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**ORIGINAL ARTICLE**

# Antimicrobial Activities of Lactic Acid Bacteria

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**ABSTRACT**
*Lactic acid bacteria (LAB) are widely distributed in nature such as in dairy, fish as well as vegetables and grains. Metabolites produced from LAB including lactic acid, acetic acid, hydrogen peroxide, bacteriocins and some low molecular weight compounds have antimicrobial activities. Four types of lactic acid bacteria (Lactobacillus plantarum subsp plantarum PTCC1745, Lactobacillus sakei subsp sakei PTCC 1712, Lactobacillus casei subsp casei PTCC 1608, Lactococcus lactis sub species lactis PTCC 1336) were obtained from collection center of fungi and bacteria, Tehran, Iran, and the antimicrobial effects of these bacterial culture supernatants (neutral and acidic) were evaluated on pathogen bacteria strains by using disc diffusion agar and well diffusion agar methods. LAB had shown a good antimicrobial ability against six pathogen bacteria. The highest inhibitory effect was shown with Lactococcus lactis subsp lactis against E. coli PTCC 1399 according to well diffusion agar with average of inhibitory zone diameter 14 mm. Also, in the comparison between two method of well diffusion agar and disc diffusion agar, the well diffusion agar was the far more sensitive than disc diffusion agar method and acidic supernatant in comparison to neutralized have more performance. According to this study the produced metabolite from lactic acid bacteria can prevent the growth of pathogen bacteria which shows the positive role of this class of bacteria in human health and it would be suggested that is class of bacteria which are abundance in dairies, should be used more.*
***Key Words:*** *Lactic acid bacteria, Antimicrobial activity, Pathogenic strains*

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## INTRODUCTION

Lactic acid bacteria (LAB) are a heterogeneous group of bacteria found widely in nature. They colonize the gastrointestinal and urogenital tracts of humans and animals, and are present in foods such as dairy products, fermented meats, fruits and vegetables. The LAB form a taxonomically diverse group of microorganisms, which can convert fermentable carbohydrates into lactic acid. The most typical LAB members are gram-positive, catalase-negative organisms of the low C+G branch, belonging to the genera *Lactobacillus*, *Lactococcus*, *Pediococcus* and *Leuconostoc* [1, 2]. LAB are among the most important groups of microorganisms used in food fermentations. There has been much recent interest in the use of various strains of LAB as probiotics, since because these bacteria, mainly *Lactobacilli* and *Bifidobacteria*, may have several therapeutic functions [3]. Probiotics are live microbial food supplements which benefit the health of consumers by maintaining or improving their intestinal microbial balance [4]. LAB produce various compounds such as organic acids, diacetyl, hydrogen peroxide, and bacteriocin or bactericidal proteins during lactic fermentations. Lactic acid bacteria exert strong antagonistic activity against many microorganisms, including food spoilage organisms and pathogens [5]. The ability of *Lactobacillus* and *Bifidobacterium* strains to adhere to the mucosal surfaces of the intestine and the subsequent long or short term colonization has long been one of the most commonly encountered criteria for the selection of probiotic strains [6, 7]. The aim of this study was to investigate the effect of lactic acid bacteria against some pathogenic bacteria.

## MATERIALS AND METHODS

### Bacterial strains and growth conditions

The standard strains used in this study were *Staphylococcus aureus* PTCC 1431, *Salmonella enterica* PTCC 1231, *Shigella dysenteriae* PTCC 1188, *Escherichia coli* PTCC 1399, *Lactobacillus plantarum* subsp *plantarum* PTCC1745, *Lactobacillus sakei* subsp *sakei* PTCC 1712, *Lactobacillus casei* subsp *casei* PTCC

1608 and *Lactococcus lactis* subsp *lactis* PTCC 1336. The strains were obtained from collection center of fungi and bacteria, Tehran, Iran. The hospital strains were from different specimens of patients referring to Razi Hospital, Rasht, Iran. These microorganisms were *Staphylococcus aureus, Escherichia coli.* Lactic acid bacteria were grown in MRS Broth (Merck; Germany) at 37ºC for 48 h. Other strains were grown in Nutrient Broth (Merck; Germany) at 37ºC for 24 h.

**Preparation of cell-free supernatants**

Strains to be tested for antimicrobial activity were incubated in MRS broth for 48 h at 37°C. Bacterial cells were removed by centrifuging the culture at 5000 g for 20 min at 4°C. The pH values of supernatants were adjusted to pH 6.5-7.0 by the addition of 1 N NaOH, The supernatants were membrane filtered (Millipore, 0.22µm) and stored at 4°C.

