



Republic Of Iraq  
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# **ANTIBACTERIAL ACTIVITIES OF LACTOBACILLUS SPP. AGAINST E.COLI**

*A Research  
Submitted to the Council of the College of Veterinary Medicine/  
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Requirements for the Degree of Bachelor of Science in Veterinary  
Medicine Sciences / Veterinary Surgery*

*BY*

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بسم الله الرحمن الرحيم

{ فَتَعَالَى اللَّهُ الْمَلِكُ الْحَقُّ <sup>قُلْ</sup> وَلَا تَعْجَلْ  
بِالْقُرْآنِ مِنْ قَبْلِ أَنْ يُقْضَىٰ إِلَيْكَ وَحْيُهُ <sup>صَلِّ</sup>  
وَقُلْ رَبِّ زِدْنِي عِلْمًا }

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## **SUPERVISOR CERTIFICATION**

I certify that this research entitled (**Antibacterial Activities of Lactobacillus Spp. against E.Coli**), was prepared by **Nariman Ali Habib** under my supervision at the College of Veterinary Medicine/University of Al-Qadisiyah in partial fulfillment of the requirements for the degree of *Bachelor* of Science in Veterinary Medicine Sciences / Veterinary Surgery.

**Lect. Mushtaq Talib Hussein**

2018

## CERTIFICATE OF INSTRUCTOR

We certify that **Nariman Ali Habib** has completed the fulfillment of his graduation research entitled **(Antibacterial Activities of Lactobacillus Spp. against E.Coli)** for the year 2017/2018 under our construction.

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*April 2018*

## **DEDICATION**

*To The Women Whom The Prophet (Peace  
And Blessings Of Allah Be Upon Him And  
His Righteous Family) Recommended, The  
Woman That Without Allah Grace And Her  
Support I Wouldn't Be Standing Here Today...  
To My Mother*

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*I would like to thank my family, especially my mother, for her encouragement, patience, and assistance over the years, who has always kept me in her prayers.*

*Nariman*

## Summary

The study was performed at Veterinary Medicine Collage, University of Al-Qadisiyah from 2017 to 2018. The present study was aimed to detect Antibacterial Activity of *Lactobscillus* spp. Against *Escherichia coli* . For such aim, yoghurt sample were inoculated at 370C for various periods of incubation. The antibacterial activity of *Lactobscillus* spp. were determined in vitro.

*E.coli* showed a high sensitivity toward antibacterial materials of *Lactobscillus* spp.

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# Chapter One

## Introduction

## 1.1 Introduction:

*Lactobacillus* is a genus of Gram-positive, facultative anaerobic or microaerophilic, rod-shaped, non-spore-forming bacteria. (Makarova *et al.*, 2006).

it produce lactic acid from fermentation of carbohydrates (Suskovic *et al.*, 2001).

The antibacterial activity of lactobacilli app appears to be strain-specific because they act against pathogenic bacteria in the gastrointestinal tract or in the food through multifunctional ways, by secreting antimicrobial substances (H<sub>2</sub>O<sub>2</sub>, lactic acid and other organic acids and bacteriocins) (Eid *et al.*, 2016),

A large number of lactobacilli isolates, procured from traditional fermented foods prepared with the dairy materials, including *L.acidophilus*, *L.plantarum* and *L.rhamnosus* had excellent antibacterial activity against *Escherichia coli* standard strain (Mashak, 2016).

Many studies referred to the efficiency of probiotics to offer a proper alternative to the use of antibiotics in the treatment of enteric infection (Marteau *et al.*, 2001) or to reduce the symptoms of antibiotic- associated diarrhea (Rastall *et al.*, 2005).

Bacteriocins from lactic acid bacteria are considered safe additives, useful to control the frequent development of pathogens as broad range of antibacterial activity. The metabolic pathway of glucose may be homofermentative or heterofermentative. In the first case two molecules of lactate are generated and in the second, lactate, ethanol and carbon dioxide are produced. Sugar fermentation followed by a reduction in pH due to the production of lactic and other organic acids that lower the pH in the human intestine, is an important factor to inhibit of undesired microorganisms

growth. The low pH makes by organic acids allowing them to break through the cell membrane and reach the cytoplasm of pathogens (**Parada *et al.*, 2007**).

