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ANTIBIOTIC RESISTANCE IN LACTIC ACID BACTERIA ISOLATED FROM FERMENTED DAIRY PRODUCTS AND BOZA

*Gamze Başbülbül**[1], *Melihcan Özteber*[1], *Haci Halil Biyik*[1]

Address(es): Gamze Başbülbül
Adnan Menderes University, Faculty of Arts and Science, Biology Department, 09010, Aydin/TURKEY. phone number: 0090 256 218 2000-1937

*Corresponding author: gbasbulbul@adu.edu.tr

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ABSTRACT

In this study, the resistance of 83 strains of lactic acid bacteria isolated from Turkish cheese, yogurt, kefir and boza samples to 6 antibiotics (gentamicin, tetracycline, chloramphenicol, erythromycin, vancomycin and ciprofloxacin) was evaluated. The 83 isolates were identified by 16S rRNA gene sequencing and according to BLAST comparisons with sequences in the data banks, those strains showing the highest similarities with the isolates were *Enterococcus faecium* (10), *Lactococcus lactis* subsp. *Lactis* (10), *Lactobacillus fermentum* (6), *Lactobacillus plantarum* (6), *Lactobacillus coryniformis (7), Lactobacillus casei* (13), *Leuconostoc mesenteroides* (14), *Pediococcus pentosaceus* (10), *Weisella confusa* (7). Antimicrobial resistance of strains to 6 antibiotics was determined using the agar dilution method. The antibiotic resistance among all the isolates was detected against chloramphenicol (31,3 % of the isolates), tetracycline (30,1 %), erythromycin (2,4 %), ciprofloxacin (2,41%), vancomycin (73,5 %, intrinsic resistance). Overall 19,3 % of the isolates showed resistance against multiple antibiotics. Antibiotic resistance genes were studied by PCR and the following genes were detected; *tet*(M) gene in *Lactobacillus fermentum* (1), *Lactobacillus plantarum* (1), *Pediococcus pentosaceus* (5), *Enterococcus faecium* (2), *Weisella confusa* (4) and the vancomycin resistance gene *van*(A) in one *Weisella confusa* strain.

Keywords: Antibiotic resistance, Lactic acid bacteria, Boza, Fermented dairy products

INTRODUCTION

Lactic acid bacteria (LAB) are a group composed of Gram positive bacteria and they excrete lactic acid into the medium as a main fermentation product (**Schleifer & Ludwig 1995)**. Many LAB species are present as contaminants on raw agricultural materials or deliberately added as starter cultures into them (**Leroy & de Vuyst, 2004; Capcarova *et al.*, 2011; Sharma *et al.*, 2013**). LAB have a long history of safe use as fermenting natural products and probiotics intended for health benefits and have acquired the "Generally recognized as safe" (GRAS) status (**Mathur & Singh, 2005**) but there is a great attention to these bacteria may serve as reservoirs of antibiotic resistance (**Thumu & Halami 2012**). The main mechanisms of horizontal transfer in bacteria in natural environments are believed to be conjugation and transduction via bacteriophages (**Kleinschmidt *et al.*, 1993**). LAB have been reported to be capable of supplying antimicrobial resistance genes to food-borne or enteric pathogens (**Gevers *et al.*, 2003**). According to "qualified presumption of safety" (QPS) concept evolved by European Food Safety Authority (**EFSA, 2014**), the presence of transmissible antibiotic resistance markers in these bacteria has become an important safety criterion.
Fermented dairy foods and beverages like boza are extensively consumed in Turkey. Therefore investigations on antibiotic resistance profiles of LAB strains common to these products are very important for food safety. In this study antibiotic resistance of LAB strains present in fermented dairy products and boza was tested by phenotypic and genotypic methods, in an attempt to contribute to the knowledge about food associated LAB.

MATERIAL AND METHODS

Isolation and identification of strains

Bacterial strains were isolated from fermented dairy products including white cheese, sheep cheese, dry cheese, yogurt, kefir and boza samples from local markets and bazaars in Aydin (Turkey). The samples were initially homogenized and then serial dilutions between 10^{-3}- 10^{-6} were inoculated to MRS agar (Merck, Darmstadt Germany) plates supplied with 100 mg/L of cycloheximide ((Sigma-Aldrich, Taufkirchen, Germany) using spread plate method. MRS agar plates were incubated anaerobically at 30 °C for 48 hours using the Gas Pack system (Merck, Darmstadt, Germany). Pure cultures of isolates obtained and then strains with gram positive and catalase negative reactions were finally used for further identification.
DNA was extracted from isolates according to phenol-chloroform method defined by **Ronimus *et al*** (1997). The following oligonucleotide primers were used to amplify the 16S rRNA gene from strains, forward primer 20F (5'- AGA GTT TGA TCC TGG CTC AG - 3') and reverse primer 1390R (5'-GAC GGG CGG TGT GTA CAA-3'). PCR was utilized under the following thermocycling conditions: pre-denaturation step at 94 °C 5 min, 35 cycles of 94 °C for 30 s, 50 °C for 30 s and 72 °C for 10 s, followed by a final extension step of 72 °C for 15 min. To determine the closest relatives of the partial 16S rDNA sequences, amplified PCR products sequenced by Macrogene (South Korea). Sequences were BLAST in GenBank database (www.ncbi.nlm.nih.gov) for species assignment.

