

Antimicrobial activity of lactic acid bacteria isolated from minced beef meat against some pathogenic bacteria

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Abstract

In this study we achieved to isolate and identify Lactic Acid Bacteria (LAB) from minced beef meat by using de Man Rogosa and Sharpe agar (MRS) medium. The influence of antimicrobial activities were obtained by using the agar well diffusion method (Muller Hinton Agar) against some members of gram positive and gram negative pathogenic bacteria involved (*Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Klebsiella pneumoniae*, *Proteus* spp., *Salmonella* spp., *Corynebacterium* spp., *Streptococcus pneumoniae* and *Staphylococcus aureus*). The results show that the LAB can inhibit these bacteria by develop a zones around the well which contain these LAB.

Keywords: Lactic Acid Bacteria, Beef meat, Antimicrobial activity.

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الفعالية ضد ميكروبية لجرثومة *Lactobacillus* المعزولة من لحم البقر المثلوم لبعض أنواع الجراثيم المرضية

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الخلاصة

تم في هذا البحث عزل وتشخيص جرثومة Lactic Acid Bacteria (LAB) من لحم البقر المثلوم باستخدام وسط de Man Rogosa and Sharpe agar (MRS). وقد تم ملاحظة فعالية ضد ميكروبية باستخدام طريقة الانتشار للحفر بالاكار وباستخدام وسط (Hinton Agar) لبعض أنواع الجراثيم الموجبة والسالبة لصبغة كرام التالية: (*Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Klebsiella pneumoniae*, *Proteus* spp., *Salmonella* spp., *Corynebacterium* spp., *Streptococcus pneumoniae* and *Staphylococcus aureus*). أظهرت النتائج أن جرثومة (LAB) تمكنت من تكوين مناطق تثبيط مختلفة حول الحفر الحاوية عليها.

Introduction

Meat is a the major source of protein and valuable qualities of vitamins for most people in many parts of the world, thus they are essential for the growth repair and maintenance of body cells and necessary for our everyday activities (1). Due to the chemical composition and biological characteristics, meats are highly perishable foods which provide excellent source for growth of many

hazardous microorganisms that can cause infection in humans and spoilage of meat and economic loss (2). The most important bacterial spoilage of meat were caused by lactic acid bacteria which are physiologically related group of fastidious and ubiquitous gram positive organisms includes many species such as *Lactobacillus*, *Leuconostoc*, *Pediococcus* and *Streptococcus* (3). As well as these bacteria are widely used for preservation of wide range of foods e.g.: meat, fermented foods and milk (4). The

preservative activity of these bacteria are due to their ability to produce a variety of antimicrobial substances as a natural competitive means to overcome other microorganisms sharing the same niche, among them, ethanol, formic acid, acetone, hydrogen peroxide, diacetyl and bacteriocins (5). The antimicrobial spectrum against competing natural flora was frequently includes spoilage bacteria and food-borne pathogens such as *L. monocytogenes* and *S. aureus* (6) and (7).

The aim of this research was to study the effect of the cell free culture LAB as an antimicrobial feature to inhibit growth of some gram positive and gram negative bacteria associated of meat spoilage. Limited studies were carried out about the important role of bacteriocins for preservation and extension of minced meat by using LAB as a biopreservation in Mosul city as a native work.

Materials and methods

Isolation of LAB

Samples of minced meat were collected randomly from different butchers shop in Mosul city, these samples were transported to the laboratory immediately using cool box (4°C) and tested directly. LAB were isolated from minced meat by adding 10 gm meat sample and mixed with 90 ml of normal saline solution (8.5 gm NaCl / L) and homogenizing for 2 min (8). Serial dilutions up to 10^{-7} were prepared and appropriate dilutions were plated onto de Man Rogosa and Sharpe agar (this medium was obtained from commercial offices in Mosul city / HiMedia Laboratories Pvt. India) (9). Duplicate plates were incubated at 37 °c for 24 hours, after growing a single colony was tested and examined morphologically and microscopically for purity and then subculture in MRS broth (10) and (11).

Preparation of Cell-Free Filtrate

We inoculated 10 ml of MRS broth with LAB and incubated at 30 °C for 48 hrs. after incubation, a cell free solution was obtained by centrifuging the bacterial culture at 6000 xg for 15 min. followed by filtration of the supernatant through 0.2 mm pore size filter thus obtaining cell free filtrate (10).

In vitro Inhibition Test

The antimicrobial activity of the isolated LAB (cell free filtrate) against (*Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Klebsiella pneumoniae*, *Proteus* spp., *Salmonella* spp., *Corynebacterium* spp., *Streptococcus pneumoniae* and *Staphylococcus aureus*), that obtained from Dep. of Biology / College of Science, was performed by the well diffusion assay. The pathogenic test bacteria were incubated in Brain heart Infusion (BHI) broth at appropriate temperature for 24 hrs. Petri dishes

containing 20 ml of Muller Hinton agar were prepared previously and inoculated with 0.1 ml of 24 hrs. broth culture of pathogenic bacteria. Once solidified the dishes were stored for 2 hrs. in a refrigerator. Four wells were made and filed using 100 µl of cell-free filtrate according to (10) that previously prepared. Incubation Petri dishes at 37°C for 24 hrs. then the diameter of the inhibition zone was measured with calipers in mm. The antimicrobial activity was determined by measuring the clear zone around the wells (10) and (12).

