ARTÍCULO DE INVESTIGACIÓN

Lactic Acid Bacteria as Inhibitory Agents of *Escherichia coli* ATCC 25922

Bacterias Ácido Lácticas como Agentes Inhibidores de *Escherichia coli* ATCC 25922

***Kevy Sandrith Ordoñez-Lozano*[*], *Claudia Milena Amorocho-Cruz*[**]**

DOI: 10.15446/rev.colomb.biote.v26n2.112781

ABSTRACT

In the search to generate new natural alternatives that are less harmful to the body, the use of Lactic Acid Bacteria (LAB) has been chosen due to its ability to grow in different conditions and generate antagonism against other pathogenic microorganisms. *Escherichia coli* has been the cause of emerging food outbreaks, especially in children and the elderly who have weak immune systems, being a risk to public health. In addition, this pathogen has been demonstrating decreased sensitivity to antibiotics. The present study sought to evaluate the antimicrobial power of 4 LAB strains isolated from food from the Huila-Colombia region against *Escherichia coli* ATCC 25922 through disc-diffusion and well-diffusion. The strains that inhibited in the presence of cells were L, D (strains isolated from coffee fermentation mass), and 12 (Huila cheese isolate), obtaining inhibition percentages of 37.27±4.15, 32.65±0.97 and 23.47±0.34 for strains L, D and 12, respectively. In the absence of cells, the organic acids of strains L and D obtained from the coffee fermentation process stood out, with inhibition percentages of 27.28±0.27 and 23.63±1.00 respectively. The findings demonstrate the effectiveness of strains native to the region, exalting those obtained from the coffee chain. We performed a multifactorial analysis between LAB and inhibition methods, finding statistically significant differences between methods with $p<0.05$. In order to know the viability of LAB under *in vitro* and *in vivo* conditions, it is important to study other parameters and evaluate the feasibility of using them as a biotherapeutic treatment.
Key words: LAB, Antimicrobial action, Functional food, Probiotic.

RESUMEN

En la búsqueda de generar nuevas alternativas naturales que sean menos dañinas para el organismo, se ha optado por el uso de Bacterias Ácido Lácticas (BAL) debido a su capacidad de crecer en diferentes condiciones y generar antagonismo contra otros microorganismos patógenos. *Escherichia coli* ha sido la causa de brotes alimentarios emergentes, especialmente en niños y ancianos que tienen sistemas inmunitarios débiles, siendo un riesgo para la salud pública. Además, este patógeno ha venido demostrando una menor sensibilidad a los antibióticos. El presente estudio buscó evaluar el poder antimicrobiano de 4 cepas de BAL aisladas de alimentos de la región Huila-Colombia frente a *Escherichia coli* ATCC 25922 mediante difusión en disco y difusión en pozo. Las cepas que inhibieron en presencia de células fueron L, D (Cepas aisladas de la masa de fermentación del café), y 12**)** Aislado del queso del Huila), obteniéndose porcentajes de inhibición de 37,27±4,15, 32,65±0,97 y 23,47±0,34 para las cepas L, D y 12, respectivamente. En ausencia de células, destacaron los ácidos orgánicos de las cepas L y D obtenidos del proceso de fermentación

[*] Agricultural Engineer, Agroindustry-USCO Research Group. Universidad Surcolombiana. Neiva, Huila, Colombia sandrith.ksol@gmail.com. https://orcid.org/0000-0002-0044-4723

[**] PhD in Biotechnology. Agroindustry-USCO research group. Agricultural Engineering Program, Universidad Surcolombiana. Neiva, Huila, Colombia. claudiamilena.amorocho@usco.edu.co. https://orcid.org/0000-0003-3986-5768

del café, con porcentajes de inhibición de 27,28±0,27 y 23,63±1,00 respectivamente. Los resultados demuestran la eficacia de las cepas nativas de la región, superando a las obtenidas de la cadena del café. Realizamos un análisis multifactorial entre BAL y métodos de inhibición, encontrando diferencias estadísticamente significativas entre métodos con $p<0,05$. Para conocer la viabilidad de las BAL en condiciones *in vitro* e *in vivo* es importante estudiar otros parámetros y evaluar la factibilidad de utilizarlas como tratamiento bioterapéutico.

