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THEME

Antibacterial activity of lactic acid bacteria isolated from Camel Milk

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NASSREDDINE

Dedication

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Dedication

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FAYCAL

Abstract

The objective of this study is to investigate the antibacterial activity of lactic acid bacteria isolated from camel milk. The physicochemical analysis of camel milk collected from the Djelfa region in central Algeria provided the following findings: the pH value was measured at 6.29, density at 1.028, acidity level at 16, dry matter content at 8.2637, fat content at 0.94, solids-not-fat (SNF) content at 6.98, protein content at 2.57, total solids at 17.4, lactose content at 3.84, conductivity at 8.58, added water content at 18.26, and a freezing point ranging from -0.4 to -0.525.

For the characterization of lactic acid bacteria microflora present in the collected camel milk, selective media (MRS and M17) were used in the isolation. Microscopic identification revealed the presence of *Lactobacillus* and *Streptococcus* isolates, which aligns with previous research indicating the presence of these bacteria in camel milk.

Camel milk is recognized for its high concentration of antibacterial molecules such as lysozymes, bacteriocins, lactoferrin, and more. To demonstrate this unique quality of camel milk, the lactic acid bacteria strains were evaluated for their antagonistic activity against four pathogenic bacteria: *Escherichia coli* (ATCC 259222), *Pseudomonas aeruginosa* (ATCC 9027), *Bacillus cereus* (ATCC 14579), and *Staphylococcus aureus* (ATCC 43300) using the disc diffusion method. The lactic acid bacteria strains exhibited significant inhibition zones against all tested pathogenic strains, with diameters ranging from 7 to 16 mm. Notably, a *Lactobacillus* strain demonstrated the largest inhibition diameter.

Keywords: Camel milk - physicochemical analysis – isolation - lactic acid bacteria

ملخص

الهدف من هذه الدراسة هو التحقيق في النشاط المضاد للبكتيريا للبكتيريا اللاكتيكية المعزولة من حليب الإبل. اعطت نتائج التحاليل الفيز وكيميائية لحليب الإبل الذي تم جمعه من منطقة الجلفة بوسط الجزائر النتائج التالية: قيمة الأس الهيدروجيني 6.29، الكثافة 1.028، مستوى الحموضة 16، قيمة المادة الجافة 8.2637، الدهون 0.94، المواد الصلبة غير الدهنية و 18.58 (SNF) 6.98 البروتين 2.57، إجمالي المواد الصلبة عند 17.4، نسبة اللاكتوز 3.8.84، الموص لاكتوز 8.58، ،.

لتوصيف بكتيريا حمض اللاكتيك الموجودة في حليب الإبل الذي تم جمعه، تم استخدام الوسائط الزراعية (MRS و M17) لعزل LAB.كشف التعريف المجهري عن وجود عزلات *Lactobacillus* و*Streptococcus* ، والتي تتماشى مع الأبحاث السابقة التي تشير إلى وجود هذه البكتيريا في حليب الإبل.

يتم التعرف على حليب الإبل لتركيزه العالي من الجزيئات المضادة للبكتيريا مثل الليزوزيمات والبكتيريوسينات واللاكتوفيرين والمزيد. لإثبات هذه الجودة الفريدة من حليب الإبل، تم تقييم سلالات LAB لنشاطها المضاد ضد أربع بكتيريا مسببة للأمراض: *Escherichia coli* (ATCC 259222), *Pseudomonas aeruginosa* (ATCC 9027), *Bacillus cereus* (ATCC 14579), and *Staphylococcus aureus* (ATCC 43300)

أظهرت سلالات LAB مناطق تثبيط كبيرة ضد جميع السلالات الممرضة المختبرة، بأقطار تتراوح من 7 إلى 16 ملم. والجدير بالذكر أن سلالة *Lactobacillus* أظهرت أكبر قطر للتثبيط.

الكلمات الرئيسية: حليب الإبل - تحليل فيزيوكيميائي - عزل - بكتيريا حمض اللاكتيك

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Abbreviations List

FAO : Food and Agriculture Organization

pH : potential hydrogen

LAB: lactic acid bacteria

TSI: triple sugar iron

ONPG: O-Nitrophenyl β-D-galactopyranoside

ATCC: American type culture collection

Sp: Species not specified

Afnor : association française de normalisation

K : Potassium

Ca: Calcium

P: Phosphorus

Mg: Magnesium

Na: Sodium**Fe:** Iron

Zn: Zinc

Cu: Copper

H_2O_2: hydrogen peroxide

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Chapter III: Results and discussion

Introduction

INTRODUCTION

Camel has been mentioned in Quran in different places and described as a miracle of almighty God. Also, prophet Muhammad (PBUH) recommended camel in his speech (hadith). As scientific research continues to explore the potential medicinal and health benefits of camel milk, it is important to conduct systematic reviews that take into account recent discoveries Due to a lack of up-to-date knowledge regarding these benefits, this study aims to collect and thematically review current information on the pharmaceutical benefits of camel milk, drawing from both religious texts such as the Holy Quran and sayings of Prophet Muhammed (PBUH) as well as modern scientific perspectives on the medicinal benefits of camel milk. According to statistics provided by the FAO, the global population of camels was 19 million, with the majority of camels residing in Africa (15 million) and the rest in Asia (4 million). Additionally, the data suggests that roughly 17 million of these camels were classified as one-humped dromedary camels, while the remaining 2 million were two-humped camels.

The camel is a highly versatile animal that can be utilized for a variety of purposes and has a high production rate. In today's world where deserts are expanding, global warming is increasing, and food and water scarcity are becoming more prevalent, camels can adapt to these challenges **(Abdurahman, 2004)**.

Moreover, under such circumstances, camel milk can be an excellent source of nutrition. Along with being a nutritious food, camel milk has been used for medicinal purposes in various cultures due to its therapeutic properties, primarily attributed to its high content of vitamin C. **(Yagil, 2017)**.

Camel milk has been shown to have potential medicinal properties and has been used in the treatment of various diseases, including but not limited to malaria, jaundice, gastrointestinal disorders, and strong cough (pneumonia), according to research studies **(Yadav, 2015)** and tuberculosis. **(Ilse and Hanwant, 2004)**.

Extensive research has confirmed the presence of numerous essential nutritional and pharmaceutical components in camel milk, such as insulin, which can regulate B-cell functions and act as an anti-hypoglycaemic agent. **(Shabo and Yagil, 2005)**.

Camel milk is an excellent source where lactic acid bacteria (LAB) can be isolated with high probiotic potential. Camel milk contains a greater amount of natural antimicrobial compounds the bovine milk **(Elagamy et *al*., 1996)**.

Introduction

The beneficial microflora found in camel milk, specifically lactic acid bacteria have the potential to be a valuable source of biological materials for use in dairy technology **(Khedid et *al.*, 2009)**.

lactic acid bacteria is currently the subject of extensive international research due to their role in the fermentation of many foods, as well as their ability to produce various antimicrobial compounds that promote probiotic properties (**Temmerman et al., 2003**), including antitumoral activity (**De Vuyst and Degeest, 1999), (Østlie et al., 2003)** reduction of serum cholesterol (**Desmazeaud, 1995; Jackson et al., 2002**) alleviation of lactose intolerance (**De Vrese et al., 2001**) stimulation of the immune system (Isolauri et al., 2000) and stabilization of gut microflora (**Gibson et al., 1997**).

Certain LAB strains that produce Exopolysaccharide (ESP) are commonly used in the production of fermented milk for improving its texture and viscosity **(Curk et *al.*, 1996)**, **(Ruas et *al.*, 2010)**. While dairy products were traditionally preserved through spontaneous fermentation, modern large-scale production techniques typically use starter systems with defined strains to ensure uniformity, safety, and quality of the final product **(Ross et *al.*, 2002)**.

The objective of this study is to investigate the antibacterial activity of lactic acid bacteria isolated from camel milk.

Chapter I
Bibliography synthesis

Camel milk refers to the milk produced by female camels. It is a nutritious beverage that has been consumed for centuries in regions where camels are prevalent, such as in parts of Africa, the Middle East, and Asia. Camel milk has gained attention for its potential health benefits and is considered an alternative to cow's milk for individuals who are lactose intolerant or have allergies to cow's milk.

Here are some key characteristics and aspects of camel milk:

I.1 Nutritional Composition

Camel milk is rich in various nutrients, including proteins, fats, carbohydrates, vitamins (such as vitamin C and B vitamins), minerals (such as calcium, iron, and potassium), and antibodies. It is lower in lactose compared to cow's milk, making it potentially easier to digest for lactose-intolerant individuals **(Farah, 1993).**

I.2 Flavor and Texture

Camel milk has a distinct flavor that is often described as slightly salty and sweet. Its texture is generally thinner compared to cow's milk, and it may have a slightly yellowish color.

