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كلية العلوم الطبيعة والحياة

Faculty of Natural and Life Sciences

قسم البيولوجيا الخلوية والجزيئية

Department of Cellular and MolecularBiology

END OF STUDY THESIS

In view of obtaining the AcademicMaster'sdegree

Major: Biological Sciences

Speciality: Applied Biochemistry

THEME

Influence of probiotic supplementation on the progression of chronic kidney disease

Presented by :

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In front of the jury composed of:

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College year: 2022/2023

Thanks

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Dedication

First of all, praise be to God who helped me to do this

Thanks to my family. If you're there, so is it. THANKS. You bring me in turn, each in your own way, a little something, encouragement, comfort, laughter.... Mom, it's you who helps Me to be positive in all circumstances, Dad, nothing that thinking of you Proud of me is enough to cheer me up.

Khaled, your sister's heart, how can I tell you how grateful I am for everything you've done for me?

My brothers and sisters, I love you very much.

I thank my friends for their help and support. The first of them is the kind ARCHI GHANIA and the quarrelsome FARHAT RABAA the companion of my life KADDOUR HANANE the flower of my life REZZAG LEBZA ZOHRA my sweet and kind friend ZOURI AHMED WAFA thank you very much for being there and for all the l help you have given me, I love you so much.

In conclusion, I will simply say that the years I spent at the Faculty of Natural and Life Sciences were enriching from a human and scientific point of view and that they will remain etched in my memory.

✍ *Ouafa*

Abstract

Abstract

The aim of the current study was the effect of probiotics on the progression of chronic kidney disease. We wanted to clarify the therapeutic benefits of probiotics. For this reason, strains of lactic acid bacteria were isolated from the intestines of camels, and based on the microbiological tests that were conducted, we selected only bacteria that possess probiotic properties. After many studies, two types of. lactic strains have been identified and they are *Pediococcusacidilactici* and *Lactobacillus acidophilus*.

20 female white Wister rats were used Equally divided into a control group and 3 treatment groups, during the first month, treated with hemi-penta-hydrate cadmium chloride analyte (5 mg /kg/day) and then treated with a dose of 0.5 ml of selected lactic acid probiotic bacteria, diluted with an amount of physiological acid water (5 ml) for a week, after examining the results of kidney analyzes and a complete blood count, it was noted that there was a significant increase in most values and abnormal tissue sections of the kidneys in the group that received hemi-penta-hydrate cadmium chloride only, with normal results recorded in the control group and in the control group. Both groups were treated with probiotics, with no undesirable results. Finally, we can see the great therapeutic benefit of probiotics.

Keywords: kidney disease, Probiotic, intestines of camels, analytical reagent cadmium chloride hemi-penta-hydrate.

Résumé

L'objectif de la présente étude était l'effet des probiotiques sur la progression de la maladie rénale chronique. Nous avons voulu clarifier les bénéfices thérapeutiques des probiotiques. Pour cette raison, des souches de bactéries lactiques ont été isolées des intestins de chameaux et, sur la base des tests microbiologiques qui ont été effectués, nous avons sélectionné uniquement des bactéries possédant des propriétés probiotiques. Après de nombreuses études, deux types de. des souches lactiques ont été identifiées et il s'agit de *Pediococcusacidilactici* et *de Lactobacillus acidophilus*.20 rats wister blancs femelles ont été utilisés répartis également en un groupe témoin et trois groupes traitement, au cours du premier mois, traités avec l'analyte de chlorure de cadmium hémi-penta –hydraté (5mg /kg/jour) puis traités par une dose de 0,5 ml de bactéries lactiques probiotiques sélectionnées, diluées avec une quantité d'eau acide physiologique (5 ml) pendant une semaine, après examen des résultats d'analyses rénales et d'une formule sanguine complète, il a été noté qu'il y avait une augmentation significative de la plupart des valeurs et des sections de tissus anormaux des reins dans le groupe ayant reçu uniquement du chlorure de cadmium hémi-pentahydraté, avec des résultats normaux enregistrés dans le groupe témoin et dans le groupe témoin. Les deux groupes ont été traités avec des probiotiques, sans résultats indésirables. Enfin, on peut voir le grand bénéfice thérapeutique des probiotiques.

Mots-clés : maladie rénale, probiotique, intestins de chameaux, réactif analytique chlorure de cadmium hémi-penta-hydraté.

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Abreviationslist

EDTA: Ethylene Diamine Tetra Acetic Acid

LAB: lactic acid bacteria

PBS: phosphate-buffered saline

UFC: Colony Forming Units

PH: Hydrogen Potential

EPS: Exo-polysaccharides

ug: microgram

HCT: Hematocrit

RBC: Red Blood Cells

PLT: Blood Platelets

WBC: white blood cell

ZOI: zone of inhibition

Mm: millimeter

MI: milliliter

C°: Degrees Celsius

RCB: Residual Cancer Burden

HGB:Hemoglobin

MCV:Mean Corpuscular Volume

MCH: Mean Corpuscular Hemoglobin

MCHC:Mean Corpuscular Hemoglobin Concentration

Summary

General Introduction

General Introduction

Probiotic microorganisms are defined as living microorganisms that confer health benefiting properties to the host when administered adequately. Probiotics exert beneficial functions mainly through producing antimicrobial peptides, assimilating dietary fibers, regulating fat storage, modulating mucosal immunity, or regulating gut microbiota **(Ku *et al*., 2016).**

For centuries, probiotics have been widely used in various functional foods, e.g., yoghurt, milk, cheese, infant formula, and dietary supplements. The most common probiotics include Lactobacilli and Bifidobacteria, which predominantly inhabit the animal or human intestinal tract. **(Chen, 2021).**

Kidney disease is a condition that affects the ability of the kidneys to filter blood and remove waste from the body. It can be caused by various factors, such as diabetes, high blood pressure, infections, inflammation, or genetic disorders. Kidney disease can lead to serious complications, such as cardiovascular disease, kidney failure, and deathA. **(Bentall ,2021)..**

According to the Global Burden of Disease study, chronic kidney disease affects nearly *10%* of the general population worldwide, and millions die each year because they do not have access to affordable treatment. (**Kovesdy& al, 2022**). The prevalence and mortality of kidney disease vary widely across regions and countries, with some areas having much higher rates than others.For example, in central and Andean Latin America, chronic kidney disease was the second and fifth ranked cause of death in 2017[6]. In contrast, in high-income Asia Pacific, it was the 23rd ranked cause of death in the same year. Kidney disease is a global health challenge that requires more awareness, prevention, and management strategies to reduce its burden and improve the quality of life of those affected by it.(**Cockwell& al, 2020**)

Probiotics are live microorganisms that can confer health benefits to the host when consumed in adequate amounts. They can modulate the gut microbiota, which is altered in patients with chronic kidney disease (CKD), and influence various metabolic and inflammatory pathways.**(Wang, 2021).**

Probiotics may have beneficial effects on kidney function and uremic symptoms by reducing the production and absorption of uremic toxins, such as indoxyl sulfate and p-cresyl sulfate, which are derived from protein fermentation by gut bacteria **(Koppe& al, 2015).**Probiotics may also improve the intestinal barrier function, decrease inflammation,

lower oxidative stress, and enhance immunity in CKD patients. **(Wang, 2021).** Several studies have investigated the effects of probiotics on CKD progression and outcomes, using different strains, doses, durations, and endpoints.**(Koppe& al, 2015).**

The objectives of our study revolve around isolation, purificationand identification of lactic acid bacteria from camel intestines, possessing probiotic propertiesand to evaluate this ability in vivo.

This study is divided into two parts, first part bibliographies contain a chapter about probiotics and chapter about kidney disease. Second practical partcontain a chapter Material andMethods and chapter about result and discussion and finally conclusion.

FIRST PART

Bibliographic synthesis

Chapter I:

PROBIOTIC

I.1.Definition

A probiotic is a living microorganism that, when ingested in sufficient quantities, exerts a positive effect on health. Probiotics are mainly bacteria and yeasts present or reintroduced into the resident intestinal flora **(Gaggia*and al.*,2010).**

There are four main groups of Probiotics :

- **The lactic ferments**

They are capable of producing acid by the fermentation of certain sugars such as lactose.

- **Bifidobacteria**

Of human or animal origin, they belong to the normal intestinal flora. They are grouped into two categories according to their morphology: Lactobacilli and shells.

- **The different yeasts of the saccharomyces type**

They are mainly used in the food industry.

- **The other sporules including Bacillus subtilis and cereus**

The concept of probiotics appeals to four important notions

✓ A probiotic is a living microorganism.
✓ A probiotic is ingested orally.
✓ A probiotic exerts a beneficial effect.
✓ A probiotic exerts its action in the balance of the intestinal flora probiotics can be considered as a means of conveying the active ingredients they contain (enzymes, wall components, immunomodulatory peptides, antibacterial substances) to their targets of action in the digestive tract **(Marteau *&* *al.*,1993 *&* 1998)**.

I.2.Classification of probiotics

Probiotics can be classified into four categories (Table 01). The first category contains the species of the genus Lactobacillus. Lactobacillus are gram-positive bacteria, classified in the Bacillota phylum and belonging to the Lactobacillaceae family (Hammes and Vogel, 1995).

They are in the form of bacilli or shells and are facultative anaerobic, immobile, non-flagellated and non-spore-forming. Lactobacillus form a large part of the lactic acid bacteria which are capable of producing lactic acid by the fermentation of certain sugars such as lactose These species colonize humans and are generally present in the gastrointestinal tract, the vaginal mucous membranes and the oral cavity (LandRouster-Stevens & al., 2005). Lactobacillus are among the most widely used probiotics in humans with more known applications, in particular the manufacture of fermented milk products such as yogurt (Klaenhammer, 1998). Among the most widely used commercial strains, we find the strains of L. acidophilus, L. casei, L. paracasei and L. johnsonii (Holzapfel and Schillinger, 2002).

The second category is composed of Bifidobacterium species. They are gram-positive, strict anaerobic, immobile bacilli and form the predominant bacterial group of the human intestinal flora (Mitsuoka, 1990). Bifidobacteria are mainly used as probiotics especially by the food industry because of their many health benefits. This is the case of the commercial strain *B. animalisssp. LactisBb12* (Mohan *and* al., 2006; Kabeerdoss*and al.*, 2011)

The third group of probiotics includes other shell-shaped lactic acid bacteria such as *Enterococcus* and *Streptococcus*. As for the fourth group, it consists of non-lactic microorganisms, in particular spore-forming bacteria (*Bacillus cereus*), bacteria belonging to the species *Propionibacterium freudenreichiias* well as certain yeasts of the *Saccharomyces* type mainly used by the agri-food industry.

Table 01: List of microorganisms considered as probiotics (Holzapfel, Haberer and al., 2001).

Lactobacillus	*Bifidobacterium*	Autres bactéries lactiques	Bactéries non-lactiques
L. acidophilus	*B. adolescentis*	*Enterococcus faecalis*	*Bacillus spp,*
L. amylovirus	*B. animalis*	*Enterococcus faecium*	*Escherichia coli* Nissle
L. brevis	*B. bifidum*	*Lactococcus lactis*	*Propionibacterium freudenreichii*
L. casei	*B. breve*	*Leuconostoc mesenteroides*	*Saccharomyces cerevisae*
L. cellobius	*B. infantis*	*Pediococcus acidilactici*	*Saccharomyces bourlardii*
L. crispatus	*B. lactis*	*Sporolactobacillus inulinus*	
L. curvatus	*B. longum*	*Streptococcus thermophilus*	
L. delbrueckii	*B. thermophilum*	*Streptococcus diacetylactis*	
L. farciminis		*Streptococcus intermedius*	
L. fermentum			
L. gallinarum			
L. gasseri			
L. johnsonii			
L. paracasei			
L. plantarum			
L. reuteri			
L. rhamnosus			

I.3.Properties and selection criteria of probiotic strains.

In order for a microorganism to be recognized as a probiotic potential, it must meet certain criteria.

First of all, it must be non-pathogenic and be recognized as safe. It must have the ability to survive and grow in the physiological conditions of the digestive tract, as well as have a good tolerance to the acidic PH encountered in the stomach and bile salts encountered in the duodenum (**Dunne *and al.*, 2001**).

Adhesion to the epithelial cells of the intestine is often cited as a selection criterion **(Guarner and Schaafsma, 1998).** The importance of this characteristic is highlighted by the fact that many probiotics do not colonize the intestine and therefore need to be fixed to have their beneficial effect. For example, bacteria of the genus Lactobacillus are more able to adhere to the intestinal cell lines HT-29 and Caco-2 than Bifidobacterium **(Thornton, 1996).**Table 2 provides the criteria most used for the selection of probiotics.

Table 02: Selection criteria for probiotics (Nousiainen*and al.*, 2004).

Criteria	**Aim sought**
Resistance to gastric acidity	Survival during passage through the stomach and duodenum
Bile salt resistance	Survival during passage through the small intestine
cid production (from glucose and lactose)	Efficient (acid barrier) production in the intestine
Adhesion to mucus and/or human epithelial cells	Efficient colonization, reduction of adhesion sites of pathogens on the surface
Production of antimicrobial substance	Inhibition of the development of pathogenic germs
Heat resistance	Survival during the transformation process
Good technological properties	Stability, growth on a large scale, survival in the product, resistance to bacteriophages

I.3.1.Resistance to gastric acidity

The survival of bacteria in gastric juice depends on their ability to tolerate low PH levels. The passage time can be from one hour to four hours depending on the individual and his diet.

Therefore, some authors propose that the probiotic strains must resist at a pH of 2.5 in a culture medium for four hours **(Ammor and Mayo, 2007).**

I.3.2. Resistance to bile salts

In the small intestine, tolerance to bile salts is an important factor that contributes to the survival of probiotics. The bacteria that arise in the acidic conditions of the stomach must then cope with the detergent action of the bile salts released in the duodenum after ingestion of fatty meals. Bacteria can reduce the emulsifying effect of salts by hydrolysing them with hydrolases, thereby decreasing their solubility **(Ammor and Mayo, 2007) (Gu*and al.*, 2008)**

I.3.3. Adhesion to epithelial cells

The ability to adhere to the intestinal layer is a recommended selection criterion for the choice of probiotics, because it is a condition for the colonization of the notches. Adhesion constitutes the first defense mechanism against the invasion of pathogenic bacteria. It is based on the realization of a set of in vitro and then in vivo tests using cells of animal and/or human origin **(Palomares *and al.*, 2007)(Reyes *and al.*, 2011).**

In addition to the adhesion power to the epithelial cells of the intestine, probiotics can attach to the mucus that covers the antherocytes or to the various microorganisms found in the gastrointestinal tract **(Lamoureux, 2000).**

I.3.4. The production of antimicrobial substances

Lactic acid bacteria synthesize molecules with bactericidal\bacteriostatic action such as organic acids, hydrogen peroxide, carbon dioxide, diacetyl and bacteriocins. These antimicrobial mechanisms have been exploited to improve food preservation **(Titiek *and al.*, 1996) (Labioui*and al.*, 2005).**

I.3.5. Antibiotic resistance

Lactic acid bacteria are naturally resistant to many antibiotics thanks to their structure and physiology. The work of (Temmerman*and al.* 2003) showed that 68.4 of the isolated probiotics have resistance to one or more antibiotics. Lactobacillus strains have been found resistant to Kanamycin 81, tetracycline 29,5, erythromycin 12 and chloramphenicol 8,5.38 Enterococcus faecium isolates have been found resistant to vancomycin.

In most cases the resistance is not transmissible, however, it is possible that the plasmid coding for antibiotic resistance is transferred to other species and genus. This is a significant reason for choosing strains lacking the resistance transfer potential (Denohue, 2004).

I.4. Probiotics and their beneficial effects on health

Several studies have demonstrated the multiple beneficial effects of probiotics, in fact, probiotics are involved in the prevention and treatment of several diarrhea, including traveler's diarrhea and diarrhea associated with taking antibiotics **(Beausoleil*and al.*, 2007;McFarland, 2007).** They are also involved in the reduction and treatment of certain gastrointestinal infections **(Salminen*and al.*, 2005).** They also contribute to the modulation of the immune system and to the strengthening of the intestinal mucosa (Table 03, Figure 01) **(Matsuzaki and Chin, 2000) (Madsen *and al.*, 2001).**

Table 03: The main beneficial effects attributed to probiotics (Salminen and al., 2004; Patterson, 2008).

Gut effects	Effects on the Immune System	Other effects
Control of the following disorders: ▪ Poor digestion of lactose. ▪ Diarrhea due to rota viruses and Diarrhea-associated with antibiotics. ▪ irritable bowel syndrome ▪ Constipation. ▪ infection par Helicobacterpylori. ▪ Bacterial proliferation in the small intestine. ▪ Inflammatory bowel diseases ▪ Prevention of necrotizing enterocolitis in newborns.	▪ Immune modulation ▪ Suppression of allergic reactions by reducing inflammation. ▪ Reduced risk of infection by common pathogens (Salmonella, Shigella)	**Reduced risk of:** ▪ Certain cancers (colorectal, bladder, cervix, breast) ▪ Coronaropathie. ▪ Urinary tract disease ▪ Upper respiratory tract infection and related infections ▪ Reduction of serum cholesterol and blood pressure.

Probiotics also improve the digestion of food and play a role in reducing the symptoms of lactose intolerance **(Nagpal*and al.*, 2007)**. Probiotics also have an antimicrobial action thanks to the production of bacteriocins**(Klaenhammer, 1988)**

Certains probiotiques ont démontré leur capacité à prévenir certaines maladies chroniques telles que la maladie de Crohn, l'obésité et le diabète **(Schultz *and al.*,2004) (Yadav*and al.*, 2007)**. D'autres travaux laissent présager qu'ils pourraient également jouer un rôle important dans la prévention du cancer du côlon **(Wollowski*and al.*,2001).**

Some probiotics have demonstrated their ability to prevent certain chronic diseases such as Crohn's disease, obesity and diabetes **(Schultz *and al.*, 2004) (Yadav*and al.*, 2007).** Other studies suggest that they could also play an important role in the prevention of colon cancer **(Wollowski*and al.*, 2001)**

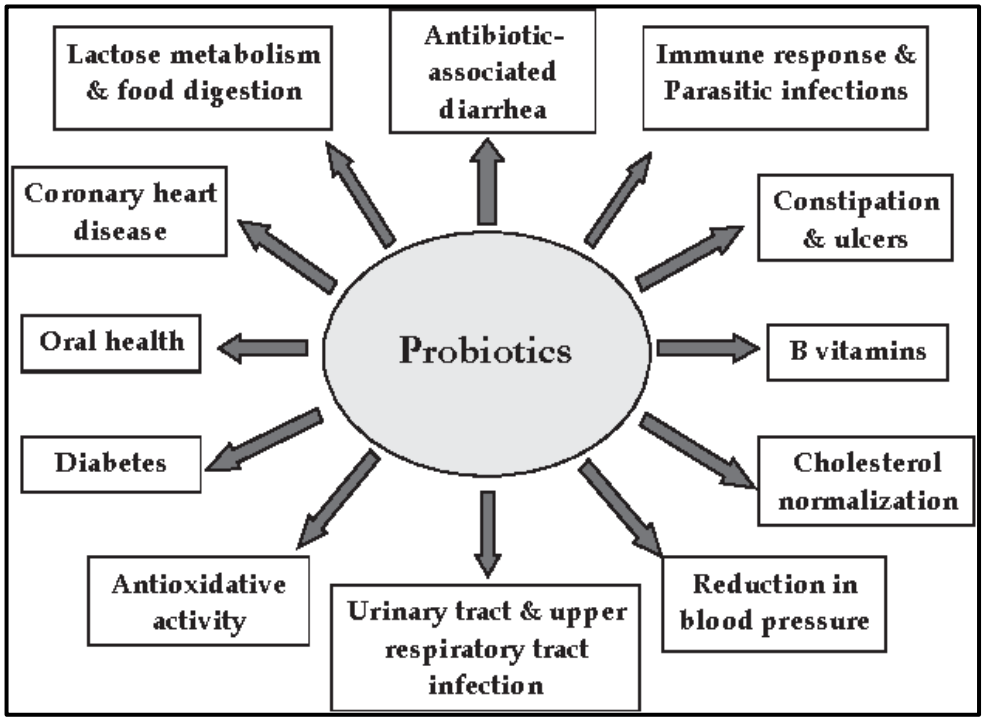

Figure 01: The main benefits of probiotics (Nagpal and al., 2012)

I.5. Role of Probiotics

✓ They participate in the activation of immunity and the reduction of allergies in subjects at risk.

