ISSN 0026-2617, Microbiology, 2011, Vol. 80, No. 2, pp. 164–171. © Pleiades Publishing, Ltd., 2011.
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EXPERIMENTAL ARTICLES

Antibiotic Resistance of Potential Probiotic Bacteria of the Genus *Lactobacillus* from Human Gastrointestinal Microbiome

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Received May 20, 2010

Abstract—Thirteen *Lactobacillus* strains isolated from the gastrointestinal microbiome of people from the territory of the former Soviet Union have been studied for resistance to 15 antibiotics of different nature, namely, penicillins, aminoglycosides, macrolides, lincosamides, tetracyclines, chloramphenicol, and rifampicin. The strains included four strains of *L. plantarum*, four of *L. helveticus*, three of *L. casei/paracasei*, one of *L. rhamnosus*, and one of *L. fermentum*. All strains showed relative sensitivity to ampicillin, chloramphenicol, rifampicin, roxithromycin, erythromycin, and azithromycin, while none of them were sensitive to all tested antibiotics. *L. plantarum* strains had the broadest resistance spectra: one strain was resistant to tetracycline and three aminoglycosides and three strains were resistant to tetracycline and five aminoglycosides; one strain demonstrated high resistance to clindamycin and two strains to lincomycin. At the same time, two *L. plantarum* strains demonstrated resistance to benzylpenicillin coupled with sensitivity to ampicillin, another β-lactam antibiotic. Such resistance was clearly not related to the β-lactamase activity and could be explained by a specific mutation in one of the penicillin-binding proteins of the cell wall. Strains of *L. helveticus, L. casei/paracasei, L. rhamnosus*, and *L. fermentum* exhibited cross resistance to two to five different aminoglycosides. A PCR test of the resistance determinants for the widely clinically used antibiotics, tetracycline, chloramphenicol, and erythromycin revealed the presence of the *tetM* gene of conjugative transposon in *L. casei/paracasei* and two *L. helveticus* strains. Nucleotide sequence analysis of the amplified *tetM* fragments demonstrated their high homology with the *tetM* genes of *Enterococcus faecalis* and *Streptococcus pneumoniae*. The strains carrying *tetM* were tested for the genes of replication and conjugative transfer of plasmids in lactic acid bacteria. The results indicated that these strains contain genes identical or highly homologous to the *rep* and *trsK* genes of the plca36 plasmid and *rep* gene of the pLH1 and pLJ1 plasmids of lactic acid bacteria. The *tetM* gene is probably not expressed in strains sensitive to the corresponding antibiotic. However, the investigated lactobacilli cannot be directly used as probiotics, as they may serve as a source of genes for antibiotic resistance in the human microbiome.

Keywords: Lactobacillus, lactic acid bacteria, probiotics, human gastrointestinal microbiome, antimicrobial agents, antibiotic resistance, plasmids, genes *tetW/O, tetM, ermT, ermB, catTC, rep,* and *trsK.*

DOI: 10.1134/S0026261711020032

Many bacteria contain genes responsible for various mechanisms of antibiotic resistance and thus survive in the presence of antibiotics in the environment [1]. One of the functions of antibiotic resistance genes is assumed to be the maintenance of intercellular (population) communication in the medium [2, 3]. Recently, the complex of the genes controlling cell resistance to antimicrobials was named resistome [4–6]. During their long period of evolution, microorganisms have developed diverse strategies to overcome various ecological problems, including the presence of toxic agents in the environment. Microorganisms resistant to antibiotics were discovered over 70 years ago, before wide clinical use of antibiotics came into practice [7]. On the other hand, decades of antibiotic application have led to the appearance of bacteria resistant to many modern antibiotic formulations. Until recently, isolates of pathogenic bacterial strains were used in studies of the resistance phenomenon. However, according to a number of authors, bacteria of the gastrointestinal microbiome (lactic acid bacteria and bifidobacteria) may act as a reservoir of bacterial genes of antibiotic resistance that in turn may be transferred through the gastrointestinal tract to pathogenic bacterial strains causing various infectious diseases [8, 9].