Antibiotic susceptibility testing

Minimal Inhibitory Concentrations (MIC) for 6 antibiotics was determined by agar dilution test using multipoint inoculator. Isolates were grown in MRS broth (Merck, Darmstadt, Germany) for 48 hours and then inoculated to LSM Agar (90% Iso-Sensitest™ Broth (Oxoid) + 10% MRS Broth (Merck)+ 1,5% Agar (Merck) (**ISO 10932**; **Klare *et al.*,2005**) plates containing gentamicin, tetracycline, chloramphenicol, erythromycin and vancomycin antibiotics (Oxoid, Hampshire, UK) with the concentration range of 0.0625-128 µg/ml or 0.0625-64 µg/ml for ciprofloxacin. The MIC was defined as the lowest concentration of antibiotic giving a complete inhibiton of visible growth in comparison to an antibiotic free control point. Breakpoints were adopted from EFSA report (2008), **Ouoba *et al.*,** 2008 and **Danielsen & Wind, 2003.**.

Detection of antibiotic resistance genes in LAB isolates

PCR amplification of antibiotic resistance genes was done in 50 µL volumes that contained 30 pmol of each specific primer (BM Labrotories, Ankara, Turkey), 2 mM $MgCl_2$, 1X Taq DNA polymerase buffer, each dNTP at a concentration of 200 µM, 1 U of Taq DNA polymerase (ABM, Canada) and 1 µL DNA used as a

template. The oligonucleotide primers and annealing temperatures are listed in Table 1. DNA fragments were amplified in a Techne TC3000 thermocycler up to 40 cycles and PCR products were separated by electrophoresis on 1,2 % agarose jel containing (0,05%, v/v) safe view solution. For *tet(M)*, *erm(C)* and *van(A)* genes positive controls were used.

Table 1 Primers, annealing temperatures and PCR conditions used for the detection of resistance genes.

Resistance gene	Primer	T_a°C	Reference
tet(M)	5'-GGTGAACATCATAGACACGC-3' 5'-CTTGTTCGAGTTCCAATGC-3'	55 °C	**(Werner et al, 2003)**
tet(K)	5'-CAATACCTACGATATCTA-3' 5'-TTGAGCTGTCTTGGTTCA-3'	52 °C	**(Werner et al, 2003)**
van(A)	5'-TTGCTCAGAGGAGCATGACG-3' 5'-TCGGGAAGTGCAATACCTGC-3'	65 °C	**(Klein et al., 2000)**
van (B)	5'-TTATCTTCGGCGGTTGCTCG -3' 5'-GCCAATGTAATCAGGCTGTC-3'	62 °C	**(Klein et al., 2000)**
cat	5'-ATGACTTTTAATATTATTRAWTT-3' 5'-TCATYTACMYTATSAATTATAT-3'	49 °C	**(Hummel et al., 2007)**
erm(A)	5'-TCTAAAAAGCATGTAAAAGAA-3' 5'-CTTCGATAGTTTATTAATATTAGT-3'	52 °C	**(Sutcliffe et al., 1996**
erm(B)	5'-GAAAAGGTACTCAACCAAATA-3' 5'-AGTAACCGTACTTAAATTGTTTAC-3'	52 °C	**(Sutcliffe et al., 1996)**
erm(C)	5'-TCAAAACATAATATAGATAAA-3' 5'-GCTAATATTGTTTAAATCGTCAAT-3'	52 °C	**(Sutcliffe et al., 1996)**

RESULTS

Identification of bacteria

Eighty-three presumptive LAB strains isolated from Turkish cheese, yogurt, kefir and boza samples. All isolates were catalase negative and Gram positive rods, cocci or pleomorphics. Strains were identified as *Enterococcus faecium* (10), *Lactococcus lactis* subsp. *Lactis* (10), *Lactobacillus fermentum* (6), *Lactobacillus plantarum* (6), *Lactobacillus coryniformis* (7), *Lactobacillus casei* (13), *Leuconostoc mesenteroides* (14), *Pediococcus pentosaceus* (10), *Weisella confusa* (7) according to BLAST results.

