Short Communication

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Antimicrobial Effects of Lactobacillus Plantarum and Lactobacillus Paracasei Isolated from Honey against Staphylococcus Aureus

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ABSTRACT

BACKGROUND AND OBJECTIVE: Lactobacilli are gram positive, catalase-negative, and found in a variety of fermented foods such as honey, as well as human normal flora. The aim of this study was to identify lactobacillus Plantarum and lactobacillus Paracasei in Iranian honey samples and to investigate the probiotic and antimicrobial properties of them against Staphylococcus aureus.

METHODS: This cross-sectional study investigated 88 honey samples from different areas in Iran at 6 months, from May to September, 2016. Samples were cultured in MRS broth and after were cultured on MRS agar. Sequencing of 16S rDNA gene was used to detect lactobacillus isolates. Then probiotic capacity (acid and bile resistance) of isolates was measured. Antimicrobial activity of lactobacillus isolates was investigated by diffusion method from wells and antibiotic resistance by disc diffusion method.

FINDINGS: From 88 honey samples, 39 Lactobacillus isolates were isolated, four L. plantarum and two L. paracasei were identified by molecular technique. Every six isolates tolerated acidity but were sensitive to bile salt. Five isolates inhibited the growth of S. aureus. The most antibiotic resistance of Lactobacillus strains was seen to vancomycin(100%), nalidixic acid(100%) and streptomycin(100%).

CONCLUSION: Iranian honey samples can be a source for different Lactobacillus species as L. plantarum and L. Paracasei which some of these species could have wonderful inhibitory effects against pathogen bacteria like S. aureus.

KEY WORDS: *Lactobacillus, Honey, Probiotics, Staphylococcus Aureus.*

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Introduction

Lactobacilli are gram-positive, catalase-negative bacilli and found in a variety of fermented foods, such as honey, as normal human flora (1-3). Honey contains glucose, fructose, vitamins, enzymes and minerals that provides a good environment for beneficial bacteria to growth such as lactobacilli (4,5). Probiotics are living microorganisms that, if consumed sufficiently, cause health effects in the host (6,7). Probiotics can play protective and therapeutic role in infectious diseases by inhibiting pathogen microorganisms (8-10). Varieties of Lactobacillus species such as L.

Plantarum and L Paracasei, Bifidobacterium, Streptococcus, yeasts and molds are used as commercial probiotics (11). Staphylococcus aureus is one of the major causes of foodborne infections and a wide range of hospital infections. Unfortunately, the treatment of Staphylococcus aureus infections is becoming more and more difficult day by day due to an increase in antibiotic resistance (12-14). That's why scientists are trying to find new therapeutic approaches, such as the use of probiotics for infections of this pathogen (15). Aween et al showed that the L. acidophilus isolated from honey inhibits Staphylococcus aureus (16). Tajabadi and colleagues in Malaysia were able to separate L. Plantarum, L. Fermentum and L.

Pentosus from the species (17). So far, there has been no study in Iran on the presence of lactobacilli in honey and the study of their probiotic and antimicrobial properties. Therefore, the aim of this study was to investigate the presence of lactobacilli in Iranian honey samples and to study the probiotic properties and their inhibitory effect on Staphylococcus aureus.

Methods

This cross-sectional study was carried out in the Department of Microbiology of Babol University of Medical Sciences during six months from April to September of 2016. 88 natural honey samples from 13 provinces were purchased directly from beekeepers. One gram of honey samples was inoculated into MRS Broth (Merck, Germany) medium and incubated at 30 ° C for three to seven days in a candle jar. The re-cultivation was then performed on a MRS agar (Merck, Germany). The MRS agar plate was incubated for two to five days in a candle jar at a temperature of 30 ° C. From each positive culture honey sample, two or three different colonies were examined and gram-positive and catalase-negative bacilli were isolated. Isolates were detected at the species level by sequencing of 16S rDNA gene.