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Table 1. List of strains used in the study, strain origin, NCBI GenBank accession number of 16S rRNA sequence

Species	Strain name	Source of isolation	NCBI GenBank accession no.
L. plantarum	CS 396	Healthy human intestine content, collection of Gabrichevskii Institute of Epidemiology and Microbiology	GU560031
L. plantarum	8-PA-3	Woman's vagina, Estonia, collection of Gabrichevskii Institute of Epidemiology and Microbiology	GU560032
L. plantarum	90-TC-4	Plant origin, Estonia, collection of Gabrichevskii Institute of Epidemiology and Microbiology	GU560033
L. plantarum	GKNM 101	Healthy human intestine content, collection of Gabrichevskii Institute of Epidemiology and Microbiology	GU560034
L. helveticus	Er 317/402 NARINE	the same	GU560035
L. helveticus	100 ash	the same	GU560036
L. helveticus	NK-1	the same	GU560037
L. helveticus	NNIE	the same	GU560038
L. casei/paracasei	GKNM 2311	the same	GU560039
L. casei/paracasei	GKNM 577	the same	GU560040
L. casei/paracasei	K_3Sh_{24}	the same	GU560041
L. rhamnosus	421-2	the same	GU560042
L. fermentum	GKNM 526	the same	GU560043

Naturally high resistance to a number of antibiotics, especially vancomycins, is characteristic of lactobacilli [10]. During the study of 500 isolates of lactic acid bacteria from various substrates and fermented products, 50% were identified as lactobacilli and genetic determinants of resistance to antibiotics were revealed almost exclusively in bacterial strains isolated from nonfermented substrates, while bacteria isolated from manufactured products were not resistant to antibiotics [11]. Typing of the genes of antibiotic resistance in 34 strains of lactobacilli identified as *Lactobacillus acidophilus*, L. *casei,* and *L. delbrueckii* subsp. *bulgaricus*, as well as in bifidobacteria isolated from industrial probiotic formulations and foodstuffs demonstrated that all strains under study were resistant to aztreonam, cycloserine, kanamycin, nalidixic acid, polymyxin B, and spectinomycin. Resistance to chloramphenicol, gentamycin, lincomycin, metronidazole, neomycin, streptomycin, tetracycline, and vancomycin varied among the strains [12]. In another, more detailed study, 115 strains of the species *L. paracasei* and *L. casei* were found to be naturally resistant to streptomycin and gentamycin. Three *L. paracasei* strains isolated from cheese were described as possessing acquired resistance to tetracycline and erythromycin. The resistance was associated with the genes *tetM* or *tetW* and *ermB*, respectively [13]. We assume that modern studies of lactobacillus biosafety should include detection of the possibility to transfer drug resistance genes. Studying antibacterial resistance gene distribution among lactic acid bacteria will help better understanding of the role of these bacteria in horizontal transfer of resistance genes and will eliminate the biosafety problem of starting and probiotic cultures of lactobacilli [8].

The goal of this study was to define the spectra of resistance and reveal the genes determining resistance to the most clinically common antibiotics in potential probiotic bacteria of the genus *Lactobacillus* from the human gastrointestinal microbiome. In addition, the potential possibility of transfer of the resistance genes was evaluated.

MATERIALS AND METHODS

Strains and cultivation conditions. In the present work, 13 strains of the genus *Lactobacillus* (obtained from the Gabrichevskii Institute of Epidemiology and Microbiology) were used isolated from the intestinal content of healthy patients. A list of the strains is presented in Table 1. Physiological, biochemical, and molecular genetic characteristics of the strains have been reported in our earlier works [14, 15]. Bacterial cultures were grown for 24–48 h in liquid and agar MPC media (HiMedia) in a thermostat at 37 ± 0.5°C. Cultivation on agar-solidified media was performed in an anaerobic culture apparatus using gas packages (bioMerieux) providing 10% CO_2 atmosphere.

Bacterial sensitivity to antibiotics was determined by the disc diffusion technique according to the methodological recommendations [16].

Total genome DNA was isolated according to [14].