It has been shown that lactobacilli are implanted in the intestinal tract there seems to be suppressive effect on other members of the microflora .associated diarrhea and colitis have been controlled (**Shahani *et al.*, 1977**).

The bacterial (pathogenic) multidrug resistance and formation of biofilm lead to the lack of activity of antibiotics in the treatment of infection, while the administration of probiotics has been seen functional in preventing and/or counteracting the biofilm-related infection (**Vuotto *et al.*, 2014**).

## **1.2 The aim of study:**

Was to detect the antibacterial activity of *lactobacillus* spp. Against *E.coli*.

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# Chapter Two

## Literature Review

**2.1 History:**

In old days, the transformation of basic food materials into fermented foods was a mystery and a miracle, cause they had no idea what caused the sudden, dramatic, and welcomed transformation. In ancient times fermentation joined smoking, drying, and freezing as basic and widely practiced food preservation techniques. Wang and Hesseltine (1979) note that " the first fermentation were discovered accidentally when salt was incorporated with the food material, the salt selected certain harmless microorganisms that fermented the product to give a nutritious and acceptable food(**Parvaneh,2011**).

In present we know that fermentation is the chemical transformation of organic substances into simpler compounds by the action of enzymes, complex organic catalysts, which are produced by microorganisms such as molds, yeasts or bacteria.. Lactic acid bacteria (LAB) are one the most applied bacteria in fermentation. These bacteria produce lactic acid due to carbohydrate fermentation and are broadly used in the production of fermented foods, from dairy to fruits and vegetables products. The reasons for a widespread use of LAB are to make food durable, to improve its safety, flavor, appearance and texture and to enhance its physiological and hygienic value because of the presence of viable cells and valuable metabolites of LAB (**Semjonovs *et al.*, 2008**).

*L. acidophilus* is important in the fermentation of many foods especially dairy products the fermentation occurs when it break down sugars and carbohydrates to produce alcohol, carbon dioxide and lactic acid (**Narendranath *et al.*, 1997**).



production of flavor, and aroma that cause organoleptic properties of fermented foods as a result of The metabolic activity of this microorganism and inhibits food spoilage bacteria (**Leroy and De Vuyst, 2004**).

Elie Metchnikoff, he was the first scientist who proposed the therapeutic use of LAB for the prevention and treatment of several pathological conditions. (**Preidis and Versalovic, 2009**).

### **2.1.1 Probiotic:**

The word 'probiotic' is derived from the Greek meaning 'for life' and it had several different meanings over the years. It was first used by Lilley and Stillwell in 1965 for describing substances secreted by one microorganism which stimulated the growth of another that mean the opposite of 'antibiotic'. However using it in this form did not persist and it was used by Sperti (1971) to describe tissue extracts which stimulated microbial growth. In 1974 Parker used it in the context . His definition was 'Organisms and substances which contribute to intestinal microbial balance'. This definition related probiotic use to the intestinal microflora but the inclusion of 'substances' gave it a wide connotation which would include antibiotics. In order to improve the definition, Fuller (1989) redefined probiotics as 'A live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance'. This revised definition stressed the need for a probiotic to be viable. At the turn of the 20th century the concept of probiotics was introduced as live microorganisms, which when consumed in adequate amount, confer a health benefit on the host. Probiotics are have widespread application for preventing and treatment of disease especially gastro intestinal disease. Mechanisms of probiotics include

remodeling of microbial communities and suppression of pathogens, (Preidis and Versalovic, 2009).

Probiotic must be differentiated from prebiotic, In 1995, Glenn Gibson and Marcel Roberfroid introduced the prebiotic concept. They defined a prebiotic as ‘a nondigestible food ingredient that beneficially affects the host by stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and this improves host health (Gibson *et al.*, 2010).