✓ Resistance to gastric acid and bile, allows probiotics to survive in the digestive tract where part of the immunity resides.

✓ Probiotics participate in the development of the immune system in infants and improve it in the elderly by increasing the number of phagocytes and Natural killer lymphocytes, first defense against an exogenous agent.

✓ They also act on immunity by colonizing the intestinal tract, thus achieving a "barrier effect" prevents, on the one hand, the colonization of the epithelium by pathogens and, on the other hand, strengthens immunity at the level of intestinal mucous membranes by increasing the production of IgA and mucus, local defenses at the level of the mucous membranes **(Makhloufi, 2012**).

I.6.Applications of Probiotics

The various products marketed as human or animal probiotics consist either of a single microorganism (so-called mono-strain products) or of an association of several species (so-called multi-strain products). Nowadays, probioticproducts are marketed in threeforms**(Patterson, 2008)** :

★ A culture concentrate added to foods and drinks based on dairy products, fruits and cereals..

★ An ingredient added to a food based on milk or soy and which is allowed to reach a high concentration by fermentation

★ Dried, concentrated, powdered, capsule or tablet cells.

Probiotics are usually associated with the dairy products of culture.la range of probiotic products now includes cheeses, ice creams and frozen yogurts as well as non-dairy foods and beverages **(Patterson, 2008)**

I.7. Mechanisms of action of Probiotics

Probiotics are currently the subject of a certain consensus in the scientific community thanks to their beneficial effects on the health of the host. Several mechanisms by which certain probiotics exert protective or therapeutic effects have been proposed. However, these modes of action have not yet been completely elucidated. Among these main mechanisms of action, we find the strengthening of the intestinal barrier, the inhibition of the adhesion of pathogens to the intestinal mucosa, the production of antimicrobial substances and the modulation of the immune system.

I.7.1. Inhibition of the adhesion of pathogens: phenomenon of competition/exclusion

Probiotics exert a direct antimicrobial action by opposing the invasion of pathogenic microorganisms in the digestive tract while preventing their adhesion to the intestinal walls **(Vanderpool*and al.*, 2008).**Indeed, there is a direct competition between probiotic strains and infectious germs to occupy the sites of adhesion to the walls of the intestine. Some probiotics have an ability to adhere to the digestive tract and can colonize it for a long time. This property could constitute an ecological advantage favoring their implantation at the intestinal walls and consequently, the inhibition of the attachment of pathogenic germs. Thus, probiotics play a role as a physical barrier against pathogenic microorganisms. This phenomenon has been observed in certain lactobacilli that adhere to intestinal villi and inhibit the attachment of enteropathogenic Escherichia coli **(Roselli*and al.*, 2006) (Collado*and al.*, 2007)**

I.7.2.Production of antimicrobial substances

Probiotics could also limit the growth of pathogens by exerting an indirect antimicrobial action. The latter is achieved through the production of various antimicrobial compounds.

I.7.2.1.The bacteriocins

These are protein compounds that respectively slow down the invasions of bacterial strains **(Klaenhammer, 1998).** These harmful substances produced by probiotics are directed against bacteria phylogenetically close to the producing strain. They act mainly on the outer membrane of the target bacteria by forming pores that lead to the release of the intracellular contents and the death of the affected bacteria. Lactobacilli and lactococci, unlike bifidobacteria strains, are most often associated with the production of bacteriocins**(Fooks and Gibson, 2002)**.Nisin, which is produced by the bacterium Lactococcuslactis, is the most documented bacteriocins.

I.7.2.2. Organic acids

Probiotic bacteria have the ability to produce organic acids that contribute to the inhibition of the growth of enterovirulant microorganisms **(Servin, 2004).** These are lactic acid and acetic acid, which are produced respectively by lactobacilli and bifido-bacteria via the fermentation of hexoses. These organic acids, produced from carbohydrates ingested during food intake, contribute to lowering the intestinal PH . Their passive diffusion through the bacterial membrane in their un dissociated form makes it possible, after their dissociation, to acidify the cytoplasm and therefore to inhibit the spread, growth and survival of acid-sensitive pathogens.

I.7.2.3. Hydrogen peroxide

Some lactic acid bacteria produce, in a humid environment, hydrogen peroxide (H_2O_2) which inhibits many pathogenic bacterial strains **(Ouwehand and Vesterlund, 2004).** The production of hydrogen peroxide accompanied by that of lactic acid makes it possible to inhibit the development of certain pathogenic species such as certain viruses such as the foot-and-mouth disease virus, certain fungi such as *Candida albicans*, or even certain bacteria such as *Escherichia coli*, etc.

I.7.3. Stimulation of the activity of the intestinal immune system

The interaction of probiotics with the immune system makes it possible to increase the immune response of the host against enteropathogenic agents. Indeed, probiotics intervene in the stimulation of adaptive immunity, such as the production of IgA-type antibodies **(Shu and Gill, 2002),** as well as innate immunity such as the production of macrophages, monocytes,

etc. **(Oelschlaeger, 2010).** Therefore, the 33 probiotics act as adjuvants by modulating a rapid response of the intestinal mucosa and thus strengthening the intestinal immune system.

I.7.4. Health claims associated with the consumption of probiotics

Known for hundreds of years, especially through traditional fermented milk products, probiotics are the subject of a real revival of interest on the part of industrialists. The latter also insist on their beneficial action, in particular on the digestive system and the balance of the intestinal flora. In addition, consumer interest in these health-promoting products is also one of the drivers of the growth of the probiotics market. But, the arrival of new rules on claims has slowed the growth of probiotics. Recently, Health Canada has developed new regulations regarding generic claims related to the consumption of probiotics **(Naimi, 2014)**

CHAPTER II:

KIDNEY DISEASE

I.1.Definition

Kidney disease means kidneys are damaged and can't filter blood the way they should. Kidney disease, also known as chronic kidney disease or CKD, causes more deaths than breast cancer or prostate cancer (NVS 2021 report of 2018 data). It is *the* under-recognized public health crisis. **(NIDDK, 2022).**

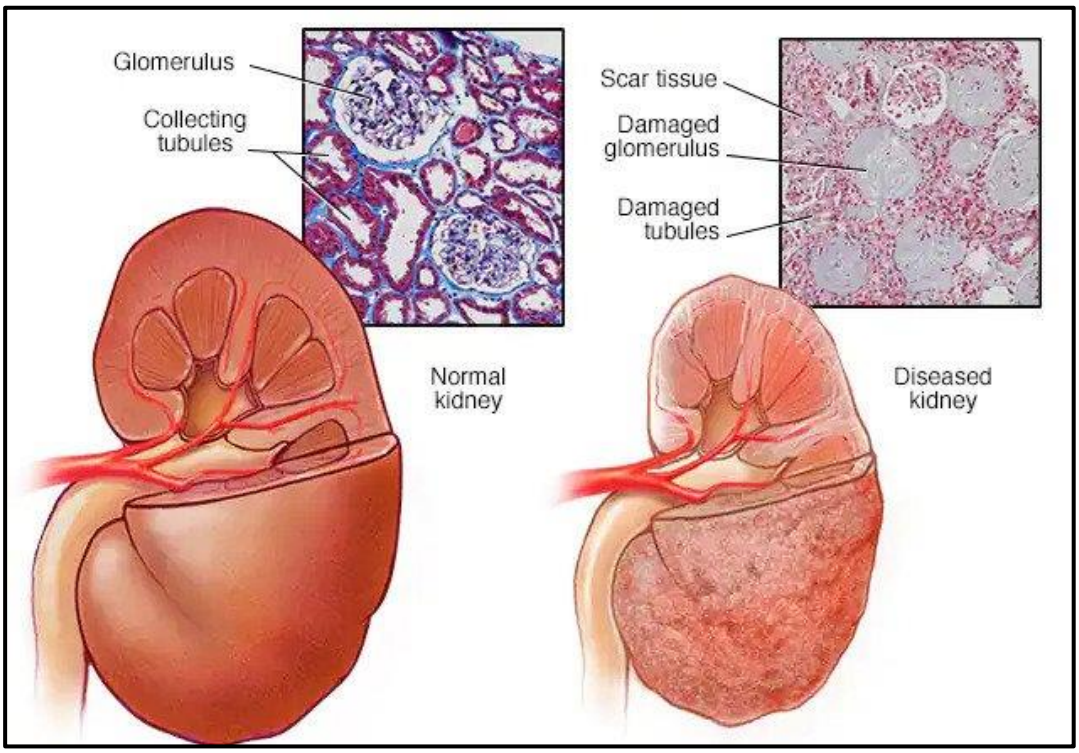

Figure 02: Kidney disease (CDC. 2022).

I.2.Types of kidney diseases

- **ChronicKidneyDisease**

Chronic kidney disease (CKD) is when lasting damage to kidneys causes them to lose their ability to filter waste and fluid out of your blood. Waste can build up in body and harm health. This damage–and kidney function–can get worse over time, and when kidneys stop working completely, this is called kidney failure or end-stage renal disease.

- **Fabry disease**

Fabry disease is a rare genetic disease that is passed down through family. It affects organs all around body, including heart, brain and kidneys, and can cause them to get less blood than they need. Over time, this can cause chronic kidney disease or kidney failure.

- **Cystinosis**

Cystinosis is a rare disorder that allows a natural chemical called cystine to build up in body and cause health problems. Kidney damage from cystinosis can cause kidney failure. People with cystinosis must take medicine to lower their cystine levels and may need a kidney transplant. Cystinosis is genetic (runs in families) and is most often diagnosed in young babies.

- **Glomerulonephritis**

Glomerulonephritis is when the tiny filters in kidneys that clean your blood (glomeruli) are damaged and lose their ability to remove waste and fluid from your blood. Over time, this can cause kidney failure. Many health problems can cause glomerulonephritis and treatment depends on the cause.

- **IgANephropathy**

IgA nephropathy is a disease that causes proteins made by immune system to build up in your kidneys and damage the tiny filters that clean your blood (glomeruli). This damage can take years to develop, and people with IgA nephropathy often do not know they have it. Over time, IgA nephropathy can lead to chronic kidney disease, kidney failure or death. There is no cure for IgA nephropathy, but medicines can slow the damage to your kidneys.

- **Lupus Nephritis**

Lupus nephritis is an autoimmune disease (a disease that causes body's immune system to attack its own tissues) that leads to pain, swelling and damage in whole body, including kidneys. This can lead to chronic kidney disease or kidney failure. The exact cause of lupus nephritis is unknown and it cannot be cured, but with treatment many people with lupus can lower their symptoms and prevent serious kidney damage.

- **aHUS**

aHUS (atypical hemolytic uremic syndrome) is a very rare genetic (runs in families) disease that causes tiny blood clots to form in the small blood vessels of your body. These clots can block the flow of blood to your kidneys and other organs and cause damage. Many people who have aHUS never have symptoms. For people who do have symptoms, they often start after a "triggering event", such as becoming pregnant or having cancer.

- **PolycysticKidneyDisease**

Polycystic kidney disease (PKD) is a genetic (runs in families) disorder that causes cysts (growths filled with fluid) to form on kidneys and other organs. These cysts can lower kidney's ability to filter fluid and waste from blood. Over time, PKD can cause kidney failure. There is no cure for PKD, but treatments can slow the growth of the cysts and prevent PKD symptoms from causing health problems.

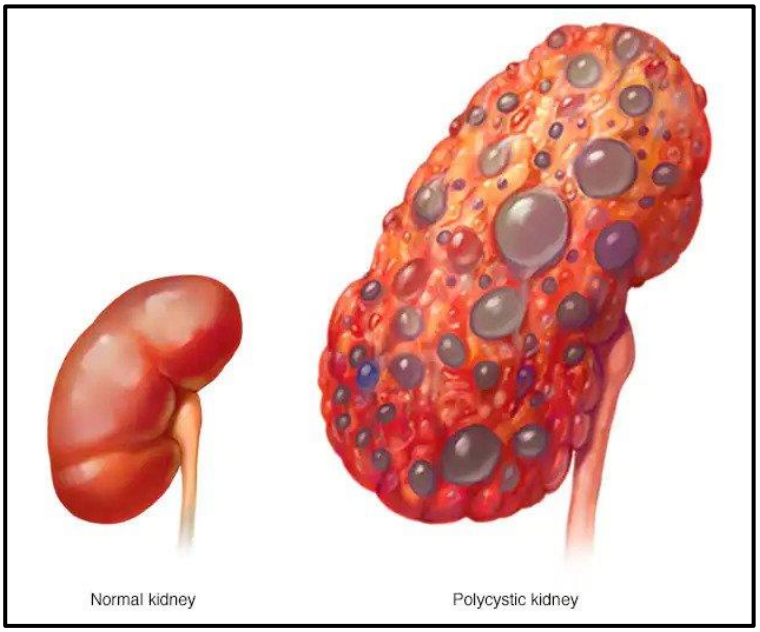

Figure 03 normal and Polycystic kidney disease(CDC. 2022).

- **Rare diseases**

There are other rare diseases that can damage kidneys and lower their ability to filter waste and fluid out of blood. This damage can lead to chronic kidney disease or kidney failure. **(American Kidney Fund, 2023)**

I.3.Symptoms

People who are at high risk of developing CKD should have regular kidney function checks. Early detection can help prevent severe kidney damage.

CKD is a slow and gradually progressive condition that causes kidney dysfunction. However, if one kidney stops functioning correctly, the other may still carry out normal functions.

A kidney may deteriorate to a certain level of dysfunction and not get any worse. Sometimes, however, the condition may progress to kidney failure.

Most people with CKD are not aware that they have it because symptoms do not usually develop in the early stages of the condition. Typically, by the time a person notices any symptoms, the condition is at an advanced stage. Damage to the kidneys at this stage is irreversible.

Symptoms of CKD can include:

- ✓ hypertension, or high blood pressure.
- ✓ anemia.
- ✓ edema, or swollen feet, hands, and ankles.
- ✓ fatigue, or tiredness.
- ✓ decreased urine output.
- ✓ bloody urine, in some cases.
- ✓ dark urine, in some cases.
- ✓ decreased mental alertness, when the condition is severe.
- ✓ aloss of appetite.
- ✓ persistent itchy skin, when the condition is severe.
- ✓ more frequent urination, especially at night, in some cases **(CDC. 2022).**

I.4.Risk factors

Factors that can increase your risk of chronic kidney disease include:

- ➢ Diabetes
- ➢ High blood pressure
- ➢ Heart (cardiovascular) disease
- ➢ Smoking
- ➢ Obesity
- ➢ Being Black, Native American or Asian American
- ➢ Familyhistory of kidneydisease

- Abnormalkidney structure
- Olderage

Frequent use of medications that can damage the kidneys **(Mayo Clinic, 2021).**

I.5.Complications

Chronic kidney disease can affect almost every part of your body. Potential complications include :

❖ Fluid retention, which could lead to swelling in your arms and legs, high blood pressure, or fluid in your lungs (pulmonary edema)

❖ A sudden rise in potassium levels in your blood (hyperkalemia), which could impair your heart's function and can be life-threatening

❖ Anemia.

❖ Heartdisease

❖ Weak bones and an increased risk of bone fractures

❖ Decreased sex drive, erectile dysfunction or reduced fertility

❖ Damage to your central nervous system, which can cause difficulty concentrating, personality changes or seizures

❖ Decreased immune response, which makes you more vulnerable to infection

❖ Pericarditis, an inflammation of the saclike membrane that envelops your heart (pericardium)

❖ Pregnancy complications that carry risks for the mother and the developing fetus

❖ Irreversible damage to your kidneys (end-stage kidney disease), eventually requiring either dialysis or a kidney transplant for survival **(sparrow.org. 2022).**

I.6.Causes

Kidneys carry out the complex system of filtration in our bodies. This involves removing excess waste and fluid material from the blood and excreting it from the body.

Kidneys filter toxins and waste from a person’s blood. However, problemscanoccur:

- if the blood flow does not reach the kidneys properly
- if the kidneys are not working properly because of damage or disease
- if an obstruction prevents urine outflow.

CKD often happens as a result of either diabetes or hypertension.

When a person has uncontrolled diabetes, sugar (glucose) accumulates in the blood and can damage the kidneys.

High blood pressure, meanwhile, can damage the glomeruli. These are parts of the kidney that filter waste products.

Some other causes of CKD may include:

♦ **Obstructed urine flow :** Blocked urine can back up into the kidney from the bladder. Blocked urine flow increases pressure on the kidneys and undermines their function. Possible causes include an enlarged prostate, kidney stones, and a tumor.

♦ **Kidney diseases:** There are many different kidney diseases, including polycystic kidney disease, pyelonephritis, and glomerulonephritis.

♦ **Kidney artery stenosis:** This causes a narrowing or blockage of the renal artery before it enters the kidney.

♦ **Heavy metal poisoning:** Lead is a common source of poisoning.

♦ **Fetal developmental problems:** This can occur if the fetus' kidneys do not develop correctly in the womb.

♦ **Systemic lupus erythematosus:** This is an autoimmune condition wherein the body's immune system attacks the kidneys as though they were foreign tissue.

♦ **Malaria and yellow fever:** These two mosquito-borne diseases may cause impaired kidney function.

♦ **Certain medications:** The overuse of certain drugs, including NSAIDs, can lead to kidney failure.

♦ **Illegal substance use:** Using substances such as heroin or cocaine can damage the kidneys.

