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# Antibacterial Activity of Probiotics (*Lactobacillus* Spp.) against Intestinal Pathogenic Bacteria

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## Abstract

This work aimed to determine the antibacterial activity of probiotic bacteria (*Lactobacillus* spp.) isolated from fermented milk products against pathogenic bacteria (*Escheria coli*, *Salmonella typhi* and *Staphylococcus aureus*). *Lactobacillus bacterium* was isolated from dairy products include fermented milk (Yoghurt). Samples collected from Khartoum state, they were cultured in MRS agar, incubated at 37°C for 72 h. Then they have been identified by biochemical tests. After that, Lactobacillus was cultured in MRS broth, incubated for 48-72 h. The antimicrobial compounds were extracted by centrifuge, then the filter paper was saturated with the extract. Three pathogenic isolates were imported. Then the antibacterial activity test was done, using agar diffusion test by placing the saturated filter paper on the culture of tested bacteria. It was observed that the gram negative bacterium (*E. coli*) was more sensitive than gram positive bacteria (*Staphylococcus aureus*).

**Keywords:** Probiotics; Antibacterial; *Lactobacillus*; Fermented milk; Intestinal pathogens

## Introduction

Probiotics bacteria, have been recognized by FAO/WHO as live microorganism, which when ingestion in a particular number, confer a health benefit on the host [1,2]. Probiotics microorganisms have two main important sources, intestinal tract of human and fermented of dairy products [3]. These microorganisms may be included in foods or be marketed as supplement, in the form of tablets, capsules and by fermentation of dairy products and foods (non-dairy products) [4].

They are occurring in the intestine of healthy persons, micro flora of human gastrointestinal [5]. Various benefits have been described, particularly those pertaining to improvements in gastrointestinal diseases [6]. Many species of the genus of *Lactobacillus* are considered as a part of commensal intestinal flora of human and animal [7]. Certain strains of this genus can play as microbial barriers and they were able to produce inhibitory compounds such as lactic acid, hydrogen peroxide and proteinaceous compounds (e.g. bacteriocins) [8]. The main important groups of probiotic microorganisms are belonged to the Lactic Acid Bacteria (LAB) *viz. Lactobacilli* species, *Enterococcus* spp. and *Bifidobacterium* [9].

These groups of probiotics are consisted of a physiological and genetically diverse group of rod-shape, gram-positive, non-spore forming bacteria [10,11]. Catalase negative, microaerophilic to anaerobic, fastidious, strictly fermentative microorganisms and their main products as the results of fermentation path way is lactic acid [12,13]. Many studies have documented that *Lactobacilli* which isolated from dairy products have long history of safe use [14]. Several benefits have been found to be associated with the consumption of these bacteria including influencing immune system, also these probiotics microorganisms have ability to secret or produce antimicrobial or inhibitory substances such bacteriocins, and hydrogen peroxide. These metabolizes, have ability to inhibit both, gram positive and negative bacteria, with strong effect against gastric in the intestinal pathogens. This paper aimed to evaluate the antibacterial efficacy of probiotics (*Lactobacilli* spp.) against intestinal pathogenic bacteria (*S. aureus*, *E. coli* and *S. typhi*).

## Materials and Methods

### Collection of samples

Dairy products including fermented and raw samples were collected, about 12 samples, obtained from local markets in Khartoum state. Samples were preserved under aseptic conditions, and then transported to microbiological lab in an ice box to analyze in the same day.

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### Isolated and identification of *Lactobacillus*

From each sample 1 ml of fermented milk were diluted with 9 ml of sterile 0.1 peptone water (Difco) and homogenized serial dilution were prepared and 100 u from 10-10 dilutions of the samples were inoculated on plates of MRS agar medium, incubated at 37°C for 48-72 h under anaerobic condition using anaerobic jar. Plates containing 30-300 colonies were then enumerated. The colonies were characterized by their shape and color. Selected isolates also characterized for gram staining and biochemical tests.

### Antibacterial activity of LAB to three pathogenic bacteria

Antibacterial activity was done on Muller Hinton Agar (MHA). *Staphyloccus aureus*, *Escherichia coli*, *Salmonella typhi* were sub-culture in MRS Broth. All bacterial cultures were diluted in 0.9% Nacl to 0.5% McFarland. A bacterial suspension of pathogenic bacteria was swabbed on (MHA) using sterile cotton. Suspensions of the LAB were dropped on the plates of MHA, the discs were placed on MHA, and then they were incubated at 370°C for 18-24 hr. The inhibition zone was measured.

## Results and Discussion

### Isolation of *Lactobacillus* bacteria

Nine samples were grown out 12 of samples shown in **Table 1**.

**Table 1:** Isolation of the *Lactobacillus* bacteria.

| Sample dilution | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | + | + | _ | + | + | + | + | _ | + | _ | _ | _ |
| 2 | _ | _ | _ | _ | + | + | + | _ | _ | _ | _ | _ |
| 3 | _ | _ | _ | _ | + | + | + | _ | _ | _ | _ | _ |

**Note:** - =non-growth; + =growth

### The identification

Form **Table 2** represented nine rod-shape, gram-positive, non-spore, catalase negative, oxidase negative, non-motility bacteria were isolated from twelve samples. Nine of them were produced acid from glucose.

