Inhibition of biofilms by the use of strains of lactic acid bacteria

Abstract

Bacterial biofilms are ubiquitous life forms that influence health and industry. Bacteria in a biofilm are more resistant to different antimicrobial treatments. They can also survive harsh conditions and resist the host's immune system. As a result, understanding the biological processes within these microbial communities is an ongoing challenge that has enabled researchers to design strategies for inhibiting biofilms using more effective methods. The objective of this review was to highlight the importance of lactic acid bacteria isolated from raw goat milk (four strains of *Leuconostoc mesenteroides* (W9, W10, LN31, and LY36) and four strains of *Entercoccus* sp. (LY22, EB12 , EB13 and EB14) and to assess their impact on the formation of the bacterial biofilm of six strains of pathogenic and /or spoilage bacteria.

This is done using quantitative measurement technology using a crystal violet capable of coloring biological membranes and measuring the absorption intensity at 490 nm after 5 hours and 24 hours of incubation.

The results showed that the strains tested presented good characteristics of their anti-biofilm activity in varying proportions depending on the incubation period and the strains studied, which indicates the production of different molecules specific to each strain for a sufficient duration.

Keywords: Anti-biofilm, *Enterococcus* sp., lactic acid bacteria, *Leuconostoc mesenteroides*, pathogenic and /or spoilage, quantification, raw goat milk.

Introduction

Biofilms are defined as microbial communities composed of structures embedded in a polymeric matrix produced by the bacteria themselves, and adhered to an inert or biotic surface (Angela *et al.*, 2020). It is the main mode of life of microorganisms in nature with eighty percent of the terrestrial microbial biomass in the state of biofilm. These so-called sessile populations frequently express different phenotypes from their non-adherent counterparts, with a particular ability to colonize new surfaces and a high tolerance to exogenous stress (Macfarlane et Dillon 2007). Biofilms are ubiquitous, they are present in most ecological niches and colonize very diverse, biotic surfaces, such as metals, soils, plants or even mucous membranes. Many human activities are thus affected by biofilms, whether in the industrial, environmental, agri-food or health sectors (William et Bloebaum, 2010). Some bacterial biofilms, such as the intestinal microbiota, also play protective and functional roles. Interactions between intestinal commensal

bacteria and beneficial bacteria are directly involved in host homeostasis (Leoni et Landini, 2014)

Biofilm refers to microorganisms living in complex three-dimensional structures composed of cells, polysaccharides, and other components such as proteins, extracellular DNA, and lipids (Azevedo *et al.,* 2021). Biofilm confers adaptive resistance and physical protection to microorganisms and plays a vital role in pathogenicity and drug resistance (Flemming et Wingender, 2010; Ciofu et Tolker-Nielsen, 2019). It is reported that 80% of chronic infections are related to biofilms (Jamal *et al.,* 2018; Redelinghuys *et al., 2020*).

The formation and development of biofilms is a complicated procedure involving different stages which can be the target of natural anti-biofilm agents for the prevention of biofilm some of the well-studied stages of biofilm development include: attachment of bacterial cells to a suitable biotic/abiotic surface, development of biofilm structure, maturation of biofilm, and dispersion (Boles et Horswill , 2008). The first two stages are highly critical in the development of biofilms and targeting one or both of these stages seems to be the ideal strategy for inhibition of biofilm formation. The attachment stage involves cytoskeletal elements (predominantly flagella, fimbriae) and lipopolysaccharides as key players. Surface signaling/communication of a group of bacteria, also termed as Quorum Sensing is a key player in the formation of biofilm. The natural anti-biofilm agents either act solely or synergistically by diverse mechanisms (Rojita *et al.,* 2020; Amana *et al.,* 2021).

The use of lactic acid bacteria as an alternative strategy for controlling biofilm formation has recently emerged in connection with the difficulties of eradicating biofilms by conventional therapeutic strategies and the renewed interest in so-called probiotic lactic acid bacteria. the mechanisms involved are of several orders: competition phenomena, co-aggregation capacities, production of "anti-biofilm" molecules, or transcriptional modifications altering initial bacterial adhesion and/or favoring the dispersion of biofilm (Vuotto *et al.,* 2014a) Intra- and interspecific interactions and competition between microorganisms within the biofilm are governed by ecological and evolutionary parameters (Rendueles et Ghigo, 2015) Bacterial interferences are present at different levels of biofilm development, they can affect primary adhesion and/or maturation via exclusion/competition phenomena, modify the composition of the matrix or improve dispersion. Antibacterial activities govern bacteria interactions "surfaces and bacteria interactions" and they are shared between commensal, pathogenic, and probiotic bacteria (Vuotto *et al.,* 2014b). According to (Hill *et al.,* 2014), probiotics have gained increasing medical attention due to their antagonistic effects against many pathogens. They are endowed

