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EGYPTIAN ACADEMIC JOURNAL OF

# BIOLOGICAL SCIENCES

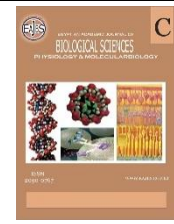
PHYSIOLOGY & MOLECULAR BIOLOGY



ISSN  
2090-0767

WWW.EAJBS.EG.NET

Vol. 14 No. 2 (2022)



## Study of the Characteristics and Antimicrobial Activity of Bacteria Isolated from Goat's Milk

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### ARTICLE INFO

#### Article History

Received:4/9/2022

Accepted:16/12/2022

Available:20/12/2022

#### Keywords:

Antimicrobial activity, Lactic acid bacteria, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853

### ABSTRACT

Lactic acid bacteria are a natural part of our environment. It has long been recognized that lactic acid bacteria have the property of producing antimicrobial substances and are used in the fermentation and bio-preservation of food. The study of lactic acid bacterial strains was carried out using raw goat milk, and the isolation of lactic acid bacteria allowed us to obtain 6 strains (Gram-positive and catalase-negative) which are then purified and preserved.

The study of all the phenotypic, biochemical and physiological characteristics (fermentation type test, growth test at pH 3, pH 9, pH 7, pH 4.5, growth test at different temperatures, the thermal resistance test and the oxygen effect test made it possible to study the criteria of isolated lactic acid bacteria. The results of the antibiogram show that strain 5 is sensitive to cephalixin. The 6 strains were also tested for their inhibitory power against three referenced pathogenic strains which are: *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, by the good diffusion method.

### INTRODUCTION

Lactic acid bacteria form a heterogeneous group composed of cocci and bacilli, the main characteristic of which is the production of lactic acid from the fermentation of sugars. Bacteria colonize various natural environments such as the surface of plants and the mucous membranes of mammals (intestinal, mouth, vagina and surface of the skin). Their use has appeared, for millennia, in the manufacture of foods such as cheeses, cold cuts, fermented drinks, sourdough bread, sauces, brines, fermented vegetables, silage, etc. They make it possible, through their metabolism, to increase the original shelf life of foodstuffs and give them a different flavor and texture (Badis *et al.*, 2005). These bacteria synthesize molecules with bactericidal and/or bacteriostatic actions such as organic acids, hydrogen peroxide, carbon dioxide, diacetyl, reuterin and bacteriocins (De Vuyst and Vandamme, 1994), these natural bacteriocins, produced by lactic acid bacteria, could be used to improve food quality and safety.

The only bacteriocin whose use is currently authorized as a food additive is nisin (E234), produced by the strain *Lactococcus lactis* (Delves-Broughton, 1990; De Vuyst *et al.*, 1996). The inhibitory activity of lactic acid bacteria can be attributed to several substances such as organic acids (lactic acid, acetic acid, etc.), CO<sub>2</sub>, reuterin, hydrogen peroxide, diacetyl and bacteriocins (Dortu et Thonart, 2009).

## MATERIALS AND METHODS

### The Objective of The Work:

The objectives of this study revolve around the following points:

Study and isolation of lactic acid bacteria from a dairy product which is goat's milk in the region of the wilaya of Sidi bel Abbès.

Our study was carried out at the level of the general microbiology laboratory in the department of biology, university of

Djilali Liabes of sidi bel Abbès, for a period of three months.

### Provenance et Collection D'échantillon:

The strains of lactic acid bacteria used in our study were well isolated from the sample which is raw goat's milk (Species: *Capra* black in color) which comes from a breeding farm located in the Faculty of Science and of nature (ITMA) in Sidi bel Abbès Algeria (Fig.1)

### Sample Collection Conditions:

- After washing with soapy water, rinsing with water then drying the udder, the milk was collected in sterile bottles (100ml/bottle).
- And transport immediately, and not exceeding 24 hours after collection.
- The samples are sent to the general microbiology laboratory in a cooler at 4°C, then are put in the refrigerator so that they can be used later, within 48 hours at most.



**Fig.1:** The method for taking samples from goat's milk (*Capra*) in the Sidi bel Abbés region.

### Biological Materials Used:

-Raw goat's milk, lactic acid bacteria are isolated directly from this milk

**Culture Media Used:** Several culture media were used (MRS medium, Mueller Hinton medium, Nutrient broth, Mannitol mobility).

