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Bioactive substance produced by lactic acide bacteria (LAB) against pathogenic bacteria

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Dedication

I’d like to dedicate this work to my dears parents SAID and FOUSIA and my grandparent MOHAMED and KHADIDJA .
Also to those who supported my only brother MOKHTAR and sisters AICHA, MARWA,SABRINA ,RIHANA and MANEL .
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I would like to thank my family and miss Daouadji soumia family for supporting me.
I’m forever grateful.

Abbreviation list

MIC : Minimum inhibitory concentration.

MDR : Multiple drug resistance.

CRE : Carbapenem resistant Enterobacterales.

CMI : Concentration minimale inhibitrice.

MRSA : Methicillin-resistant *Staphylococcus aureus*.

VRE: vancomycin-resistant *enterococc*.

CRE: Carbapenem-resistant Enterobacterales.

ESBL : Extended-spectrum β-lactamases.

EPS : Extracellular polymeric substances.

LAB : Lactic acid bacteria.

PBP: penicillin-binding protein.

PCR: Polymerase chain reaction.

CFS: cell free supernatant.

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E.coli BLSE (1), *L.monocytogenes* ATCC (2), *P.aeruginosa* (PA) (3), *K. pneumonia* (KP) (4), *B.subtilis* ATCC 6633 (5), *S.aureus* (MRSA) (6).

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Introduction

The bacterial antibiotic resistance pose a serious problem for a global public health, where the abusive use of antibiotics accelerates the process of appearance of antibiotic-resistant bacterial strains **(Zhang *et al*., 2015).** Recently, the increased antibiotic resistance has encouraged the scientific searchers to investigate a news alternative therapeutic option by use of several compounds with antibacterial property such as plant compounds, bacteriophages and potential bactericidal compounds against multi-resistance (MDR) pathogenic bacterial strains **(Singh *et al*., 2010 ; Holmes *et al*., 2016)**.

The lactic acid bacteria (LAB) present in different ecological system such as milk and dairy products, meat and meat products cereals, plants, which was characterized by their antibacterial activity towards many pathogenic microorganisms, due the pres-ence of the important molecules like organic acids, di-acetyl, hydrogen peroxide, acetoin, 2,3-butanediol, acetaldehyde, benzoate, bacteriolytic enzymes, bacteriocin, reuterin, etc... **(Mayo *et al*., 2010)**. Therefore, the production of bacteriocin by the LAB has received more attention and has interested many searchers **(Bekuma and Ahmed, 2018)**.

Bacteriocins from LAB are recognised for their ability to prevent microbial contamination and infections **(García *et al*., 2010).** Several small peptide bacteriocins that are isolated and purified and have emerged as an alternative to antibiotics, due to their broad-spectrum antimicrobial activity in very low concentrations **(Parada *et al*., 2007).** Bacteriocins from LAB are classified based on their structural, physiochemical, molecular characteristics and antibacterial activity **(Klaenhammer, 1993).** as: Class I bacteriocins – lantibiotics (e.g. Nisin), small (<5 kDa), membrane-active peptides; Class II bacteriocins – small, heat-stable, non-lanthionine-containing peptides; and Class III bacteriocins – large, heat-labile bacteriocins. Class II bacteriocins are divided into subclasses: IIa (pediocin-like bacteriocins, antilisterial activity); IIb (two peptide bacteriocins, e.g. lactococcin G); IIc (cyclic bacteriocins); and IId (single peptide, non-pediocin- like, linear bacteriocins). Class II bacteriocins act either by cell wall hydrolysis or dissipating membrane potential of the target organism **(Jin *et al*., 2010).**

Biofilm-associated infections on implantable medical devices caused by pathogenic strains, which have negative impacts on public health and medicine, are a major concern **(Lindsay and Von Holy, 2006)**. Alternatives are an immediate requisite to combat drug resistant pathogens **(Liu *et al*., 2015)**. Bacteriocins and antimicrobial peptides can be used as antibiofilm agents for combating infections as well as battling drug resistance.

This study was carried out on the screening of a wide range of indigenous lactic acid bacteria from traditional fermented dairy products (Jben and kamaria) from southern Algeria for

their antagonist activity against MDR pathogens and clinical isolates.The objectives of this study were to explore novel and atypical ecological niches, isolate LAB with potential applications as hurdles against biofilm forming pathogens and spoilage strains.

I Antimicrobial resistance

1. Generality

A bacterium is defined as being clinically resistant to an antimicrobial agent when the drug – after recommended dosing – does not reach a concentration at the site of infection that is able to effectively inhibit the growth of the bacterium or to kill it. This definition takes into account the pharmacological parameters relevant for systemic therapy of the antimicrobial agent in the patient species concerned. It also considers the minimum inhibitory concentration (MIC) of the causative bacteria to the antimicrobial agent applied. These factors, along with the results of clinical efficacy studies, play key roles in the definition of clinical breakpoints **(CLSI , 2011).**In general, antimicrobial resistance in bacteria can be either intrinsic or acquired **(Schwarz *et al*., 2006).**

2.Different mechanisms of resistance to antimicrobials

2.1.Intrinsic resistance

Bacteria may be inherently resistant to an antimicrobial.This passive resistance is a consequence of general adaptive processes that are not necessary linked to a given class of antimicrobials. An example of natural resistance is *Pseudomonas aeruginosa*, whose low membrane permeability is likely to be a main reason for its innate resistance to many antimicrobials **(Yoneyama and Katsumata, 2006)**.Other examples are the presence of genes affording resistance to self-produced antibiotics, the outer membrane of Gram-negative bacteria, absence of an uptake transport system for the antimicrobial or general absence of the target or reaction hit by the antimicrobial **(Wright, 2005)**.

2.2.Acquired resistance

Active resistance, the major mechanism of antimicrobial resistance, is the result of a specific evolutionary pressure to develop a counterattack mechanism against an antimicrobial or class of antimicrobials so that bacterial populations previously sensitive to antimicrobials become resistant **(Wright, 2005)**. This type of resistance results from changes in the bacterial genome. Resistance in bacteria may be acquired by a mutation and passed vertically by selection to daughter cells. More commonly, resistance is acquired by horizontal transfer of resistance genes between strains and species. Exchange of genes is possible by transformation, transduction or conjugation **(Rachakonda and Cartee, 2004)**.

The major mechanisms of active antimicrobial resistance (Figure 1) are (1) prevention of accumulation of antimicrobials either by decreasing uptake or increasing efflux of the antimicrobial from the cell *via* a collection of membrane-associated pumping proteins, (2) qualitative drug target site alteration, which reduces the affinity for antimicrobials either by mutation or by target modification, or quantitative drug target alteration by overproduction of the target and (3)

inactivation of antibiotics either by hydrolysis or by modification **(Yoneyama and Katsumata**, 2006).

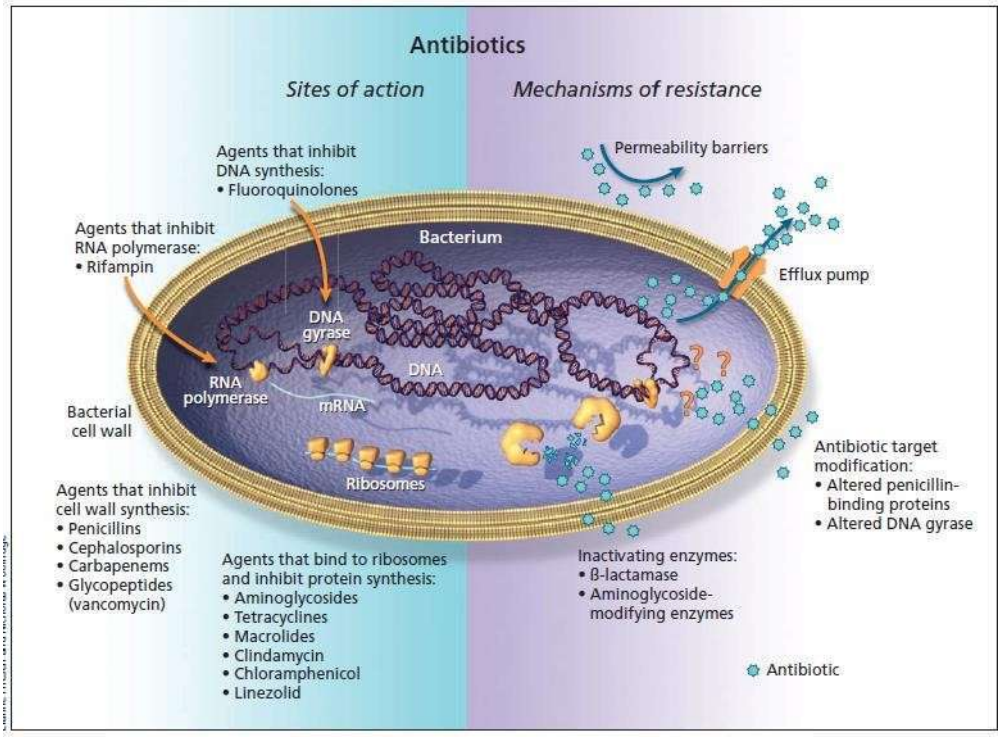

Figure1. Sites of action and potential mechanisms of bacterial resistance to antimicrobial agents. Modified **(Neu, 1992).**

3.Common Antibiotic-Resistant Bacterial Species

MDR gram-negative bacteria including *A.baumannii*, *Pseudomonas aerugionosa*, extended-spectrum beta-lactamase (ESBL)-producing Enterobacteria, and carbapenem-resistant Enterobacteria (CRE) are considered the main causative agents of nosocomial infections. **(Teerawattanapong *et al.*, 2017).** Methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant *Enterococcus* (VRE) have been reported recently to be the most common bacterial pathogens, and besides, hospitals have also been isolated from foods of animal origins, water, and animals **(Vivas *et al.*, 2019)**.

