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Antimicrobial Activity of *Lactobacillus* spp. Isolated from Commercial Yoghurts against Pathogenic Bacteria

Ahmad Tajehmiri[1], Reza Kazemi Darsanaki[2], Mina Nasiri Moslem[3], Zahra Lozoumi[3], Masoud Hassani Kolavani[3] and Morteza Azizollahi Aliabadi[3]*

[1]Medical Biology Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran.
[2]Young Researchers and Elites Club, Lahijan Branch, Islamic Azad University, Lahijan, Iran.
[3]Department of Microbiology, College of Microbiology Science, Lahijan Branch, Islamic Azad University, Lahijan, Iran.

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***Lactobacillus* spp. are well known probiotics with beneficial effects to human health. The aim of this study was to determine antimicrobial properties of *Lactobacillus* spp. isolated from commercial yoghurts. For this reason, 30 samples were collected from market and pour plate technique was used to isolate the organisms. Then, they were detected by using biochemical tests according to Bergey's Manual. Antimicrobial effects of these bacterial culture supernatants were evaluated on pathogen bacteria strains (*Staphylococcus aureus* PTCC 1431, *Salmonella enterica* PTCC 1231, *Shigella dysenteriae* PTCC 1188 and *Escherichia coli* PTCC 1399) by using disc diffusion agar and well diffusion agar methods. *Lactobacillus acidophilus*, *Lactobacillus Plantarum*, *Lactobacillus delbrueckii* sub sp. *bulgaricus* and *Lactobacillus casei* species were identified in commercial yoghurts. *Lactobacillus* spp. had shown a good antimicrobial ability against four pathogen bacteria. The highest inhibitory effect was shown with *L. acidophilus* against *S. enterica* PTCC 1231 according to well diffusion agar with average of inhibitory zone diameter 15 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.**

Key words: Lactic acid bacteria, Yoghurt, *Lactobacillus*, Antimicrobial activity.

Lactic acid bacteria (LAB) are known microorganisms that have probiotic properties. They can produce inhibitory compounds such as lactic acid, bacteriocin, hydrogen peroxide, diacetyl and acetaldehyde. These compounds are able to inhibit the growth of pathogenic microorganisms[1,2]. They colonize the gastrointestinal and urogenital tracts of animals, humans and are present in foods such as dairy products, fruits, fermented meats and vegetables. The LAB form a taxonomically diverse group of microorganisms, which can convert fermentable carbohydrates into lactic acid[3,4]. Probiotics are defined as live microorganisms which when administered in adequate amounts confer a health benefit on host. Most probiotics commercially available today belong to the genera *Lactobacillus* and *Bifidobacterium*[5]. In milk and dairy products such ascheeses, yoghurts and fermented milks, Lactobacilli are naturally present or added intentionally, for technological reasons or to generate a health benefit for the consumer and yoghurt is one of the best-known foods that contain probiotics[6]. The present study is aimed to

* To whom all correspondence should be addressed.
Tel.: +989119281236;
Email: Morteza_azizollahi@yahoo.com

isolate *Lactobacillus* spp. from commercial yoghurts and determine their inhibitory effect against some pathogenic bacteria.

MATERIALS AND METHODS

Isolation and Identification of *Lactobacillus* spp.

A total of 30 different commercial yoghurts collected from Northern of Iran. All samples were transferred to the laboratory under refrigeration and stored at 4°C until their analysis. Samples were used directly and also diluted to 10^{-1}, 10^{-2} and 10^{-3} using sterile peptone water. 1 ml aliquot of the samples and dilutions were plated into selective medium Man- Rogosa-Sharpe medium (MRS) agar (Merck; Germany). After incubation, individual colonies were selected and transferred into sterile broth media. The selected colonies were purified by streak plate technique. The isolates were examined according to their colony morphology, catalase reaction and gram reaction, different temperature, production of NH_3 from arginine and were confirmed according to Bergey's Manual. Gram positive and catalase negative bacilli colonies were taken as *Lactobacillus* spp. stored in glycerol culture and kept for further investigation at -20°C [7,8].

Preparing supernatant of *Lactobacillus* spp.

Lactobacilli were inoculated in MRS broth medium and incubated in anaerobic at 37°C for 48 h. 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 1N NaOH, the supernatants were membrane filtered (Millipore, 0.22µm) and stored at 4°C.

Standard Strains

The standard strains used in this study were *Staphylococcus aureus*PTCC 1431, *Salmonella enterica* PTCC 1231, *Shigella dysenteriae* PTCC 1188 and *Escherichia coli* PTCC 1399. The strains were obtained from collection center of fungi and bacteria, Tehran, Iran.

Antimicrobial assay

Disc diffusion agar and well diffusion agar methods used to detect antimicrobial activities of supernatants produced from *Lactobacillus* spp. 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) 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 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)[5,9].