**Antibiotic Discs:** To study the behavior of lactic acid bacteria towards antibiotics, five discs (applied microbiology laboratory) were used to carry out an antibiogram on a solid medium, this is Gentamicin (GEN 10µg), Cephalexin (CL 30 µg), Lincomycin (LIN 1530 µg)

**Study of Antimicrobial Activity:**The 6 strains were also tested for their inhibitory

power against three referenced pathogenic strains which are: *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923. *Pseudomonas aeruginosa* ATCC 27853, by the well diffusion method.

## RESULTS

### Bacteria isolation

During this study, total of (6) strains of lactic acid bacteria were isolated from raw goat milk on a selective medium, which is the MRS medium. After determining their characteristics, the strains are designated by a code consisting of three to four letters and a number, The isolated strains named: S1, S2, S3, S4 and S5 and S6 (Table 1).

**Table 1:** The codes of the lactic strains isolated.

Isolation medium	Isolated strain code
SRM medium	S 1
	S 2
	S 3
	S 4
	S 5
	S 6

**Purification and Characterization of Isolates:**

The characterization of the isolates according to their macroscopic appearance makes it possible to describe the appearance of the colonies and to determine the criteria relating to the colonies of lactic acid bacteria (size, pigmentation, contour, appearance,

viscosity).

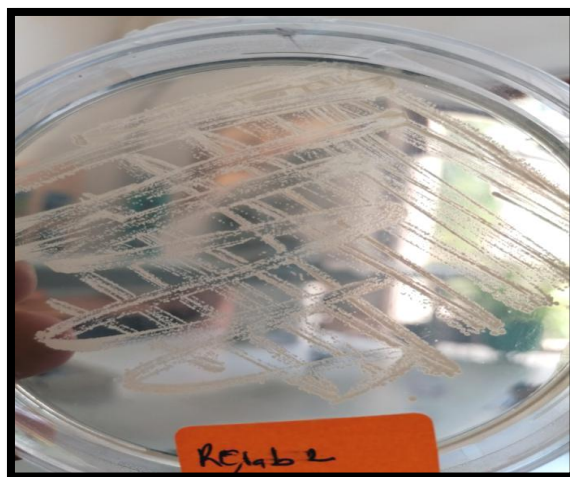
-White colonies with a circular shape of regular contours, of very small sizes approximately (0.5 and 1mm).

-Other cream-colored colonies with circular shapes of irregular contours, of sizes approximately (1 to 3 mm).

These colonies are obtained from Petri dishes containing solid MRS medium after 72 hours of incubation at 37°C, and subcultured in MRS broth and incubated at 37°C for (48 to 72 hours) in anaerobiosis.

**Macroscopic Characterization:**

The macroscopic observation of the lactic isolates on the solid medium shows the existence of small colonies of about 1m in diameter, of the lenticular shape of whitish or milky colors, with a smooth surface and a regular circular outline (Fig. 2).



**Fig..2:** Macroscopic appearance of colonies of isolated bacteria obtained after 27 hours of incubation at 37°C on a solid MRS medium.

**Observation on MRS Broth:**

The macroscopic observation of the growth of lactic acid bacteria in the liquid MRS medium is characterized by the appearance of cloudiness at the bottom of the tube with a transparent zone 5mm thick on the surface of the liquid medium (Fig. 3).



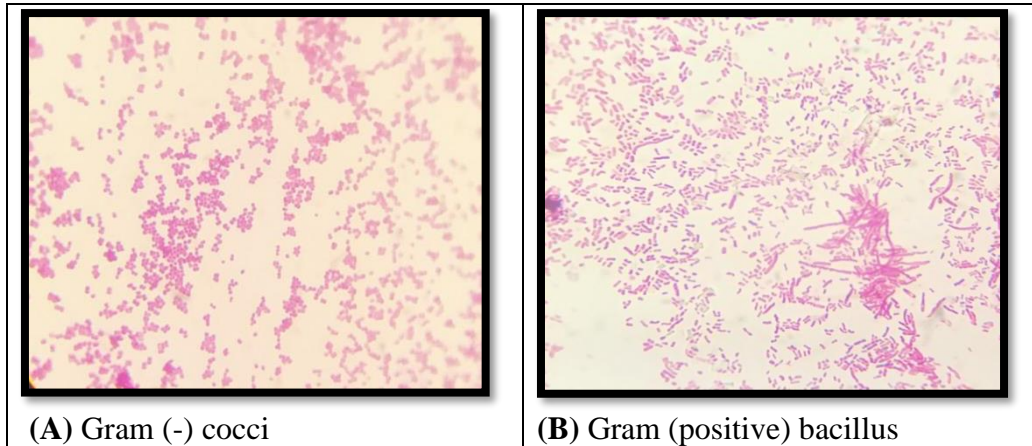
**Fig.3:** Macroscopic appearance of isolated bacteria obtained after 2 hours of incubation at 37°C in MRS broth.