Table 2. Primers used in PCR analysis to test the presence of the genes responsible for resistance to antimicrobial preparations

Gene	Organism	GenBank accession no.	Primer title*	Primer nucleotide sequence, 5'–3'	Expected fragment length
catTC	*Lactobacillus reuteri*	U75299	CatTC-PF CatTC-NR	CGACGGAGAGTTAGGTTATTGG- GATAAGGCACTCCATCGAACTGACCATC	607 bp
ermB	*Enterococcus faecalis*	Y00116	ErmB-PF ErmB-NR	ACAGGTAAAGGGCATTTAACGACG TGGAACATCTGTGGTATGGCG	438 bp
ermT	*Lactobacillus reuteri*	M64090	ErmT-PF ErmT-NR	TTGAGATTGGTTCAGGGAAAGGTC GCAACCTTTTAGCAAATCCATATTCC	303 bp
tetM	*Enterococcus faecalis*	X04388	TetM-PF TetM-NR	CCACCGAATCCTTTCTGGGC CCGAGCAGGGATTTCTCCAC	444 bp
tetO	*Bifidobacterium thermophilum*	AM710601	TetO-NF TetO-PR	TCAATCGTTCCAAAATGCGG CTAACTTGTGGAACATATGCCGAACC	506 bp
*tetW***	*Bifidobacterium longum*	DQ060146	TetW384F TetW589R	CAAGATCGACCAGGCTGGCG GGCTGATTGGTTCTCCTGCG	206 bp

Notes: * To select primers, the known nucleotide sequences of DNA probes and primers to antibiotic resistance genes were used completely or partially [17, 18].

** Known primers TetW348F and TetW589R [18] were used.

Primer designations: F, forward, R, reverse.

Primer selection for molecular genetic analysis of the lactobacilli strains. The primers to reveal antibiotic resistance genes were selected using the online NCBI/Primer-BLAST tool (http://www.ncbi.nlm.nih.gov/tools/primer-blast/). The annotated nucleotide sequences of antibiotic resistance genes from *L. reuteri* and *Enterecoccus faecalis*, which is closely related to lactobacilli, as well as from bifidobacteria *Bifidobacterium thermophilum* and *B. longum*, were used. The common parameter in the choice of primers was annealing temperature of 64°C (for two of the known primers, the annealing temperature was 68 and 65°C). Parts of the single-chain DNA probes used previously for studying the occurrence of 90 antibiotic resistance genes in gram-positive bacteria including lactobacilli [17] were used as forward primers to the genes *catTC, ermB, ermT*, and *tetM*. Reverse primers were chosen for each forward primer at a distance of 200–600 bp apart (Table 2). To reveal the genes *tetW* and *tetO*, as well as the hybrids *tetW/32/O, tetO/W*, and *tetO/W/32/O/W/O* in PCR, the primers TetO-NF and TetO-PR containing part of the published nucleotide sequence of the primer *tetO* 1917R [18] (Table 2) were used along with the reported pair of primers TetW384F and TetW589R. The pair TetW348F and TetW589R was specific to all of the above-mentioned *tet* genes (including hybrid ones) with the exception of *tetO* and *tetM*, while the TetO-NF and TetO-PR pair was specific to the genes *tetO, tetW/32/O*, and *tetO/W/32/O/W/O*.

We also constructed primers for the three known lactobacillus plasmid genes. Homologous regions of the *rep* genes of plasmids pLH1 and pLJ1 from *L. helveticus* were used as targets to design primers L.h.repF and L.h.repR; a fragment of the *rep* gene of the plasmid plca36 from *L. casei* identical to the supposed *rep* genes of other lactobacilli (*L. gasseri* MV-22, *L. salivarius* UCC118 plasmid pSF118-44, *L. antri* DSM 16041, *L. hilgardii* ATCC 8290, *L. rhamnosus* LMS2-1, *L. paracasei* subsp. *paracasei* 8700:2), in L.c.rep36F and L.c.rep36R primer pair design; and fragments of gene *trsK* of the plasmid plca36 from *L. casei* (the gene product is assumed to be a conjugation coupling protein) homologous to the genes of other lactobacilli (*L. paracasei* subsp. *paracasei* 8700:2, *L. plantarum* WCFS1 plasmid pWCFS103, *L. helveticus* DSM 20075, *L. buchneri* ATCC 11577), in the design of primers L.c.CP36F and L.c.CP36R. The sequences of the used primers are presented in Table 3.

