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Antibiotic Resistance of lactic Acid Bacteria Used in Russian Dairy and Probiotic Products

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Abstract. About 60 species of lactic acid bacteria (LAB) are used or are suitable for the use by food industry. Most of them are used for the production of the well accepted by the consumers fermented food products, probiotic products and biologically active supplements. Majority of the technologically used microorganisms getting in the gastrointestinal tract with food can survive in it and there they interact with its commensal and transient micro flora, including horizontally transferring genes. However, there are very few informations about the spectrum of antibiotic resistance as well as about the nature of the detectable resistance of LAB used by food industry. This communication presents the results of antibiotic resistance study of 30 strains of *Lactobacillus*. The studied LAB strains were isolated from the food products consumed in Russian Federation. The sensitivity of the studied strains to antibiotics was tested by using agar diffusion method with paper disks containing doxycycline, streptomycin, gentamicin, tetracycline, ciprofloxacin, lincomycin, chloramphenicol, vancomycin, erythromycin, imipenem, ampicillin and cefalotin. Among the strains isolated from commercially prepared foods – 87.5 % were resistant to vancomycin, what may be due to gene transmission mechanism. The next phase of planed research will be devoted to molecular identification of genetic determinants resistance in food isolates of *Lactobacilli*.

Keywords: Antibiotic resistance; Lactic Acid Bacteria (LAB); fermented milk products "non commercial" strains.

Introduction. Russian food industry presently uses about 60 species of lactic acid bacteria (LAB). They are used for the production of the well accepted by Russian consumers fermented food products, probiotic foods and biologically active additives.

Most of the technologically useful LAB getting into the gastrointestinal tract with food, can survive in it and there they may interact with its commensal and transient micro flora exchanging sometimes genes.

The evolution of antibiotic resistant food borne pathogens has been amply documented in recent years (Teuber and Perreten, 2000; Threlfall et al., 2000; White et al., 2002).

Antibiotic resistance has been shown to occur rarely in bacteria collected before the antibiotic era (Hughes and Datta, 1983). It was shown that lactobacilli could potentially serve as both donors and recipients of the genes that confer the resistance to antibiotics. They can also be recipients of genes of virulence factors discovered also in pathogenic bacteria (Mathur, Singh, 2004). Shortly after the introduction of each new antimicrobial compound, emergence of antimicrobial resistance is observed (Levy, 1997).

It is known that many bacteria from genus *Lactobacillus* are sensitive to clinically used antibiotics such as penicillin, ampicillin, erythromycin, chloramphenicol, kanamycin, but they are resistant to polymyxin, gentamicin (Danielsen and Wind, 2003). Multi Drug Resistances (MDR) of lactobacilli was detected in these strains to be associated with resistance to phages, heavy metals and biocides (Wang and Lee, 1997). Resistances to antimicrobial agents are observed in all kinds of LAB. Consequently, it is important to consider this during approval of the use of LAB strains by the industry.

However, very few information about the spectrum of antibiotic resistance as well as about the nature of detectable resistance to antibiotics of LAB used by Russian food industry are available. For example, pathogens may harbor extra chromosomal DNA, determining some features of metabolism, as well as antibiotic resistances (Nicas et al., 1989; Johnson et al., 1990; Woodford et al., 1995). R-plasmids do not exist in LAB but LAB may harbor extra chromosomal DNA what determines some aspects of metabolism, as well as antibiotic resistance. Thus, all the plasmids of *L. acidophilus* have a molecular weight larger/smaller than 5 MD. It is believed that these plasmids are unable of independent infection/migration, since lactobacilli do not possess pilis necessary for conjugation.

The purpose of this study was to evaluate the phenotypic sensitivity of *Lactobacillus* strains used and proposed to be used for production of probiotic dairy products and dietary supplements of foods by Russian industries in respect to the most widely used in medicine antimicrobials.

Materials and methods. We tested the strains of LAB, forming part of technological micro flora of industrially manufactured fermented milk products and food supplements, as well as so-called “wild” strains, isolated from naturally fermented products (home-made cottage cheese and milk drinks, pickled vegetables and fruits – fermented apples, sour cabbage), obtained also from non industrial producers.

30 cultures of *Lactobacillus* genus, isolated by a double dilution technique on MRS broth were studied. Taxonomic characterization of the studied strains was achieved by an analysis of cultural morphological and biochemical properties with the use of panels, containing 49 API CHL 50 (BioMerieux, France) tests. According to the obtained results of biochemical identification, the studied strains could be classified in 8 species.