**Microscopic Appearance:****Gram Stain:**

Gram staining reveals that these strains are all Gram-positive (Fig. 4) among the 6 strains isolated four isolates had a circular shape that is cocci. The rest appears

in the form of rods, it is bacilli with different modes of association: isolated, in pairs, in chains of variable length and in clusters. The morphological criteria are presented (Table 2).



**Fig.4:** Microscopic appearance of lactic acid bacteria after Gram staining (G×100).

**Table 2:** Presentation of the morphological and biochemical criteria of the 6 presumed isolates of lactic acid bacteria isolated from raw goat's milk.

Strain Code	Morphological and biochemical criteria	Macroscopic appearance of colonies	Microscopic appearance		Catalase test	
			The shape of the cells	Gram stain		
S1		Small size (2mm), round, milky whitish, regular outline	Cocci Short-chain	+	-	
S2		Small (1mm), round, whitish, milky, regular contour	Cocci In clusters	+	-	
S3		Small (1mm), round, bumpy, whitish, milky, translucent	Cocci In clusters	+	-	
S4		Medium (2mm), whitish, milky, round, regular edge	Bacillus	+	-	
S5		Medium (2mm), whitish, milky, round, regular edge	Bacillus	+	-	
S6		Small (1mm), round, whitish, regular outline	Cocci Scattered and in clusters	+	-	

+: positive; - : negative.

**Results of Physiological Tests:**

The results of this test revealed that all the strains isolated are catalase negative (Fig.5).



**Fig 5:** Catalase test results of isolated lactic acid bacteria.

**Fermentation Type Research:**

The 6 strains isolated and tested are all homofermentative, no gas production (CO<sub>2</sub>) in the bells was recorded (Fig. 6).



**Fig.6:** Results of the fermentation-type lactic acid bacteria test.

**Mannitol Mobility Test:**

After incubation of the test tubes, it is observed that all the bacteria have developed throughout the spike without invasion of the medium, generally, they are immobile (Fig.7).

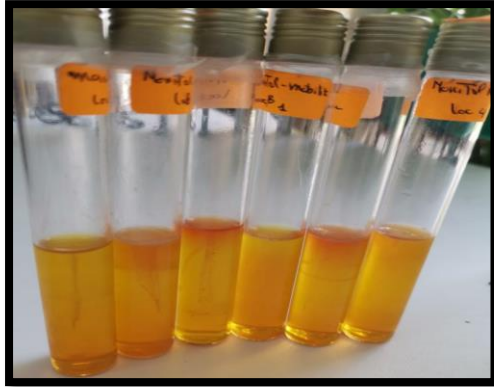


Fig.7: Mannitol mobility test results

**Growth Results at Different Temperatures:**

The study of the growth of bacterial isolates at the following temperatures: 30°C, 37°C, 45°C and incubation for 24 hours, indicates that all the strains isolated (S1, S2, S3, S4, S5 and S6) are capable to grow on MRS broth at the temperatures already mentioned.

**Heat Resistance Test:**

The majority of the isolated strains are heat-resistant, growth is observed on the MRS broth after the heat treatment (Fig. 8).

