الجمهورية الجزائرية الديمقراطية الشعبية

République Algérienne Démocratique et Populaire
Ministère de l'Enseignement Supérieur et de la Recherche Scientifique
Université Ibn Khaldoun –Tiaret–
Faculté Sciences de la Nature et de la Vie
Département de Biologie

Mémoire de fin d'études

En vue de l'obtention du diplôme de Master académique

Domaine : Sciences de la Nature et de la Vie

Filière : Sciences biologiques

Spécialité : Infectiologie

Présenté par :

Akram Merchadi

The influence of pro, para and postbiotics derived from lactic acid bacteria on pathogens with biofilm-forming abilities.

Soutenu publiquement, le :

Jury :	**Grade**
President : Dr. Mohamed Abbes	**« MCA »**
Supervisor: Dr. Badra Boubakeur	**«MCA »**
Co-supervisor: Dr. Moustapha Drabo.	
Examiner : Dr. Djamila Mezouar	**«MCA »**

Année universitaire 2023-2024

ACKNOWLEDGEMENT

Praise be to Allah the Almighty for giving me the courage and will to start and finish this thesis. I would like to express my sincere thanks to my supervisors, Dr. Barda Boubakeur and Dr. Moustapha Songalo Drabo, who honored me by guiding our work. They graciously supervised and guided me throughout its development, for which we are deeply grateful for their advice, availability, and hard work.

My heartfelt thanks go to the thesis committee members, Dr. Mohamed Abbas and Dr. djamilaMezouar , for agreeing to evaluate this thesis.

I also extend my thanks to the staff of the Department of Nature and Life Sciences at Ibn Khaldoun University in Tiaret.

To all the laboratory engineers, especially those in Microbiology, we appreciate their assistance throughout my work.

I would also like to thank all my teachers who accompanied me during my university studies.

THANKS

First and foremost, I thank God for giving me the strength and will to accomplish this modest work.

I dedicate this modest work to:

- The two most dear people in the world, whom I can never thank enough for their help, encouragement, support, sacrifices, and patience throughout my life: My dear parents, may God grant them good health and long life.

- All my family members

- My friends: Hamid, Morsli, Ghano. Basset And more and more.

I wish you all the best in yours life.

AKRAM

Tables list

List of figures

ABBREVIATION LIST

LAB:	**Lactic acid bacteria**
PRO:	**Probiotics**
PRE:	**Prebiotics**
POST:	**Postbiotics**
T°:	**Temperature**
B.S:	**Bile salts**
pH:	**Potential of hydrogene**
OD:	**Obtic dencity**
CFU:	**Colony forming units**
FAO:	**Food and agriculture organisation**
WHO:	**World Health Organisation**
STREPT:	***Streptococcus thermophilus***
LACT:	***Lactobacillus plantarum***
P. *Aeroginosa:*	***Pseudomona aeroginosa***
B. *cereus:*	***Bacillus cereus***
E.coli:	***Echerichia coli***
STAPH:	***Staphilococcus aureus***

Abstract

The lactic acid bacteria are widely used and generally considered safe due to their ability to produce various beneficial compounds. In this study, the two bacteria Streptococcus thermophilus and Lactobacillus plantarum 299v were examined. The main goal was to characterize and assess the probiotic potential of these two strains by studying their tolerance to different pH levels, bile salts, temperature, and their ability to form biofilms through autoaggregation. Their antibacterial effect were also investigated. The two strains demonstrated promising results. They were able to survive in a range of temperatures and resist various pH levels and bile salt concentrations. They also exhibited high autoaggregation and coaggregation capacities, indicating strong biofilm formation. In conclusion, this study suggests that the two strains possess probiotic properties. However, further studies are still needed to fully confirm their effectiveness and safety.

Résumé

Les bactéries lactiques sont largement utilisées et généralement considérées comme sûres en raison de leur capacité à produire divers composés bénéfiques. Dans cette étude, les deux bactéries *Streptococcus thermophilus* et *Lactobacillus plantarum* 299v ont été examinées. L'objectif principal était de caractériser et d'évaluer le potentiel probiotique de ces deux souches en étudiant leur tolérance à différents niveaux de pH, aux sels biliaires, à la température et leur capacité à former des biofilms par auto-agrégation. Les deux souches ont donné des résultats prometteurs. Elles ont pu survivre dans une gamme de températures et résister à différents niveaux de pH et concentrations de sels biliaires. Elles ont également présenté des capacités d'autoagrégation et de coagrégation élevées, ce qui indique une forte formation de biofilms. Leur pouvoir antibactérien a aussi été evalué. En conclusion, cette étude suggère que les deux souches possèdent des propriétés probiotiques et un pouvoir antibactérien considérable. Toutefois, d'autres études sont encore nécessaires pour confirmer pleinement leur efficacité et leur sécurité.

الملخص

تستخدم بكتيريا حمض اللبن بشكل واسع وعمومًا تُعتبر آمنة بسبب قدرتها على إنتاج مركبات مفيدة متنوعة. في هذه الدراسة الحالية، تم دراسة اثنين من بكتيريا هما ستربتوكوكيس ثيرموفيليس ولاكتوباسيليس بلونتاريوم ، وكان الهدف الرئيسي هو توصيف وتقييم القدرة الحيوية للسلالتين من خلال دراسة تحملهما لمستويات مختلفة من الحموضة، وتحملهما لأملاح الصفيحة، بالإضافة إلى مقاومتهما لدرجات الحرارة المختلفة، وقدرتهما على تكوين الغشاء الحيوي المظهر في القدرة على الاندماج الذاتي. كما تم تقييم قدرتها على تثبيط بعض البكتيريا الضارة.

أظهرت السلالتان نتائج واعدة، حيث يمكنهما العيش في درجات حرارة مختلفة ويمكنهما مقاومة مستويات مختلفة من الحموضة وأملاح الصفيحة. كما أظهرا قدرات أعلى في الاندماج الذاتي والاندماج المشترك. كما كان لهما تاثبر معتبر على البكتيريا الضارة المستخدمة.

في الختام، أظهرت هذه الدراسة أن السلالتين لديهما خصائص بروبيوتيكية و ضد بكتيرية معتبرة، ومع ذلك، هناك حاجة إلى المزيد من الدراسات لتأكيد فعاليتهما وسلامتهما.

Sommaire

General introduction

Lactic acid bacteria are beneficial microorganisms used in the fermentation industry to develop efficient starter cultures. They are prevalent in traditional fermented foods and are known for their protective role against pathogens, GRAS (Generally Recognized As Safe) status, and diverse metabolites. However, some genera, such as *Streptococcus* and *Enterococcus*, include pathogenic species. Therefore, understanding their taxonomy, metabolism, and molecular biology is essential to maximize their benefits while minimizing risks **(Bogsan et *al.*, 2015; Kos et *al.*, 2003; FAO, 2001).** The food industry's evolution and the demand for high-quality products without synthetic stabilizers have increased interest in lactic acid bacteria and their metabolites. These bacteria enhance nutritional, sensory, and sanitary food qualities. Probiotic lactic acid bacteria are used in functional foods, vaccines, and pharmaceuticals, requiring robust strains that can withstand processing conditions and survive digestion to benefit health. Key traits include resistance to cold and heat, adhesion to intestinal cells, and biofilm formation for producing valuable metabolites like exopolysaccharides **(Boubakeur et *al.*, 2018; O'Grady and Gibson, 2005; Gregirchak et *al.*, 2019; Kos et *al.*, 2003).**

Probiotics, recognized by the FAO and WHO as beneficial live microorganisms when consumed in sufficient quantities, include LAB, bifidobacteria, *Bacillus*, and *Saccharomyces* (**Fenster et *al.*,2019)**. Found in a variety of foods, beverages, and dietary supplements like yogurt, kefir, sauerkraut, and kimchi, probiotics are known to reduce the risk of conditions such as antibiotic-associated diarrhea, colic, infectious diarrhea, allergies, and respiratory infections **(Ouwehand, 2016)**. They support a healthy gut microbiota, which in turn enhances immune function and brain health, fueling interest in LBPs **(Johasen et *al.*, 2020)**. The global probiotics market is expanding rapidly, with substantial growth projected in the coming years.

Although the exact benefits differ from strain to individual, the accumulation of evidence acts in favor of the potential for probiotics to be a very valuable tool for the promotion of general health and well-being. Our main objective is to develop a more effective strategy for controlling negative bacterial biofilm. In specific, we aim to investigate different biotic formulations derived from lactic acid bacteria as new anti-bacterial agents.

Review thesis

I. Probiotic review

I.1 Over view of probiotics and prebiotics

The most reliable way to influence gut microbiota composition is through live microbial dietary supplements, known as probiotics. with evidence of humans consuming live bacteria over 2,000 years ago. However, it was in the early 20th century that **Metchnikoff (1908)** first scientifically investigated probiotics. He theorized that the normal gut flora could have negative effects on the host and that consuming 'fermented milk' could counteract this. The term "probiotics", derived from the Greek words "pro" and "biotos" meaning "for life," was first used by **Kollath (1953)** to describe the recovery of health in malnourished patients through various supplements.

In **1954 Vergin** proposed that the microbial imbalance caused by antibiotic treatment could be rectified by a diet rich in probiotics, a notion regarded as one of the earliest references to modern probiotics. Kolb also identified the harmful effects of antibiotic therapy and suggested using probiotics as a preventive measure **(Vasiljevic and Shah, 2008).Lilly and Stillwell (1965)** further defined probiotics as microorganisms that support the growth of other microorganisms This idea dates back to antiquity **(Rastall et *al*., 2000)**.

The definitions of probiotics have become more precise, considering mechanisms of action, sites of action, delivery methods, and techniques. Although the definition has broadened to include health benefits from new mechanisms, dead microorganisms are not considered probiotics, even though some physiological benefits have been associated with them **(Sanders, 2008).** In the context of food, probiotics are defined as viable preparations in foods or dietary supplements intended to enhance human and animal health. Numerous microbial species are classified as probiotics, but lactic acid bacteria (LAB) are the most crucial for the gastrointestinal ecosystem **(Holzapfel et *al*.; 2001).**

According to the **FAO/WHO (2002)** working group on the evaluation of probiotics in food, probiotics are live microorganisms that, when taken in sufficient quantities, provide health benefits to the host **(Sanders, 2008; Schrezenmeir and De Vrese, 2001)**. Metchnikoff had earlier suggested that Lactobacilli could combat intestinal putrefaction and contribute to

longevity. While these beneficial microorganisms may not always reside in the gut, they should enhance the overall health of humans and animals **(Holzapfel et *al*., 2001; Belhadj et *al*., 2010**

FAO/WHO (2001),defined probiotics as"live microorganisms that, when administered in adequate amounts, confer a health benefit on the host .In **2002**, the World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations (FAO) formalized the definition of the term "probiotic" to prevent any misconceptions.

Prebiotics must first and foremost be clearly distinguished from probiotics. Indeed, they are not considered microorganisms. In reality, they are simple non-digestible molecules derived from foods that can stimulate the growth and activity of certain intestinal bacterial strains. Therefore, they represent a significant energy source for the microorganisms that make up the gut flora and for probiotics. They are generally found in large quantities in foods such as wheat, rye, leek, onion, artichoke, and banana **(Raphaëlle, 2015).**

I.2 Selection criteria for probiotics

I.2.1 General criteria

The criteria for selecting probiotics are summarised in the table below **(Bessila,Messaoudi, 2021).**

Table 1: Criteria for probiotic screening

❖ **SAFETY CRITERIA**	• History of non-pathogenicity (GRAS) • Strain characterized by phenotypic and genotypic methods • Strain deposited in an international culture collection • No potential for transmission of antibiotic resistance genes • No dehydroxylation of bile salts
❖ **FUNCTIONAL CRITERIA**	• Tolerance to gastric acidity • Tolerance to bile • Antagonism against pathogens • Adhesion to various intestinal cell lines and/or mucus • Stimulation of the immune system
❖ **TECHNOLOGICAL CRITERIA**	• Stability during production processes and in the finished product • Preservation of probiotic properties after production

I.2.2 Factors needed for the selection

I.1.1.1 Evaluation of safety

Safety evaluation is crucial to ensure that probiotic strains do not pose any hazards to human health. Antibiotic resistance profiles can be analyzed using molecular methods such as polymerase chain reaction and DNA sequencing to identify resistance genes. Additionally, virulence factors should be examined to confirm that probiotic strains do not possess traits linked to pathogenicity. Toxicity assessments are conducted

to evaluate potential adverse effects, including cytotoxicity, and acute or chronic toxicity . Furthermore, allergenicity tests aim to determine if probiotic strains can provoke allergic reactions, using techniques like enzyme-linked immunosorbent assay to detect allergenic proteins**(keerthi et *al* 2023).**

I.1.1.2 Evaluation of stability

Stability testing evaluates the capacity of probiotic strains to maintain viability, functionality, and product quality throughout storage and consumption. Acid tolerance can be assessed by exposing probiotic cells to simulated gastric fluid and measuring their survival using plate count or molecular techniques **(Lebeer et *al*., 2008).**

Similarly, bile tolerance can be evaluated using simulated intestinal fluid. lyophilization is a common preservation method, and the viability and stability of freeze-dried probiotics can be assessed by rehydrating the samples and evaluating their survival using enumeration methods or viability assays Optimizing **(keerthi et *al* 2023).** freeze-drying techniques is crucial to minimize damage to probiotic cells and maximize their survival and functionality during rehydration and storage.

