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Antimicrobial Resistance Patterns of Probiotic Strains Isolated from Probiotic Yogurts of Iran

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ABSTRACT

Background: Probiotics mainly belong to *Lactobacillus* spp. and they are useful for humans in appropriate amounts. The present study aimed to determine antibiotic resistance patterns of such bacteria isolated from probiotic yogurts of Iran.

Methods: Probiotic bacteria were isolated from 7 yogurt samples and the isolates were identified by conventional methods and then confirmed using polymerase chain reaction (PCR) technique. Antibiotic susceptibility test was performed by disc diffusion test in order to determine isolates antibiotic resistance patterns.

Results: From 7 yogurt samples, 8 isolates were recovered and PCR assay also showed that the isolates belong to *Lactobacillus delbrueckii* subsp. *bulgaricus*. Antibiotic susceptibility testing showed that three isolates from three brands were resistant to vancomycin and gentamicin. Other strains were susceptible to all tested antibiotics.

***Conclusion*:** Low resistance rate to antibiotics among Iranian probiotic bacteria indicate that the consumption of their products is safe but their clearance following antibiotic therapy can be unfavorable. Hence, applying probiotic strains with non-transferrable resistance elements in probiotic yogurts can be a useful strategy to make stable probiotic products.

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Introduction

Probiotic bacteria are a group of microorganisms that are beneficial for human health when used at appropriate amounts (1). These bacteria mainly belong to lactic acid metabolic group including *Lactobacillus* spp., *Bifidobacterium* spp. and *Enterococcus* spp. and are an important part of human and animal's intestinal microbiota (2, 3). *Lactobacillus* spp. as the main genus of probiotic bacteria produce bacteriocins, which are small peptides that can kill and eliminate pathogenic bacteria in gastrointestinal tract (GIT) (4). Different criteria are considered in selection of probiotic species for human consumption including resistance to acid and bile, ability to colonize the gastrointestinal tract and adherence to host epithelial tissues (5, 6). Probiotic bacteria are also able to inhibit the growth of some food-borne gastrointestinal pathogens such as *Salmonella* spp., and *Listeria monocytogenes* (7).

Nowadays, resistance to antibiotics is increasing among bacterial species and the use of probiotics to treat resistant bacterial infections have been recently suggested (8). However, the application of lactic acid bacteria could results to antibiotics resistance among floral bacteria by gene transfer mechanism (9). Consumption of probiotic food products may also result in the transfer of resistance genes to pathogenic bacteria, which can lead to impairment in their treatment (10).

The present study aimed to isolate probiotic bacteria from the Iranian probiotic yogurts and also to decipher the abundance of the antibiotic resistance properties in such beneficial bacteria.

Materials and Methods

Samples and culture condition

Totally, 7 brands of Iranian yogurts (AK (A), AS (A), AZ (A), AH (A), AM (A), AM (B) and AL (A)) were assessed. The yogurt samples were cultured in MRS broth (Merck, Darmstadt, Germany) and incubated at 37 ºC for 3 days under microaerophilic condition. About of 100 microliters of cultured bacteria were transferred into 1cc PBS 1X buffer with pH 3 and incubated at room temperature (10). After 3h, the acidic treated bacteria were cultured in MRS agar (Merck, Darmstadt, Germany) and incubated at 37 °C under microaerophilic condition. Growing colonies were examined by microscopic and phenotypic methods and then subjected to confirm by Polymerase Chain Reaction (PCR) method.

PCR

Single colonies were cultured in MRS broth (Merck, Darmstadt, Germany) and DNA was extracted using DNA extraction kit according to manufacturer's instrument (FAVORGEN, Taiwan). The quality of extracted DNAs was observed by agarose gel electrophoresis.

Three pairs of specific primers were designed using Primer3 software (11) and their sequences are presented on Table 1.

Polymerase chain reaction carried out in a final 25ul reaction mixture containing 12.5 µl master mix (CinnaGen, Iran), 10 pmol of both forward and reverse primers (Bioneer, Korea) and 50 ng DNA. The PCR reactions were run as below: initial denaturation at 95 °C, 5 min; 30 cycles of 94 °C for 30s, 60 °C for 30s and 72 °C for 30s; and final extension at 72°C for 5 min using a thermocycler (Eppendorf Thermal cycler, Germany).

The PCR products were electrophoresed on 1% (W/V) agarose gel (Sigma-Aldrich, USA) and visualized under UV illumination (UviTec Cambridge) after staining with a DNA Safe Stain (CinnaGen, Iran).

Antibiotic susceptibility testing

Antimicrobial susceptibility testing was performed according to the Clinical and Laboratory Standards Institute (CLSI 2017) and by the Kirby Bauer‘s’ disc diffusion method. Tested antibiotic discs (Mast Group Ltd., Merseyside, UK) were include: tetracycline (T30C),

chloramphenicol (C30C), penicillin (PG10C), erythromycin (E15C), vancomycin (VA30C), gentamicin (GM10C), ampicillin (AP10C), rifampicin (RP5C) and clindamycin (CD2C).

Results

Isolated strains

Totally 8 strains of *Lactobacillus* were isolated from 7 yogurt samples. The results of PCR method showed that the all of strains belonged to *L. delbrucki* subsp. *bulgaricus* (Figure 1).

Antibiotic susceptibility testing

Antimicrobial susceptibility test carried out according disc diffusion method. The most of strains were susceptible to most tested antibiotics. Resistance to GM and VA were also observed among 2 strains. The results are also summarized on Table 2.