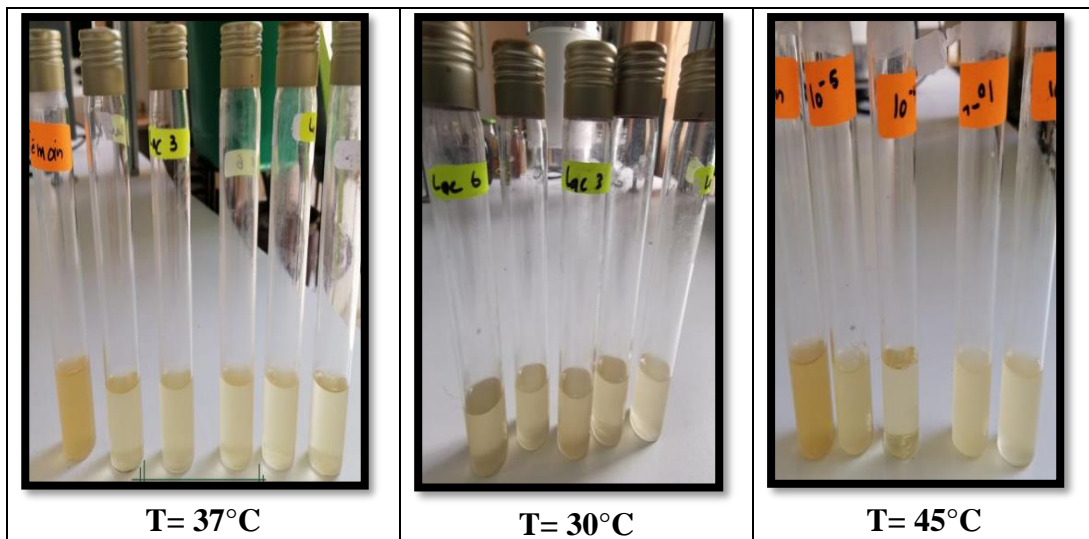


Fig.8: Growth test results at different temperatures

**Oxygen Effect:**

After incubation of the strains on the MRS agar medium in aerobiosis, growth

was observed for most of the strains tested in aerobiosis (Fig. 9).

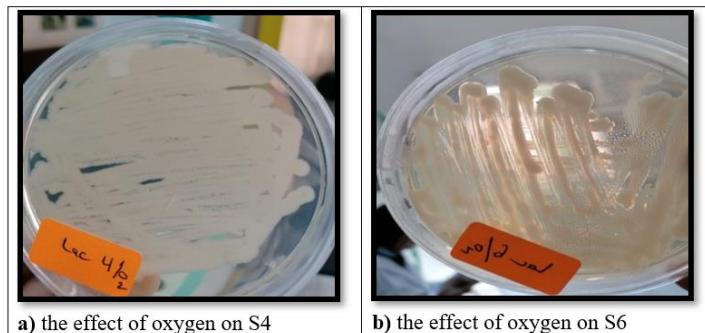
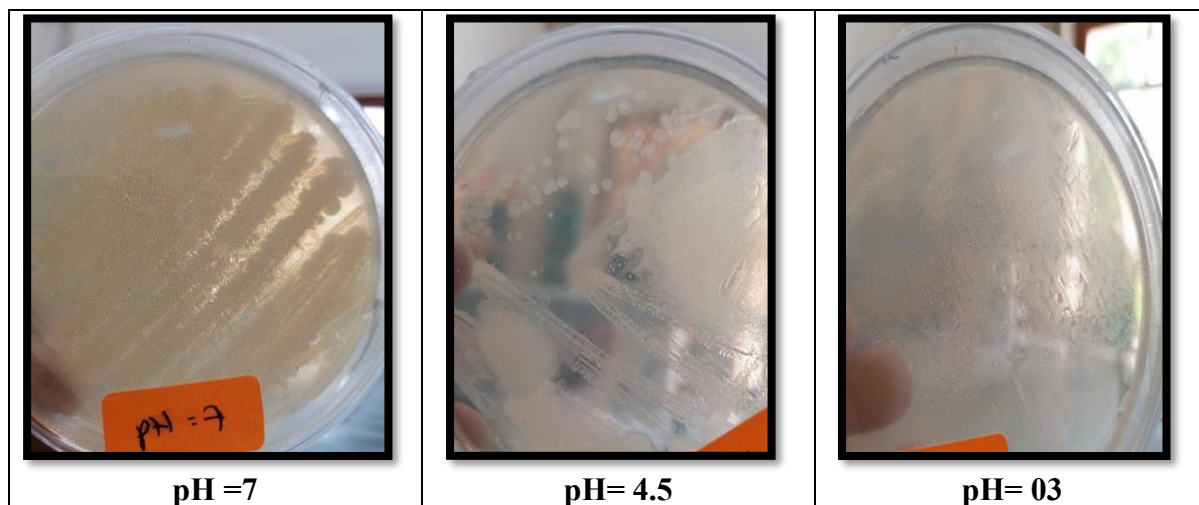


Fig.9.: Effect of oxygen on S4 and S6.