3.1. Methicillin-resistant *S. aureus*

S. aureus is a gram-positive ubiquitous strain known to produce several virulence factors that facilitate disease causation and help rapidly develop antimicrobial resistance against antimicrobial agents used for its control, a feature that increases the importance of this microorganism as a pathogen. **(Chang *et al.*, 2003)**.

Since its emergence in 1961, MRSA has spread worldwide, and infections caused by this microorganism are regarded as one of three major infectious diseases threatening human health. This bacterium apart from causing infections in cutaneous lesions can result in severe cases of pneumonia, meningitis, endocarditis, septicemia, and even systemic infections, with risk of death **(Togneri *et al*., 2017).**Compared to infections caused by *S. aureus* strains sensitive to methicillin, those caused by MRSA usually have more severe clinical manifestations and are the most difficult to treat, as methicillin resistance indirectly affects other virulence factors and enhances the pathogenesis of the bacterium. **(Schlievert *et al*., 2010)**.

As is well established by several authors, methicillin resistance in *S. aureus* is mediated by a penicillin-binding protein (PBP2A) encoded by the mecA gene, that is carried on a mobile genetic element designated staphylococcal cassette chromosome mec (SCCmec) **(David and Daum, 2010)**.

3.2.Vancomycin-resistant *Enterococcus*

Enterococci are indigenous flora of the gastrointestinal tracts of animals and humans, and the species *E. faecium* and *E. faecalis* have heightened interest because of their ability to cause serious infections and their intrinsic resistance to antimicrobials, including Vancomycin **(Silva *et al*., 2011)**.VRE faecium (VREfm) has disseminated rapidly in hospitals in many parts of the world since 2012. In contrast, vancomycin resistance has been reported considerably less frequently in *E. faecalis* globally **(Raven *et al*., 2016)**.

Resistance to vancomycin is determined by one of nine resistance determinants (vanA, B, C, D, E, G, L, M, and N), but the vanA and vanB genotypes predominate worldwide.These genetic determinants could be carried in the mobile genetic element, such as Tn1546, mostly located on conjugative plasmids (variants of the vanA and vanB-type) or located on the chromosome (vanC) **(Iweriebor *et al*., 2015)**.

3.3. Carbapenem-resistant *enterobacteria* (CRE)

The emergence of CRE is one of the major public health problems in the world. Carbapenems are important for the empirical treatment of critically ill patients at the risk of multiresistant bacterial infection.44 They have been used as a drug of choice for the treatment of infections caused by ESBL producing enterobacteriaceae for years, which is also one of the main factors responsible for the emergence of CREs through selective pressure **(Braun *et al*., 2014)**.

Among the *Enterobacteriacea*, *K. pneumoniae* is the most common bacterium exhibiting carbapenem resistance followed by Enterobacter species. Others such as *E. coli* have been reported less frequently. CREs have emerged in recent decades, but have become one of the major concerns of hospital infection control services. High prevalence of infections by these bacteria is present in

several countries on all continents, leading to an important restriction in treatment options **(Lerner *et al*., 2015)**.

3.4. Carbapenem-resistant *A. baumannii*

The genus Acinetobacter consists of gram-negative, aerobic cocobacillus that are ubiquitous, immobile, nonfermenting, catalase positive, and oxidase negative. The *A.calcoaceticus-A. baumannii* complex is responsible for most of the community or hospital-acquired infections **(Chusri *et al*., 2014)**.

The different species of Acinetobacter present in diverse natural habitats can be isolated from the soil, water, vegetables, and animal and human hosts. They are part of the commensal flora of human skin and mucous membranes. *A. baumannii* can survive in a variety of settings in the hospital environment: in dialysis machines, mechanical ventilation systems, water sources, skin and mucous membranes of health professionals and patients, medicinal preparations,and disinfectants **(Vahdani *et al*., 2011)**.

This microorganism has progressively accumulated resistance to penicillins, cephalosporins, quinolones, and aminoglycosides. Consequently, carbapenems have become the therapy of choice for serious infections. The mechanisms of resistance of *A. baumannii* can be intrinsic or acquired and are mediated by several factors, such as loss of membrane permeability and, more significantly, the production of betalactamases, enzymes that degrade betalactam antibiotics. Betalactamases are the most important cause of bacterial resistance, mainly in gram-negative bacilli **(Gusatti *et al*., 2009)**.

3.5. Multidrug-resistant *Pseudomonas*

P. aeruginosa is a non fermenting gram-negative bacillus,widely distributed in nature and in hospital environment.Responsible for nosocomial infections, it is one of the most important opportunistic pathogen causing bloodstream infection, urinary tract infection, and ventilator-associated pneumonia, especially in critically ill patients receiving intensive care **(Biswal *et al*., 2014)**.Moreover, it is also highly resistant to many currently used drugs making it a major public health concern **(Utchariyakiat *et al*., 2016)**.

P. aeruginosa is intrinsically resistant to several antimicrobials and has great versatility to acquire new genes that confer resistance to many other drugs. The antibiotic resistance of this bacterium is mainly due to the low cell wall permeability of this microorganism, which restricts the uptake of antibiotics, associated with wide resistance mechanisms, such as efflux pumps and enzymes, which modify or degrade antibiotics and drug targets **(Lambert, 2002)**.

4.Selective antibiotic pressure

Selective pressure refers to the environmental conditions that allow organisms with certain characteristics to survive and proliferate. Exposure to an antibiotic, for example, may inhibit or kill the majority of the bacterial population who are susceptible. However, a resistant subset of organisms may not be inhibited or killed by the antibiotic (Figure 2). These bacteria may be intrinsically resistant to the antibiotic, or they may have acquired resistance. Thus, antimicrobial use selects for the emergence of resistant strains of organisms that may then proliferate and become predominant **(McGowan, 1983)**.Indeed, antimicrobial resistance in health care facilities and the community is largely determined and magnified by the selective pressure of antimicrobial use **(Gaynes, 1995)** .

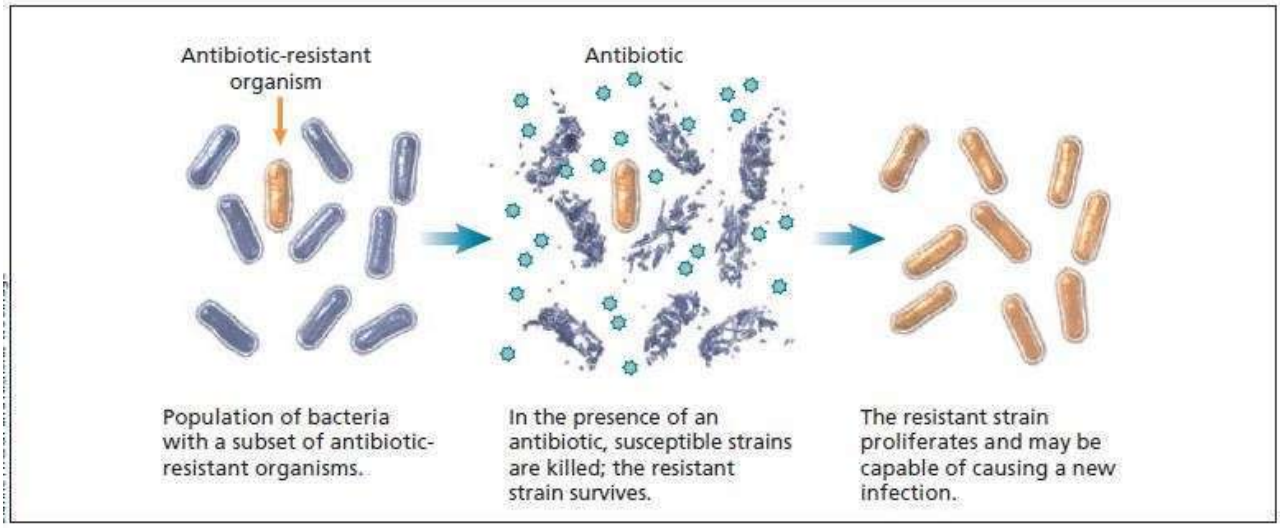

Figure 2. Effect of selective antibiotic pressure in bacteria **(Mulvey and Simor, 2009).**

5.Biofilm formation by pathogenic bacteria

Bacteria can unite and organize themselves in an extracellular matrix and forming a thin layer called a "biofilm"**(Rhoads *et al*., 2008).**

Biofilm is an organized aggregate of microorganisms living within an extracellular polymeric matrix that they produce and irreversibly attached to fetish or living surface which will not remove unless rinse quickly **(Hurlow *et al*., 2015).** Formation of extracellular polymeric substances (EPS) occurs in the attachment stage of a biofilm to the surface. Whether a microbial biofilm will form on an inanimate or solid surface or not is a consequence of the formation of an exopolysaccharide matrix, which provides strength to the interaction of the microorganisms in the biofilm **(Brandas *et al*., 2005).**

Typically 5-35% of the biofilm volume is constituted by the microorganisms while the remaining volume is extracellular matrix. This extracellular matrix is partially or mostly composed

of proteins **(Sun *et al*., 2005)**.Some important nutrients and minerals are trapped from the surrounding environment through the scavenging system, created by the extracellular matrix **(Costerton *et al*., 1994)**.

