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Original article

ANTIBIOTIC SUSCEPTIBILITY OF *LACTOBACILLUS PLANTARUM* STRAINS, ISOLATED FROM KATAK

L. DOBREVA[1], M. KOPRINAROVA[2], A. BRATCHKOVA[1] & S. DANOVA[1]

[1]Department of General Microbiology, The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria; [2]Institute of Molecular Biology "Acad. Roumen Tsanev", Bulgarian Academy of Sciences, Sofia, Bulgaria

Summary

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Several *Lactobacillus* species are accepted as microorganisms with Qualified Presumption of Safety (QPS) in the EFSA's list. One of them, *Lactobacillus plantarum* is a widely distributed species with a proven probiotic potential and technological relevance. In addition, every strain must complete several requirements, before implementation. Antibiotic susceptibility is one of EFSA's important criteria regarding the safety of probiotics. The reason is to avoid any possibility of antibiotic resistance genes transfer to opportunistic pathogens in the gut. In the present study 14 *Lactobacillus plantarum* strains were assessed for susceptibility to 21 antibiotics from different groups. A high number of resistant strains was determined toward 12 antibiotics (penicillins – penicillin, piperacillin; IIIth generation cephalosporins – cefotaxime, ceftriaxone, ceftazidime; glycopeptides – vancomycin; tetracyclines – tetracycline; aminoglycosides – gentamicin; macrolides – clarithromycin; quinolones – nalidixic acid, ciprofloxacin, levofloxacin). Concerning the other tested antibiotics, strain-specific antibiotic-sensitivity patterns were observed. Antibiotic resistance was also discussed as an advantage in the selection of probiotic strains, however only when it is not transferable. Estimated susceptibility patterns of some of tested candidate probiotic strains are also important, considering the use of the latter as agents accompanying antibiotic therapy

Key words: antibiotic susceptibility, *Lactobacillus* spp., probiotics

INTRODUCTION

Recently, antibiotic resistance has received more attention worldwide due to the increased possibility for emergence of some resistant bacteria. The complex inter-connections and interaction between humans, drugs and the environment sometimes result in appearance of bacterial antibiotic resistance (Barbosa & Levy, 2000; O'Brien, 2002). Thus, several selection pressures from the environment can result in variation in different geographic regions (McCormick *et al.*, 2003). The

mechanism of antibiotic resistance most often occurs through horizontal gene transfer (McCormick *et al.*, 2003). In this aspect, pathogenic bacteria have gained great attention, due to direct threat (Neut *et al.*, 2017), however scarce information exists to friendly bacteria, such as *Lactobacillus* (Horowitz *et al.*, 1994). The reason is that lactic acid bacteria (LAB) have obtained a generally regarded as safe status – GRAS (Generally Recognised as Safe) or QPS (Qualified Presumption of Safety), according to FDA and EFSA, respectively. They have been extensively used in food preservation throughout history and are naturally abundant in fermented food. LAB are a great part of the microbiota of mouth, gastrointestinal tract, urogenital tract etc.

Several *Lactobacillus* strains have been considered beneficial since they confer health benefits on humans and animals and are considered probiotics (Casas & Dobrogosz, 2000). The term probiotic is derived from the Greek words "*pro* – for" and "*bio* – life". Probiotics are usually living microorganisms that, when taken at the required dose, provide benefits to the health of the host (Hill *et al.*, 2014). They naturally benefit the health of the host. Moreover, *Lactobacillus* species are well-known starters and some of them emerge naturally as fermented microflora. *Lactobacillus plantarum* is a widespread species and a major participant in fermentations in the plant, dairy, meat products and often used as probiotics (Danova & Georgieva, 2013).

Recently, a possible co-application of probiotic and antibiotic is widely discussed. The advantage of such a form of combined therapy is recognised and widely used for preventing antibiotic-associated diarrhoea and induced dysbiosis.