I.3 Biofunctuality of probiotics

I.3.1 Inhibitory substances production

Probiotics exert antibacterial effects against pathogenic and food spoilage gram-positive and negative bacteria through production of antibacterial substances such as bacteriocins, organic acids, hydrogen peroxide, among others **(Arauz e*t al*., 2009; Razdan et *al*., 2012; Bajaj et *al*., 2014; Dec et *al*., 2014)**. Probiotic-derived antibacterial substances, known as **postbiotics,** show their effects individually or synergistically to inhibit the growth of pathogens. Probiotics have been reported to produce different **bacteriocins (Arauz et *al*., 2009).** *Lactobacillus planatarum* produces **lactolin (Vila et *al*., 2010).** Bacteriocin produced by probiotic strain *Lactobacillus salivarius UCC118*, protect the mice against infection with the invasive food borne pathogen *Listeria monocytogenes*. Lactobacilli and bifidobacteria have been shown to inhibit a broad range of pathogens, including *E. coli*, *Salmonella*,*Helicobacter pylori*,

Listeria monocytogenes and *Rotavirus***(Bermudez-Brito et *al.*, 2012).** Bacteriocins produced by Gram-positive bacteria have a narrow activity spectrum and act only against closely related bacteria, however, some bacteriocins inhibit food-borne pathogens like *Listeria monocytogenes* **(Nielsen et *al.*, 2010)**. Several *Bifidobacterium* strains have been reported to produce a unique bacteriocin which is active towards Gram-positive bacteria. Two *Bifidobacterium* strains exhibited a strong killing activity against several pathogenic bacteria, including *Salmonella entericaser.Typhimurium SL1344* and *E. coli C1845***(Bermudez-Brito et *al.*, 2012).** Twenty *Lactobacillus* strains inhibited enteropathogenic *Yersinia enterocolitica* while two strains *Lactobacillus casei* C1 and *Lactobacillus plantarum* C4 inhibited *Salmonellaenterica serovar Typhimurium* and *Listeria monocytogenes* in addition to *Y. enterocolitica*. Mechanism of inhibition was decrease in pH resulting from dextrose fermentation by lactobacilli.

The common mechanisms of bacteriocin-mediated killing include the destruction of target cells by pore formation and/or inhibition of cell wall synthesis. Bacteriocin production confers producing strains with a competitive advantage within complex microbial environments as a consequence of their associated antimicrobial activity, and at the same time inhibits pathogens in gastrointestinal tract **(Nielsen et *al.*, 2010; Hassan et *al.*, 2012).**

However, protective effects of these probiotic strains and their postbiotics could not be established in mouse experimental infection models against *S. Typhimurium*. Although *L. plantarum* C4 showed partial protective effect that was attributable to an immunostimulatory mechanism. Thus, in vitro study of antibiosis may provide useful information on the probiotic potential of Lactobacillus strains **(Bujalance et *al.*, 2014).**

The table below illustrates the comparison of Probiotics, Prebiotics, and Postbiotics **(Ji et *al* ,2023).**

Table 2: Comparison between probiotics,prebiotics,and postbiotics

PROBIOTICS	PREBIOTICS	POSTBIOTICS
Live microorganisms that, when consumed in sufficient quantities, provide a health benefit to the host.	Non-digestible food components that promote the growth and function of beneficial gut bacteria.	A formulation of non-living microorganisms and their constituents that provides a health advantage to the intended host.
Microorganisms such as bacteria or yeast, frequently sourced from fermented foods like yogurt.	Usually, dietary fibers or similar carbohydrates.	Usually, metabolic by products produced by probiotic bacteria. They can be isolated and administered independently of live bacteria.
They can inhabit the gut, improving its microbial equilibrium, and generate postbiotics.	Supply nutrients for beneficial bacteria, encouraging their proliferation and functionality	May not directly alter the composition of the microbiota but can still have positive effects on the overall health .
Susceptible to environmental factors such as temperature and stomach acidity	Typically robust and unaffected by temperature variations or stomach acidity.	Durable; not prone to damage from temperature fluctuations, stomach acid, or digestive enzymes.
May induce infections in individuals with weakened immune systems.	Excessive intake may result in gastrointestinal discomfort	Usually considered safe, but the consequences of increased doses are not completely understood

I.3.2 Competition for nutrients

Competition for nutrients serves as one of the mechanisms for preventing the colonization of pathogens in the human gut. When beneficial bacteria are present, they consume more nutrients, leaving fewer resources available for pathogenic bacteria, potentially leading to their starvation and subsequent inability to survive. This competitive exclusion occurs in two main ways.

Probiotics outcompete pathogens for nutrients, leading to the exclusion of pathogens and providing protection to the host. Competition for nutritional substrates among probiotics, intestinal pathogens, and the microbiota may play a significant role in maintaining gut health. **(Hojo et *al.*, 2007).**

health-promoting bacteria inhibit the growth of pathogens by consuming the nutrients and energy sources required for their proliferation in the gut environment. Secondly, beneficial bacteria produce various organic acids and volatile fatty acids through their metabolism and fermentation processes. These organic acids lower the pH of the gut to levels below what is conducive for the growth of pathogenic bacteria such as Salmonella and *E. coli* **(Bermudez-Brito et *al.*, 2012).**

I.4 Probiotics consumption health benefits

I.4.1 Impact of Probiotics on Digestive Health

Probiotics, by producing and/or enhancing the activity of various digestive enzymes, significantly improve digestion and intestinal absorption, especially in individuals with enzyme deficiencies. For instance, the lactase from yogurt bacteria (*Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*) enhances lactose digestion in the small intestine compared to standard milk, even in lactose-intolerant individuals. This improved lactose absorption and tolerance in yogurt is due to the mechanical protection provided by the cell walls of *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus*, which shield the bacterial lactase from gastric acidity. The cell walls are then broken down by bile salts in the small intestine, allowing increased enzymatic hydrolysis of lactose **(Burgain et *al.*, 2012).**

I.4.2 Immune Responses modulation

The effects of fermented milks on immune responses are now well-documented thanks to double-blind, placebo-controlled clinical studies. These effects are largely attributed to the high quantities of live bacteria, known as probiotics, present in these products. The primary site of these immune responses is the intestine, which is the body's main immune organ. Immune responses are diverse. Innate responses involving phagocytosis and the destruction of foreign elements and abnormal cells expressing viral or tumor proteins. These responses are carried out by circulating monocytes, Natural Killer cells, and macrophages and dendritic cells found in all tissues. These cells also act as sentinels, alerting other immune cell populations through "lock-and-key" type cellular contacts and the secretion of cytokines. Subsequently, T and B cells generate antigen-specific responses, known as adaptive responses, which can be either cellular or humoral (involving the production of antibodies of different isotypes,) and endowed with immunological memory **(Moreau, 2006).**

I.5 Formulation and development of probiotic product

Formulation is essential in the development of probiotic products as it guarantees the viability, stability, and effectiveness of probiotic strains during storage and consumption. Key formulation considerations crucial for successful probiotic product development include:

I.5.1 Selection of Suitable Carriers and Excipients

Probiotic strains require an appropriate carrier matrix to ensure protection during processing and storage. These carriers can be food-grade materials such as milk, yogurt, fruit purees, or prebiotic fibers. Excipients, including cryoprotectants and protective agents, can be incorporated to boost probiotic viability and stability during these processes **(Gupta et *al.*, 2018).** The selection of carriers and excipients is influenced by the specific probiotic strains, the desired product format, and the intended application.

I.5.2 Encapsulation and Microencapsulation Techniques

Encapsulation and microencapsulation techniques are employed to protect probiotic cells from environmental stresses, such as heat, acidity, and bile salts. Encapsulation involves entrapping probiotic cells within a protective matrix, whereas microencapsulation involves the encapsulation of individual cells within micro-sized particles. These techniques provide a

physical barrier that shields probiotic cells, improves survival during processing, and facilitates targeted delivery in the GI **(Champagne et *al.*, 2011).**

I.5.3 Shelf-Life Stability and Storage Conditions:

Probiotic products need to preserve their viability and functionality throughout their shelf life. Factors like temperature, humidity, and oxygen exposure can significantly impact probiotic viability and product stability. Ensuring proper packaging, optimal storage conditions, and accurate expiration date determination are crucial for maintaining the quality and efficacy of probiotic products **(Ouwehand et *al.*, 2017).** Conducting stability studies is necessary to evaluate the survivability and activity of probiotic strains during storage.

Chapter II :

materials and methods

MATERIAL AND METHODS

I. Objectives

The general objective of this study is to leverage two lactic acid bacteria strains to create a product that can be used for both industrial and pharmaceutical/probiotic purposes.

The specific objectives are:

- ✓ isolation and characterisation of probiotic potential of two lactic acid bacteria;
- ✓ Evaluation of the probiotic, parabiotic and postbiotic effect on pathogenic bacteria.

II. Duration and location work

The work had been done at ibn khaldoun university faculty of life and nature science, Microbiology laboratory from **18/02/2024 to 28/03/2024.**

III. Material

III.1 Strains used

III.1.1 . Lactic strains

- *Streptococcus thermophilus* species belongs to streptococcus genus ;to streptococcaceae family ;to lactobacillales order , to the class of bacilli. It was isolated form natural yogurt.
- *Lactobacillus plantarum 299v* species belongs to lactiplantibacillus genus ; to lactobacillaceae family ; to the class of bacilli**.** It was offered by Dr Hafidha Khadem.

II.1.2. Pathogenic strains

The pathogenic strains used are: *S. aureus*, *P. aerugenosa*, *Bacilus cerius*. They are offered by Dr. Badra Boubakeur.

III.2 Laboratory equipment

The laboratory equipment used in this study is shown in the table below.