**Growth Test at Different pH Values:**

The culture of the lactic strains in different pH shows in the results that there is

bacterial growth of the strains at pH=4.5, pH=7, and pH=9. At pH=3 we noticed an absence of growth (Fig.10).



**Fig.10:** The results of the growth of lactic acid bacteria at different pH.

The results of the physiological and biochemical criteria of lactic acid

bacteria isolated from raw goat milk are mentioned in Table 3.

**Table 3:** The physiological and biochemical criteria of lactic acid bacteria isolated from raw goat milk.

	Criteria	T= °C			pH				O <sup>2</sup> effect	fermentative type	Mannitol Mobility	T=62 °C
		30	37	45	9	7	4.5	3				
Strains	S1	+	+	+	+	+	+	-	+	Homo	Immobile	+
	S2	+	+	+	+	+	+	-	+	Homo	Immobile	+
	S3	+	+	+	+	+	+	-	+	Homo	Immobile	+
	S4	+	+	+	+	+	+	-	+	Homo	Immobile	+
	S5	+	+	+	+	+	+	-	+	Homo	Immobile	+
	S6	+	+	+	+	+	+	-	+	Homo	Immobile	+

+ Presence of growth, - absence of growth.

**Antibiogram Test: (resistance of bacteria to antibiotics):**

Antibiogram results indicate that strain 5 is sensitive to cephalaxin and strain 4

is sensitive to lactic gentamicin are sensitive to antibiotics. The results of the antibiogram are mentioned in Table (4).

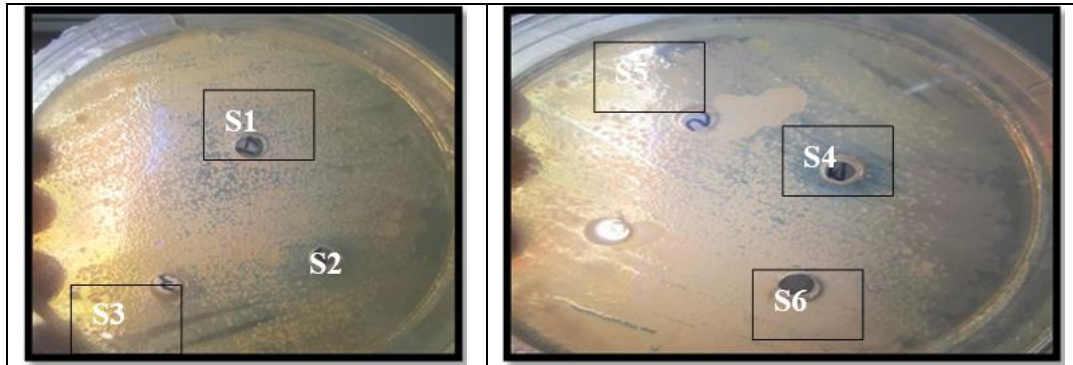
**Table 4:** The results of the antibiogram of the lactic strains isolated S: sensitive bacteria, R: resistant bacteria by mm.

Antibiotic	Charge disc	Disc symbol	The strains tested								
			MRS Solid Medium						Mueller-Hinton		
			S1	S2	S3	S4	S5	S6	S4	S5	S6
Cephalaxin	30µg	CL	R 0	R 0	R 30	R 30	S 35	R 25	R 32	R 28	R 25
Cephalaxin	10µg	GEN	R 14	R 0	R 24	R 21	R 20	R 20	S 30	R 25	R 25
Lincomycin	15µg	LIN	R 0	R 0	R 0	R 0	R 0	R 0	R 25	R 0	R 0

### Results of the Antagonistic Activity by The Good Diffusion Method on MRS Medium:

Our results show that S2 and S4 strains have antimicrobial activity against

*Escherichia coli* ATCC 25922 and S4 and S5 strains have antimicrobial activity against *Pseudomonas aeruginosa* ATCC 27853 (Table 5 and Fig. 11).



**Fig.11:** The results of the inhibitory effect of the lactic strains on the solid MRS medium against the E-coli strain by the good diffusion method.