Phenotypic Profile of Antibiotic Resistance

Results obtained from the MIC's of tested bacteria are presented in Table 2. None of the tested bacteria were found to be resistant to gentamicin. Only two of the eighty three strains, *Enterococcus faecium* GL-21 and *Weisella confusa* GL-33 were resistant to erythromycin. The most prevalent resistances were tetracycline and chloramphenicol (30,1 % and 31,3 respectively). Twenty five LAB strains including eight of the ten pediococci, two of the ten *E. faecium* and all of the *W. confusa*, displayed phenotypic resistance to tetracycline with MIC values of 8 µg/ml or higher. For chloramphenicol four of the thirty-two lactobacilli, eight of the ten pediococci, all *Weisella* strains, two of the ten Enterococci and five of the thirteen *Leuc. mesenteroides* were found to be resistant with MIC values ranging between 8 and 16 µg/ml. *Weisella* and pediococci seemed like most resistant genera to tetracycline and chloramphenicol. For erythromycin, all LAB isolates displayed MIC values of 2 µg/ml or lower and evaluated as susceptible except for *E. faecium* GL-21 with MIC value of 32 µg/ µL and *W. confusa* GL-33 with of 16 µg/ml. *Lactococcus lactis* subsp. *Lactis* strains isolated in the study did not show any resistance to the tested antibiotics. Obtained LAB strains were intrinsically resistant to vancomycin except for those belonging to genera *Enterococcus* and *Lactococcus* which they were found to be susceptible to this antibiotic. E. *faecium* GLM-160 and *E. faecium* GLM-161 showed resistance to ciprofloxacin, *P.pentosaceus* strains were inherent resistant.

Detection of antibiotic resistance genes in LAB isolates

All of the isolated LAB strains were tested by PCR for the presence of the most frequently detected AR genes; *tet*(M), *tet*(K), *van*(A), *van*(B), *cat*, *erm*(A), *erm*(B) and *erm*(C) regardless of whether or not they are phenotypically resistant to antibiotics. *tet*(M) was detected in twelve of the tetracycline resistant LAB strains, while *van*(A) detected in one of the seven vancomycin resistant *Weisella* isolates. *Lb. fermentum* GL-9 and *Lb. plantarum* which are phenotypically susceptible to tetracycline, were found to carry the *tet*(M) gene (Table 3).

Table 2 LAB species isolated in the study, numbers and MIC ranges

Isolated LAB species	Gentamicin	Tetracycline	Chloramphenicol	Erythromycin	Vancomycin	Ciprofloxacin
***Lb. fermentum* (n=6)**	0.25-8	4-16	2-8	≤0,0625-0,25	128-≥128	4-16
***Lb. plantarum* (n=6)**	≤0,0625-4	2-32	1-8	≤0,0625-0,125	≥128	1-16
***Lb. coryniformis* (n=7)**	1-4	4-32	≤0,0625-4	≤0,0625-0,5	≤0,0625-≥128	0,5-8
***Lb. casei* (n=13)**	0,25-8	0,125-8	1-16	≤0,0625-0,5	≥128	0,25-2
***P. pentosaceus* (n=8)**	0,5-4	4-32	8-32	≤0,0625-0,125	≥128	16-32
***P. parvulus* (n=2)**	0,25	8-16 r	2-4	≤0,0625	≥128	1-2
***E. faecium* (n=10)**	8-32	0,125-16	4-16	0,125-32	0,25-1	≤0,0625-4
***L. lactis* subsp. *lactis* (n=10)**	0,25-4	≤0,0625-0,5	1-4	≤0,0625-0,125	0,125-0,25	0,5-2
***W. confusa* (n=7)**	0,5-8	8-32	16	0,125-16	≥128	0,25-0,5
***Leuc. mesenteroides* (n=14)**	0,125-2	0.0,5-4	2-4	≤0,0625-0,125	≥128	0,5-4

Table 3 PCR positive LAB strains and their MIC values

LAB strain	Gene	MIC value (µg/mL)
***Lb. fermentum* GL-9**	*Tet* (M)	8
***Lb. plantarum* GLM-209**	*Tet* (M)	32
***P. pentosaceus* GL-20**	*Tet* (M)	16
***P. pentosaceus* GL-62**	*Tet* (M)	32
***P. pentosaceus* GL-20**	*Tet* (M)	16
***P. pentosaceus* GL-63**	*Tet* (M)	32
***E. faecium* GL-83**	*Tet* (M)	16
***E. faecium* GL-89**	*Tet* (M)	16
***W.confusa* GL-31**	*Tet* (M)	8
***W.confusa* GL-55**	*Tet* (M)	8
***W.confusa* GL-74**	*Tet* (M)	8
***W.confusa* GL-56**	*Van* (A)	≥128
***W.confusa* GL-76**	*Tet* (M)	8

DISCUSSION

In the present study, 83 LAB isolates from Turkish fermented foods and boza were isolated and identified by 16S rRNA gene sequencing. After bacterial identification, the strains were evaluated by phenotypic and genotypic methods for their antibiotic resistance profiles.