Different types of components are present in extracellular polymeric substances: protein in majority (>2%); other constituents,such as polysaccharides (1-2%); DNA molecules (<1%), RNA (<1%); ions (bound and free), and finally 97% of water. The flow of essential nutrients inside a biofilm is attributed to the water content **(Lu and Collins, 2007).**

6.Steps in biofilm formation

Genetic studies tell us about the formation of biofilm that it occurs in many steps. It requires special type of signaling, known as quorum sensing, between the microorganism cells.Also, it requires transcription of different set of genes compared to those of planktonic forms of the same microbial organisms **(Federle and Bassler, 2003)**.In addition, there are channels in the biofilm that separate the micro colonies. Mechanical stability of a biofilm is attributed to the visco elastic features of the EPS Matrix **(Shaw *et al*., 2004)**.Formation of biofilm is complex but according to different researchers it occurs in few common steps: initial contact/attachment to the surface, followed by micro-colony formation, maturation and formation of the architecture of the biofilm, and finally detachment/dispersion of the biofilm.Each of these steps will be discussed below **(Sutherland, 2001).**

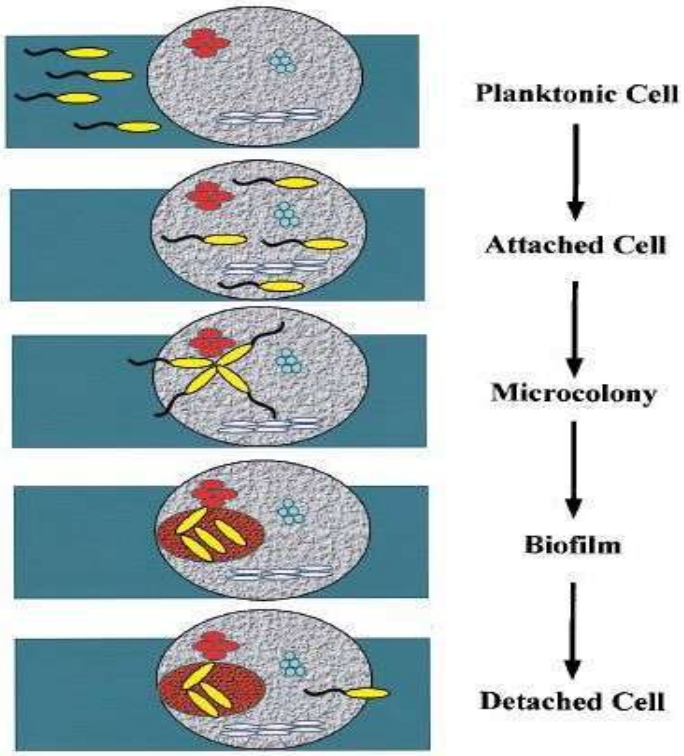

Figure 3. Schematic representation of step of a new bacterial species are taking place in forming of a biofilm on a rock previously colonized with multiple species of bacteria **(Watnick and Kolter, 2000)**.

The yellow bacteria represent an aquatic species that swims towards the rock using polar flagella, forms random loose attachments to the rock, migrates over the surface to form a microcolony, and finally produces exopolysaccharide to form a three-dimensional biofilm. When environmental conditions become unfavorable, some of the bacteria may detach and swim away to find a surface in a more favorable environment .

7.Infections associated with biofilm

It is estimated that about 65% of all bacterial infections are associated with bacterial biofilms **(Lewis, 2001).**These include both, device- and non-device-associated infections. Data for device related infections have been estimated for several devices, such as: 2% for breast implants; 2% for joint prostheses; 4% for mechanical heart valves; 10% for ventricular shunts; 4% for pacemakers and defibrillator, and about 40% for ventricular-assisted devices **(Darouiche, 2004).** Native valve endocarditis (NVE) is an inflammation caused by interaction of bacteria with the vascular endothelium and pulmonic valves of the heart. This is usually the result of *Streptococci*, *Staphylococci*, gram negative bacteria, and/or fungal infections **(Kokare *et al.*, 2009).**

In this condition microbial cells gain access to the heart and blood through the gastrointestinal tract, urinary tract and/or through the oropharynx. As the intact valve endothelium gets damaged by

the microorganisms that attach to it, even after the bacteria have been cleared by the immune system a non-bacterial thrombotic endocarditis (NBTE) develops at the injury location,as a result a thrombus formation occurs, a condition where platelets, red blood cells and fibrin are aggregated **(Donlan and Costerton, 2002)**.

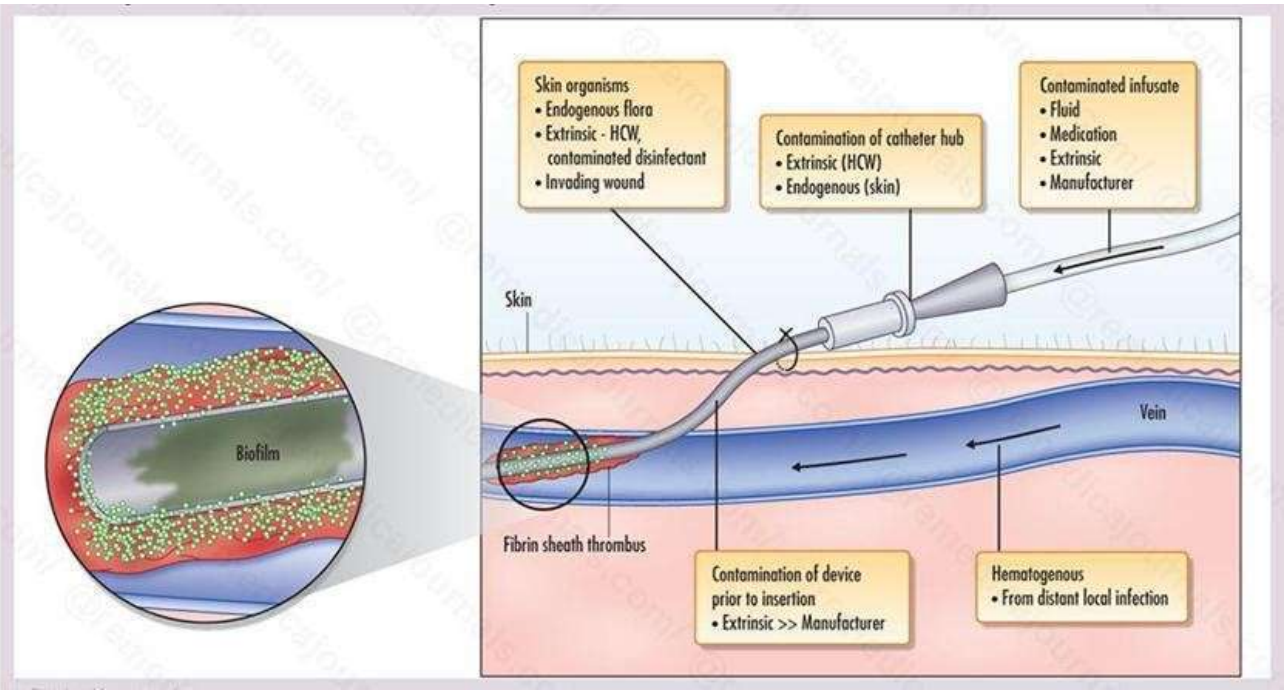

Figure 4. Biofilm formation on venous catheter **(James *et al*., 2011).**

II. Lactic acid bacteria

1. Generality

The term lactic acid bacteria (LAB) was gradually accepted in the early 20th century **(Van Reenen and Dicks, 2011)**. Other terms such as "milk acidifying bacteria" and "producing lactic acid"had already been used for the same bacteria, causing slight confusion. This ended with the publication of a monograph on lactic acid bacteria written by **Orla-Jensen (1919).**

Lactic acid bacteria (LAB) are a group of phylogenetically diverse Gram-positive bacteria characterized by some common morphological, metabolic and physiological traits.