**Table 5:** the spectrum of antibacterial activity of lactic strains by the good diffusion method

Strains	Antagonist activity by the good diffusion method		
	Diameter of inhibition zones in mm		
Indicators	<i>Pseudomonas aeruginosa</i> ATCC 27853	<i>Escherichia coli</i> ATCC 25922.	<i>Staphylococcus aureus</i> ATCC 25923.
S 1	00 mm	00 mm	00 mm
S 2	00 mm	10 mm	00 mm
S 3	00 mm	00 mm	00 mm
S 4	12 mm	10 mm	00 mm
S 5	29 mm	00 mm	00 mm
S 6	00 mm	00 mm	00 mm

### DISCUSSION

In our study, 6 strains of lactic acid bacteria were isolated from raw goat milk. They were cultured and isolated on MRS medium which is a rich medium that offers lactic acid bacteria different sources of nutrients that facilitate the growth of bacteria such as glucose and nitrogen, peptones and tween80. Due to the nutritional requirements of lactic acid bacteria, culture media must be very rich in sugars, nitrogenous materials and especially in growth factors (Pilet M-F, et al., 2005). The study of the main morphological, biochemical and physiological characteristics showed a diversity of genera and species isolated from raw goat milk. according to their microscopic appearance are cocci and

other Gram-positive rod-shaped strains, and non-spore-forming negative catalase (Teuber M. and Geis A., 2006). Phenotypic identification results for strain five (S5) indicate that this rod-shaped, Gram-positive, catalase-negative strain, according to (Hammes and Hertel, 2006) these bacteria are lactobacillus which is capable of fermenting glucose without gas evolution is homofermentative. According to Raynaud, (2006) lactic acid bacteria are Gram-positive microorganisms, non-spore-forming, non-motile, anaerobic but aerotolerant and do not possess catalase. All lactic acid bacteria have a fermentation metabolism allowing them, by using fermentable sugars, to produce mainly lactic acid but also other organic acids (acetic



acid, formic acid.). Test results of lactic acid bacteria growth at different temperatures indicate that there are strains that can grow at 45°C and others that are heat resistant. (Béal, 1989; Béal *et al.*, 2008) show that the optimum temperatures for activity are between 22° and 45°C depending on the bacterial species. The optimum growth temperature is generally lower than the optimum temperature for the production of lactic acid but higher than the temperature recommended for the production of bacteriocins (Béal, 1989; Béal *et al.*; 2008). The fermentation type test shows that all the strains are homofermentative, and immobile according to the classification of Kandler and Weiss (1986), certain species of the genus *Lactobacillus*, constituting group I of the lactobacilli, are included in this group. They ferment glucose into lactic acid exclusively via glycolysis and do not ferment pentoses. Homofermentative lactic acid bacteria include species of lactococci, pediococci, as well as certain lactobacilli. This pathway leads under optimal growth conditions to the production of two molecules of lactate and two molecules of ATP by glucose molecule consumed (Thompson and Gentry-Weeks, 1994).

The results of the growth of lactic strains to different pHs obtained to indicate the presence of growth at pH = 9, pH = 7, and the absence of growth at pH = 3; In lactococci and thermophilic streptococci, growth is greatly reduced at a pH below 5 and arrested at a pH below 4.5. So, lactic acid bacteria must be able to maintain a pH close to neutrality during growth because they are strong producers of lactate (Canteri, 1997). The antibiogram test indicates that the majority of the strains are resistant to the antibiotics used (Cephalexin 30µg, Gentamicin, 10µg, Lincomycin, 15µg), with diameters less than 35mm, and strain 5 and sensitive to Cephalexin with a diameter equal to 35mm. The study of antimicrobial activity shows that the S2 and S4 strains have antimicrobial activity against *Escherichia coli* ATCC 25922 and the S4 and S5 strains have antimicrobial activity against

*Pseudomonas aeruginosa* ATCC 27853. according to (Zacharof and Lovitt, 2012), lactic acid bacteria synthesize bacteriocidal agents that vary in their spectrum of activity. Many of these agents are structural protein bacteriocins.

## CONCLUSION

Lactic acid bacteria have had a primary interest in food for at least four thousand years man has used these bacteria for the fermentation of food and to develop preferentially in various ecosystems but also to be used as bio-conservatives. These bacteria play an important role in the prevention and treatment of bacterial and viral diseases through different mechanisms. The exploration of naturally occurring antimicrobials is receiving increasing attention due to the alarming frequency of bacterial resistance that threatens human life.

Our present study has confirmed the importance and diversity of lactic acid bacteria present in goat milk, so it is interesting to exploit the lactic acid bacteria present in human or animal ecosystems.

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