The resistance of the studied *Lactobacillus* spp. to antibiotics was variable according to species and antibiotic tested. While none of the lactobacilli were resistant to gentamicin, erythromycin or ciprofloxacin, some *Lb. fermentum* and *Lb. coryniformis* strains were detected as resistant to tetracyclin and *tet*(M) gene was found in *Lb. fermentum* GL-9 as a silence resistance gene. Chloramphenicol resistance was more common in *Lb. casei* strains (GL-72, GLM-210, GLM-212) than other lactobacilli, only one *Lb. fermentum* was resistant to chloramphenicol. It has been shown that *Lactobacillus* spp. are generally susceptible to chloramphenicol, erythromycin and tetracycline (**Zhou *et al*., 2005; Rojo-Bezares *et al*., 2006; D'Aimo *et al*., 2007, Bujnakova *et al*., 2014**). However, resistant strains to these agents, and several genes providing resistance have also been identified. For example a chloramphenicol resistance cat gene has been found in *Lactobacillus plantarum* (**Ahn et al., 2002**), *tet*(M) gene in *Lactobacillus fermentum* isolates (**Gfeller *et al*., 2003**). **Cataloluk and Gogebakan (2004)** reported that six isolates of *Lactobacillus casei* from dairy products and human origin carried *tet*(M) genes. A previous study conducted with home-made Spanish cheese reported that *L. plantarum, L. acidophilus, L. brevis* and *L. casei* strains were resistant to tetracycline and chloramphenicol (**Herrero *et al*., 1996**).

Lactobacillus spp, *P.pentosaceus, W. confusa* and *L. mesenteroides* isolates showed very high MIC values (128 µg/ml or higher) for vancomycin. *Leuconostoc, Weissella, Pediococcus, Lactobacillus*, and *Erysipelothrix* are involved in clinically relevant, non-enterococcal intrinsic glycopeptide resistant Gram positive organisms (**Klein *et al*. 2000; Lahtinen *et al*., 2012**). Many strains of *Lb. plantarum, Lb. casei, Lb. salivarius, Lb. leichmannii, Lb. acidophilus* carry intrinsic resistance towards vancomycin which is due to the presence of D-alanine: D-alanine ligase-related enzymes **(Elisha and Courvalin 1995)**. Intrinsic vancomycin resistance of *Lactobacillus, Leuconostoc* and *Pediococcus* species has been used to separate them from other Gram-positive bacteria on vancomycin supplemented selective media **(Simpson *et al*. 1995).**

Among the Pediococci isolates, only two were susceptible to tetracycline and *tet*(M) gene was detected in four resistant *Pediococcus pentosaceus* strains. As indicated in the literature, data on the antibiotic susceptibility of *Pediococcus* spp. isolated from food are very scarce (**Ammor *et al*., 2007**). **Hummel *et al.*** (2007) investigated antibiotic resistances of 45 lactic acid bacteria those belong to the genera *Lactobacillus, Streptococcus, Lactococcus, Pediococcus* and *Leuconostoc*. One of the *Pediococcus* strain *P. pentosaceus* strain BFE 7436 was found to show low resistance to tetracycline. However, it was reported that neither of the genes those encode the ribosomal protection proteins [*tet*(M), *tet*(Q), *tet*(S) or *tet*(W)] nor the genes those encode the tetracycline efflux pumps [*tet*(K) or *tet*(L)] were found.

While chloramphenicol resistance seems to be common in *P. pentosaceus* strains isolated in our study, cat gene could not be detected in any of these resistant strains. It has been reported chloramphenicol usually active against *Pediococcus pentosaceus* isolates [**Gevers *et al*., 2000; Sabir *et al*., 2010**) and susceptibility levels are thought to be species-dependent (**Ammor *et al*., 2007**). The ciprofloxacin MICs were 16-32 µg/ml for *P. pentosaceus*. **Danielsen et al., (2005)** reported that ciprofloxacin resistance in *Pediococcus pentosaceus* was likely to be an intrinsic property of the species.

Enterococcus faecium strains isolated in our study were found to be resistant to different antibiotics. *E. faecium* GL-21 showed high level of resistance to erythromycin (32 µg/ml), and also chloramphenicol resistant which was isolated from boza, but none of the *erm*(A), *erm*(B), *erm*(C) or cat genes could be detected. Two of the four *E. faecium* strains, GL-83 and GL-89 were resistant to tetracycline and carried *tet*(M) gene.

