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ORIGINAL RESEARCH ARTICLE

Antimicrobial Activity of Lactic Acid Bacteria Isolated From Goat Meat against Various Pathogenic Bacteria

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ABSTRACT

In this present study, Lactic Acid Bacteria (LAB) was isolated and identified from goat 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* spp., *Bacillus* spp., *Klebsiella* spp and *Staphylococcus* spp). This study revealed that higher inhibition growth was found in *Escherichia coli* (50µl) in the paper disc and agar diffusion test.

Key words: Lactic Acid Bacteria, Goat meat and Antimicrobial activity.

1. INTRODUCTION

Meat is 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]. This study was undertaken to understand the ability of *in vitro* antagonistic effect

2. MATERIALS AND METHOD

ISOLATION OF LAB

Meat samples were collected from market, these samples were transported to the laboratory immediately using cool box (4°C) and tested directly. LAB were isolated from goat 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 [5]. Serial dilutions up to 10^{-7} were prepared and appropriate dilutions were plated onto de Man Rogosa and Sharpe agar. Duplicate plates were incubated at 37°C for 24 hours, after growing a single colony was tested and examinated morphologically and microscopically for purity and then subculture in MRS broth [6,7].

PREPARATION OF CELL-FREE FILTRATE

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 rpm for 15 min followed by filtration of the supernatant through 0.2 mm pore size filter thus obtaining cell free filtrate [6].

IN VITRO INHIBITION TEST

The antimicrobial activity of the isolated LAB (cell free filtrate) against (*Escherichia coli*, *Pseudomonas* spp, *Bacillus* spp, *Klebsiella* spp, *Staphylococcus* spp), was performed by the well diffusion assay and paper disc assay. Petri dishes containing 20 ml of nutrient agar were prepared previously and inoculated with 0.1 ml of 24 hrs broth culture of pathogenic bacteria. Five wells were made and filled using 10µl, 20µl, 30µl, 40µl, and 50µl of cell-free filtrate according to that previously prepared. Incubation Petri dishes at 37°C for 24 hrs. Then the diameter of the inhibition zone was measured with measuring scale (mm). The antimicrobial activity was

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determined by measuring the clear zone by using Hi Antibiotic zone scale around the wells [7,8].

ANTIMICROBIAL ACTIVITY:

PAPER DISC ASSAY:

Paper disc assay was adopted for evaluation of antibacterial activity LAB (cell free filtrate). Muller Hinton agar medium was prepared and seeded with one ml of fresh goat meat spoilage and pathogenic strains. *Escherichia* spp, *Pseudomonas* spp, *Bacillus* spp, *Klebsiella* spp and *Staphylococcus* spp were prepared separately. The seeded medium was poured in sterile petriplate and the selected was prepared in different concentration. Paper disc dipped in sterile water served as control. Different concentrations like 10µl, 20µl, 30µl, 40µl, 50µl were added in paper disc and incubated for 24hrs at 37ºC. After incubation period the diameter of inhibition zone formed around the paper disc were measured by using HiAntibiotic zone scale and expressed in mm.

WELL DIFFUSION METHOD:

Well diffusion method was followed for evolution of antibacterial activity of LAB (cell free filtrate). Sterilized Muller Hinton agar medium was prepared and poured in sterile petriplates and allowed for solidification. The stainless steel cork borer used to make the 1 cm diameter well. Different concentrations like 10µl, 20µl, 30µl, 40µl, 50µl were added in well and incubated at 37ºc for 24 hrs, after which they were observed for the zone of inhibition. Diameter of inhibition zones was calculated and expressed in mm.

3. RESULT AND DISCUSSION

The antimicrobial activity of LAB isolates were tested against some pathogenic bacteria are summarized in (**Table 1**) by using paper disc diffusion and well diffusion method (**Table 2**). The zones of inhibition against some of the pathogenic bacteria under study as the results indicate, the diameters of the inhibition zones were varied it ranged between 10 to 27 mm. This revealed that the LAB inhibited all the pathogenic bacteria tested according to [8] 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 *Escherichia 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 [6] .As the results indicate, the diameters of the inhibition zones were higher in *E.coli* (recorded 24mm) for paper disc diffusion and 27mm was recorded in agar well diffusion method. This revealed that the LAB inhibited all the pathogenic bacteria tested according to[8] 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. Nigeria Olotu in 2007 tested the strain of LAB isolated from cow milk samples used to inhibit growth of some pathogenic bacteria by the same procedure and the results indicated the inhibitory effects on *E. coli* and *Pseudomonas aeroginosa* but not for *Bacillus cereus*, *Klebsiella pneumonia* and *Staphylococcus aureus*[9]. 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 aerouginosa* with the exception of *Candida albicans* and *Proteus vulgaris* [7]. 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 [10] .

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

Organism tested	Area of inhibition zone (mm)					
	Culture filtrate of *Lactococcus lactis* (µl)					
	Control	10	20	30	40	50
Escherichia coli	-	10.00	12.33	14.00	15.80	24.00
Pseudomonas spp	-	11.00	14.33	15.00	17.33	18.00
Bacillus spp	-	13.00	15.66	16.00	18.66	20.00
Klebsiella spp	-	13.00	15.80	17.33	18.00	22.00
Staphylococcus spp	-	12.00	14.33	17.33	20.00	22.00

Table 2: Antimicrobial activity of *Lactobacillus* spp. against some pathogenic bacteria that detected in supernatant fluid of MRS broth by agar well diffusion assay

Oranism tested	Area of inhibition zone (mm)					
	Culture filtrate of *Lactococcus lactis* (µl)					
	Control	10	20	30	40	50
Escherichia coli	-	10.00	12.33	13.00	16.33	27.00
Pseudomonas spp	-	11.00	14.33	16.66	17.00	23.00
Bacillus spp	-	13.00	15.66	17.33	18.00	20.00
Klebsiella spp	-	13.00	14.33	16.33	18.00	22.00
Staphylococcus spp	-	10.00	12.00	16.33	18.00	23.00

CONCLUSION

This study showed that the Lactic acid bacteria isolated from goat milk samples are capable of inhibiting pathogenic and spoilage microorganisms. These Lactic acid bacterial isolates were widely used in the food industry and

are generally regarded as safe (GRAS). Hence, they may be considered as natural preservatives acceptable by the food industries.

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