This group includes cocci or rods, nonsporing, microaerofilic or facultatively anaerobic, lacking off cytocroms and catalase *sensu stricto*, producing lactic acid as the major end-product during the fermentation of carbohydrates (at least 50%) and characterized by a G + C content smaller than 55 mol%. Recent taxonomic studies suggested that the group should include the following genera: *Streptococcus sensu stricto*, *Lactococcus*, *Enterococcus*, *Pediococcus*, *Leuconostoc*, *Lactobacillus*, *Carnobacterium*, *Aerococcus*, *Tetragenococcus*, *Vagococcus*, *Oenococcus* and *Weissella* **(Stiles and Holzapfel, 1997).**

Because they have complex nutritional requirements for amino acids, vitamins, peptides, salts, fatty acids and carbohydrates, lactic acid bacteria are present every where in nature and they are generally associated with nutrient-rich habitats such as different food products (milk, beverages, meat products, plant products...). They also exist in the digestive system of humans, they belong to the normal flora of the gut, the mouth and the vagina **(Audisio and Apella, 2010) .**

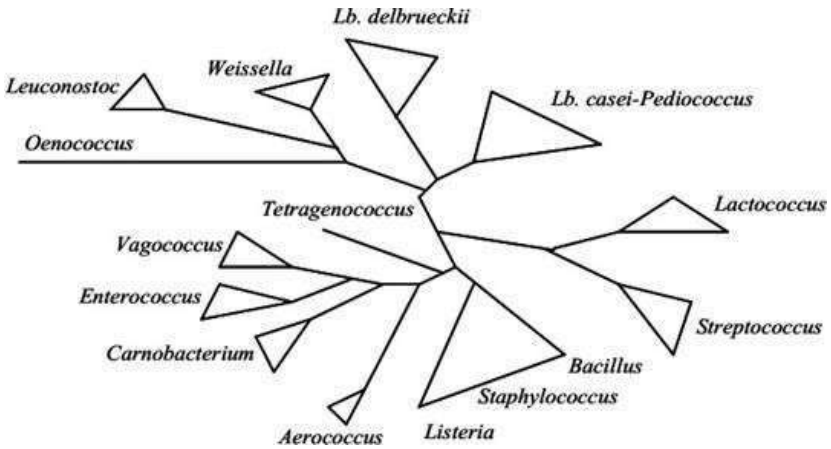

Figure 5. Phylogenic tree of lactic acid bacteria and comparison with the genera *Aerococcus, Bacillus*, *Listeria* and *Staphylococcus* **(Axelsson, 2004).**

2. Classification

The classification of lactic acid bacteria is essentially based on the composition of the bacterial cell wall, including the nature of fatty acids, such as lactobacillic acid (C19: 0) and unsaturated fatty acids (C14: 0, C16: 0, C18 : 0) **(De Ambrosini et al., 1996).**

A second classification of lactic acid bacteria is focused on the different models of glucose fermentation, divided into 3 groups **(McLeod et al., 2008).** Group I contains bacteria carrying out exclusively homofermentation, composed mainly of *Lactobacillus.* Group II includes heterofermentation bacteria and includes *Leuconostoc*, *Oenococcus, Weissella* and some species belonging to the genus Lactobacillus. Group III includes species belonging to the genus *Lactobacillus* and the majority of species belonging to the genus *Enterococcus, Lactococcus* and *Streptococcus* and presents an intermediate position between group I and II, the two glucose fermentation pathways (homofermentation, heterofermentation) **(McLeod *et al*., 2008).**

The second edition of Bergey's manual of systematic bacteriology **(Vos *et al*., 2009)** classifies lactic acid bacteria into two distinct branches: Firmicutes and Actinobacteria. The branch of Firmicutes contains the most important genera: *Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Pediococcus, Streptococcus and Weissella* which belong to the order Lactobacillales, characterized by a low GC content (31-49%). The Actinobacteria phylum contains the genus Bifidobacterium, characterized by a high GC content (58-61%) **(Horvath *et al*., 2009).**

DNA / DNA hybridization studies, and ribosomal RNA sequences have become essential elements in the identification and taxonomic classification of lactic acid bacteria **(Vandamme *et al*., 1996).** Currently, with the technologies of PCR and automatic DNA sequencing of the 16S rRNA gene, species identification has become a simpler practice.

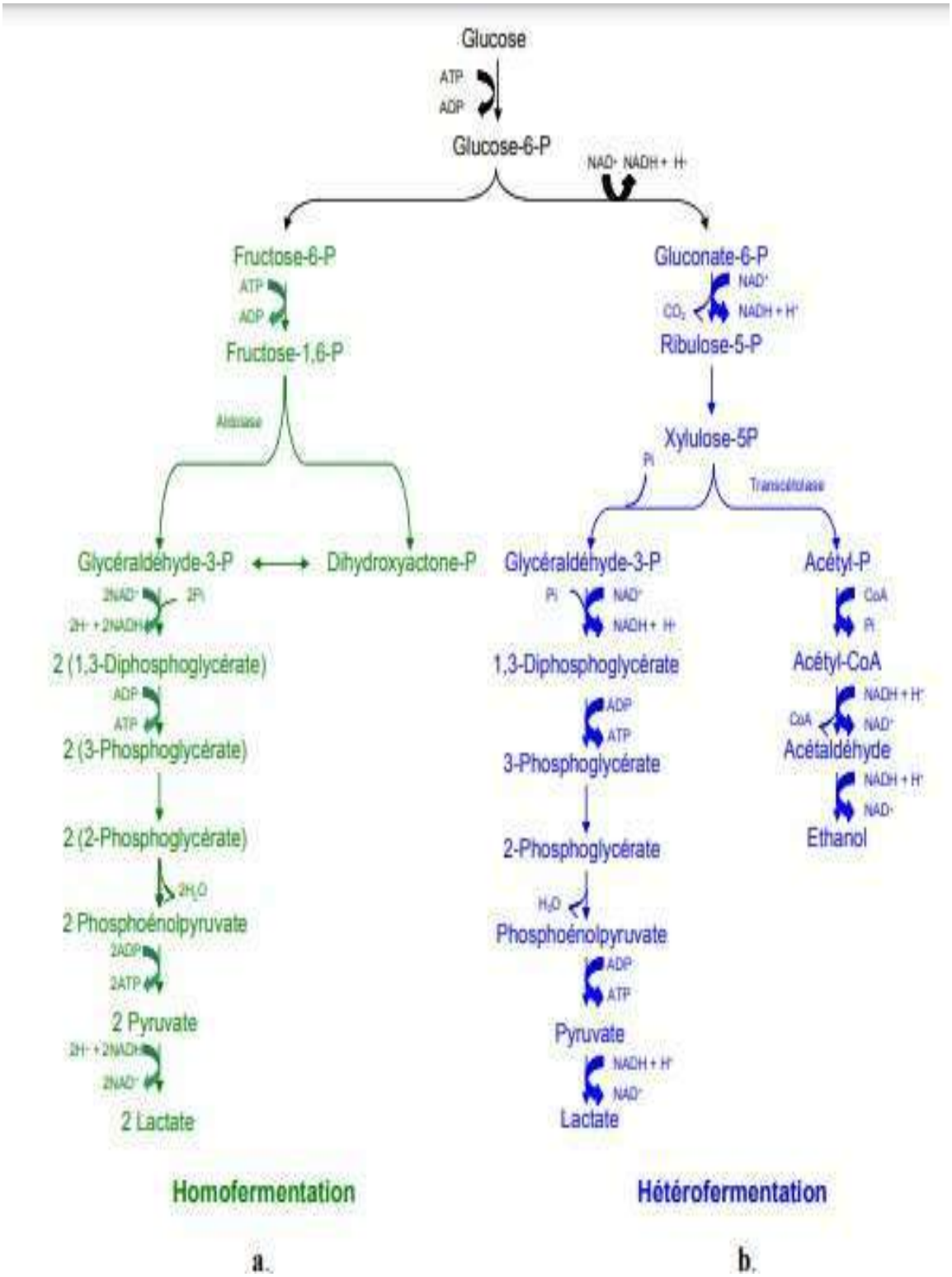

Figure 6. Schematic representation of the main fermentation pathways of hexoses in lactic acid bacteria **(Kandler, 1983).**

3. Antimicrobial properties of lactic acid bacteria

The antagonist activity of lactic acid bacteria is mainly due to the production of different organic and non-organic compounds able to inhibit the growth of certain pathogens

.

3.1. Organic acids

Lactic acid bacteria produce different types of organic acids. During the production of organic acids, the pH value of the culture medium decreases, thus inducing the inhibition of the flora.

3.2. Hydrogen peroxide

Catalase, an enzyme required to break down hydrogen peroxide into oxygen and water, is absent in lactic acid bacteria. This results in an accumulation of this compound which can be an inhibitor of various microorganisms. Inhibition occurs by oxidation of membrane lipids of target strains and by destruction of cellular protein structures **(Strus *et al.*, 2006).**

3.3. Carbon dioxide

It is essentially formed during heterolactic fermentation. Under anaerobic environmental conditions, carbon dioxide inhibits aerobic microorganisms. Its accumulation in the lipid bilayer causes a dysfunction of membrane permeability **(Ammor *et al.*, 2006).**

3.4. Diacetyl

Diacetyl is an essential aromatic compound. Numerous bacteria of the genera *Lactococcus*, *Leuconostoc*, *Lactobacillus* and *Pediococcus* can synthesize it **(Leveau and Bouix, 1993).** The inhibitory power of diacethyl vis-à-vis yeasts, Gram-positive and negative bacteria is less sensitive **(El -Ziney *et al.*, 1998).**

3.5. Reuterine

Reuterin (or 3-hydroxypropionaldehyde) is an intermediate metabolite with antimicrobial potency. Reuterin is produced during the anaerobic fermentation of glycerol by certain species of *Lactobacillus* and other genera of non-lactic bacteria such as *Bacillus*, *Klebsiella*, *Citrobacter*, *Enterobacter* and *Clostridium* **(El-Ziney et al., 1998).**

III. Bacteriocin

1. Generality

Bacteriocins are a heterogeneous group of ribosomally synthesized peptides or proteins displaying antimicrobial activity against other bacteria **(Klaenhammer, 1993).**

They are protein toxins produced by bacteria and certain members of archaea to inhibit the growth of similar or closely related bacterial strains **(Bemena *et al*., 2014).** These molecules haveantimicrobial activity against pathogenic and deteriorating bacteria, justifying their biotechnologicalpotential. If the bacteriocins produced by a bacterium inhibit other bacteria belonging to the samespecies, they are generally considered to be narrow-spectrum bacteriocins. In contrast, if theyinhibit bacteria belonging to another genus, they are considered to be broadspectrum bacteriocins.