Total DNA PCR analysis. The reaction mixtures contained 0.5 μg of total DNA of the analyzed strain, 20 pmoles of both forward and reverse primers, 200 μM dNTPs, 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris–HCl (pH 8.8), and 1.25 activity units of *Taq*-polymerase in a total volume of 50 μl. PCR was performed in an Omn-E apparatus (Hybaid). Each reaction included (1) 5 min of DNA denaturation at 94°C; (2) 35 cycles consisting of 30 s of DNA denaturation at 94°C, 40 s of primers annealing at 64°C for antibiotic resistance genes and at 56°C for genes *rep* and *trsK*, 1 min of polymerase reaction at 72°C; and (3) 4 min of final DNA elongation at 72°C. PCR products were separated by electrophoresis at 5 V/cm in horizontal 0.7% agarose gel containing 0.5 μg/ml of ethidium bromide in Tris–acetate buffer. To assess the DNA fragment size, GeneRuler™ DNA (Fermentas) was used as a standard DNA marker.

Nucleotide sequence determination. DNA sequencing was performed at the Genome center for

Table 3. Primers to detect the genes of replication and conjugation transfer

Gene	Organism and plasmid	GenBank accession no.	Primer title*	Primer nucleotide sequence, 5'–3'	Expected fragment length
rep	*Lactobacillus casei* plca36	CP000935	L.c.rep36F L.c.rep36R	GGTATTAATGTATGGCGAGC GTCCAACTTTTGAGTATCTATAC	536 bp
rep	*Lactobacillus helveticus* pLH1	AJ222725	L.h.repF L.h.repR	TTACGTCTACTTCACGATTGA CTTTATTTGTCCCCATTTTGG	355 bp
trsK	*Lactobacillus casei* plca36	CP000935	L.c.CP36F L.c.CP36R	GAAACGGAGAACAGCATTGT CAAATTCATCAAGGATAAAGTC	704 bp

collective use (Engelhardt Institute of Molecular Biology, Russian Academy of Sciences) using the ABI PRISM^{R} BigDye^{TM} Terminator v. 3.1 reagent kit and ABI PRISM 3730 (Applied Biosystems) automated DNA sequencer. Sequence identities were established basing on the statistical significance of matching in alignment with Clustal X software package (http://www.ebi.ac.uk/Tools/clustalw/index.html).

Genome analysis. A gene search based on homology to the sequences of known antibiotic resistance genes of interest was performed within the complete genome sequences of *L. helveticus* and *L. casei* available in GenBank (BLAST software package). For the annotated species of lactobacilli (*L. helveticus* DPC 4571, *L. casei* ATCC 334, and *L. casei* BL23), we also searched by gene names on the NCBI server (http://www.ncbi.nlm.nih.gov).

RESULTS AND DISCUSSION

Sensitivity of lactobacilli strains to antibiotics determined by the disc diffusion method. Fifteen antibiotics of different groups (penicillins, aminoglycosides of the first, second and third generations, macrolides, lincosamides, chloramphenicol and amikacin) were used in the study. The results are presented in Table 4. Antibiotic resistance of the strains was evaluated by the size of growth inhibition zones around the discs containing antibiotics. According to this criterion, all studied strains were susceptible to macrolide antibiotics (azithromycin and roxithromycin), as well as to chloramphenicol and rifampicin. Most cultures exhibited sensitivity to erythromycin, except for the intermediately sensitive *L. plantarum* strains CS 396, 90-TC-4, 8-PA-3, gKNM 101, and *L. helveticus* Er 317/402 NARINE. As for lincosamide antibiotics, all tested strains were sensitive to clindamycin. Most of the strains appeared to be sensitive to lincomycin, while *L. plantarum* CS 396 and 90-TC-4 were resistant to it.