A number of organisms are used as probiotics including *Lactobacillus*, *Streptococcus*, *Leuconostoc*, *Pediococcus*, *Bifidobacterium*, and *Enterococcus*, the main species that have probiotic characteristics are *Lactobacillus acidophilus*, *bifidobacteria*, and *Lactobacillus casei*. (Krishnakumar and Gordon, 2001, Holm, 2003)

**Table (2.1) : Prebiotics and Probiotics. (Sánchez *et al.*, 2017).**

| Prebiotics   | Probiotics   |
|--|--|
| Non-living, non digestible compounds such as certain carbohydrates   | Live microorganisms  |
| Serve as food for beneficial bacteria within the gut   | Bacteria or Yeast  |
| Available as food supplements and naturally occurring in certain foods, which include many fruits and vegetables | Available as supplements and in certain foods containing live cultures (yogurt, cheese, etc)                                     |
| Prebiotics aid digestion and may support the treatment of several chronic digestive disorders.                   | Probiotics may support the treatment of several chronic GI disorders, such as Irritable bowel syndrome, lactose intolerance, etc |

## **2.2 Lactobacillus:**

### **2.2.1 General Characteristics:**

*Lactobacillus* They are Gram positive, non motile, non spore forming, catalase negative, peroxidase positive, cytochrome absent, facultative anaerobes, aerolerant, nutritionally demanding bacteria. They are negative for nitrate reduction, gelatin liquefaction, indol and H<sub>2</sub>S production, they are acid tolerant with strictly fermentative metabolism (**Kalender and Weiss , 1986**).

### **2.2.2 Antimicrobial Properties:**

*Lactobacillus* bacteria produce organic acids like lactic and acetic acids, hydrogen peroxide and bacteriocins as antimicrobial substances. the main organic acids produced by *Lactobacillus* are Acetic, and lactic acids Other acids produced in small quantities include citric acid, hippuric acid, orotic acid and uric acid (**Lankaputhra and Shah, 1998a**). Lactic and acetic acids account for over 90% of the acids produced. Lowering of pH because of production lactic acid or acetic acid produced by these bacteria in the gut has a bacteriocidal or bacteriostatic effect . *Lactobacillus* species occupy the limited mucosal layer space and epithelial cell surface to prevent the growth of pathogenic microorganisms that can cause diarrhea (**Burr et al.,2005**). *Lactobacillus* species also control the pathogenic microbes by the production of antimicrobial agents, such as bacteriocins or bacteriocin-like molecules, organic acids, and hydrogen per oxide. Production of these antimicrobial agents suppresses the growth of harmful microorganisms .(**Ng et al., 2009**).

**2.2.3 Effectiveness against diarrhea :**

One of the main applications of *lactobacillus* has been treatment and prevention of diarrhoea. Selected probiotic strains are also effective against antibiotic associated diarrhoea. The strongest evidence of a beneficial effect of defined strains of probiotics has been established using *Lactobacillus rhamnosus* GG and *Bifidobacterium lactis* BB-12 for prevention and treatment of diarrhoea in children and animal mainly caused by several causes. (Saavedra *et al.*, 1994; Guandalini *et al.*, 2000)

There is some evidence that certain *lactobacillus* strains can inhibit the growth and adhesion of a range of enteropathogens (Cocconier *et al.*, 1993). There is also high evidence that *lactobacillus* strains can prevent traveller's diarrhoea (Hilton *et al.*, 1997).

A major problem compaine with antibiotic treatment is appear of diarrhoea, ., disruption of indigenous microflora because of antibiotic treatment leads to an increase in bad bacterial number and toxin production, which causes symptoms of diarrhoea. Administration of exogenous lactobacillus is required to return the balance of flora. Lactobacillus have proved to be useful as a prophylactic regimen with antibiotic-associated diarrhoea and for treatment after onset of antibiotic induced diarrhea (Armuzzi *et al.*, 2001).

### **2.2.4 Bacteriocins:**

Bacteriocins are proteins or complexed proteins biologically active with antimicrobial action against other bacteria, principally closely related species. It is produced by bacteria and not termed antibiotics to avoid confusion and concern with therapeutic antibiotics, which can potentially illicit allergic reactions in humans and other medical problems (**Deraz *et al*, 2005**).

In recent years bacterial antibiotic resistance has been considered a problem because of the extensive use of classical antibiotics in treatment of human and animal diseases (**Roy, 1997; Lipsitch *et al*, 2000; Yoneyama and Katsumata, 2006**). As a consequence, multiple resistant strains appeared and spread causing difficulties in use of antibiotics as growth promoters. So, the continue development of new classes of antimicrobial agents has become of increasing importance for medicine (**Kumar and Schweiser, 2005; Fisher *et al*, 2005**).

to control their abusive use in food and feed products, one plausible alternative is the application of some bacterial peptides as antimicrobial substances in place of antibiotics of human application.. (**Deraz *et al*, 2005**).