Interestingly, bacteriocin-producing bacterial cells are resistant to their antimicrobial peptides, which are mediated by specific immunity proteins produced by host cells **(Juturu and Wu, 2018)**.

The genes encoding bacteriocin production and immunity are generally organized in operonclusters and may reside on mobilizable elements such as chromosome in conjunction with transposons or on a plasmid **(Zacharof and Lovitt, 2012)**.

LAB-bacteriocins may contribute to reducing the frequency at which resistant bacterial populations develop and to improving the hygienic quality and shelf-life of food **(Vignolo *et al*., 2000).**

2.Bacteriocin classes

Bacteriocins were classified according to primary structures, molecular weight, post-translational properties and genetic characteristics. According to Klaenhammer (1993),four classes of bacteriocins have been distinguished. Subsequently,different classification schemes for bacteriocins have been proposed, taking into account new subclasses,based on the mechanism of biosynthesis and the antibacterial activity of the molecules **(Alvarez-Sieiro *et al*., 2016)**.

2.1. Class I Bacteriocins/Lantibiotics.

Lantibiotics are small (<5 kDa) heat-stable peptides that are highly post translational modified and that contain characteristic polycyclic thioether amino acids such as lanthionine, methyl-lanthionine,and unsaturated amino acids such as dehydroalanine and 2-amino isobutyric acid. Lantibiotics are further subdivided into two types, depending on the difference in charge **(Kaur, 2015)**.The lantibiotics are divided into two subgroups, A and B, differing according to their structural characteristics and their mode of inhibition **(Dierksen *et al*., 2000)** .

Type A-lantibiotics such as nisin and lacticin 3147 are flexible screw-shaped molecules with a positive charge of 2–4 kDa which causes the formation of pores in the cell membrane of the target organism and thus lead to depolarization of the target species cytoplasmic membrane **(Kaur ,**

2015).

Type B lantibiotics include globular peptides that are negatively charged or without net charge, are smaller than type A, and contain up to 19 amino acids **(Avonts and De, 2001)** .

2.2. Class II Bacteriocins.

Class II bacteriocins or non-lantibiotics are relatively small molecules (<10 kDa) ranging in size from 30 to 60 amino acids. They are thermostable and do not undergo post-translational modification. This class comprises the largest subgroup of bacteriocins: class IIa (pediocin-like), characterized by a close activity against Listeria monocytogenes **(Fimland *et al*., 2005)** .

Bacteriocins in subclass IIb include lactacin F and lactococcin G. These are two-component bacteriocins, in which two distinct peptides act synergistically to generate an antimicrobial effect **(Kaur, 2015)**. The third subclass IIc contains circular bacteriocins such as gassericin A, circularin A, and carnocyclin A **(Cotter *et al*., 2005)**. These peptides carry two transmembrane segments that facilitate the formation of pores in the target cells **(Kawai *et al*., 2004)** .

2.3. Class III Bacteriocins.

This class includes bacteriocins which have a high molecular weight (> 30 kDa). They are thermolabile pro teins that act in a different way from other classes of bacteriocins. Colicin is the most characteristic of this class **(Lazdunski, 1995)** . It generally contains three domains, including receptor binding, translocation and the lethal domain **(Riley, 1993)**. Some of the colicins, megacins (from *Bacillus megaterium*), klebicin (from *Klebsiella pneumonia*), helveticin I (from *Lactobacillus helveticus*), and enterolysin (from *Enterococcus faecalis*) are members of this group **(Kaur ,2015)** .

Another proposed additional class (class VI) is defined as complex bacteriocins containing lipid or carbohydrate moieties. Little is known about the structure and function of this class, which includes leuconocin S **(Balakrishnan *et al*., 2000)** and lactocin 27 as an example **(Tomas *et al*., 2004)** .

Table 1. Classification of bacteriocins adapted from (Gulluce *et al*., 2013).

Classification	Features	Subcategories	Examples
Class I bacteriocins (lantibiotics)	Lanthionine or peptides containing β-lanthionine	Type-A (linear molecules)	Nisin, subtilin, epidermine
		Type-B (globular molecules)	Mersacidin
Class II bacteriocins	Heterogeneous class of small thermostable peptides	Subclass IIa (antilisterial pediocine bacteriocins type)	Pediocin, enterocin, sakacin
		Subclass IIb (composed of two peptides)	Plantaricin, lactacin F
		Subclass IIc (other bacteriocins)	Lactococcin
Class III bacteriocins	Large thermolabile peptides		Helveticin J, millericin B

3.Mode of action of bacteriocins

The bacteriocins often act on the target cells in two steps: adsorption of the bacteriocin at the cell surface, followed by the formation of pores on the plasma membrane of the target cell

(Guyonnet *et al.*, 2000), causing a permeability of this one and thus cell death **(Bauer and Dicks, 2005)** .

Their mechanisms of action on the target cell are varied, and can be divided into three types: bacteriostatic action that leads to slowing down or stopping growth, without cell death, bactericidal action during which bacteria die while keeping their physical integrity (no cell lysis) and bacteriolytic action that leads to dissolution of the bacterial cell **(Taale *et al.*, 2016).**

Certain bacteriocins, and in particular many of those that inhibit Gram-positive bacteria, work by attacking the cell envelope. Certain class I bacteriocins inhibit lipid-II on the cell membrane, thus eliminating the synthesis of peptidoglycan.Other bacteriocins form pores to inhibit or kill their target bacteria (Figure 7). For example, class II bacteriocins such as lactococcin A bind to the pore-forming receptor mannose phosphotransferase system (Man-PTS) **(Cotter *et al.*, 2013)**.

It has been shown that some members of class I or lantibiotic bacteriocins, such as nisin, have a dual mode of action. They can bind to lipid-II, the main transporter of peptidoglycan subunits from the cytoplasm to the cell wall, and therefore prevent correct cell wall synthesis, leading to cell death **(Cotter *et al.*, 2005)**.

Many bacteriocins that inhibit Gram-negative bacteria (and thus need to be transported through the outer and, in many cases, inner membranes before functioning) control their target bacteria by interfering with DNA, RNA, and protein metabolism (Figure 8) **(Cotter *et al.*, 2013)**.

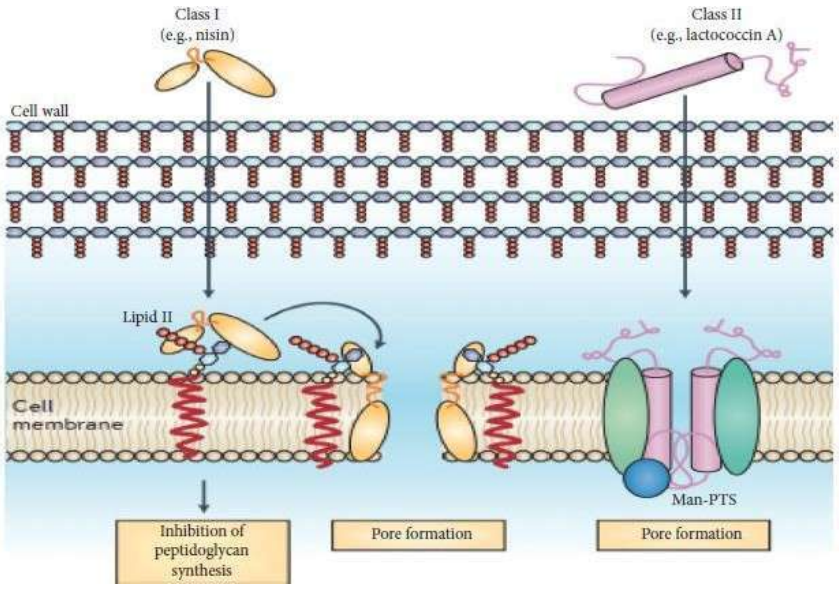

Figure 7.Mechanism of action of bacteriocins on Gram-positive bacteria **(Cotter *et al.*, 2013)**.

