Antimicrobial Effect of Lactic Acid Bacteria against Common Pathogenic Bacteria

Mohammad Mohammaddoost Chakoosari (MSc)
Young Researchers and Elites Club, Lahijan Branch, Islamic Azad University, Lahijan, Iran

Mohammad Faezi Ghasemi (PhD)
Assistant Professor of Microbiology, Department of Microbiology, Islamic Azad University of Lahijan, Guilan, Iran

Akreza Masiha (MSc)
Department of Biotechnology, Faculty of Basic Sciences, Lahijan Branch, Islamic Azad University, Lahijan, Iran

Reza Kazemi Darsanaki (MSc)
Young Researchers and Elites Club, Lahijan Branch, Islamic Azad University of Lahijan, Guilan, Iran

Abolfazl Amini (MSc)
Laboratory Sciences Research Center, Golestan University of Medical Sciences, Gorgan, Iran

Corresponding Author: Mohammad Mohammaddoost Chakoosari
Email: Chakoosari@hotmail.com
Tel: +98911335181
Address: Islamic Azad University of Lahijan, Guilan, Iran

Received: 19 Mar 2013
Revised: 10 Aug 2014
Accepted: 14 Aug 2014

ABSTRACT

Background and Objective: Probiotics are living microorganisms that have beneficial effects on the health of digestive system. The aim of this study was to evaluate the antimicrobial ability of acidic and neutral supernatants (culture supernatant) of lactic acid bacteria against common bacterial pathogens.

Methods: Four species of lactic acid bacteria (Lactobacillus plantarum PTCC1745, Lactobacillus PTCC1608, Lactobacillus Saki PTCC1712 and Lactobacillus Lactis PTCC1336) were obtained from the microbial collection of Iranian Research Organization for Science and Technology in Lyophilized form. The antimicrobial activity of neutral and acidic supernatants against bacterial pathogens was investigated using the Disk and Well Diffusion Agar methods.

Results: Lactic acid bacteria showed good antimicrobial ability against six pathogenic bacteria with the highest inhibitory effect observed in Lactococcus lactis against E. coli PTCC1399 through well method with an average diameter of 14 mm inhibition zone. In this study, the well diffusion method was far more sensitive compared to the disk method and acidic supernatants showed higher antimicrobial efficiency compared to neutral types.

Conclusion: The metabolites produced by lactic acid bacteria are able to inhibit the growth of pathogenic bacteria that can be an important and practical solution for the prevention and treatment of infections and ultimately improve human health.

Keywords: Lactobacillus; Lactococcus; Probiotic; Antibacterial.
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 in 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 nutrient broth medium with turbidity equivalent to 0.5 McFarland (0.5 McFarland). Wells were created using a sterile Pasteur pipette on the medium and 0.1ml of both supernatants (acidic and neutralized with NaOH) of lactic acid bacteria were added to the wells. After medium dried out, the plates were incubated for 24 h at 37°C. Then diameter of growth inhibition by lactic acid bacteria against each pathogenic bacterium was measured by a millimeter ruler.

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 dysentry 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

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 Staphylococcus aureus PTCC 1431</th>
<th>Clinical Staphylococcus aureus</th>
<th>Salmonella enterica PTCC 1231</th>
<th>Shigella dysentry PTCC 1188</th>
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<tr>
<td>Lactic acid bacteria</td>
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<tr>
<td>Lactobacillus Saki subspecies Saki</td>
<td>12.66</td>
<td>11.33</td>
<td>12</td>
<td>10.33</td>
<td>13</td>
</tr>
<tr>
<td>Lactobacillus casei subspecies casei</td>
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<td>11</td>
<td>12</td>
<td>11.66</td>
<td>12</td>
</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>
<th>Clinical E.coli Staphylococcus aureus PTCC 1431</th>
<th>Clinical Staphylococcus aureus</th>
<th>Salmonella enterica PTCC 1231</th>
<th>Shigella dysentry PTCC 1188</th>
</tr>
</thead>
<tbody>
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<td>Lactic acid bacteria</td>
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<tr>
<td>Lactobacillus Saki subspecies Saki</td>
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<td>8.66</td>
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<tr>
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</tr>
<tr>
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<td>9</td>
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<td>8.33</td>
</tr>
<tr>
<td>Lactococcus lactis subspecies lactis</td>
<td>10.66</td>
<td>9.66</td>
<td>11</td>
<td>8.33</td>
<td>11.66</td>
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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.

Medical Laboratory Journal, Nov-Dec 2015; Vol 9: No 5
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* 10.8 mm) (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). Ogunganwo 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**

I’d like to express my gratitude to Ms. Elham Alikhani and Sheida Akbari for their cooperation in conducting this study.

**CONFLICT OF INTEREST**

There are no conflicts of interest.

**REFERENCES**