Table 3: laboratory equpements

equipment	• Autoclave • Bunsen burner • Balance • pH meter • Centrifuge • Thermal magnetic stirrer • Incubator • Hotplate • Microscope • Spectrophotometer (uv) • Water bath • Vortex
glassware	• Petri dish • Becher • Pasteur pipette • test tube
Chemical products and media culture	• PBS(phosphate buffered saline) • MRS broth agar • MRS liquid • alcohol
coloring	• fuchsin • methylene bleu • lugol • gentian violet • red congo • purple cristal

IV. Methods

IV.1. Experimental protocol

The experimental approach towards conducting the above-mentioned research can, therefore, be summarized in the following

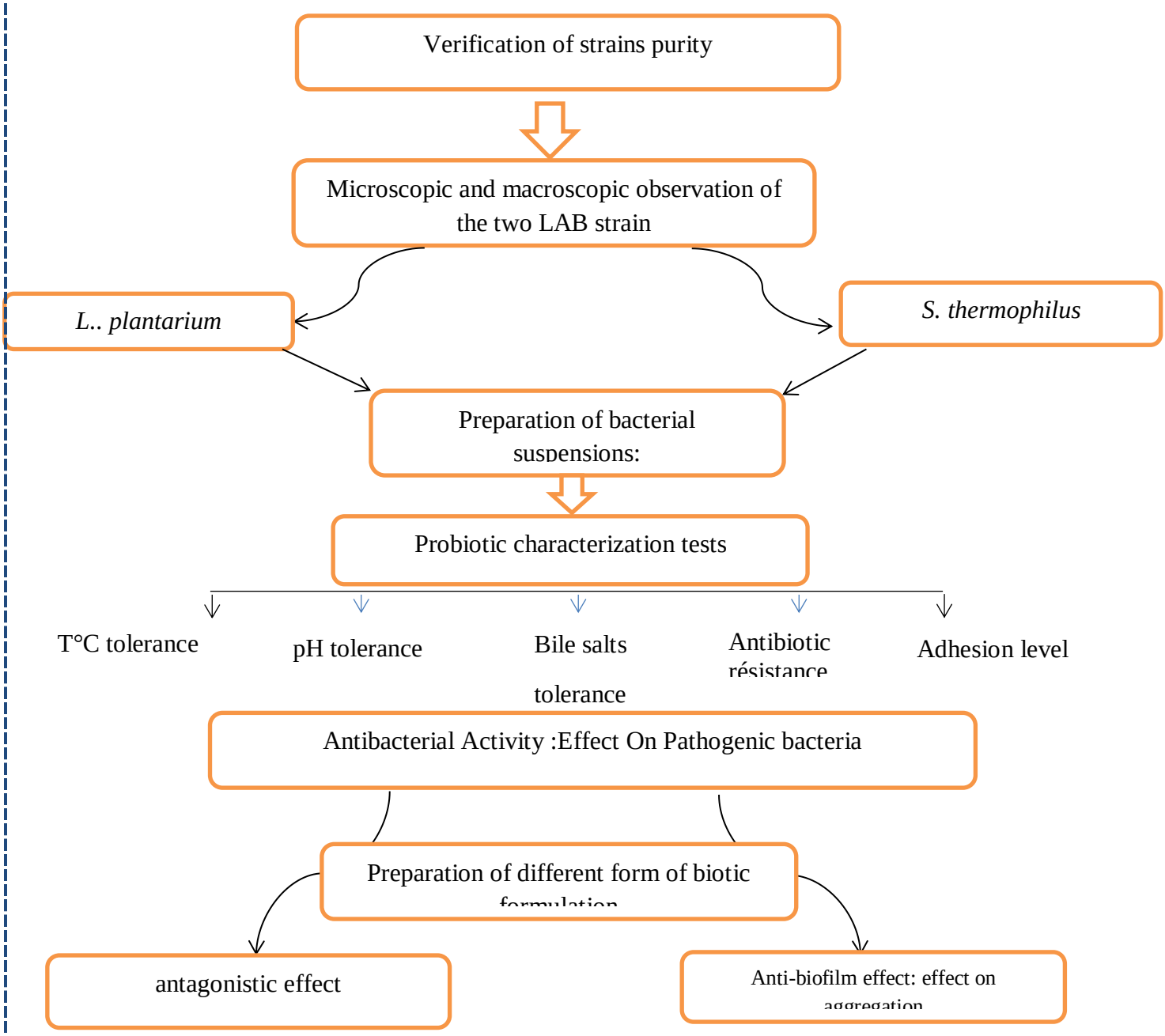


Figure 01 : Experimental protocol

IV.2. Preparation of inocula

To make sure that the strains are pure , at 42°C /37°C in aerobic state , culture were grown on modified MRS agar (MRS+GLUCOSE).every test needs an 18 hours culture before its been done , using a Pasteur pipette colonies were recovered in liquid MRS , then adjusted to 10^8 CFU/ml (OD=0.11) at 570 nm wavelength. Modified protocol (BIOCHROM Libra s6) **(Andrew et *al*.,2008).**

We did all the tests using two strains of lactic acid bacteria at specific temperature for each of them *(streptococcus thermophilus* at 42°C ; *lactobacillus plantarum229v* at 37°C).

IV.3. Secreening of the probiotic potential of lactic strains

IV.3.1. Bile salts tolerance

The test used a modified protocol based on the work of Anukan and Koyama (2007). This involved adding 1 ml of inoculum (containing 107 colony-forming units per ml) into MRS liquid media supplemented with 0.05%, 1%, and 2% bile salts. After incubating the samples for 24 hours at 37°C, the optical density (OD) was measured at 570 nm.

The percentage of growth inhibition (GI) was then calculated using the following formula:

$$\mathbf{GI\ \% = [(\log N_0 - \log N_t)/\log N_0]. 100}$$

N_0= cell number without bile salts after 3h incubation

N_T=cell number with bile salts after 3h incubation

IV.3.2. Tolerance to pH

The **Anukan and Koyama (2007)** protocol was adopted to assess the resistance of our strains to gastric pH. By adding HCL; the MRS broth were adjusted to pH=2 , PH=3. After that the medium was inoculated with 1 ml of a young culture (10^8 CFU/ml). After incubation of 3h, the growth was assessed at 570 nm the and the result was compared to control culture atpH=7.

IV.3.3.Temperature resistance

The modified protocol based on the work of Haddaji et *al*. (2015) was adopted. This involved adding a young culture suspension (containing 108 colony-forming units per ml) to a series of liquid MRS media samples. The samples were then incubated under the following conditions:

- 37°C for 24 hours
- 60°C for 2 hours
- 90°C for 30 minutes

After incubation, the optical density (OD) of the samples was measured at 570 nm and compared to control samples.

IV.3.4. Autoaggregatrion

For this test, we adopted a modified protocol based on the work of Zommiti et al. (2017). This involved the following steps:

- A 24-hour young culture was centrifuged for 15 minutes at 6000 rcf.
- The cell pellet was washed 2 times with PBS and then resuspended in PBS.
- The optical density (OD) of the cell suspension was adjusted to be greater than 108 CFU/ml.
- 1 ml of the cell suspension was added to 9 ml of PBS buffer to create a decimal dilution, and the mixture was shaken.

The OD of the diluted samples was measured at 570 nm over a 4-hour period, with measurements taken every 1 hour. Care was taken to sample from the surface without disturbing the series of tubes.

The percentage was calculated using a formula provided.

$$\mathbf{Agg\ (\%) = [\ 1 - (\ A_T / A_0\ ) . 100\ ]}$$

Were: A_0 = 0h absorbance time

A_T = absorbance at 1h 2h 3h 4h

IV.3.5. Co-aggregation (pro+pro), (pro+pathogens)

The coagregation of our lactic strains with each other and with pathogenic germswas achieved by adopting the following protocol of **Zommiti et *al* .(2017).**

For *Lactobacillus plantarum* with *Streptococcus thermophilus* we followed this protocol , as we did the same method when working with the *couples Lactobacillus plantarum (LP)* with *bacillus cereus(BC)* then with *Pseudomonas aeroginosa (PA)* sequentially and *Streptococcus thermophilus (ST)* with *bacillus cereus(BC)* and*Pseudomonas aeroginosa(PA)* sequentially.

- After 24h of incubation, two lactic strains were washed with PBS two times separately.
- Then centrifuged at 6000 rcf/ 15 min.
- The culture suspended in PBS buffer and fixed at $10^8 <$ (CFU/ ml), the same for each strain.
- After obtaining a decimal suspension of the two strains together suspended in PBS buffer , the optic dencity were measured at 570 nm every hour until 4 h .
- The % calculation formula is :

$$(\%) = [\, 1 - A_t / A_0 \,) \,.\, 100 \,]$$

Were: A_0 = absorbance at 0h

A_t =absorbance every hour (1h , 2h , 3h , 4h).

IV.3.6. Antibiotic resistance

For this test, we adopted the protocol described by Boubakeur et *al*. (2022), which involved testing the following five antibiotics: Gentamicin (10 µg), Tetracycline (30 µg), Chloramphenicol (30 µg), Colistin (10 mg), Cefepime (30 µg), Metronidazole (5 µg).

After 24 hours of incubation at 37°C, the inhibition zones (in millimeters) around each antibiotic were measured.

IV..3.7. Antagonistic effect

The study adopted a modified protocol from Boubakeur et *al*. to evaluate the antagonistic effect of two bacterial strains (*S. thermophilus* and *L. plantarum 299v*) against two pathogenic strains (*E. coli* and *Staphylococcus aureus*).

The key steps were:

- Working with 18-hour young bacterial cultures.
- Standardizing the cell concentrations to 10^8 CFU/mL for the S. thermophilus and L. plantarum strains, and 10^6 CFU/mL for the pathogenic E. coli and S. aureus strains.
- Spreading the pathogenic bacterial suspensions evenly over Petri dishes.
- Creating holes in the Muller-Hinton agar plates and filling them with 150 µL of the S. thermophilus and L. plantarum bacterial suspensions separately.
- Incubating the plates for 24 hours and then observing the results.

The goal was to assess the antagonistic activity of the S. thermophilus and L. plantarum strains against the pathogenic E. coli and S. aureus strains.

IV.4. Antimicrobial effect of the biotic lactic formulation (probiotic; postbiotic; parabiotic)

The protocol of Jiménez-Esquilínet et *al*. (2005) and Elleuch et *al*.(2010) were adopted for all of the following tests. The pathogen strains used in this test are *Psudoonas aeroginosa and Bacillus cerues.* The tested bacteria for its antimicrobial effect are *Lactobacillus plantarum 299v and Streptococcus thermophilus.*

IV.4.1. Probiotic effect

To evaluate the antagonistic effect of the lactic strains culture against the pathogenic bacteria, the following steps were taken:

- An 18-hour incubation was used to obtain a fresh, young bacterial culture.
- The bacterial culture was centrifuged at 6000 rpm for 15 minutes and washed twice with PBS.
- The washed bacterial cells were resuspended and adjusted to a concentration of 10^7 CFU/ml.
- 250 µl of the 10^7 CFU/ml bacterial suspension was spread onto Muller-Hinton agar Petri dishes.
- A separate suspension of pathogenic bacteria was also prepared using the same centrifugation and washing steps, but the final concentration was adjusted to 10^6 CFU/ml.
- 100 µl of the 10^6 CFU/ml pathogenic bacterial suspension was added to the Muller-Hinton agar plates in the holes/wells created.

- The inoculated plates were incubated for 24 hours under optimal conditions for the pathogenic bacteria.
- After incubation, the zones of inhibition around the bacterial suspensions were measured.

IV.4.2. Parabiotic effect

For Evaluating parabiotic (killed-cell) antagonistic effect, the following steps are adopted:

- Obtain an 18-hour bacterial culture.
- Kill the 18-hour bacterial culture by exposing it to 90°C for 15 minutes with shaking every 5 minutes.
- Centrifuge and wash the heat-killed bacterial cells with PBS.
- Resuspend the washed, heat-killed cells and standardize the concentration to 10^7 CFU/mL.
- Spread 250 µL of the 10^7 CFU/mL heat-killed bacterial suspension onto Muller-Hinton agar Petri dishes.
- Create holes/wells in the agar and add 100 µL of the 10^6 CFU/mL pathogenic bacterial suspension to the holes.
- Incubate the plates for 24 hours under optimal conditions for the pathogenic bacteria.
- Measure the zones of inhibition around the heat-killed bacterial suspensions.

IV.4.3. Postbiotic effect

The goal of this protocol is to evaluate the antagonistic effect of the bacterial metabolites and extracellular compounds (postbiotics) present in the cell-free supernatant against the pathogenic bacteria. The adopted steps are:

- Obtain an 18-hour bacterial culture.
- Standardize the bacterial concentration to 10^7 CFU/mL.
- Centrifuge the 10^7 CFU/mL bacterial suspension at 6000 rcf for 15 minutes to separate the cells from the supernatant.
- Collect the supernatant (cell-free fraction) and discard the bacterial cell pellet.