As for aminoglycoside antibiotics, only *L. helvetica* Er317/402 NARINE culture was sensitive to amikacin, a third-generation aminoglycoside; *L. plantarum* 8P A3 and *L. helveticus* 100ash were sensitive to a second-generation aminoglycoside, gentamycin; and all strains were resistant to first-generation aminoglycosides (kanamycin and neomycin). All strains, except for *L. helveticus* NK-1, were sensitive to streptomycin. The natural resistance of anaerobes to aminoglycosides is ascribed to the dependence of the antibiotics' transport across the cytoplasm membrane on the electron transporting system, which is absent in anaerobes. For the same reason, facultative anaerobes are significantly more resistant to aminoglycosides under anaerobic conditions than under aerobic ones. Bacteria of the genus *Lactobacillus* are facultative anaerobes; all strains are capable of growth under anaerobic conditions, and resistance to aminoglycosides and tetracycline is strain-specific (Table 4). Strain behavior toward penicillin antibiotics was not uniquely defined. All strains, without exception, turned out to be sensitive to ampicillin, while sensitivity to benzylpenicillin was an individual trait, with some strains being highly resistant and others sensitive to this antibiotic (Table 4). As for tetracycline, all four *L. plantarum* strains (CS 396, 90-TC-4, 8-PA-3, and gKNM 101), *L. helveticus* NNIE strain, and *L. casei* BT-24/88 exhibited resistance, while the rest of the strains were sensitive to this antibiotic.

Therefore, all the studied strains exhibited relative susceptibility to ampicillin, chloramphenicol, rifampicin, roxithromycin, erythromycin, and azithromycin. None of the strains was sensitive to all antibiotics. *L. plantarum* strains displayed the widest spectrum of resistance, with one strain being resistant to tetracycline and three aminoglycosides and three strains being resistant to tetracycline and five aminoglycosides; moreover, one strain was highly resistant to clindamycin and two strains to lincomycin. At the same time, two *L. plantarum* strains were characterized by resistance to benzylpenicillin, yet were sensitive to another β-lactam antibiotic, ampicillin. Apparently, resistance of this kind is not linked to β-lactamase activity and may be caused by specific mutations of the penicillin-binding proteins of the cell wall.

Total DNA PCR analysis to test the presence of antibiotic resistance genes. Molecular genetic analysis of the lactobacilli was performed to test the presence of the clinically important genes of antibiotic resistance, that is *tetW/O* and *tetM*, which determine resistance to tetracycline; *ermT* and *ermB*, to erythromycin; and *catTC*, to chloramphenicol. The presence of the

Table 4. Antibiotics resistance of probiotic *Lactobacillus* strains of human gastrointestinal microbiome

no.	Antibiotic	Strain/Antibiotics sensitivity*												
		L. plantarum CS 396	*L. plantarum* 8-PA-3	*L. plantarum* 90-TC-4	*L. plantarum* GKNM 101	*L. helveticus* Er 317/402	*L. helveticus* 100 ash	*L. helveticus* NK-1	*L. casei/paracasei* GKNM 2311	*L. casei/ paracasei* GKNM 577	*L. helveticus* NNIE	*L. casei/paracasei* K_3Sh_{24}	*L. rhamnosus* 421-2	*L. fermentum* GKNM 526
Penicillins														
1.	Benzylpenicillin	–	+	+	–	–	–	–	–	–	ND	–	–	–
2.	Ampicillin	–	–	–	–	–	–	–	–	–	ND	–	–	–
First-generation aminoglycosides														
3.	Streptomycin	+	+	+	+	+	–/+	–	+	+	ND	–/+	+	+
4.	Neomycin	+	–/+	+	+	+	–/+	+	+	+	ND	–/+	+	–/+
5.	Kanamycin	+	+	+	+	+	+	+	+	+	ND	–/+	+	+
Second-generation aminoglycosides														
6.	Gentamicin	+	–	+	+	+	–	+	+	+	ND	+	+	+
Third-generation aminoglycosides														
7.	Amikacin	+	+	+	+	–	+	+	+	+	ND	+	+	+
Tetracyclines														
8.	Tetracycline	+	+	+	+	–/+	–	–	–	–	+	–/+	–	–
Macrolides														
9.	Erythromycin	–/+	–/+	–/+	–/+	–/+	–	–	–	–	+	–	–	–
10.	Azithromycin	–/+	–	–/+	–/+	–	–	–	–/+	–	ND	–	–	–
11.	Roxythromycin	–	–	–	–	–	–	–	–	–	ND	–	–	–
Lincosamides														
12.	Lincomycin	+	–/+	+	–/+	–	–/+	–/+	–/+	–	ND	–	–/+	–
13.	Clindamycin	–/+	+	–	–	–	–/+	–/+	–	–	ND	–	–	–
Chloramphenicols														
14.	Chloramphenicol	–	–	–	–	–	–	–	–	–	+	–	–	–
Ansamycins														
15.	Rifampicin	–	–	–	–	–	–	–	–	–	ND	–	–	–