with anti-biofilm properties, of which they show promise in the treatment of infections of the mouth, wounds, and vagina in clinical trials and in *in vitro* studies (Vuotto *et al.,* 2014b). Pour certains probiotiques, cette activité bénéfique est renforcée lorsqu'elle est cultivée sous forme de biofilm (Rieu *et al.,* 2014). Pathogens also exhibit anti-biofilm properties when they compete with other bacteria to reach new ecological niches [(Hill *et al.,* 2014).

For example, (Kang *et al.,* 2006) reported that dextran production of lactic acid bacteria strains isolated from healthy oral cavities inhibits oral biofilm formation.

While the genus *Leuconostoc* is a heterofermentative type of lactic acid bacteria which are commonly used as starter bacteria in some dairy fermentation processes. Some strains of *Leuconostoc* spp. such as *Leuconostoc mesenteroides* are used as lactic ferments for the manufacture of cheese and butter. Additionally, several strains of *Leuconostoc* spp. including *Leuconostoc mesenteroides* and *Leuconostoc gelidum* produce bacteriocins. Several strains of *Leuconostoc* spp. are known to possess the ability to produce extracellular polysaccharides, such as dextran, when cultured in the presence of sucrose (Ates, 2015).

Hence, the demand for the research on the use of probiotics bacteria is growing for environment-friendly approach. Moreover, probiotic bacteria produce important enzymes and nutrients which are used for improving the growth of host organisms as well as fighting against pathogens (Nayak et Mukherjee, 2011; Hernández-González *et al.,* 2021; Benítez-Chao *et al.,* 2021). Hence, the probiotics strains are used to treat disease, and reduce pathogenic microbial population environment. Probiotics are well-defined as microbes which are live when directed in tolerable volumes that deliberate a healthiness advantage to the host. Moreover, it visualizes that the probiotic bacteria have the capability to produce acids, enzymes, inhibitory compounds and other bio-molecules (Moni *et al.,* 2020).

The occurrence of many biofilm-based human infections and their multiple antimicrobial resistance is a major concern in medicine and human health. The elevated rate of resistance to antibiotics in biofilm leads to the discovery and characterization of novel natural anti-biofilm agents. The structure and function of natural anti-biofilm agents from various sources have been exploited to develop numerous advanced therapeutic strategies showing increased activity, stability, and reliability. Here, we continue to analyze the efficacy of specially targeted lactic acid bacteria against pathogenic biofilms without disturbing the natural microflora. From this point of view, the study was aimed at testing the anti-biofilm efficiency of the insulated

probiotic isolates from raw goat milk vis-à-vis pathogenic and/or alteration bacteria in various in vitro conditions.

2. Materials and methods

2.1. The origin of the strains used

This article studies the antibiofilm potential of 4 strains of *Leuconostoc mesenteroides* (LN31, LY36, W9 and W10) and 4 strains of *Enterococcus* spp. (EB12, EB13, EB14 and LY22) were isolated from raw milk samples from goats collected in the northwestern region of Algeria in the Saïda town then they were identified phenotypically and by the MALDI-TOF technique and after having proven their antagonistic capacities against pathogenic bacteria and/or alterations which are reused by this study in the sessile state: biofilm.

The pathogenic and/or spoilage bacteria used are *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Salmonella montevideo* ATCC 3581, *Pseudomonas aerogenosa* ATCC 27853 (from the biology laboratory of the University of Saïda) and *Listeria ivanovii* ATCC 19119, *Listeria innocua* ATCC 33090 (from the applied microbiology laboratory (LMA) of the University of Oran ES –Senia).