Palabras clave: BAL, Acción Antimicrobiana, Alimento Funcional, Probiótico.

Recibido: febrero 2 de 2024 **Aprobado:** noviembre 3 de 2024

INTRODUCTION

The population has been affected worldwide by a serious public health problem due to Foodborne Diseases (FBD) (Mora, *et al*., 2022). The consumer's health is put at risk by ingesting food and/or beverages contaminated with pathogenic microorganisms due to poor handling, which produces in the consumer different gastrointestinal symptoms, a consequence also brought about by the low content of intestinal microbiota, which generates low defenses in the immune system, and facilitates that pathogenic microorganisms colonize the gastrointestinal tract making it susceptible to diseases produced by toxins generated by pathogenic microorganisms (Zamudio-Vázquez *et al*., 2017) such as diarrhea and vomiting, among the mildest; but there are also others such as septic shock, hepatitis, headaches, fever, double vision, and complications that can lead to death (Gonzales and Rojas 2005; Fernandez *et al*., 2021).

E. coli is Gram-negative, belongs to the enterobacteria, and is one of the best-studied microorganisms. It is widely distributed in soil and water, and, although it is a harmless resident of the gastrointestinal tract, several studies have documented that strains of *E. coli* have been implicated in diarrhea, extraintestinal diseases, and infectious pathologies as common as prostatitis, hepatobiliary infection, abscesses, among others, causing morbidity and mortality worldwide (Sauca *et al*., 1997; Croxen *et al*., 2013; Villacres *et al*., 2022).

Antibiotics are commonly used to fight infections caused by bacteria. Antibiotics are molecules formed occasionally by substances generated during the metabolism of bacteria and fungi mainly, and they can also be elaborated by compounds obtained through chemical synthesis capable of inhibiting or eliminating the growth of pathogenic bacteria (De la Fuente-Salcido *et al*., 2015).

However, over the years it has become evident that gram-negative (pathogenic) bacteria have developed various antibiotic resistance mechanisms such as transduction, conjugation, transformation, transfer, enzymatic modification of antibiotics, efflux pumps, changes in the permeability of the outer membrane and alterations in the site of action. In addition, there are gram-positive species such as *Staphylococcus aureus*, *Enterococcus faecium*, *Enterococcus faecalis* and *Streptococcus pneumoniae*, which are microorganisms that have also presented multiple mechanisms of resistance to the agents used so far (Lozano *et al.,* 2017). This multi-resistance represents a challenge that hinders the therapeutic management of diseases caused by these microorganisms (Gallego-Maldonado *et al*., 2019).

Indeed, *E. coli* has acquired great antibiotic resistance, which is a natural biological phenomenon due to mutations caused by the stimulation of its environment or its adaptation for survival, besides the great capacity of bacteria to horizontally transfer their genetic material, which has been an emerging problem worldwide (Grehs *et al*., 2021; Peñaloza *et al*., 2021).

Due to the resistance generated by this bacterium, other treatment and control alternatives have been sought. For some time, probiotics have been used, among which we find mostly LAB, yeasts, and some molds. The standing out bacteria genera are *Lactobacillus*, *Bifidobacterium*, *Lactococcus*, *Carnobacterium*, *Enterococcus*, *Streptococcus*, *Pediococcus*, *Propionibacterium*, *Leuconostoc* and *Bacillus* species, yeasts such as *Saccharomyces*, and fungi such as *Aspergillus*. Their use can provide the host with certain positive effects such as maintaining the balance of the intestinal microbiota, inhibiting the growth of pathogenic bacteria, synthesizing and improving the bioavailability of nutrients, promoting good digestion such as lactose assimilation, reducing the effect of allergens, reducing cholesterol, stimulating the immune system, increasing resistance to infection (Castillo-Escandón *et al*., 2019; Corrales & Arias, 2020), protecting against gastrointestinal, urogenital, and respiratory tract diseases, balancing the immune system, preventing and treating dermatological diseases, and protecting against colon cancer.