In a study undertaken by **Temmerman *et al*. (2003**), a total of 29 *E. faecium* strains were isolated from different European probiotic products and antibiotic resistance was detected against tetracycline (24% of the isolates), erythromycin (97% of the isolates) and chloramphenicol (34% of the isolates). The resistance of *Enterococcus* species isolated from Turkish white cheese samples to 13 antibiotics is studied by Citak and coworkers (2004) and 96% of *E.faecium* isolates were found to be resistant to erythromycin, whereas 76% and 44% were resistant to chloramphenicol and tetracycline respectively. Huys and coworkers (2004) have found that 24% of *Enterococcus* isolates from European cheeses displayed phenotypic resistance to tetracycline with MIC ranges of 16 to 256 µg/ml. *E. faecium* GLM-160 and GLM-161 were resistant to ciprofloxacin with MIC values of 4 µg/ml. Similarly, ciprofloxacin resistance has been described among *E. faecium* isolates from different food sources at varying degrees.**(Franz *et al*., 2001; Drahovska *et al*., 2004; Johnston and Jaykus, 2004; Belicova *et al*., 2007; Valenzuela *et al*., 2008**)

W. confusa is present in some fermented vegetables and milk (**Bjorkroth *et al*., 2002**), as well as in human faeces of healthy individuals (**Walter *et al*., 2001**) or in some bacteremia cases (**Kumar *et al*., 2011**; **Harlan *et al*.**, 2011), In *Weisella confusa* strains from KoKo (fermented millet porridge) of Africa origin, one of these strains was found to be resistant to tetracycline (**Ouoba *et al*., 2008**). All the strains belonging to *Weisella confusa* species in our study showed multiresistant to tetracycline and chloramphenicol. Strain GL-33 which has the highest MIC value for tetracycline was also eryrthromycin resistant.

Ayeni *et al*., (2011) reported that *Weisella confusa* isolates obtained from Nigerian dairy products and cow's intestine are chloramphenicol resistant and authors argued that their results could indicate the presence of specific chloramphenicol resistance genes in *Weisella*.

While the most *Leuconostoc* species those have been investigated previously were reported to be susceptible to chloramphenicol, like to rifampicin, erythromycin, clindamycin and tetracycline (**Swenson *et al*.; 1990; Florez *et al*., 2005**) *Leuc. mesenteroides* strains isolated in our study were found to be resistant to chloramphenicol.

CONCLUSION

Knowledge on the antibiotic resistance of LAB is still limited and it is hard to evaluate the resistance profiles because of the heterogeneity of this group. Our findings suggest that the LAB strains belonging to the genera *Pediococcus, Leuconostoc, Weisella, Enterococcus* are generally resistant to clinically relevant antibiotics such as tetracycline and chloramphenicol. The authors concluded that prevalence of antibiotic resistant LAB in traditionally fermented dairy products and boza is high and these kinds of foods should be monitored carefully.

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REFERENCES

AHN C., COLLINS-THOMPSON D., DUNCAN C., STILES M.E. 1992. Mobilization and location of the genetic determinant of chloramphenicol resistance from *Lactobacillus plantarum* caTC2R. *Plasmid* 27, 169–176.

AMMOR, M.S., FLÓREZ, A.B., MAYO, B. 2007. Antibiotic resistance in non-enterococcal lactic acid bacteria and bifidobacteria. *Food Microbiol* 24, 559-70. http://dx.doi.org/10.1016/j.fm.2006.11.001

AYENI, F.A., SÁNCHEZ, B., ADENIYI, B.A., LOS REYES-GAVILÁN, C.G., MARGOLLES, A., MADIEDO, P.R. 2011. Evaluation of the functional potential of Weissella and Lactobacillus isolates obtained from Nigerian traditional fermented foods and cow's intestine. *Int J Food Microbiol* 147, 97-104. http://dx.doi.org/10.1016/j.ijfoodmicro.2011.03.014

BELICOVÁ, A., KRZKOVÁ, L., KRAJCOVIC, J., JURKOVIC, D., SOJKA, M., EBRINGER, L., DUINSKÝ, R. 2007. Antimicrobial susceptibility of Enterococcus species isolated from Slovak Bryndza cheese. Folia Microbiol 52(2):115-9.

BERGE, A.C.B., ATWILL, E.R., SISCHO, W.M. 2005. Animal and farm influences on the dynamic of antibiotic resistance in faecal *Eschrichia coli* in young dairy calves. *Preventive Veterinary Medicine*, 69, 25-38. http://dx.doi.org/10.1016/j.prevetmed.2005.01.013

B, ROJO-BEZARES, B, SAENZ, Y POETA, et al. 2006. Assessment of antibiotic susceptibility within lactic acid bacteria strains isolated from wine. *Int J Food Microbiol* 111(3), 234-240. http://dx.doi.org/10.1016/j.ijfoodmicro.2006.06.007

BJORKROTH, K.J., SCHILLINGER, U., GEISEN, R., WEISS, N., HOSTE, B., HOLZAPFEL, W.H., KORKEALA, H.J., VANDAMME, P. 2002. Taxonomic study of *Weissella confusa* and description of *Weissella cibaria* sp. nov., detected in food and clinical samples. *Int J Syst Evol Microbiol* 52. 141–148.