I.3 Therapeutic Potential

Research suggests that camel milk may have potential therapeutic properties. It has been investigated for its antimicrobial, anti-inflammatory, and immune-boosting effects. Some studies have shown positive outcomes in managing conditions such as diabetes, autoimmune disorders, and gastrointestinal issues. However, further research is needed to fully understand and validate these potential benefits. **(Salminen et *al.*, 2004).**

I.4 Availability and Consumption

In regions where camels are prevalent, camel milk is consumed both traditionally and commercially. It can be consumed fresh or processed into various products like cheese, yogurt, and ice cream. In other parts of the world, camel milk is gaining popularity as a specialty or niche product, available in certain markets or through online sources. **(Breulmann et *al.*, 2007).**

I.5 Chemical composition

Camel milk typically has a salty taste, opaque white color, and normal odor. Its chemical composition showed in Table 1. The milk is composed of 90% water and 10% total solids. Camel milk is known for its high levels of iron and calcium, as well as other components such as insulin, whey acidic protein, peptidoglycan recognition protein, B-lactoglobulin, casein micelles, whey, and Omega-7. In comparison to cow milk, camel milk has higher concentrations of these substances. Researchers have reported that peptidoglycan recognition protein may have anti-cancer properties and could help control metastasis in breast cancer patients. **(Mal et *al.*, 2006).**

Table 1. Chemical composition of camel milk (Mal et *al.*, 2006).

Parameters (quantity)	Camel milk
Water (%)	90
Total solid (%)	10
Fat (%)	2
Insulin (µu/ml)	40.5
Pantothenic acid (Mg/ml)	0.9
B-lacto-globulin (Mg/ml)	0
Whey acidic protein (Mg/ml)	157
Peptidoglycan recognition protein (Mg/ml)	107
B-Lacto albumin (Mg/ml)	3500
Kappa casein (%)	5
Casein micelle (µm)	320
Whey protein (%)	1
Omega-6 (%)	3.5
Omega-7 (%)	11.6

I.6 Mineral profile

Table 2 shows the mineral content of camel milk during two stages of lactation, revealing a noticeable increase in mineral quantity during the later stages. Camel milk is known for its moderate amounts of iron and calcium, as well as its high acidity due to vitamin C, which lowers the pH and promotes nutrient absorption in the intestine **(Soliman, 2005)**. Additionally, camel

milk contains zinc, an essential mineral that helps maintain and improve the immune system. Notably, camel milk contains higher levels of trace minerals compared to other mammals, as reported by several studies **(Singh et *al*., 2006; Mirmirani et *al*., 2017)**.

Table 2. Mineral profile of camel milk in different stages (Singh et *al*., 2006).

Mineral	Early lactation	Late lactation
Ca	94.06±0.75mg	97.32±0.51
P	41.68±0.55mg%	47.17±0.52mg%
Mg	11.82±0.22mg%	13.58±0.31mg%
Na	29.70±0.53 mEq/L	35.49±0.89mEq/L
K	50.74±0.51mEq/L	71.86±1.43mEq/L
Fe	1.00±0.12mg/dl	-
Zn	2.00±0.02mg/dl	-
Cu	0.44±0.04mg/dl	-

I.7 Camel milk benefits

Due to its low fat and cholesterol content and rich supply of vitamins, minerals, and insulin, camel milk has been identified as a potential treatment for diabetes **(Khalesi et *al*., 2017)**. Several studies have reviewed the potential benefits of camel milk in managing diabetes. We can produce from camel milk many dairy products showed in **(Fig 1).**

Figure 1. Dairy product from camel milk.

- ✓ Production of cheese from camel milk is difficult due to the poor coagulability of the milk **(Breulmann et *al*., 2007)**.
- ✓ Camel milk yogurt Producing camel milk yogurt with a texture and consistency similar to that of bovine milk yogurt is challenging, as camel milk does not coagulate easily. As a result, the curd formed is often less firm, fragile, and heterogeneous, consisting of dispersed flakes, as reported by **(Attia et *al*., 2001)**.
- ✓ Camel milk powder: Currently, spray drying technology has been used to produce camel milk powder in countries such as Saudi Arabia, UAE, India, and Pakistan **(El-Agamy, 2017; Kamal-Eldin et *al*., 2022)**. Camel milk ice cream: Ice cream and frozen desserts are popular and widely consumed dairy products, especially in countries with hot climates such as the Middle East **(Muthukumaran et *al*., 2022)**.

I.8 Lactic acid bacteria effect against pathogenic bacteria

The ability of LAB to exhibit antibacterial activity against pathogenic bacteria stems from a multitude of factors. This antimicrobial effect arises from the combined influence of diverse biological elements derived from their metabolic activities.

✓ Lactic acid bacteria (LAB) lower the pH of the culture medium by producing organic acids. Homofermentative LAB mainly produce lactic acid, while heterofermentative LAB produce lactic acid along with other substances like acetic acid. This acid production inhibits the growth of pathogenic bacteria. LAB's remarkable tolerance to low pH levels, both inside and outside the cells, contributes to this inhibition. Lactobacillus, being well-adapted to acidic pH conditions, exhibits particularly strong antibacterial activity **(Cotter and Hill, 2003; Salminen et *al.*, 2004; Dworkin et *al.*, 2006)**.

✓ One important factor that has been recognized for a long time as a major contributor to the antimicrobial activity of LAB, especially lactobacilli, is hydrogen peroxide (H_2O_2). However, its effectiveness is diminished in this case due to the anaerobic incubation of the lactic bacteria **(Mami et *al.*, 2008)**.

✓ Lactic acid bacteria (LAB) possess the capability to produce peptides or proteins called bacteriocins. These bioactive compounds demonstrate antimicrobial properties that are usually specific to closely related bacterial species, without causing harm to the producing strain. Bacteriocins exhibit significant diversity in terms of their molecular weight, biochemical characteristics, and mode of action. While their range of activity can vary from narrow to broad, they primarily target Gram-positive bacteria **(Bayoub et *al.*, 2006; Salminen et *al.*, 2004)**.

Camel milk stands out for its remarkable antimicrobial activity when compared to cow's milk. This exceptional quality can be attributed to the presence of specific components such as lactoferrin, immunoglobulin, lysozyme, lactoperoxidase, and vitamin C **(Saboui et *al.*, 2009; Konuspayeva et *al.*, 2004)**.

It is important to note that the antibacterial activity of LAB can vary depending on the strain, environmental conditions, and interactions with other microorganisms. Additionally, the antibacterial activity of LAB is typically targeted towards specific bacteria and may not be effective against all pathogens.

Research on the potential antibacterial activity of lactic acid bacteria (LAB) has been extensively conducted, exploring various strains and their effectiveness against different pathogens. Here are some key findings from studies investigating LAB's antibacterial activity:

- *Lactobacillus spp*.: *Lactobacillus species*, such as *Lactobacillus acidophilus*, *Lactobacillus casei*, and *Lactobacillus plantarum*, have been shown to possess antibacterial properties against a range of pathogens. These include *Escherichia coli*, *Salmonella spp*., *Staphylococcus aureus*, and *Helicobacter pylori*. The antibacterial activity of *Lactobacillus species* is attributed to the production of organic acids, hydrogen peroxide, and bacteriocins.
- *Bifidobacterium spp*.: *Bifidobacterium species*, particularly *Bifidobacterium bifidum* and *Bifidobacterium breve*, have demonstrated antibacterial activity against several pathogens, including *Clostridium difficile*, *Enterococcus faecalis*, and *Shigella flexneri*. *Bifidobacterium spp*. exert their antibacterial effects through the production of organic acids, as well as competing for adhesion sites and nutrients.
- *Streptococcus salivarius*: *Streptococcus salivarius*, commonly found in the oral cavity and gastrointestinal tract, has been shown to inhibit the growth of *Streptococcus mutans*, a major contributor to dental caries. This antibacterial activity is attributed to the production of bacteriocins and hydrogen peroxide.
- *Pediococcus spp*.: *Pediococcus species*, such as *Pediococcus acidilactici* and *Pediococcus pentosaceus*, have exhibited antibacterial effects against various pathogens, including *Listeria monocytogenes*, *Bacillus cereus*, and *Enterococcus faecium*. *Pediococcus spp*. produce bacteriocins, organic acids, and hydrogen peroxide, contributing to their antibacterial activity.
- *Weissella spp*.: *Weissella species*, including *Weissella cibaria* and *Weissella confusa*, have shown antibacterial properties against pathogens like *Staphylococcus aureus*, *Salmonella enterica*, and *Vibrio cholerae*. *Weissella spp*. produce organic acids, bacteriocins, and antimicrobial peptides, contributing to their antibacterial activity.

I.9 Challenges

Despite its potential benefits, there are some challenges associated with camel milk. One major challenge its limited availability, as camel populations are concentrated in specific regions. Additionally, the high cost and logistical difficulties of milk extraction and transportation contribute to its limited accessibility in many areas.

