Antimicrobial Effect of Lactic Acid Bacteria against Common Pathogenic Bacteria

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INTRODUCTION

Lactic acid bacteria such as Lactobacillus, Lactococcus and Bifidobacterium are food supplements with beneficial effects on the host that can help balance the intestinal flora (1). The mechanism of these bacteria on promotion of health can be attributed to settling in the intestine and proliferation, regulation and control of intestinal microflora, reinforcement of intestinal mucosal wall, affecting the permeability of the intestinal mucosa, prevention of pathogenic bacteria adhesion to the intestinal mucosa, prevention and treatment of gastrointestinal disorders, antibiotic activity against pathogens, regulation of bacterial enzyme activity, preventing the side effects of antibiotic treatment, the change in protein diet, basic digestion of proteins, vitamin synthesis, improved calcium absorption, lactose metabolism, reduced lactose intolerance, improved digestion abilities, enhancing the nutritional value of products, preventing allergies, reduced blood cholesterol, improved immunity, anti-cancer effects and non-beneficial bacteria growth inhibition (2-5). Lactic acid bacteria by producing lactic and acetic acid, bacteriocin, hydrogen peroxide, diacetyl, acetaldehyde and ammonia are capable of reducing the environmental pH and inhibit the growth of many microorganisms (6). Nowadays, with the increased antibiotic resistance and side effects caused by using chemical drugs, the use of alternative therapies seems necessary. The previous studies have well established that these bacteria and their produced metabolites can have wide therapeutic applications as well as a positive role in pathogen inhibition (7). The aim of this study was to evaluate the anti-microbial ability of neutralized and acidic culture supernatant fluid of lactic acid bacteria on common bacterial pathogens.

MATERIAL AND METHODS

In this study, four standard strains (Lactobacillus plantarum subspecies plantarum PTCC1745, Lactobacillus casei subspecies casei PTCC1608, Lactobacillus Saki subspecies Saki PTCC1712 and Lactobacillus lactis subspecies lactis PTCC1336) were obtained from the microbial collection of Iranian Research Organization for Science and Technology (IROST). All four strains were then cultured on liquid MRS medium and incubated for 24 h at 37°C anaerobically. Purified lactic acid bacteria in liquid MRS medium and incubated for 24 h with NaOH were neutralized the culture supernatant. Four common pathogenic bacteria strains, including Staphylococcus aureus PTCC 1431, Escherichia coli PTCC 1399, Shigella dysenteri PTCC 1188 and Salmonella enterica PTCC 1231 were obtained from the microbial collection of IROST.

RESULTS

Well diffusion agar: During this procedure, the Lactococcus lactis subspecies lactis had the highest inhibitory effect against E. coli PTCC 1399 with acidic supernatant with an average diameter of 14 mm inhibition zone. Lactobacillus Saki subspecies...
Saki PTCC 1712 and Lactobacillus plantarum subspecies plantarum PTCC 1745 with neutralized supernatant had the least inhibitory effects, respectively, against Shigella dysentery PTCC 1188 and clinical Staphylococcus aureus, Staphylococcus aureus PTCC 1431 with an average diameter of 8.33 mm inhibition zone. Other bacteria also showed a high inhibitory ability against pathogenic bacteria with 8.66 – 13.66 mm inhibition zone. It has to be noted that acidic supernatant showed higher inhibitory ability compared to neutralized supernatant (Tables 1 and 2). Disk diffusion agar: In this method, Lactobacillus plantarum PTCC1745 showed the highest inhibitory effect against Salmonella centrifuged for 25 min at 4°C, and 3500 rpm. IN to obtain the culture supernatant, the bacteria were NaOH was used to enterica subspecies PTCC 1231 with acidic supernatant with an average diameter of 12.33 mm inhibition zone. The other bacteria also showed high inhibitory ability with 7.66-11.66 mm inhibition zone against pathogenic bacteria while acidic supernatant had higher inhibitory ability compared to neutralized supernatant (Tables 1 and 2). The comparison of Disk and Well methods showed that Well method had better and higher results while lactic acid bacteria showed more inhibitory effects. Acidic supernatants had higher inhibitory abilities compared to neutralized supernatants.