The sensitivity of studied strains to antibiotics was tested by agar diffusion. The discs coated with doxycycline, streptomycin, gentamicin, tetracycline, ciprofloxacin, lincomycin, chloramphenicol, vancomycin, erythromycin, imipenem, ampicillin, cefalotin were placed on agar. Agarized MRS broth was poured into 90 mm diameter and 20 ml volume Petri dishes to obtain a 4.0 ± 0.5 mm high horizontal agar layer. Suspensions of dairy cultures (5×10^8 cfu/ml) were applied on the agar surfaces. Four to five antibiotic-coated disks were placed on the agar surface 15 min after its inoculation with the tested LAB suspension. The Petri dishes were incubated over 18-24 h. Sizes of LAB growth inhibition areas were used according to as relevant criteria of antibiotic resistance/sensitivity.

The studied cultures were subdivided into sensitive (S), intermediate resistant (IS), resistant (R) on the basis of the observed inhibition area.

Results and Discussion. As shown in Table 1, 44 % of cultures, isolated from industrially prepared products were sensitive to studied antibiotics.

Among the resistant cultures, 21 % and 12.5 %, respectively, were considered as intermediately resistant, what implies the formation of transmissible antibiotic resistance in these cultures.

Table 1. Sensitivity of lactobacilli, isolated from industrial and manufactured natural fermented products

Antimicrobial agent	Number of cultures from the studied ones (abs., %)					
	Industrially manufactured products, n = 24			Naturally fermented products, n = 6		
	Sensitive	Intermediately resistant	Resistant	Sensitive	Intermediately resistant	Resistant
Vancomycin	2/24 (8,3 %)	1/24 (4,2 %)	21/24 (87.5 %)	0	0	6/6 (100 %)
Chloramphenicol	19/24 (79.2 %)	3/24 (12.5 %)	2/24 (8.3 %)	6/6 (100 %)	0	0
Gentamicin	0	4/24 (16.7 %)	20/24 (83.3 %)	1/6 (16,7 %)	0	5/6 (83.3 %)
Streptomycin	0	1/24 (4.2 %)	23/24 (95.8 %)	0	0	6/6 (100 %)
Erythromycin	17/24 (71 %)	5/24 (21 %)	2/24 (8 %)	6/6 (100 %)	0	0
Lincomycin	10/24 (41.6 %)	7/24 (29.2 %)	7/24 (29.2 %)	6/6 (100 %)	0	0
Cefalotin	2/24 (8 %)	18/24 (75 %)	4/24 (17 %)	1/6 (16,7 %)	5/6 (83.3 %)	0
Tetracycline	20/24 (83.4 %)	2/24 (8.3 %)	2/24 (8.3 %)	6/6 (100 %)	0	0
Ampicillin	6/24 (25 %)	16/24 (66.6 %)	2/24 (8.4 %)	6/6 (100 %)	0	0
Imipenem	22/24 (91.6 %)	1/24 (4.2 %)	1/24 (4.2 %)	6/6 (100 %)	0	0
Ciprofloxacin	7/24 (29.2 %)	4/24 (16.6 %)	13/24 (54.2 %)	2/6 (33 %)	4/6 (67 %)	0
Doxycycline	22/24 (91.6 %)	0	2/24 (8.4 %)	6/6 (100 %)	0	0
On the average, %	44.1 ± 2.1	21.8 ± 1.1	34.1 ± 1.8	64.0 ± 3.3	12.5 ± 0.32	23.5 ± 1.3

We studied the sensitivity/resistance of the tested cultures to a wide range of antibiotics such as chloramphenicol, gentamicin, and tetracycline. All the cultures, isolated from naturally fermented products turned out to be chloramphenicol- and tetracycline- sensitive.

From the total number of cultures isolated from industrially manufactured products, 2 cultures exhibited a resistance to chloramphenicol and 2 cultures to tetracycline.

Twenty-two industrially manufactured cultures and 5 “wild” cultures were resistant to gentamicin aminoglycoside. Twenty-three strains of LAB isolated from industrially manufactured products and all cultures (6) isolated from home fermented products were resistant against streptomycin. Most probably this is a natural resistance to aminoglycosides, which may be explained by the fact that the transport of these antibiotics through cytoplasmic membrane in LAB is connected with electron transport systems absent in anaerobes (Mathur, Singh, 2004).

Tested LAB were quite resistant to vancomycin an antibiotic considered to be a marker of transmissible type antibiotic resistances, i.e. being of an extra chromosomal origin. As observed in Table 1, from the 24 cultures isolated from industrially manufactured products, 3 cultures were sensitive to this antibiotic. This may be due also to the presence of antibiotic sensitivity transmissible genes in technological fermented milk bacteria as well as to their potential ability to participate in a gene transfer and to transmission of this resistance to GI tract symbiotic cultures

(Ruoff et al., 1988; Swenson et al., 1990; Handwerger et al., 1994; Klein et al., 1988; Salminen et al., 1998).