2.2. Methods

The evaluation of the biofilm formation of six pathogenic bacteria and/or alterations (*Listeria inocoua* ATCC 33090, *Listeria ivanovii* ATCC 19119, *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 25923) was evaluated in the presence of four strains of *Leuconostoc mesenteroides* (W 9, W10 , LN 31 and LY 36) and four strains of *Entercoccus* sp. (LY22, EB12, EB13 and EB14) on a microplate to test several strains at the same time with a very simple protocol, this test is based on the technique of quantification by the use of crystal violet which has the ability to color the biofilm (Zimmer *et al.,* 2014). This absorbed color is directly correlated to the density of the biofilm formed, and its solubilization allows its quantification (Musk *et al.,* 2005; Ebert *et al.,* 2021). To evaluate this activity, we followed the method of (Bulgasem *et al.,* 201)5 with a few modifications (Figure1):

A bacterial suspension of the selected strains was prepared by centrifugation at 4000 rpm for 15 min from an 18-hour culture, of which the 1st row of the plate was considered as a control (BHI medium only), the 2nd row contained 50µl of untreated bacteria (107 CFU/ml) plus 50µl of BHI in each well. Then, a volume of 50 µl of BHI plus 50 µl of the producing strain was

inoculated into each well, supplemented with 50 µl of lactic supernatant. Then, the plates were incubated for 5 and 24 hours at 37°C.

After incubation the plates were washed 3 times with sterile PBS, this buffer is aspirated with a piece of sterile absorbent paper in an inverted position. Then the cells are fixed with absolute ethanol for 15 min. Ethanol is aspirated and the microplates were stained by adding crystal violet (0.1% w/v) to each well and incubated for 20 min. After staining, a 2nd rinse (three times with PBS) is performed to remove excess crystal violet. Finally, the wells were filled with 33% acetic acid. Finally, the absorbance of each well was measured at 630 nm using a microplate reader, whose biofilm inhibition percentages were calculated according to equation (1):

$$\text{Antibiofilm (\%)} = (1-(AM/AC))\times 100 \quad \ldots\ldots(1)$$

AM: Absorbance of the well containing lactic supernatant + target strain

AC: Absorbance of the well containing only the target strain.

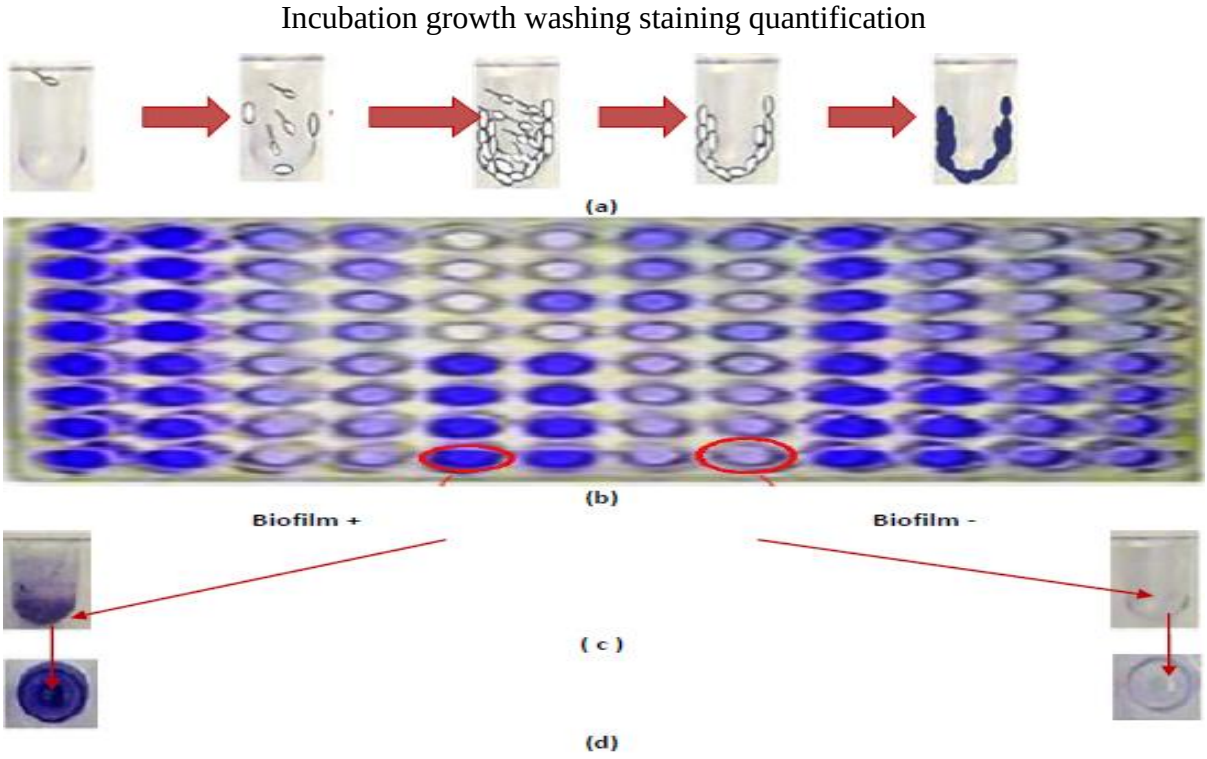

Figure 1. Formation of biofilm in microplates (Bellifa, 2014).

a) Stages of biofilm formation in microplate wells
b) Crystal violet staining of 96 wells of the Elisa microplate.
c) Side view.
d) View from above.

