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Antimicrobial Activity of some *Lactobacillus* Species against Intestinal Pathogenic Bacteria

Hamid Tebyanian[1,a], Afsaneh Bakhtiari[2,b], Ali Karami[1,c], Ashraf Kariminik[3,d,*]

[1]Research Center for Prevention of Oral and Dental Disease, Baqiyatallah University of Medical Sciences, Tehran, Iran

[2]Medical Faculty, Midwifery Department, Babol University of Medical Sciences, Babol, IR Iran

[3]Department of Microbiology, Kerman Branch, Islamic Azad University, Kerman, Iran

[a]tebyan.hamid@yahoo.com, [b]afbakhtiari@gmail.com,[c]ali.karami@gmail.com, [d]a.kariminik@iauk.ac.ir

*Corresponding author

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Abstract. Probiotics have antibacterial effects against pathogenic bacteria in the gut while maintaining the balance of intestinal flora such as *Lactobacillus*. This study aimed to evaluate the antimicrobial activity of four *Lactobacillus* species against intestinal pathogenic. Four different species of *Lactobacillus* (*Lactobacillus bulgaricus* (PTCC 1332), *Lactobacillus casei* (PTCC 1608), *Lactobacillus plantarum* (PTCC 1058) and *Lactobacillus Fermentum* (PTCC 1638)) were experimented to investigate the inhibitory activity against 4 bacterial enteric pathogens (*Escherichia coli, Staphylococcus aureus, Shigella dysenteriae* and *Salmonella paratyphi A*) which were separately inoculated in MRS medium (de Man, Rogosa and Sharpe medium) for 48 hours at 37 °C and pH 7. Our results showed that enteropathogens growth was stopped in the presence of all *Lactobacillus* and inhibition zone was between 12 and 32 millimeter. It can be concluded that these four *Lactobacillus* strains had potential antimicrobial compounds against human enteric pathogens and should be further studied for their human health benefits.

Introduction

The gastrointestinal tract makes a complex system which functions in concert with the local microbiota as a physical and practical obstacle which keeps the host from attack by uninvited and dangerous microorganisms [1]. The mucosal surface of the gastrointestinal tract faces the external environment [2-4]. The gut is a muscular tissue which releases the acid and enzymes for digesting food. Human stomach has three regions: the cardia, the fundus/corpus, and the antrum [3, 5, 6]. Specific secretory cell phenotypes include acid-secreting parietal cells, mucus neck cells, and pepsinogen-secreting zymogenic cells in the fundus and corpus and gastrin-secreting cells and gland cells [7].

The human gastrointestinal system has a various number of bacteria which are normal flora and their numbers are 10^{10} to 10^{12} of 100 different species [8]. The composition and diversity of normal flora are different in during life and different ages [9]. A healthy intestine is one that maintains an important balance of bacteria such as *lactobacilli*, *Bacteroides*, *clostridia*, *streptococci* and *coliform*. Conditions such as climate, stress, excessive alcohol use, high-fat diets, meat, sugar, genetic disorders, chlorine and fluoride in drinking water, antibiotics, inadequate food, exposure to environmental toxins and many others factors could change the balance of our intestinal flora [10-14].

Probiotics live in the intestines and connect to epithelial cells such as *Lactobacillus*, *Bifidobacterium* and yeast such as *Saccharomyces cerevisiae* which they cause to prevent the replace in pathogens and performance a vital role in health [15, 16]. *Lactobacillus* sp. quickly colonized in intestinal epithelial which they disorder growth and proliferation of enteropathogens with producing bacteriocin and lactic acid and also reducing pH [10, 17, 18]. And also, *Lactobacillus* plays a critical role in the immune system, such as local control immune responses,

allergic and inflammation diseases by increasing the activity of macrophages and immunoglobulin IgA production [19-21]. The present study was carried out to identify, and characterize some lactic acid bacteria as potential probiotics with antibacterial activity against microorganisms that are a pathogen and the probiotic properties were investigated through *in vitro* assays.

Materials and Method

Collection of commercial probiotic bacteria

In this study, four *Lactobacillus* strains were obtained from Persian Type Culture collection, Tehran, Iran including *Lactobacillus bulgaricus* (PTCC 1332), *Lactobacillus plantarum* (PTCC 1058), *Lactobacillus fermentom* (PTCC1638), *Lactobacillus casseii* (PTCC 1608) (Table 1).