Table 2 presents data on sensitivity of various species of *Lactobacillus* cultures, isolated from the studied products, to the 12 tested antibiotics.

Table 2. Sensitivity of various species of *Lactobacillus* cultures to antimicrobial agents

Species of *Lactobacillus*	Quantity	Antimicrobial agent											
		Vancomycin	Chloramphenicol	Gentamicin	Streptomycin	Erythromycin	Imipenem	Lincomycin	Cefalotin	Tetracycline	Doxycycline	Ampicillin	Ciprofloxacin
L. rhamnosus	n =7	0/7	7/7	0/7	0/7	5/7	7/7	5/7	1/7	7/7	7/7	2/7	5/7
L. acidophilus	n=6	1/6	2/6	0/6	0/6	5/6	5/6	3/6	1/6	4/6	5/6	1/6	1/6
L. plantarum	n=5	0/5	5/5	0/5	0/5	5/5	5/5	4/5	1/5	5/5	5/5	4/5	1/5
L. paracasei	n=5	1/5	4/5	0/5	0/5	2/5	4/5	0/5	0/5	4/5	4/5	1/5	1/5
L. fermentum	n=2	0/2	2/2	1/2	0/2	1/2	2/2	1/2	0/2	1/2	2/2	1/2	0/2
L. delbrueckii ssp. *delbr.*	n=2	1/2	1/2	0/2	0/2	2/2	1/2	1/2	0/2	1/2	1/2	1/2	1/2
L. sakei	n=1	0	1	0	0	1	1	0	0	1	1	1	0
L. curvatus	n=1	0	1	0	0	1	1	1	0	1	1	1	0
L. brevis	n=1	0	1	0	0	1	1	0	0	1	1	0	0

A comparison of sensitive and resistant cultures among the studied LAB food isolates did not exhibit any dependence on species. The cultures of all LAB species, as determined by biochemical methods (except for *L. rhamnosus*) were resistant to vancomycin and to the latest generation of germicides such as cefalotin and ciprofloxacin.

Conclusions. The antibiotic resistance of LAB cultures, isolated from industrially manufactured and naturally fermented Russian products was studied.

The cultures, isolated from naturally fermented (“wild”) products are globally 1.5 times more sensitive to the most clinically relevant antibiotics, than cultures, isolated from industrially manufactured products. Among the cultures, isolated from industrially manufactured products, 87.5 % were resistant to vancomycin, what can be caused by transmissible mechanisms. The next stage of the studies will be devoted to the analysis of genetic resistance determinants in *Lactobacillus* food isolates.

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УДК 57

Антобиотикорезистентность бактерий рода Lactobacillus, используемых при производстве молочных и пробиотических пищевых продуктов в России

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Аннотация. Сегодня насчитывается около 60 видов лактобактерий, используемых и потенциально пригодных для пищевой промышленности. Большинство из них применяется для производства наиболее распространенных в питании населения кисломолочных продуктов, пробиотических продуктов и биологически активных добавок к пище (БАД). Многие из технологических микроорганизмов, поступая в желудочно-кишечный тракт (ЖКТ) человека с пищей, способны выживать в нем и взаимодействовать с его комменсальной и транзиторной микрофлорой, в том числе обмениваться генными элементами. В настоящее время очень мало данных как о спектре антибиотикочувствительности, так и о природе выявляемой резистентности лактобактерий для пищевой промышленности.

В работе было использовано 30 штаммов лактобактерий, входящих в состав заквасочной микрофлоры различных кисломолочных продуктов и биологически активных добавок к пище промышленного изготовления, отобранных в торговой сети. И штаммы, выделенные из продуктов естественной ферментации (творог домашний, кисломолочный напиток, а также сквашенные при участии полезной микрофлоры овощи и фрукты: моченые яблоки, капуста квашеная), приобретенные у частных лиц в Центральной России. Чувствительность штаммов к антимикробным препаратамиспытывали методом агародиффузии. Тестировали доксициклин, стрептомицин, гентамицин, тетрациклин, ципрофлоксацин, линкомицин, левомицетин, ванкомицин, эритромицин, имипенем, ампициллин, цефалотин, нанесенные на диски. Результаты исследования показали, что среди штаммов, выделенных из промышленно приготовленных продуктов, 87,5% обладали антибиотикоустойчивостью к ванкомицину, которая может быть обусловлена трансмиссивным механизмом. Следующий этап исследования будет посвящён анализу генетических детерминант резистентности у пищевых изолятов Lactobacillus.

Ключевые слова: Устойчивость к антибиотикам; молочнокислых бактерий (LAB), кисломолочные продукты «некоммерческих» штаммов.