Due to the increased application of lactobacilli as probiotics, EFSA has developed a number of requirements, concerning their safety and functionality. Every commercial probiotic should be able to obtain QPS status. Even though QPS is a status attributed to species, genome content varies widely between species, including those from *Lactobacillus* genus (Broadbent *et al.*, 2012; Raftis *et al.*, 2014). The bacteria with infectious history and strains that may possess virulence or antibiotic resistance genes should not be used to prevent gene transmission to other species (EFSA, 2012a).

The human and animal GIT, due to the immense amount of bacteria and the close contact between them, is a possible place for a gene transfer. The main hazard is antibiotic resistance determinants transmission from commensal bacteria and the emergence of resistance to common microbial infections, impairing successful antibiotic treatment (Snydman, 2008). Therefore, the lack of acquired or transferable resistance factors need to be justified for candidate probiotics and starter cultures, so they can obtain QPS status (EFSA, 2012a). In the context of co-administration of probiotics with antibiotics, probiotic's resistance toward the antibiotic used may also be discussed as desired. Before the general application for commercial use, the resistance's nature should be clarified.

The antibiotic resistance genetic determinants are often plasmid-associated genes. Their passage occurs through horizontal gene transfer. Plasmid carried genes and conjugative transposons could be passed from one LAB to other. They have been commonly found in many strains (Teuber *et al.,* 1999). This is considered acquired resistance since it is found in strains that are typically susceptible. The transmissible genes have been

determined in strains belonging to the species *Lactobacillus fermentum*, *Lactobacillus reuteri* and *L. plantarum* (Tannock *et al.*, 1994; Fons *et al.*, 1997). In the opposite, antibiotics resistance of numerous LAB strains have been considered as intrinsic (natural) and non-transmissible (Adams & Marteau, 1995; Salminen *et al.*, 1998). The difference between intrinsic/plasmid antibiotic resistance should be determined. The strains, with a plasmid-derived resistance, should not be used as probiotic products in animal and human products, while intrinsically resistant strains could be useful during antibiotic treatment in patients with unbalanced microbiota (Salminen *et al.*, 1998). Since lactobacilli are widely used as starters and probiotics and co-administered with antibiotics in therapy, they are obtained in great quantity and thus are able to interact with the host microbiota. Therefore, they should be carefully checked for lack of transferable genes and should not add up to the total genes for antibiotic resistance (EFSA, 2012b) in food and gut microbiome. According to the safety criteria of LAB, intrinsic and acquired resistance differences should be carefully distinguished. This is an important requirement for the safety assessment of each newly characterised candidate probiotic and/or starter LAB.

With this aim, the antibiotic susceptibility of 14 *L. plantarum strains*, newly isolated from homemade samples of the Bulgarian dairy product katak to 21 antibiotics was characterised. The antibiotics belong to the groups of inhibitors of cell wall synthesis; inhibitors of protein synthesis and nucleic acid synthesis and were tested on the group of pre-selected lactobacilli.

MATERIALS AND METHODS

Lactobacillus strains and culture conditions

Fourteen strains, newly identified as *Lactobacillus plantarum* strains (unpublished data), were included in the present study. They were part of the laboratory collection of lactic acid bacteria (LAB), isolated from different habitats. All 14 strains were isolated from a homemade sample of a traditional dairy product katak from Lukovit, Bulgaria.

The 14 *Lactobacillus* cultures were stored at −20 ^{o}C in MRS broth supplemented with glycerol (20% v/v) and were pre-cultivated twice at 37 °C in De Man Rogosa Sharpe (MRS) broth (Hi-Media Pvt. Ltd., India), prior to assays. MRS agar, pH 6.5 (Hi-Media Pvt. Ltd., India) was used to cultivate them for antibiotic susceptibility tests. All media were sterilised by autoclaving at 121 °C for 20 min.

In vitro antibiotic susceptibility tests

Twenty-one antibiotics, divided into 3 groups according to their mode of action were included in the study:

(1) Inhibitors of cell wall synthesis: aminopenicillins – amoxicillin (10 μg/disk, HiMedia); penicillins – ampicillin (10 μg/disk) penicillin (15 μg/disk), piperacillin (100 μg/disk); carbapenems – meropenem (10 μg/disk); II generation cephalosporins – cefuroxime (30 μg/disk); III generation cephalosporins – ceftriaxone (30 μg /disk), ceftazidime (30 μg/disk), cefotaxime (30 μg/disk); glycopeptides – vancomycin (5 μg/ disk) – all from BB-NCIPD Ltd.