♦ **Kidney injury:** Sustaining a sharp blow or another physical injury to the kidneys can cause damage.**(Newman, 2023).**

I.7.Stages of chronic kidney disease

The five stages of CKD refer to how well your kidneys are working. Kidney disease can get worse in time. In the early stages (Stages 1–3), your kidneys are still able to filter waste out of your blood. In the later stages (Stages 4–5), your kidneys must work harder to filter your blood and may stop working altogether.

The goal at each stage of CKD is to take steps to slow down the damage to your kidneys and keep your kidneys working as long as possible.

Each stage is based on the eGFR number and has different symptoms and treatments.

Table 04: Stages of chronic kidney disease (American, 2023).

Stage of CKD	eGFR result	What it means
Stage 1	90 or higher	- Mild kidney damage - Kidneys work as well as normal
Stage 2	60-89	- Mild kidney damage - Kidneys still work well
Stage 3a	45-59	- Mild to moderate kidney damage - Kidneys don't work as well as they should
Stage 3b	30-44	- Moderate to severe damage - Kidneys don't work as well as they should
Stage 4	15-29	- Severe kidney damage - Kidneys are close to not working at all
Stage 5	less than 15	- Most severe kidney damage - Kidneys are very close to not working or have stopped working (failed)

- **Stage 1 of CKD**

Stage 1 CKD means you have a normal eGFR of 90 or greater and mild damage to your kidneys. Your kidneys are still working well, so you may not have any symptoms. You may have other signs of kidney damage, such as protein in your urine.

- **Stage 2 of CKD**

Stage 2 CKD means your eGFR has gone down to between 60 and 89, and you have mild damage to your kidneys. Most of the time, your kidneys are still working well, so you may not have any symptoms. You may have other signs of kidney damage, such as protein in your urine or physical damage.

Stage 3 of CKD

Stage 3 CKD means you have an eGFR between 30 and 59 and mild to moderate damage to your kidneys. Your kidneys do not work as well as they should to filter waste and extra fluid out of your blood. This waste can build up in your body and begin to cause other health problems, such as high blood pressure and bone disease. You may begin to have symptoms, such as feeling weak and tired or swelling in your hands or feet.

Stage 3 CKD is split into two substages based on your eGFR:

★ Stage 3a means you have an eFGR between 45 and 59

★ Stage 3b means you have an eGFR between 30 and 44

With treatment and healthy life changes, many people in Stage 3 do not move to Stage 4 or Stage 5.

Stage 4 of CKD

Stage 4 CKD means you have an eGFR between 15 and 29 and moderate to severe damage to your kidneys. Your kidneys do not work as well as they should to filter waste out of your blood. This waste can build up in your body and cause other health problems, such as high blood pressure, bone disease and heart disease. You will likely have symptoms such as swelling of your hands and feet and pain in your lower back.

This is the last stage before kidney failure. It is important to have regular visits with a nephrologist (kidney doctor) to take steps to slow kidney damage and plan ahead for possible treatments for kidney failure.

Stage 5 of CKD

Stage 5 CKD means you have an eGFR less than 15 and severe damage to your kidneys. Your kidneys are getting very close to failure or have already failed (stopped working). Because your kidneys have stopped working to filter waste out of your blood, waste products build up in your body, which can make you very sick and cause other health problems. When your kidneys fail, treatment options to survive include dialysis or a kidney transplant. **(American Kidney Fund , 2023)**

I.8.Prevention

To reduce your risk of developing kidney disease :

✓ **Follow instructions on over-the-counter medications:** When using nonprescription pain relievers, such as aspirin, ibuprofen (Advil, Motrin IB, others) and acetaminophen (Tylenol, others), follow the instructions on the package. Taking too many pain relievers for a long time could lead to kidney damage.

✓ **Maintain a healthy weight:** If you're at a healthy weight, maintain it by being physically active most days of the week. If you need to lose weight, talk with your doctor about strategies for healthy weight loss.

✓ **Don't smoke:** Cigarette smoking can damage your kidneys and make existing kidney damage worse. If you're a smoker, talk to your doctor about strategies for quitting. Support groups, counseling and medications can all help you to stop.

✓ **Manage your medical conditions with your doctor's help:** If you have diseases or conditions that increase your risk of kidney disease, work with your doctor to control them. Ask your doctor about tests to look for signs of kidney damage.**(Mayo Clinic, 2021)**

I.9.Treatment of kidney disease

The best treatment of kidney disease is facilitated by early detection, when the disease can be slowed or stopped. Early treatment includes diet, exercise, medications, lifestyle changes, and treating risk factors like diabetes and hypertension. However, once kidneys fail, treatment with dialysis or a kidney transplant is needed.

- Dialysis comes in two forms: hemodialysis (HD) or peritoneal dialysis (PD). Both forms remove wastes and extra fluid from your blood. Patients receive hemodialysis usually 3–4 times a week, either at home or at a dialysis center. During hemodialysis, your blood is pumped through a dialysis machine, where it is cleaned and returned to your body. With peritoneal dialysis, your blood is cleaned inside your body every day through the lining of your abdomen using a special fluid that is periodically changed. Peritoneal dialysis can be done at home, at work, at school, or even during travel. Home dialysis is an increasingly popular mode of treatment, and is associated with better outcomes.
- A kidney transplant places a healthy kidney into your body from a deceased donor or from a living donor, such as a close relative, spouse, friend, or generous stranger. A kidney

transplant, however, is a treatment, not a cure. Anti rejection and other medications are needed to maintain the transplant. Per the United States Renal Data System (USRDS), more than 22,000 (22,393) kidney transplants were performed in the United States in 2018. The active waiting list remains substantially larger than the supply of donor kidneys, which presents a continuing challenge.

Although it is very important for patients who are nearing the need for dialysis or kidney transplantation to be cared for by a nephrologist, in 2018, 38.8% of incident (newly occurring) KFRT patients (18–44 years) had received little or no pre-KFRT nephrology care.**(National Kidney Foundation., 2022)**.

SECOND PART

Experimental study

Chapter I

Material and Methods

The practical part of our work was carried out at the level of the "pedagogical laboratory of the faculty of natural and life sciences of the EchahidHammaLakhdar university- ElOued, For a period of 50 separate days, From the 6th of February to the 25th of February, and from the 20th of March to the 20th of April 2023.

The objectives of this study revolve around the following points: isolation, purification and identification of lactic acid bacteria from camel intestines, possessing probiotic properties and to evaluate this ability in vivo.

I. Materials

I.1. Biological material

I.1.1 Camel intestine

The sample used in this study was provided by the Royal Abattoir, of the wilaya of El-Oued, it is 100 g of the small intestine of male dromedary from the Targuie population.

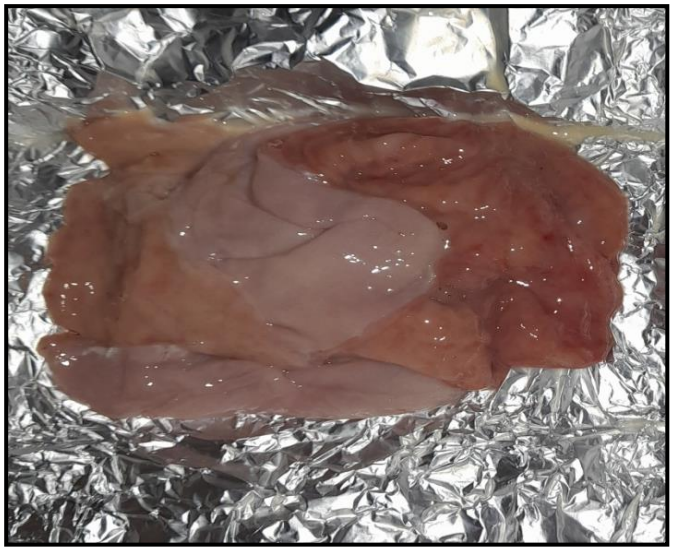

Figure 04 : Camel intestine (Original picture, 2023)

I.1.2 The rats

A total of 20 adult female albino rats, weighing 160-240g, were obtained from Pasteur Institute, Algeria. They were placed and kept in the animal house of the Molecular and Cellular Biology Department, University of El-Oued, Algeria. Animals were adapted for 10 days under the same laboratory conditions of photoperiod (12 h light/12 h dark) with relative humidity and room temperature, of Standard rat food and tap water were available ad libitum for the duration of the experiments **(Southon and Johnson , 1984).**

I.1.3. Micro-organisms

Pathogenic strains used in the detection of antibacterial activity from the Pasteur Institute. Table 5 shows the genus, species and code of these bacteria.

Table 05 : Genus, species and code used strains.

Genus and species	Code	Gram	Origin
Staphylococcus aureus	**ATCC 44300**	**+**	**Pasteur Institute**
Pseudomonas aeruginosa	**ATCC 9027**	**-**	**Pasteur Institute**
Escherichia coli	**ATCC 25922**	**-**	**Pasteur Institute**

I.2. Laboratory materials

- **Apparatus**

✓ Optical microscope of the OPTIKA type linked to a camera.
✓ SIGMA type horizontal centrifuge.
✓ Oven type (MEMMERT and LABTECHLTB-060M)
✓ Electric scale KERN EMB 2200-O type.
✓ Analytical balance type KERN ABJ/ABS.
✓ Hot plate magneticstirrers.
✓ Autoclave casserole.
✓ Refrigerator.
✓ The pH/MV meter.
✓ Haute UV.
✓ Vortex mixer .

Our study also requires the use of materials such as Pasteur pipettes, micropipettes, a sterile swab, 5 and 10 ml syringes, mortar and other glassware (bucher , Erlenmeyer flask, test tube, test tubes, graduated pipette...)

I.3. Chemical reagents and solvents

Chemicals and reagents		Utilization
Colorants	Gentian Violet	Gram coloration
	Fuchsin	
	Lugol	
Othersproducts	Oxygenated water H2O2 (10V)	For the realization of the catalase test
	NaOH (0.1N)	To adjustcrop ph
	Physiological water	For the preparation of dilutions and smears
	Distilled water	For the preparation of culture media and thus for rinsing during Gram staining
	Immersion oil	To increase the resolution of the microscope
	Ethanol	For discoloration during Gram staining
	NaCl	Test of different concentrations of NaCl

I.4. Culture mediums

- ✓ The M17 agar (M17 agar) **(Terzaghi and Sandine, 1975).**
- ✓ The M17 broth **(Terzaghi and Sandine, 1975)**
- ✓ La gélose MH **(Muller Hinton)(Mueller and Hinton , 1941)**

All culture media used in this study are sterilized at 120°C for 20 minutes.

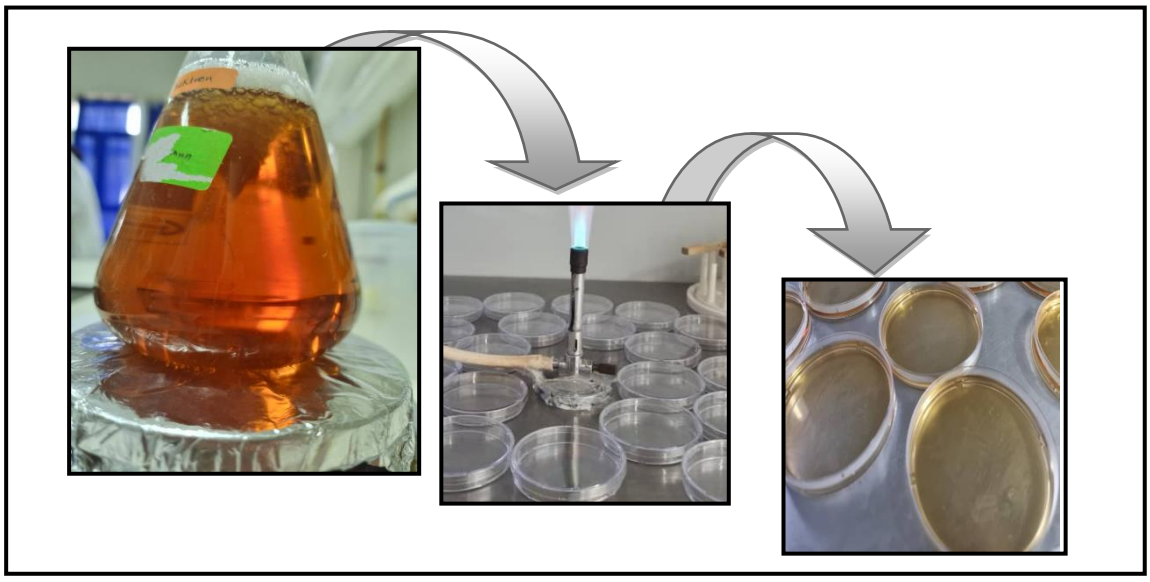

Figure 05: Preparation of culture medium (Original picture, 2023)

II.Methods

II.1. Isolation and purification of lactic acid bacteria

II.1.1.Isolation of strains

Lactic acid bacterial strains were isolated from camel intestines, after washing them thoroughly in tap water and then soaking them in saline water (NaCl)for 10 minutes, after which we ground them well to ensure that the bacteria were well obtained.weighing 1g of the sample and serially diluted with 10 ml sterile physiological water. After homogenization, 10^{-4} serially diluted samples were spread on M17 agar medium. The plates were incubated for 48 h at 37°C.for*pediococci* and *lactococci* and at 45°C/72h for *lactic streptococci***(Badis*and al.* 2005).**

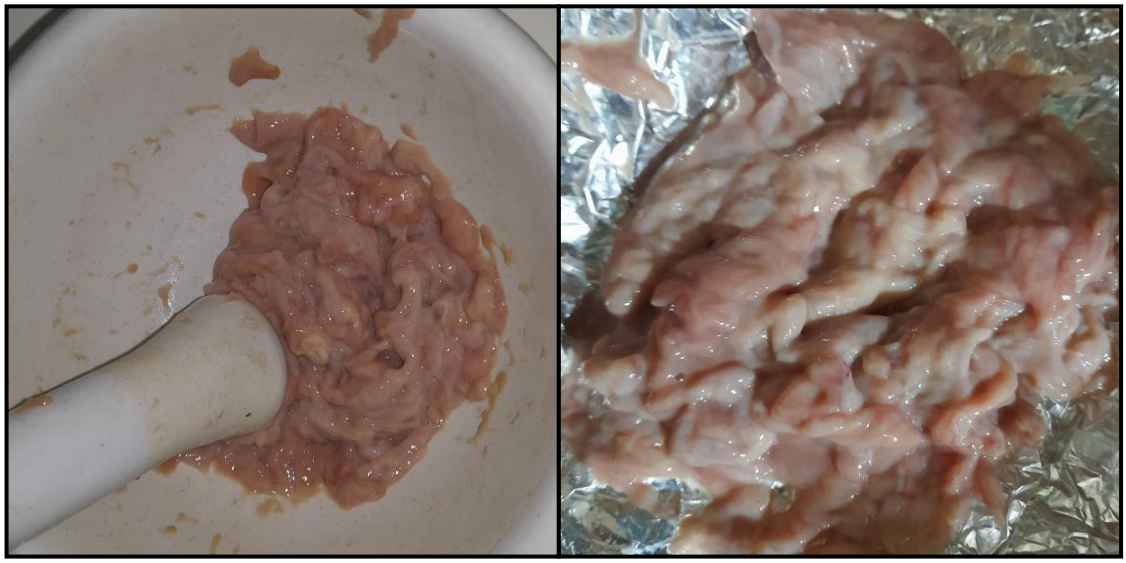

Figure 06: Grinding camel intestines (Original picture, 2023)

II.1.2. Purification of strains

Purification consists of carrying out successive subcultures on agar and broth 17, with incubation at 37°C for 24 hours, until colonies of the same size, shape and color are obtained, indicating the purity of the strains**(Idoui*and al*. 2009).**

In this present work, 5 characteristic colonies of lactic acid bacteria are chosen at random and transplanted into M17 broths. After incubation at 37°C for 48 hours, the colonies are taken up in M17 broth and then subcultured onto Petri dishes containing M17 agar previously poured and then incubated at 37°C for 48 hours.**(Bousebta*and al*., 2021)**

II.1.3.Conservation of strains

- **Short-termpreservation**

To ensure good continuity of work, the strains must be stored in adequate conditions.**(Lairini*et al*. 2014)**

The short-term conservation of pure strains is carried out on solid M17 medium. After growth at the optimum temperature, the cultures are maintained at 4°C and the strains are renewed by transplanting every 4 weeks **(Brahimi, 2015)**

- **Long-termpreservation**

The long-term storage of the strains is carried out on a medium enriched with 0.05% yeast extract and 20% glycerol at -20°C. If necessary, the strains are subcultured before use **(Belarbi, 2011 ;Chentouf, 2015).**

II.2. Pre-identification of isolates

The pre-identification of purified strains is established for lactic acid bacteria based on morphological and biochemical characters (shape, Gram staining, catalase, growth at different temperatures, sensitivity to Nacl) **(Lairini*et al*. 2014).**

II.2.1.Morphological study

❶ Macroscopicexamination

Consists of observing the cultural characteristics of bacteria isolated from cultures obtained on M17 agar by the naked eye. The colonies are differentiated by their appearance, shape, color, size and by their odor **(Bekhouche and Boulahrouf, 2005)**

❷ Microscopicexamination

Microscopic observation was carried out using an optical microscope at magnification (G×100). It consists of studying the Gram, the shape of the cells and the mode of regrouping by doing Gram staining **(Zantar*et al*. 2013)**

II.2.2.Biochemical and physiological characterization

The selected strains were subjected to physiological and biochemical characterization

according to the dichotomous keys proposed by**Guiraud, (2003) and Holzapfel and Wood, (2014)**

a. Catalase test

Catalase allows the transformation of hydrogen peroxide into oxygen and water according to the following reaction.

$2H_2O_2$ ——— Catalase ———> $2H_2O + O_2$

An isolated colony of the strain was placed on a clean glass slide then a drop of H_2O_2 was added, mixed and observed. The presence of gassing (O2) means that the strain is able to break down H_2O_2, hence Catalase (+), while the absence of gassing (O2) indicates that it is catalase (-) **(Ahirwar*et al.* 2017).**

b.Growth at different temperatures

This test is carried out for the differentiation between mesophilic lactic strains and thermophilic strains **(Bousebta*et al*. 2021).** After inoculation of the M17 broth with the pure cultures, the tubes are incubated at 10°C for 5 to 7 days, and at 40°C and 45°C for 24 to 48 hours for all the cultures**.** The turbidity of the medium indicates growth **(Bennani*et al.*2017).**

c. Growth at different concentrations of NaCl

Growth on salted media was carried out by inoculating the lactic isolates in M17 broth containing different concentrations of NaCl : 2%, 4% and 6% , then incubated at 37°C/ 24h to 48h. The turbidity of the medium reveals the growth **(Ammor*et al*. 2005).**

d. API 10 S Gallery

The API 10S gallery is a standardized system for the identification of bacteria according to biochemical characters.

It includes 10 microtubes containing dehydrated substrates. The microtubes are inoculated with a bacterial suspension which reconstitutes the tests. The reactions produced during the incubation period are reflected by spontaneous color changes or revealed by the addition of reagents.

These reactions are read using the reading table and identification is obtained by consulting the list of profiles using the identification software.