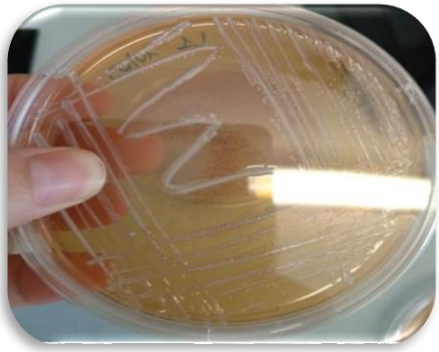
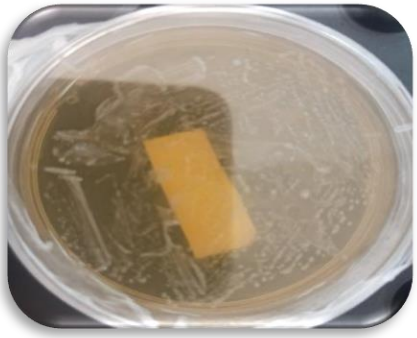
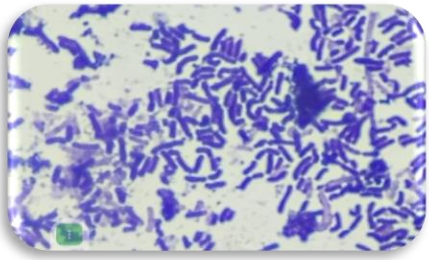
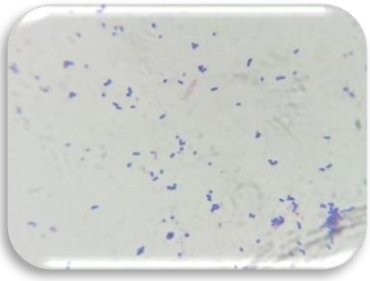
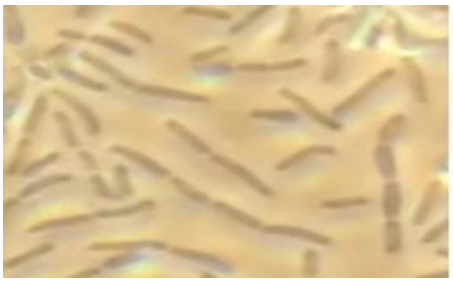
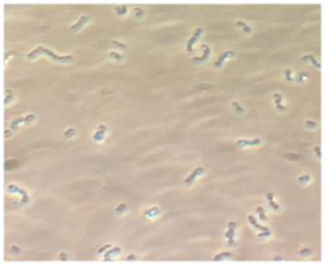
- Fill the holes/wells in the Muller-Hinton agar Petri dishes containing the pathogenic bacterial lawn with 250 µL of the cell-free supernatant.
- Incubate the inoculated plates for 24 hours under optimal conditions for the pathogenic bacteria.
- After incubation, measure the zones of inhibition around the supernatant-filled wells.

chapter III:

Results and discussion

I. Result Of Verification Of Strain Purity

The following tables shows the macroscopical and microscopical observations of the two studied souches.

Characteristics	*L. plantarum* 299v	*S. thermophilus*
Macroscopic Aspect (photo prise par Boubakeur)		
Microscopic Aspect « Gram staining» (photo prise par Boubakeur, 22/04/2024)	Gram-positive& purple cells color 	
Contrast microscopic treatment (photo prise par Boubakeur, 03/05/2024)		
Association Mode	Short chains of bacilli	Long chains of Cocci

II. Probiotic screening

II.1. Bile salts tolerance

To evaluat the bacterial resistance of S*treptococcus thermophilu s*and *Lactobacillus plantarum* to the bile salts, we compared their ODs before and after 3h of incubation at different concentration of bile salts (0.05% ; 0.10%; 0.20%). The results are illustrated in the figure 1.

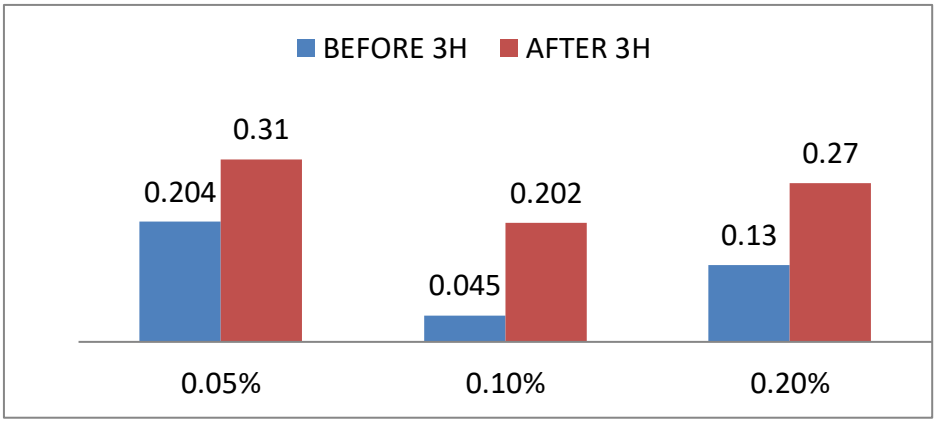


Figure 1: evaluation of *S. thermophilus* resistance bile salts

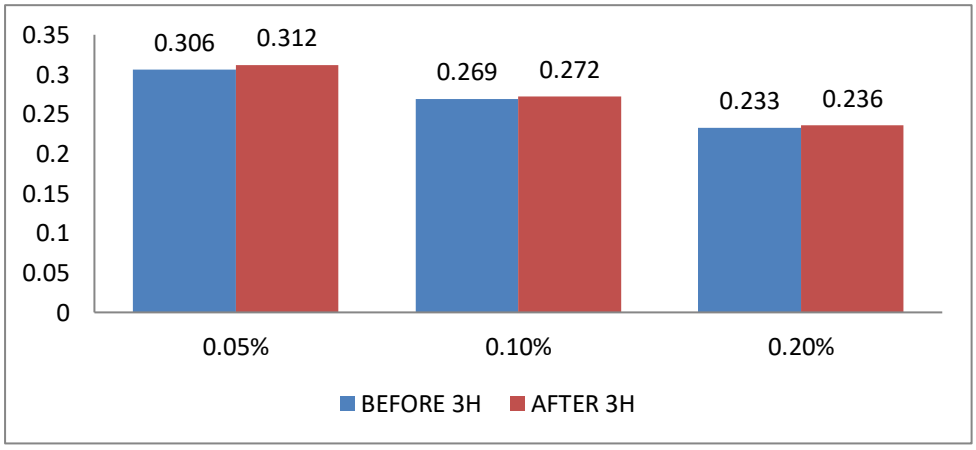


Figure 2: evaluation of *L.plantarum 299v* resistance to bile salts

Based on these results,the ODs increased from 0.204, 0.04 and 0.13 up to 0.31, 0.202 and 0.27 respectively at 0.05, 0.10 and 0.20% of bile salts for *Streptococcus thermophilus.* For *Lactobacillus plantarum, the ODs were* increased from 0.306, 0.269 and 0.233 up to 0.312, 0.272 and to 0.236 respectively at 0.05, 0.10 and 0.20% of bile salts.

Comparing this study to other one which finds that probiotic effect necessarily needs bile salts tolerance as one of the top important criteria **(Begley et *al* ., 2005)**. According to a study had been done by **boubakeur et *al*.(2021),**they found out that two experimental lactic strains (*S.thermophilus* and *E.durans*) had survived at those concentration when the tolerance of *S. thermophilus* to bile salts was much higher than *E.durans* and more viable This confirms our results where we noted a considerable resistance of *S. thermophilus*par rapport à *Lactobacillus plantarum*. Cellular homeostasis can be interrupted by cell membrane phospholipids and their proteins, as a result of secretions of bile salts, which are involved in the fat solubilisation process.**(Boubakeur et *al* ., 2021)**. **Bagci et *al.* (2019)** suggests the ability of these two bacteria to persist and live in the upper gastrointestinal tract; he assessed the characteristics of these bacteria isolated from human milk to find a high tolerance to 0.5% bile. Moreover **Mirlohi et *al*(,2009)** noticed a hight rates of growth for *L.plantarum* when comparing it to other tested strains like *L.rhamnosus* . *L.plantarum* showed the best ability of multiplication when adding 0.3% of oxigal.

II.2. Antibiotic effect

The performance of the two lactic strains in relation to the various antibiotics assayed is outlined in Table 03.

Table04: antibiotic effect on *S.thermophilus* and *L. plantarum*

	MT^5	CT_{10}	FEP30	C_{30}	TE30	CN_{10}
S.Thermophulus	R	R	R	1.9 mm	0.7 mm	R
L. Plantarum	R	R	R	/	R	R

Mt: METRONIDAZOLE CT: COLESTINE FEP: CHLOROPHINICOL

TE: TETRACYCLINE CN: GENTAMICINE C : CEREPINE

As we can see, there is resistance of *S. thermophilus* to all antibiotics except C30 and TE30, which produced two zones of inhibition measuring 1.9mm (for C30 cerpegin) and 0.7mm (for TE30 tetracycline).On the other side for l.plantarum there is a resistance for all kinds of tested antibiotics.

It is known that every lactic bacterium react differently agaist antibiotics. **Tosi et al. (2007)** demonstrated that *S. thermophilus* strains from different environments were susceptible to all six antibiotics tested, including gentamicin, although some showed resistance to tetracycline. In another investigation, **Abamecha et *al.* (2015)** found that 34% of *Enterococcus* isolates were resistant to gentamicin, 64% to tetracycline, and 34% to chloramphenicol. According to **Terzić-Vidojević et *al.* (2015)**, antibiotic resistance varies among lactic strains and geographical regions. Their study revealed that 29% of *Enterococcus* isolates, including *Enterococcus durans*, were susceptible to tested antibiotics like gentamicin, tetracycline, and chloramphenicol. Additionally, nearly 59% of these isolates demonstrated resistance to two or more antibiotics. The main concern regarding resistance dissemination is the presence of antibiotic resistance genes on mobile genetic elements. **Tosi et *al.* (2007)** suggest that when choosing microorganisms as food additives, priority should be given to those with the least resistance, which requires further characterization to understand the genetic basis of resistance .moreover ;according to **Guidone et *al*(.2014)** who found that only *L.plantarum* c17 and s85 strains had a resistant to tetracycline and erythromycin respectively , when L.plantarum strains inhibited or not at breakpoint stage for specified antibacterial we can classify it as susceptible to antibacterials.s.thermophilus in lotr of times shows a hight resistance to gentamicine ; kanamycine ; streptomycine ; trimethoprim ; sulphadazine , in other hand it is sensitive to chloramphenicol; tetracycline ; cephalothin ; ciprofloxacine **(Ammor et *al*.,2007) .**

II.3. pH resistance

The results of the effect of gastric pH on *S. thermophilus* and *L. plantarum* are shown in the figure below.

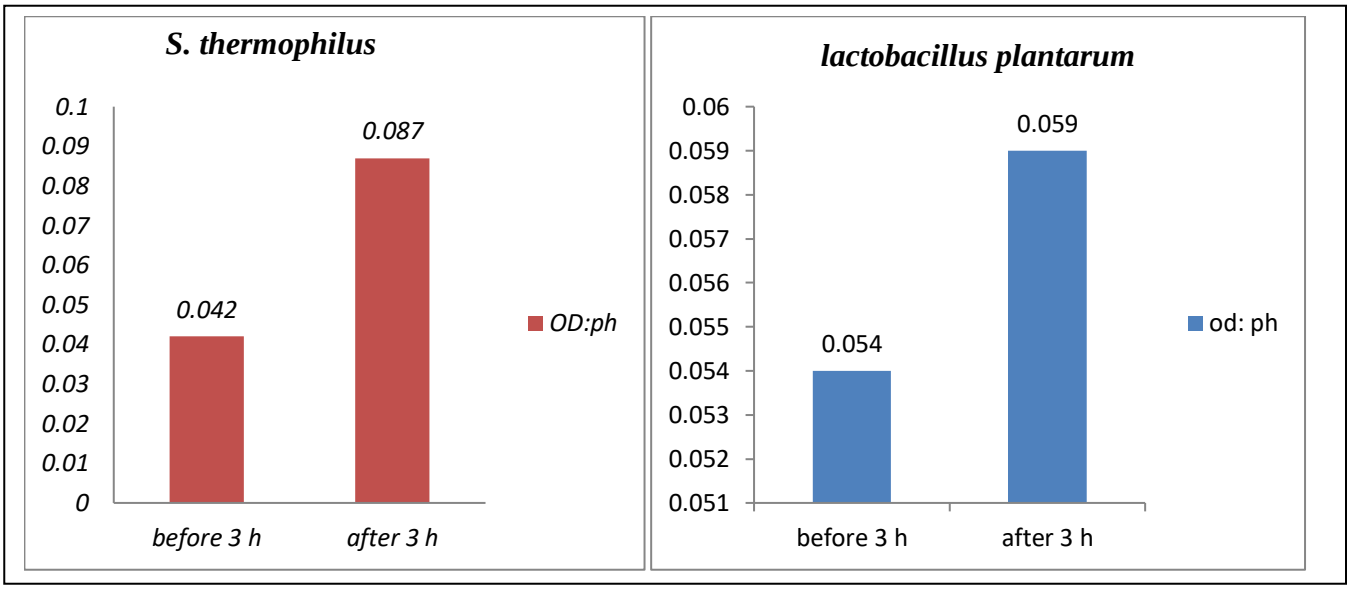


Figure 3: Evaluation of *S. thermophilus* and *L. plantarum* resistance at pH2 and pH3 after 3h of inoculation

After three hours of the bacterial incubation at pH 2, we noticed that there is a decrease in the optic density of *S. thermophilus* witch means that the amount of strain is going down comparing to its amount before 3 hours (from 0.194 down to 0.087). For *Lactobacillus plantarum*, the OD increased from 0.164 up to 0.169, which means that the strain has been multiplied without been effected by this pH.