(2) Protein synthesis inhibitors: tetracyclines – tetracycline (30 μg/disk, Oxoid, UK), doxycycline (30 μg/disk, HiMe

dia); amphenicols – chloramphenicol (30 μg/disk, HiMedia); aminoglycosides – gentamicin (10 μg/disk) and streptomycin (300 μg/disk); macrolides – clarithromycin (15 μg/disk) and erythomycin (15 μg/disk) – all from BB-NCIPD Ltd.

(3) Inhibitors of nucleic acid synthesis: quinolones – nalidixic acid (30 μg/disk); ciprofloxacin (5 μg/disk); levofloxacin (5 μg/disk); rifampicins – rifampin (5 μg/disk) – all from BB-NCIPD Ltd.

The Bauer Kurby disk method (Bauer *et al.*, 1966) was used to determine the antibiotic susceptibility to antibiotics from different groups. Petri dishes (9 cm) were seeded with 100 μL of active 24-hour cultures (0.5 MacFarland standard) and overlaid with 15 mL melted MRS agar (2% v/v), cooled to about 37–42 °C, mixed gently. Upon agar solidification, the antibiotic paper disks were dispensed and placed on the agar surface. The Petri dishes were cultivated for 24 h at 37 °C in anaerobic conditions (BBL GasPack anaerocult system). The diameters of inhibition zones (around the disks) in mm were measured and the results (average of 3 readings) were expressed as S (sensitive), I (intermediate) and R (resistant) as per Clinical Laboratory Standards Institute Performance Standards for Antimicrobial Disk Susceptibility (CLSI, 2006).

RESULTS

Strain-specific antibiotic susceptibility patterns were obtained for each of the tested 14 strains (Table 1). Overall, antibiotic susceptibility pattern varied between tested lactobacilli and antibiotics (Fig. 1–3).

Antibiotic susceptibility of lactobacilli to inhibitors of cell wall synthesis

Into the group of beta-lactam antibiotics (Table 1), all 14 tested strains showed resistance towards penicillin, and resistance to piperacillin was highly prevalent – 10 out of 14 strains. The majority of *Lactobacillus plantarum* isolates from katak were found susceptible to ampicillin, amoxicillin, and meropenem (Fig. 1). High resistance to IIIth generation cephalosporins (cefotaxime, ceftriaxone, ceftazidime) and the glycopeptide antibiotic vancomycin was also detected.

Antibiotic susceptibility of lactobacilli to inhibitors of protein synthesis

Significant difference within the group of 14 lactobacilli was observed with regard to antibiotics inhibiting protein synthesis (Fig. 2) A high number of resistant strains was observed against the aminoglycoside gentamicin (Fig. 2). Between 70–92% of the tested strains were found susceptible to doxycycline, tetracycline, clarithromycin, erythromycin and chloramphenicol (Table 1).

Antibiotic susceptibility of lactobacilli to inhibitors of the nucleic synthesis

High resistance was detected to Ist, IInd, IIIth generation quinolones – nalidixic acid, ciprofloxacin, levofloxacin (Fig. 3). Four of the strains were resistant toward rifampin (Table 1).

DISCUSSION

Before application each candidate probiotic strain has to be individually assessed, first *in vitro*. With this aim, we selected 14 *Lactobacillus* strains, isolated from

Table 1. Antibiotic susceptibility patterns of 14 newly characterised *L. plantarum* strains from a homemade fermented dairy product katak