DNA extraction was performed by boiling procedure (18). To perform PCR, primers were used for the 27F (5'-CTCGTTGCGGGACTTAA-3 ') and 1522R (5'-GCAGCAGTAGGGAATCTTC-3') and the PCR products were sequenced and blast in the NCBI (19, 20). To test the probiotic capacity of Lactobacillus isolates (survival in acidic conditions), one ml of suspension containing 109 CFU/ml of each isolate was added to 9 ml of PBS with a pH of 3.3 and the survival bacterial count at time of zero and after 3 hours incubation was counted on a MRS agar. Isolates that are less than 106 CFU/ml after three hours consider as resistant to acid (21).

To investigate the resistance of each isolate of lactobacillus to bile, two tubes were prepared containing one 9 milliliters of MRS broth containing 0.3% (W/V) bile (Oxgall, Sigma, Germany) and the other containing MRS broth without bile (control) to the both tubes 90 µl of freshly isolated Lactobacillus isolate were added. The growth rate of the isolates at 0 and 8 hours after incubation was measured by a spectrophotometer at 630 nm (22,23).

Resistance of isolates to bile was calculated using Cinh coefficient formula. Isolates with an inhibitory factor of less than 0.4 are considered as resistant to bile (24). The antimicrobial activity of Lactobacillus isolates was investigated by diffusion method from wells. At first, a suspension of 107 CFU/mL was prepared from Staphylococcus aureus ATCC 25923 and cultured as lawn cultivation on the nutrient agar. The supernatant of Lactobacillus isolates was isolated in MRS broth with centrifuge (13000 RPM-10 min) and 100 µl of it was added to each well, and the plates were incubated for 14 to 15 hours at 37 ° C. The diameter of the inhibition-growth zone around the wells was measured and the isolates with the diameter of the inhibition zone <11 mm as negative, 16-11 mm as an inhibitor of medium (+), 17-22 mm as a strong inhibitor (++) and> 23 mm were classified as very potent inhibitors (+++). Positive control strain L Rhamnosus GG was also used (25).

The experiments were repeated twice. Antimicrobial resistance of isolates was investigated by disc diffusion method on a Mueller-Hinton Agar (Merck, Germany) containing 10% MRS broth. The diameter of the inhibition zone of discs was measured

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and interpreted according to previous studies, so that the isolates (≤15 mm) were resistant, (16-16 mm) moderate and (≥ 21 mm) sensitive (26). Antibiotics of cefotaxime (30 µg), nalidixic acid (30 µg), vancomycin (30 µg), cotrimoxazole (25 µg), ciprofloxacin (5 µg), streptomycin (10 µg), amikacin (30 µg) Gentamicin (10 µg), erythromycin (15 µg), tetracycline (30 µg) and ampicillin (10 µg) were used.

Results

Of the 88 natural honey samples, 16 samples (18.18%) were positive for Lactobacillus cultivation. The highest number of samples collected from Mazandaran province then from Tehran province, but the Lactobacillus plant positive in Tehran province was higher than Mazandaran province, so that in Mazandaran province, out of a total of 40 samples, only 8 samples (20%) and in Tehran province, out of 25 samples, 8 samples (32%) were positive. The rest of the samples belonging to other provinces were all negatively affected by microbial culture.

At total of 39 isolates of Lactobacillus were identified by phenotypic method and PCR at genus level, among them, 6 isolates including four isolates L. Plantarum and two isolated L. Paracasei was identified by sequencing the 16S rDNA gene at the species level (Fig 1). In the acid resistance test, all six isolates tolerated acidic conditions, and in the bile resistivity test, each of the six isolates obtained a suppression factor of 0.4 and was in the group of bacteria sensitive to bile (Table 1).

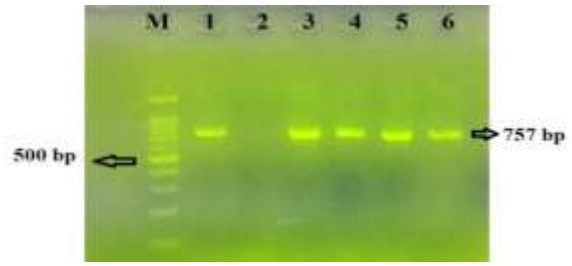


Figure1. PCR products of 16S rDNA gene of Lactobacillus Plantarum and lactobacillus Paracasei isolated from honey, M: Ladder 100 bp, Wells 1 and 2: Positive and negative control samples, Wells 3-6: Lactobacillus isolates