BUJNAKOVA, D., STRAKOVA, E., KMET, V. 2014. In vitro evaluation of the safety and probiotic properties of Lactobacilli isolated from chicken and calves. *Anaerobe* 29, 118-127. http://dx.doi.org/10.1016/j.anaerobe.2013.10.009

CAPCAROVÁ, M., WEIS, J., HRNČÁR, C., KOLESÁROVÁ, A., PETRUŠKA, P., KALAFOVÁ, A., PÁL, G., 2011. Effect of Probiotic Supplementation on Selected Indices of Energy Profile and Antioxidant Status of Chickens. *J Microbiol Biotech Food Sci* 1 (2) 225-235.
CATALOLUK, O., GOGEBAKAN, B.. 2004. Presence of drug resistance in intestinal lactobacilli of dairy and human origin in Turkey. *FEMS Microbiol Lett* 236. 7-12. http://dx.doi.org/10.1111/j.1574-6968.2004.tb09620.x
CITAK, S., YÜCEL, N., ORHAN, S. 2004. Antibiotic resistance and incidence of *Enterococcus* species in Turkish white cheese. *Int J Dairy Technol* 57 (1). 27-32. http://dx.doi.org/10.1111/j.1471-0307.2004.00122.x
D'AIMMO, M.R., MODESTO, M., BIAVATI, B. 2007. Antibiotic resistance of lactic acid bacteria and *Bifidobacterium* spp. isolated from dairy and pharmaceutical products. *Int J Food Microbiol* 115 (1). 35-42.http://dx.doi.org/10.1016/j.ijfoodmicro.2006.10.003
DANIELSEN, M., WIND, A. 2003. Susceptibility of *Lactobacillus* spp. to antimicrobial agents. *Int J Food Microbiol* 82. 1-11. http://dx.doi.org/10.1016/S0168-1605(02)00254-4
DANIELSEN, M., SIMPSON, P.J., O'CONNOR, O.B., ROSS, R.P., STANTON, C. 2007.Susceptibility of Pediococcus spp. to antimicrobial agents. *J Appl Micr* 102, 384-389. http://dx.doi.org/10.1111/j.1365-2672.2006.03097.x
DRAHOVSKÁ, H., SLOBODNÍKOVÁ, L., KOCÍNCOVÁ, D., SEMAN, M., KONCEKOVÁ, R., TRUPL, J., TURNA, J. 2004. Antibiotic resistance and virulence factors among clinical and food enterococci isolated in Slovakia. *Folia Microbiol* 49(6):763-8. http://dx.doi.org/10.1007/BF02931562
ELISHA, B.G., COURVALIN, P. 1995. Analysis of genes encoding dalanine:d-alanine ligase-related enzymes in Leuconostoc mesenteroides and Lactobacillus spp. *Gene* 152, 79–83.http://dx.doi.org/ 10.1016/0378-1119(94)00692-L

EFSA 2008. Technical guidance Update of the criteria used in the assessment of bacterial resistance to antibiotics of human or veterinary importance Prepared by the Panel on Additives and Products or Substances used in Animal Feed. European Food Safety Authorization 732. 1-15.
EFSA 2004. EFSA Scientific Colloqium Summary Report. QPS: qualified presumption of safety of microorganisms in food and feed. European Food Safety Authority, Brussels, Belgium
FRANZ, C.M.A.P., MUSCHOLL-SILBERHORN, A.B., YOUSIF, N.M.K, VANCANNEYT, M., SWINGS, J., HOLZAPFEL, W.F. 2001. Incidence of Virulence Factors and Antibiotic Resistance among Enterococci Isolated from Food. Appl Environ Micr 67 (9), 4385–4389. http://dx.doi.org/10.1128/AEM.67.9.4385-4389.2001