In recent years, camel milk has attracted attention and interest from researchers, consumers, and the food industry due to its unique nutritional profile and potential health benefits. However, it is important to note that individual responses to camel milk can vary, and

more scientific studies are needed to fully understand its effects and establish evidence-based recommendations **(Saad et *al*., 2013).**

Experimental part

Chapter II

Materials and methods

II.1 Objective of the work

The aim of our study is to achieve the following objectives:

1. Physicochemical analyse of camel milk.
2. Isolate and identification of lactic acid bacteria from camel milk.
3. Evaluate the antibacterial activity of the isolated strains against pathogenic bacteria.

II.2 Duration and place of the work

Our experimental study was conducted at the microbiological laboratory of Ibn Khaldoun University, specifically at the Faculty of Natural and Life Sciences and the laboratory of animal Hygienic and pathology in Tiaret. The study was carried out from 06/02/2023 to 20/03/2023.

II.3 Materials and Product

The materials and products used in our work are shown in the following table

Table 3. Equipment and products used in different microbiological analyses.

Glassware	Name of equipment	Products	Culture media	Other
-Beakers (400ml,600ml) -Petri dish -Sterile Pasteur Pipette -Graduated pipette -Sterile flasks (250ml) -Test tube -Watch glass -Tubes -Water bath	-Autoclave -Shaker magnetics stirrer -Incubator Spectrophotometer -Cuvette -Balance -Refrigerator -Oven -Bunsen Burner -Lacto scan -pH meter -Thermo-Lactodensimeter	-NaOH Solution -Hcl -H_2O_2 -Distilled water -Peptone water Buffered	-M17 agar -Mrs agar -M17 broth -Muller Hinton agar	-Cooler -Forceps -Magnetic bar -Platinum loop -Micropipette tips -Filter paper -Sterile knife -Mortar and pestle

II.4 Sample collection

On February 9th, 2023 at 8:00 am, milk was collected from the Mosran region (Djalfa, Hassi Bah bah). it's shown in this **(Fig 2).**

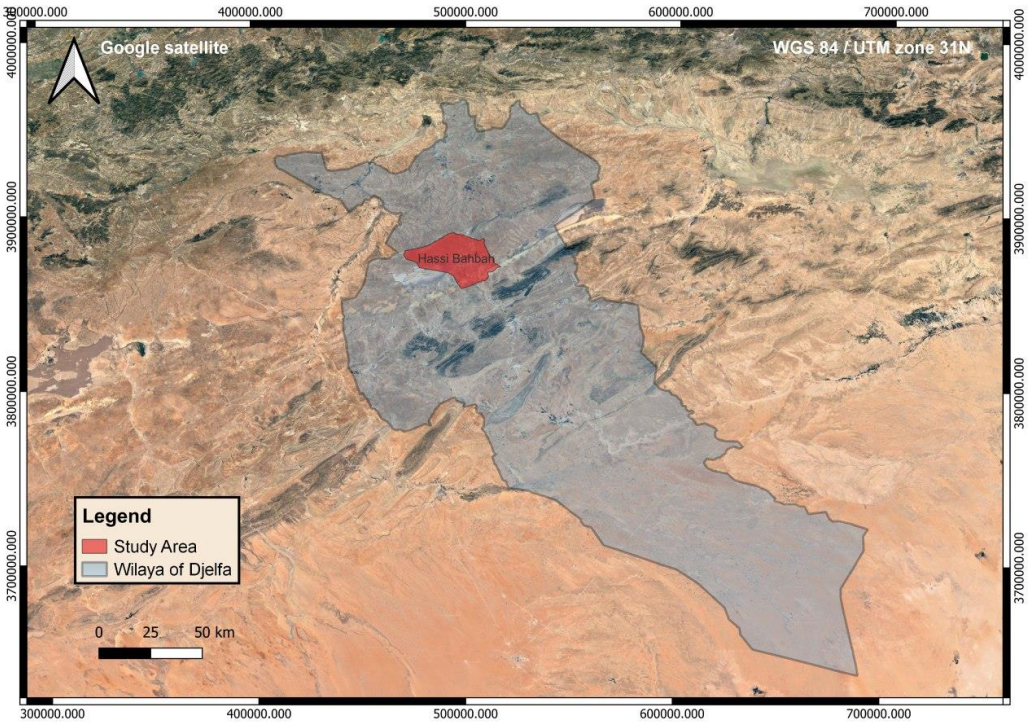

Figure 2. Area zone collection.

The milk was transported in a cooler and stored under favourable temperature conditions in a glass bottle. **(Fig 3).**

Figure 3. Material transport of milk.

The temperature and pH of the milk were measured immediately using a thermometer and pH paper. The weather conditions at the time of collection were 2°C **(Fig 4).**

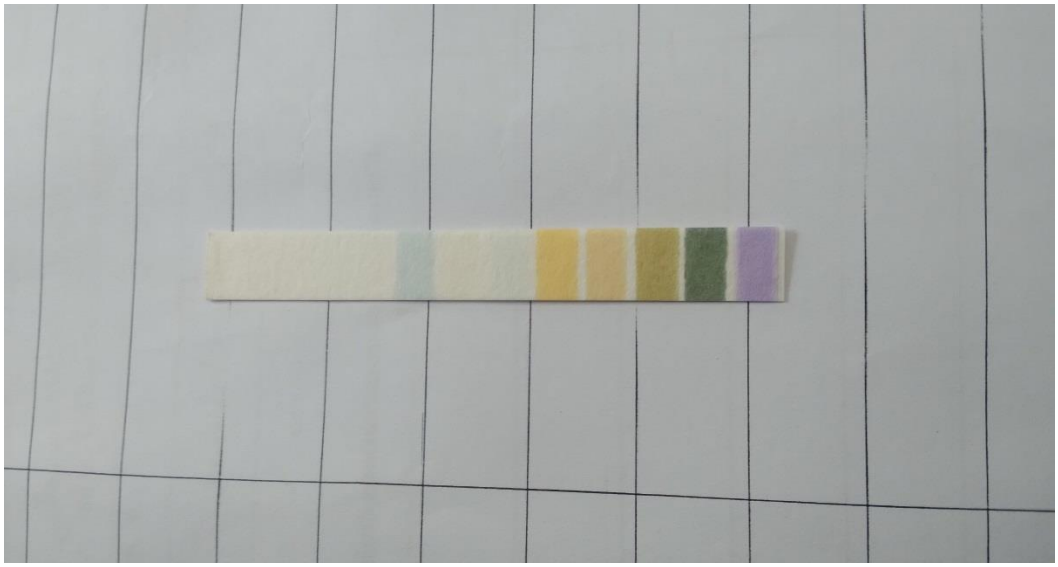

Figure 4. pH paper.

II.5 Biological material

In the current study, Pathogenic bacteria were used as indicator microorganisms to assess the inhibition of lactic acid bacteria. These pathogenic strains were chosen for their ability to serve as indicators of bacterial contamination and potential risks.

By studying the interactions between pathogenic and lactic acid bacteria, researchers hope to better understand how these microorganisms affect food safety and develop strategies to prevent contamination and infection.

The bacterial that we used were ATCC reference stains: *Escherichia coli* (ATCC 259222*)*, *Pseudomonas aeroginosa* (ATCC 9027), *Bacillus cereus* (ATCC 14579) and *Staphylococcus aureus* (ATCC 43300), from the laboratory of Microbiology, Faculty of Natural and Life Sciences, Tiaret University.

II.5.1 Probiotic

Lactobacillus Rhamnosus GG is a probiotic that forms part of the normal human intestinal microflora, genetically modified with a capsule that protects it from the acidity of unfavourable conditions (product marketed in France).