Bacteriocins differ from most therapeutic antibiotics in being proteinaceous agents that are rapidly digested by proteases in the human digestive tract. (**Saavedra *et al*, 2004**).

## **2.3 Escherichia coli :**

### **2.3.1 Characteristics :**

*Escherichia coli* also known as *E. Coli* is a gram-negative, facultatively anaerobic, rod-shaped, coliform bacterium of the genus *Escherichia* that is

commonly found in the lower intestine of warm-blooded organisms .(**Tenaillon *et al.*,2010; Singleton,1999**)

### **2.3.2 Properties :**

Most *E. coli* strains are harmless, but some serotypes can cause serious food poisoning in their hosts, and are occasionally responsible for product recalls due to food contamination.(**Vogt and Dippold ,2005**) The harmless strains are part of the normal flora of the gut, and can benefit their hosts by producing vitamin K2,(**Bentley and Meganathan ,1982**) and preventing colonization of the intestine with pathogenic bacteria, having a symbiotic relationship.( **Hudault *et al.*,2001; Reid *et al.*,2001**)

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# Chapter three

## Materials & Methods

The study was performed at Veterinary Medicine Collage, University of Al-Qadisiyah from 2017 to 2018.

### **3.1 Materials and Methods:**

#### **3.1.1 Materials:**

##### **3.1.1.1 Instruments and Equipments:**

The instruments and equipments used in the present study are listed in Table (3.1) :

**Table (3.1): Instruments and Equipments used in the Study**

| <b>Equipment</b>                 | <b>Manufacturing company/country</b> |
|----------------------------------|--------------------------------------|
| <b>Autoclave</b>                 | Gallen Kaamp / England               |
| <b>Incubator</b>                 | Gallen Kaamp/ England                |
| <b>Compound Light microscope</b> | Olympus/ Japan                       |
| <b>Platinum wire-loop</b>        | John Bolten/ England                 |
| <b>Anaerobic jar</b>             | Sigma Aldrich/US                     |
| <b>Sensitive balance</b>         | Kern PCB/Germany                     |
| <b>Petridish</b>                 | England                              |



**3.1.1.2 Chemical and biological materials:**

The chemical and biological materials used in this work were listed in Table (3.2).

**Table (3.2): Chemical and biological materials with their remarks**

| <b>Materials</b>                                   | <b>Manufacturers company/ state</b> |
|--|-------------------------------------|
| Gram stain   | Oxiod/UK                            |
| Hydrochloric acid                                  | BDH                                 |
| Hydrogen Peroxide (H <sub>2</sub> O <sub>2</sub> ) | BDH                                 |
| Kovac' s reagent                                   | Himedia                             |

**3.1.1.3 Culture media:**

Culture media used in this study were listed in table (3.2). They were prepared according to the manufacturer's instruction on their containers and sterilized according to the suitable method.

**Table(3.3) : Culture Media**

| <b>Media</b>                | <b>Company/state</b> |
|-----------------------------|----------------------|
| <b>Muller-Hinton agar</b>   | Oxoid/ England       |
| <b>Blood base agar</b>      | Himedia/ India       |
| <b>MRS agar</b>             | Himedia              |
| <b>Nutrient broth</b>       | Himedia/ India       |
| <b>Peptone water medium</b> | Himedia              |
| <b>Simmon citrate agar</b>  | Himedia              |

**3.1.2 Methods:****3.1.2.1 Stains:****3.1.2.1.1 Gram's staining:**

The isolated bacteria were examined by using gram staining . According to Collins et al., technique, and were observed under light microscope.

**3.1.2.2 Laboratory preparation of culture media:**

Culture medium listed in table (3.2) were prepared according to the manufacture instructions, autoclaved at 121 °C for 15 min, and used in appropriate tests.