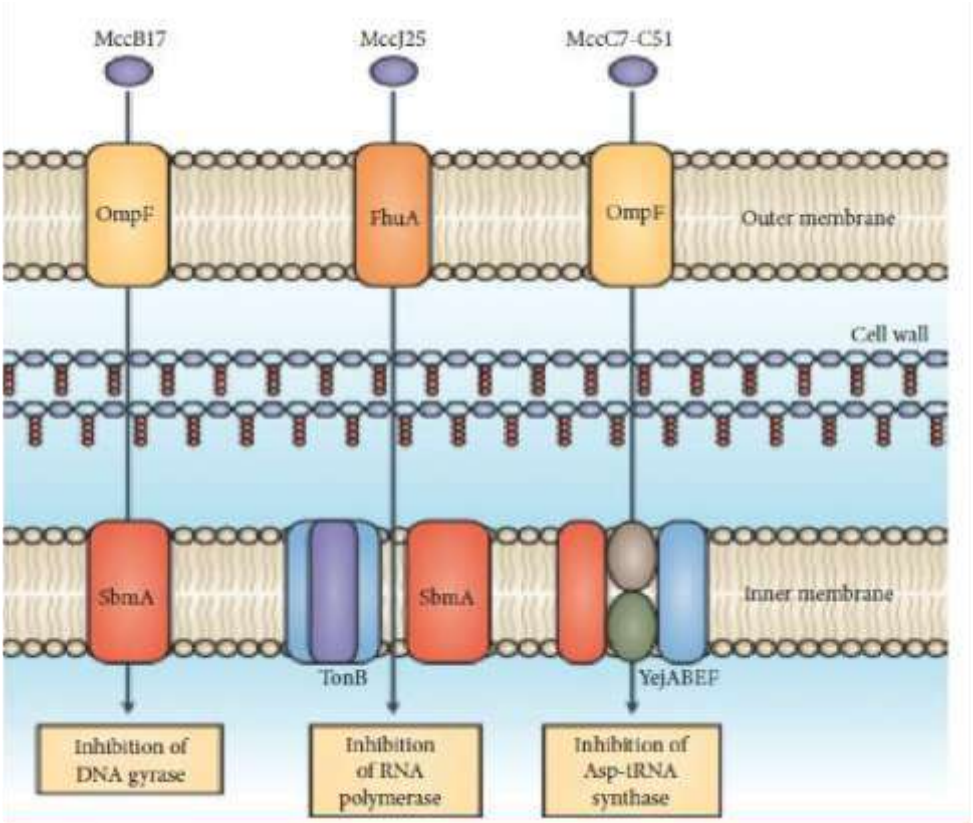

Figure 8 . Mechanism of action of bacteriocins on Gram-negative bacteria **(Cotter *et al*., 2013)**

4.Comparison between Bacteriocins and Antibiotics

Bacteriocins can be considered "designer drugs" that target specific bacterial pathogens. *Escherichia coli* and other members of the *Enterobacteriaceae* family are the few examples of Gram-negative bacteria and lactic acid bacteria; *Bacillus* species belong to Gram-positive bacteria which produce bacteriocins **(Prabhakar *et al*., 2013)**.

When they are in comparison, bacteriocins have a ribosomally synthesized nature, while antibiotics are produced by multiple enzymes complexes. Often bacteriocins exhibit bactericidal or bacteriostatic effects on a narrow spectrum of bacteria, but traditional antibiotics have a wider spectrum.

Besides, most bacteriocins are more effective against their target bacteria than antibiotics at lower concentrations **(Gulluce *et al*., 2013)**.Bacteriocins are often considered more natural because they are believed to have been present in many of the foods consumed since ancient times. Bacteriocins are inactivated by enzymes, such as trypsin and pepsin, found in the gastrointestinal tract and therefore do not alter the microbiota of the digestive tract **(Balciunas *et al*., 2013)**.

1. Origin of strains

The acid lactic bacteria test strains (Table 2) were isolated from artisanal cheese in **2019** by Mr Bouricha , they were stored at -20 ° C in MRS broth supplemented with 20% glycerol.

Table 2. Different strains of lactic acid bacteria tested.

Strains	Identification
LAB7	*Lactococcus lactis subsp lactis*
LAB8	*Leuconostoc mesonteroide*
LAB14	*Lactococcus raffinolactis*
LAB18	*Lactococcus lactis subsp lactis*
LAB24	*Lactococcus lactis subsp lactis*
LAB31	*Leuconostoc mesonteroide subsp crémoris*
LAB79	*Lactococcus lactis*
LAB85	*Lactococcus lactis*
LAB86	*Leuconostoc crémoris*
LAB87	*Leuconostoc sp*
LAB96	*Lactococcus diacetilactis*
LAB77	*Entérococcus durans*
LAB c1	*Ln.mésenteroides subsp mésenteroides*
LAB c2	*Ln.mésenteroides subsp mésenteroides*
LAB c10	*Leuconostoc gelidum*
LAB c12	*Leuconostoc fallax*

The antibacterial activity of the **16** lactic acid strains is tested against 6 bacteria multi-resistant to antibiotics, making parts of the collection of strains from **Mrs** DJELLOUL-DAOUADJI (Table 3).

Table 3. Bacterial strains used in this study and their resistance profiles

Target strains	Resistance
E.coli BLSE	Cephalosporin resistance 3rd generation
L.monocytogenes ATCC 13932	/
P.aeruginosa (PA)	Cephalosporin resistance 3rd generation
K. pneumoniae (KP)	Cephalosporin resistance 3rd generation
B.subtilis ATCC 6633	/
S.aureus (MRSA)	Methicillin resistance

2.Revivification

Revivification of the lactic acid bacteria was carried out by successive subcultures on MRS broth at pH = 6.5 until good bacterial growth was obtained. The incubation was carried out in an oven at 37 ° C for 24 hours.

3. Determination of antibacterial activity

The antibacterial activity of the lactic acid strains against the target strains was studied using their **young** spot cultures or their culture supernatant (native) by the well method.

3.1. Spot test

The antibacterial activity of (LAB) against pathogenic strains was demonstrated by the direct antagonism test. After **pouring** the MRS agar into the dishes, a volume of 5 µl of the suspension bacterial (10^6 CFU / ml). each lactic strain was placed in spots on the MRS agar.

The **dishes** were incubated at 37 ° C for 18h. After the incubation period, the dishes were covered with 10 ml of nutrient agar (GN), previously inoculated with the target strain, then re-incubated at 37 ° C. After 24 hours of incubation, the presence or absence of inhibition around the spots is noted (Schillinger and Lucke., 1989).

3.2. Well testing

In order to test the antibacterial activity of the culture supernatant of lactic acid strains with respect to target strains, cultures of lactic acid strains prepared in broth MRS were centrifuged at **12000g / for 30 min at 4 ° C**. After centrifugation, we have collects the supernatant and we tested its activity against multi target strains resistant (Table 3). The protocol is as follows:

One milliliter (1 ml) of the suspension of the target strains (10^6 CFU / ml) is inoculated with mass in 20 ml of Mueller Hinton agar. After solidification of the agar, wells of 6mm in diameter are produced in the agar. The wells were subsequently filled with 100µl of native supernatant, and the **dishes** were refrigerated for 2 hours at 4 ° C. After this period, the dishes were incubated at 37 ° C for 24 h. Antibacterial activity is revealed by the presence of zones of inhibition around the wells (Barefoot and Klaenhammer wells, 1983).

4.Adhesion of the LAB and spoilage isolates to polystyrene tissue culture plates (TCP)

The semi-quantitative method of adhesion to polystyrene tissue culture plates described by O'Toole and Kolter (1998) was used with some modifications **(Mohapatra and Jeevaratna, 2019).** Briefly, 100 µl of each culture (LAB isolates, 10^8 CFU/ml and spoilage isolates, 10^6 CFU/ml) in MRS and nutritious broth (NB) respectly (NB supplemented with 2% sucrose) were added to the wells of sterile 96-well polystyrene tissue culture plates . Cultures were decanted and wells were washed twice with sterile distilled water to remove the non adherent cells. The adherent cells in each well were fixed with 200 µl of ethanol , and after 15 min the plates were emptied and left to

dry, then they were stained for 45 min with 200 µl of 0.1% crystal violet. The stained biofilms were rinsed three times.

with 200 µl of distilled water. The content of each well (125 µl) of each well was then transferred to new sterile microplate, and the amount of biofilm was quantified .

5. Inhibition of the spoilage isolates by the LAB forming biofilms

LAB and spoilage isolates at 10^8 CFU/ml, and 10^6 CFU/ml, respectively were grown in MRS and NB, as appropriate medium for biofilm formation, and incubated at 37 °C for 18 h. After which, CFS, obtained by centrifugation (8000 × g, 20 min, 4 °C) were filtered with 0.22 µm-pore-size .The 96-well polystyrene microplates were inoculated with 50 µl of overnight spoilage cultures and 50 µl of the LAB neutralized CFS. The microplates were left stirring for 15 min before to be incubated for 24 h at 37 °C in a humid atmosphere. Afterwards, the microplates were rinsed three times with 200 µl of distilled water. The adherent cells in each well were fixed with 200 µl of ethanol. After 15 min, the plates were emptied and left to dry,then were stained for 20 min with 200 µl of crystal violet . The stained biofilms were rinsed three times with 200 µl of distilled water. The content of each well (125 µl) was transferred to new sterile microplate. The amount of biofilm was quantified by **observation of the color intensity**.

Results

1. Demonstration of antibacterial activity

1.1. Spot test

Among the 16 strains of lactic acid bacteria, 6 showed an antagonistic effect against all the resistant bacteria tested.The obtained results indicated that the measured average diameter of the inhibition zone was around 6-27 mm with differently inhibitory effects twards clinical pathogen bacteria (Figure 9,table 4).