II.6 Experimental protocol

Our work steps are showed in the following figure

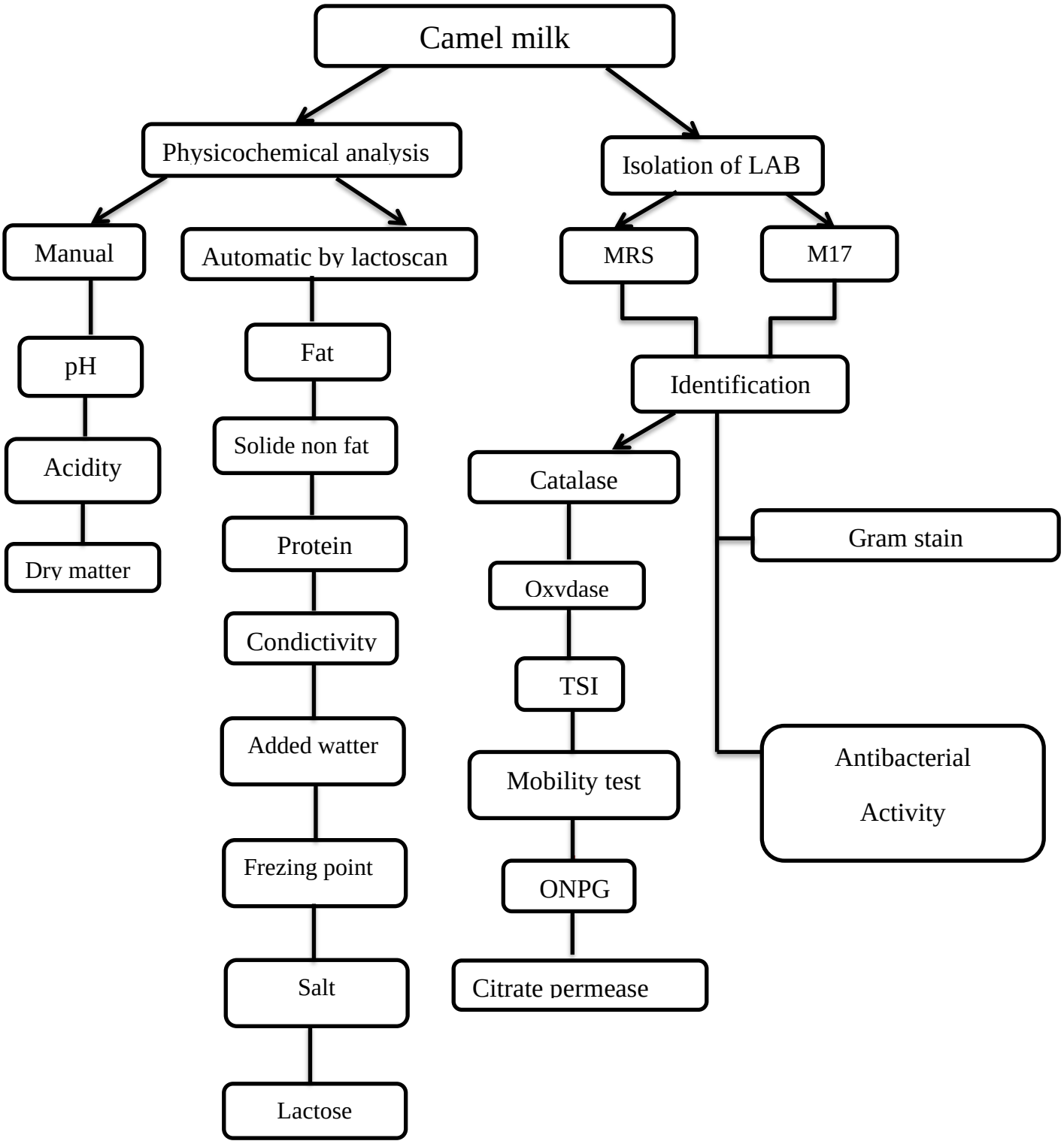

Figure 5. Protocol of work

II.7 Physicochemical characteristics

We have several physicochemical characteristics that we can studied on camel milk such as:

II.7.1 pH Measurement

The pH (hydrogen potential) of the samples was measured using a pH meter (INOLAB, pH 720, Germany). Prior to taking measurements, the pH meter was calibrated by adjusting the pH reading based on a solution of known pH (standard pH solution) **(Fig 6).**

The pH measurement was conducted following the principle of measuring the pH at a temperature of 20°C. An electrode was immersed in a beaker containing 100 ml of milk, and the pH value was directly read from the device. The procedure for pH measurement was carried out in accordance with the method described by **(Sboui et *al*., 2009)**.

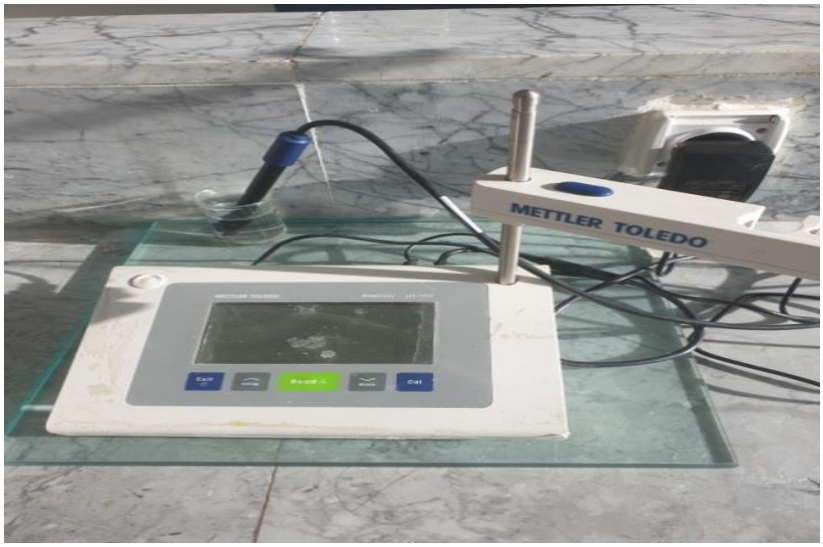

Figure 6. pH meter

II.7.2 Density

The density of the substance was measured as a physical parameter using a density meter in accordance with the **(AOAC, 2000)** method. Density is a measure of the mass contained in a given volume of a substance and is commonly expressed in units of grams per milliliter (g/ml) or kilograms per liter (kg/L).

II.7.3 Dornic acidity

Dornic acidity is a measure of the acidity or acid content in milk. It is determined by titrating the milk sample with a standard sodium hydroxide (NaOH) solution using phenolphthalein as an indicator.

To measure the dornic acidity of milk:

1. Prepare a standard solution of sodium hydroxide (NaOH) with a known concentration. This solution will be used to neutralize the acidic components in the camel milk.

- Weigh a predetermined amount of camel milk into a beaker.
- Add a few drops of phenolphthalein indicator to the milk. The indicator will exhibit a pink color in the presence of acid.
- Gradually add the standard NaOH solution to the milk while continuously stirring. Monitor the color change of the indicator. The pink color will disappear once all the acid in the milk has been neutralized.
- Record the volume of the NaOH solution used to neutralize the milk.
- Repeat the titration process three times and calculate the average volume of NaOH solution required to neutralize the milk **(AOAC, 2000)**.

II.7.4 Dry matter

Dry matter determination involves the following steps:

- Obtain a clean and dry container, such as a beaker or evaporating dish. Weigh the container and record its weight.
- Pour a measured quantity of the liquid sample into the container and record the combined weight of the container and liquid.
- Apply gentle heat to the container and liquid using a hot plate or similar heat source. Stir the mixture occasionally until all the liquid evaporates, leaving behind only solid matter.
- Allow the container to cool down to room temperature. Weigh the container and its contents again to determine the weight of the dry solid matter.

By subtracting the initial weight of the container from the final weight with the dry solid matter, you can determine the weight of the dry matter in the sample. This method is commonly used to assess the proportion of solids present in a liquid sample **(AOAC, 2000)**.

II.7.5 Parameters physical chemical by lacto scan

The LactoScan is an automated instrument used for the analysis of various physical and chemical parameters in milk and dairy products. It offers a quick and reliable method for assessing the composition and quality of milk. Some of the parameters that can be analysed using the LactoScan include **(Fig 7).**

- Fat Content: The LactoScan measures the fat content in milk through the principles of infrared spectroscopy. It provides accurate and precise measurements of the fat percentage.
- Protein Content: The instrument also determines the protein content in milk using infrared technology. It enables the assessment of the protein concentration in the sample.
- Lactose Content: Lactose, the milk sugar, can be quantified using the LactoScan. It provides information about the lactose content, which is essential for individuals with lactose intolerance.
- Total Solids: The LactoScan calculates the total solids in milk by measuring the combination of all solid components, including fat, protein, lactose, and minerals.
- Freezing Point: The freezing point of milk can be determined by the LactoScan. It is a useful parameter for assessing the dilution or adulteration of milk with water.
- pH Value: The LactoScan can measure the pH value of milk, which provides information about its acidity or alkalinity.
- Conductivity: The instrument also analyzes the conductivity of milk, which is influenced by factors such as temperature, mineral content, and presence of contaminants.

The LactoScan offers automated and efficient analyses for these physical and chemical parameters, providing valuable insights into the composition and quality of milk. Its accurate and reliable results make it a valuable tool in dairy industry and research settings.

Figure 7. Automatic analyzer lactoscan

II.8 Bacteriology characteristics

II.8.1 Isolation of lactic acid bacteria

The isolation and purification of lactic acid bacteria (LAB) involved a decimal dilution series using 1ml of milk, either directly or after incubation at 30 °C and 37 °C until coagulation. The milk samples were mixed with 9 ml of sterile physiological water solution (0.85% w/v) to obtain an initial dilution 1, which was then subjected to further dilution up to 10^{-4} **(Khedid et *al*., 2009)**. Next 100µl of each dilution was spread onto MRS and M17 solid medium plates, and incubated anaerobically in desiccator jars at either 30°C or 37°C for 48 to 72 hours. After incubation, individual colonies were selected and transferred to tubes containing 5ml of sterile MRS broth for purification. Homogeneous isolates were obtained through repeated subculturing on appropriate agar media. Microscopic examination was conducted to confirm the purity of the isolated bacteria **(Thapa et *al*., 2006).**

Here is a general protocol for using MRS and M17 media to culture lactic acid bacteria from camel milk :

- Label the bottom of each agar plate with the sample information and the type of medium used.
- Using a sterile loop or swab, aseptically streak the milk sample into the surface of the MRS and M17 agar plates. You can also spot inoculate the agar plates by placing a small drop of milk onto the surface of the agar and spreading it out with the sterile loop or swab.
- Incubate the plates upside down at 37 °C for 48-72 hours, or until visible colonies have formed.
- After incubation, examine the plates for colony morphology, counting the colonies if necessary, and identifying the lactic acid bacteria based on their morphology, growth characteristics, and biochemical tests.