Probiotics are live microorganisms, which consumed in adequate amounts confer health benefits to the host according to the WHO. Bacteria that ferment carbohydrates have been widely studied, producing especially lactic acid in the case of those that are homofermentative, which is why they are called "Lactic Acid Bacte-

ria" (LAB), highlighting *Lactobacillus* and *Bifidobacterium*; these microorganisms are biotherapeutics. One of the most important characteristics of a probiotic is resistance to the conditions of the gastrointestinal system, the ability to adhere to the intestinal epithelium and colonize it for optimal growth (Zamudio-Vázquez *et al*., 2017; Cucalón and Blay, 2020; López *et al*., 2023). LABs can preserve dairy products due to different acid metabolites, among which organic acids such as lactic acid and other compounds such as bacteriocins are found. They are also attributed to preventing the growth of pathogenic microorganisms, stable at different pH and temperatures. The above shows that they are compounds with interesting potential for the food industry, increasing the production of functional foods, including probiotics (Heredia-Castro *et al*., 2017; Fahrurrozi *et al*., 2019).

Given the situations experienced by many populations due to the consumption of contaminated water and poorly handled food, alternatives other than antibiotics are sought since bacteria have generated resistance and adaptability to this type of medicine. Therefore, in this research, we experimented with and evaluated the LAB of Colombian products such as coffee, cheese, and breast milk and their antimicrobial behavior against *E. coli* ATCC 25922.

MATERIALS AND METHODS

Native strains

LAB strains (JC, L, D, 12) stored at -20°C of the Food Microbiology Laboratory collection (Faculty of Engineering, Universidad Surcolombiana) from previous research were activated. LABs L and D were strains isolated from coffee fermentation and came from the research project (Ladino, 2017); LAB 12 came from farmer´s cheese in the work done by Cortés-Macias *et al*. (2016), and LAB JC was obtained from breast milk in the study of Cortés & Amorocho, (2022). The procedure for streaking a plate for isolated colonies involved gently drawing a loopful of inoculum numerous times across the surface (Pollack, et al., 2012) of on Man, Rogosa, and Sharpe agar medium (MRS, Conda, Spain). They were then incubated (Heratherm, IMH60-S, Thermo Scientific, Germany) at 37° C for 24 h, and after that, a triple streak seeding was performed with a platinum loop in MRS medium, the colonies were isolated for surface reseeding with MRS, and again incubated (Heratherm, IMH60-S, Thermo Scientific, Germany) for 24 h. comes

Bacterial strain Escherichia coli ATCC 25922

The pathogen of the reference strain *E. coli* ATCC 25922 (KWIK STIK), was activated by extracting the strain's hydrating liquid, performing a triple streak seeding in PCA medium (Plate Count Agar, Conda, Spain), for the isolation of a pure culture; the plate was incubated (Heratherm, IMH 60-S, Thermo Scientific, Germany) at 37°C for 24 h.

For the order of pathogen growth, part of a pure colony was taken, mass seeding was performed in PCA medium (Plate Count, Conda, Spain), incubated (Heratherm, IMH60-S, Thermo Scientific, Germany) at 37°C for 24 h, a portion of plate growth was taken and deposited in 9 ml of peptone water (Merck, Germany). By using the vortex, it was shaken up to a Mc. Farland 0.5. Next, serial dilutions were performed with volumes of 1000 µl. A volume of 100 µl was surface seeded on plates. Incubation was carried out at 37°C for 24 h. In all cases, seeding was performed in duplicate. Gram staining was used to identify morphology and membrane structure.

Evaluation of the antimicrobial activity of native strains (LAB) against Escherichia coli ATCC 25922

The effect of LAB in the presence and absence of cells was evaluated against the pathogen *Escherichia coli*. The range was measured with a ruler (mm).

Disk Method

The activity of LAB in the presence of cells was determined by the disc diffusion technique (Figure 1). The pathogenic bacterial culture was inoculated and adjusted to 0.5 McFarland scale on standard method agar medium - PCA (Plate Count Agar, Conda, Spain). On the other hand, on MRS agar plates with the growth of each lactic strain, 7 mm diameter discs (JC, L, D, 12) were cut using a sterile punch, a well was made as positive control, which were 50 µl of the antibiotic Ciprofloxacin (Bioquímico Pharma S.A, Colombia), as negative control a disc without growth of 7 mm was deposited, considering the %IH (percentage of inhibition) a value higher than the dimension of the negative control. This procedure was performed in duplicate and the plates were incubated (Heratherm, IMH60-S, Thermo Scientific, Germany) at 37°C for 24 h (Amorocho-Cruz CM, 2011) (Figure 1).