**3.1.3 Standard bacteria:**

1. Escherichia coli

**3.2 Experiment design :**

Home made yogurt used in the current study. In order to isolate the lactic acid bacteria (LAB)...The media for this experiment was prepared using 6.2mg de Man, Rogosa and Sharpe (MRS) powder in 100 ml distal water and mixed until all the media homogenously together. Then into autoclave for sterilization for 15 minute and 121°C. After the media prepared and poured into petri dishes using two petri dishes for this experiment .The samples were cultured on (MRS) agar.The agar well diffusion method was used to determine the antimicrobial property of the LAB isolates. and inoculated in anaerobic jar were all put in the incubator for 24 hours in 37°C .After 24 hours the bacterial grow harvested. Bacterial suspension were made with 1 cc distal water. two slides were prepared using few drops from the suspension and stained with Gram stain .Using two petri dishes containing muller Hinton agar two of them cultured with Escherichia coli spreading over the surface of muller Hinton agar plates with a sterile cotton swab each one of these two containing five wells with diameter of 16 mm . The bacterial suspension were poured into each well.

**3.2.1 Identification of lactic acid bacteria:****3.2.1.1 Catalase test:**

A pure bacterial colony was taking by germs loop conveyor and placed on a clean glass slide, one drop of hydrogen peroxide were added to it. Formation of gas bubbles indicate positive result (**Nelson and George, 1995**).

**3.2.1.2: Carbohydrate fermentation test:**

Carbohydrate fermentation broth was inoculated with bacterial growth and incubated for 1-2 days at 37°C. Fermentation test was considered positive result when color of broth be changed to yellow ( **Collee *et al.*, 1996**).

**3.2.2 Identification of Lactobacillus:**

At the first step after isolation, morphological characteristic tests were done which included gram staining and physiological characteristics of LAB tests such as catalase.

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# Chapter Four

## Results & Discussion

#### 4.1 Isolation and identification of *Lactobacillus* spp. from home made yogurt

Result showed that after the suspected isolates were subjected to the microscopic examination by Gram staining, they all were found to be gram positive rods mainly grouped in chains and. Such characteristics are similar to those described by the common bacteria were *Lactobacillus* spp. and colonies were isolated from home made yogurt with typical characteristics white, small with entire margin, *Lactobacillus* showed negative result to motility test because they did not possess flagella were negative and catalase were negative. All isolates were isolated from fermented milk were found to ferment lactose. This result was resample to that obtained by (Kandler and Weis, 2005)

**Table(4.1):Biochemical tests**

| Biochemical tests             | Results                                  |
|-------------------------------|--|
| Motility test                 | -ve                                      |
| Gram stain                    | +ve                                      |
| Colonies shape and characters | white, small with entire margin colonies |
| Acid production               | +ve                                      |

#### 4.2 Antibacterial activity of *Lactobscillus* spp. in vitro :

The result showed the antimicrobial activity of *Lactobscillus* spp. against pathogenic bacteria (*E. coli*). Well diffusion method was used to estimation of this activity. *Lactobscillus* spp. had several types of antimicrobial material as lactic acid. This activity was performed after inocubation 24 hours inhibition zone appeared where the inhibitory zone against *E. coli* ranged from (10-11) mm

*E.coli* was showed the high sensitivity to the antibacterial action of *Lactobscillus* spp.. This was in agreement with (Manab *et al.*, 2011) and (Detha *et al.*, 2013) who reported that *E. coli* had the high zone of growth inhibition.