Regarding incubation at a pH of 3 after three hour, an increase in the growth of both strains was noted. The OD is going up from 0.042 to 0.188 and from 0.054 up to 0.059 respectivly for *S. thermophilus* and *L. plantarum*. Those results leads us to say that this bacteria have the ability to resist the two concentration of pH 3.

According to **Boke et *al* .(2010),** after studing and evaluating the viability or serviving in conditions of pH value below 3 (pH <3). They found that sensitivity to acid is much more significant in strains that produce low levels of EPS, while resistance to this type of acidity can be explained by the protective role of EPS. In general, LAB strains have a system to detect stress and activate defense, which gives them the ability to adapt to different variable conditions and environmental changes **(Chen et *al*., 2019)**. According to **Singhal et *al*.,(2021)** who noticed that all *L.plantarum* strains did not survive at ph=1.5 after 3 hours , in pH=2 the viability reduced to

half , in pH=3 both strains still a live .Moreover, **Tavakoli et *al*.,(2017)** found that there is no difference when decreasing pH from 5 to 2.4 on different isolats of L*actobacillus* while increasing it from 2.3 to 8 the biomass get down to more than 4 log CFU/ml , lactobacillus plantarum had the best survival rate at ph =2.2 .

II.4. Autoaggregation

The experiment is looking at the sedimentation behavior of the bacteria over a 4-hour timeframe, with measurements taken at hourly intervals to track the sedimentation rate. The results are illustrated in the figure below.

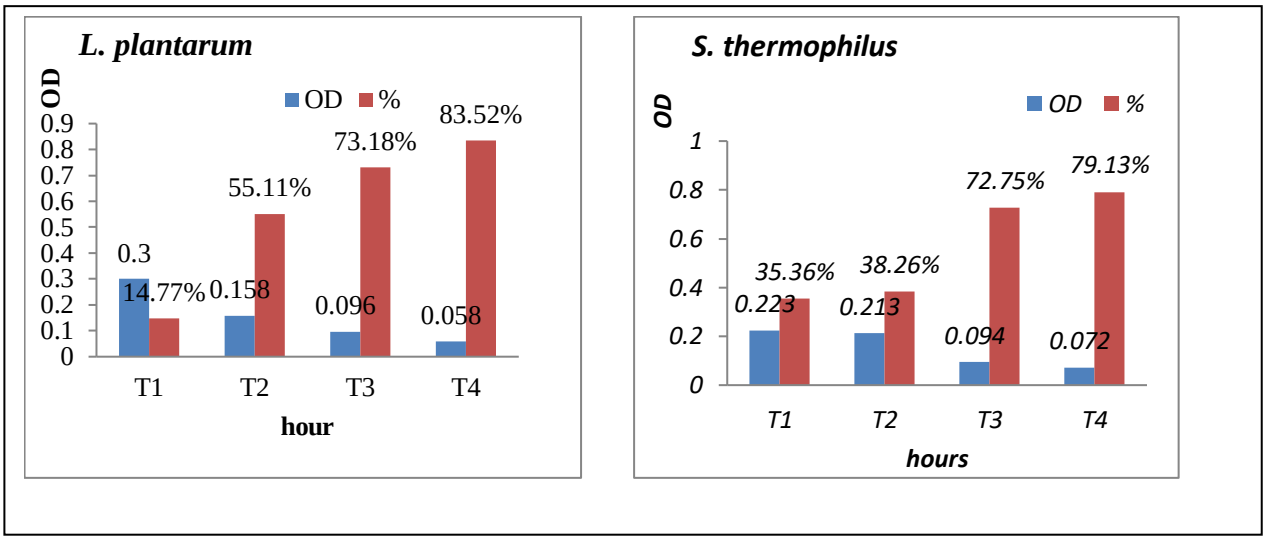


Figure 4: Autoaggregation ability of *L.plantarum* and *S.thermophilus*

We can realize that the OD is going down while the percentage of aggregation is rising up every passing hour, which leads us to say that the bacteria(*S.thermophilus* and *L.plantarum*) have a really important sedimentation rate.

In the study of **Balakrishna(2013),** the auto-aggregation of four chosen strains and five indicator strains was examined based on sedimentation properties. Three phenotypes were observed: strongly autoaggregating (Agg+) strains with high autoaggregation rates (≥80%), forming a sediment and clear supernatant; non-autoaggregating (Agg-) strains with low autoaggregation rates (<10%) and persistent turbidity; and mixed (Agg+/-) strains with moderate autoaggregation rates (20-70%), showing both sediment and turbidity aggregation ability is often connected to cell adherence properties **(Boris et *al*., 1997; Del Re et *al*., 1998).** Aggregation can

occur among microorganisms of the same strain (autoaggregation) or between different strains (coaggregation), playing a key role in various ecological environments. A link between autoaggregation and adhesion ability has been noted in some bifidobacteria species **(Perez et *al.*, 1998). Collado et *al*. (2007)** found that lactic acid bacteria (LAB) autoaggregation was associated with their adhesion ability. twenty *Lactobacillus* strains demonstrated autoaggregation rates between 24.16% and 41.39% after 5 hours of incubation at 37°C. LGG, utilized as the positive control, exhibited the highest autoaggregation rate at 41.39%. Aggregation is essential for biofilm formation and is generally associated with cell adherence properties, which are crucial for the bacteria's survival and persistence in the gastrointestinal tract (Vlková et al., 2008; Ferreira et al., 2011).tuncer and tuncer (2014) reported 49.55% autoaggregation of streptococcus thermophulis st8.01 strain . aggregation rate was different in all 6 strains that varies between 98.8% and 8.8% that could be a result of the autoaggregation of strain **Taj et *al*.,2022).**

II.5. Coaggregation

The figures below are showing the coaggregation of probiotics strains(*S.thermophilus* and *L.plantarum*) with each other and with other pathogene strains (P*seudomonas aeroginosa* , *Bacillus cereus*).

Witch reveals of an important result which is in all figures we can notice that the OD is decreasing by time while the percentage of sedimentation is increasing, that means the two associated bacteria has a good rate of sedimentationhowever **Tuo et *al*.(2013)** found that All tested strains exhibited some degree of coaggregation with *E. coli* O157, although this property varied by strain. The strain *Lactobacillus casei* 137 demonstrated the highest coaggregation ability at 61% with *E. coli* O157 In comparison, the coaggregation ability of LGG was 21.81%. Coaggregation may be essential for eliminating pathogens from the gastrointestinal tract (Todorov et al., 2008). Lactobacillus strains can form a barrier that prevents pathogenic bacteria from colonizing through coaggregation (Ferreira et al., 2011). When probiotic strains coaggregate with potential pathogens, they can produce antimicrobial substances nearby, which inhibit the growth of harmful bacteria in the gastrointestinal tract **(Reid et *al.*, 1988)**.according to **kapse et *al*.,(2024)** a s.thermophilus strain demonstrate a reaching rate of coaggregation up to 50% . and shown its ability to coaggregate with all tested pathogens .

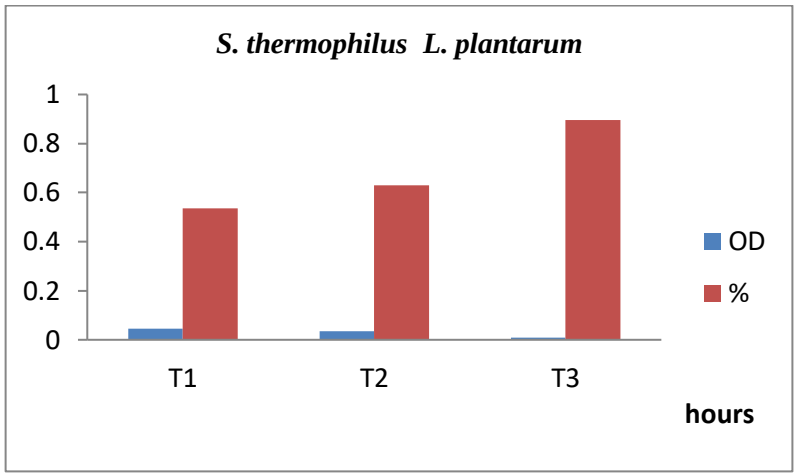


Figure 5: Coaggregation capacity of *L. plantarum* with S. *thermophilus*

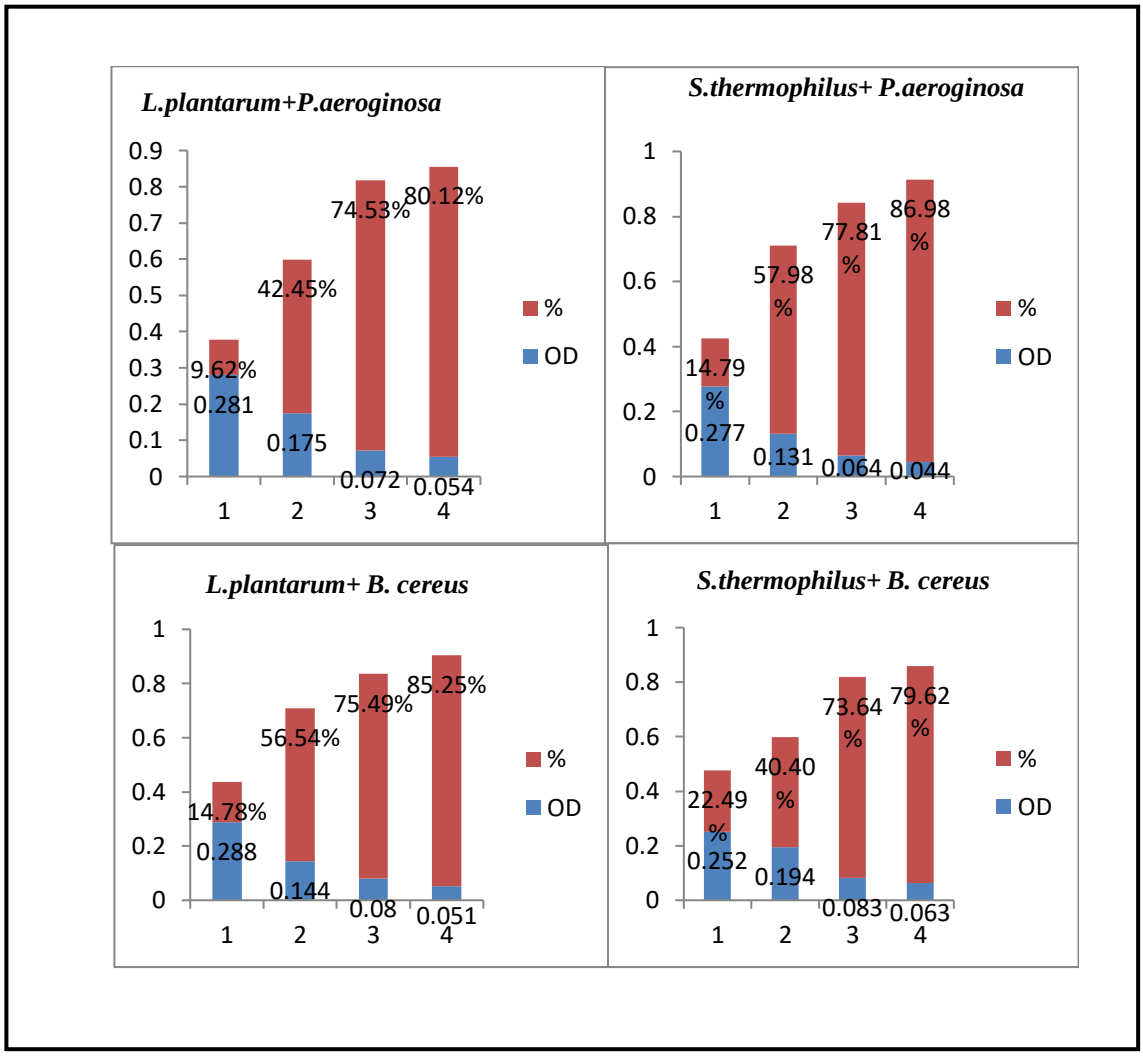


Figure 6: Coaggregation capacity of *L.plantarum* and *S. thermophilus* with P.aerugenosa and B. cerius.