Agar-well Method

Using the agar-well diffusion technique, the behavior of LAB against the pathogen in the absence of cells was observed (Figure 2). For LAB cell filtration, in each case a portion of them (MRS) was extracted, deposited in tubes containing MRS broth, and incubated (Heratherm, IMH 60-S, Thermo Scientific, Germany) at 37° C. After 24h in 2 ml tubes (Eppendorf NEST, China) was added the MRS broth with growth to be taken to centrifuge (Herauspico 17, Thermo Scientific, Germany) at 6000 rpm for 10 minutes. The supernatant was taken, and finally, with

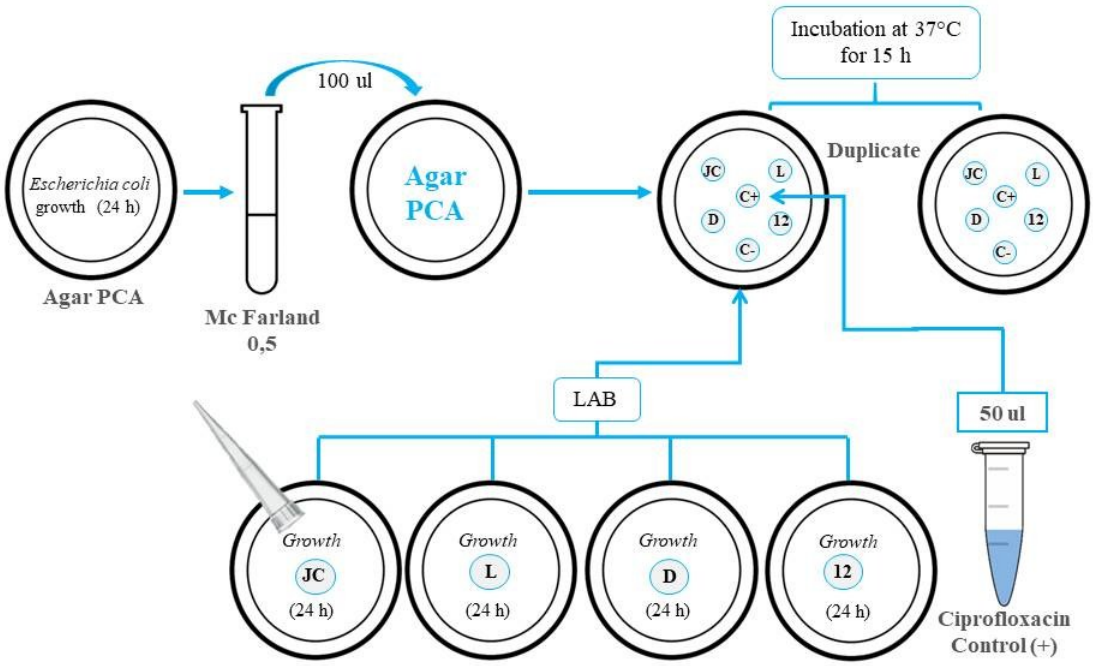

Figure 1. Diagram of the LAB disk diffusion method against *Escherichia coli* ATCC 25922.

the help of cellulose acetate (CA) membrane filters with a porosity of 0.2 µm (Sartorius stedim - Minisart, Germany), the cell-free supernatant was obtained.

The pathogenic bacterial culture was adjusted to 0.5 McFarland scale, and 100 µl were taken to spread on PCA agar. In the inoculated culture medium plate, six 7 mm diameter wells were sectioned with a sterile punch (Amorocho-Cruz CM, 2011). 50 µl of BAL supernatant was added to the respective well, the antibiotic Ciprofloxacin (Bioquímico Pharma S.A, Colombia) was used as a positive control, and the negative control well was left empty, this procedure was performed in duplicate. The plates were incubated (Heratherm, IMH60-S, Thermo Scientific, Germany) at 37°C for 24h (Amorocho-Cruz CM, 2011) (Figure 2). The %IH (percentage inhibition) was considered to be the value that is above the dimension of the negative control.