II.9 Biochemical analyses

II.9.1 Catalase Test

The catalase test is used to detect the presence of the catalase enzyme in bacteria. A small portion of a suspicious colony is diluted in oxygenated water on a sterile slide. If the enzyme is present, bubbles of gas (oxygen) will be released, indicating its activity. This test is commonly used in microbiology to identify and characterize bacteria. The reaction can be

represented as: $2H_2O_2$ + Catalase → $2H_2O + O_2$. The rapid formation of bubbles confirms a positive result **(Delarras, 2007); (Karen, 2010).**

II.9.2 Oxidase Test

The oxidase test is conducted to determine the presence of the oxidase enzyme in bacteria. A disc is placed in a hemolysis tube and moistened with distilled water. A portion of the bacterial colony under investigation is spread onto the disc. After approximately 10 minutes, a dark purple coloration will appear, which eventually turns black, indicating a positive oxidase test **(Delarras, 2007).**

II.9.3 TSI Test

The TSI (Triple Sugar Iron) test is used to detect the fermentation of lactose, glucose, and sucrose by bacteria. The bacterial culture is inoculated by a deep puncture in the bottom of a tube and a central streak on the slant. The tube is then incubated at 37°C for 24 hours. The test is performed alongside the mobility test. If the bacterium being studied is capable of mannitol fermentation (Mannitol+), the sugar fermentation will cause the medium to turn yellow due to acidification **(Leveau, 1980).**

II.9.4 Mobility Test

The motility test assesses the ability of bacteria to move. The reduction product of D-mannose, mannitol, is used to test both mannitol fermentation and bacterial mobility. The strains being studied are inoculated into the medium by central puncture and incubated at 30°C±1°C for 18 to 24 hours. A yellow color change in the medium indicates mannitol fermentation, while a cloudy appearance due to diffusion in the agar indicates bacterial mobility. The test results are interpreted accordingly **(Guiraud, 1998).**

II.9.5 ONPG Test

The ONPG (O-Nitrophenyl β-D-galactopyranoside) test is performed to detect the presence of the beta-galactosidase enzyme in a bacterial culture. A bacterial culture is inoculated into a tube of sterile ONPG broth, which contains the ONPG substrate. The tube is then incubated at 37°C for several hours **(Mille, 1972).**

II.9.6 Citrate Permease Test

The citrate utilization test determines whether a bacterium can utilize citrate as a carbon source. A selected colony is used to inoculate the surface of a medium with tight streaks on one

half of the plate, leaving the other half as a control. The plate is then incubated at 37°C for 24 hours. A positive result is indicated by a blue color change of the indicator, as the medium becomes alkaline due to citrate utilization. A negative result shows no growth and results in a green coloration of the medium **(Guiraud, 2003).**

II.10 Gram Staining

Gram staining is a technique used to differentiate bacteria into two groups: Gram-positive and Gram-negative. The steps involved in Gram staining are as follows: a heat-fixed smear of the bacterial culture is prepared on a clean glass slide, which is then flooded with crystal violet stain for 1 minute. The slide is rinsed with water and flooded with iodine solution for 1 minute. Excess iodine is rinsed off. **(Forbes et *al*., 2007).**

II.11 Antibacterial activity

The antibacterial activity of an extract was assessed using the agar diffusion method, as described by (**Celiktas et *al*., 2007; Sacchetti et *al*., 2005)**

The antimicrobial properties of lactic acid bacteria were tested against pathogenic bacteria such as *Escherichia coli* (ATCC 259222), *Pseudomonas aeroginosa* (ATCC 9027), *Bacillus cereus* (ATCC 14579), and *Staphylococcus aureus* (ATCC 43300) Briefly, cells were resuspended in saline (1–2x10^8 cells/mL for bacteria (0.5 Mc Farland) and spread on the petri dishes of Mueller-Hinton Agar (MH) **(Bouziane et *al*., 2007).**

To obtain the supernatant, *Lactobacillus spp Streptococcus ssp* were removed from two Petri dishes and transferred to tubes containing M17 broth medium, also probiotic waq transfered to M17 broth after the tubes weres incubated at 37°C dring 18 h. The mixture was then centrifuged at 3000 rpm for 10 minutes to separate the supernatant and pellet. The empty tubes were filled with the supernatant. Sterile discs with a diameter of 6 mm were impregnated with with 0.5 mL of the supernatant and deposed in to Muller Hinton agar medium, and the probiotic served as a positive control. The antibacterial activity was determined by measuring the diameter of the clear zone (no bacterial growth) around the disc in millimeters **(Bouziane et *al*., 2007).**

Chapter III

Results and discussion

III.1 Physic-chemical result

The physic-chemical composition of camel milk analysed is summarised in the table below.

Table 4. Results of physicochemical characteristics by manual work

Parameter	Result	Standard
Ph	6.29	(6,6_6,8)(FAO,2006)
Density (g\ml)	1.028	1,028 et 1,034 à 20°C normes FIL-AFNOR
Acidity (°D)	16	L'idéal 15-17 (afnor)
Dry matter	8.2637%	8-13% (afnor)

Table 5. Results of physicochemical characteristics by lactose

Parameters	Result	Standard	Interpreting
Fat	0.94	0.1- 45%±0.10%	In the standard
Solide-non-fat	6.98	3-15±0.15%	In the standard
Protein	2.57	2-7±0.15%	In the standard
Conductivity	8.58	3-14(mS/cm) ±0.05%(mS/cm)	In the standard
Total solids	17.4	0-50%	In the standard
Frezing point	-0.425	-0.4 a 0.7°C	Minimum of standard
Salt	0.57	0.4-1.5% ±0.05%	In the standard
Lactose	3.84	0.01-6±0.2%	In the standard
Added water	18.26	0-70±3	In the standard

III.1.1 Physico-chemical characteristics

III.1.1.1 pH

The pH of our study is mentioned as 6.29, and it is compared with the pH result of the standard which is between (6.6 and 6.8). showed in figure

Based on the comparison, it appears that the pH of the camel milk in our study is slightly lower than the specified standard range of 6.6 to 6.8. A lower pH value indicates a higher level of acidity in the milk compared to the standard. There can be several factors contributing to this

variation, including differences in diet, environmental conditions, or the analytical methods used for pH measurement.

In our study, the measured pH value for camel milk is 6.29, while the reference pH value is 6.65 **(Debbouz et *al*., 2014).** This comparison suggests that the camel milk in our sample is slightly more acidic than the reference value. The pH and acidity of milk are influenced by factors such as the content of casein, mineral salts and ions, hygienic conditions during milking, total microbial flora and its metabolic activity, and milk handling practices. These factors can contribute to variations in the pH levels observed in different samples of camel milk **(Fig 8)**

Figure 8. pH result

III.1.1.2 Density

Upon comparing the values, it is evident that the specific density of our camel milk sample is 1.028, which aligns with the lower limit of the specified normal density range (1.028 to 1.034). This indicates that our sample falls at the lower end of the standard range.

In contrast, the density result of 1.032 reported by **(Debbouz et *al*., 2014),** is higher than the specific density of our camel milk sample, suggesting a slight difference between the two measurements.

On the other hand, the density result of 1.026 reported by **(Siboukeur, 2007)** is lower than the specific density of our camel milk sample, indicating a more pronounced difference between the two values.

It is important to note that several factors can influence the density of milk, including the fat content, temperature, solid content, and other variables **(Saboui et *al*., 2009).** These factors can contribute to variations in density measurements observed between different samples of camel milk **(Fig 9)**

Figure 9. Density result

III.1.1.3 Acidity

The titratable acidity of camel milk is less acidic; in our result, the dornick acidity is 16 D°, compared to the norm of **(AFNOR),** so our result is in the normal range recommended for consumption.

Our result of 16 °D is significantly lower than the sample with an acidity of **(Siboukeur, 2007)** acidity= 41.9°D. So there is a significant difference between the two measurements, with higher acidity in the 41.9°D sample.

In our experiment, the result is very close to the sample with an acidity of **(Debbouz et *al*., 2014).** The two measurements are quite similar with almost identical dornic acidity. This indicates consistency between the two samples in terms of titratable acidity **(Fig 10)**.

The pH and acidity of milk are influenced by various factors, including the levels of casein, mineral salts, and ions present in the milk. Additionally, hygienic conditions during milking, the overall microbial flora, and its metabolic activity, as well as the proper handling of the milk, can also impact the pH and acidity levels **(Saboui et *al*., 2009).**

Figure 10. Equivalent point (acidity)

III.1.1.4 Dry matter

The dry matter content of our camel milk sample, which is 85.15041 g/L, falls within the recommended range set by the standard (afnor). This confirms that our sample complies with the standard requirements for dry matter content.