According to the study of **balakrishna.,(2013),** all chosen lactic strains exhibited aggregation with the indicator pathogenic strains, but coaggregation percentages were strain-specific. Coaggregation, which serves as a natural defense mechanism against pathogens **(Spencer and Chesson, 1994),** was observed in LAB species known for producing inhibitors and forming barriers against pathogen colonization **(Reid et *al*., 1988; Spencer and Chesson, 1994; Boris et *al*., 1997).** Results revealed strain-specific coaggregation percentages influenced by incubation conditions **(Collado et *al.*, 2008).** Intestinal isolates from clown fish enhanced

pathogen attachment to mucus (Vine et al., 2004), while Cl. butyricum hindered pathogen adherence to epithelial cells **(Pan et *al*., 2008).**

II.6. Thermoresstance

Here we are sinking to know if the bacteria has a resistance to deferent temperatures (37°C, 42°C, 60°C, 90°C). The results are indicated in the figures below.

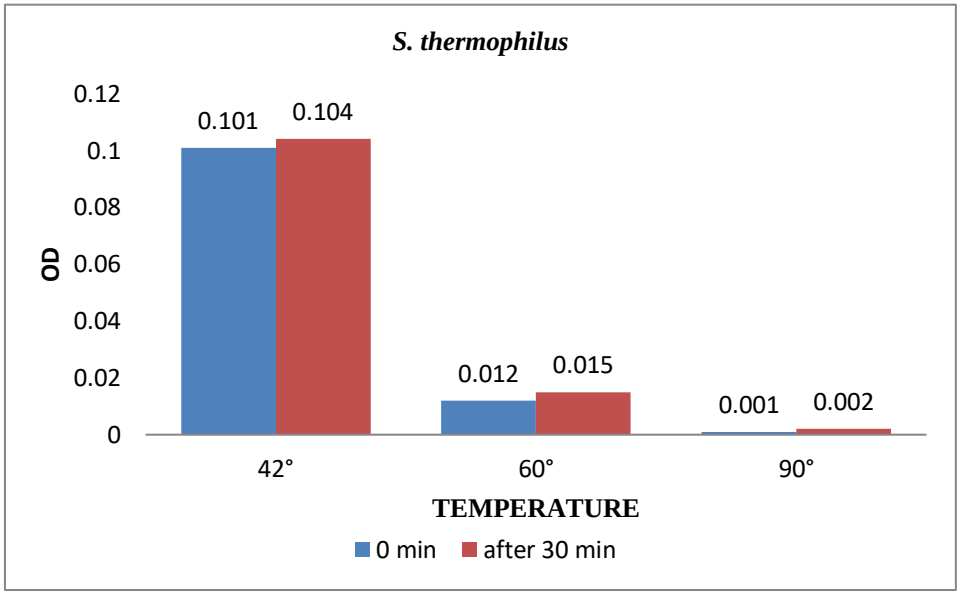


Figure 7: *S. thermophilus* resistance to variable temperatures

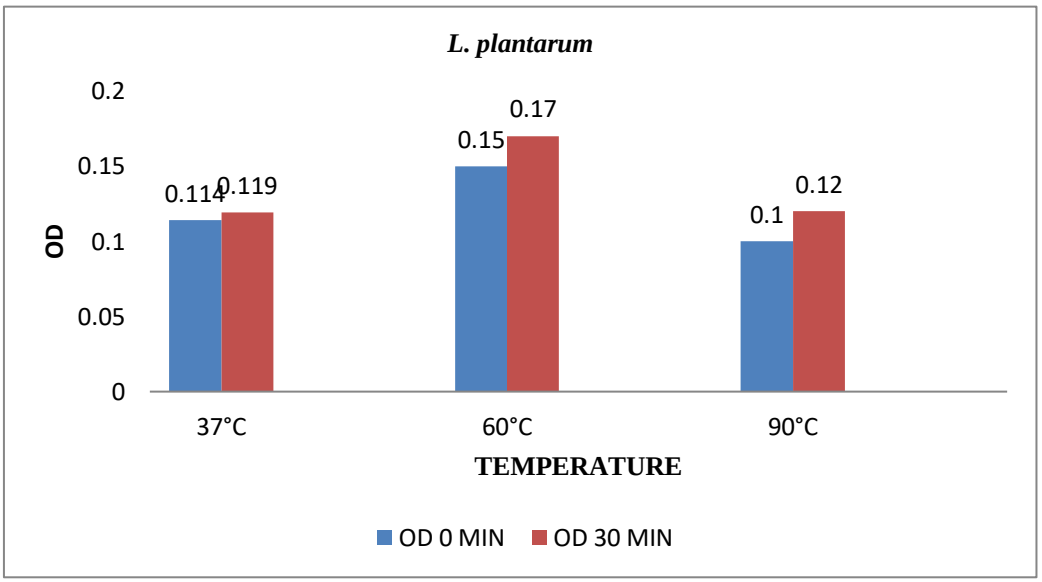


Figure 8: *L.plantarum* resistance to temperature

According to these results, There was an increased rate in optical density (OD) observed at all three temperatures (42°C, 60°C, 90°C) for *S. thermophils.* This indicates bacterial multiplication and growth occurring at these high temperatures which demonstrates the resistance and ability of the *Streptococcus thermophilus* bacteria to survive and proliferate even under these high temperature conditions.

The same results were obtained for our strai « *L. plantarum*»; the optical density increased at all three temperatures tested 37°C, 60°C, and 90°C. This demonstrates that the *L. plantarum* bacteria were also able to grow and multiply even at these elevated temperatures. The increasing OD values indicate the bacteria were able to proliferate and increase in number under these high temperature conditions.

The most commonly known fact is that most bacterial species face a lot of variations, and temperature is the first variable condition **(Varmanen and Savijoki, 2011)**. *Streptococcus thermophilus* can multiply in temperature ranges between 20 to 52°C and could adapt to heat shock, there are notable survival rates. When increasing from +42 to +52°C, they noticed a low growth of *S. thermophilus*, which was attributed to its protein synthesis profile, indicating a low secretion of polypeptides because of the temperature change effect **(Auffray et *al*., 1995).** We can say the same about *L. plantarum*. The optimal temperature for most bacteria is on average between +35 and +37°C, and few could be higher than +45°C **(Jobin et *al*., 1998).** Bacteria respond to sudden temperature changes by fast modification of gene expression, which ends with the secretion of a large group of heat shock proteins (HSPs) **(Lim and Gross, 2011).** Heat shock proteins could be found in different bacteria, and this response shows the incredible differences in the regulation of bacterial genes, including LAC **(Auffray et *al*., 1995).**

II.7. Antimicrobial effect of *S. thermophilus* and *L. plantarum*

The results illustrated in the figure below show the antimicrobial effect of both *Lactobacillus plantarum* and *Streptococcus thermophilus* against *E. coli*, as measured by the zone of inhibition.

For *Lactobacillus plantaru*m 299v, the postbiotic and probiotic suspensions exhibited inhibition zones of 1.3 mm and 0.9 mm respectively against *E. coli.* In contrast, the parabiotic suspension showed virtually no inhibition zone (only 0.1 mm) against E. coli

For *Streptococcus thermophilus*, the postbiotic and parabiotic suspensions had inhibition zones of 0.8 mm and 0.7 mm respectively against *E. coli*. However, the probiotic suspension completely lacked any inhibition zone against *E. coli* .

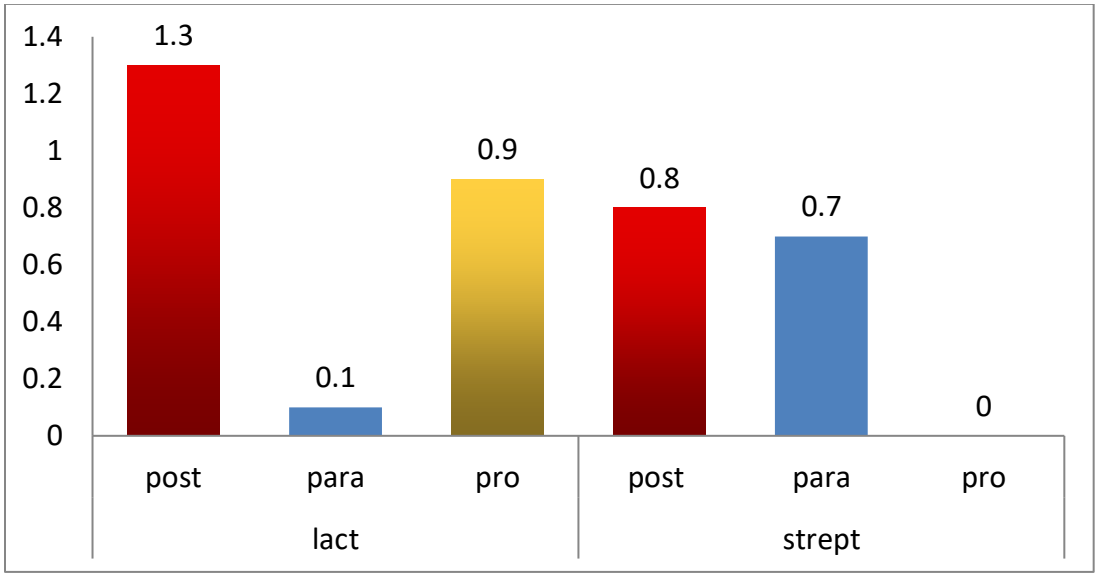


Figure 9: Antimicrobial effect of *L.plantarum* and *S. thermophilus* against *E.coli*

The antimicrobial effect of the biotic formulation on *Staphylococcus aureus* are illustrated in the figure below

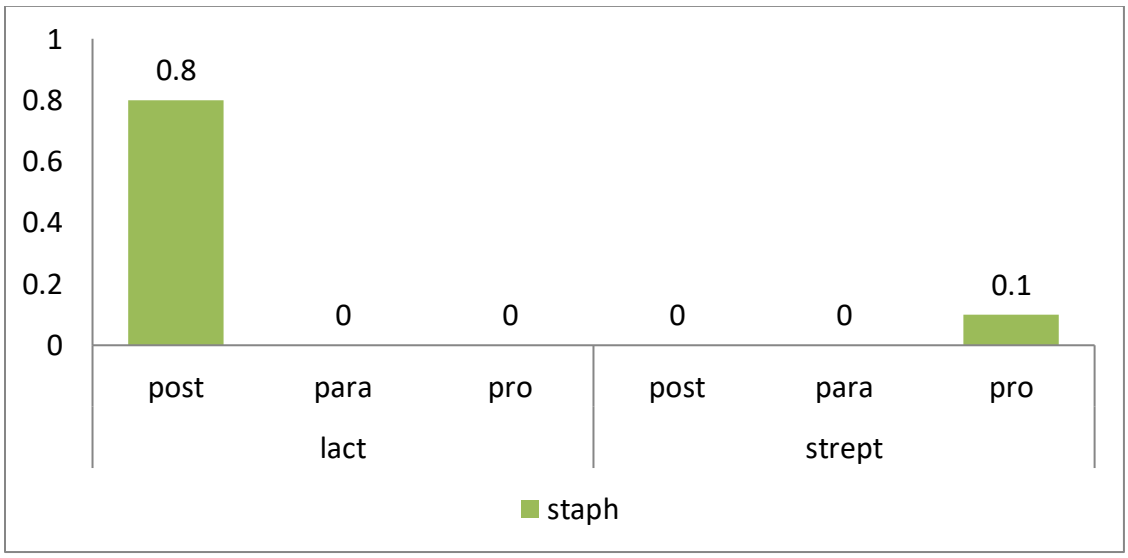


Figure 10: Antimicrobial activity of *L.plantarum* and *S.thermophilus* against *Staphilococcus aureus*

For *Lactobacillus plantarum*, an inhibition zone was observed only with the postbiotic suspension, with a diameter of 0.8 mm. However, there were no observable inhibition zones for the parabiotic or probiotic suspensions of *Lactobacillus plantarum* against *Staphylococcus aureus*