Of the six Lactobacillus isolates examined, L. Plantarum H15 had no inhibitory effect on Staphylococcus aureus. Isolates L Plantarum H46, L Plantarum H47 and L Parasitic H14 had moderate inhibitory effect on this pathogen, and L Paracasei H13 and L Plantarum H59 showed a strong inhibitory effect on Staphylococcus aureus. Strong inhibitory effect of these two isolates was similar to l. Rhamnosus GG positive control isolate (Table 2). The highest antimicrobial resistance was observed for vancomycin (100%), nalidixic acid (100%),streptomycin (100%), cotrimoxazole (83.3%),gentamicin (83.3%), amikacin (66.66%), and Ciprofloxacin (66.66%).

Table 1. Resistance of Lactobacillus Plantarum and lactobacillus Paracasei isolates, isolated from honey to acid and bile.

Isolates	Code	Resistance to acid		Resistance to bile
		h_0	h_3	Cinh
L Plantarum	H15	10^8×5.7	10^8×5.4	0.56
L Plantarum	H59	10^8×6.3	10^8×6.4	0.83
L Plantarum	H46	10^8×8.1	10^8×8.3	0.72
L Plantarum	H47	10^9×2.2	10^9×2.1	0.68
L Paracasei	H13	10^9×1.8	10^9×1.8	0.85
L Paracasei	H14	10^8×6.8	10^8×6.8	0.99

h0: Number of bacteria counted at zero, h3: Number of bacteria counted after 3 hours

Table 2. Antibacterial activity of Lactobacillus Plantarum and Lactobacillus Paracasei isolated from honey against Staphylococcus aureus ATCC 25923.

Isolates	Code	*Staphylococcus aureus mm*
L Plantarum	H15	0*(-)
L Plantarum	H59	20(++)
L Plantarum	H46	14(+)
L Plantarum	H47	16(+)
L Paracasei	H13	18(++)
L Paracasei	H14	16(+)
L Rhamnosus	GG	18(++)

*** Isolates with a diameter of the inhibition zone <11 mm were considered as negative, 16-to 11-mm were considered as medium inhibitors (+), 22-17 mm were considered as strong inhibitors (++) and> mm23 were considered as very strong inhibitors (+++).**

Discussion

The present study showed that L Plantarum and L Paracasei isolated from honey can inhibit the growth of pathogenic bacteria such as Staphylococcus aureus. Aween et al. in Malaysia, of 13 samples isolated from

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honey, isolated 32 isolates of acidic lactic bacteria, of which 6 L. Acidophilus isolates were evaluated for anti-microbial activity against different pathogens, with the highest inhibitory effect on Staphylococcus aureus (16). In Malaysia, Tajabadi et al. investigated 92 isolates of lactobacillus in phenotypic and molecular terms, from 10 samples of honey from honey bee, most of which were L. Plantarum, L Fermentum and L Pentosus was (17). The present study, similar to the above two studies, was able to isolate lactobacillus from honey samples. However, the type of Lactobacillus species detected is different from the present study, which can indicate the diversity of lactobacillus species in honey samples in different regions of the world.

It has been determined that L Paracasei, L Rhamnosus, L Acidophilus, l. Casei and L Fermentum isolated from sources such as goat's milk, human vagina, and etc, can inhibit Staphylococcus aureus (14, 27-29). In this study, all isolates were susceptible to acidbut resistant to bile. In a study by Kelanne et al. in Finland, all lactobacilli isolated from honey and fruit were susceptible to acid but resistant to bile (30). It seems that the reason for the difference between the present study and Kelanne study is time of tests. In the present study, after three hours' exposure to the acid, and in the Kelanne study, after seven days of exposure to the acid, the isolates survivance was studied. In the Kelanne study, the isolates survivance after three hours' exposure to the bile but in the present study after 8 hours' exposure to the bile was checked. Lactobacillus isolates of probiotic candidates should not have antibiotic resistance genes that can be transmitted to other bacteria. The most antibiotic resistance to vancomycin, nalidixic acid and streptomycin was observed in this study. The study of Davoodabadi et al in Iran showed that the isolated lactobacilli of the stool sample (92.59%) were resistant to streptomycin and vancomycin (31). It has been shown that lactobacilli are intrinsically resistant to vancomycin and aminoglycosides, and this chromosomal resistance is un-transferable and its safety is not worrying (32,33).