3. Results and Discussion

Biofilms are recognized by the public health community as an important source of pathogens (Rendueles et Ghigo,2015; Wingender et Flemming , 2011). They are implicated in specific infectious diseases such as osteomyelitis, otitis media, peridontitis and dental caries (Angela *et al.*, 2020) and in chronic diseases such as lung infections in patients with cystic fibrosis. They are also implicated in nosocomial infections due to opportunistic pathogens, in particular the urinary tract, the lower respiratory tract, etc.

Pathogens have several mechanisms of resistance to external control factors, including drug target alteration, changes in cell permeability, genetic mutations, and acquisition of mobile genetic elements (Beatson et Walker,2014; Santajit et Indrawattana, 2016).

Including that Foodborne pathogens can attach to plastic surfaces and form a biofilm, making the use of plastic cutting boards and cooking raw foods extremely prone to cross-contamination (Roy *et al.*,2022; Zara *et al.*, 2020; Lee *et al.*,2020). Additionally, compared to glass and SS surfaces, which are hydrophilic materials, plastic is more likely to allow *Salmonella* germs to stick to them (Roy *et al.*,2022, Kim *et al.*, 2022).

Furthermore, these pathogens exhibit an adaptive mechanism in the form of biofilm development, which is a collection of microbial populations covered by self-produced polymeric substances (De la Fuente-Núñez *et al.*, 2013). The biofilms serve as a physical barrier against antimicrobial agents (Mah et O'Toole, 2001). Biofilms also cause the formation of a small population of persister cells, which are the primary source of persistent and recurring infection (Khan *et al.*, 2020).

Considering biofilms as a community of microorganisms, attached to a biotic or abiotic surface, which undergoes profound changes during the transition between the planktonic state and the biofilm state. They can involve a single type of microbial species or a complex set of different species. The principle of this test is based on measuring the absorbance of the color correlated with the density of the biofilm formed. The classification of biofilm formation according to the criteria of (Stepanovic *et al.*, 2007) is based on the optical density (OD) values obtained for the strains (Table 1).

Table 1. Classification of biofilm formation according to (Stepanovic *et al.*, 2007).

Average of OD values	**Formation de biofilm**
BOD ≤TOD	Absence
TOD <BOD ≤2TOD	Low
2TOD ≤ BOD ≤4TOD	Moderate
BOD >4DOT	Strong

TOD: the average OD of the controls (BHI without bacterial inoculation);
BOD: the average OD of BHI inoculated with pathogenic bacteria without treatment).

The measurement of absorbance (A) at 490 nm gave us the following values (Table 2).

Table 2. Quantification of indicator bacteria biofilms

	Absorbance (Ac) at 490 nm			
	After 5 hours of incubation		After 24 hours of incubation	
	Average OD read	Characteristic of biofilm	Average OD read	Characteristic of biofilm
Salmonella menteridea	0.090±0.003	Low	0.142±0.003	Moderate
Eeschrechia coli	0.106±0.008	Low	0.140±0.004	Moderate
Pseudomenas aerogenosa	0.070±0.001	Low	0.114±0.003	Low
Staphylococcus aureus	0.080±0.001	Low	0.090±0.006	Low
Listeria innocua	0.122±0.002	Low	0.167±0.003	Moderate
Listeria ivanovii	0.100±0.002	Low	0.125±0.004	Low
Control (BHI without bacterial inoculation)	0,064±0.001		0.066±0.002	

Our study shows that the formation of biofilms by the selected pathogenic strains is greater after 24 hours of incubation than after 5 hours (Figures 2 and 3). This is explained according to (Abedi *et al.*, 2013), by several factors responsible for the resistance associated with the biofilm, in particular the density and the physiological state of the cells, but also the physical structure of the biofilm. However, the aspect of biofilm formed during the two incubation periods is between weak and moderate. This can be explained according to (Abedon, 2015) by the chemical composition of the culture medium, the nature of the adhesion surface (the hydrophobic surfaces are favorable to bacterial adhesion"), the topography of the surface (amplitude of the roughness "nano- and micro-topography), organization of the roughness

(isotropic / anisotropic), porosity (micro- and macro porosity), chemistry and composition of the surface and surface charge (ionic tension).