FLO´ REZ, A.B., DELGADO, S., MAYO, B. 2005. Antimicrobial susceptibility of lactic acid bacteria isolated from a cheese environment. *Can J Microbiol* 51, 51–58. http://dx.doi.org/10.1139/w04-114
GEVERS, D., DANIELSON, M., HUYS, G., SWINGS, J. 2003. Molecular characterization of tet(M) genes in *Lactobacillus* isolates from different types of fermented dry sausage. *Appl Environ Microbiol* 69, 1270–1275. http://dx.doi.org/10.1128/AEM.69.2.1270-1275.2003
GEVERS, D., HUYS, G., DEVLIEGHERE, F., UYTTENDAELE, M., DEBEVERF, J., SWINGS, J. 2000. Isolation and identification of Tetracycline Resistant Lactic acid bacteria from pre-packed sliced meat products. *Syst Appl Microbiol* 23, 279-284. http://dx.doi.org/10.1016/S0723-2020(00)80015-6
GFELLER, K.Y., ROTH, M., MEILE, L., TEUBER, M. 2003. Sequence and genetic organization of the 19.3-kb erythromycin- and dalfopristin resistance plasmid pLME300 from *Lactobacillus fermentum* ROT1. *Plasmid* 50, 190–201. http://dx.doi.org/10.1016/j.plasmid.2003.08.001
HARLAN, N.P., KEMPKER, R.R., PAREKH, S.M., BURD, E.M., KUHAR, D.T. 2011. Weissella confusa bacteremia in a liver transplant patient with hepatic artery thrombosis. Transpl Infect Dis.13 (3) : 290-3. http://dx.doi.org/10.1111/j.1399-3062.2010.00579.x
HERRERO, M., MAYO, B., GANZALES, B., SUAREZ, J.E. 1996, Evaluation of technologically important traits in lactic acid bacteria isolated from spontaneous fermentations. *J Appl Bacteriol* 81, 565–570. http://dx.doi.org/10.1111/j.1365-2672.1996.tb03548.x
HUMMEL, A.S., HERTEL, C., HOLZAPFEL, W.H., FRANZ, C.M.A.P. 2007. Antibiotic resistances of starter and probiotic strains of lactic acid bacteria. *Appl Environ Microbiol* 73, 730-739. http://dx.doi.org/10.1128/AEM.02105-06
HUYS, G., D'HAENE, K., COLLARD, J.M., SWINGS, J. 2004. Prevalance and molecular characterization of tetracycline resistance in *Enterococcus* isolates from food. *Appl Environ Microbiol* 70. 1555-1562. http://dx.doi.org/10.1128/AEM.70.3.1555-1562.2004
ISO 10932:2010 (Milk and milk products — Determination of the minimal inhibitory concentration (MIC) of antibiotics applicable to bifidobacteria and non-enterococcal lactic acid bacteria (LAB).
JOHNSTON, L.M., JAYKUS L.A., 2004. Antimicrobial Resistance of *Enterococcus* Species Isolated from Produce. *Appl Environ Micr* 70 (5). 3133–3137. http://dx.doi.org/10.1128/AEM.70.5.3133-3137.2004

KLARE, I., KONSTABEL, C., MULLER-BERTLİNG, S., REİSSBRODT, R., HUYS, G., VANCANNEYT, M., SWİNGS, J., GOOSSENS, H.,WITTE, W. 2005. Evaluation of new broth media for microdilution antibiotic susceptibility testing of *Lactobacilli, Pediococci, Lactococci*, and *Bifidobacteria*. *Appl Environ Microbiol* 71. 8982–8986. http://dx.doi.org/10.1128/AEM.71.12.8982-8986.2005
KLEIN, G., HALLMANN, C., CASAS, I.A., ABAD, J., LOUWERS, J., REUTER, G. 2000. Exclusion of vanA, vanB and vanC type glycopeptide resistance in strains of *Lactobacillus reuteri* and *Lactobacillus rhamnosus* used as probiotics by polymerase chain reaction and hybridization methods. *J Appl Microbiol* 89, 815-824. http://dx.doi.org/10.1046/j.1365-2672.2000.01187.x
KLEINSCHMIDT, J., SOEDING, B., TEUBER, M., NEVE, H. 1993 Evaluation of horizontal and vertical gene transfer and stability of heterologous DNA in *Streptococcus thermophilus* isolated from yogurt and yogurt starter cultures. *Syst Appl Microbiol* 16. 287-295. http://dx.doi.org/10.1016/S0723-2020(11)80482-0
KUMAR, A., AUGUSTİNE, D., SUDHİNDRAN, S., KURİAN, A.M., DİNESH, K.R., KARİM, S., PHİLİP, R. 2011. Weissella confusa: a rare cause of vancomycin-resistant Gram-positive bacteraemia. *J Med Microbiol* 60, 1539-41. http://dx.doi.org/10.1099/jmm.0.027169-0
LAHTİNEN, S., OUWEHAND, A.C., SALMİNEN, S., WRİGHT, A.V. 2011. Lactic acid bacteria, Microbiological and Functional Aspects, Fourth Edition, CRC Press, USA, 798 p. ISBN 978-1-4398-3677-4.
LEROY, F., DE VUYST, L. 2004. Lactic acid bacteria as functional starter cultures for the food fermentation industry. *Trends Food Sci Technol* 15. 67-78. http://dx.doi.org/10.1016/j.tifs.2003.09.004
MATHUR, S., SINGH, R. 2005. Antibiotic resistance in food lactic acid bacteria. *Int J Food Microbiol* 105. 281-295. http://dx.doi.org/10.1016/j.ijfoodmicro.2005.03.008
OUOBA, LII., LEI, V., JENSEN, L.B. 2008. Resistance of potential probiotic lactic acid bacteria and bifidobacteria of African and European origin to antimicrobials: determination and transferability of the resistance genes to other bacteria. *Int J Food Microbiol* 121. 217-224. http://dx.doi.org/10.1016/j.ijfoodmicro.2007.11.018
RONIMUS, R.S., PARKER, LE., MORGAN, H.W. 1997. The utilization of RAPD-PCR for identifying thermophilic and mesophilic *Bacillus* species. *FEMS Microbiol Lett* 147. 75-79. http://dx.doi.org/10.1016/S0378-1097(96)00507-1
SABIR, F., BEYATLI, Y., COKMUS, C., ONAL-DARILMAZ, D. 2010. Assessment of Potential Probiotic Properties of Lactobacillus spp., Lactococcus spp., and Pediococcus spp. strains Isolated from Kefir. *J Food Sci* 75 (9). 568-573. http://dx.doi.org/10.1111/j.1750-3841.2010.01855.x
SCHLEIFER, K.H., LUDWIG, W., 1995. Phylogenetic relationships of lactic acid bacteria. In: Wood, BJB., Holzapfel, wh (Eds). The lactic acid bacteria. Vol.2: The genera of lactic acid bacteria. Blackie Academic and Professional, Glasgow, pp. 7-18. http://dx.doi.org/10.1007/978-1-4615-5817-0_2
SHARMA, N., GARCHA, S., SINGH, S. 2013. Potential of Lactococcus lactis subsp. lactis MTCC 3041 as a biopreservative. J Microbiol Biotech Food Sci, 3 (2) 168-171.
SIMPSON, W.J., TAGUCHI, H.1995. The genus *Pediococcus*, with notes on the genera *Tetratogenococcus* and *Aerococcus*. In: The Genera of Lactic Acid Bacteria 125–172. Blackie Academic & Professional, London. ISBN 075-14-0215-X.

