}, with values between 1,6x10^{12} CFU/mL and 3,6x10^{14} CFU/mL at 37 °C and values between 1,6x10^{12} CFU/mL and 1,0x10^{13} CFU/mL at 45 °C. The optimal growth temperature for *L. casei* ranges from 35 °C to 40 °C and can tolerate temperatures between 2 °C and 50 °C[40, 41]. The temperature fluctuation in lactic acid production between 29 °C and 42 °C for *L. casei* is reported as not significant[40].

Microencapsulation presented the following results after 90 days of storage: 100 % viability; 84,64 % efficiency; 4,0 % humidity; 99,8 % solubility; 2 min with 22 seconds of wettability; 0,617 water activity; and a particle size ranging between 2,1 µm and 5,28 µm (see figure 3).

The results after exposing the microencapsulated *L. casei* against recreated gastrointestinal conditions are presented in Table 1.

The production of *L. casei* exopolysaccharides in MRS medium at different temperatures and times was positive and was determined by the presence of precipitate in the evaluated samples.

In the assays conducted to evaluate the adhesion capacity of *L. casei*, the quality of negative controls (slides without bacterial cells) and positive controls (*S. aureus*) was initially assessed to verify the purity of the slides and the quality of negative controls. Thus, the adhesion results for *L. casei* demonstrated its adhesion capacity (see figure 4).

Table 1. Bacterial growth of microencapsulated Lactobacillus casei under recreated gastrointestinal environments.

Continuum conventional	**Discontinuous conventional**		
CFU/mL	Lysozyme 10 min	Pepsin+NaCl+ HCl 90 min	Pancreatin+bile+bile salt +NaCl+NaOH 150 min
9,12x10^{11}	1,6x10^{12} CFU/mL	1,33x10^{12} CFU/mL	7,58x10^{11} CFU/mL

CFU: Colony Forming Units
Source: authors.

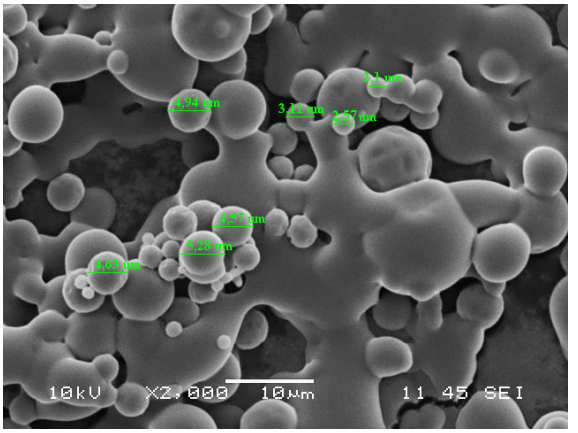


Figure 3. Scanning electronic microphotography of microencapsulated Lactobacillus casei.
Source: authors.

Discussion

Bacteria of the genus *Lactobacillus* resistant to ciprofloxacin, erythromycin, gentamicin, and vancomycin have been detected in isolates from dairy products such as yogurt, white cheese, tulum cheese, cokelek, camız cream, and kefir, dairy products that were collected from several supermarkets in Turkey[42]. Other authors isolated lactic acid strains from neonatal calves (20 to 25 days old) taken from the duodenum, jejunum, and colon, which were reported to be resistant to vancomycin and sulfonamides[43]. Researchers revealed that some resistant LAB (*L. casei, L. plantarum* and *L. helveticus*) to vancomycin and sulfonamide[44], ciprofloxacin, tetracycline, and erythromycin carried chromosomal genes (gyrA, tetM, and ermB), which confer resistance to such antibiotics, and these resistance genes are not transferable because they are at chromosome level[44, 45]. The microorganism are not accepted by any regulatory body to be used as a probiotic if it is shown that there is exogenous resistance and that it is easily transmissible[43, 44], since some strains of lactic acid bacteria could transmit antimicrobial resistance genes to pathogenic bacteria[46].

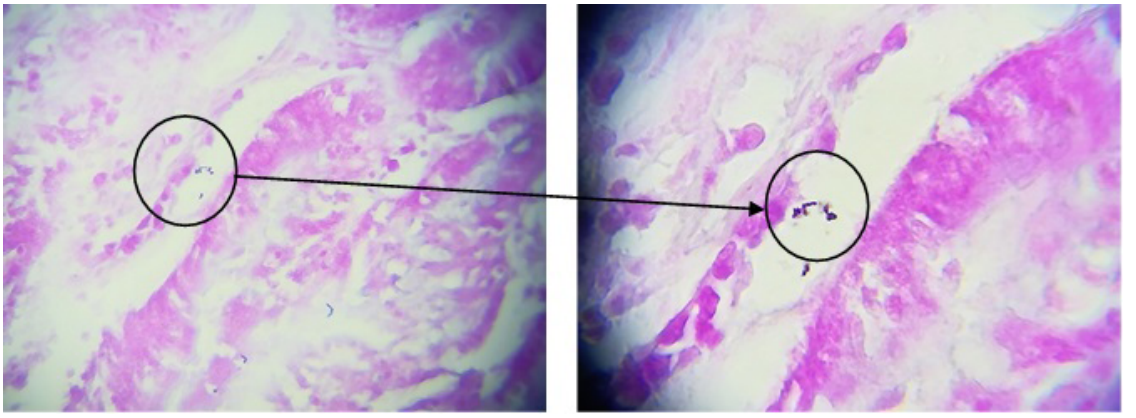


Figure 4. Adhesion Lactobacillus casei on the mucin from stomach porcine-type III medium (Sigma-Aldrich).
Source: authors.