To provide more information, we explain the formation of biofilms by all tested bacteria after 5 hours of incubation with low ratios to by the relative importance of the five cellular characteristics in the formation of the biofilm which are: exopolysaccharide, flagella, N-acyl-homoserine lactones (AHL) of quorum sensing (QS) signaling molecules, extracellular protein, swarming motility (Li *et al.*, 2009).

while the bacteria move in the liquid medium thanks to the force of the flow, the gravitation and/or the movements of their flagella. When bacteria are in the vicinity of a surface, physico-chemical attractive forces intervene and lead to a reversible interaction with the surface. Secondly, as the cells divide, the number of bacteria associated with the surface increases and adhesion becomes irreversible (O'Toole, 2011) This transition towards irreversible adhesion corresponds to the synthesis of structures on the surface of the bacterium, which is accompanied by a profound modification of the gene expression profile. Biofilms are ubiquitous in nature, having the ability to adhere to virtually any surface, and are difficult to eradicate (*Ren et al.,* 2004).

After 24 hours of incubation, the biofilm goes through the third stage which is characterized by the formation of microcolonies composed of both the initial bacteria which divide and bacteria which attach themselves to the biofilm in formation. Finally, the maturation stage corresponds to the development of microcolonies and the structuring of the biofilm: the microcolonies develop into pillars of variable thickness within which the cells are embedded in the extracellular matrix. The spaces between the microcolonies become the channels of the biofilm inside which the nutritive fluids can circulate. Some bacteria can detach from the mature biofilm and enter the dissemination phase. This last step allows the colonization of new surfaces (Roux et Ghigo, 2006)

the quantitative variation in the formation of biofilm after 24 hours of incubation is interpreted by the relative importance of the flagella on the capacity of formation of biofilm was great. It was found that the formation of biofilm is linked to the presence of a flagellum in several bacterial species, such that our results showed that the amount of biofilm formed by the five bacterial strains tested after 24 hours of incubation was, in order from greatest to least: *Listeria innocua* > *Salmonella montevideo* > *Eeschrechia coli* > *Listeria ivanovii* > *Pseudomenas*

aerogenosa; our results are similar to those of (Gav´ın *et al.*, 2002), indicating that the participation of flagella in biofilm formation is a universal phenomenon (Li *et al.*, 2009).

Without neglecting the role of extracellular polysaccharides which are a major component of bacterial biofilms, bacterial swarming is a flagella-driven movement in the presence of extracellular slime (a mixture of carbohydrates, proteins, peptides, surfactants, etc.) by which bacteria can spread as a biofilm over a surface (Somma *et al.*, 2020) It can also be hypothesized that the movement of swarms, such as extracellular proteins, is an indirect factor affecting biofilm formation. For microorganisms themselves, the formation and development of biofilms is multifactorial and complex (Van Houdt et Michiels, 2005).

We attribute the formation of biofilm by *Staphylococcus aureus* although it is a bacterium without a flagellum which are essential components of this step, to the nature of the (BHI) extracellular milieu. So, confirmed by the studies of (Rohde *et al.*, 2010), that BHI Broth was significantly more effective in biofilm formation. Proteins especially rich in leucine, proline, serine, and aspartate are abundant in BHI broth since these amino acids may be essential for the production of adhesins such as fibronectin‑binding protein and clumping factors which are necessary for adherence. The presence of lipids such as choline and sphingosine in BHI may have added advantage in biofilm formation and provide resistance from desiccation. Further, it is a source of sugars such as inositol/myoinositol which cannot be fermented by *Staphylococcus aureus* leading to resistance in pH fall, which, in turn, may be needed for robust biofilm architecture (Ashish *et al.*, 2017).

As we add that the quantitative variation of biofilms of indicator bacteria formed either after 5 hours or after 24 hours of incubation by quorum sensing (QS) which is a mode of communication and perception used by bacteria. It is based on the production of small molecules, auto inductors (AI), which can diffuse through the membrane or be transported outside the cell (Bassler et Losick, 2006) AIs, whose concentration is proportional to the number of bacteria, serve as a molecular indicator of bacterial density. From a certain concentration of these molecules, a cellular response is triggered by the activation and repression of effective genes only when the cell density is high, to set up specific phenotypes, such as the formation of biofilm, virulence, the production of exopolysaccharides, exoproteases and siderophores. Many QS-induced factors are secreted into the cell environment. They have a global interest in the bacterial community to provide nutrients to the population or for the transition from the planktonic mode of life (that is to say in suspension) to a sessile mode, called

biofilm, more frequently encountered in the natural environment (Filloux et Vallet, 2003). The QS would not only serve to count the bacterial population, but also to perceive the diffusion of the factors secreted in the medium, to optimize the efficiency of their production (West *et al.*, 2012). To better integrate this different information, bacteria have developed QS systems involving several AIs, of different stabilities and solubilities, perceived by specific receptors that are interconnected (Cornforth *et al.*, 2014).