- **Preparation of the inoculums**

A single well-isolated colony on agar medium is removed using a pipette, young cells (18 to 24 hours) are preferentially used.

The bacterial suspension is carefully homogenized in the medium. It must beusedextemporaneously.

- **Gallery inoculation**

Using a pipette, 1 to 4 morphologically identical colonies are picked and suspended in physiological saline.

The test tubes (and not the cupules) are filled with the previous suspension to avoid the formation of air bubbles at the bottom of the tubes. The tip of the pipette is placed on the side of the well, tilting the box loaded with the bacterial suspension slightly forward.

The tubes and wells of the tests which bear a frame such as GLU were filled with the suspension and the wells of the underlined tests such as ADH and URE were also filled with the suspension on which a layer of paraffin oil was added (anaerobiosis).

The test tube incubation box should be filled with a little water to avoid desiccation during incubation at 35°C for 24 hours.

After incubation, the gallery will be read and the results compared to the reading table.

On the results sheet are noted all spontaneous reactions or revealed by the addition of reagents **(Boukhemisand Boutersa, 2015).**

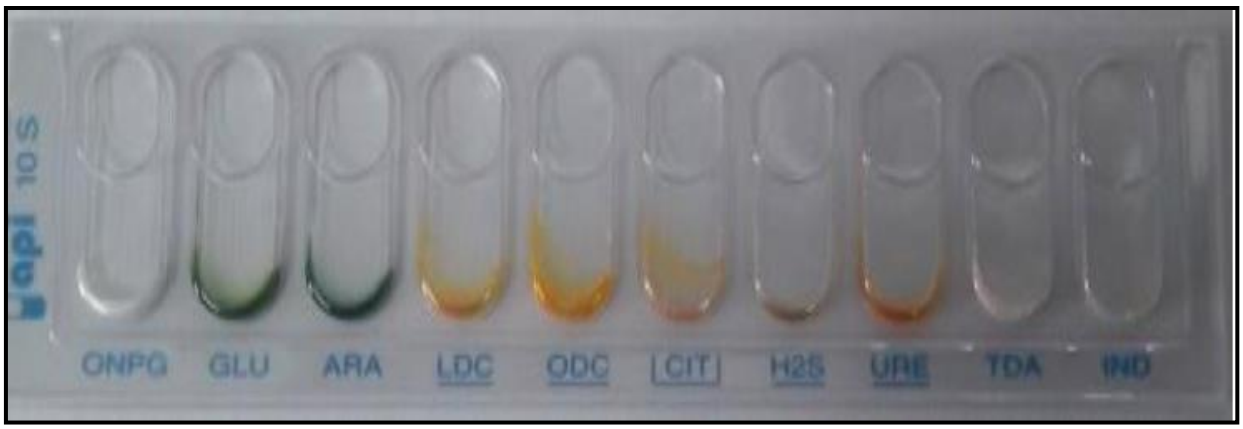

Figure 07 : Gallery API 10 S before use (Original picture, 2023)

II.3.Selection of the probiotic strains

II.3.1. Acidity tolerance

In order to evaluate the capacity of the strains to survive in an environment similar to that of the stomach (acid), we opted for the modified protocol described by**Riane*et al*. (2019)** with minor modifications. Before carrying out this test, a bacterial pellet recovery step was carried out by centrifugation at 2500 rpm/10min of the young culture, followed by two washes with PBS, the final bacterial pellet was suspended in a volume of 5 ml of M17 broth already adjusted to pH2, pH3 and pH4 An aliquot of each solution is taken immediately, at time (t: 0 min) and after 3 hours of incubation at 37°C. After exposure to different acid pH and at t=0 and t=3h.

II.3.2. Bile salt tolerance

To know the ability of the mixture of strains to grow in the presence of bile salts, we used the method described by **Riane*et al.* (2019)** with minor modifications. Before carrying out this test, a bacterial pellet recovery step was carried out by centrifugation at 2500 rpm/10min of the young culture, followed by two washes with PBS, the final bacterial pellet was suspended in a volume of 5 ml of M17 broth with 0.3%, 0.5% and 1% bile salts. Growth of bacterial cells was carried out under the same conditions as for acid stress at t=0 and t=3h.

II.3.3. Antibacterial activity

The principle of detection of antimicrobial activity is based on the diffusion of the antimicrobial agent in solid or semi-solid culture media to inhibit the growth of sensitive indicator microorganisms. Demonstration of antimicrobial activity is carried out on a few strains representing species of interest **(Guiraud, 1998).**

After obtaining the pure colonies, a colony was taken from each of the boxes tested and was poured into a tube containing 4 ml of the different media (M17 or MH). The tubes were incubated in an oven at 37°C for 24 hours for the MH medium and for 48 hours for the M17 medium. 10 min, and recovery of the upper part **(Aissous*and al,* 2021).**

The MH agar was poured into Petri dishes, after solidification, the indicator strains were spread by sterile cotton swab on the surface. After drying, drops of supernatant of the strains tested were deposited on the surface by the micropipette and were dried. Incubation was

carried out at 37°C for 24 h **(Basli*et al.* 2012).**The indicator bacteria (*Salmonella Typhi*, *Escherichia coli* and *Staphylococcus aureus*)**.**

II.3.4. Antibiotic sensitivity

An antibiogram is a laboratory technique aimed at testing the sensitivity of a bacterium to one or more antibiotics. The principle consists of placing the culture of bacteria in the presence of antibiotics and observing the consequences on its development and survival.

There are three types of interpretation depending on the diameter of the circle surrounding the antibiotic disc: sensitive, intermediate or resistant strain. Mueller Hinton agar is a basic medium which allows the realization of the standard antibiogram. It is poured into Petri dishes. The surface of the agar is dried for 15 minutes at 37°C.

The inoculum is prepared using 3 to 5 colonies isolated and picked then placed in a tube containing nutrient broth. The latter is steamed for 30 min then a drop of inoculum is homogenized in a tube containing physiological saline.

Sowingisdone:

- by flooding: flooding is done with 5 ml of the suspension on Mueller Hinton agar, left in contact for 30 seconds then left to dry for 15 minutes at 37°C.
- by swabbing (Kirby method): the medium is inoculated in very tight streaks in 3 passages by rotating 60°.

The antibiotic discs are placed on the agar with sterile metal forceps. The boxes are incubated for 24 hours at 37°C. The reading must be done within the recommended time limits: 18 to 24 hours for the diffusion method for fast growing bacteria and 2 to 3 days for difficult growing species. The circular zone of inhibition is measured by the diameter in millimeters according to various means (ruler, compass or caliper) **(Boukhemis and Boutersa, 2015).**

II.4. In vivo evaluation of probiotic properties

II.4.1. Induction of renal insufficiency

After the 10-day adaptation phase, the mice were randomly divided into 4 groups, the first was a control group, and the second, third and fourth were poisoned with the analytical reagent cadmium chloride at a dose of 5 mg/kg/day, for one month. He received a normal diet

during the poisoning. After this step, the rats of the third and fourth groups were treated with a dose of 0.5 ml of probiotic lactic acid bacteria diluted with a quantity of physiological water (5 ml), the diet of the three target groups was modified in fasting them for 16 hours each day before gastrulation .force-feeding for a week.

➢ **Infection Protocol**

The adult rats were randomly divided into four groups, each containing 5 rats as follows:

- **Group I :**it acted as a witness to the experiment as he was treated with pure water.
- **Group II :** Mix a dose of 5 mg/kg/day of analytical reagent cadmium chloride hemi-pentahydrate in drinking water daily for one month.
- **Group III:** During the first month, she was treated with the analytical reagent cadmium chloride hemi-penta-hydrate (5 mg/kg/day) and then treated with a dose of 0.5 ml of the first selected probiotic lactic acid bacteria, diluted with a quantity of physiological acid water (5 ml), for one week.
- **Group IV:**For the first month, she was treated with the analytical reagent cadmium chloride hemipentahydrate (5 mg/kg/day) then treated with a dose of 0.5 ml of the second selected probiotic lactic acid bacteria, diluted with a quantity of physiological water (5 ml), for one week.

The body weight of the rats was recorded before and after the experiment.

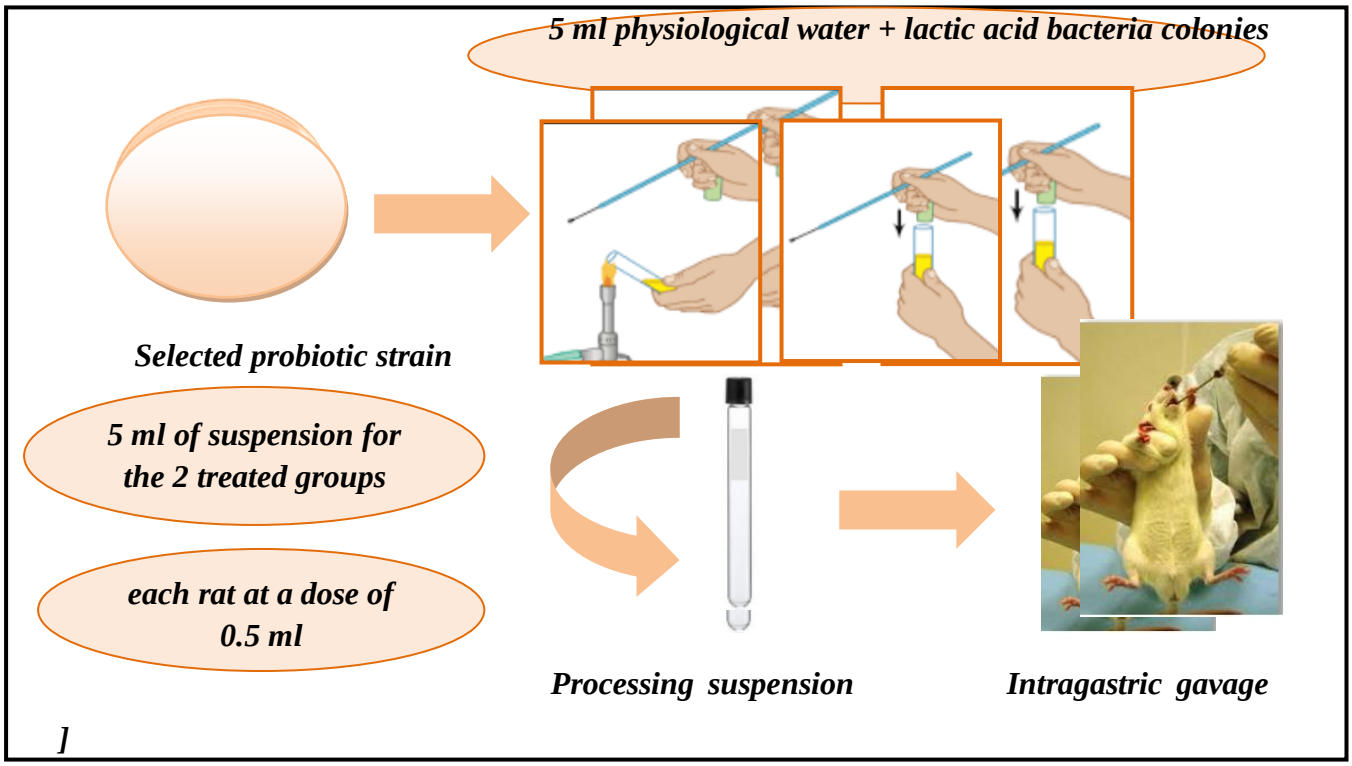

Figure 08: Rat treatment protocol with the selected probiotic strains

At the end of the experiment, the mice were slaughtered after fasting for 16 hours and, under light chloroform anesthesia, we obtained blood, kidney tissue for medical analysis and histological sections for microscopic observation.

II.4.2. Blood sampling

Blood was collected in heparinized tubes and centrifuged for 15 minutes at 3,500 rpm to separate plasma from constituents. Plasma samples are stored at -20°C for later determination of plasma levels for certain biochemical and hematological analyses.

Blood analysis was performed Parameters included: white blood cell (WBC) count, lymphocyte and granulocyte counts, and some biochemical parameters: urea et creatinine.

II.4.3. Renal histology

After the sacrifice of the rats, the part of the kidney tissues was removed and immersed infixative (10% formaldehyde solution)until the time of preparation of slices, Histological sections of rat kidneys were made in the faculty laboratory.

II.4.3.1. Steps in making histological sections

❶ Sampling

After the rats were slaughtered and dissected, part of the kidneys were removed with great care and stored in a box containing diluted formalin.

❷ fixation and conservation

The fixation is a primordial and essential step in order to avoid damage, necrosis and infection of the sample, it consists in coagulating the proteins of the sample and making it hard suitable for microtome cutting.

The fixation is carried out immediately after taking the sample to be observed by immersing the biological material in diluted formalin**(Hambli and Djabrohou,2022)**

❸ Dehydration

The purpose of this step is to eliminate the intracellular water, by passing the samples through alcohol baths of increasing concentrations (from diluted alcohol at 50° to absolute alcohol at 100°). This step prepares the embedding, since paraffin is hydrophobic **(Babaali, and Ben Teghri , 2022).**

❹ Inclusion

The embedding medium used is paraffin (opaque white resin). The sample is bathed in molten paraffin (heated at 56°C for 4 hours in an oven) which then infiltrates all the cells. Its purpose is to allow the production of thin sections (with a thickness of 2 to 5 μm) **(Babaali, and Ben Teghri , 2022).**

❺ Confection of histological sections

The passage of paraffin blocks in a microtome makes it possible to produce slices of section (sections) of 2 to 5 μm in thickness arranged in series, the latter are placed in a water bath to facilitate their bonding on slides of glass that we label and place in metal slide holders.**(Taleb and Tibhirt ,2021)**

❻ Coloration

Staining consists of placing the slide rack in different reagents for specific periods. The most commonly used is hematoxylin (or hematein), which stains the nuclei blue, followed by eosin, which stains the cytoplasm pink (hematein-eosin or hematin-eosin H.E). This routine staining then makes it easier to observe by analyzing the architecture of cells, their nucleus, their cytoplasm and extracellular constituents. Sections will only be ready to receive stains after the following two steps: depara-ffinization and hydration. This step is performed automatically in a staining automaton.**(Bensbia,2022).**

❼ Mounting and Microscopic Observation

Its purpose is to produce a histological slide ready to be observed under an optical microscope, for this we put a few drops of synthetic resin on the coverslip which must be carefully mounted on the colored slide in order to avoid air bubbles and the crushing of the cut and at the end the blades were left to dry. Then we moved on to observation under an optical microscope.**(Taleb and Tibhirt ,2021)**

III. Statistical analysis

The experimental results are expressed as mean ± standard deviation of triplicate measurements. Results were evaluated using EXCEL 2010 software differences are considered significant when $p<0.05$**.**

Chapter II

Results and discussion

I. Isolation and identification of lactic acid bacteria

After having had a problem of contamination of our dish previously inoculated by the dilutions, we succeeded in isolating five lactic strains from camel intestines. Three out of five isolated colonies were obtained from dishes incubated at 37°C with dilutions 10^{-3} and 10^{-2} and stock solution, two from plates incubated at 45°C with 10^{-1} dilutions and stock solution.

After counting the colonies, we obtained 34 colonies at 10^{-3} dilution, 66 coloniesat 10^{-2} and 114 isolates at the stock solution. From boxes incubated at 30°C we obtained 81 coloniesat 10^{-2} dilution and 106 coloniesat the stock solution. From boxes incubated at 45°C.

The number of lactic acid bacteria obtained is lower than expected, some authors admit through their work that compared with products of animal origin, plants generally contain a low number of lactic acid bacteria **(Nguyen-The and Carlin, 1994)**

The identification of lactic isolates by using tests relating to cultural, morphological (macroscopic and microscopic), physiological and biochemical characteristics, the preliminary tests showed that the isolates possessed the characteristics of lactic acid bacteria.

I.1.Purification of strains

The colonies were purified, separating similar colonies in color, shape and size from the five colonies obtained, were subcultured onto M17 Agar and incubated at 30ºC for 24 hours. for further testing.

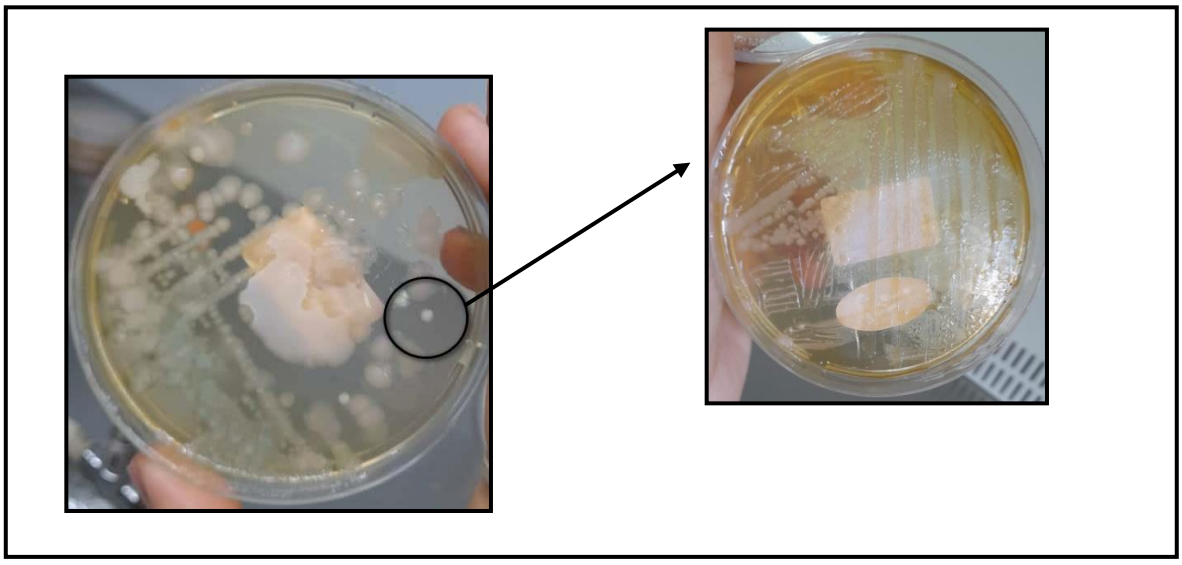

Figure 09: Purification of isolated strains.

I.2.Pre-identification of isolates

I.2.1.Macroscopic examination

Macroscopic observation of the colonies of isolated lactic strains revealed to us the color, size and morphology of these colonies. The resultswere as follows:

- **Strain 01:** Circular, medium-sized, rounded, smooth colonies, white in color with a regular outline.
- **strain 02:** Small smooth rounded colonies of gray color with distinct outline.
- **strain 03:** Large, rounded colonies of transparent yellow color, smooth, with a distinct outline.
- **strain 04:** Small rounded smooth colonies of white color with a regular outline.
- **strain 05:**Circular, medium-sized, domed, smooth, yellowish colored colonies with a distinct outline.