Counting of growth units

The plate colony counts for the dilutions used were recorded and the following equation (1) was used to determine the order of pathogen growth.

$$UFC * ml^{-1} = \frac{NC}{Vol*ND} \quad (1)$$

Where **NC**: number of growing colonies on plate. **Vol**: seeding volume (0.1 ml) and **ND**: colony count dilution.

Calculation of the inhibition zone (%)

Equation (2) was used to determine the inhibitory capacity of LAB strains (Corzo Barragán, 2012):

$$\%IH = \left(\frac{ØH_{ext} - ØH_b}{ØH_{cp} - ØH_b}\right) * 100 \quad (2)$$

Where **ØHext**: Halo of the extract, **ØHb**: Halo of the blank (7 mm) and **ØHcp**: Halo of the positive control.

Statistical analysis

The statistical analysis was performed using StatGraphics software (Centurion XVI Version 16.1.03). A multifactorial ANOVA analysis of variance was performed with a 95.0% confidence level for the results of antimicrobial activity of LAB in the presence and absence of cells obtained in the laboratory, thus determining how the factors involved (LAB and diffusion methods: discs and wells) significantly affected or did not affect the percentage of inhibition, allowing a comparison between them. Additionally, the adequacy of the model was verified by means of the assumptions of normality of the residuals with normal probability plot (Montgomery, 2010).

RESULTS AND DISCUSSION

LAB native strains

It was confirmed according to Ladino, 2017, Cortes-Macias *et al*, 2016, Cortes & Amorocho, 2022, that the

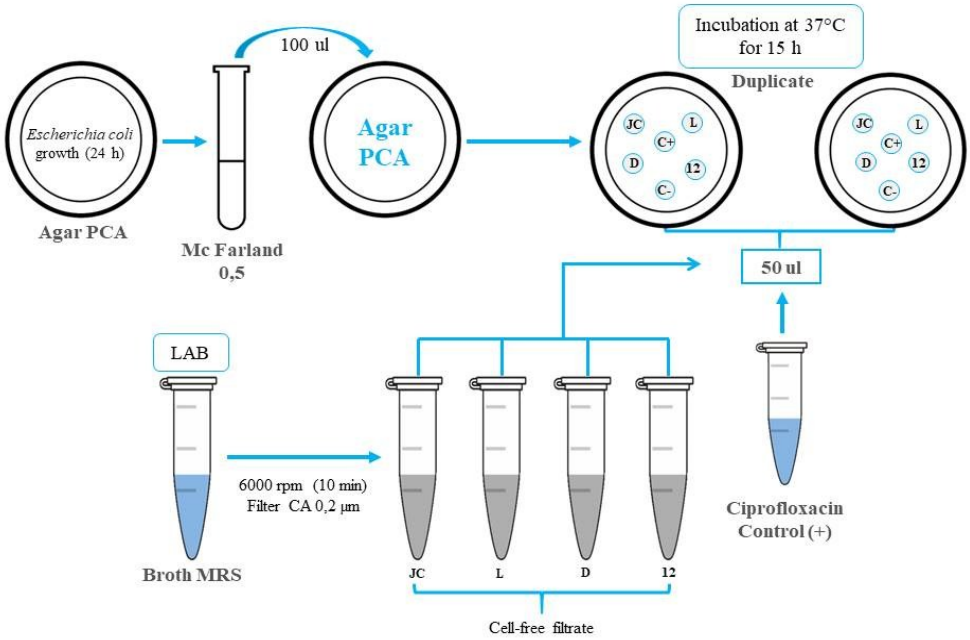

Figure 2. Diagram of the LAB well diffusion method against Escherichia coli ATCC 25922.

native strains JC, L, D, and 12 show the morphology described by them, as indicated below (Table 1).

Escherichia coli ATCC 25922

The morphological characteristics of the bacterial strain *Escherichia coli* ATCC 25922 were observed in the suspension (McFarland scale 0.5), circular colonies were obtained, with irregular edges (Lobulate-spiculated) and with the appearance of continuing to expand, without elevation - flat, milky white color, opaque density, shiny surface, with a diameter ranging between 1.5 and 3 mm. Through the microscope, straight bacilli were observed, with rounded smooth edges, with a length of 4.2 µm, and a width of approximately 1.6 µm, Gram-negative.