The dry matter content of our camel milk sample, which is 85.15041 g/L, exhibits a notable contrast to the samples of (**Saboui, 2009)** 120.056 g/L and **(Ould Moustapha and Ould Hamadi, 2016),** 119.438 g/L**.** These results indicate a higher concentration of dry matter in those samples.

The total dry matter content of milk also varies depending on the lactation stage **(Bengoumi et *al.*, 1994).** It decreases during the month following calving and then increases due to the rise in fat and protein levels. **(FAO, 1995).**

III.1.1.5 Lactoscan interpreting

According to the standards our results are therefore within the range of standards, so thesample is consumable as shown in **(Fig 11)**.

Figure 4. Result of lactoscan

III.2 Isolation of lactic acid bacteria

First of all, camel milk inoculation is performed in two culture media (MRS, M17). The MRS medium is used for the growth of *Lactobacillus*, *Pediococcus*, and *Leuconostoc*. The M17 medium is used to facilitate the growth of *Lactococcus*, *Enterococcus*, and *Streptococcus*. **(Dworkin et *al.*, 2006).**

Following the seeding of the milk and its subsequent dilution (10^{-2},10^{-4}) in the designated isolation medium, the Petri dishes are incubated at a temperature of 37°C for 24 hours to facilitate the growth of LAB (lactic acid bacteria). This incubation process occurs in an anaerobic environment to reduce the formation of H_2O_2 (hydrogen peroxide).

The outcome of our study did not yield the isolation of lactic bacteria in the MRS medium from the analyzed milk sample. This could be attributed to its exceptionally strong resistance to bacterial multiplication in the initial stages of its presence. **(Kamoun, 1995).**

The catalase test is performed on the bacterial colonies present in the M17 plate. The catalase test results in two isolated bacterial colonies showed a positive reaction. The remaining colonies are preserved for further identification tests.

III.2.1 Macroscopic characteristics

To determine the macroscopic characteristics of the observed colonies, we performed a description of their size, shape, color, contour, and appearance, and conducted the catalase test. As showen in **(Fig 12)**

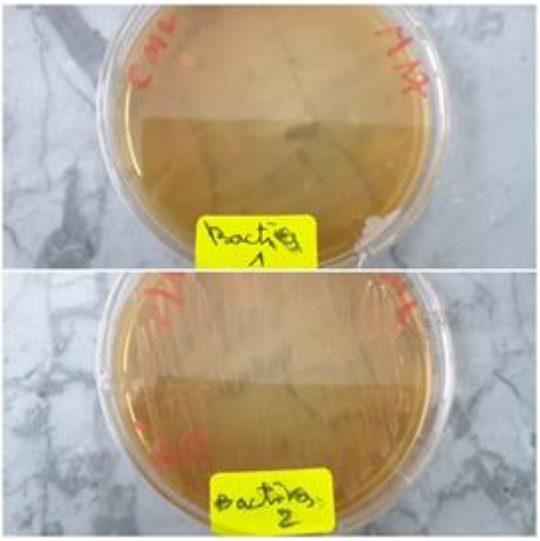

Figure 12. Lactic acid bacteria isolated result

III.2.2 Microscopic characteristics

After Gram staining, all isolates are examined under an optical microscope. The shape, Gram type, and cellular arrangement have been described.

The classification of bacteria is primarily determined by their shape and their affinity for dyes, as well as their arrangement (monotrichous, diplococci, chains). As shown in **(Fig 13).**

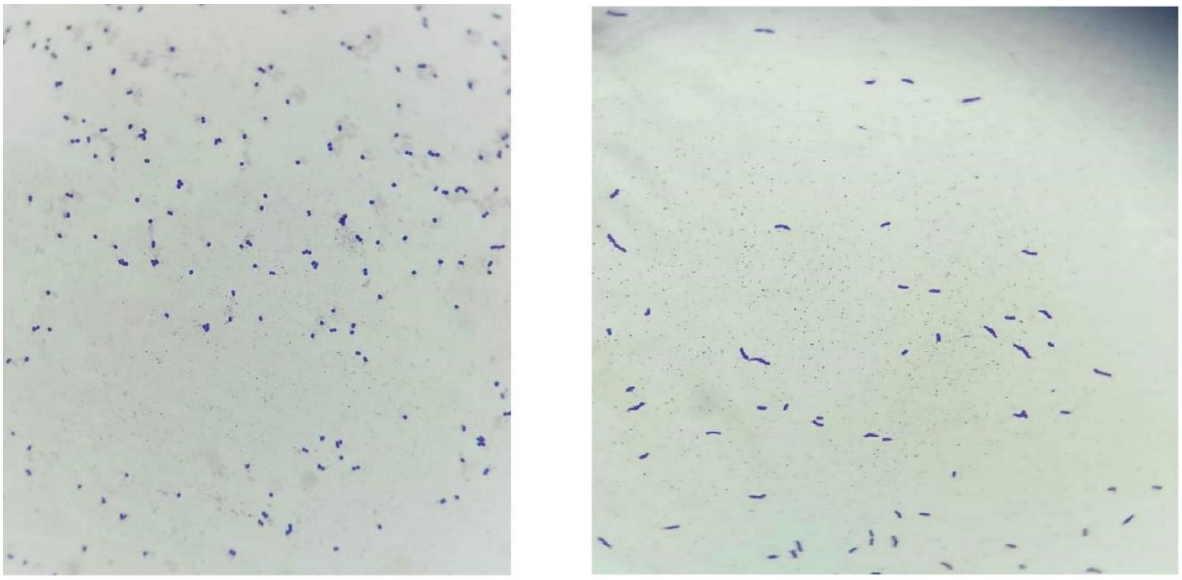

Figure 13. Microscopic observation result

–A- Bacteria coccus shape/ -B- Bacteria bacillus shape

III.3 Biochemical characteristics interpreting

III.3.1 Oxidase test

In the oxidase test, there is no color change, indicating that these isolates were negative for oxidase test (showing oxidase activity). In the case of two bacterial strains.

III.3.2 TSI test

In this test, both of the bacterial strain exhibits a positive outcome, characterized by a brown color change. The TSI test is commonly used to differentiate enteric bacteria based on their ability to ferment sugars, produce gas, and produce acid.

III.3.3 Mobility test

The outcome of this test has shown a negative result in the mobility test which indicate that, these bacterial strains are considered non-motile bacteria. They lack structures like flagella or cilia that enable movement. Instead, they rely on other means of dissemination, such as being carried by external factors like air, surfaces, or other organisms.

III.3.4 ONPG test

In this particular test, the appearance of a yellow color change in the disc indicates a positive result for the strain, indicating the degradation of lactose. However, for the second bacterial strain, the result is negative, suggesting that lactose degradation did not occur.

III.3.5 Citrate permease

The findings of this study reveal that both bacterial strains demonstrate a negative result, a negative reaction in the citrate permease test suggests that these bacteria do not possess the necessary enzyme or transport system to utilize citrate. They rely on other carbon sources for growth and energy, results are shown in the following table 6 and figures 14.

Table 6. Biochemical identification of the isolates

Parameter	*Lactobacillus spp*	*Streptococcus spp*
Catalase	-	-
Oxidase	-	-
TSI	+	+
Motility	-	-
ONPG	+	-
Citrate permease	-	+
Gram stain	+	+

Figure 5. Biochemical tests results

III.4 Antibacterial activity

In this study, the disc diffusion method on agar was chosen as the preferred method to assess the antibacterial activity. This method was selected due to its simplicity and its ability to provide a comprehensive evaluation of the antibacterial effects of LAB **(Polak-Berecka et *al*., 2009).**

The LAB strains that were isolated in this study were tested against both a probiotic containing 10 billion CFU (colony-forming units) and several pathogenic bacterial strains. The pathogenic strains included *Escherichia coli* (ATCC 259222), *Pseudomonas aeruginosa* (ATCC 9027), *Bacillus cereus* (ATCC 14579), and *Staphylococcus aureus* (ATCC 43300). These bacteria are commonly found in milk as contaminants, contributing to its deterioration and posing potential health risks to consumers. They can originate from various sources, such as the environment, fecal contamination, or infected udders, leading to their presence in milk **(Robinson, 2002).**

The results obtained from the study are presented in the accompanying table 7 and figures 15, 16, 17, which provide a comprehensive overview of the findings.