Antibiotics		Tested *Lactobacillus* strains													
		L 1	L 2	L 3	L 4	L 5	L 6	L 7	L 8	L 9	L 10	L 11	L 12	L 13	L 14
Cell wall synthesis inhibitors	*Penicillins (beta-lactams)*														
	Ampicillin	S	I	S	S	R	I	S	I	S	S	I	S	R	S
	Penicillin	R	R	R	R	R	R	R	R	R	R	R	R	R	R
	Piperacillin	R	S	I	S	R	R	R	R	R	R	R	I	R	R
	Aminopenicillins														
	Amoxicillin	I	I	I	R	S	I	I	S	I	I	S	S	I	I
	Carbapanems														
	Meropenem	S	I	S	S	S	I	S	S	S	S	I	I	I	S
	II generation cephalosporins														
	Cefuroxime	S	S	S	R	S	I	I	I	S	I	S	S	I	I
	III generation cephalosporins														
	Ceftriaxone	S	R	R	R	R	R	R	R	R	R	R	R	R	R
	Ceftazidime	R	R	R	R	R	R	R	R	R	R	R	R	R	R
	Cefotaxime	R	R	R	R	R	R	R	R	R	R	R	R	R	R
	Glycopeptides														
	Vancomycin	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Protein synthesis inhibitors	*Tetracyclines*														
	Doxycycline	S	I	S	S	S	I	I	I	S	S	I	R	I	S
	Tetracycline	S	I	I	I	S	I	R	R	S	R	I	I	R	S
	Aminoglycosides														
	Gentamicin	S	R	R	R	R	R	R	R	R	R	R	R	R	R
	Streptomycin	S	S	S	I	S	S	S	S	I	S	I	S	S	S
	Amphenicols														
	Chloramphenicol	R	S	S	S	S	S	I	S	S	S	I	S	I	S
	Macrolides														
	Clarithromycin	R	I	I	S	I	I	I	I	S	I	I	I	I	S
	Erythromycin	R	I	I	I	I	I	I	I	I	R	R	I	I	I
Nucleic acid synthesis inhibitors	*I generation quinolones*														
	Nalidixic acid	R	R	R	R	I	R	R	R	R	R	R	R	I	R
	II generation quinolones														
	Ciprofloxacin	R	R	R	R	R	R	R	R	R	R	R	R	R	R
	Levofloxacin	R	R	R	R	R	R	R	R	R	R	R	R	R	R
	Rifampicins														
	Rifampin	R	S	R	I	S	S	S	S	R	S	R	S	S	S

Legend: Black colour- resistant, white colour – sensitive and grey colour – intermediate.

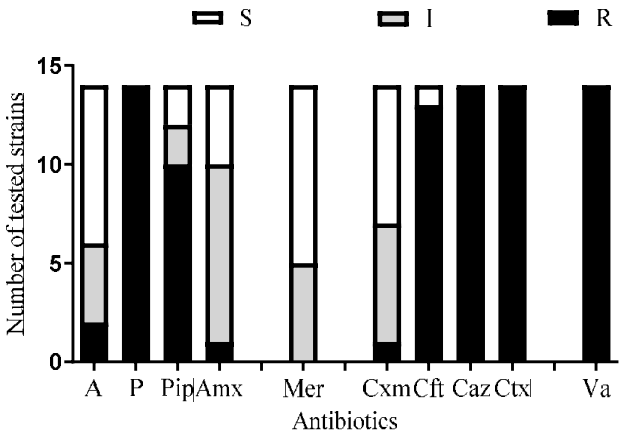

Fig. 1. Antibiotic susceptibility of 14 *L. plantarum* strains to antibiotics – inhibitors of cell wall synthesis: beta-lactams: penicillins (ampicillin, A; penicillin, P; piperacillin, Pip); aminopenicillins (amoxicillin, Amx); carbapenems (meropenem, Mer); II generation cephalosporins (cefuroxime, Cxm); III generation cephalosporins (ceftriaxone, Cft; ceftazidime, Caz; (cefotaxim, Ctx); glycopeptides (vancomycin, Va).

