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 of 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. After incubation period the diameter of inhibition zone formed around the colonies of the producer strain was 0.5 mm or larger. Similar study was 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* but not for *Bacillus cereus, Klebsiella pneumonia* and *Staphylococcus aureus*. Many studies were carried out in Nigeria, Adeskan in 2008 using poultry meat to isolate LAB and study its antimicrobial activity against several pathogenic bacteria under study as the results indicated the inhibitory effects on *E. coli* and *Pseudomonas aeruginosa* with the exception of *Candida albicans* and *Proteus vulgaris*. 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**

<table>
<thead>
<tr>
<th>Organism tested</th>
<th>Culture filtrate of <em>Lactobacillus lactis</em> (µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>-</td>
</tr>
<tr>
<td><em>Pseudomonas</em> spp</td>
<td>-</td>
</tr>
<tr>
<td><em>Bacillus</em> spp</td>
<td>-</td>
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<tr>
<td><em>Klebsiella</em> spp</td>
<td>-</td>
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<tr>
<td><em>Staphylococcus</em> spp</td>
<td>-</td>
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</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Organism tested</th>
<th>Area of inhibition zone (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Culture filtrate of <em>Lactobacillus lactis</em> (µl)</td>
</tr>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>-</td>
</tr>
<tr>
<td><em>Pseudomonas</em> spp</td>
<td>-</td>
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<tr>
<td><em>Bacillus</em> spp</td>
<td>-</td>
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<tr>
<td><em>Klebsiella</em> spp</td>
<td>-</td>
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<tr>
<td><em>Staphylococcus</em> spp</td>
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</table>

**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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