Antimicrobial activity of native LAB strains against Escherichia coli ATCC 25922

The antimicrobial activity performed on each of the LAB in the presence and absence of cells had a concentration of 6.60 ± 0.43 Log10 CFU/ml of the bacterial strain *Escherichia coli* ATTC 25922.

Disk Method

The results obtained in the evaluation of LAB in the presence of cells are shown in Table 2, where inhibition is expressed as mean ± standard deviation.

As can be seen, the formation of LAB's inhibition halos occurs only in three of the strains L, D, and 12, with a larger zone in strain L with a value of 37.27%. The action of the antibiotic is confirmed in the positive control with a halo of 56 mm.

Agar-well Method

The results obtained in the absence of cells are shown in Table 3, where inhibition is expressed as mean ± standard deviation:

The substances produced (organic acids) by strains L and D stand out for their antimicrobial activity inhibiting *E. coli* ATCC 25922, while the metabolites of strain 12 did not inhibit the pathogen. The strain JC, as in the previous method, had no inhibitory activity. Therefore, it is inferred that the metabolites produced by each LAB are different and it is considered that the antimicrobial activity is strain-dependent and is not a characteristic conferred to a group of LABs from the same origin. The action of the positive control is reaffirmed in this method.

In the research conducted by Leal, B. & Amorocho, C.M., (2017) LABs obtained from farmer´s cheese, artisanal, and commercial yogurt from Huila-Colombia were evaluated against *E. coli*. In the cell presence method, they obtained the highest inhibition diameters varying between 10-17.7 mm, which is confirmed in the *in vitro* test performed in this research, also finding that the areas of highest inhibition are shown in the presence of cells, L, D, and 12 with percentages of 37.27%, 32.65%

Table 1. Macroscopic and microscopic characterization of native strains.

STRAIN	CHARACTERIZATION	DESCRIPTION
JC	Macroscopic	Colonies rounded, with smooth edges, milky white, opaque, concave, shiny-moist appearance, size about 1 mm.
	Microscopic	Cocci uniform, in the form of clusters, with diameters ranging from 0.9 to 1 µm.
L	Macroscopic	Circular colonies, with smooth rounded edges, creamy white, translucent, concave surface, moist-glossy appearance, size approximately 1.8 mm.
	Microscopic	Elongated bacilli with rounded smooth ends, palisade-shaped, measuring approximately 2.5 µm long and 0.86 µm wide.
D	Macroscopic	Milky-white colonies, with a diameter of 4 mm and a defined circular shape.
	Microscopic	Short bacilli with smooth ends were observed - no clustering.
12	Macroscopic	This strain has rounded colonies of white color at the periphery, milky in the center, with a diameter of 3 mm.
	Microscopic	Bacilli, with smooth edges.

Table 2. Zone of inhibition of LAB with the disc diffusion method

Inhibition zone	BAL strains				
	JC	L	D	12	C. positive
	Escherichia coli ATTC 25922				
	Volume 50 µl				
Dimeter (mm)	-	25.25 ± 1.77	23.0 ± 0.71	18.5 ± 000.	56.0 ± 0.71
Porcent (%)	-	37.27 ± 4.15	32.65 ± 0.97	23.47 ± 0.34	100 ± 0.00

Table 3. Zone of inhibition of LAB with the well diffusion method.

Inhibition zone	BAL strains				
	JC	L	D	12	C. positive
	Escherichia coli ATTC 25922				
	Volume 50 µl				
Dimeter (mm)	-	20,0 0,14 ±	18,3 0,04 ±	-	56,0 0,07 ±
Porcent (%)	-	27.280,27 ±	23,631.00 ±	-	1000,00 ±

and 23.47% respectively. However, the organic acids of strains L and D also inhibited the growth of the pathogen *E. coli* in the cell-free methodology. The literature highlights the importance of the presence of cells to adhere to and colonize the epithelial cells of the gastrointestinal tract and thus compete with pathogens such as *E. coli* to improve the eradication rate of the pathogen (Santacroce *et al.*, 2019).