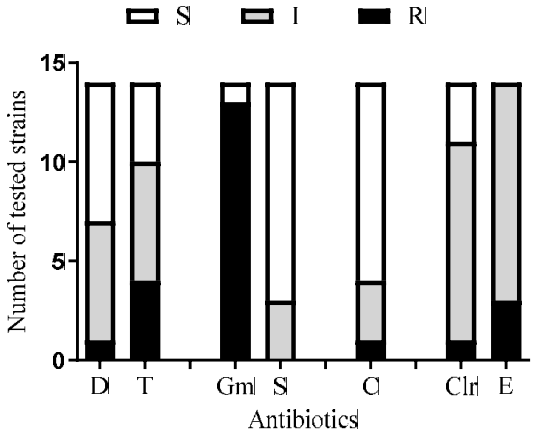

Fig. 2. Antibiotic susceptibility of 14 *L. plantarum* strains to antibiotics – inhibitors of protein synthesis: tetracyclines (doxycycline, D; tetracycline, T); aminoglycosides (gentamicin, Gm; streptomycin, S); amphenicols (chloramphenicol, C); macrolides (clarithromycin, Clr; erythromycin, E).

traditional Bulgarian "katak". They were identified as *L. plantarum* (unpublished data). This fermented milk product is famous with a long shelf life, up to one year without preservatives (Danova & Georgieva, 2013). However, limited data exist on its autochthonous lactic acid microbiota, which is probably responsible for such stability and safety of the product. All lactobacilli originated from one sample of homemade katak and observed variety in the spectrum of antibiotic susceptibility was unexpected.

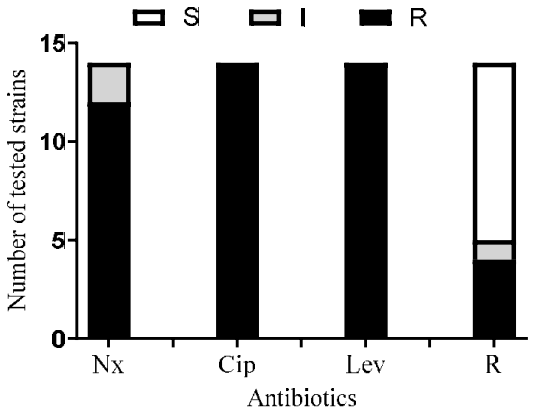

Fig. 3. Antibiotic susceptibility of 14 *L. plantarum* strains to antibiotics – inhibitors of nucleic synthesis: quinolones (nalidixic acid, Nx; ciprofloxacin, Cip; levofloxacin, Lev); rifampicins (rifampin, R).

Antibiotics in current human/animal use have some limitations, concerning their spectrum of antibacterial activity. Therefore, 21 different antibiotics were included in the *in vitro* tests. Only 4 out of 21 antibiotics did not inhibit the growth of all 14 lactobacilli, while for the other 17, a strain-specific variety was observed (Table 1). A higher number of resistant strains was found towards the group of antibiotics inhibitors of cell wall synthesis. Even though generally *Lactobacillus* have been found to be susceptible to penicillins (ampicillin and penicillin) (Ammor *et al.* 2007), we revealed high prevalence of resistance towards penicillin and piperacillin. Our results confirm the report of Zarazaga *et al.* (1999). Different studies have shown penicillin's resistance in *L. plantarum* strains from different habitats: fermented vegetables (Pulido *et al.*, 2005; Lapsiri *et al.*, 2011); home-made Spanish (Herrero *et al.*, 1996) and other cheeses (Flórez *et al.*, 2005; Belleti *et al.*, 2009). Data for *Lactobacillus* spp. from yogurt (Savadogo *et al.*, 2010) and different fermented milk products (Yüksekdap & Beyatli, 2008) are also reported. In addition, widespread penicillin resistance has been observed in probiotic and starter lactic acid bacteria (Charteris *et al.*, 1998; Danielsen & Wind, 2003).

Resistance to β-lactams is a disturbing and increasingly spread phenomenon. A supposed mechanism according to Condon (1983) is cell wall impermeability. Other mechanisms implied are non-specific, such as multidrug transporters (Putman *et al.*, 2001) and defective cell wall autolytic systems (Kim *et al.*, 1982). Currently, there are no evidence and reports on *Lactobacillus* suggesting the transferability of resistance genes for β-lactam antibiotics (Devika *et al.*, 2019).