We obtained strains S1, S2 and S3 from dishes incubated at 37°C and strains S4 and S5 from dishes incubated at 45°C. This difference explains why in a solid medium, observation reveals many morphological aspects of the colonies, unlike in a liquid medium, the heterogeneity is weaker **(Tahlaiti , 2019).**

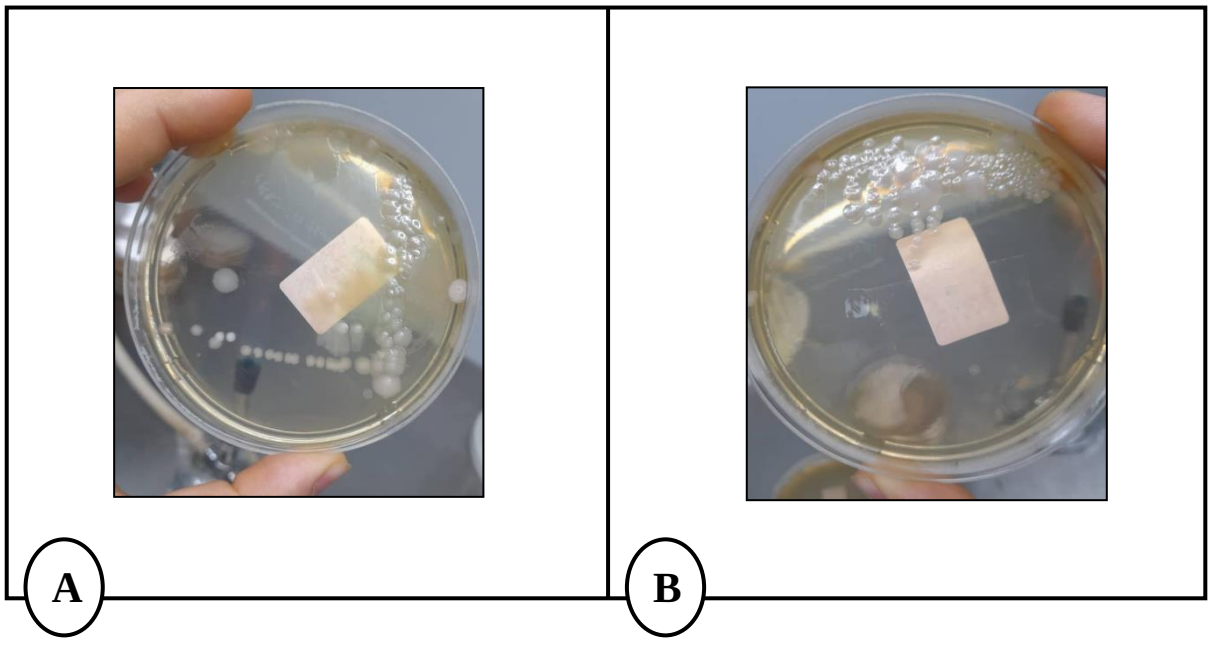

Figure 10:Macroscopic appearance of colonies of isolated strains(A =incubated at 37°C /B =incubated at 45°C)(Original picture, 2023)

I.1.2.Microscopic examination

After Gram staining, microscopic observation showed the presence of Gram-positive strains, in the form of diplococci, bacilli and cocci, the majority of the strains studied were diplococcic.

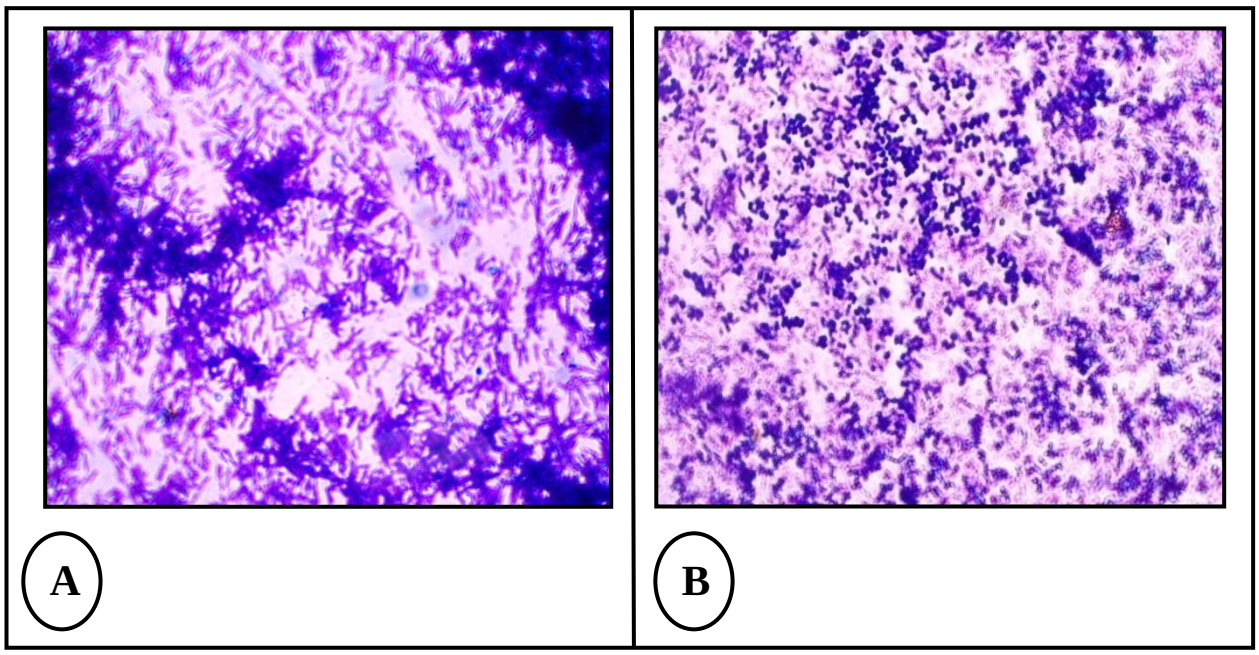

Figure11 :Microscopic appearance of cells after Gram staining (×100). (A Bacillus/ B Cocci) (Original picture, 2023)

Table 06 : Results of pre-identification of isolates.

Strain	Macromorphology (Appearance of colonies)	Micromorphology (Forms of bacteria)	gram stain
S_1	• circular, medium-sized, rounded. • smooth colonies • white	cocci	+
S_2	• smallrounded • smooth colonies • gray	diplococci	+
S_3	• large, rounded • smoothcolonies • transparent yellow	diplococci	+
	• smallrounded • smooth colonies	diplococci	+

S_4	• white		
S_5	• circular, medium-sized, domed. • Smooth colonies • Yellowish	bacilli	+

+ *:positive*

I.3.Biochemical and physiological tests

In this part, the approach used for the identification of our isolates is essentially based on phenotypic characterization. The latter is based on morphological, biochemical and physiological criteria. Results are shown in the following table.

Table07: Physiological and biochemical criteria of lactic acid bacteria strains.

Strain		**S_1**	**S_2**	**S_3**	**S_4**	**S_5**
Temperature	**10 °C**	**-**	**-**	**-**	**-**	**-**
	45 °C	**+**	**+**	**+**	**+**	**+**
NACL	**2 %**	**+**	**+**	**+**	**+**	**+**
	4 %	**+**	**+**	**+**	**+**	**+**
	6 %	**+**	**+**	**+**	**+**	**+**
Test catalase		**+**	**+**	**+**	**+**	**+**
ONPG		**Yellow (+)**	**Yellow (+)**	**Yellow (+)**	**Yellow (+)**	**Yellow (+)**
GLU		**Yellow (+)**	**Yellow (+)**	**Yellow (+)**	**Yellow (+)**	**Yellow (+)**
ARA		**Yellow (+)**	**Yellow (+)**	**Yellow (+)**	**Yellow (+)**	**Yellow (+)**
LDC		**Red (+)**	**Orange(+)**	**Orange(+)**	**Orange(+)**	**Yellow (-)**

ODC	**Yellow (-)**	**Yellow (-)**	**Orange(+)**	**Orange(+)**	**Yellow (-)**
CIT	**Yellow (-)**	**Yellow (-)**	**Yellow (-)**	**Yellow (-)**	**Yellow (-)**
H2S	**Noir (+)**	**Noir (+)**	**Noir (+)**	**Noir (+)**	**Noir (+)**
URE	**Yellow (-)**	**Yellow (-)**	**Yellow (-)**	**Yellow (-)**	**Red (+)**

+ : positive / - :negative

- **Test catalase**

The presence of air bubbles after the addition of hydrogen peroxide indicates that these bacteria are catalase positive. Analysis of our results showed that all isolates are Gram-positive and catalase-positive, although most catalase results obtained in previous lactic acid bacteria studies were catalase-negative**(Saaddjaballah and Medkour (2015)**.

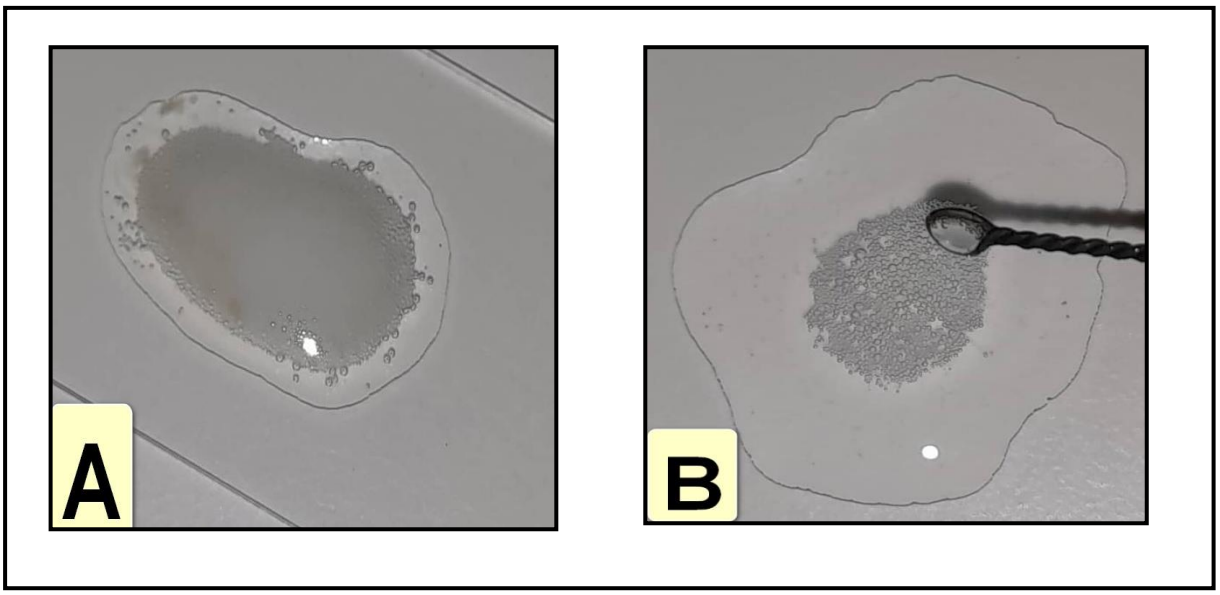

Figure 12: Positive catalase test result for the selected lactic strain (Original picture, 2023)

- **Growth at differentTemperatures**

This test identifies thermophilic and mesophilic bacteria. The table shows that theisolates (S1, S2, S3, S4, S5) have able to grow at 45°C but not at 10°C, This explains it as thermophilic.

Growth at different concentrations of NACL

Thefollowing table shows that all isolates are resistant to concentrations of 2, 4 and 6% NaCl, with strain growth at all three concentrations being very good.

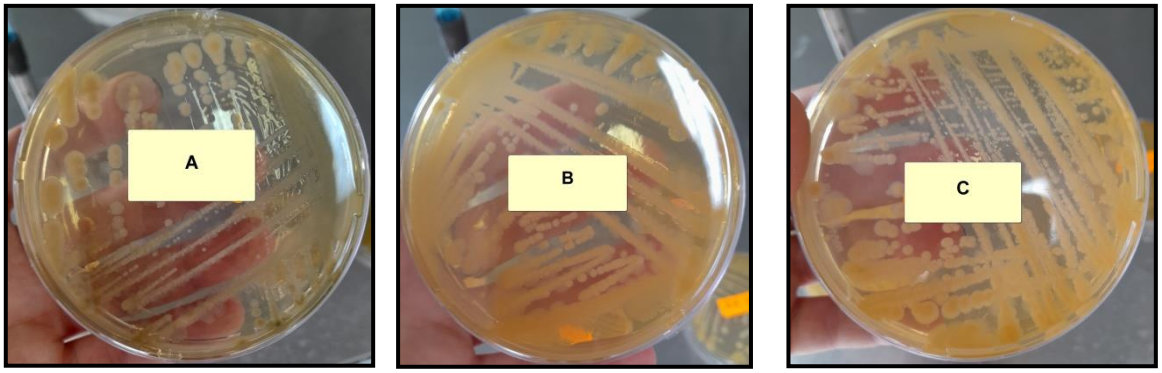

Figure13 : Growth of the strain at different concentrations of NaCl (A →2% B →4%C6%)(original picture, 2023)

API 10 STest (analytical profile index)

Through the table of results, we conclude that most of the tests were positive, unlike the negative results, which were few and mainly in the CIT, URE and ODC test.

The results of this test were determined by the color change of each tube (as shown in the document).TDA and IND tests have not been performed, but most studies related to lactic strains are positive.

Figure 14:API S10 test results(original picture, 2023)

The table shows that the isolates (S_1, S_2, S_3, S_4) belong to the genus Pedio-coccus. they form round colonies which under the optical microscope reveal a tetrad shape. These homo-fermentative isolates are able to grow at 45°C but not at 10°C and grow at the different concentration of NaCl**(Adjoudj*etal.*2020 ;Carr*et al.* 2002).**

Isolate (S_5) are Gram + bacilli. These isolates are attachedto the genus lactobacillus based on Gram stain. These strains are able to grow at 45°C and show growth at 45°C and show positive growth at 2% NaCl, and negative growth at 10°C **(Ghazi *and al.*, 2006).**

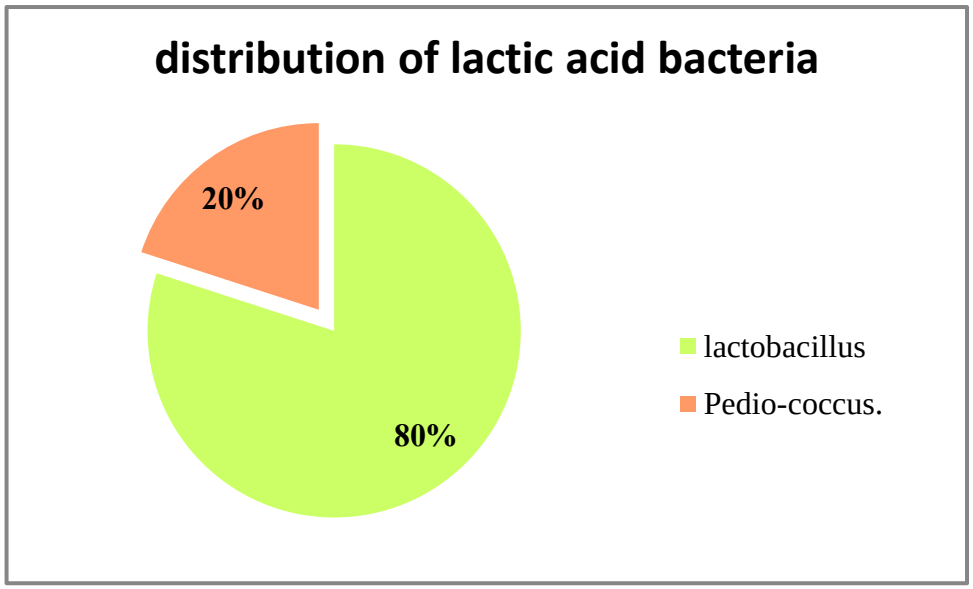

Figure 15: Distribution of genus of lactic acid bacteria.

Based on morphological, microscopic and biochemical criteria; our isolates can probably regress to the following strains:

☒ S1, S2, S3, S4: ***Pediococcusacidilactici***

☒ S5:***Lactobacillus acidophilus***

II. Selection of strains with probiotic properties

II.1.Resistance to different degrees of pH

Resistance to stomach acidity is one of the main selection criteria for probiotic strains. The pH of the human stomach varies from pH 1 to pH4.5, the digestion of food can take up to 3 hours. A low pH tolerance test is essential to predict the survival of probiotic strains in the stomach environment **(Kusharyati*et al.* 2020).**

Lactic acid bacteria isolates S_2 and S_5 were tested for their ability to tolerate acidic conditions in M17 broth with pH settings of 2, 3 and 4 for 3 hours. The results obtained are shown in the table :

Table07: Effect of acid pH on the viability of lactic strains (log CFU/ml)

	PH=02		**PH = 03**		**PH = 04**	
	0h	**3h**	**0h**	**3h**	**0h**	**3h**
Pediococcusacidilactici	4.76±3.98	4.68±3.86	4.71±4.04	4.80±4.07	4.55±3.70	4.94±4.09
Lactobacillus acidophilus	4.74±3.79	4.60±3.75	4.83±3.86	4.61±3.70	4.83±3.95	4.85±4.01

At pH 2, pH 3 and pH 4, the two strains show fairly similar growth at T=0 and during the 3 h incubation period.According to the results obtained, it can be seen that the two strains have a high viability in the presence of pH 4, this is consistent with the work of **(Muller*et al.* 2009);(Azat*et al.* 2016).** In addition, *Lactobacillus acidophilus*has the highest number of viable cells at 0 h, unlike *Pediococcusacidilactici*,which recorded the highest growth number at pH3, And this also has an effect on its viability.

At pH 2, the two strains showed a large proportion of viable cells at T0 but decreased after incubation, on the contrary at pH 4 the growth increased and reached the highest value. The results obtained are consistent with those of(**Linh*et al*.2017)** who tested the tolerance of certain probiotic strains to artificial intestinal gastric juices; they found a decrease with pH2 compared to pH4.

Most bacteria grow more slowly at low pH levels because the presence of acids damages them and reduces their viability. However, lactic acid bacteria have the ability to regulate their cytoplasmic or intracellular pH around neutral pH, even when cultured or maintained at low extracellular pH **(Jannah *et al.* 2018).**

II.2.Resistance to bile salts

Bile salt tolerance is an essential factor because it determines the survival of probiotics in the duodenum. Bile salts present in the intestinal tract disrupt the cell membrane of bacteria, however, probiotics have the ability to tolerate 0.05-0.3% bile **(Hassan *et al.* 2020).**

Bile salts are surfactant chemicals produced in the liver for cholesterol metabolism. Bile salts damage the bacterial cell membrane leading to cell death. For this reason, it is important for the probiotic strain to tolerate bile salts to pass the intestinal tract (**Bindu and Lakshmidevi, 2020).**The results of the tolerance of strains S_2 and S_5 in the presence of 0.1%, 0.3% and 0.5% bile salts are illustrated in the following table:

Table08 : Effect of different bile salt concentrations on the viability of lactic strains (log CFU/ml)

	0.1%		**0.3%**		**0.5%**	
	0h	**3h**	**0h**	**3h**	**0h**	**3h**
Pediococcusacidilactici	2.73	2.48	2.89	2.59	2.58	2.72
Lactobacillus acidophilus	2.46	2.54	2.84	2.70	2.57	2.43

In our study, the concentration of 0.3% bile salts was chosen because it is considered the physiologically appropriate concentration of animal bile in many studies **(Reuben *et al.* 2019).**The physiological bile concentration in humans is between 0.1 and 0.5% **(Denohue, 2004).**At 0.1%, 0.3%, 0.5% bile salts, both strains show positive growth and converge at T=0 and during the 3 hour incubation period.