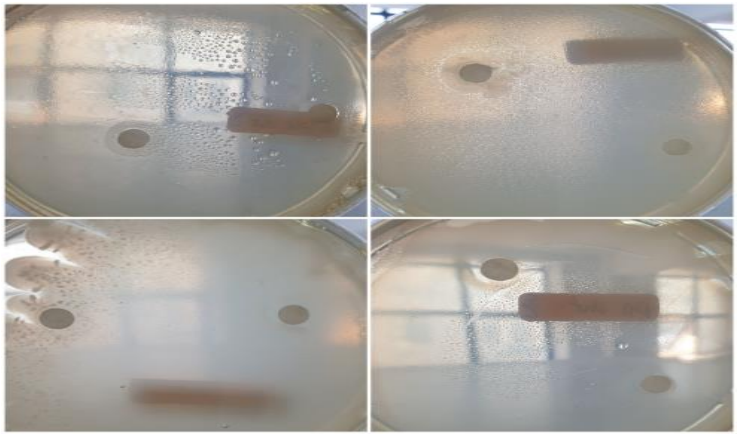

Figure 15. Probiotic against for pathogenic bacteria

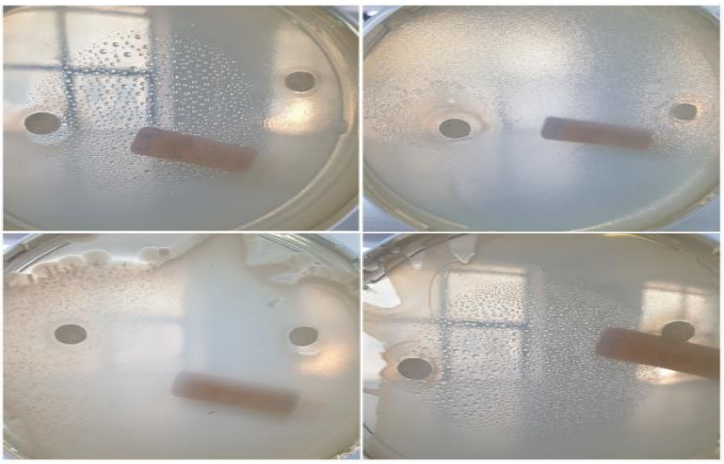

Figure 6. *Streptococcus* ssp against for pathogenic bacteria

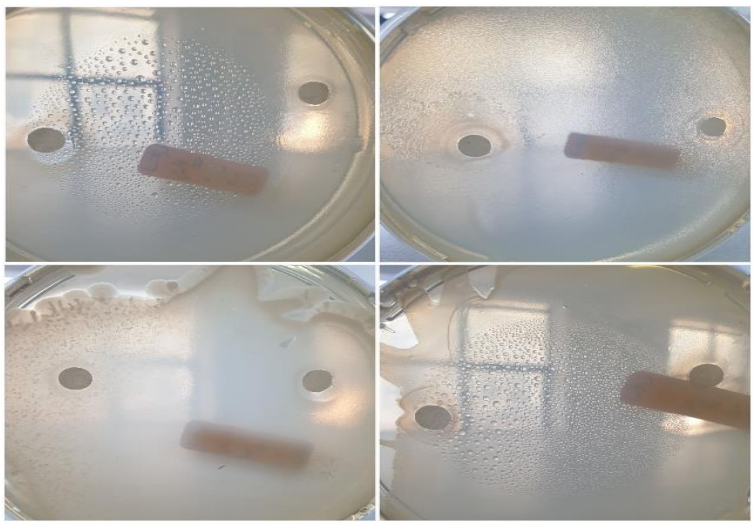

Figure 7. *Lactobacillus ssp* against for pathogenic bacteria

Table 7. Results and inhibition zone diameter (mm)

Strains / **Bacteria**	*Lactobacillus spp*	*Streptococcus spp*	Probiotic
E. coli	+ sensible (12 mm)	+ sensible (09mm)	+ sensible (12mm)
P. aeruginosa	+ sensible (11mm)	-Resistable (00mm)	+ sensible (7mm)
B. cereus	+ sensible (12mm)	-Resistable (00mm)	+ sensible (8mm)
S. aureus	+ sensible (16mm)	+ sensible (10mm)	+ sensible (14mm)

Interpreting

In this study, the disc diffusion method on agar was chosen as the preferred method to assess the antibacterial activity. This method was selected due to its simplicity and its ability to provide a comprehensive evaluation of the antibacterial effects of LAB **(Polak-Berecka et *al*., 2009).**

The LAB strains that were isolated in this study was tested against both a probiotic containing 10 billion CFU (colony-forming units) and several pathogenic bacterial strains. The pathogenic strains included *Escherichia coli* (ATCC 259222), *Pseudomonas aeruginosa* (ATCC 9027), *Bacillus cereus* (ATCC 14579), and *Staphylococcus aureus* (ATCC 43300). These bacteria are commonly found in milk as contaminants, contributing to its deterioration and posing potential health risks to consumers. They can originate from various sources, such as the environment, fecal contamination, or infected udders, leading to their presence in milk **(Robinson, 2002).**

The results obtained from the study are presented in the accompanying table and figures, which provide a comprehensive overview of the findings.

Based on the findings, all the bacterial colonies isolated from Petri dish 1 exhibited sensitivity to the tested pathogenic bacteria, including *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus cereus*, and *Staphylococcus aureus*. This suggests that the isolated bacterial strains may possess antibacterial properties or mechanisms that inhibit the growth of these pathogens. These results are consistent with a previous study conducted by **(Rahmeh et *al*., 2019)**, which supports the validity of the observations.

However, an interesting and unexpected observation was made in Petri dish 2. The colonies in this dish displayed resistance against Bacillus cereus and Pseudomonas aeruginosa, which contradicts the findings reported by **(Rahmeh et *al*., 2019).** This discrepancy may

indicate the presence of unique bacterial strains or variations in the tested conditions that led to different outcomes.

The use of probiotics allowed for the investigation of potential antibacterial activity against the pathogenic strains. By testing the effects of probiotics on the growth of these pathogens, researchers can determine if the probiotics possess inhibitory properties. The results of this study contribute to the understanding of the antibacterial potential of the isolated bacterial strains and their relevance in combating pathogenic bacteria.

The results obtained from the study have important implications for understanding the antibacterial activity of the isolated bacterial strains. The fact that all the colonies from Petri dish 1 showed sensitivity to the tested pathogenic bacteria suggests that these strains possess antimicrobial properties. This finding is promising and highlights the potential of these bacterial strains for applications in combating pathogenic infections.

The consistency of these results with a previous study by **(Rahmeh et *al*., 2019**) adds credibility to the findings and suggests that the observed antibacterial activity is reproducible. It also indicates that the antimicrobial potential of these strains is not an isolated occurrence but is likely to be a characteristic of these particular bacterial isolates.

On the other hand, the resistance observed in colonies from Petri dish 2 against Bacillus cereus and Pseudomonas aeruginosa is intriguing and requires further investigation. The contrasting results compared to the study **(Rahmeh et *al*., 2019)** raise questions about the underlying mechanisms or factors influencing the antibacterial activity of the bacterial strains in this specific context. Future studies could focus on exploring the reasons for this discrepancy and elucidating the possible factors responsible for the observed resistance.

The use of probiotics in determining the presence of antibacterial activity against pathogenic strains provides valuable insights into their potential therapeutic applications. Probiotics, which are beneficial microorganisms, have been increasingly recognized for their ability to inhibit the growth of harmful bacteria. By assessing the inhibitory effects of probiotics on the tested pathogenic strains, the study contributes to our understanding of their potential as alternative treatments for bacterial infections.

Overall, these findings contribute to the field of antibacterial research by shedding light on the antimicrobial properties of the isolated bacterial strains and their potential applications in combating pathogenic bacteria. Further investigation is warranted to explore the mechanisms underlying the observed resistance and to validate the use of these strains as potential therapeutic agents.

Lactic acid bacteria (LAB) are known to exhibit antibacterial activity, which can be attributed to several mechanisms:

- Production of organic acids: LAB are capable of fermenting sugars and converting them into various organic acids, such as lactic acid, acetic acid, and propionic acid. The low pH created by these organic acids inhibits the growth of pathogenic bacteria, as many pathogens are sensitive to acidic conditions. The acidic environment created by LAB can disrupt the pH homeostasis and metabolic processes of the target bacteria, leading to their inhibition or death. **(Corsetti and Settanni, 2007)**.
- Production of bacteriocins: LAB are well-known producers of bacteriocins, which are antimicrobial peptides with specific activity against certain bacteria. Bacteriocins can directly kill or inhibit the growth of closely related bacteria by disrupting their cell membranes or interfering with essential cellular processes. Some examples of bacteriocins produced by LAB include nisin, pediocin, and lacticin. **(Cui et *al*., 2012).**
- Competition for nutrients and adhesion sites: LAB can compete with pathogenic bacteria for nutrients and adhesion sites in the gastrointestinal tract and other body surfaces. By utilizing available nutrients and occupying adhesion sites, LAB can prevent the growth and attachment of harmful bacteria, thereby reducing their pathogenic potential. **(Ouwehand et *al*., 2016)**.
- Production of hydrogen peroxide: Some LAB strains have the ability to produce hydrogen peroxide (H_2O_2) as a metabolic by product. H_2O_2 exhibits antimicrobial properties by causing oxidative stress and damaging the DNA and proteins of target bacteria.
- Modulation of the immune system: LAB can interact with the host's immune system and stimulate the production of immune-modulating compounds, such as cytokines and antimicrobial peptides. These immune responses can enhance the host's defense mechanisms against pathogenic bacteria. **(Sengun et *al*., 2009).**
- Combination effects: Some studies have explored the synergistic effects of combining different LAB strains or LAB with conventional antibiotics. These combinations have shown enhanced antibacterial activity against pathogens, suggesting potential applications in combination therapy. **(Parvez et *al*., 2006).**

Overall, LAB demonstrate promising antibacterial activity against a range of pathogens through various mechanisms, including the production of organic acids, hydrogen peroxide, bacteriocins, and competition for nutrients and adhesion sites. Further research is ongoing to

explore their potential as natural alternatives or adjuncts to conventional antibiotics in various applications, such as probiotics, food preservation, and healthcare settings.