* Resistance was assessed by the size of cell growth inhibition zone around the antibiotic-containing disc: +, high resistance; –/+, low, insignificant resistance; –, susceptibility; ND, not determined.

above-mentioned genes responsible for resistance to antibiotics was tested by PCR using the primers listed in Table 2. As follows from the experimental results (Fig. 1), most of the strains lacked the genes *catTC, ermB, ermT, tetM, tetO, tetW/32/O, tetO/W*, and *tetO/W/32/O/W/O*. However, three strains possessed genes of tetracycline resistance; notably, fragments of genes homologous to *tetM* were detected in *L. casei/paracasei* strain K_3Sh_{24}, *L. helveticus* strain NNIE, and *L. helveticus* strain Er 317/402. The *tetM* gene revealed in 3 of the 13 studied strains is rather widespread among bacteria of the genus *Lactobacillus*. For example, in a study of 123 strains of lactic acid bacteria tested for the presence of antimicrobial resistance genes (*tetM, tetO, tetK, ermA, ermB, ermC, aac (6')-Ie, aph (2")-Ia, mecA*, and *blaZ*), the genes *tetM* and *ermB* were detected in 59 isolates; 11 among them were identified as *Lactobacillus plantarum*; 28 as *Lactococcus garvieae*; 6 as *L. salivarius*; and single isolates as *Lactococcus lactis* subsp. *lactis*, *Lactobacillus johnsonii, L. reuterii, L. crispatus*, and *L. brevis*. The *tetM* gene was shown to be highly homologous (99%) to the *tetM* gene earlier described in human pathogens *Listeria monocytogenes* and *Neisseria meningitidis* [19].

Determination and analysis of the nucleotide sequence of gene fragments. Determination and comparative analysis of the nucleotide sequence of the *tetM* gene fragments obtained from strains *L. casei/paracasei* K_3Sh_{24}, *L. helveticus* NNIE, and *L. helveticus* Er 317/402 confirmed the *tetM* gene identification by a 400-bp-long coding sequence (99.8–100% matches with each other and with the annotated genes of *Enterococcus faecalis* and *Streptococcus pneumoniae*).

Total DNA PCR analysis to test the presence of replication and conjugation genes homologous to the plasmid genes of lactobacilli. Plasmids are genetic structures that are rather easily transferred between bacterial cells either of the same species or of different species and even genera. Plasmids carry various genes, including those of antibiotic resistance. Therefore, the strains used as probiotics should preferably contain no plasmids.

In the three strains that were shown to contain genes homologous to those coding for factors of antibiotic resistance, we have searched for genes characteristic of lactobacilli plasmids.

Data on over 60 lactobacilli plasmids were found on the site of NCBI (http://www.ncbi.nlm.nih.gov/genomes/genlist.cgi?taxid=1&type=7&name=Plasmids). From 1 to 7 plasmids have been described for various species and 16 plasmids for *L. plantarum*. The plasmids belong to the sigma and theta replication types, the latter being predominant. Most plasmids are 2–40 kbp long.