A. Antibiofilm activity after 5 hours of incubation

Foodborne pathogenic bacteria, among other microorganisms as well, can easily attach to various surfaces encountered within food processing and create biofilms on them, resisting this way the antimicrobial action of common sanitizing agents and other harsh environmental conditions, such as desiccation and nutrients deprivation (Bridier *et al.*, 2015). As thus, new efficient antibiofilm approaches are needed to combat these detrimental biofilms, ensuring the safety of our food supply. In the past years, the bioprotective action of LAB and some of their purified metabolites, such as bacteriocins, exopolysaccharides (EPS), and biosurfactants, contained in their cell-free culture supernatants (CFSs), are included among those approaches that have been successfully tested (Cornforth *et al.*, 2018; Castellano *et al.*, 2017; Riaz *et al.*, 2020). Recently, food-based probiotics have assumed great significance for their nutritional and therapeutic potential (Ministero, 2013) Probiotics are defined by the World Health Organization (WHO) as "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host" (Backhed *et al.*, 2012). During the past few decades, lactic acid bacteria (LAB), a popular member of probiotics, have been extensively used in humans and animals for various purposes to enhance nutrient utilization, to modulate both the innate and the adaptive immune systems, and to inhibit the growth of numerous pathogenic microorganisms (Bernaola *et al.*, 2013; Goldenberg *et al.*, 2013).

with the aim of using beneficial bacteria to combat the formation of undesirable biofilms represents an innovative therapeutic strategy and to test the prevention of the formation or disruption of undesirable biofilms and the effect on bacterial cells in a sessile state, we have selected the most efficient strains in planktonic bacterial inhibition to see their effects on the biofilm form, the results of this test showing that the maximum inhibitions of the biofilms after 5 hours were for the W9 strain of *Leuconostoc* sp. against *Salmonella montevideo* and *Listeria innocua* (with 36.65±0.9% and 46±0.21%; respectively). While the two LY22 strains of *Enterococcus* sp. and Leuconostoc sp. strain W9. inhibited *Escherichia coli* biofilm by similar percentages equal to (39.12±0.09%). However, *Leuconostoc* sp. prevented the formation of

Pseudomonas aeruginosa, *Staphylococcus aureus* and *Listeria ivanovii* biofilms with respective ratios of (39.75±0.2%, 44.5±0.24% and 54.50±0.15%); (Fig. 2) Similar studies provided by (Mingkun *et al.*, 2022) showed that biofilm formation of *S. mutans* was significantly reduced when co-cultured with *Leuconostoc mesenteroides*. isolated from fermented foods.

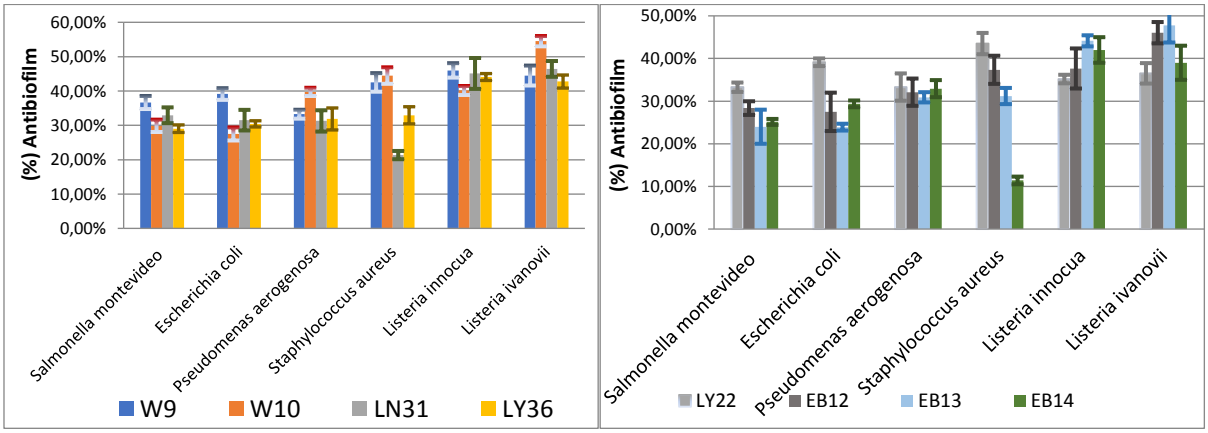

Figure 2. Anti-biofilm formation activity after 5 hours of incubation at 37°C.
a) Using strains W9, W10, LN31 and LY36 of *Leuconostoc* sp.
b) By using strains LY22, EB12, EB13 and EB14 of *Enterococcus* sp.