Conclusion

Milk plays a vital role in our daily diet, thanks to its balanced composition of essential nutrients, vitamins, and minerals. In our study, we investigated the factors influencing the physicochemical composition of camel milk. The microflora of Algerian camel milk consists of two types of bacteria: *Lactobacillus* and *Streptococcus*.

Through meticulous analysis, we discovered that lactic acid bacteria possess the remarkable ability to inhibit the growth of bacteria that can compromise milk quality and pose health risks to consumers. When combined with the inherent antimicrobial properties of camel milk, the lactic microflora acts as a defense mechanism against milk spoilage, particularly in hot climates. This dual defense not only extends the shelf life of milk but also holds therapeutic potential.

Within the Lactobacillus genus, a specific bacterium has shown exceptional effectiveness in inhibiting the growth of various tested pathogenic bacteria. It has also demonstrated resilience in acidic conditions and resistance to bile salts, indicating its potential as a beneficial probiotic.

To further explore the potential of camel milk, we propose the following actions: performing genotypic methods for reliable identification of isolates, studying the technological capabilities of strains to produce a variety of camel milk products, confirming the probiotic effects of isolates through additional in vitro and in vivo tests, and investigating the characteristics of lactic acid bacteria strains and their mechanisms of inhibiting pathogenic bacteria through lactoferrin, bacteriocins, and low pH.

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Appendix

Appendix 01:

Composition of isolation culture media

MRS agar (Fluka) (Quantity in g)

Peptone	10
Meat extract	8.0
Yeast extract	4.0
D(+)-Glucose	20
Dipotassium hydrogen phosphate	2.0
Sodium acetate trihydrate	5.0
Triammonium citrate	2.0
Magnesium sulfate heptahydrate	0.2
Manganese sulfate tetrahydrate	0.05
Agar	15
Tween80	1ml
Distilled water qsp	1L

pH 6.5 +/- 0.2 at 37°C. Autoclave at 121°C for 15 min.

M17 agar (Pronadisa) (Quantity in g)

Sodium glycerophosphate	19
Soybean peptone	5.0
Meat extract	5.0
Lactose	5.0
Meat peptone	2.5
Casein peptone	2.5
Yeast extract	2.5
Ascorbic acid	0.5
Magnesium sulfate	0.2
Bacteriological agar	12.75
Distilled water qsp	1L

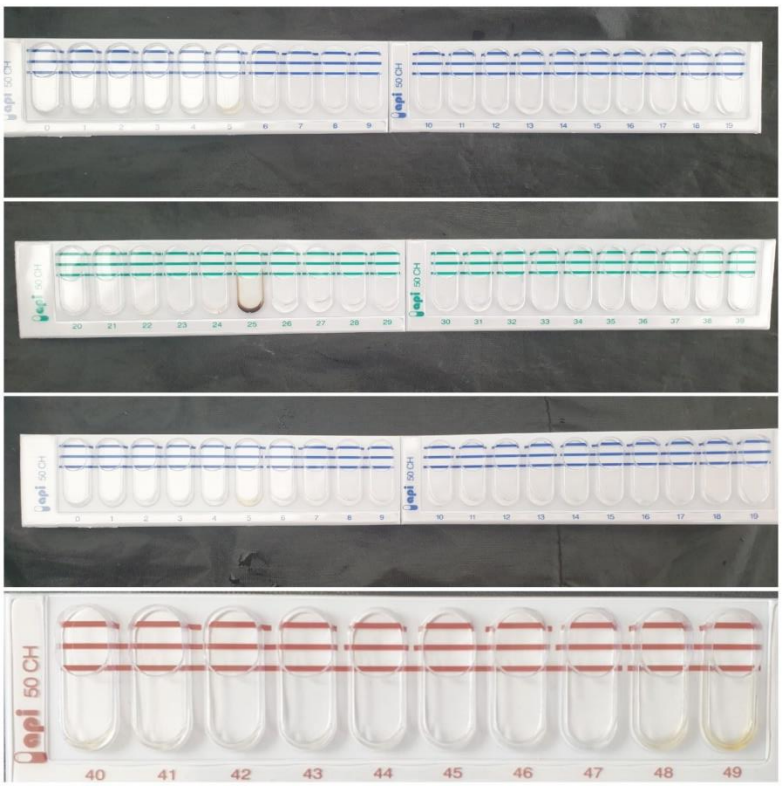
Final pH: 6.9 ± 0.2 at 25°C. Autoclave at 121°C for 15 min.

Appendix 02:

API 50 CH

The API 50 CH gallery consists of 50 microtubes that facilitate the study of substrate fermentation, which belongs to the carbohydrate and derivative family, including heterosides, polyalcohols, and uronic acids. To initiate fermentation, the gallery is inoculated with $N^0 1$ bacterial suspension. Over the incubation period, the fermentation process produces acid in anaerobic conditions, which causes a color change in the capsules. (Bio Mérieux, 2010)

The first tube is used as a negative control as it does not contain an active ingredient. To produce a suspension of opacity, several colonies from an 18-hour culture were added to API NaCl 0.85% medium (5mL) ampoules using a spectrophotometer (0.99). Similarly, the API 50 CH gallery inoculum was prepared in an API 50 CHB/E medium ampoule. The identification of the API 50 CH gallery was made using web-based identification software. The software identified the results of medium acidification, which was indicated by a color change of phenol red (pH indicator). (Bio Mérieux, 2010).



API 50

Appendix 03 : Lactoscan

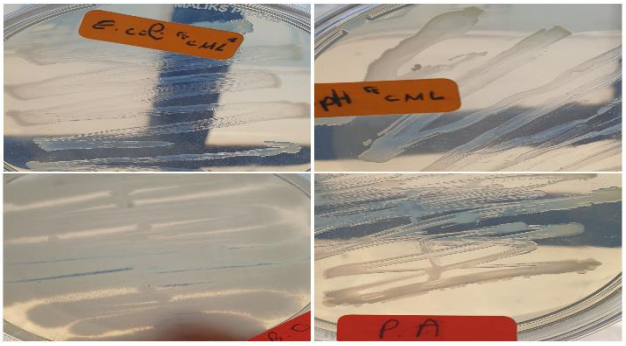
Operation mode

- 15 ml of the sample is poured into the sample holder of the analyser;
- The sample holder is placed in the cavity of the analyser and then press "Enter"
- The Lacto scan performs the analysis in 50 seconds;
- Cleaning is done automatically.

symbols representation of lactose

Parameters	Symbols
Fat	F
Solide-non-fat	SNF
Protein	P
Conductivity	C
Total solids	TS
Freezing point	FP
Salt	S
Lactose	L
Added water	a_w

Appendix 04 : Pictures



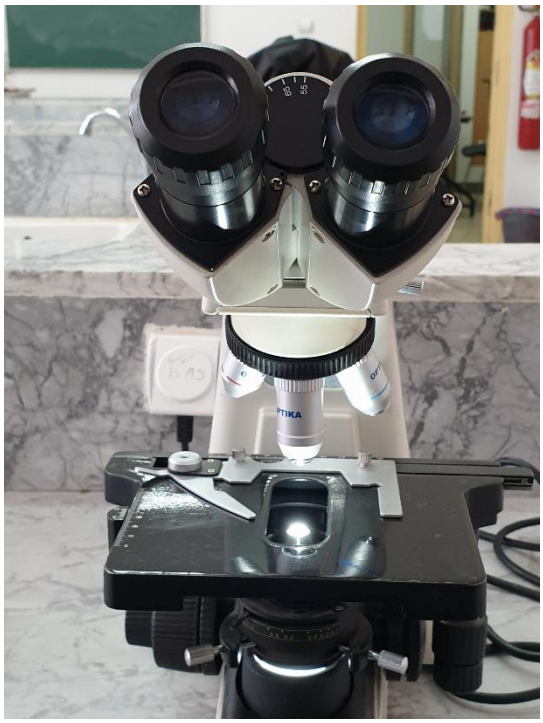
4 Pathogenic bacteria

Probiotic 10 milliards

Spectophotometer uv/vis

Autoclave

Oven



Microscope x 100

Desiccator