The present study showed that Iranian honey samples could be a source of indigenous lactobacilli and some of these isolates have an antimicrobial potential especially against Staphylococcus aureus and may be used as a probiotic candidate for prevention and treatment of infections caused by this pathogen, which requires further investigation.

Conflict of Interest: No conflicts of interest.

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References

1.Paul DV, George MG, Dorothy J, Noel RK, Wolfgang L, Fred AR. Bergey's Manual of Systematic Bacteriology. 2nd ed. Springer-Verlag, New York; 2009. P. 465-511.

2.Sonomoto K, Yokota A. Lactic acid bacteria and bifidobacteria: current progress in advanced research. Horizon Scientific. 2011. P. 68-80. Avaible From: https://www.caister.com/lactic-acid-bacteria

3.Holt JG, Krieg NR, Sneath PH, Staley JT, Williams ST. Bergey's manual of determinative bacteriology. Baltimore: Williams and Wilkins; 1994. P. 121-30.

4.Nakano S, Noguchi T, Takekoshi H, Suzuki G, Nakano M. Maternal-fetal distribution and transfer of dioxins in pregnant women in Japan, and attempts to reduce maternal transfer with Chlorella (Chlorella pyrenoidosa) supplements. Chemosphere. 2005;61(9):1244-55.

5.Khismatullina N. Apitherapy: Guidelines for more effective use: Mobile; 2005. Avaible From: https://books.google.com/books/about/Apitherapy.html?id=77RFMwEACAAJ.

6.Wright AL, Bauer M, Naylor A, Sutcliffe E, Clark L. Increasing breastfeeding rates to reduce infant illness at the community level. Pediatrics. 1998;101(5):837-44.

7.Araya M, Morelli L, Reid G, et al. Guidelines for the evaluation of probiotics in food. ftp fao org/es/esn/food/wgreport2 pdf>(pp 1e11). 2002.

8.Kaur IP, Chopra A, Chopra K. Probiotics in paediatric disorders. Int J Pharma Med. 2006;20(1):37-48.

9.Blaut M. Relationship of prebiotics and food to intestinal microflora. Eur J Nutr. 2002;41(1):11-6.

10.Fuller R. Probiotics in human medicine. Gut. 1991;32(4):439-42.

11.Kopp-Hoolihan L. Prophylactic and therapeutic uses of probiotics: a review. J Am Diet Assoc. 2001;101(2):229-38.

12.Jamali H, Paydar M, Radmehr B, Ismail S, Dadrasnia A. Prevalence and antimicrobial resistance of Staphylococcus aureus isolated from raw milk and dairy products. Food Control. 2015;54:383-8.

13.Roussel S, Felix B, Vingadassalon N, Grout J, Hennekinne JA, Guillier L, et al. Staphylococcus aureus strains associated with food poisoning outbreaks in France: comparison of different molecular typing methods, including MLVA. Front Microbiol. 2015;6:882.

14.Karska-Wysocki B, Bazo M, Smoragiewicz W. Antibacterial activity of Lactobacillus acidophilus and Lactobacillus casei against methicillin-resistant Staphylococcus aureus (MRSA). J Microbiol Res. 2010;165(8):674-86.

15.Gan BS, Kim J, Reid G, Cadieux P, Howard JC. Lactobacillus fermentum RC-14 inhibits Staphylococcus aureus infection of surgical implants in rats. J Infect Dis. 2002;185(9):1369-72.

16.Aween MM, Hassan Z, Muhialdin BJ, Eljamel YA, Al-Mabrok ASW, Lani MN. Antibacterial activity of lactobacillus acidophilus strains isolated from honey marketed in malaysia against selected multiple antibiotic resistant (mar) gram-positive bacteria. J Food Sci. 2012;77(7):364-71.