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 was added to the sterile paper discs and incubated at 37°C for 48 h. After incubation antimicrobial activity 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 AND DISCUSSION

All isolates were catalase-negative, gram positive and oxidase negative. These isolates were identified as *L. acidophilus*, *L. Plantarum*, *L.casei* and *L. delbrueckii* subsp. *bulgaricus* by observing their colony morphology, physiological and as well as some biochemical characteristics. Their characteristics have shown in Table 1. Antimicrobial effects of these bacterial culture supernatants were evaluated on pathogen bacteria strains (*S.aureus* PTCC 1431, *S.enterica* PTCC 1231, *Sh.dysenteriae* PTCC 1188 and *E. coli* PTCC 1399) by using disc diffusion agar and well diffusion agar methods. The antimicrobial activity of *Lactobacillus* spp. are given in Table 2 and 3. All of *them* exhibited antimicrobial activity against tested bacteria. Moreover, *L. acidophilus* had highest antimicrobial activity against to tested microorganisms. In general, bacteria with an average inhibition power of 11.85 mm produced a good capacity for inhibiting of pathogenic bacteria.

Lactobacilli are a part of normal flora, contained antimicrobial substance that has inhibitory effect on growth of pathogens and play an important role in human health by improving

Table 1. Identification of *Lactobacillus* spp. based on biochemical and morphological tests

Strains	Growth at			catalase	motility	NH_3 from arginine	Sugar fermentation									
	15 ºC only	45 ºC only	15 and 45 ºC				Cello-biose	lac-tose	Mann-itol	Raffi-nose	galac-tose	Meleb-iose	suc-rose	Mal-tose	Man-nose	Treha lose	Sorb-itol	Ascu-lin
L. Plantarum	+	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
L.casei	+	-	-	-	-	-	+	+	+	+	+	-	+	+	+	+	+	+
L. delbrueck. iisubsp *bulgaricus*	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
L. acidophilus	-	+	-	-	-	-	+	+	-	-	+	-	+	+	+	-	-	+

gastrointestinal system and boosting immune system[6]. Many studies have focused on antimicrobial activity release of many compounds produced by *Lactobacillus* spp. to prevent the growth of pathogenic bacteria. In study by Savadogo *et al*. demonstrated the inhibitory performances of some lactic acid bacteria from burkinafaso fermented milk against a wide range of pathogenic organisms including *S. aureus, E. coli, Enterococcus faecalis* and *B. cereus*[11]. In study by Ali *et al*. fourteen isolates, identified as two *Lactobacillus* spp. eleven *Bifidobacterium* spp. and one *Streptoococcus* spp. LAB had shown a good antimicrobial and antifungal activity[12]. Gilliland and Speck 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*)[13]. Ryan *et al.* reported that *Lactobacillus* spp. showed a broad inhibitory spectrum against the indicator organisms tested[14]. In study by Kaboosi, Three kinds of bioyoghurts (ProFeel, Evolus and Gefilus) purchased from Helsinki supermarkets were tested. Probiotics, isolated from Evolus, had no activity against *E. coli.* Although, Gefilus probiotics killed the test bacteria of *E. coli* and *S.typhi*, they were only inhibitory for *S. aureus* and were not active against *P. aeruginosa*[15]. Tufail *et al.* showed that, some *L. bulgaricus* strains which were isolated from yoghurts had antibacterial potential against some food borne pathogen and spoilage microorganisms especially *V. cholera* and *E. coli*, because of significant characteristic of bacteriocin production[16]. In study by Ahmed, showed that all LAB species exhibit antagonistic effect on both gram-positive and gram-negative bacteria (*E. coli, Klebsiella pneumonia, Proteus mirabilis, Pseudomonas aeruginosa, Staphylococcus aureus* (MRSA), *Bacillus subtilis*)[17]. During this study, *Lactobacillus* spp. had shown a good antimicrobial ability against four pathogen bacteria. The highest inhibitory effect was shown with *L. acidophilus* against *S. enterica* PTCC 1231 according to well diffusion agar with average of inhibitory zone diameter 15 mm. At presence, with increasing of the antibiotic resistance and side effects of chemical drugs, it seems, we need to use alternative remedies. According to this study the produced metabolite from *Lactobacillus* spp. can

Table 2. Antimicrobial activity of isolates *Lactobacillus* spp. by well diffusion agar

Isolates	Diameter zone of Inhibition (mm) Tested microorganisms			
	Sh. dysenteriae PTCC 1188	*S. enterica PTCC 1231*	*E. coli* PTCC1399	*S.aureus* PTCC1431
L. acidophilus	13	15	13.2	11
L. Plantarum	12	14	13	10
L.casei	11.5	11	11.5	8.5
*L. delbrueckii*subsp. *bulgaricus*	11.5	12.5	12.5	9.5
Mean±SD	12±0.70	13.12±1.75	12.55±0.76	9.75±1.04

Table 3. Antimicrobial activity of isolates Lactobacillus spp. by disc diffusion agar

Isolates	Diameter zone of Inhibition (mm) Tested microorganisms			
	Sh. dysenteriae PTCC 1188	*S. enterica PTCC 1231*	*E. coli* PTCC1399	*S.aureus* PTCC1431
L. acidophilus	12.5	14.2	13	10
L. Plantarum	11	13	12.2	8.5
L.casei	9	12	11.5	8
*L. delbrueckii*subsp. *bulgaricus*	9.5	11.5	11	8
Mean±SD	10.5±1.58	12.67±1.19	11.92±0.87	8.62±0.95

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.

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