A study in Valledupar (Colombia) in the year 2019, where *S. aureus* strains were isolated from coastal cheese expressed resistance to tetracycline, chloramphenicol, penicillin and erythromycin[47]. It is explained that the mecA gene integrated into the chromosome of the *S. aureus* strain resistant to methicillin is responsible for this characteristic[48]. This property has been improved and multiplied gradually; therefore, infection of this microorganism in the population has increased[49]. The antibiograms obtained in the present investigation are like those mentioned above with some exceptions due to the fact that resistance to some antibiotics is determined by the origin of the strains evaluated, both lactic and pathogenic.

The effectiveness of *L. casei* on the control of *S. aureus* has been demonstrated by indicating that the inhibition produced by lactic strains responds to various mechanisms of LAB survival[50]. One of the inhibition mechanisms is the result of organic acids (lactic acid and acetic acid), which is based on the ability of the non-dissociated form of organic acid to cross the cell membrane and cause lysis[51].

Furthermore, the identification of the caseicin bacteriocin produced by *L. casei*, which affects the biosynthesis of proteins and DNA[52]. *L. casei* has a significant inhibitory effect on *S. aureus*, with the agar disc method being the most effective. Antibiotic resistance profiles are essential to ensure the safety of probiotic strains. These results support the potential application of *L. casei* as a probiotic agent in the prevention of *S. aureus* infections.

The values for cell duplication reported in different investigations are variable, 14,47 minutes for *L. plantarum* in MRS medium[53], 64,38 minutes and 48,41 minutes for *L. gasseri* evaluated in MRS and PRO medium[54], 0,98 hours and 42,42 minutes for *L. platarum* in the media MRS and PRO[55]. In the determination of kinetic parameters of lactic acid bacteria, authors record different values referring to the conditions of microencapsulation, storage period and packaging since they can interfere with each one of the evaluated items.

Around a specific velocity, *L. plantarum* evaluated in MRS and MSL bacterial media (molasses, whey, and yeast) presented values of 0,61 μmax (h^{-1}) and 0,56 μmax (h^{-1}), respectively[56]. Moreover, *L. casei* in culture medium with aloe vera reported 2,7 μmax (h^{-1}) and 2,9 μmax (h^{-1}) in MRS medium[57]. In a culture medium with inulin, values of 0,79 max (h^{-1}) and 0,307 max (h^{-1}) were recorded for the lactic strains *L. acidophilus* and *L. casei*, respectively[58], which are data that are similar to those obtained in this study.

The generations per hour and maximum harvest are parameters that, like those mentioned above, vary depending on culture medium, bacterial strain and factors involved in bacterial growth. Thus, 4,62 data points for the number of generations per hour have been found, with a maximum harvest of 10,3 Ln CFU/150 μL[59].

Data have been reported for protein production and sugar consumption for several lactic species, as follows: *L. gasseri* in MRS and PRO culture media of 0,66 mg/L at 20 hours and 3,12 mg/L at 16 hours[58], on the other hand, *L. plantarum* in MRS and PRO culture media of 1,61 and 1,47 mg/L at 16 hours[54] for protein production. Furthermore, sugar consumption was recorded in MRS and PRO media in the exponential phase 1,79 mg/L (20 hours) and 2,043 mg/L (16 hours), respectively, for *L. gasseri*[60], and for *L. plantarum,* a sugar consumption of 6,98 mg/L in the exponential phase (11:50 hours)[59].

There are reports of lactic acid percentage in *L. platarum* evaluated in MRS medium ranging from 0,17 % in zero hours to 0,41 % in 24 hours[55]. These values are far from those obtained in the study; however, the increase in lactic acid was evidenced. Similarly, it is considered that to obtain good bacterial growth in LAB, the pH has to approach to 5,5 and it is possible that they resist a pH of about 2,0[61]. The bacteriostatic effects of LAB are considered to be related to the production of lactic and acetic acid, as derived from the fermentative metabolism of carbohydrates; in addition, the pH of the medium decreases as an effect of the concentration of organic acids, which correlates with the inhibition of pathogenic microorganisms[62].

It should be noted that the study compares the use of various sources of carbon and nitrogen; therefore, the variables obtained from fermentation kinetics will be affected. Various sources of nutrients influence bacterial metabolic processes, affecting CFU population, bacteriocin synthesis, exopolysaccharides, and antimicrobial activity[62, 63].