Three strains in which we have detected genes coding for resistance against tetracycline were previously identified as *L. casei/paracasei* and *L. helveticus*

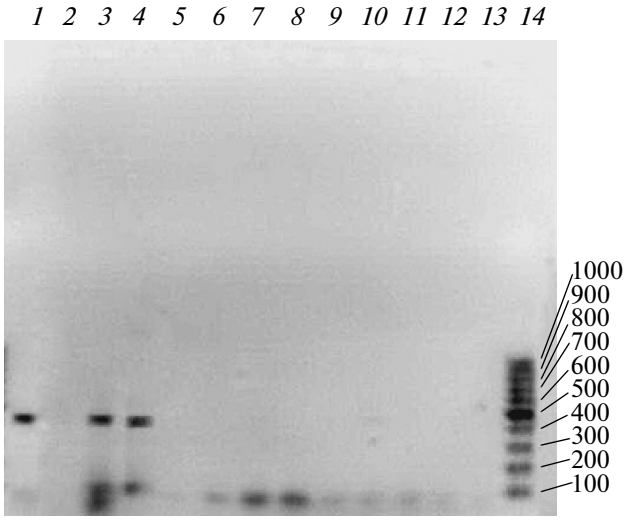

Fig. 1. Electrophoresis picture of the results of *tetM* gene detection in the lactobacillus strains *L. helveticus* Er 317/402 (*1*), *L. helveticus* 100ash (*2*), *L. casei/paracasei* K_3Sh_{24} (*3*), *L. helveticus* NNIE (*4*), *L. fermentum* gKNM 526 (*5*), *L. casei/paracasei* gKNM 577 (*6*), *L. plantarum* 8-PA-3 (*7*), *L. plantarum* 90-TC-4 (*8*), *L. plantarum* CS396 (*9*), *L. helveticus* NK-1 (*10*), *L. plantarum* gKNM 101 (*11*), *L. rhamnosus* 421-2 (*12*), *L. casei/paracasei* gKNM 23 11 (*13*), and marker DNA (*14*).

strains (see Table 1 and references [14, 15]). Two plasmids have been described for *L. helveticus*—pLH1, 19 kbp, and pLJ1, 3 kbp—both of the theta replication type. Four plasmids have been reported in *L. casei*—pSMA23, 3 kbp; pYIT356, 5 kbp; plasmid 1, 29 kbp; and plca36, 36 kbp; the replication type of pSMA23 is RC; of pYIT356 and plca36, theta; and unidentified for the plasmid 1. The plasmid plca36 contains traβregion of 26 genes presumably involved in the process of DNA transfer via conjugation [20]. Using the constructed primers, we investigated the presence of the genes homologous to the replication genes of plasmids pLH1 and pLJ1 from *L. helveticus* and plca36 from *L. casei*, as well as to the gene *trsK* encoding the binding conjugation protein of plca36 plasmid from *L. casei* in the three strains of lactobacilli. The results are presented in Fig. 2. As follows from the data, a gene identical to the *rep* gene of plasmids pLH1 and pLJ1 from *L. helveticus* was detected in three studied strains of lactobacilli from human intestinal microbiome. A gene homologous to the *rep* gene of plca36 plasmid from *L. casei* was detected in strain *L. casei/paracasei* K_3Sh_{24} and a gene homologous to the *trsK* gene of plca36 plasmid from *L. casei* also in all three strains. Therefore, it is highly probable that these strains contain plasmids—moreover, conjugative plasmids. Since the studied strains were found to contain the gene determining tetracycline resistance (*tetM*), this gene is probably localized in the plasmids. Indeed, there are some works evidencing the presence of antibiotic resistance genes in plasmids of lactobacilli. For