Culture condition of commercial probiotic bacteria

Commercial probiotic bacteria were cultivated in MRS medium and four enteropathogenic bacteria *(Staphylococcus aureus, Salmonella paratyphi A, Shigella dysentraei* and *Escherichia coli)* had been isolated from clinical samples The Agar overlay method was used for the antibacterial survey [22]. In this way, 1.5×10^8 ml of enteropathogenic bacteria were prepared which was equivalent to 0.5 of broth McFarland in normal saline. *Lactobacillus* sp. were pointy inoculated in four parts of MRS and incubated for 24 hours at 30°C at 5% carbon dioxide conditions in carbon dioxide gas jar. After incubation period and the creation of the colony of *Lactobacilli*, each plate overlayed with 7 ml of semi-solid of Trypticase Soy with inoculated bacteria separately. All plates were incubated at 37°C for 24 hours [22, 23].

Table 1. Bacterial Strains, Media and Culture Condition.

Bacterial Species	Strain	Origin	Media/Atmosphere/Temperature
Commercial probiotic strains			
Lactobacillus bulgaricus	PTCC 1332	Persian Type Culture	MRS/anaerobic/37^{o}C
Lactobacillus plantarum	PTCC 1058	Persian Type Culture	MRS/anaerobic/37^{o}C
Lactobacillus fermentom	PTCC 1638	Persian Type Culture	MRS/anaerobic/37^{o}C
Lactobacillus casseii	PTCC 1608	Persian Type Culture	MRS/anaerobic/37^{o}C

Antibacterial sensitivity

Antibacterial activity of each probiotic strains against different strains of enteropathogenic bacteria was studied by measuring the diameter inhibition zone around cultivation spot of *Lactobacillus* [22, 24]. Since, *Lactobacillus* produces lactic acid which it affects in reducing pH, a liquid culture of *Lactobacillus* in MRS broth was centrifuged for half an hour at 2700 rpm to assess the effects of acidic on inhibitory feature and achieved supernatant pH was neutralized by NaOH 0.1 normal. Then, antibiogram tests were done by good diffusion and inhibitory activity was studied as mentioned above [23].

Statistical analysis

The analysis was performed with using the SPSS software, Version 22.0. We used ANOVA tests to recognize the level of statistical.

Results

All used of probiotic strains had antagonistic effects against various pathogenic bacterial strains (Table 2). Inhibition zones were observed around the cultural point of *Lactobacillus* strains and they were 12 to 32 mm. And also, it was demonstrated that neutralizing acid pH (6 to 6.5) of achieved supernatant from *Lactobacillus* was not as an antagonistic effect on their properties (Figure 1).

Table 2. Mean Inhibition Zone (millimeter) of Antagonistic effects of probiotic strains against various pathogenic bacterial strains.

Commercial probiotic strains	***L.plantarum***	***L. casei***	***L.fermentum***	***L.bulgaricus***	
Bacterial Species					**Std. Deviation**
S.aureus	20.8	27.4	29.2	27.8	±3.746999
S.paratyphi A	27.8	26.2	24.2	26.2	±1.474223
Sh.dysanteriae	18.8	24.2	25.4	26.8	±2.288376
E.coli	16	19.8	24.6	24.6	±1.340398

According to the two-way ANOVA, *Lactobacillus* and enteropathogenic bacteria were at 1% significance meaning level and P-values of less than 0.05 were considered to be significant which four *Lactobacillus* strains could be considered as potential antimicrobial probiotic strains against human enteric pathogens.