The tested strains were also found to be resistant toward III generation cephalosporins – cefotaxime; ceftriaxone, ceftazidime (Fig. 1). Resistance towards cephalosporins is found across numerous *Lactobacillus* spp. (Abriouel *et al.*, 2015) including isolates from different types of cheeses (Danielsen & Wind, 2003). Charteris *et al.* (1997) reported a high level of resistance toward IInd generation cephalosporins. However, our strains with the

exception of a single strain were susceptible to cefuroxime (Fig. 1).

All tested strains from katak tolerated well the glycopeptide antibiotic vancomycin (Fig. 1 and Table 1). Such a feature, which is widespread among *Lactobacillus* spp., is considered chromosomally encoded (Holliman & Bone, 1988; Nicas *et al.*, 1989) and thus is non-transmissible. Such intrinsic resistance could be preferred because antibiotics often cause alteration in the microbiome, dysbiosis and induce antibiotic-associated diarrhoea or other health issues. Therefore, it is desired that the probiotic remains viable and not affected by the antimicrobials applied (Neut *et al.*, 2017).

Lactobacilli are most often susceptible to different antibiotics such as cell wall inhibitors penicillins (Danielsen & Wind, 2003), as well as to low concentrations of most inhibitors of protein synthesis (e.g. chloramphenicol, macrolides and tetracyclines) (Ammor *et al.*, 2007). On the other side, they tend to be resistant to protein cell wall inhibitors – aminoglycosides (kanamycin, gentamicin and streptomycin) (Ammor *et al.*, 2007), intrinsically resistant to cell wall inhibitors – glycopeptides (vancomycin and teicoplanin) (Charteris *et al.*, 1998; Danielsen & Wind, 2003) and some of them to cephalosporins (cefuroxime, ceftriaxone and cefoxitin) (Danielsen & Wind, 2003; Belleti *et al.*, 2009). In addition, they also show intrinsic resistance to inhibitors of nucleic acid synthesis – quinolones (e.g. ciprofloxacin and nalidixic acid) (Hummel *et al.*, 2007).

The tested strains revealed high resistance >90% toward gentamicin (Fig. 2) and 30% to tetracycline. Commonly acquired resistance genes in probiotics and lactobacilli isolated from fermented food are the genes *Tet* (M), *Tet* (S) for tetracycline resistance (Thumu & Halami, 2012). Chloramphenicol resistance is dependent on *cat* genes (Ahn *et al.*, 1992, Hummel *et al.*, 2007). Tetracycline and chloramphenicol resistance is a frequent phenotype that is due to acquired resistance genes. Neut *et al.* (2017) have detected the same pattern for *Lactobacillus* spp. from fermented food. *L. plantarum* strains from katak demonstrated high resistance toward tetracycline, but not to chloramphenicol (Fig. 2). Supposedly, due to the mobile gene homology of mobile genetic elements, lactobacilli from fermented food could have acquired resistance genes from staphylococci and enterococci (Abriouel *et al.,* 2015).

Clarithromycin resistance has been observed in commercial probiotic *L. plantarum* strains (Sharma *et al.*, 2015). However, other probiotic strains from commercial dairy products were susceptible (Billah *et al.*, 2010), and intermediate and high susceptibility was determined for 13 out of the 14 tested *L. plantarum* (Fig. 2). Clarithromycin is applied in *Helicobacter pylori* treatment and co-administration with probiotic is often recommended.

With regard to inhibitors of nucleic acid synthesis, resistance to quinolones e.g. ciprofloxacin is common (Hummel *et al.,* 2007). This is confirmed by our results showing resistance of lactobacilli from katak to nalidixic acid, ciprofloxacin, levofloxacin (Fig. 3). Resistance towards nalidixic acid was observed for 12 *L. plantarum* strains (Cebeci *et al.*, 2003) and is also in line with the findings of Horowitz *et al.* (1994) and Charteris *et al.* (1998) for *Lactobacillus spp*. The resistance towards quinolones is due to intrinsic factors such as the structure of cell wall, efflux mechanism and permeability. A lack of sensitivity to rifampicin is a result of mutations (Ezekiel & Hutchins,

1968) and is therefore not likely to be transferable.

Marketed probiotic strains have been found resistant toward levofloxacin (Neut *et al.*, 2017) and our results revealed the same characteristic (Fig. 3).