B. Antibiofilm activity after 24 hours of incubation

(Fig. 3) presents the inhibition of biofilms after 24 hours of incubation, by the same inhibitor strains used against biofilms after 5 hours, whose maximum inhibitions were for the W9 strain against the adhesion of (*Salmonella montevideo*, *Staphylococcus aureus* and *Listeria innocua*) by percentages equal to (18.54±0.9%, 27.07±0.7% and 35±0.21%). However, strain W10 inhibited the formation of *Listeria ivanovii* biofilm by a percentage of 30.02±0.09%. While the two EB13 strains of *Enterococcus* sp. and *Leuconostoc* sp. strain LN31. prevented the formation of *Escherichia coli* biofilm by similar percentages equal to (19.88±0.09%). Simultaneously, analysis in our study showed weak aggregation of *Escherichia coli* cells in the biofilm after treatment with *Leuconostoc* sp. strain LN31. This suggests the active role of certain metabolites such as enzymes or dispersal signal molecules that may have contributed to biofilm inhibition (Hemila , 1996; Hemilä and Chalker 2013) plus the role of peptides released by these bacteria that induce curvature in the lipid bilayer and pore generated by both peptides and phospholipid headgroups that make up the cells of biofilm-forming bacteria (Jia *et al.,2021).* While the LY36 strain of *Leuconostoc* sp. inhibited *Pseudomonas aeruginosa* biofilm with a percentage of (15.69±0.2%). Indeed, there is no specific mechanism by which Lactic acid bacteria prevents the biofilm formation; however, several studies have proposed that probiotics can influence the

expression of genes involved in quorum sensing, cell adhesion, virulence factors, and the formation of biofilms .Lactic acid bacteria also secretes a variety of extracellular inhibitory substance, which includes extracellular substance, exopolysaccharides (Maidens *et al.*, 2013), biosurfactants (US Department of Health & Human Services, 2013), bacteriocins (Parrino , *et al.*, 2019), different enzymes(Petrof *et al.*, 2013), and anti-quorum compounds Specifically, several studies have reported that bacteriocin may decrease the formation of biofilms due to growth inhibition (Parrino , *et al.*, 2019).