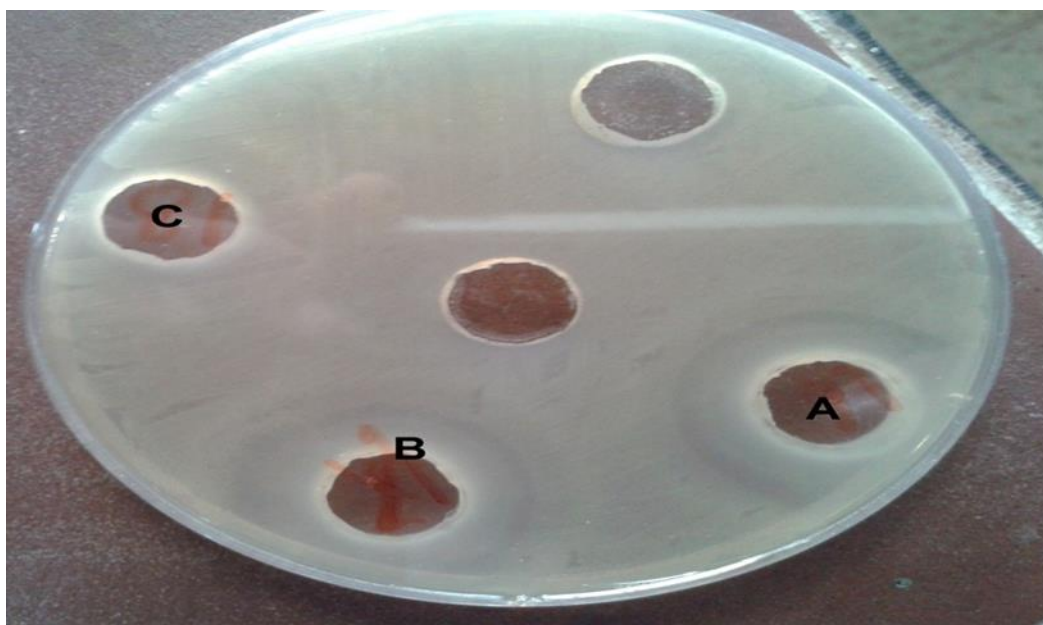
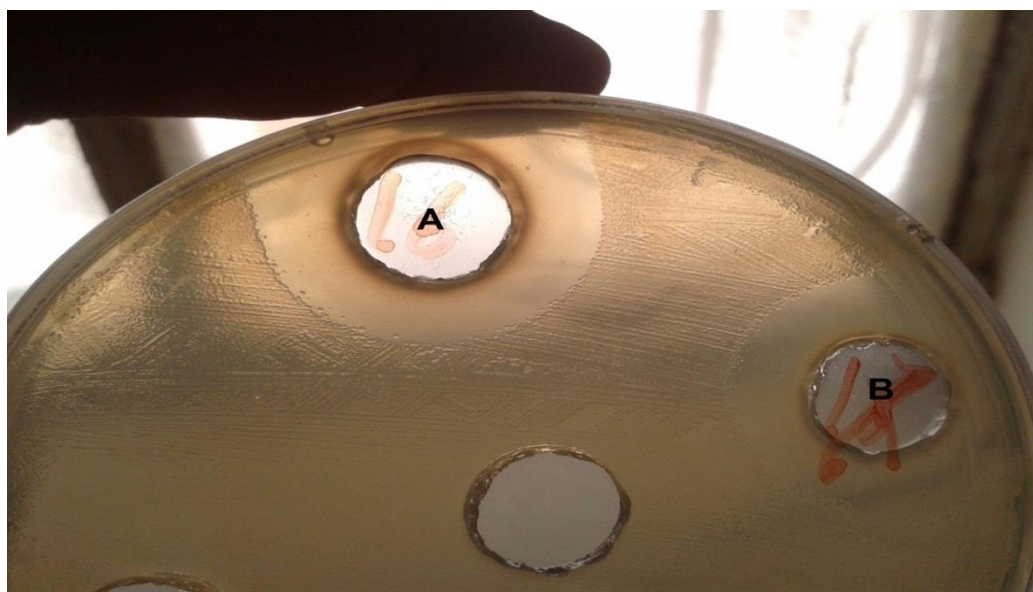
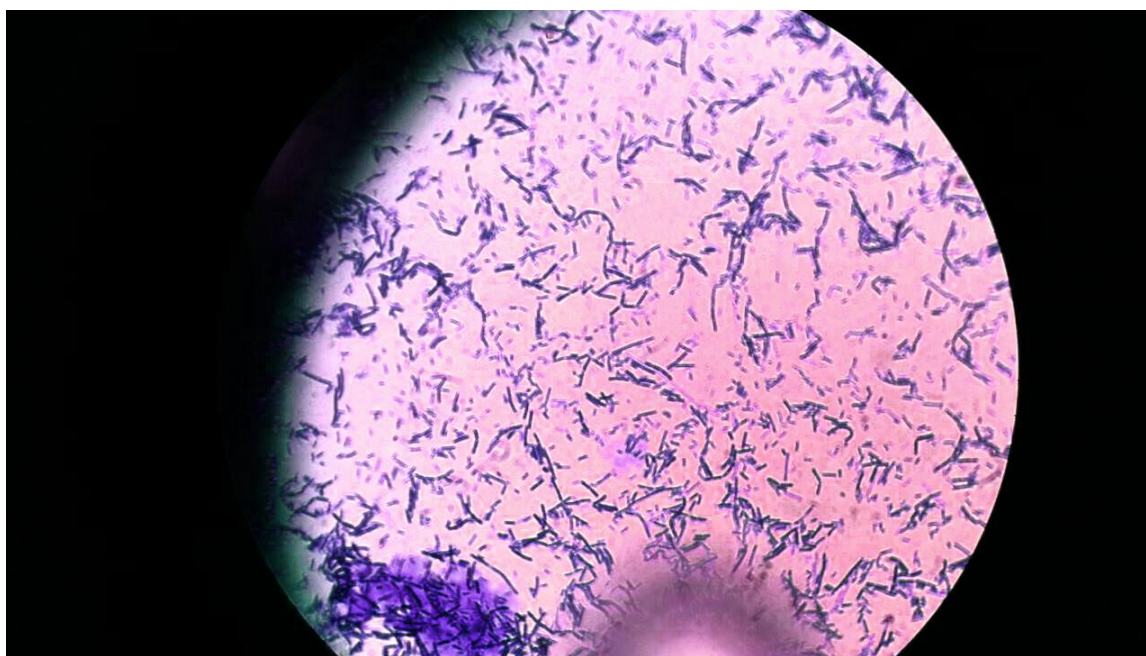