17.Tajabadi N, Mardan M, Saari N, Mustafa S, Bahreini R, Manap MYA. Identification of Lactobacillus plantarum, Lactobacillus pentosus and Lactobacillus fermentum from honey stomach of honeybee. Braz J Microbiol. 2013;44(3):717-22.

18.Antonsson M, Molin G, Ardö Y. Lactobacillus strains isolated from Danbo cheese as adjunct cultures in a cheese model system. Int J Food Microbiol. 2003;85(1):159-69.

19.Yun JH, Lee KB, Sung YK, Kim EB, Lee H-G, Choi YJ. Isolation and characterization of potential probiotic lactobacilli from pig feces. J Basic Microbiol. 2009;49(2):220-6.

20.Hilmi HTA, Surakka A, Apajalahti J, Saris PEJ. Identification of the most abundant Lactobacillus species in the crop of 1-and 5-week-old broiler chickens. Appl Environ Microbiol. 2007;73(24):7867-73.

21.Tsai C-C, Lin P-P, Hsieh Y-M. Three Lactobacillus strains from healthy infant stool inhibit enterotoxigenic Escherichia coli grown in vitro. Anaerobe. 2008;14(2):61-7.

22.Gilliland SE, Walker DK. Factors to consider when selecting a culture of Lactobacillus acidophilus as a dietary adjunct to produce a hypocholesterolemic effect in humans. J Dairy Sci. 1990;73(4):905-11.

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[DOI: 10.18869/acadpub.jbums.20.3.44]

23.Belviso S, Giordano M, Dolci P, Zeppa G. In vitro cholesterol-lowering activity of Lactobacillus plantarum and Lactobacillus paracasei strains isolated from the Italian Castelmagno PDO cheese. J Dairy Sci Tech. 2009;89(2):169-76.
24.Gopal PK, Prasad J, Smart J, Gill HS. In vitro adherence properties of Lactobacillus rhamnosus DR20 and Bifidobacterium lactis DR10 strains and their antagonistic activity against an enterotoxigenic Escherichia coli. Int J Food Microbiol. 2001;67(3):207-16.
25.Rammelsberg M, Radler F. Antibacterial polypeptides of Lactobacillus species. J Appl Microbiol. 1990;69(2):177-84.
26.Hashemi SMB, Shahidi F, Mortazavi SA, Milani E, Eshaghi Z. Potentially probiotic Lactobacillus strains from traditional Kurdish cheese. Probiotics Antimicrob Proteins. 2014;6(1):22-31.
27.Ocaña VS, Pesce de Ruiz Holgado AA, Nader-Macías ME. Growth inhibition of Staphylococcus aureus by H2O2-producing Lactobacillus paracasei subsp. paracasei isolated from the human vagina. FEMS Immunol Med Microbiol. 1999;23(2):87-92.
28.Anas M, Eddine HJ, Mebrouk K. Antimicrobial activity of Lactobacillus species isolated from Algerian raw goat's milk against Staphylococcus aureus. World J Dairy Food Sci. 2008;3(2):39-49.
29.Gilliland S, Speck M. Antagonistic action of *Lactobacillus acidophilus* toward intestinal and foodborne pathogens in associative cultures. J Food Prot. 1977;40(12):820-3.
30.Kelanne N. Applicability of fructophilic lactic acid bacteria in food industry. 2012:55-100.
31.Davoodabadi A, Dallal MMS, Foroushani AR, Douraghi M, Harati FA. Antibacterial activity of Lactobacillus spp. isolated from the feces of healthy infants against enteropathogenic bacteria. Anaerobe. 2015;34:53-8.
32.Tomás MSJ, Duhart CIS, De Gregorio PR, Pingitore EV, Nader-Macías ME. Urogenital pathogen inhibition and compatibility between vaginal *Lactobacillus* strains to be considered as probiotic candidates. Eur J Obstet Gynecol Reprod Biol. 2011;159(2):399-406.
33.Saarela M, Mogensen G, Fonden R, Mättö J, Mattila-Sandholm T. Probiotic bacteria: safety, functional and technological properties. J Biotechnol. 2000;84(3):197-215.

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