According to the results obtained, it can be seen that the two strains have a high viability in the presence of all the concentrations tested in bile salts, in particular at 0.5% for strain 2 and 0.1% for strain 5 with an increase in the number of bacterial cell growth even

after 3 hours of incubation. Moreover, our results are close to those obtained by**Oyewole*et al.*(2018)**, unlike the studyby**Jannah*et al*. (2014)**, they found that lactic acid bacteria isolated from the gastrointestinal tract unable to survive 0.3% bile salts, but were able to survive 0.1% bile salts.

According to**Gowri and Ghost (2010),** the normal level of bile salts in the intestine is around 0.3%. At this concentration, all the strains tested proved to be tolerant to bile salts. However, our study proved that increasing or decreasing the bile concentration, we get an approximate cell growth rate at a 0.3% of the bile concentration.

This resistance to bile salts is due to the fact that bile plays a major role in the emulsification of lipids and thus facilitates their digestion. It is also able to affect the cellular integrity of bacteria by acting on their plasma membrane **(Begley *et al.* 2005).**

According to **Daoudou*et al.*(2011),** bile resistance in some strains is associated with the specific enzyme activity of bile hydrolase (BSH), which helps hydrolyze conjugated bile, thereby reducing its toxic effects. BSH activity is most commonly found in microorganisms isolated from the gut or feces of animals.

II.3.Antibacterial activity

Strains isolated from camel intestine were tested for their ability to inhibit pathogenic bacteria, Gram-negative *Salmonella.sp, E.coli*, and Gram-positive such as *S.aureus*.

For the detection of lactic strains producing antimicrobial protein substances. This method makes it possible to bring the supernatant of the isolates into contact with the indicator pathogenic strains. Inhibitory activity results in the appearance of zones of inhibition around the wells **(Tahlaiti, 2019).**

The results recorded showed a total absence of zones of inhibition around the wells despite numerous repetitions. This lack of inhibition may be caused by poor bacterial growth. These results agree with those of **(Ammor*et al.* 2005)**, or that the supernatant is not too concentrated to allow visualization of inhibition **(Dalache, 2006).**

According to **(Todorov*et al.*2011),** the antagonistic activity against pathogenic bacteria is due to the action of low pH or to a synergistic action between the antibacterial substances synthesized by the strains of lactic acid bacteria, and a variation of 0.5 in the pH can cause a significant decrease in the activity of bacteriocins.

II.4.Antibiotic sensitivity

Antibiotic activity is one of the important selection criteria for probiotics, and for effective probiotics, strains must have resistance that promotes their survival in the gastrointestinal tract of animals **(Oyewole*et al*. 2018; Tsega*et al*. 2019).**

Antibiotics are usually added to bird feed (drinking water or food) in sub-therapeutic doses as disease control treatments as well as for their growth promoting (growth factor) effects. This routine often exposes the natural gut microflora to traces of antibiotics that accumulate over time and confer antibiotic resistance to lactic acid bacteria **(Oyewole*et al.* 2018).**

In our study, we investigated the susceptibility of our isolates to antibiotics (Oxacillin, Amoxicillin, Gentamicin, Ofloxacin and Penicillin G) using the disk diffusion method on M17 agar. The results of this test were good, are mentioned in thefollowing table :

Table09: Antibiogram of selected lactic strains (cm).

	Oxacilline (1µg)	**Amoxicillin (10µg)**	**Gentamicin (10µg)**	**Ofloxacin (5µg)**	**Penicillin G (10µg)**
Pediococcusacidilactici	R	R	S(0.9cm).	S (2.2cm).	R
Lactobacillus acidophilus	R	R	R	S (1.2cm).	R

***R**:résistance**S :** sensible*

Examination of the antibiogram shows that *Pediococcusacidilactici*is resistant to antibiotics (Oxacillin, Amoxicillin, and Penicillin G) but is sensitive to (Gentamicin and Ofloxacin).

*Lactobacillus acidophilus*is sensitive to one out of five antibiotics tested. The resistance of *Lactobacillus* to antibiotics has been found to be mainly intrinsic and carried by chromosomal genes that are not transferable between bacteria **(Ammor*et al.* 2008; Casodo*etal* . 2014).**

Antibiotic resistance can be divided into two types: intrinsic and acquired. Intrinsic antibiotic resistance is native (natural), encoded in the chromosomes and cannot be transmitted to other species. Certain components of the cell membrane make bacteria resistant to antibiotics. For the second type (acquired resistance), it is plasmid-encoded and transferable between species, hence the emergence and transfer of natural gut microbiota genes **(Davidson *et al.*2019).**

Intrinsic resistance to certain antibiotics is an advantage for probiotics, where the cell walls of bacteria become more rigid so that they are not damaged by antibiotics. However, the acquired resistance of lactic acid bacteria to antibiotics is not preferred because genes can be transferred to pathogenic bacteria by conjugation to make them resistant, but it can have advantages or good effects in the case of acquired resistance. to probiotics **(*Daoudou et al.* 2011; Davidson *et al.* 2019).**

III.Study of the in vivo evaluation of probiotic properties

III.1.Body weight and organs

The average weight of the rats used, at the beginning to the end of the experiment varied as shown in the following table

Table 10: Body weight of rats before and after carrying out the experimental protocol

Groups	Group I	Group II	Group III	Group IV
Body weight (Before)	176.75± 24.25	205.5±7.5	197 ±25	194 ±11.5
Body Weight (After)	202.75 ± 20.375	185.25 ± 12.625	191 ± 13	202 ± 7

In general, despite the difference in the number of rats before and after the experiment, the weights were recorded and the difference determined.

Where we noticed a significant increase both in the control group and in the two groups that received treatment, unlike the affected group, we recorded a decrease in the weight of the compared rats, all rats were fed with a regular diet. The food was renewed every day, and the drink every 2/3 days, so that the weight loss of the rats in the infected group can only be interpreted as proof of infection.

Table 11: Organ (kidneys) weights of rats after completion of the experimental protocol

Group	Group I	Group II	Group III	Group IV
Organ weight	1.1385 ± 0.1495	1.40375 ± 0.280125	1.07675 ± 0.05475	1.195 ± 0.098

After making a comparison between the groups in the weight of the limbs, we noticed that the size of the kidney was similar in all the groups, except for the affected group, whose weight was greater, Kidney size is also an important parameter for clinical evaluation of kidney diseases **(Jovanović*et al.* 2020).**

III.2.hematological markers

Table 12: hematological markers of different groups of rats

	Groups of rats			
	Group I	**Group II**	**Group III**	**Group IV**
WBC count ($x10^9$ /L)	8.725± 1.5375	10.725± 3.075*	9.525±0.1875[b]	7.8±1.15
Lymphocytes ($x10^9$ /L)	5.5625 ± 0.76875	7.2 ± 2.1*	5.425 ± 0.1875 [b]	4.65 ± 0.75
Granulocyte ($x10^9$ /L)	2.025 ± 0.1375	2.25 ± 0.65**	3.05 ± 0.1	2.45 ± 0.25[c]
RCB ($x10^{12}$ /L)	6.47 ± 0.11	6.99 ± 0.16***	7.005 ± 0.11	6.6 ± 0.8
HGB (g/L)	139 ± 3.5	147 ± 7*	146.25 ± 0.875	133.5 ± 12.5[b]
HCT	0.35275 ± 0.005625	0.3845 ± 0.011**	0.37725 ± 0.00475	0.361 ± 0.044[b]
MCV (fL)	54.225 ± 0.0875	55.025 ± 1.5625*	53.3 ± 0.4 [b]	54.35 ± 0.35
MCH (pg)	21.55 ± 0.175	21.075 ± 0.9375**	21 ± 0.25	20.15 ± 0.45[c]
MCHC (g/L)	388.75 ± 12.375	382.5 ± 6.5**	386.25 ± 2.75[a]	370.5 ± 10.5
PLT ($x10^9$ /L)	801 ± 313	731 ± 229.5*	971.5 ± 47.5 [a]	888.5 ± 42.5[a]

:p<0.05, **:p<0.01, ***:p<0.001: significantly different from control group.

a: p<0.05, b: p<0.01,c: p<0.001: significantly different from (probiotics) group.

The results obtained by analyzing the hematological parameters of the rats of the group treated with 5 mg/kg body weight/day with hemi-penta-hydrate cadmium chloride analyte(the second group) compared to the control rats (the first group): there are significant differences ($P < 0, 05$) in the values of total WBC/lymphocyte/HGB/MCV/PLT . And it showed a significant difference ($P < 0.01$) in the proportion of Granulocyte /HCT/MCH/MCHC, as for the group of rats treated with probiotics (Groups III and IV), the results showed normal levels in the analysis of some blood parameters and others with significant differences ($P < 0.001$) compared to control mice.

Regarding blood markers, there is a significant ($p < 0.05$) increase in leukocytes and lymphocytes/HGB/MCV/ and an increase ($p < 0.01$) in granulocytes/HCT/MCH in both, followed by a significant ($p < 0.01$) decrease in MCHC in .with a decrease ($p < 0.05$) in PLT, in the placebo group compared to the control group. This indicates the detrimental effect of cadmium chloride hemipentahydrate, as we explain the deficiency of immune cells by its immunosuppressive effect**(Sharma Jaballi et al.2014).**

We have noticed an increase in the respiratory rate in rats treated with cadmium chloride hemipentahydrate, and this may indicate that the organs need oxygen despite the high percentage of hemoglobin and red blood cells, given that hemoglobin is responsible for transporting oxygen**(Krantz and Jacobson.1970).**

We also note that there is a significant decrease ($p < 0.01$) in the number of white blood cells and immune cells in the groups treated with probiotics (the Group III), and a significant convergence in the values of (the group IV) compared to the control group, Probiotics they stimulate the host's immune system and enhance the host's immune response, thus helping the host to resist various diseases, which is extremely important for the improvement of human health**(Ezendamandvan Loveren.2006),** probiotics have proven that it is possible to enhance both innate and adaptive arms of the host immune system**(Artis,2008).**

We also noticed a significant increase ($p < 0.01$) In MCV, a significant increase ($P < 0.05$) in PLT, and a significant decrease ($P < 0.05$) in MCHC, and there was no difference in the rest of the values, in the third group compared to the control group.

For the fourth group, most of the values are similar compared to the control group, only a significant increase ($p < 0.01$) in MCH/MCV and significantly ($P < 0.05$) in PLT.

III.3.Renal check

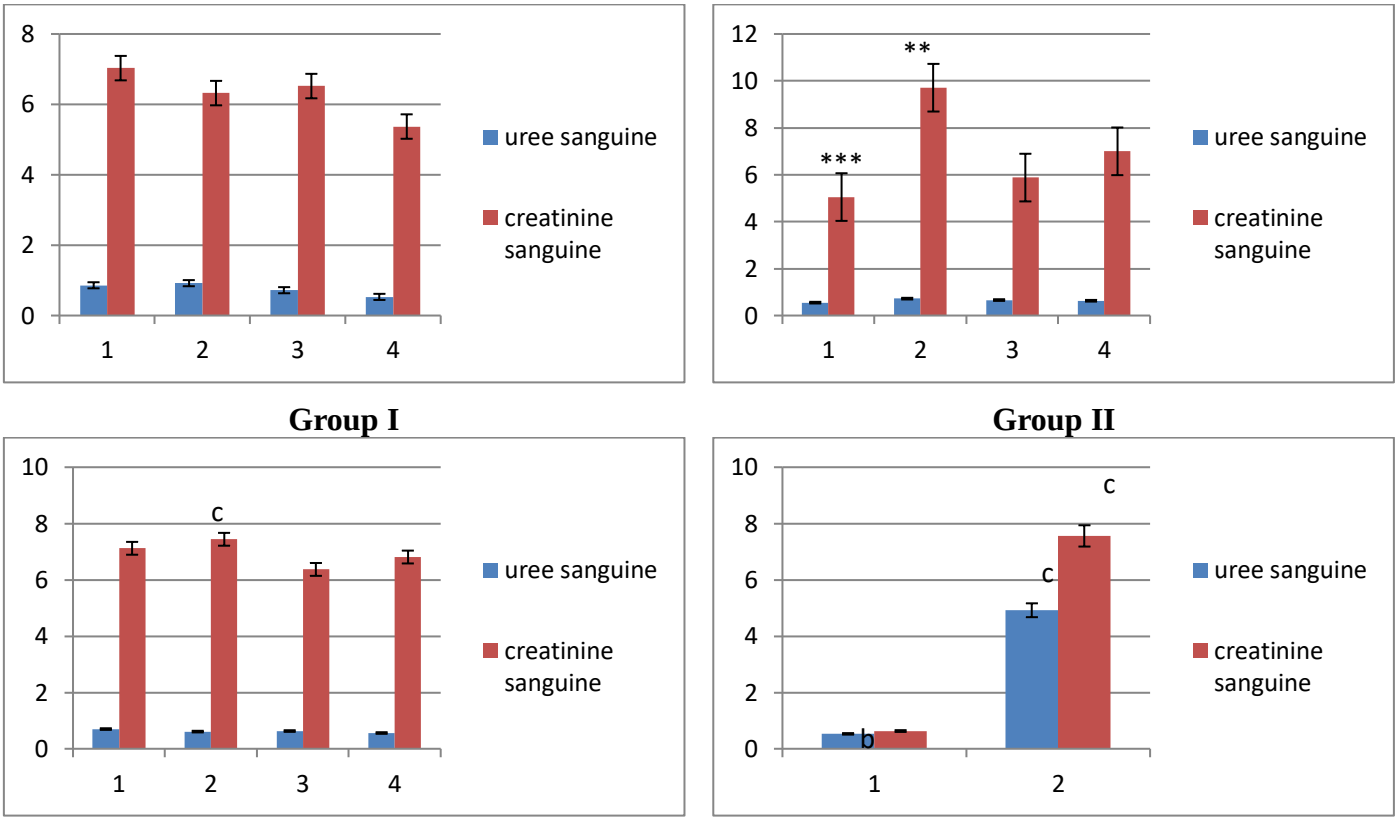

Figure 16: Renal check markers of different groups of rats

***:p<0.05, **:p<0.01, ***:p<0.001: *significantly different from control group*.**

a: p<0.05, b: p<0.01,c: p<0.001: *significantly different from (probiotics) group.*

urée sanguine: blood urea nitrogen

créatinine sanguine :blood creatinine

According to the results shown in the figure, an increase ($p < 0.001$) in plasma levels of urea and creatinine was observed in rats treated with cadmium chloride hemi-penta-hydrate assay compared to rats of the control group. The results also showed relatively similar values in plasma levels of urea and creatinine in both probiotic-treated mice (Groups III and IV) compared to control mice.

In addition, the kidney plays an important role in the homeostasis of the body ensuring the filtration of toxic waste from the bloodstream and their excretion in the urine**(Jaballi et al.2017).**The role of kidney in excretion of xenobiotics predisposes it to structural and/or functional damage**(O'Shaunessy,2010).**

There was a significant increase ($p < 0.001$) in the blood urea values and creatinine levels in the rats (the second group), compared with those of the control group. However, the

mice that took probiotics (the group IV), showed a significant decrease in these values Urea Creatinine ($p < 0.001$) compared to those of the (Groups III) which was closer to the control group.

The probiotics showed a positive effect on the levels of urea and creatinine in the blood, when compared to the levels of the control group.Usually, these compounds are filtered out by the kidneys with little or no tubular reabsorption**(Pritchard et al.2009).** Their high levels in the blood usually indicate problems with kidney function **(Ben Saadet al.2017).** Creatinine is formed in the body by non-enzymatic dehydration of creatine synthesized by the liver and stored in muscle **(Weber et al.2002).** A high plasma creatinine level (associated with a high urea level) reflects a decrease in glomerular filtration. Urea comes from the destruction of proteins**(Yengkhom et al.2019).** It is completely filtered by the glomeruli. Its blood level reflects the overall functioning of the kidneys. In our experimental study, an increase in plasma concentrations of urea and creatinine was noted in rats treated with cadmium chloride hemipentahydrate. This increase is usually related to kidney damage or dysfunction, or even nephrotoxicity**(Weber et al.2002).**

The first aim of administering probiotics during CKD is URS removal. Therefore, as the production of URS, mainly generated by protein degradation, could not be completely

blocked by a low-protein diet, reducing the conversion of amino acids into trimethylamine n-oxide, p-cresyl sulfate, or IS by modeling intestinal microbiota could be considered as an additional beneficial intervention**(Laetitia Koppe et al.2015).**

The second aspect is whether probiotics may control chronic inflammation, where biomarkers of inflammation are inversely correlated with kidney function**(Gupta et al.2012).**recently demonstrated in a mouse model of acute kidney injury that probiotic treatment increased plasma short-chain fatty acids and protected mice from kidney ischemia reperfusion injury through modulation of inflammation**(Andrade-Oliveira et al.2015).**

The third question is whether probiotics might improve renal function. Because of the potential beneficial effect of probiotics (reducing inflammation and uremic toxins) it is possible that renal function improves during treatment**(Laetitia Koppe et al.2015).**

Further beneficial effects of probiotics may occur. Indeed, probiotics are able to improve constipation in CKD patients **(Nakabayashi et al.2010).**

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General conclusion

General conclusion

Probiotics are microorganisms that have a positive effect on human and animal health. The great importance of probiotics was the starting point of our study and its application at the human medical level through its effect on kidney diseases chronic.

At the Royal Slaughterhouse in Al-Wadi State, a sample was taken from the small intestine of a male camel, with the aim of isolating and selecting lactic acid strains with probiotic properties, after carrying out identification and isolation tests of lactic acid strains and evaluation of their properties (effects) in vitro, followed by an in vivo study. This approach carried out through the following steps:

Isolation of lactic acid bacteria strains, purification, morphological identification of the strains isolated by the usual test: Gram test, we carried out several biochemical and physiological characterization tests of these strains by the catalase test, gallery test Api10 s and resistance to different NaCl concentration, temperature. The selection of the probiotic strains was carried out by the following tests, the tolerance to acidity, to bile salts, and the resistance to certain numbers of antibiotics, we also studied their antibacterial activities, in the end our evaluated this aptitude in vivo.