SUTCLIFFE, J., GREBE, T., TAIT-KAMRADT, A., WONDRACK, L. 1996. Detection of erythromycin-resistant determinants by PCR. *Antimicrob Agents Chemotherapy* 40 (11), 2562-2566.
SWENSON, J.M., FACKLAM, R.R., THORNSBERRY, C. 1990. Antimicrobial susceptibility of vancomycin-resistant Leuconostoc, Pediococcus, and Lactobacillus species. *Antimicrob Agents Chemotherapy* 34, 543–549. http://dx.doi.org/10.1128/AAC.34.4.543
TEMMERMAN, R., POT, R., HUYS, B., SWINGS, J. 2003. Identification and antibiotic susceptibility of bacterial isolates from probiotic products. *Int J Food Microbiol* 81 (1), 1-10. http://dx.doi.org/10.1016/S0168-1605(02)00162-9
THUMU, S.C.R., HALAMI, P.M. 2012. Acquired resistance to Macrolide-Lincosamide-Streptogramin Antibiotics in Lactic Acid Bacteria of Food Origin. *Indian J Microbiol* 52 (4), 530-537. http://dx.doi.org/10.1007/s12088-012-0296-5
VAY, C., CİTTADİNİ, R., BARBERİS, C., HERNA´N RODRI´GUEZ, C., MARTI´NEZ, H.P., GENERO, F., FAMİGLİETTİ, A. 2007. Antimicrobial susceptibility of non-enterococcal intrinsic glycopeptide-resistant Gram-positive organisms, *Diagnostic Microbiology and Infectious Disease* 57, 183–188. http://dx.doi.org/10.1016/j.diagmicrobio.2006.08.014
VALENZUELA, A.V., OMAR, N.B., ABRIOUEL, H., LOPEZ, R.L.,ORTEGA, E., CANAMERO, M.M., GALVEZ, A. 2008. Risk factors in enterococci isolated from foods in Morocco: Determination of antimicrobial resistance and incidence of virulence traits. Food Chem Toxicol46 (2008) 2648–2652. http://dx.doi.org/10.1016/j.fct.2008.04.021

WALTER, J., HERTEL, C., TANNOCK, G.W., LIS, C.M., MUNRO, K., HAMMES, W.P. 2001 Detection of Lactobacillus, Pediococcus, Leuconostoc and Weissella species in human feces by using group-specific PCR primers and denaturing gradient gel electrophoresis. *Appl Environ Microbiol* 67, 2578–2585. http://dx.doi.org/10.1128/AEM.67.6.2578-2585.2001

WERNER, G., WILLEMS, R.J., HILDEBRANDT, B., KLARE, I., WITTE, W. 2003. Influence of transferable genetic determinants on the outcome of typing methods commonly used for *Enterococcus faecium*. *J Clin Microbiol* 41. 1499-1506. http://dx.doi.org/10.1128/JCM.41.4.1499-1506.2003

ZHOU, J.S., PILLIDGE, C.J., GOPAL, P.K., GILL, H.S. 2005. Antibiotic susceptibility profiles of new probiotic *Lactobacillus* and *Bifidobacterium* strains. *Int J Food Microbiol* 98 (2),211-217. http://dx.doi.org/10.1016/j.ijfoodmicro.2004.05.011