According to the multifactorial ANOVA statistical analysis applied to the LAB factors and methods, the 3 P-values obtained are <0.05 (Table 4), so these factors have a statistically significant effect on %IH with a 95.0% confidence level. Therefore, it is inferred that the antimicrobial effect of LAB against *E. coli* ATCC 25922 shows significant differences ($p<0.05$) between LAB and methods. As it is also evident in the means of the percentage of inhibition in each of the methods (presence and absence), it is observed that the presence of the cells of the evaluated LAB strains proved to be more effective than organic acids in the inhibition of *E. coli*, being the disc method (presence), the method with the highest percentage of

Table 4. P-value result of the evaluated factors LAB and Methods.

	Method	LAB	Interactions
P-value	0.0000	0.0000	0.0001

inhibition with statistically significant differences ($p<0.05$). The verification of the normality assumption for the application of ANOVA was satisfactory.

It should be noted that the antimicrobial effect of the LAB strains under study against the pathogen *E. coli* ATCC 25922 is dependent on these strains, so it cannot be attributed that all native strains obtained from breast milk, coffee, and farmer´s cheese have the same antimicrobial activity against the pathogen *E. coli* ATCC 25922. The tests performed are qualitative; however, they may be useful in future *in vivo* research.

It is important to determine the bactericidal capacity against pathogenic microorganisms to know the probiotic potential of a LAB. LABs have different mechanisms that generate antagonism with other microorganisms, such as modification of the intestinal environment by producing inhibitory compounds, reducing pH levels, production of bacteriocins (nisin, plantaricin, lacticin), hydrogen peroxide, metabolites (organic acids) such as acetic, formic, succinic, lactic acid, among others, in addition to the competition for nutrients (Wan *et al*., 2019; Van *et al*., 2020).

The results obtained show that cells from lactic acid strains (L, D, and 12) inhibit *E. coli* ATCC 25922 under *in vitro* conditions while, in the well method, the organic acids that inhibit *E. coli* ATCC 25922 are those isolated from coffee fermentation (L and D). Lopez *et al*., 2021, using isolates from raw milk and cheese, found that the organic acids of their LAB little inhibited *E. coli* ATCC 25922, contrary to the power of these against *S. aureus* ATCC 25923. Similarly, in the study by Moreira, 2019, it was appreciated that their strains of *L. Lactis* did not inhibit *E. coli* ATCC 25922, showing resistance in both cases. On the other hand, in the disc diffusion method used in the research of Lucumi-Banguero *et al*., 2021, the isolates of meat masses showed bactericidal capacity against *E. coli* with small inhibition halos. Similarly, Jurado *et al*., 2015 found in a related study that *Lactobacillus lactis* ATCC 11454 inhibited *E. coli* ATCC 25922 with halos around 2.6 cm in commercial MRS medium, which shows high similarity with the results of this research.

In other investigations, *E. coli* was susceptible to bacteriocins, and organic acids produced by *L. plantarum* (Fajardo-Argoti *et al*., 2021). Ramirez *et al*., 2021 also mentioned that within their results, *L. plantarum* strains have an antagonistic effect against *E. coli* as well as another pathogen *S. aureus*, mainly attributed to the pH of the supernatant, which was in a range between 4 and 4.5 since *Staphylococcus* showed a growth at pH higher than 5, while *E. coli* supported more acidic pH values due to the low pH of the supernatant.

On the other hand, the tests carried out in the present research allowed the identification of the LAB strains with the highest antimicrobial power. The obtained from coffee fermentation L and D stand out, which respectively managed to inhibit in higher proportion the pathogenic microorganism with inhibition diameters of 2.5 and 2.3 cm. These values are higher than those published by Narvaéz *et al*., 2017, which obtained 1.4 and 1.6 cm from native strains QR2 and QJ2 isolated from goat milk cheese, in addition to a lactic acid production of 0.9 and 0.74%, respectively, at 48 h.

Authors such as Rodríguez and García-Godos (2017), observed that their LABs isolated from *Molle* chicha produced antimicrobial substances capable of inhibiting pathogens such as *Escherichia coli*, *Salmonella typhimurium*, *Staphylococcus aureus*, and the yeast *Candida albicans*, with diameters of 14 mm, 15 mm, 15 mm, and 14 mm, respectively. Other findings, such as those published by Cervantes-Elizarrarás (2019), found that 50% of LAB strains isolated from mead, pulque, and blackberry had inhibitory activity against *E. coli* and *S. aureus*. The P24-2 strain identified as *L. plantarum* JCM 1149 showed higher inhibition against both pathogens with a halo of 3.33±0.35 cm.