Discussion

In the present study, 7 brands of probiotic yogurts were selected and the only *L. delbrucki* subsp. *bulgaricus* isolated from all 7 yogurt brands.

Tolerance to different conditions such as resistance to acid (pH 3) and bile and high durability in the products are the properties that commonly used in probiotic products (13, 14). We applied an acidic condition (pH 3) for all samples in in order to evaluate the tolerance of strains against acidic environment. The acidic tolerance test showed that *L. delbrucki* subsp. *bulgaricus* is the only species that is acid resistant and therefore isolated from the examined products. These results are not consistent with manufacturer's claims that introduce L. acidophilus as a probiotic organism into probiotic yogurts. Probiotic yogurts may have two species but the only one of them was stable to acid (pH 3) and hence, only one species isolated following acidic condition.

Based on the results of antibiotic susceptibility testing; the only two strains were resistant to vancomycin and gentamicin. The results are similar to the results of other researches (15, 16). The use of antimicrobial resistant probiotics can increase the risk of antibiotic resistance genetic elements transfer to sensitive strains into gastrointestinal tracts and it may also interferes with treatment of some infections (17, 18)

Resistance to antimicrobial agents was low in tested probiotic bacteria, which can have some advantages and disadvantages. One of the advantages is antibiotic resistance transferring to the microbial flora is completely excluded, (19) but they can eliminated after antibiotics treatment (20). It is believed that probiotic bacteria with intrinsic resistance to antimicrobial agents could be beneficial for microbiota to restore in gut after antibiotic treatment (21).

Conclusion

In conclusion, studying the persistence of different probiotic species against acidic condition with more accurate methods. Finally, consumption of probiotic yogurts in Iran can help the normal functioning of the body's flora due to their low antibiotic resistance rate.

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Table 1. Primers used in study.

Species	Forward(5'→3')	Revers(5'→3')	Amplicon size(bp)
L. delbrucki subsp.*bulgaricus*	AAG TCC TCG TTT GGT GGT TG	ATT CCT TGA CGT TGG TCA GC	164
L .acidophilus	GCT TGC CGA TAC ATT CTC CG	CCG CGA CGA CCT ACT CTT AT	213
L. casei	TCA TGA CTG CGA CGG AAT TA	TGA AGC CGT GAC TGC AAA T	317

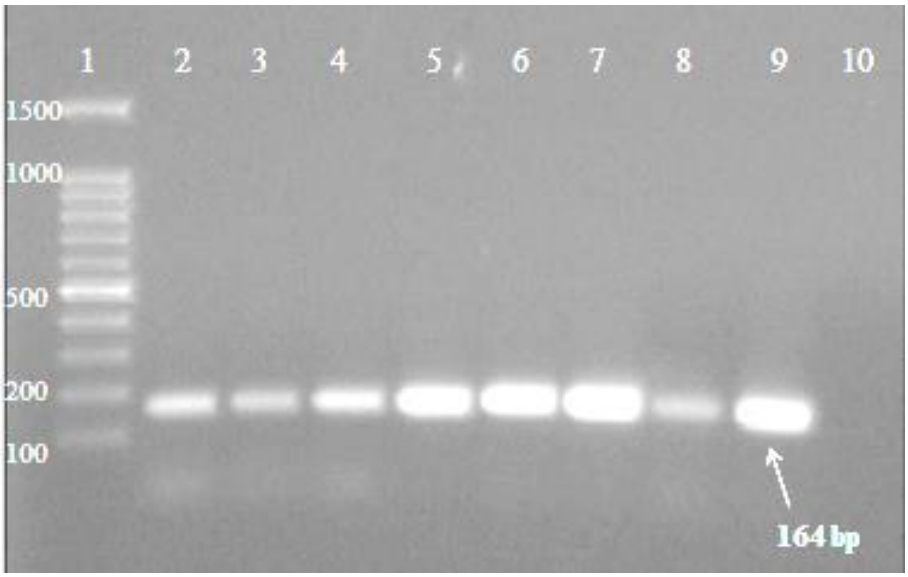

Figure 1. Electrophoresis of PCR products in agarose gel 1%. lane1: DNA ladder100bp; lanes 2-9: PCR products; lane 10, negative control.

Table 2. Results of disc diffusion testing.

		Antibiotics								
Yogurt brands	**Isolated species**	**Ampicillin**	**Chloramphenicol**	**Clindamycin**	**Erythromycin**	**Penicillin**	**Rifampicin**	**Tetracycline**	**Gentamicin**	**Vancomycin**
AK(small)	*L. delbrucki* subsp. *bulgaricus*	S	S	S	S	S	S	S	S	S
AK(A)	*L. delbrucki* subsp. *bulgaricus*	S	S	S	S	S	S	S	R	R
AL(A)	*L. delbrucki* subsp. *bulgaricus*	S	S	S	S	S	S	S	S	S
AS(A)	*L. delbrucki* subsp. *bulgaricus*	S	S	S	S	S	S	S	R	R
AH(A)	*L. delbrucki* subsp. *bulgaricus*	S	S	S	S	S	S	S	S	S
AZ(A)	*L. delbrucki* subsp. *bulgaricus*	S	S	S	S	S	S	S	S	S
AM(A) (High fat)	*L. delbrucki* subsp. *bulgaricus*	S	S	S	S	S	S	S	S	S
AM(B) (Low fat)	*L. delbrucki* subsp. *bulgaricus*	S	S	S	S	S	S	S	S	S

Conflict of interest

The authors declare that there are no conflicts of interest.

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