Figure (4.1 ): Inhibitory activity of *Lactobscillus* spp. against *E. coli*



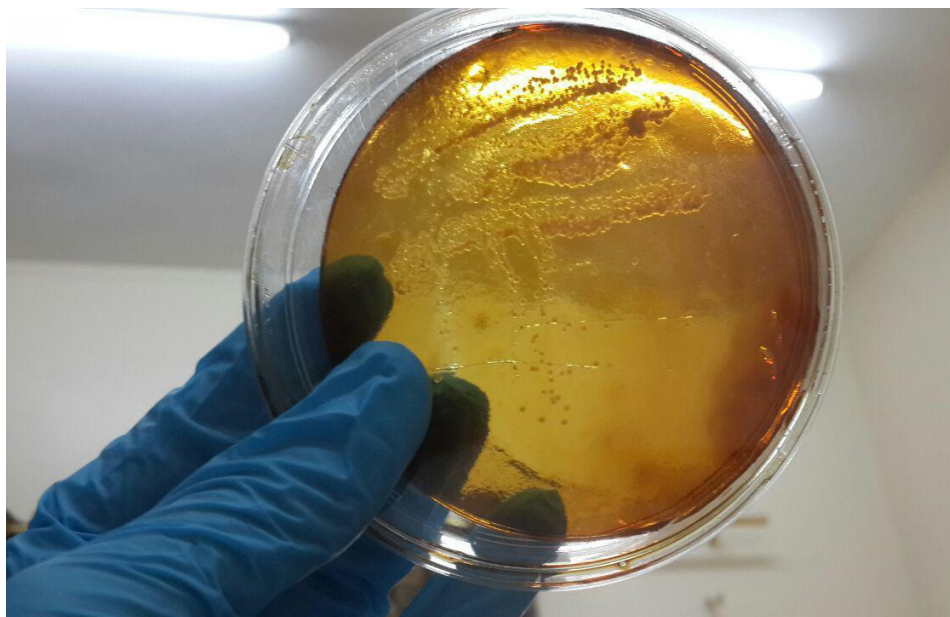


**Figure (4.2): Antibacterial activity of *Lactobacillus* spp. (A) and (B) against *E. coli*.**



**Figure(4.3) show lactobacillus spp under microscope which show gram positive**





**Figure(4.4) show growing of lactobacillus spp on MRS agar after  
24hours**



# Chapter five

## Conclusion & Recommendation

**5.1 Conclusions:**

1. *Lactobscillus* spp. had the ability to kill or inhibition of bacterial growth.
2. The antibacterial materials of Lactic acid bacteria were effective against pathogenic bacteria as *E. coli*

**5.2 Recommendations:**

1. Reduce the use of drugs or/and the tendency to use probiotics as an effective alternative to drugs that have similar work action (for example using probiotic in capsules as a treatment for related cases of *E.coli* infection )
2. Conduct accurate research to see if Probiotics are specific in action against pathogenic *E.coli* (strain specific) or not

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