The best antibacterial activity against *P.aeruginosa* (PA) was recorded with the LAB c1. Concerning, *L.monocytogenes* AT*CC* 13932 the best activity was recorded with strains LAB 14,LAB 86,LABc1, LABc2, LABc10, LABc12. In addition, the weakest activity with regard to the *K. pneumonia* (KP), strain was recorded in strains LAB8, LAB31, and for *S. aureus* MRSA are LAB7, LAB31.Strains LAB77, LAB79 showed no antagonistic effect against *B.subtilis* ATCC 6633, the same for strain LAB24 against *S. aureus* MRSA .A first, the isolated, selected LAB, *L.raffinolactis* LAB 14 and *Leuconostoc mesenteroides* subsp. *cremoris* LAB86 has manifested a excellent inhibition zones against all clinical pathogen bacteria.

Table 4. Illustration of the inhibitory effect of the isolated antagonistic lactic acid bacteria (LAB7, LAB8. LAB14, LAB18, LAB24, LAB31, LAB77,LAB 79, LAB85, LAB86, LAB87, LAB96, LABc1, LABc2, LABc10, LABc12) against pathogen bacterial *E.coli* BLSE, *S.aureus* (MRSA), *P.aeruginosa* (PA), *K. pneumonia* (KP), *B.subtilis* ATCC 6633, *L.monocytogenes* AT*CC* 13932

Isolated bacterial LAB	**Code**	**Inhibitory effect of the isolated antagonistic lactic acid bacteria against pathogen bacterial**					
		E.coli BLSE	*B.subtilis* ATCC6633	KP	PA	*L.monocytogenes* ATCC13932	MRSA
Lactococcus lactis subsp lactis	LAB7	10	12	00	09	11	06
Leuconostoc mesonteroide	LAB8	12	09	06	00	10	08
Lactococcus raffinolactis	LAB14	13	14	16	17	27	18
Lactococcus lactis subsp Lactis	LAB18	12	10	00	14	10	14
Lactococcus lactis subsp Lactis	LAB24	10	12	10	13	11	00
Leuconostoc mesonteroide subsp crémoris	LAB31	12	03	06	12	10	06
Entérococcus durans	LAB77	18	00	10	14	11	14
Lactococcus lactis	LAB 79	15	00	12	15	11	12
Lactococcus lactis	LAB 85	11	06	10	00	09	20
Leuconostoc mesenteroides subsp. *Cremoris*	LAB 86	12	15	10	18	25	18
Leuconostoc sp	LAB 87	06	10	16	00	15	17
Lactococcus diacetilactis	LAB 96	15	05	13	14	11	21

Ln.mésenteroides subsp Mésenteroides	LAB c1	14	20	11	26	21	21
Leuconostoc lactis	LAB c2	15	13	19	10	23	11
Leuconostoc gelidum	LABc10	17	16	14	19	27	16
Leuconostoc fallax	LABc12	20	12	15	14	22	22

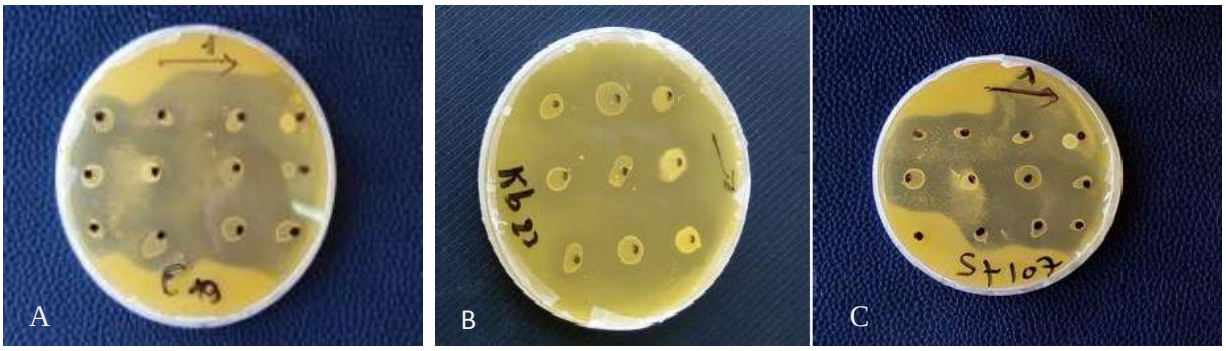

Figure 9 .Antibacterial activity of the isolated lactic acid bacteria against such pathogen bacterial growth of *E.coli* BLSE (A) , *K. pneumonia* (KP) (B) and *S. aureus* MRSA (C) by the using agar diffusion method, inoculated on the MRS solid culture medium, incubated at temperature of 30°C for 24 hours.

1.2. Well testing

The results of the antibacterial activity of the native culture supernatant of the strains lactic acid with respect to the target strains is presented in Figure 10. The best antibacterial activity was recorded with the culture supernatants of strains of LAB14 and LAB86 against *L.monocytogenes* ATCC13932 , *S.aureus* MRSA and *P. aeruginosa* (PA) respectively. The weakest antibacterial activity concerns the supernatants of the strains LABc1, LABc2, LABc10, LABc12.

Figure10. Representing antibacterial activity of *Leuconostoc mesenteroides* subsp. *cremoris* (LAB86) against *Staphylococcus aureus* MRSA by the well method.

2.Biofilm formation by pathogenic bacteria

After coloration by crystal violet, we note the intensity of the coloration for the Six multiresistant pathogenic bacteria E.coli BLSE (1), L.monocytogenes ATCC (2), P.aeruginosa (PA) (3), K. pneumonia (KP) (4),B.subtilis ATCC 6633 (5), S.aureus (MRSA) (6). The results obtained (figure 11)showed that the pathogenic bacteria (MDR) tested are strongly adherent.

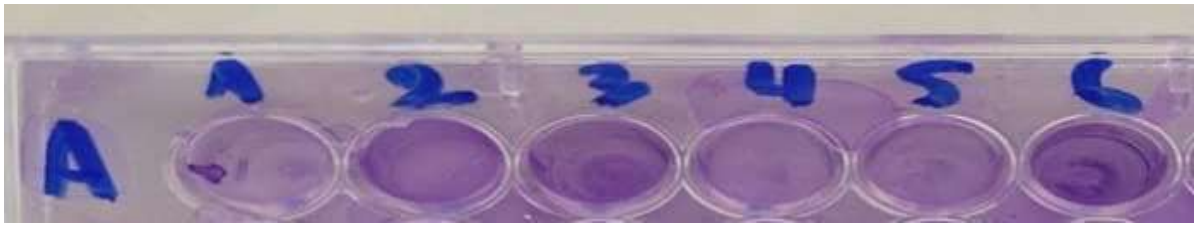

Figure 11. Microtiter plate method showing none, strong, moderate, and weak biofilm producers differentiated by crystal violet stain in 96-well tissue culture plate. *E.coli* BLSE (1), *L.monocytogenes* AT*CC* 13932 (2), *P.aeruginosa* (PA) (3), *K. pneumonia* (KP) (4), *B.subtilis* ATCC 6633 (5), *S.aureus* (MRSA) (6).

Table 5. The results of biofilm formation for sixpathogens bacteria (1, 2, 3, 4, 5 and 6).

\\\\	1	2	3	4	5	6
Biofilm formation	**+**	**+**	**++**	**+**	**+**	++

3. Antibiofilm activity of the bioactive substance against pathogenic bacteria

The following table 6 and figure 12 showed the results of four kinds of the supernatant which containing bacteriocin (B, C,D and E) against six pathogenic bacteria *E.coli* BLSE (1), *L.monocytogenes* ATCC (2), *P.aeruginosa* (PA) (3), *K.pneumonia* (KP) (4), *B.subtilis* ATCC 6633 (5), *S.aureus* (MRSA) (6). by crystal violet stain in 96-well tissue culture plate.

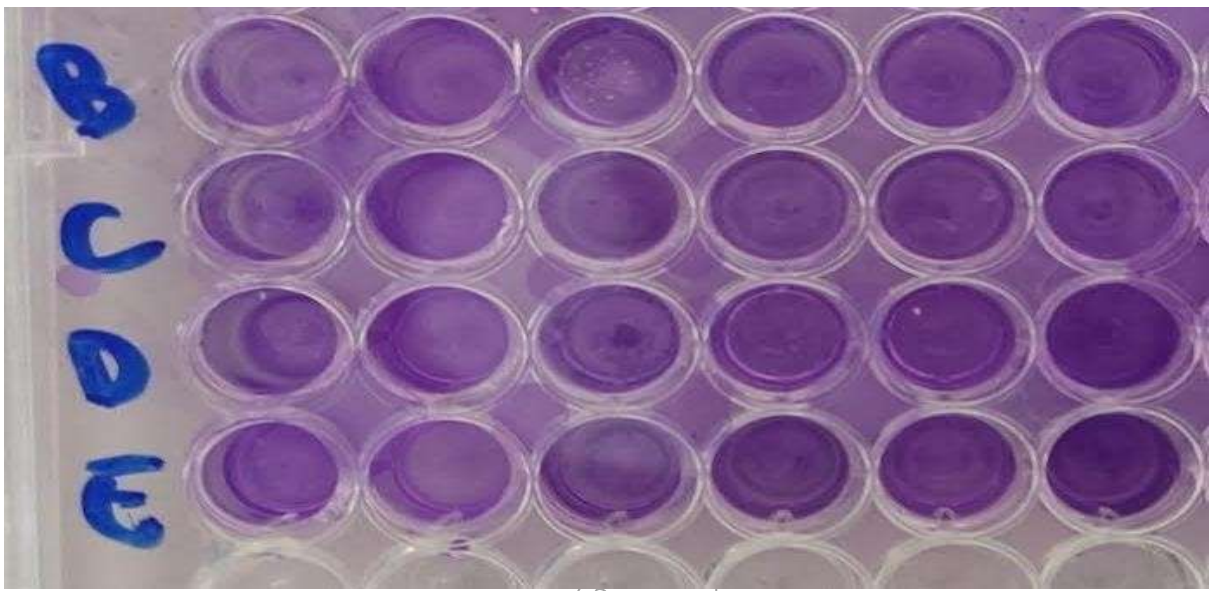

Figure12 . Microtiter plate method showing none, strong, moderate, and weak biofilm of four bacteriocin (B, C ,D and E) against six pathogenic bacteria (1 , 2, 3, 4, 5 and 6) producers differentiated by crystal violet stain in 96-well tissue culture plate.