For *Streptococcus thermophilus*, the probiotic suspension exhibited a small inhibition zone of 0.1 mm against Staphylococcus aureus. But there were no inhibition zones seen for the postbiotic or parabiotic suspensions of *Streptococcus thermophilus*against *Staphylococcus aureus*

However, According to **boubakeur et *al*.,(2021).** The study demonstrated notable inhibitory effects of lactic acid bacteria against two opportunistic pathogens, *E. coli* and *S. aureus*. *S. thermophilus* showed inhibition rates of 13–9.84% against *E. coli* and 12–9% against *S. aureus*. Lactic acid bacteria are widely employed in food preservation for their ability to produce antimicrobial substances, which enhance food safety andextend shelf life**. Akpinar et *al*. (2011)** reported that all strains of *S. thermophilus* displayed antimicrobial activity against *K. pneumoniae*. Moreover, strains SL4 and SY2 of *S. thermophilus* exhibited antimicrobial effects against all bacteria tested, including *Staphylococcus aureus* and *Escherichia coli*. Some *S. thermophilus* strains produce a bacteriocin named thermophilin, which effectively combats several types of bacteria responsible for food spoilage. *Enterococcus*, *Lactococcus*, and *Pediococcus*, along with other lactic acid bacteria, are widely used natural preservatives due to their ability to produce antimicrobial metabolits such as organic acids, hydrogen peroxide, antimicrobial enzymes, and bacteriocins **(Wu et *al*., 2014).**according to **Tavakoli et *al*.2017** who reveled In his stud that all selected strains, except for Lactobacillus plantarum MT.ZH293 and *Lactobacillus pentosus* MT.ZH693, inhibited the growth of *Staphylococcus aureus and *Pseudomonas aeruginosa*. L. pentosus MT.ZH693 was the only strain unable to inhibit Enterococcus hirea, while both L. plantarum MT.ZH293 and Lactobacillus casei MT.ZH493 did not prevent Salmonella enterica growth. L. casei MT.ZH493 inhibited Escherichia coli without a clear halo, but L. plantarum MT.ZH293 did not. Other strains showed strong inhibition against E. coli.

The inhibitory effects are mainly due to metabolites like organic acids, hydrogen peroxide, and bacteriocins produced by probiotic bacteria. Previous studies have reported the antagonistic

potential of various probiotic lactobacilli strains against human pathogens such as Staphylococcus aureus, Salmonella typhimurium, Escherichia coli, and Enterococcus faecalis. Overall, the lactic strains studied have significant therapeutic and prophylactic potential against infectious diseases.

Conclusion

Probiotics have become an increasingly important part of human health and wellbeing due to their beneficial effects. The present study aimed to identify and characterize new lactic acid bacterial strains, and to evaluate their biotic effects - including probiotic, parabiotic, and postbiotic activities - as well as their biofilm formation capabilities.

The study demonstrated the probiotic and antimicrobial potential of Streptococcus thermophilus and Lactobacillus plantarum strains. Previous results had shown these strains possess desirable characteristics, such as tolerance to bile salts, adaptability to different pH and temperature conditions, and notable auto-aggregation capacity.

Based on these findings, the investigated bacterial strains could be recommended as potential probiotic culture starters to help prevent the growth of pathogenic bacteria within the gastrointestinal tract. The multi-faceted biotic effects of these strains highlight their promise as useful probiotics for human health applications.

Recommandation

To better exploit the multifaceted biotic properties of the *Streptococcus thermophilus* and *Lactobacillus plantarum* strains, and position them as promising probiotics with broad applications for promoting human health and well-being, the following recommendations should be considered:

❖ **Further Evaluation and Characterization:**

- Conduct additional *in-vitro* and *in-vivo* studies to fully elucidate the probiotic, parabiotic, and postbiotic mechanisms of action of the *Streptococcus thermophilus and Lactobacillus plantarum* strains.
- Assess their safety, stability, and survivability under different storage and gastrointestinal conditions.

❖ **Potential Applications:**

- Develop the strains as probiotic supplements or functional food ingredients to promote gut health and prevent gastrointestinal infections.
- Explore their use as protective cultures in food processing and preservation to inhibit the growth of foodborne pathogens.
- Consider incorporating the strains into synbiotic formulations with prebiotic substrates to enhance their probiotic efficacy.

REFERENCES

References

1 A.E. Jiménez-Esquilín, T.M. Roane, Antifungal activities of actinomycete strains associated with high-altitude Sagebrush Rhizosphere, J. Ind. Microbiol. Biotechnol. 32 (2005) 378–381

2 Ammor MS, Flórez AB, Mayo B (2007). Antibiotic resistance in non enterococcal lactic acid bacteria and Bifidobacteria. Food Microbiol.,24: 559-570

3 Andrew J.M. (2008), BSAC standardized disc susceptibility testing method (version 7), Journal of Antimicrobial Chemotherapy, 62, pp. 256–278

4 Anukam K. C., Koyama, T. (2007), Bile and acid tolerance of Lactobacillus plantarum KCA1: A potential probiotical agent, International Journal Dairy Science , 2 (3), pp. 275–280

5 ARAUZ, L.J., JOZALAA, A.F., MAZZOLAB, P.G., VESSONI, PENNAA, T.C. 2009. Nisin biotechnological production and application: a review. Trends Food Sci. Technol. 20, 146–154.

6 BAJAJ, B.K. RAZDAN K., CLAES I.J.J., LEBEER, S. 2014a . Probiotic attributes of the newly isolated lactic acid bacteria from infants'gut. J. Microbiol. Biotechnol. Food Sci. (submitted)

7 Bauer A. W. (1966), Antibiotic susceptibility testing by a standardized single disc method, Am J clin pathol, 45(4), pp. 149–158–24.

8 Belhadj, H., Harzallah, D., Khennouf, S., Dahamna, S., Bouharati, S., and Baghiani, A. (2010). Isolation, identification and antimicrobial activity of lactic acid bacteria from Algerian honeybee collected pollen. Acta Hort. (ISHS) 854: 51 – 58

9 BERMUDEZ-BRITO, M., PLAZA-DÍAZ, J., MUÑOZ-QUEZADA, S., GÓMEZ-LLORENTE, C., GIL, A. 2012. Probiotic Mechanisms of Action. Ann. Nutr. Metab. 61, 160–174

10 Bessila, R & messaoudi,L. (2021). Proprites probiotics des bacteries lactiques. these de master. Université de larbi tebessi-tébessa faculté des science exactes et des sciences de la nature et de la vie. P27

11 Boubakeur b.,khadem h., drabo m.s ., ali a., meddah a.t (2022). Probiotic properties and exopolysaccharids production of streptococcus thermophilus CNRZ 477 and enterococcus durans NCBI 53345 10(4). P 856 .

12 BUJALANCE, C., JIMÉNEZ-VALERA, M., MORENO, E., RUIZ-LÓPEZ, M.D., LASSERROT, A., RUIZ-BRAVO, A. 2014. Lack of correlation between in vitro antibiosis and in vivo protection against enteropathogenic bacteria by probiotic lactobacilli. Res. Microbiol. 165, 14-20

13 Burgain J., Gaiani C., Jeandel C., Cailliez-Grimal C., Revol A.-N., Scher J. « Maldigestion du lactose : formes cliniques et solutions thérapeutiques ». Cahiers de Nutrition et de Diététique. septembre 2012.47(4), p. 201-209. In : (Place des probiotiques dans le traitement de diverses pathologie intestinales [Thèse de doctorat] Université de Limoges.)

14 champagne, C. P., Gardner, N., & Roy, D. (2011). Challenges in the addition of probiotic cultures to foods. Critical Reviews in Food Science and Nutrition, 51(6), 483-494

15 Charteau N, Deschamps AM, Hadj Sassi A (1994). Heterogeneity of bile salts resistance in the lactobacillus isolates of a probiotic concosortium. Lett Appl Microbiol. 18:42A (Abstract).

16 Collado, M. C., I. Surono, J. Meriluoto, and S. Salminen. 2007b. Indigenous Dadih lactic acid bacteria: Cell-surface properties and interactions with pathogens. J. Food Sci. 72:M89–M93.

17 DEC, M., PUCHALSKI, A., URBAN-CHMIEL, R., WERNICKI, A. 2014. Screening of Lactobacillus strains of domestic goose origin against bacterial poultry pathogens for use as probiotics. Poult Sci. 2014 93, 2464-2472

18 Elleuch, M. Shaaban, S. Smaoui, et al., Bioactive secondary metabolites from a new terrestrial Streptomyces sp. TN262, Appl. Biochem. Biotechnol. 162 (2010) 579–593.

19 ELLI, M., ZINK, R., RYTZ, A., RENIERO, R., MORELLI, L., 2000. Iron requirement of Lactobacillus spp. in completely chemically defined growth media. J. Appl. Microbiol. 88, 695–703.

20 Ferreira, C. L., L. Grześkowiak, M. C. Collado, and S. Salminen. 2011.In vitro evaluation of Lactobacillus gasseri strains of infant originon adhesion and aggregation of specific pathogens. J. Food Prot.74:1482–1487.

21 Gupta, V., Garg, R., & Probiotics, P. (2018). Encapsulation of probiotics for safety and targeted delivery: A review. Journal of Functional Foods, 40, 17-30

22 Haddaji N., Krifi B., Lagha R., Khouadja S., Bakhrouf A. (2015), Effect of high temperature on viability of Lactobacillus casei and analysis of secreted and Groel proteins profiles, African Journal of Bacteriology Research, 7(3), pp. 29–35.

23 HASSAN, M., KJOS, M., NES, I.F., DIEP, D.B., LOTFIPOUR, F. 2012. Natural antimicrobial peptides from bacteria: characteristics and potential applications to fight against antibiotic resistance. J. Appl. Microbiol. 113, 723- 726

24 HOJO, K., NAGAOKA S., MURATA S., TAKETOMO N., OHSHIMA T., MAEDA N. 2007. Reduction of vitamin K concentration by salivary Bifidobacterium strains and their possible nutritional competition with Porphyromonas gingivalis. J. Appl. Microbiol. 103, 1969-1974

25 Holzapfel, W.H., P. Haberer, R. Geisen, J. Björkroth, U. Schillinger (2001) Taxonomy and important features of probiotic microorganisms in food nutrition. Am. J. Clin. Nutr. 73, 365S-373S

26 Ji,j., jin,w., liu,s., jiao,z., li,x. (2023). Probiotics, prebiotics, and postbiotics in health and disease. Review, p7

27 Keerthi,r.s.s ., pokharel,B., abunamous,zhh.(2023). Article. Probiotics: production, characterization, types, and health benefits. P27

28 Kollath, W. (1953) Ernährung und Zahnsystem. Deutsche Zahnärztliche Zeitschrift 8: 7-16.

29 Lebeer, S., Verhoeven, T. L., Francius, G., Schoofs, G., Lambrichts, I., Dufrêne, Y., ... & Vanderleyden, J. (2008). Identification of a gene cluster for the biosynthesis of a long, galactose-rich exopolysaccharide in Lactobacillus rhamnosus GG and functional analysis of the priming glycosyltransferase. Applied and Environmental Microbiology, 74(17), 5559-5568

30 Lilly D.M, and R.H. Stillwell (1965) Probiotics: growth promoting factors produced by microorganisms. Science. 147: 747–8.

31 Metchnikoff, E. (1908). The Prolongation Of Life, New York, G.P. Putnam's Sons, 376 p

32 Mirohli.m ; t. zotta ; r.p.ross ; c.stanton ;m.c.ros ;e. parente ; a. ricciardi.,(2014). functional properties of lactobacillus plantarum strains : a multivariate screening study. P 8.10

33 Moreau, J.R., IWATA, M., EKSTEEN, B., SONG, S.Y., JUNT, T., SENMAN, B., OTIPOBY, K.L., YOKOTA, A., TAKEUCHI, H., RICCIARDICASTAGNOLI, P., RAJEWSKY, K., ADAMS, D.H., VON ANDRIAN, U.H. 2006. Generation of gut-homing IgA-secreting B cells by intestinal dendritic cells. Sci. 314, 1157–1160

34 n.kapse , v.pisu ,p.k.dhakephalkar , p. margale,s.wagh , s.dagar.;(2024). Unveiling the probiotic potential of streptococcus thermophilus mcco0200: insight from in vitro studies corroborated with genome analysis.p.11

35 n.singhal, n.s.singh ,s.kumar ,j.s.virati.,(2021). Rhizospheric lactobacillus plantarum strains exhibit bill salt hydrolysis, hypocholesterolemic and probiotic capabilities in vitro. P 6;7.