L. casei indicated a peptide equivalent to the VAL-TIR-VAL chain at a concentration of 0,56 mg/mL and the detection of lactic acid within the supernatant, characterized by its measured values 27,7 g/L and 29,62 g/L, similar to the value of 30,21 g/L in supernatant of *L. casei*[23]. The amount of peptides and amino acids produced by fermentation may be affected by differentiations within the framework of proteolytic metabolism compounds, where nutritional requirements, intracellular peptidases, and their regulatory methods affect the release into the environment[64].

Researchers reported values of *L. plantarum* microencapsulated as follows: 83,3 % viability, 88,4 % efficiency, 7,97 % and 5,23 % humidity, 0,4 % water activity, 1 min with 56 seconds wettability, and 96 % solubility, and microcapsule dimensions of 35,68 μm and 3,47 μm[60, 65]. The results cited are like those reported in the present research and are within stable ranges, except for water activity, which despite having a high value did not affect the results

of the other parameters. The interaction between the binary matrix and the microencapsulated bacterium demonstrates stability in the structural composition over a 90-day storage period.

The authors evaluated the microencapsulation of *Bifidobacterium* BB-12 by spray drying using reconstituted skim milk, inulin and oligofructose as wall material, the authors noted that inulin exerted a protective effect on the *bifidobacteria* in the encapsulation process and explained this by the possible thermoprotective function that this component exerted on the bacteria subjected to the drying procedure[66].

In the literature review, values close to those obtained are found for *L. platarum* strains with growths between 2,0x10^{9} CFU/150 µL and 3,0x10^{12}CFU/150 µL, and for microencapsulated *L. reuteri,* a bacterial growth equal to 2,2x10^{11} under conditions like those assessed in this investigation[65]. The tests carried out with LAB in the gastrointestinal category depend on the animal species and its physiology. Generally, probiotic strains have to be analyzed under simulated gastrointestinal tract systems in order to determine tolerance to conditions similar to those established by the gastric environment, such as antimicrobial enzymes (lysozymes), low pH, and bile salts[67].

In one study, a new synbiotic functional drink powder was designed with extracts of grape pulp, pomegranate and beet peels, encapsulated *Lactobacillus* casei (quince gum and sodium alginate), the probiotic survival rate in functional drink powders containing free *Lactobacillus* casei was 42,16 % and increased to 86,40 % and 87,56 % in powders containing microcapsules with sodium alliginate and microcapsules with probiotic sodium alliginate-quince gum, respectively. The production yield (10,95-13,16 %), moisture percentage (4,94-5,17 %), solubility (85,25-88,29 %) and wettability (21,56-22,12s), together with the recommended bacterial survival of 10^{7} CFU/g during the 60-day storage period made the powdered beverage a functional symbiotic product[68].

The LAB could produce EPS, whose function is to protect bacteria from factors such as environment and gastrointestinal conditions[14]. The importance of EPS-producing strains is highlighted in the food industry as polymers that improve the viscosity and texture of products[69] and the extraordinary properties of the biopolymers they produce. They do not involve any danger to health, are generally recognized as safe (GRAS), and have antioxidant activity, antibiotic activity, and antitumor activity[70].

Various standards have been put forward for the identification of promising probiotics, such as the ability to adhere to intestinal cells. Due to this adherence capacity, the persistence of probiotic strains in the intestine can be enhanced, allowing them to exert beneficial functions such as balancing mucosal immunity, enhancing cytokine production, IgA secretion, inhibitory substance production, phagocytosis, maturation of intestinal epithelial cells, and nutrient absorption[53].

It should be noted that the intestinal microbiota consists of consortia of bacteria that exert relevant defense activities and are not attached to the epithelium. Instead, they remain active in the intestinal lumen, aiding in waste elimination and neutralization of toxins and pathogens. In this regard, several investigations have indicated that *Lactobacillus* strains possess the ability to impede the attachment of pathogens by hindering their establishment through competitive exclusion, a finely tuned mechanism reliant on both probiotic and pathogenic bacterial strains[22].

Cellular adhesion is a multifaceted procedure that encompasses the interaction between the probiotic strain and mucus, thereby adding complexity to the interplay of long-distance electrostatic and van der Waals forces, in addition to other interactions at shorter ranges[53].

Conclusions

L. casei presented inhibitory action on *S. aureus* in the methods used. The halo of major inhibition was presented by the method of agar discs with 4.3 mm. The microencapsulated material presented good properties after being stored for a period and subjected to gastrointestinal conditions. These properties are reflected in the different stability tests carried out. The shape of the capsule is circular, with diameters between 2.1 µm and 5.28 µm. The kinetic parameters indicated a logarithmic growth phase in hours 18 (PRO mean) and 15 (MRS mean), with bacterial growths equal to 28.48Ln CFU/mL

and 22.44Ln CFU/mL, respectively, and adhesion of *L. casei* on the mucin from stomach porcine-Type III medium.

Acknowledgements

The authors thank the research group PROBIOTEC-FORAPIS of the University of Nariño for the funding of this research project.

Conflict of interest

The authors state that they have no conflict of interest of a financial, professional, academic, or personal nature, which may influence in the presented results.

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