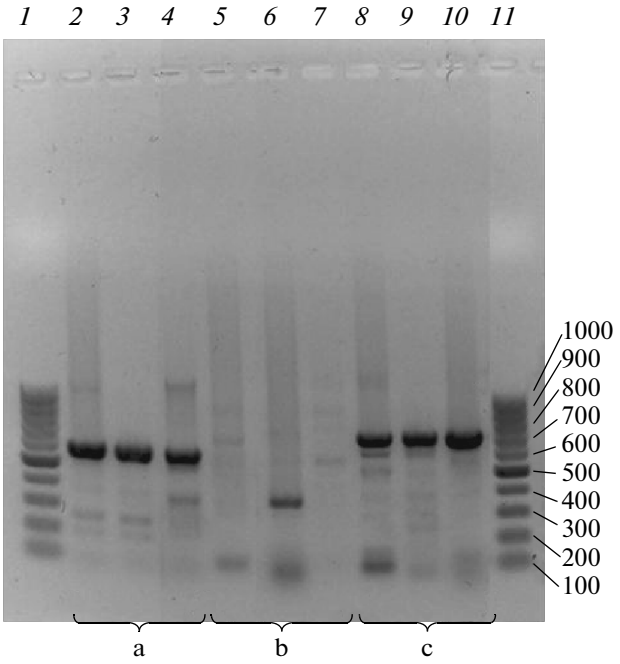

Fig. 2. Electrophoresis picture of the results of the testing for the presence of the replication and conjugation transfer genes in *Lactobacillus* strains. (a) *rep* gene of *Lactobacillus casei* plca36, (b) *rep* gene of *Lactobacillus helveticus* pLH1, and (c) *trsK* gene of *Lactobacillus casei* plca36. DNA marker (*1, 11,*), L. *helveticus* NNIE (*2, 5, 8*), *L. casei/paracasei* K_3Sh_{24} (*3, 6, 9*), and *L. helveticus* Er 317/402 (*4, 7, 10*).

example, *ermB* was revealed in the plasmid pLEM3 of *L. fermentum* [21], as well as in pTE80 and pTE15 of *L. reuteri* [22], and the genes *tetW* and *lnuA*, in pLR581 and pLR585 plasmids of *L. reuteri* [23].

Genome analysis. We analyzed the genomes to reveal the genes of resistance to tetracycline, erythromycin, and chloramphenicol (*tetW/O/M, ermT/B* and *catT/C*) in three strains of lactobacillus: *L. helveticus* DPC 4571, *L. casei* ATCC 334, and *L. casei* BL23. The complete genome nucleotide sequence of these strains is available in the NCBI database, while their species attribution is identical to the taxonomic position of the strains carrying resistance genes revealed in this study. Genomes of *L. helveticus* DPC 4571 and *L. casei* BL23 consist of a single chromosome, while *L. casei* ATCC 334 also contains a plasmid (plasmid 1). As shown by bioinformatics analysis, no *tetW/O/M, ermT/B*, or *catT/C* genes are present in the genomes of the analyzed strains, either in chromosomes or in the plasmid.

In our work, information on resistance of potential probiotic bacteria of the genus *Lactobacillus* from human intestinal microbiome to widespread clinically used antibiotics, as well as the results of gene typing to test the presence of antibiotic resistance genes, was obtained and analyzed. Three strains (*L. casei/paracasei* K_3Sh_{24}, *L. helveticus* Er 317/402, and *L helveticus* NNIE) out of the thirteen analyzed were found to contain the tetracycline resistance gene (*tetM*). However, according to our data, strains *L. casei/paracasei* K_3Sh_{24} and *L. helveticus* Er 317/402 showed low resistance to tetracycline (Table 4); high resistance was only observed in the case of *L. helveticus* NNIE. The *tetM* gene is probably not expressed in cells of the sensitive strains relevant. On the contrary, all four studied tetracycline-resistant strains of *L. plantarum* did not contain genes of resistance against this antibiotic. The study demonstrates difficulties associated with the testing of antibiotic resistance, since strains may contain silent genes or utilize other mechanisms of resistance. Similar results were obtained in work [24], where the results of the study of antibiotic sensitivity for 45 strains of probiotic bacteria of the genera *Lactobacillus, Streptococcus, Lactococcus, Pediococcus*, and *Leuconostoc* were compared with the presence of the genes determining antibiotic resistance. The presence of the genes homologous to plasmid genes of replication and conjugation in strains *L. casei/paracasei* K_3Sh_{24}, *L. helveticus* NNIE, and *L. helveticus* Er 317/402 indicates the possible presence of plasmids in these strains and, consequently, the possibility of conjugative transfer of resistance genes to other bacteria. Therefore, strains *L. casei/paracasei* K_3Sh_{24}, *L. helveticus* NNIE, and *L. helveticus* Er 317/402 are not feasible for direct use as probiotics, since they may act as a source of antibiotic resistance genes in the human microbiome.

ACKNOWLEDGMENTS

The work was supported by government contract no. 02.522.12.2009.

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