The 05 isolated strains were characterized by their form of Cocci/bacillus, gram positive, catalase positive. These 05 isolates are retained and have undergone physiological and biochemical tests for the identification of the species. The species revealed are: *Pediococcusacidilactici*, *Lactobacillus acidophilus.*

Our study then developed around these strains isolated from the small intestine of a male camel, by examining certain probiotic properties, after carrying out the various tests to determine the most efficient strain, the results which were obtained in the selection tests (antibacterial activities against E. coli, pseudomonas and S. aureus, acidity tolerance, bile salt tolerance, antibiotic resistance.) This helped us to choose (*Pediococcusacidilactici*, *Lactobacillus acidophilus*).

In vivo results from infected rats showed a significant increase in kidney size with some swelling, unlike the probiotic treated groups, kidney size was normal. A slight increase in the body weight of the rats was also observed for the treated groups and a decrease in the infected group.

The results of comprehensive blood and kidney census analyzes showed an increase in most values and abnormal tissue sections of the kidneys in the infected group, with normal results and tissue sections recorded in the probiotic-treated groups.

Thanks to these results, we conclude this work by the importance of lactic acid bacteria which have the property of probiotics and their positive impact on the development of chronic renal failure.

This work opens the door to other complementary studies and perspectives that seem necessary to carry out.

- ✓ Extend the range of pathogenic strains tested.
- ✓ Study the effect of probiotics on hepatitis.
- ✓ The application of this study at the level of immunological diseases.

References

References

Agüero, N. D. L., Frizzo, L. S., Ouwehand, A. C., Aleu, G., &Rosmini, M. R. (2020). Technological characterisation of probiotic lactic acid bacteria as starter cultures for dry fermented sausages. *Foods*, *9*(5), 596.

Ahirwar, SS, Gupta, MK, Gupta, G., & Singh, V. (2017). Dépistage, isolement et identification des espèces de lactobacilles des caries dentaires des enfants. International Journal Of CurrentMicrobiology and Applied Sciences, 6 (1), 497-503.

Aissous, S., Bibet H. et Merikhi N., (2021). Screening des souches bactériocinogènes à partir de tractus digestif de la mouette rieuse .Mémoire Master. Université Mohammed Seddik Ben Yahia, Jijel. p28.

American Kidney Fund. (2023, A). *Stages of kidney disease*. American Kidney Fund. Retrieved May 7, 2023, from https://www.kidneyfund.org/all-about-kidneys/stages-kidney-disease

American Kidney Fund. (2023, B).*Types of kidney diseases* . American Kidney Fund. Retrieved May 7, 2023, from https://www.kidneyfund.org/all-about-kidneys/types-kidney-diseases

Ammor M., Mayo B. (2007).Selection criteria for lactic acid bacteria to be used as functional starter cultures in dry sausage production.*Meat Science*.76:138-146.

Ammor, S., Florez, A.B., Van Hoek, A.H.A.M., De Los Reyes-Gavilan, C.G., AartsHenk, J.M., Margolles, A. et MAyo, B. (2008). Molecular characterization of intrinsic and Acquired Antibiotic Resistance in Lactic Acid Bacteria and Bifidobacteria. J. Mol. MicrobiolBiotechnol, 14:6-15.

Ammor, S., Rachman, C., Chaillou, S., Prévost, H., Dousset, X., Zagorec, M., & Chevallier, I. (2005). Identification phénotypique et génotypique de bactéries lactiques isolées d'une petite structure de production de saucissons secs traditionnels. Microbiologie alimentaire, *22* (5), 373-382.

Andrade-Oliveira, V., Amano, M. T., Correa-Costa, M., Castoldi, A., Felizardo, R. J. F., De Almeida, D. R. A., . . . Câmara, N. O. S. (2015). Gut Bacteria Products Prevent AKI Induced by Ischemia-Reperfusion. Journal of the American Society of Nephrology, 26(8), 1877–1888. https://doi.org/10.1681/asn.2014030288

Artis, D. (2008). Epithelial-cell recognition of commensal bacteria and maintenance of immune homeostasis in the gut. Nature Reviews Immunology, 8(6), 411–420. https://doi.org/10.1038/nri2316

Azat, R., Liu, Y., Li, W., Kayir, A., Lin, D., Zhou, W., Zheng, X. (2016). Probiotic properties of lactic acid bacteria isolated from traditionally fermented Xinjiang cheese. Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology), 17(8), 597–609

Babaali, A., BenTeghri, F.,(2022). Impact de la lumière artificielle sur le rythme de la sécrétion du cortisol. Mémoire Master .Universite Yahia Fares de Medea.

BADIS, A; LAOUABDIA, S; GUETARNI, D; KIHAL, M & OUZROUT, R.(2005). Caractérisation phénotypique des bactéries lactiques isolées à partir de lait cru de chèvre de deux populations caprines locales "Arabia et kabyle". Sciences & Technologie, 23, 30-37 p

Basli A, Chibane M, Mdani K et Oukil N (2012). Activité antibactérienne des polyphénolsextraits d'une plante médicinale de la flore d'Algérie : *Origanumglandulosum*. Desf.Phytothérapie, 10 (1):2-9.

Beausoleil, M., N. Fortier, et al. (2007). "Effect of a fermented milk combining *Lactobacillus acidophilus* Cl1285 and *Lactobacillus casei*in the prevention of antibiotic-associated diarrhea: a randomized, double-blind, placebo-controlled trial." *Canadian Journal*

Begley,M..Hill,C.,andGaham,C.G.M.(2006). Bile salt hydrolase activity in probiotic. Appl. EnvironementalMicrobiology. 72 (33): 1729-1738.

Bekhouche, F., &Boulahrouf, A. (2005). Etudes quantitative et qualitative des bactérieslactiques de lait cru produits par des vaches locales appartenant à six stations d'élevage deConstantine. Sciences & Technologie. C, Biotechnologies, 38-45.

Belarbi, F. (2011). Isolement et sélection des souches de bactéries lactiques productrices desmétabolites antibactériennes. Mémoire de Magister, Université d'Oran

Bennani, S., Mchiouer, K., Rokni, Y., & Meziane, M. (2017). Caractérisation et identification des bactéries lactiques isolées du lait cru de vache marocain. Journal plus vert des sciences biologiques, 25, 4934-4944.

Bensbia, S.,(2022). Histopathologie des tumeurs cérébrales : à propos de 15 cas dans la région de Bordj Bou Arreridj. Mémoire Master.Université Mohamed El Bachir El Ibrahimi B.B.A.

Bentall A. (2021). Chronic kidney disease. On https://www.mayoclinic.org/diseases-conditions/chronic-kidney-disease/symptoms-causes/syc-20354521

BOUKHEMIS, A., BOUTERSA A., (2015). Identification et anti biorésistance de souches*d'Escherichia coli* et de *Klebsiellapneumoniae*des infections urinaires à l'aide des moyens classiques et des moyens automatisés. Mémoire Master.Université des Frères Mentouri Constantine.

Bousebta, K.,Boudekhane, K.,&Boulhoulou, M. (2021). Identification des bactéries lactiques isolées à partir du lait de chèvre et évaluation de leurs caractéristiques technologiques. Mémoire Master. Université Mohammed Seddik Ben Yahia, Jijel, 20 p.

Brahimi, S. (2015). Isolement et caractérisation biotechnologiques des bactéries lactiquesisolée à partir des margines d'olives « AMOREDJ » fermentés .Thèse de Mgister, Universitéd'Oran.

Carr F.J., Chill, D., & Maida, N. (2002). The Lactic Acid Bacteria: A Literature Survey. Critical Reviews in Microbiology, 28, 281-370.

CasadoMuňoz, M., Benomar,N.,Lavilla, L., Lerma, L.,Gâlvez, A.,etAbriouel, H. (2014). Antibiotic résistance of *Lactobacillus pentosus* and *Leuconostocpseudomesenteroïdes* isolated from naturally fermented Aloreňa table olives throughout fermentation process. International Journal of Food Microbiology, 172: 110118.

Centers for Disease Control and Prevention. (2022, February 28).*Chronic kidney disease basics*. Centers for Disease Control and Prevention. Retrieved May 7, 2023, from https://www.cdc.gov/kidneydisease/basics.html

Chen, J., Chen, X., & Ho, C. L. (2021). Recent development of probiotic Bifidobacteria for treating human diseases. *Frontiers in Bioengineering and Biotechnology*, *9*, 1371.

Chentouf, H. (2015). Effet des substances antimicrobiennes produites par Leuconostoc mesenteroides isolées à partir du lait cru de chamelle d'algérie sur la croissance de Listeria

monocytogenes dans les produits alimentaires. Thèse de doctorat, Université d'Oran1-Ahmed Ben Bella .

Cockwell, P., & Fisher, L. A. (2020). The global burden of chronic kidney disease. *The Lancet*, *395*(10225), 662-664.

Collado, M. C., Meriluoto, J., &Salminen, S. (2007). Role of commercial probiotic strains against human pathogen adhesion to intestinal mucus. *Letters in AppliedMicrobiology*, *45*(4), 454-460.

Dalache, F. (2006). Effets inhibiteurs des bactéries lactiques. Bactériocines de *Lactococcus*etd'*Enterococcus* : mise en évidence d'un support plasmidique. Thèse de Doctorat en microbiologie moléculaire et génétique. Université d'Oran Es-Senia.

Danielsen, M., & Wind, A. **(2003).** Susceptibility of Lactobacillus spp. to antimicrobial agents. International journal of food microbiology, 82(1), 1-11

Daoudou, B., Leopold, T. N., Augustin, M., & Moses, M. C. (2011). Assessment of physiological properties of some lactic acid bacteria isolated from the intestine of chickens use as probiotics and antimicrobial agents against entero pathogenic bacteria. Innovative Romanian Food Biotechnology, (8), 33-40.

Davidson, A. L., Lase, E., Lister, I. N. E., &Fachrial, E. (2019, July). JProbiotic activity of lactic acid bacteria isolated from several commercial fermentation product in Medan, North Sumatera. In Journal of Physics: Conference Series (Vol. 1230, No. 1, p. 012060). IOP Publishing.

Denohue(2004). Safety of nevelprobioticbactiria.In :Lacticacidbacteria :microbiological and functional aspects (Salminen S., Wright A.V.et ouwehand A.) 3eEd.,Marcel Dekker, In *New York*. 531-546.

Denohue, D.C., (2004). Safety of novel probiotic bacteria In: Lactic acid bacteria microbiological and functional aspects (SalminensS, Wrigh, A.V.et Ouwehand, A).3éEd.Marcel Dekker NY 531- 546.

Dunne C., Omahony L., Murphy L.,Thornton G., Morrissey D., Ohalloran S., Feeney M.,FlynnS.,Fitzgerald G., Daly C., Kiely B., Osullivan G.C.,Shanaham F and Collins J.K(2001).*In vitro* selection criteria for probiotic bacteria of human origin: correlation with in vivo findings *Am.J.Clin.Nutr*.,**73:** 386-392.

Ezendam, J., & van Loveren, H. (2006). Probiotics: immunomodulation and evaluation of safety and efficacy. *Nutrition Reviews, 64*(1), 1-14.

Fooks, L. J., & Gibson, G. R. (2002). In vitro investigations of the effect of probiotics and prebiotics on selected human intestinal pathogens. *FEMS Microbiology Ecology, 39*(1), 67-75.

Fukao, M., Yajima, N. (2012). Assessment of Antibiotic Resistance in Probiotic Lactobacilli,
Antibiotic Resistant Bacteria- A Continuous Challenge in New York. Pana(Ed). 503-512. Fuller,R., (1989). A review: Probiotics in man and animals. J.Appl.Bacteriol., 66, 365-378.

Gaggia F., MattarelliPandBiavatiB(2010).probiotics and prebiotics in animal feeding for safe food production. *International Journal of Food Microbiology***,** 141 Supple 1 : 15-28.

Ghazi, F., Henni, D. E., Benmechernene, Z., &Kihal, M. (2009). Phenotypic and wholecell protein analysis by SDS-PAGE for identification of dominants lactic acid bacteria isolated from Algerian raw milk. World Journal of Dairy Food Science, 4(1), 78-87.

Gowri, S., Ghost, A.R.(2010).*Pediococcus ssp*. - a potential probiotic isolated from Khadi (an Indian fermented food) and identified by 16 S rDNA sequences analysis. Afr J Food Sci.4:597-602.

Gu R X., Yang Z.Q., Li Z.H., Chen S.L and Luo Z. L (2008). Periodic properties of lactic acid bactria isolated from stool samples of longevons people in regions of mutant ;Xirijiang and Bama ; Guangxi ; *Anaerobe* . 14: 313-317.

Guarner, F., &Schaafsma, G. J. (1998). Probiotics. *International journal of food microbiology, 39*(3), 237-238..

Guiraud, J.P. (1998). Microbiologie alimentaire, Techniques d'analyse microbiologiques. Dunod.

Guiraud, J-P., (2003). Microbiologie Alimentaire. Technologyand Documentation, Dunod.Paris, 90-292.

Gupta, J., Mitra, N., Kanetsky, P. A., Devaney, J., Wing, M. R., Reilly, M. P., . . . Raj, D. S. (2012). Association between Albuminuria, Kidney Function, and Inflammatory Biomarker Profile in CKD in CRIC. Clinical Journal of the American Society of Nephrology, 7(12), 1938–1946. https://doi.org/10.2215/cjn.03500412

Hambli, M.,Djabrohou S,.(2022). Variations des paramètres biochimiques, hématologiques et histologiques chez les femmes enceintes à faible risque. Mémoire Master .Université Laarbi Ben Mhidi Oum El Bouaghi.

Hammes, W. P. and R. F. Vogel (1995).The genus*Lactobacillus*. The genera of lactic acid bacteria, *Springer***:** 19-54.

Hassan, M. U., Nayab, H., Shafique, F., Williamson, M. P., Almansouri, T. S., Asim, N., Akbar, N. (2020). Probiotic properties of *Lactobacillus helveticus*and *Lactobacillus plantarum*isolated from traditional Pakistani yoghurt. *BioMed Research International*, **17,** 8889198.

Holzapfel, W. H. and U. Schillinger (2002)."Introduction to pre-and probiotics."*Food Research International*.**35**(2): 109-116.

Holzapfel, W. H., & Wood, B. J. (Eds.). (2014). Lactic acid bacteria: biodiversity and taxonomy. John Wiley and Sons.

Holzapfel, W. H., P. Haberer, et al. (2001). "Taxonomy and important features of probiotic microorganisms in food and nutrition."*The American journal of clinical nutrition.***73**(2): 365s-373s.

Idoui, T., Boudjerda, J., Leghouchi, E., &Karam, N. E. (2009). Lactic acid bacteria from"Sheep'sDhan", a traditional butter: Isolation, identification and major technological traits. *Grasas y aceites*, *60*(2), 177-183.

Izerghouf, T. etHabhoub, S.(2021). Activité antimicrobienne et protéolytiques des bactéries lactique isolées du lait et des produits laitiers fermentés. Mémoire Master.Université de Larbi Tébessi , Tébessa. p 55.

Jaballi, I., Saad, H. B., Bkhairia, I., Kammoun, I., Droguet, M., Magné, C., . . . Amara, I. B. (2017b). Increasing maneb doses induces reactive oxygen species overproduction and nephrotoxicity in adult mice. Toxicology Mechanisms and Methods, 27(5), 382–393. https://doi.org/10.1080/15376516.2017.1300617

Jannah, S. N., Dinoto, A., Wiryawan, K. G., &Rusmana, I. (2014). Characteristics of lactic acid bacteria isolated from gastrointestinal tract of Cemani chicken and their potential use as probiotics. Media Peternakan, 37(3), 182-189.

Jannah, S. N., Saraswati, T. R., Handayani, D., &Pujiyanto, S. (2018, May). Antibacterial Activity of Lactic Acid Bacteria Isolated from Gastrointestinal Tract of "AyamKampung" Chicken Against Food Pathogens. In Journal of Physics: Conference Series (Vol. 1025, No. 1, p. 012082). IOP Publishing.

Jovanović, D., Gasic, B., Pavlovic, S., &Naumovic, R. (2013). Correlation of kidney size with kidney function and anthropometric parameters in healthy subjects and patients with chronic kidney diseases. *Renal failure*, *35*(6), 896-900.

Kabeerdoss J., R. S. Devi et al. (2011). "Effect of yoghurt containing *Bifidobacterium* lactis Bb12® on faecal excretion of secretory immunoglobulin A and human beta-defensin 2 in healthy adult volunteers."Nutr J**10**: 138.

Klaenhammer T. R (1988). "Bacteriocins of lactic acid bacteria."*Biochimie***70**(3): 337-349.

Klaenhammer T. R (1998). "Functional activities of *Lactobacillus* probiotics: genetic mandate." International Dairy Journal.**8**(5-6): 497-505.

Koppe, L., Mafra, D., &Fouque, D. (2015). Probiotics and chronic kidney disease. *Kidney international*, *88*(5), 958-966.

Koppe, L., Mafra, D., &Fouque, D. (2015). Probiotics and chronic kidney disease. Kidney International, 88(5), 958–966. https://doi.org/10.1038/ki.2015.255

Koppe, L., Mafra, D., &Fouque, D. (2015b). Probiotics and chronic kidney disease. Kidney International, 88(5), 958–966. https://doi.org/10.1038/ki.2015.255

Kovesdy, C. P. (2022). Epidemiology of chronic kidney disease: an update 2022. *Kidney International Supplements*, *12*(1), 7-11.

Krantz, S., & Jacobson, L. (1970). Morphological identity of the hematopoietic stem cell. Erythropoietin and the Regulation of Erythropoiesis; University of Chicago Press: Chicago, IL, USA, 98-102.

Kusharyati, D.F., Pramono, H., Rayandini, D., Manshur, T. A., Dewi, M. A., Khatimah, K., Rovik, A.(2020). *Bifidobacterium* from infant stool: the diversity and potential screening. *Biodiversitas* Journal of Biological Diversity, **21(6),** 2506-2513.

Labioui H.,ElmoualdiL., Elyachioui M et Ouhssine M (2005). Sélection de souches de bactéries lactiques antibactériennes.*Bull.Soc. Pharm.Bordeaux*.**144 :**237-250.

Lairini, S., Beqqali, N., Bouslamti, R., Belkhou,R., &Zerrouq,F. (2014). Isolement des bactéries lactiques à partir des produits laitiers traditionnels Marocains et formulation d'un lait fermenté proche du Kéfir.Afrique science. 10(4) (2014) 267 – 277.

Lamoureux L (2000). Exploitation de l'activité β-galactosidase de culture de bifidobactéries en vue d'enrichir des produits laitiers en galacto-oligosaccharides. *National Library canada.*23-47.

Land, M. H., K. Rouster-Stevens, et al. (2005). "*Lactobacillus* sepsis associated with probiotic therapy." *Pediatrics.***115**(1): 178-181.

Madsen, K., A. Cornish, et al. (2001). "Probiotic bacteria enhance murine and human intestinal epithelial barrier function." *Gastroenterology.***121**(3): 580-591.

Makhloufi, K. M. (2012). *Caractérisation d'une bactériocine produite par une bactérie lactique Leuconostocpseudomesenteroides isolée du boza* (Doctoral dissertation, Université Pierre et Marie Curie-Paris VI).