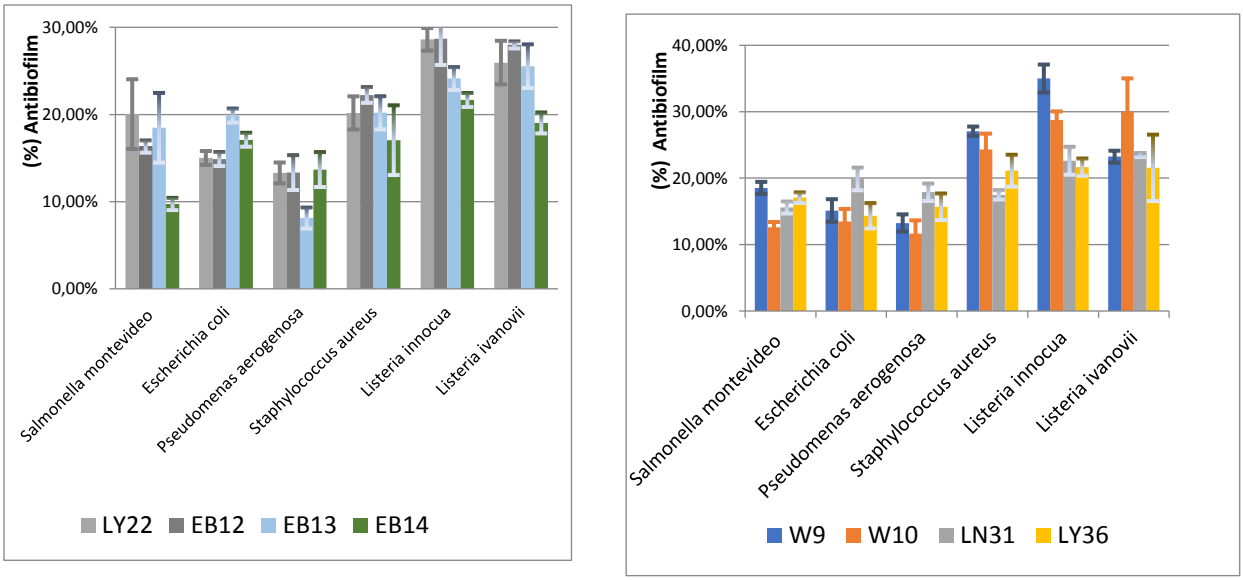

Fig. 3: Anti-biofilm formation activity after 24 hours of incubation at 37°C.
a) Using strains W9, W10, LN31 and LY36 of *Leuconostoc* sp.
b) By using strains LY22, EB12, EB13 and EB14 of *Enterococcus* sp.

Inhibition of the formation of biofilms of pathogenic bacteria by our strains of LAB: Different strategies are envisaged to prevent or inhibit the development of the biofilm. For example, by limiting the adhesion stage, the transition from the planktonic form to the biofilm form, the maturation stage or intercellular exchanges. But also, by reactivating dormant cells or by promoting the dispersion of bacterial cells in the biofilm (Brackman et Coenye, 2015). According to our results, it is justified that the percentages of inhibition of the formation of bacterial biofilm are more significant after 5H of incubation than after 24H, by what the cells of the young biofilms (of 5H) are still under discussion the reversible phase of adhesion, during this phase the bacteria are easily suppressed by the application of minimum forces (Watnick et Kolter, 2000) and even by simple washing(Strevett et Chen, 2003) and do not develop thick biofilms (Valeriano et Oliveira, 2012) While the variation in the inhibition of the formation of biofilms is influenced by several criteria among the, it is cited that *Listeria innocua*, is a ubiquitous bacterium, it can develop in any type of environment such as within the food industry

and able to form biofilms on abiotic surfaces. The work of (Musk *et al.,* 2005) has shown that this bacterium is capable of forming biofilms with a high cell density.

The differentiation of the sensitivity of the bacterial biofilms formed can be linked to several mechanisms such as adhesion, the synthesis of inhibitory molecules by our strains tested, nutritional competition and the difficulty in establishing and developing on the surface in community with other bacteria. While *Staphylococcus aureus* has mechanisms that allow it to naturally resist many antibacterial agents, colonize inert surfaces and form protective biofilms (Essoh, 2014). For example, the opportunistic pathogen bacterium *Pseudomonas aeruginosa* produces and perceives four distinct AIs, each of which is perceived by a specific regulator (Lee et Zhang, 2015) This functioning allows bacteria to have a combinatorial mode of communication to set up a precise response to the signals they perceive and thus adapt the lifestyle most conducive to their survival (Cornforth *et al.,* 2014).

The reduction in the adhesion of the indicator strains in the presence of supernatant of the strains of lactic acid bacteria could be due to secreted metabolites that reduce the hydrophobicity of the bacteria (Ljungh et Wadstrom , 2006)

It is still known that the expression of many of these traits by pathogenic bacteria is adjusted by quorum sensing (QS) mechanisms, through which bacteria coordinate their behavior by sensing not only their own population density but also those of their surroundings (Giaouris et al., 2015) Interestingly, lactic acid bacteria species have been shown to secrete metabolites with anti-QS activity (Kiymaci *et al.,* 2018).

Undoubtedly, the application of lactic acid bacteria and/or their purified (or semi purified) metabolites against foodborne pathogenic biofilms is considered an environmentally friendly approach (limiting the use of synthetic chemicals), while the use of such metabolites at subinhibitory concentrations for planktonic growth of the target bacteria (mainly through interference with cell-to-cell interactions) is believed to exert less selective pressure to the latter and therefore limit the likelihood for resistance development. This last is quite important considering the great resistance numerous pathogens are currently displaying against some of the most common antibiotics and/or other sanitizers (Hutchings et Truman 2019).

Conclusion

The attachment and bacterial biofilm formation abilities depend on many factors, such as inherent biological characteristics and environmental factors. Therefore, finding methods to

remove bacterial biofilms is a significant challenge. In this context, lactic acid bacteria isolates were suitable inhibitors of pathogenic biofilms. Therefore, future studies should focus on exploring optimal parameters for the use of these isolates for the prevention of biofilm formation or early elimination.

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