E.coli BLSE (1), *L.monocytogenes* AT*CC* (2), *P.aeruginosa* (PA) (3), *K. pneumonia* (KP) (4), *B.subtilis* ATCC 6633 (5), *S.aureus* (MRSA) (6).

LAB86(B), LAB 14(C), LABc1 (D), LABc2 (E).

Table 6. Table 6.The results of four bacteriocin (B, C ,D and E) against six pathogenic bacteria (1 , 2, 3, 4 , 5 and 6).

\\\\	1	2	3	4	5	6
B	+	+	+	+	+	+
C	+	+	+	+	+	+
D	++	+	++	++	++	+++
E	++	+	++	+++	+++	+++

Strong biofilm = +++ ,Moderate biofilm = ++ , Weak biofilm = +, No biofilm = -

E.coli BLSE (1), *L.monocytogenes* AT*CC* (2), *P.aeruginosa* (PA) (3), *K. pneumonia* (KP) (4), *B.subtilis* ATCC 6633 (5), *S.aureus* (MRSA) (6).

LAB86(B), LAB 14(C), LABc1 (D), LABc2 (E).

The occurrence of a rapid emergence of bacterial resistance threat the longevity of antibiotics. Furthermore, the nosocomial infections caused by multidrug resistant Gram-negative pathogens bacteria presented as a major burden to both patients and healthcare systems, which was accompanied with increased annual mortality **(Meade *et al*., 2020).**

The produced bacteriocin by LAB has explored a huge potential as food preservatives and the production for next generation antibiotics, which can be used as target for the MDR pathogens **(Perez *et al*., 2014).**

In the present work, 16 strains of lactic acid bacteria (LAB) were identified to test their antibacterial activity against multi-resistant strains. The activity of the latter was revealed by applying two different tests (spot and well test). The results obtained showed significant activity against target strains with areas very distinct inhibitions, the diameter of which varies between 06 to 27 mm and this depending on the strain studied.

Lack of activity antibacterial does not necessarily mean that the substance is absent or not active enough, but this may be due to poor diffusion of it, in the environment, because it is not polar or well made up of non-polar compounds.

The investigated antibacterial activity of the LAB isolates against MDR bacterial growth hat indicated that among 16 isolates, only 4 bacterial strains have manifested important inhibition and used for further characterization. Both bacterial strains *L. raffinolactis* LAB14 and *Leuconostoc mesenteroides* subsp. *cremoris* LAB86, LABc1, LABc2 were excellent candidates for inhibition of various Gram-positive and negative bacteria.

Mokdad *et al*. **(2020)** has reported that *Leuconos-toc mesenteroides* subsp. *cremoris* has showed a important potentiel for inhibition of pathogens bacteria such as *L. monocytogenes L. innocua L. ivanovii S. aureus B. thermosphacta M. luteus L. sakei E. coli P. aeruginosa.*

The ability of bacteria to biofilms has attracted considerable interest from scientists. Biofilms are a multi-microbial community integrated into a polymer matrix, attached to a biotic or abiotic surface **(Miquel *et al*., 2016).**

The study of the antibiofilm activity of four lactic acid bacteria, isolated selected antagonists (LAB14, LAB86, LABc1 and LABc2) is demonstrated for the reduction of biofilm formation and the binding of pathogenic bacteria

The results obtained showed that the pathogenic bacteria tested are strongly adherent. The presence of the culture supernatant of the LAB86 and LAB14 strains reduced the adhesion of the bacteria *S.aureus* and *P.aeruginosa*, However, the supernatant of LABc1 and LABc2 showed no influence on adhesion of pathogenic bacteria.

Bacteria adhered to the surface of the wells are revealed by a crystal violet staining Notably, the

genus Leuconostoc was investigated for the antibiofilm potential, and showed considerable inhibition against biofilm formation of *Pseudomonas aeruginosa* (PA) and *Staphylococcus aureus* MRSA respectively **(Salman *et al.*, 2015).**

Previous work carried out by **Ait Ouali** *et al*. **(2014)** have shown that the culture supernatants of lactic acid bacteria are able to prevent the adhesion of the *S. aureus* SA3 strain, and the formation of biofilm.

Pimentel-Filho *et al*. **(2014)** showed that HC5 biovin and nisin inhibited the adhesion of *S. aureus* bacteria and biofilm formation. (Todoriki et al. 2001) have shown that the antimicrobial substances contained in the culture supernatant of lactic acid bacteria are responsible for the anti-adhesion activity.

Bacteriocin produced from *L .raffinolactis* (LAB14) and *Leuconostoc mesenteroides* subsp. *cremoris* (LAB86) showed broad-spectrum antibacterial activity against numerous Gram-positive and Gram-negative pathogens.Considering all the results obtained, we can conclude that the LAB14 and LAB86 could be used as an alternative to antibiotics .

The evaluation of the anti-biofilm activity of the neutralized supernatant of LAB86 and LAB14 showed a significant inhibition of biofilm formation vis-à-vis many Gram-positive and negative bacteria, which explains the biofilm formation of pathogens is strongly related to the probiotic and pathogenic strain.

The above work has opened an opportunity for potential biotechnological and clinical applications of bacteriocins. Further complete characterization of the purified bacteriocins is required to determine and confirm the novelty of these bioactive antibacterial compounds.

The results obtained by this study remain preliminary, they must be supplemented by a series of other tests, namely:

-Confirmed, isolate components from the strains supernatant

-Expand the study of the activity on other Gram-negative and positive bacteria.

- anti-biofilm tests using pure bacteriocin to confirm the results obtained.

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Abstract

The objective of this work was to study the antagonistic power of 19 strains of lactic acid bacteria, vis-à-vis multi-resistant strains. For this and initially, the spot test and the well test are implemented. These tests revealed the inhibitory activity of the lactic acid bacteria strains tested against the target strains by their power to produce antimicrobial substances. Secondly, a test of the antibiofilm effect is carried out between the MDRs and the culture supernatant of the lactic acid bacteria. It is concluded that the bacteriocins produced by L. raffinolactis (LAB14) and Leuconostoc mesenteroides subsp. cremoris (LAB86) exhibit therapeutic potential against pathogenic bacteria forming biofilm, which contributes to pathogenicity and antibiotic resistance.

Key words:- Multi-resistant bacteria, antagonism, lactic acid bacteria, antimicrobial substances, biofilm.

Résumé

L'objectif de ce travail était l'étude du pouvoir antagoniste de 19 souches de bacteries lactiques, vis-à-vis des souches multi-résistantes. Pour cela et dans un premier temps, le test des spots et le test des puits sont mis en oeuvre. Ces tests ont permis de révéler l'activité inhibitrice des souches lactiques testées à l'égard des souches cibles par leur pouvoir de produire des substances antimicrobiennes. Dans un deuxième temps, un test de l'effet antibiofilm est réalisé entres les MDR et le surnageant de culture des bactéries lactiques. On conclu que les bacteriocines produites par *L.raffinolactis* (LAB14) and *Leuconostoc mesenteroides* subsp. *cremoris* (LAB86) présentent un potential therapeutique vis –à-vis des bactéries pathogenes formant le biofilm , qui contribue à la pathogenicité et à l'antibiorésistance.

Mots clés :- Bactéries multi-résistantes, antagonisme, bactéries lactiques, substances antimicrobiennes ,biofilm.

ملخص :-

الهدف من هذا العمل هو دراسة التأثير المضاد لـ 19 نوع من بكتيريا حمض اللاكتيك ضد سلالات متعددة المقاومة.

أولاً تم إجراء إختبارين و هم إختبار البقعة و إختبار البئر ، و كشف هذين الإختبارين عن النشاط المثبط لسلالات حمض اللاكتيك ضد السلالات المستهدفة من خلال قدرتها على إنتاج مواد مضادة للميكروبات ثانيًا ، يتم إجراء اختبار لتأثير المضاد الحيوي بين سلالات متعددة المقاومة و محلول بكتيريا حمص اللاكتيك الطافي و نستنتج أن البكتريوسينات التي تنتجها البكتيريا *L.raffinolactis* (LAB14) و *Leuconostoc mesenteroides* subsp cremoris (LAB86) و قد عرضت بكتيريا القدرة العلاجية فيما يتعلق بالبكتيريا المسببة للأمراض التي تشكل الأغشية الحيوية ، والتي تساهم في الأمراض ومقاومة المضادات الحيوية.

كلمات البحث :- بكتيريا متعددة المقاومة ، بكتيريا حمض اللاكتيك ، مواد مضادات الميكروبات.