**Antimicrobial assay**

Disc diffusion agar and well diffusion agar methods used to detect antimicrobial activities of supernatants produced from lactic acid bacteria. The plates were poured with 20 ml Mueller Hinton Agar (Merck; Germany). The Standard strains (*S. aureus* PTCC 1431, *S. enterica* PTCC 1231, *Sh. dysenteriae* PTCC 1188, *E. coli* PTCC 1399) and hospital strains (*S. aureus, E. coli*) were adjusted to a density of $10^9$ CFU/ml by adding sterile water and spread on the surface of MHA.

**Well Diffusion Agar**

Agar well diffusion method is widely used to determine the antimicrobial activity against different types of pathogenic microorganisms. Wells of 7 mm in diameter were cut into these agar plates and 100 µl of the supernatants (neutral and acidic) were placed into each well. The culture plates were incubated at 37°C for 48 h and the zones of inhibition measured in diameter (mm) [8, 9].

**Disc Diffusion Agar**

In this method sterile paper discs (6 mm-Himedia) were placed over MHA agar plates seeded whit indicator strains. 100 µl of culture supernatant (neutral and acidic) was added to the sterile paper discs and incubated at 37°C for 48 h. After incubation antimicrobial activity (mm) was measured in diameter (mm) around the paper discs [10]. Antimicrobial tests were done in thrice and the mean values were recorded. Statistical analyses were performed using SPSS software.

## RESULTS

The lactic acid bacteria were shown the different antimicrobial activities. All four LAB strains supernatants (neutral and acidic) effectively inhibited the growth six pathogen bacteria. The results obtained from the agar well diffusion assay are shown in Table 1, 2. The highest inhibitory effect was shown with *Lactococcus lactis* subsp *lactis* against *E. coli* PTCC 1399 with average of inhibitory zone diameter 14 mm.

Table 1. Antibacterial activity of lactic acid bacteria against pathogenic bacteria (Neutral- in millimeter)

| Pathogenic Bacteria / Lactic Acid Bacteria | *Sh. dysenteriae* PTCC 1188 | *S. enterica* PTCC 1231 | *S. aureus Clinical* | *S. aureus* PTCC 1431 | *E. coli Clinical* | *E. coli* PTCC 1399 |
|---|---|---|---|---|---|---|
| *Lactobacillus sakei* subsp *sakei* PTCC 1712 | 8.33 | 10 | 9 | 10 | 8.66 | 9.66 |
| *Lactobacillus casei* subsp *casei* PTCC 1608 | 9 | 9.33 | 8.66 | 8.66 | 9 | 9.33 |
| *Lactobacillus plantarum* subsp *plantarum* PTCC1745 | 10 | 9.33 | 8.33 | 8.33 | 9 | 10.33 |
| *Lactococcus lactis* subsp *lactis* PTCC 1336 | 11.33 | 10 | 9 | 11.66 | 11 | 10.66 |

Table 2. Antibacterial activity of of lactic acid bacteria against pathogenic bacteria (Acidic- in millimeter)

| Pathogenic Bacteria / Lactic Acid Bacteria | *Sh. dysenteriae* PTCC 1188 | *S. enterica* PTCC 1231 | *S. aureus Clinical* | *S. aureus* PTCC 1431 | *E. coli Clinical* | *E. coli* PTCC 1399 |
|---|---|---|---|---|---|---|
| *Lactobacillus sakei* subsp *sakei* PTCC 1712 | 12 | 12.33 | 13.33 | 13 | 12 | 12.66 |
| *Lactobacillus casei* subsp *casei* PTCC 1608 | 12.66 | 12.66 | 13 | 12 | 12 | 12.33 |
| *Lactobacillus plantarum* subsp *plantarum* PTCC1745 | 12.33 | 12.33 | 13.66 | 13.33 | 13.66 | 13.66 |
| *Lactococcus lactis* subsp *lactis* PTCC 1336 | 12.33 | 12.66 | 13.33 | 13.66 | 13.66 | 14 |

The results obtained from the agar disc diffusion assay are shown in Table 3, 4. The highest inhibitory effect was shown with *Lactobacillus plantarum* subsp *plantarum* against *S. enterica* PTCC 1231 with average of inhibitory zone diameter 12.33mm. Also, in the comparison between two method of well diffusion agar and disc diffusion agar, the well diffusion agar was the far more sensitive than disc diffusion agar method and acidic supernatant in comparison to neutralized have more performance.