36 NIELSEN, D.S., CHO, G.S., HANAK, A., HUCH, M., FRANZ, C.M., ARNEBORG, N. 2010. The effect of bacteriocin-producing Lactobacillus plantarum strains on the intracellular pH of sessile and planktonic Listeria monocytogenes single cells. Int. J. Food Microbiol. 141, S53–S59.

37 Raphaëlle, M.-S. (2015). les probiotiques.Thèse de doctorat . Université de lille 2, faculté des sciences pharmaceutiques, p109.

38 Rastall R. A., R. Fuller, H. R. Gaskins, G. Gibson (2000). Colonic functional foods. In: Gibson G. and Williams CM. (eds.) Functional Foods: Concept to Product. Woodhead Publishing Limited, Cambridge; pp. 72-95

39 RAZDAN, K., PARIHAR, J., BAJAJ B.K. 2012. Isolation and characterization of a lipolytic and phytase producing probiotic for potential application in poultry feed. Online J. Anim. Feed Res. 2, 369-377

40 Reid, G., J. A. McGroarty, R. Angotti, and R. L. Cook. 1988. Lactobacillus inhibitor production against Escherichia coli and coaggregation ability with uropathogens. Can. J. Microbiol. 34:344–351.

41 Sanders M. E. (2008). Probiotics: Definition, Sources, Selection, and Uses. Clinical Infectious Diseases. 46:S58–61.

42 Schrezenmeir J., and M. De Vrese (2001) probiotics, prebiotics, and symbiotics – approaching a definition. Am. J. Clin. Nutr. 73:361S-364S.

43 Taj.r , t. masud , a.sohail , s. sammi , r.naz , b.k.khamal , m.a.nawaz.;(2022).invitro screening of eps producing streptococcus thermophilus shain for theire nprobiotic potential from dahi .p 8.

44 Todorov, S. D., Von Mollendorff, J. W., M. Moelich, E., Muller,N., Witthuhn, R. C., & Dicks, L. M. T. (2009). Evaluation of potentialprobiotics properties of Enterococcus mundtii, its survival in bozaand in situ bacteriocin production. Food Technology Biotechnology,47, 178–191.

45 Tosi L, Berruti G, Danielsen M, Wind A, Huys G, Morelli L. 2007. Susceptibility of Streptococcus thermophilus to antibiotics. Antonie van Leeuwenhoek 92:21–28.

46 Tuncer, B. O., & Tuncer, Y. (2014). Exopolysaccharide producer Streptococcus thermophilus ST8.01 strain; a potential probiotic culture. GIDA, 39(4), 195–202.

47 Vasiljevic and N.P. Shah, (2008) Review: probiotics — from Metchnikoff to bioactives, International Dairy Journal 18, pp. 714–728

48 Vergin F. (1954) Anti- and probiotika. Hippokrates 25: 16–19

49 VILA, B., ESTEVE-GARCIA, E., BRUFAU, J. 2010. Probiotic microorganisms: 100 years of innovation and efficacy; modes of action. World's Poult. Sci. J. 65, 369-380

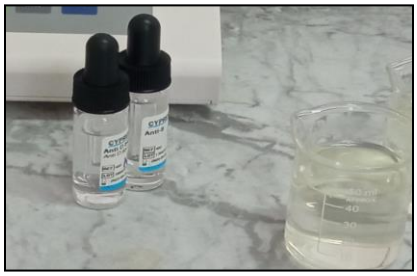
50 Vlkova, E., Rada, V., Smehisova, J., & Killer, J. (2008). Auto-aggregation and co-aggregation ability in Bifidobacteria and Clostridia. Folia Microbiology, 53, 263–269.

51 Vlková, E., V. Rada, M. Šmehilová, and J. Killer. 2008. Auto-aggregation and co-aggregation ability in bifidobacteria and clostridia.Folia Microbiol. (Praha) 53:263–269.

52 y.tuo ; yu.h ; l.ai ; z.wu ; b. guo ; w.chent ;.(2013).aggregation and adhesion properties of 22 lactobacillus strains. P 4254.

53 Zommiti M, Connil N, Hamida J. Ben, Ferchichi M (2017) Probiotic characteris-tics of Lactobacillus curvatus DN317, a strain isolated from chicken ceca.Probiotics Antimicrob Proteins 9(4):415

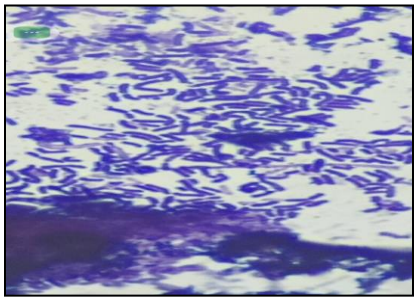
APPENDIX



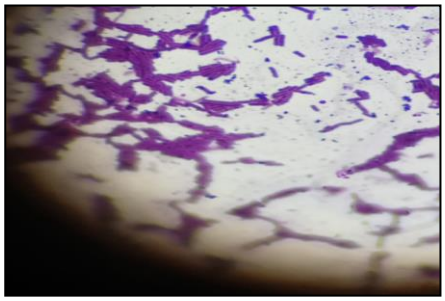
PICTURE OF HCL NAOH

PH MEASURING STRIPS

PICTURE OF PH METER

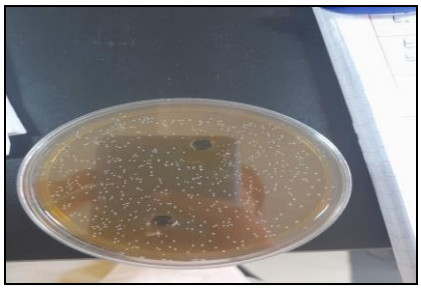


MICROSCOPIC PICTURE OF *L.PLANTARUM*

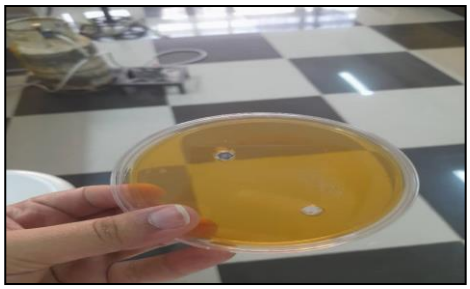


MICROSCOPIC PICTURE OF *S.TERMOPHILUS*

PICTURE OF SPECTROPHOTOMETRE

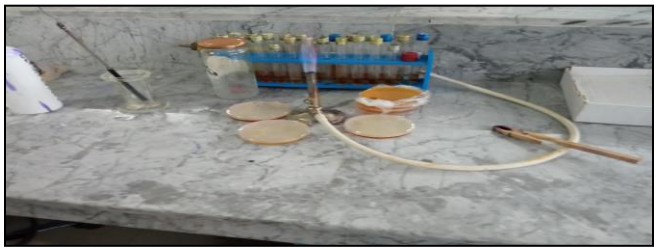


BACTERIAL PATHOGENIC STRAIN

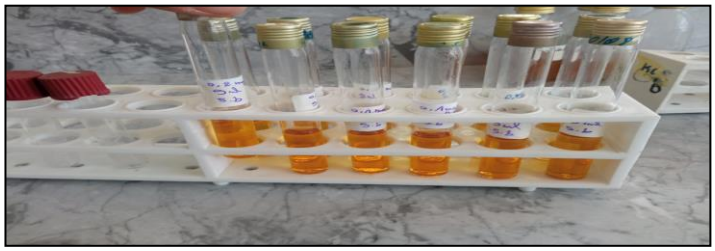


BACTERIAL STRAIN PICTURE

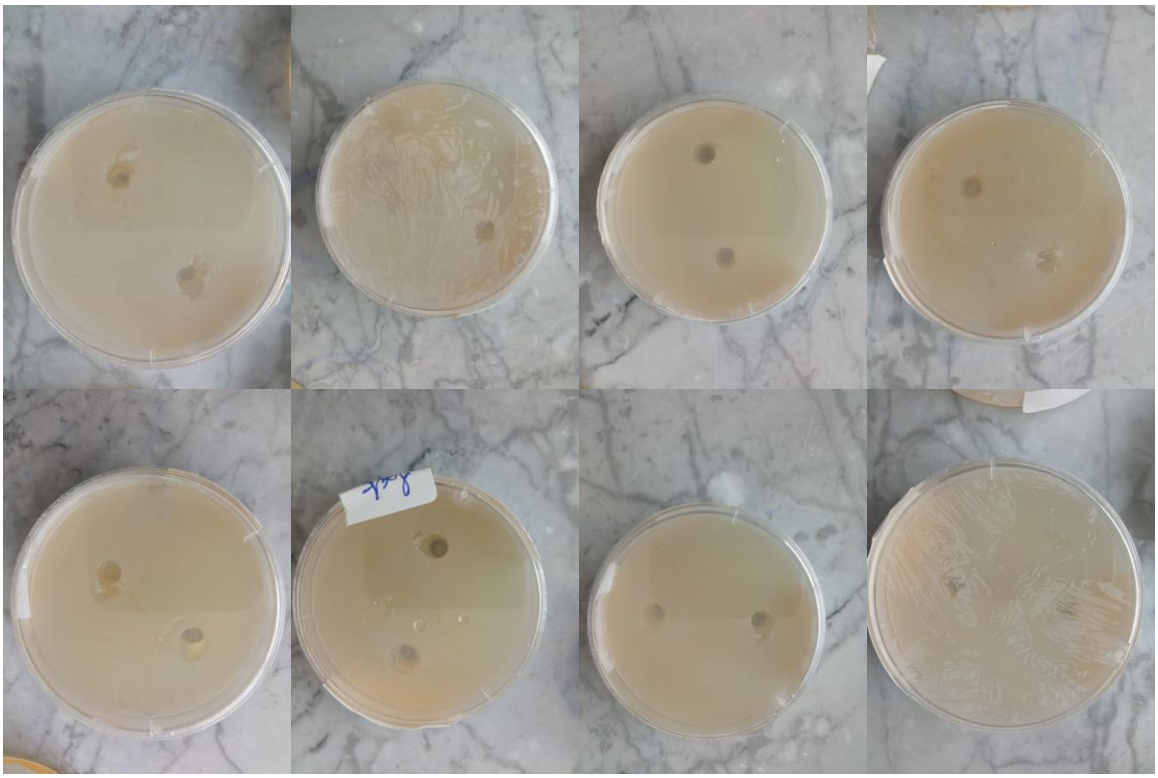
CENTREFUGE PICTURE



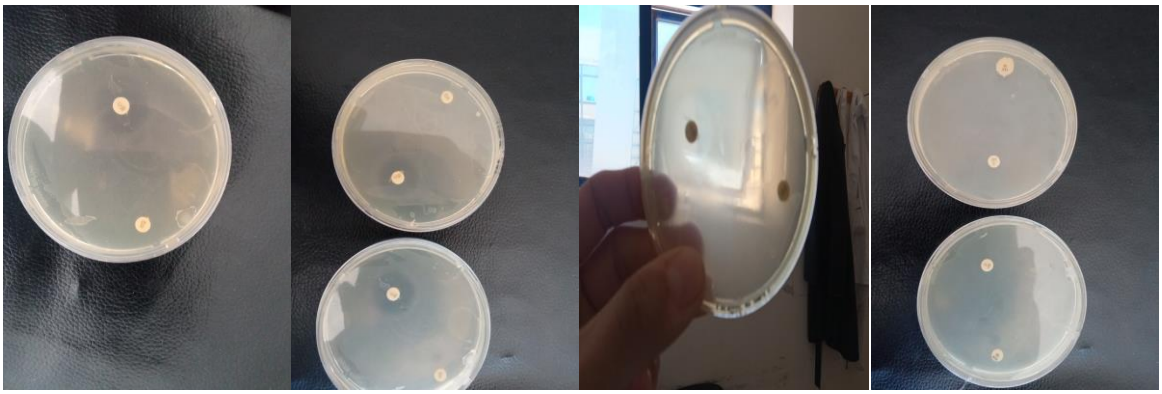
PICTURE OF WORKING AREA (ZONE)



Bill salts testing tubes



Photos of antibacterial test results



Photos of antibiotic test results