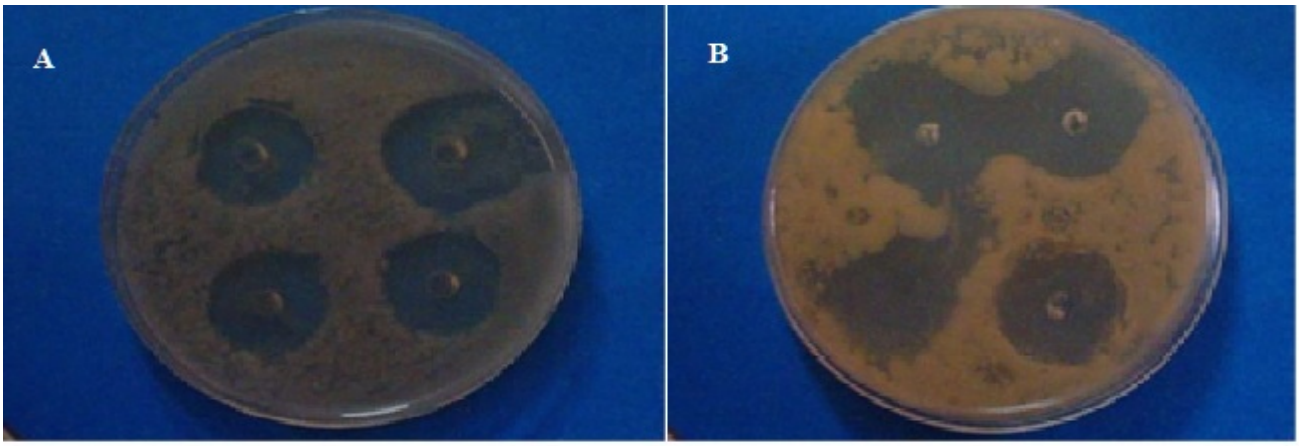

Figure 1. A; antibacterial activities of 4 *Lactobacillus* strains against *S. aureus*. B; Neutralizing pH test of and antibacterial activity of *Lactobacillus* sp.

Discussion

Probiotics are living organisms which they not only destroy pathogenic microorganism but also they help to balance the intestinal microbial and their strengthening is effective in maintaining the health [25]. Gastrointestinal infections mainly are associated with changing or preserving natural flora [19]. Therefore, several studies have been carried out to evaluate antagonistic properties and effect of probiotic microorganisms [26].

The obtained results of present study showed that four different used *Lactobacillus* were the most important probiotic organism which they have growth inhibitory effects against different isolates of Gram-positive and Gram-negative bacteria. *Staphylococcus aureus* was the most susceptible bacteria. In a similar study, Savadogo and his colleagues reported that gram-positive bacteria are more sensitive than gram-negative groups [27]. Jamalifar and colleagues researched on screening of *Lactobacillus* strains against *Pseudomonas aeruginosa* and they found that some *lactobacilli* such as *Lactobacillus acidophilus* showed significant inhibitory activity against the multidrug resistant clinical isolates of *Pseudomonas aeruginosa* [28].

The neutralizing results of pH revealed that antagonist property of *Lactobacillus* had not be related to lactic acid production which our result was similar to Park and his colleagues and Ammor's findings [22, 23]. Drago and colleagues studied on effective antagonist of probiotics bacteria such as *Lactobacillus, Bifidobacterium* and *Saccharomyces boulardii* on bacteria such as *Escherichia coli*, *Listeria monocytogenes, Vibrio cholerae* and *Salmonella* and also, their effective were related to other mechanisms such as bacteriocins, H_2O_2 and Diacetyl production of probiotic bacteria [29, 30].

In this research, we found that enteropathogens bacteria (*Escherichia coli, Staphylococcus aureus, Shigella dysenteriae* and *Salmonella paratyphi A*) growth were inhibited in the presence of all *Lactobacillus* and inhibition zone was between 12 and 32 millimeter. Dasari and colleagues studied on *Lactobacilli* and different pathogenic bacteria for finding of the production of hydrogen peroxide and antimicrobial compounds along with probiotic properties and they found that *Lactobacillus* producing antimicrobial compounds which prevents the growth of cervical pathogens, revealing that the hypothesis of preventing vaginal infection [31].

Several studies showed that probiotic application in reducing diarrhea duration in children [24, 32]. Probiotics can treat viral diarrhea which caused by Rotavirus in children and prevents loss of water and electrolytes from the body [33, 34]. Davoodabadi and colleagues researched on antimicrobial activity of *Lactobacillus* strains against five diarrhea genic *E. coli* pathotypes and they found that *Lactobacillus* strains with human origin had a mild inhibitory activity against the diarrhea genic *E. coli* [35].

Suvarna and Baghi have introduced useful some probiotics in the treatment of allergies and Eczema [18]. In the most cases, irradiation caucus diarrhea and gastrointestinal side effects in the abdominal area. Consumption of probiotic products has a significant role in the prevention of these complications before operation [36]. According to our results, *Lactobacillus* can be considered as an important and effective factor in treating of intestinal infections and also, *L.fermentum* and *L.bulgaricus* had significant inhibition against human enteric pathogens.

Conclusion

These *Lactobacillus* strains had potential antimicrobial compounds against human enteric pathogens (*Escherichia coli, Staphylococcus aureus, Shigella dysenteriae* and *Salmonella paratyphi A*) and it might be used as bioprotective agents to control the intestinal pathogenic and also, their antimicrobial effects can be evaluated *in-vivo*.

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Conflict of Interest

There is no conflict of interest.

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