Marteau P et Ramb J .C (1998). Probiotique en gastroentérologie, base rationnel,effetsdémontrés et perspectives.*Hepato-Gastro* N°4 ;Vol 5 .

Matsuzaki T. and Chin J(2000). "Modulating immune responses with probiotic bacteria."*Immunology and cell biology***.78**(1): 67-73.

Mayo Clinic. (2021, September 3).*Chronic kidney disease*. Mayo Clinic. Retrieved May 7, 2023, from https://www.mayoclinic.org/diseases-conditions/chronic-kidney-disease/symptoms-causes/syc-20354521#:

McFarland, L. V. (2007)."Meta-analysis of probiotics for the prevention of traveler's diarrhea."*TravelMédicine and Infections Disease.***5**(2): 97-105.

Mitsuoka, T. (1990)."Bifidobacteria and their role in human health."*Journal of IndustrialMicrobiology***.6**(4): 263-267.

Mohan, R., Koebnick, C., Schildt, J., Schmidt, S., Mueller, M., Possner, M., ...&Blaut, M. (2006). Effects of Bifidobacterium lactis Bb12 supplementation on intestinal microbiota of preterm infants: a double-blind, placebo-controlled, randomized study. *Journal of Clinical Microbiology*, *44*(11), 4025-4031

Muller, J. A., Ross, R. P., Fitzgerald, G. F., Stanton, C. (2009). Manufacture of probiotic bacteria. In: Charalampopoulos, D., Rastall, R. A. (eds.). Prebiotics and Probiotics Science and Technology. Springer, New York, NY. 725-760.

Nagpal R., A. Kumar et al. (2012). "Probiotics, their health benefits and applications for developing healthier foods: a review." *FEMS microbiology letters.***334**(1): 1-15.

Nagpal R., H. Yadav et al. (2007). "Potential of probiotics and prebiotics for symbiotic functional dairy foods: an overview." *International Journal of Probiotics and Prebiotics.* **2**(2/3): 75.

Naimi, S. (2014). *Isolement, caractérisation et étude" in vitro" de l'activité anti-inflammatoire de différentes souches probiotiques* (Doctoral dissertation, Université Laval).

Nakabayashi, I., Nakamura, M., Kawakami, K., Ohta, T., Kato, I., Uchida, K., & Yoshida, M. (2010). Effects of synbiotic treatment on serum level of p-cresol in haemodialysis patients: a preliminary study. Nephrology Dialysis Transplantation, 26(3), 1094–1098. https://doi.org/10.1093/ndt/gfq624

National Kidney Foundation. (2022, May 24). *Kidney disease: The basics*. National Kidney Foundation. Retrieved May 7, 2023,fromhttps://www.kidney.org/news/newsroom/fsindex#:

Newman , T. (2023).*Chronic kidney disease: Symptoms, stage, treatment, and more*. Medical News Today. Retrieved May 7, 2023, fromhttps://www.medicalnewstoday.com/articles/172179

Nguyen, C.and F. Carlin. (1994). The microbiology of minimally processed fresh fruits andvegetables. Crit.Rev. Food Sci. Nutr., 34:371.0.

Nguyen, H. L., Linh, H. Q., Krupa, P., La Penna, G., & Li, M. S. (2022). Amyloid β Dodecamer Disrupts the Neuronal Membrane More Strongly than the Mature Fibril: Understanding the Role of Oligomers in Neurotoxicity. *The Journal of Physical Chemistry B*, *126*(20), 3659-3672.

NIDDK (2022).*Kidney disease* - National Institute of Diabetes and Digestive and Kidney Diseases. Retrieved May 7, 2023, from https://www.niddk.nih.gov/health-information/kidney-disease#:

Nousiainen J., Javanainen P., Setala J. Et Wright A.V. (2004). *"Lactic acid bacteria as animal probiotics". In: Lactic* acid bacteria: microbiological and functional aspects (Salminen S., Wright A.V. etOuwehand A.). *3e Ed., Marcel Dekker, Inc*. New York. 547-560. In (Etude de quelques propriétés probiotiques des quelques souches Lactobacillus isolées de lait chamelle et de chèvre [Mémoire de fin d'études] Université Abdel Hamid Ibn Badis).

O'Shaughnessy, K. M. (2010). Principles of clinical pharmacology and drug therapy. In Oxford University PresseBooks (pp.1449–1476).https://doi.org/10.1093/med/9780199204854.003.10

Oelschlaeger, T. A. (2010). Mechanisms of probiotic actions–a review. *International journal of medical microbiology*, *300*(1), 57-62.

Ouwehand, A. C., &Vesterlund, S. (2004). Antimicrobial components from lactic acid bacteria. *FOOD SCIENCE AND TECHNOLOGY-NEW YORK-MARCEL DEKKER-*, *139*, 375-396.

Oyewole, O. F., Maria, C. O., Tope, P. S., &Funmi, O. O. (2018). In vitro Study of Potential Probiotic Lactic Acid Bacteria Isolated from The Gut of Chickens in Abeokuta, Nigeria. Alexandria Journal for Veterinary Sciences, 58(1).

Palomares I. C., Prérez-Morales R etAcedo-félisc E (2007). Evaluation of probiotic properties *in Lactobacillus isolated froms mall intestine of piglets .Rev. Latinoan. Microbiol.*49 (3-4):46-54.

Patterson (2008). Probiotiques : bien faits au- delà des fonctions nutritionnelles de base .*AAFC.*1-4.

Pritchard, J., Burn, C. C., Barr, A., &Whay, H. R. (2009). Haematological and serum biochemical reference values for apparently healthy working horses in Pakistan. Research in Veterinary Science, 87(3), 389–395. https://doi.org/10.1016/j.rvsc.2009.05.003

Reuben, R. C., Roy, P. C., Sarkar, S. L., Alam, R. U., &Jahid, I. K. (2019). Isolation,
characterization, and assessment of lactic acid bacteria toward their selection as poultry probiotics. BMC microbiology, 19(1), 1-20.

Reyes-GavilanC.G.,Suarez A.,Fermandez –Garcia M.,Margolles A., Gueimonde Met Ruas-Madiedo P (2011). Adhésion of bile –adapted *Bifidobactérium strains* to the

HT296MTX cell line is modifiedaftersequential gastro-intestinal challenge simulated in vitro usinghumangastricandduodenaljuices. Res. Microbiol.**162:** 514-519

Riane, K., Sifour, M., Ouled-Haddar, H., Idoui, T., Bounar, S., Boussebt, S. (2019). Probiotic properties and antioxidant efficiency of *Lactobacillus plantarum* 15 isolated from milk. The Journal of Microbiology Biotechnology and Food Sciences**, 9(3),** 516-520.

Ridlon, J. M., Harris, S. C., Bhowmik, S., Kang, D. J., &Hylemon, P. B. (2016). Consequences of bile salt biotransformations by intestinal bacteria. *Gut microbes*, *7*(1), 22-39.

Roselli, M., Finamore, A., Britti, M. S., &Mengheri, E. (2006). Probiotic bacteria Bifidobacterium animalis MB5 and Lactobacillus rhamnosus GG protect intestinal Caco-2 cells from the inflammation-associated response induced by enterotoxigenic Escherichia coli K88. *British Journal of Nutrition*, *95*(6), 1177-1184.

Saad, H. B., Gargouri, M., Kallel, F., Chaabene, R., Boudawara, T., Jamoussi, K., Amara, I. B. (2016). Flavonoid compounds from the red marine algaAlsidiumcorallinumprotect against potassium bromate-induced nephrotoxicity in adult mice. Environmental Toxicology, 32(5), 1475–1486. https://doi.org/10.1002/tox.22368

Salminen S., Gorbach S., lee Y.K.et Benno Y(2004). Human studies on probiotic : Wheat is scientifically proventoday .In: Lactic acid bacteria : microbiogical and functional aspects (SalminenS.,Wrightt.A.V.etouwehand A.).3e Ed., *Marcel Dekker, Inc, Newyork*.515-530.

Salminen, S. J., M. Gueimonde, et al. (2005). "Probiotics that modify disease risk."*The Journal of nutrition.***135**(5): 1294-1298.

Schultz M., A. Timmer et al. (2004)."*Lactobacillus* GG in inducing and maintaining remission of Crohn's disease." *BMC gastroenterology.***4** (1): 5.

Servin, A. L. (2004). Antagonistic activities of lactobacilli and bifidobacteria against microbial pathogens. *FEMS microbiology reviews*, *28*(4), 405-440.

Sharma, G. D., Rasania, S., Dadhaniya, P., Patel, C., &Vachhani, K. (2015). Assesment of 28 days repeated adminstration toxicity profile of dexamethasone palmitate injection. Journal of NPA, 27(1), 9–19. https://doi.org/10.3126/jnpa.v27i1.12145

Shu, Q., & Gill, H. S. (2002). Immune protection mediated by the probiotic Lactobacillus rhamnosus HN001 (DR20™) against Escherichia coli O157: H7 infection in mice. *FEMS Immunology & Medical Microbiology*, *34*(1), 59-64.

Southon, S., Gee, J. M., & Johnson, I. T. (1984). Hexose transport and mucosal morphology in the small intestine of the zinc-deficient rat. British Journal of Nutrition, 52(2), 371.

sparrow.org. (2022).*End-stage renal disease*. Sparrow. Retrieved May 7, 2023, from https://www.sparrow.org/departments-conditions/conditions/end-stage-renal-disease

Tahlaiti H., (2019). Etude des propriétés technologiques et inhibitrices de bactéries lactiques isolées à partir de blé fermenté. Thèse de doctorat,Université Abdelhamid Ibn Badis Mostaganem.

Taleb, N., Tibhirt, K.,(2021). Isolement des kystes Toxoplasmique /Sarcocystis à partir des coupes histologiques de cœur et masséter chez les bovins, caprins et ovins dans la wilaya de Tizi-Ouzou.Mémoire Master. UniversiteMouloudMammeri de Tizi-ouzou.

TemmermanR.,Pot B.,Huys G and Swings J (2003). Identification and antibiotic susceptibility of bacterialisolates from probiotic products.*Int.J.Food Microbiol*.**81 :**1-10

Terzaghi, B.E.et Sandine, W.E. (1975). Improved medium for lactic *Streptococci* and theirbacteriophages. Appl. Environ.Microbiol. 29,807-813

Thornton, G. M. (1996). *Probiotic bacteria: selection of Lactobacillus and Bifidobacterium strains from the healthy human gastrointestinal tract; characterisation of a novel Lactobacillus-derived antibacterial protein* (Doctoral dissertation, NUI).

Titiek F.D.,Endang S.R., DjokowetSlamet S (1996). Antimicroial Substance Produced by *Lactobacillus*sp.TGR-2 isolated from Growol. *Indonesian.FoodNutr.Prog*.**3(2):**29-34.

Todorov SD, Dicks LMT . (2005). Characterizationofbacteriocins produced by lactic acid bacteria isolated from spoiled black olives. Journal of Basic Microbiology 45: 312-322.

Tsega, K. T., Maina, K., &Berhane, N. (2019). Characterization of Lactic Acid BacteriaIsolated from the Gastrointestinal Tract of Ethiopian Rhode Island Red Chicken and theirPotential Use as Probiotics.

Vanderpool, C., Yan, F., & Polk, B. D. (2008). Mechanisms of probiotic action: implications for therapeutic applications in inflammatory bowel diseases. *Inflammatory bowel diseases*, *14*(11), 1585-1596.

Wang, M. (2021). Protecting the kidney with probiotics. *Nature Reviews Nephrology*, *17*(10), 633-633.

Weber, M., Dindo, D., Demartines, N., Ambühl, P. M., &Clavien, P. (2002). Kidney Transplantation from Donors without a Heartbeat. The New England Journal of Medicine, 347(4), 248–255. https://doi.org/10.1056/nejmoa020274

Weber, M., Dindo, D., Demartines, N., Ambühl, P. M., &Clavien, P. (2002b). Kidney Transplantation from Donors without a Heartbeat. The New England Journal of Medicine, 347(4), 248–255. https://doi.org/10.1056/nejmoa020274

Wollowski, I., G. Rechkemmer, et al. (2001)."Protective role of probiotics and prebiotics in colon cancer."*The American journal of clinical nutrition.***73(2):** 451s-455s.

Yadav H., S. Jain et al. (2007)."Antidiabetic effect of probiotic dahi containing*Lactobacillus acidophilus* and*Lactobacillus casei* in high fructose fed rats." *Nutrition.***23(1):** 62-68.

Yengkhom, R., Singh, P., Muwel, N., Raje, K., Handique, B., &Venkateswaran, K. (2019). Supplementation of Brown Seaweed (Turbinariaconoids) Powder and its Effect on Blood Metabolites and Mineral Profile in Adult Goats. Indian Journal of Animal Nutrition. https://doi.org/10.5958/2231-6744.2019.00019.7

Zantar, S., El Galiou, O., Zerrouk, H. M., &Laglaoui, A. (2013). Elaboration d'un fromage de chèvre semi-affiné à partir d'une sélection de souches lactiques autochtones isolées du lait de chèvres du Nord du Maroc. Options Méditerranéennes A, 108(1) ,191-197.

Annexes

Annex 01 :

Culture media:

Midfielder M17 (Terzaghi and Sandine)

For 1 liter of medium:

Tryptone.....2.50g

Meat peptic petone 2.50g

Soy papain petone 5.00g

Autolytic yeast extract 2.50g

Meat extract 5.00g

Lactose 5.00g

Sodium glycerophosphate 19.00g

Magnesium sulfate 0.25g

Ascorbic acid 0.50g

Bacteriological agar 15.00g

pH 7.2

Sterilization at 120°C for 20mm

Mid MH (Muller Hinton) (Mueller and Hinton, 1941)

For 1 liter of medium:

Peptones …… ..11.0g

Meat extract……..…….1.0 g

Sodium chloride…….……75 g

Mannitol…….……...10.0g

Phenol red……..…….…..0.025 g

Agar ……..……..15g

pH 7.2

Sterilization at 120°C for 20mm

Chemical products:

analytical reagent cadmium chloride hemi-pentahydrate

Assay%.....Min99.0

PH value (50g/L,25°C°)……4.0 - 6.5

Insoluble matter in water%.....Max0.005

Sulfate(SO4)%.....Max0.01

Total nitrogen(N)%.....Max0.002

Sodium(Na)%.....Max0.005

Calcium(Ca)%.....Max0.01

Iron(Fe)%.....Max0.0002

Copper(Cu)%.....Max0.002

Zinc(Zn)%.....Max0.002

Lead(Pb)%.....Max0.01

Annex02:

Table: Blood sugar markers of different groups of rats(mg/dl).

Rats / Groups	Rat01	Rat02	Rat03	Rat04
Group01	1.76	1.8	1.13	2.21
02Group	2.71	2.69	1.99	2.38
Group03	1.63	1.26	2.22	1.81
Group04	1.87	1.63	/ /	//

Annex03:

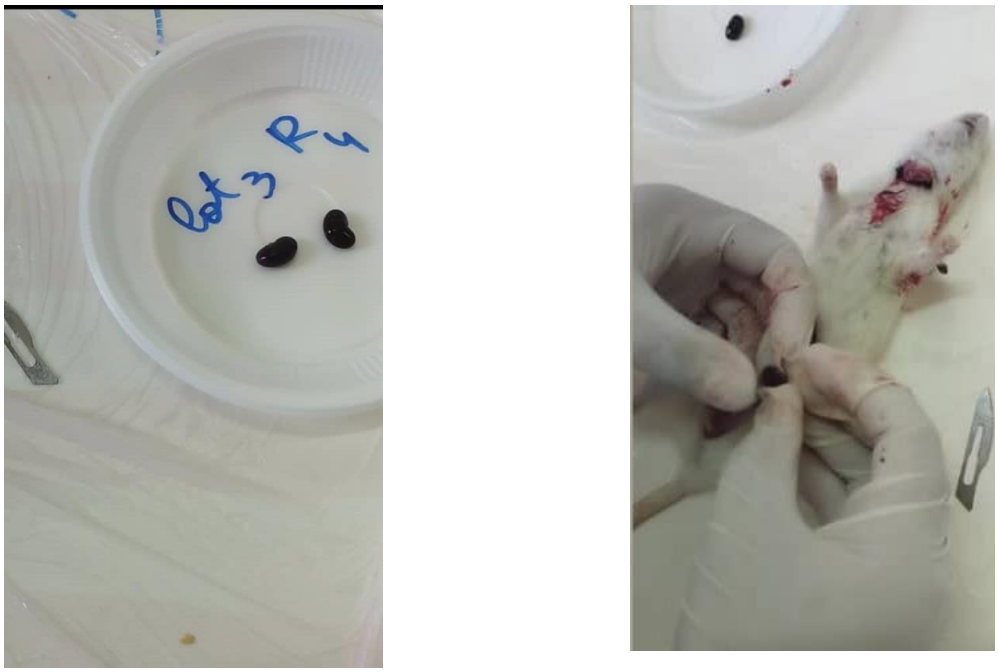

Figure 17:Kidney removal operation(Original picture, 2023)

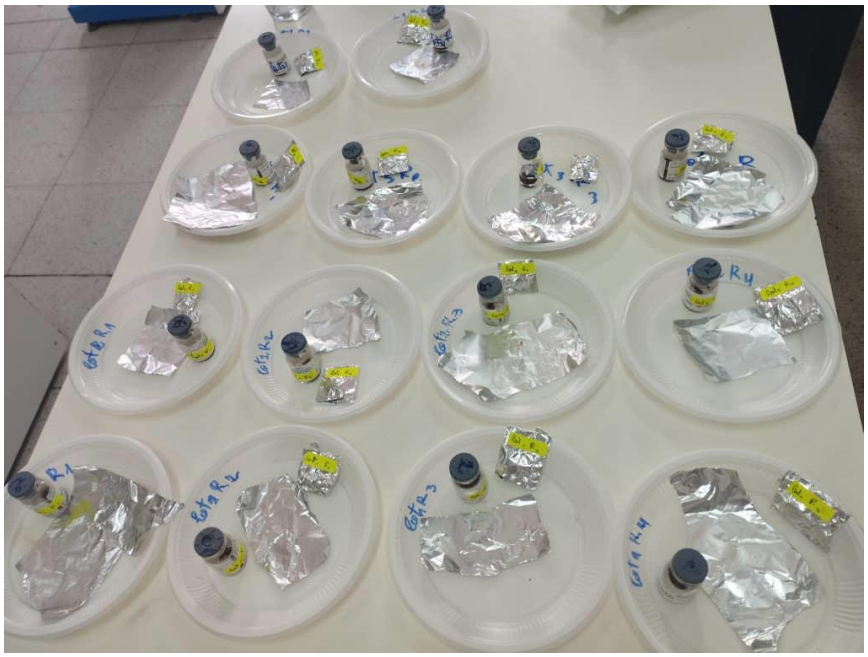

Figure18:Prepare samples for histological sections (Original picture, 2023)

Figure 19:bacteria strains(Original picture, 2023)