Table 3. Antibacterial activity of lactic acid bacteria against pathogenic bacteria (Acidic - in millimeter)

| Pathogenic Bacteria / Lactic Acid Bacteria | *Sh. dysenteriae* PTCC 1188 | *S. enterica* PTCC 1231 | *S. aureus Clinical* | *S. aureus* PTCC 1431 | *E. coli Clinical* | *E. coli* PTCC 1399 |
|---|---|---|---|---|---|---|
| *Lactobacillus sakei* subsp *sakei* PTCC 1712 | 8 | 8.33 | 8.33 | 9 | 8 | 7.66 |
| *Lactobacillus casei* subsp *casei* PTCC 1608 | 7.66 | 9 | 8 | 7.66 | 7.66 | 8 |
| *Lactobacillus plantarum* subsp *plantarum* PTCC1745 | 9.33 | 8 | 9 | 8.33 | 7.66 | 9.33 |
| *Lactococcus lactis* subsp *lactis* PTCC 1336 | 9 | 7.66 | 8.66 | 8.33 | 8.33 | 9.66 |

Table 4. Antibacterial activity of lactic acid bacteria against pathogenic bacteria (Acidic - in millimeter)

| Pathogenic Bacteria / Lactic Acid Bacteria | *Sh. dysenteriae* PTCC 1188 | *S. enterica* PTCC 1231 | *S. aureus Clinical* | *S. aureus* PTCC 1431 | *E. coli Clinical* | *E. coli* PTCC 1399 |
|---|---|---|---|---|---|---|
| *Lactobacillus sakei* subsp *sakei* PTCC 1712 | 11 | 11 | 11 | 11.66 | 10.33 | 11.33 |
| *Lactobacillus casei* subsp *casei* PTCC 1608 | 10.66 | 11.33 | 11 | 10.33 | 11.66 | 11 |
| *Lactobacillus plantarum* subsp *plantarum* PTCC1745 | 10.66 | 12.33 | 10.33 | 11.66 | 11 | 11.66 |
| *Lactococcus lactis* subsp *lactis* PTCC 1336 | 11.33 | 11.66 | 11 | 11.33 | 11.33 | 10.66 |

## DISCUSSION

Lactic Acid Bacteria are one of the most important groups of microorganisms to mankind, being involved in the production of valuable foods including fermented milk products, bread and cereals and vegetables [11]. During this study, all four LAB strains supernatants (neutral and acidic) effectively inhibited the growth six pathogen bacteria. Many studies confirm positive role of LAB in inhibitory pathogenic bacteria. In study by Savadogo *et al*. [5] demonstrated the inhibitory performances of some lactic acid bacteria from burkina faso fermented milk against a wide range of pathogenic organisms including *S. aureus, E. coli, Enterococcus faecalis and B. cereus.* Gilliland and Speck [12] had earlier reported that lactobacilli showed stronger antibacterial properties against gram positive bacteria (*S. aureus* and *Clostridium perfringens*) than Gram negative bacteria (*E. coli* and *S. typhimurium*). Huttunen et al. [13] reported that Lactic acid bacterial strains are potentially promising because they generate bactericidal bioactive peptides (bacteriocins) and enzymes that are able to control bio-film formation and the growth of the pathogens. Schillinger and Lucke [14] reported that some *L. plantarum* and *L. sake* strains from meat and meat products has inhibitory effects against several bacteria. In study by Ogunbanwo *et al*. [15] the selected *Lactobacillus* strains (*L. plantarum* F1 and *L. brevis* OG1) produced bacteriocin, which showed inhibitory activity against Gram positive and Gram-negative strains (*E. coli, E. faecalis, B. cereus, S. aureus, Sh. dysentery, Sh. flexneri* and *Listeria monocytogenes*). Alvarado *et al*. [16] showed that only 25 out of 94 isolated LAB strains from traditional Mexican foods are able to show inhibition against pathogenic microorganisms. LAB are found in dairy products, plants, meat products, sewage, manure humans and animals. These kinds of bacteria have positive effects on immune system by inhibition of pathogen attachment to epithelial cells, changing the receptor of bacterial toxins, producing antimicrobial substances such as acid, bacteriocins, fatty acid and aromatic compounds and competition for food. The results of our study showed, that of lactic acid bacteria had good antimicrobial effects. At presence, with increasing of the antibiotic resistance and side effects of chemical drugs, it seems, we need to use

alternative remedies. Lactobacilli and their produced metabolites can have therapeutic application in future.

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