Table 1 - Inhibition amount of lactic acid bacteria against pathogens in millimeters (acidic type)

<table>
<thead>
<tr>
<th>Bacterial Pathogens</th>
<th>E. coli PTCC 1399</th>
<th>Clinical E. Coli</th>
<th>Staphylococcus aureus PTCC 1431</th>
<th>Clinical Staphylococcus aureus</th>
<th>Salmonella enterica PTCC 1231</th>
<th>Shigella dysentery PTCC 1188</th>
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<tr>
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<td>Disk</td>
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<tr>
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<td>12</td>
<td>11</td>
<td>12.66</td>
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<tr>
<td>Lactococcus lactis subspecies lactis</td>
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<td>10.66</td>
<td>13.66</td>
<td>11</td>
<td>12.66</td>
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</tr>
</tbody>
</table>

Table 2 - Inhibition amount of lactic acid bacteria against pathogens in millimeters (neutralized type)

<table>
<thead>
<tr>
<th>Bacterial Pathogens</th>
<th>E. coli PTCC 1399</th>
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<tr>
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DISCUSSION
Lactic acid bacteria reduce pH and inhibit the growth of many bacteria by producing lactic and organic acids. These bacteria due to production of antimicrobial compounds such as bacteriocins can be used as natural preservatives (bacteriocins are ribosomal synthetized proteins which are secreted by bacteria and act as antibiotics) (9). It was demonstrated in this study that the metabolites Produced by lactic acid bacteria, isolated by Centrifugation in neutralized and acidic forms can prevent the growth of pathogenic bacteria. In Boris study, the Lactobacillus isolated from dairy products inhibited the growth of Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Salmonella typhimurium and Bacillus subtilis with the highest inhibitory effect on Staphylococcus aureus(10). In Kazemi et al.

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study, antimicrobial activity of three *Lactobacillus* strains against three strains of pathogens (*Staphylococcus aureus*, *Salmonella typhi* and *E. coli*) were investigated and the *Lactobacillus* strains showed acceptable inhibition ability against pathogens (*Lactobacillus plantarum* against *Salmonella typhi*-10.8mm) (9). Coconnier et al. stated that the use of *Lactobacillus fermentum*, *Lactobacillus casei*, *Lactobacillus acidophilus* and *Lactococcus lactis* supernatant has inhibitory effects on a wide range of pathogenic bacteria (11). Ogunbanwo et al. investigated the antimicrobial activity and bacteriocin production of two probiotic types on several pathogenic bacteria and found the highest inhibitory effect on *Bacillus cereus* (12). Ouwehand et al. reported that the antimicrobial effects on gram-positive probiotic bacteria are more than the Gram-negatives (13). Savadogo et al. studied the invito antimicrobial activity of lactic acid bacteria against standard strains of *Bacillus cereus*, *Enterococcus faecalis*, *Escherichia coli* and *Staphylococcus aureus* and reported inhibitory zones of 8 - 12 mm diameter (14). Strus et al. investigated the anti-pathogenic properties of *Lactobacillus* strains against anaerobic pathogens of the digestive system and demonstrated their positive impact against these pathogens (15).

In our study also, lactic acid bacteria showed good inhibitory effects on pathogenic bacteria. The created inhibition zones against pathogenic bacteria in the disk method were larger than the well method in all cases. Moreover, the acidic supernatants showed greater inhibition ability compared to the neutral supernatant.

**CONCLUSION**

The results were favorable and all species were able to inhibit the pathogenic bacteria with variable inhibition amounts based on the type of performed procedure (disk and plate) and the supernatant (acidic and neutral).

**ACKNOWLEDGMENTS**

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**CONFLICT OF INTEREST**

There are no conflicts of interest.

**REFERENCES**