Previous research by Cortés-Gaona & Amorocho-Cruz. (2022) and Jiménez-Espinosa & Amorocho-Cruz (2021) showed the antagonistic capacity of strains isolated from food from the Huila-Colombia region (L, D, 12, and JC) to inhibit other pathogens like *Shigella sonnei* ATCC 25931, and *Staphylococcus aureus* ATCC BAA-977, which is why it is important to make a molecular identification, as well as to identify and quantify the different organic acids produced by these LAB. These results will be essential for future research in *in vivo* tests.

Cortés & Amorocho, 2022 described that each LAB showed different susceptibility profiles, being most sensitive to antibiotic K (Penicillin). The strain JC was the most prevalent in resistance to this antibiotic, and D was the most susceptible, with an inhibition halo of 55 mm. On the other hand, strain L showed less susceptibility to antibiotics S (Ciprofloxacin) and P (Ceftriaxone) with inhibition halos of 26 and 28 mm, respectively. May-

Torruco and collaborators (2020) found in their results that *L. acidophilus, L. casei, L. mesenteroides,* and *B. bifidum* were sensitive to Penicillin and Ceftriaxone, thus corroborating the susceptible behavior of *Lactobacillus acidophilus, which* coincides with the biochemical characterization of strain JC.

L. kunkeei SS and *L. rahamnosus* SS 73, isolated from the intestinal tract of bees, showed susceptibility to antibiotics Ceftriaxone and Ciprofloxacin in the study conducted by Rodriguez *et al*. 2021. On the other hand, strains of *Lactococcus lactis* showed sensitivity to the antibiotic Penicillin (Moreira, 2019), while authors such as Vallejo *et al*. (2018) found that LAB of the genus La*ctococcus spp,* isolated from cocoa mucilage type Nacional (EET103) and Trinitario (CCN-51), did not show susceptibility to antibiotics such as Oxytetracycline and Penicillin. Jurado, H., & Guzman, M., (2015) found that LAB of the genus *Lactobacillus casei* showed resistance to Penicillin. Other studies, like those of Cabana, 2021 also mention the resistance of *Lactobacillus spp* to Penicillin.

Due to the incorrect or excessive use of antibiotics, some microorganisms have developed certain adaptive capacities by generating mechanisms that allow them to survive the attack (Barrantes *et al.,* 2022). That is why these research results are relevant for using LAB as an alternative in treatments. According to Amorocho (2011), this criterion is important for the selection and assurance of the use of new probiotic strains in possible applications as an adjuvant in treatments for gastrointestinal infections, in addition to ruling out the possibility of transmission of genes resistant to pathogenic microorganisms that may affect the digestive ecosystem (May-Torruco *et al*., 2020).

CONCLUSIONS

LAB strains isolated in the fermentation process of coffee, farmer's cheese, and breast milk showed antimicrobial activity in the presence of cells and their organic acids, against the reference pathogen *E. coli* ATCC 25922. It is a parameter to consider a strain as a probiotic. However, it is essential to carry out other studies such as biochemical and molecular identification of the strains, their viability under gastrointestinal conditions, and adherence to the intestinal epithelium among others. In addition, it is important to emphasize the rigorous and continuous evaluation of the safety of probiotics, as well as to establish adequate regulations governing their production and use. This will not only ensure the well-being of consumers, but will also help to preserve the integrity of existing medical treatments. The antimicrobial capacity exhibited under *in vitro* conditions against *E. coli* ATCC 25922 could be an option for future control and biotherapeutic treatment against this pathogen. In addition, it is important to emphasize the rigorous and continuous evaluation of the safety of probiotics, as well as to establish appropriate regulations governing their production and use. This will not only ensure the well-being of consumers, but will also help to preserve the integrity of existing medical treatments. The results obtained in this research will support the application of LAB isolates from products of the Huila-Colombia region, in addition to the breast milk isolate, which promotes breastfeeding in the first months of life to highly develop the immune system of neonates.

ACKNOWLEDGMENTS

We thank the VIPS office of the Universidad Surcolombiana, Neiva, for funding this project.

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