**Table 2:** Identification of lactic acid bacteria.

| Sample no. | Isolate no. | Gram staining | Shape | Endospore staining | Catalase test | Oxidase test | Motility test | OF | (Glucose acid) | Tetrad formation | Genus |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1 | + | Sphere | - | - | - | - | F | + | - | Strtp |
| 2 | 1 | + | Sphere | - | - | - | - | F | + | - | Strtp |
| 4 | 1 | + | Sphere | - | - | - | - | F | + | - | Strtp |
| 5 | 2 | + | Rod | - | - | - | - | F | + | - | Lac |
| 6 | 2 | + | Rod | - | - | - | - | F | + | - | Lac |
| 6 | 3 | + | Rod | - | - | - | - | F | + | - | Lac |
| 7 | 1 | + | Sphere | - | - | - | - | F | + | - | Strtp |
| 7 | 2 | + | Sphere | - | - | - | - | F | + | - | Strtp |
| 9 | 1 | + | Sphere | - | - | - | - | F | + | - | Strtp |

**Note:** Strtp: *Streptococcus*; Lac: *Lactobacillus*

## Antibacterial activity

Lactobacilli bacteria were cultured in pure MRS Broth, incubated for 72 hours, the antimicrobial was extracted from bacteria, by means of a centrifuge and then the filter paper was saturated with the extract and placed in the culture of pathogen bacteria (*E. coli, S. aureus, S. typhi*) and it was observed that there is a corona around that filter paper (inhibition zone) with different diameters shown in **Table 3** (**Figures 1-4**).

**Table 3:** The diameter of inhibition zone of *Lactobacilli* against the pathogenic bacteria *Lactobacillus* spp. 1 and Lactobacillus spp. 2.

| | | | | | | |
|---|---|---|---|---|---|---|
| *E. coli* | 25 | 26 | 30 | 20 | 25 | 20 |
| *Salmonella typhi* | 8 | 6 | 8 | 8 | 7 | 6 |
| *Staph. aureus* | 20 | 15 | 22 | 21 | 19 | 20 |

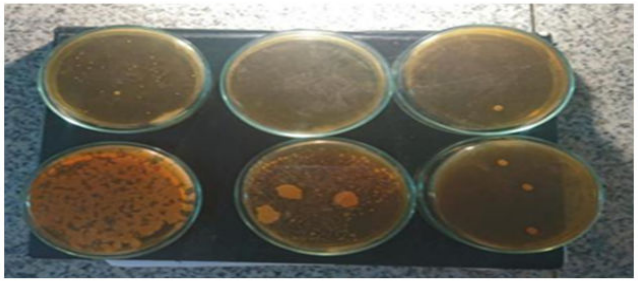

**Figure 1:** Culture of isolated LAB.

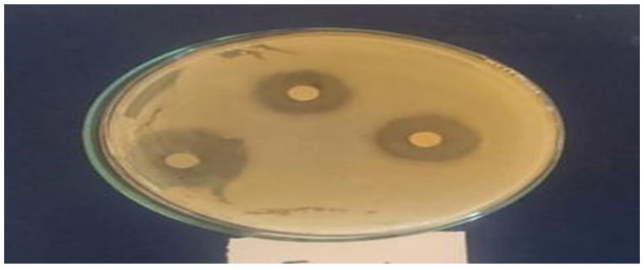

**Figure 2:** Inhibition zone of *Lactobacillus* against *E. Coli*.

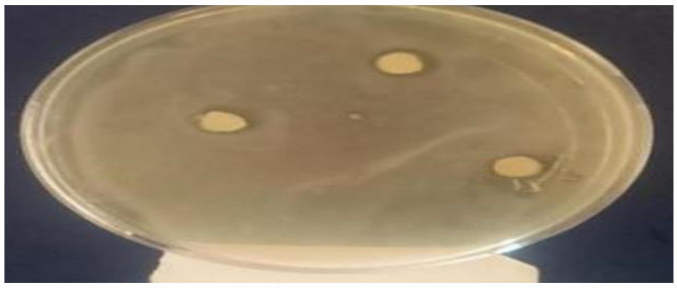

**Figure 3:** Inhibition zone of Lact*obacillus* against *Salmonella typhi*.

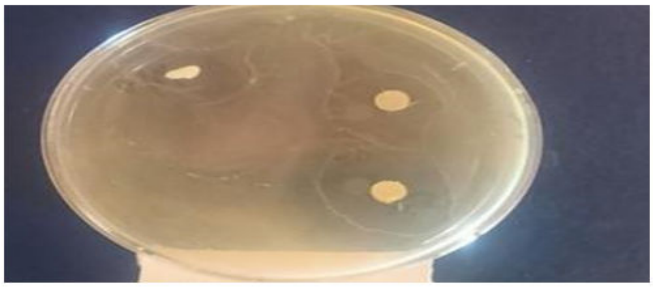

**Figure 4:** Inhibition zone of Lactobacillus against *Staphylococcus aureus*.

On the assay of antimicrobial activity of LAB (*Lactobacillus* spp.) against three pathogenic bacteria showed that the (LAB, *Lactobacillus* isolates were capable to inhibit the pathogen. This results confirmed by Sridevi, et al., This indicate that LAB produced antibacterial metabolizes such as organic acid and bacteriocin during growth. *E. coli* was more sensitive bacteria; in contrast *Salmonella typhi* was have low activity.

## Conclusion

Two LAB isolates were isolated from fermented milk. Of that, tow isolates (*Lactobacillus*) showed to inhibit to all three pathogenic bacteria, including *E. coli*, *S. aureus* and *Salmonella typhi*. The antimicrobial activity result obtained confirmed that the LAB (*Lactobacillus*) possesses an antibacterial activity. The antibacterial activity was more obvious against the gram negative bacteria *E. coli* then gram positive one, *S. aureus*.

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