Results

We isolates lactic acid bacteria (LAB) from minced meat on MRS media depending on their positive gram stain, negative for indole, catalase, motility and spore forming with rods shape, these characters were according to (13). The antimicrobial activity of LAB isolates were tested against some pathogenic bacteria are summarized in (table 1) by using agar well diffusion assay, and figure (1) illustrate the zones of inhibition against some of the pathogenic bacteria under study.

Table 1: Antimicrobial activity of *Lactobacillus* spp. against some pathogenic bacteria that detected in supernatant fluid of MRS broth by agar well diffusion assay.

Pathogenic bacteria	Zone diameter (mm)
<i>Bacillus cereus</i>	4 mm
<i>Staph. aureus</i>	2.5 mm
<i>Proteus</i> spp.	2.8 mm
<i>E. coli</i>	1.8 mm
<i>Pseudomonas aeruginosa</i>	2 mm
<i>Streptococcus pneumoniae</i>	1.8 mm
<i>Klebsiella pneumoniae</i>	1 mm
<i>Salmonella</i> spp.	0.75 mm
<i>Corynebacterium</i> spp.	0.6 mm

Discussion

As the results indicate, the diameters of the inhibition zones were varied it ranged between 0.6 to 4 mm. This revealed that the LAB inhibited all the pathogenic bacteria tested according to (12) whose mentioned that inhibition was scored positive if the width of the clear zone around the colonies of the producer strain was 0.5 mm or larger. Similar study was carried out in Morocco by Kalalou whose studied the activity of LAB on some gram positive and negative pathogenic bacteria such as *E.coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Bacillus cereus* and the inhibition zones were in the range of 1.4 to 2.8 cm (10).

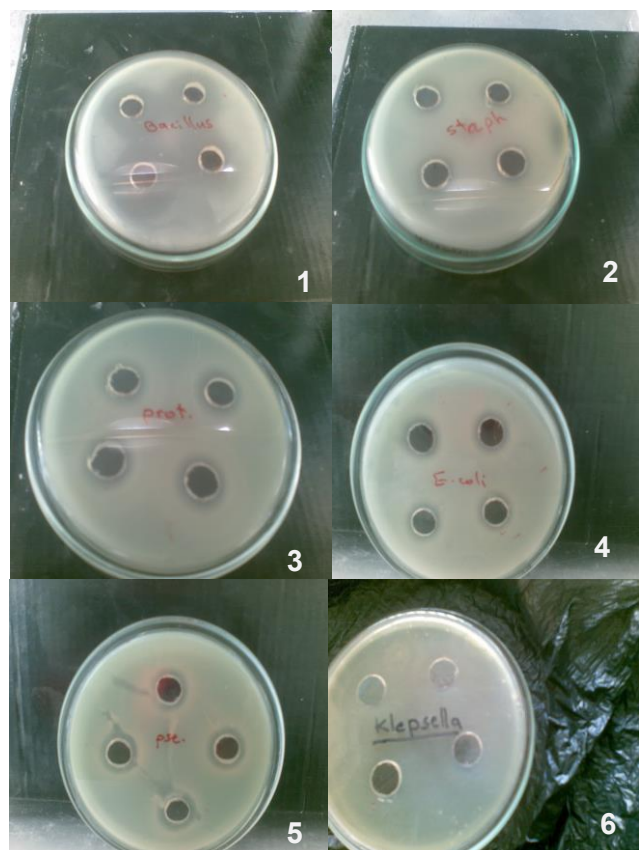


Figure 1: Inhibition Zones of LAB against some pathogenic bacteria under study. 1. *Bacillus cereus*, 2. *Staph. aureus*, 3. *Proteus* spp., 4. *E.coli*, 5. *Pseudomonas aeruginosa*, 6. *Klebsiella pneumoniae*.

Also in Nigeria Olotu in 2007, strain of LAB isolated from cow milk samples was tested to inhibit growth of some pathogenic bacteria by the same procedure and the results indicated the inhibitory effects on *E. coli* and *Pseudomonas aeruginosa* but not for *Bacillus cereus*, *Klebsiella pneumonia* and *Staphylococcus aureus* (14).

Many studies were carried out in Nigeria, Adeskan in 2008 using poultry meat to isolate LAB and study its antimicrobial activity against several microorganisms. The results showed that LAB inhibited *Staph. aureus*, *E.coli*, *Pseudomonas aeruginosa* with the exception of *Candida albicans* and *Proteus vulgaris* (11).

LAB isolated from meat are probably the best candidates for improving the microbiological safety of these foods and act as a barrier to inhibit spoilage and /or growth of pathogenic bacteria and the biopreservation

techniques for meats is in progress (15). Inhibition variety of bacteria by LAB due to a combination of many factors produced by LAB e.g. production of lactic acid which reduce PH of the meat and also other inhibitory substances such as bacteriocins which are responsible for the most antimicrobial activity (16).

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