CONCLUSION

The present study is a part of the safety assessment of newly isolated lactobacilli from a not well-studied fermented milk product – katak. The EFSA requires every strain intended for human/animal consumption to be tested for resistance to ampicillin, vancomycin, gentamicin, kanamycin, streptomycin, tetracycline, erythromycin, clindamycin, and chloramphenicol. In this aspect, tested *L. plantarum* strains L2, L3, L9 and L14 are preselected as more sensitive, only except for vancomycin and gentamicin resistance. In addition, the exclusion of strains that could carry antibiotic resistance genes is required as ingested in large numbers, they could possibly transfer antibiotic resistance determinants to the intestinal microbiota. The data from our study revealed that transmissible resistance genes could be possibly present only in limited number of strains. The *L. plantarum* L1, L10 and L11 showed undesirable for QPS resistance to erythromycin and L1 – to chloramphenicol; they will be subject to additional tests. The minimal inhibitory concentration will be determined and only the strains, under the EFSA's MIC border values could be further explored.

The tested 14 *L. plantarum* also revealed atypical penicillin resistance. Overall, the majority of pre-selected strains from katak completed *in vitro* safety criteria of EFSA and did not present a reservoir of antibiotic resistance genes. As a part of autochthonous lactic acid microbiota of the traditional fermented product, they probably may contribute to the safety quality of katak.

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REFERENCES

Abriouel, H., M. Casado Muñoz, L. Lavilla Lerma, B. Pérez Montoro, W. Bockelmann, R. Pichner, J. Kabisch, G. S. Cho, CMAP Franz, A. Gálvez & N. Benomar, 2015. New insights in antibiotic resistance of *Lactobacillus* species from fermented foods. *Food Research International*, **78,** 465–481.

Acar, J. F. and F. W. Goldstein, 1991. Disk susceptibility test. In: *Antibiotics in Laboratory Medicine,* 3rd edn, Williams & Wilkins, London, Baltimore, pp. 17– 52.

Adams, M. R. & P. Marteau, 1995. On the safety of lactic acid bacteria from food. *International Journal of Food Microbiology,* **27**, 263–264.

Ahn, C., D. C. Thompson, C. Duncan & M. E. Stiles, 1992. Mobilization and location of the genetic determinant of chloramphenicol resistance from *Lactobacillus plantarum* ca TC2R. *Plasmid* , **27**, 169–176.

Ammor, M. S., A. B. Flórez & B. Mayo, 2007. Antibiotic resistance in nonenterococcal lactic acid bacteria and bifidobacteria. *Food Microbiology*, **24**, 559–570.

Barbosa, T. M. & S. B. Levy, 2000. The impact of antibiotic use on resistance development and persistence. *Drug Resistance Updates*, **3**, 303–311.

Bauer, A. W., W. M. M. Kirby, J. C. Sheris & M. Tuck, 1966. Antibiotic susceptibility testing by a standardized single disk

method. *The American Journal of Clinical Pathology*, **45**, 493–496.

Bayer, A. S., A. W. Chow, N. Concepcion & L. B. Guze, 1978. Susceptibility of 40 lactobacilli to six antimicrobial agents with broad Gram-positive anaerobic spectra. *Antimicrobial Agents and Chemotherapy*, **14**, 720–722.

Belletti, N., G. Monica, B. Benedetta Erasmo, N. T. Giulia & G. Fausto, 2009. Antibiotic resistance of lactobacilli isolated from two Italian hard cheeses. *Journal of Food Protection*, **72**, 2162–2169.

Billah, Md. M., M. Z. Hoque, F. Akter, K. M. Hossain, M. S. M. Rahman & K. M. D. Islam, 2010. Isolation, identification and analysis of probiotic properties of *Lactobacillus* spp. from selective regional yoghurts. *World Journal of Dairy & Food Sciences*, **5**, 39–46.

Broadbent, J. R., E. C. Neeno-Eckwall, B. Stahl, K. Tandee, H. Cai, W. Morovic, P. Horvath, J. Heidenreich, N. T. Perna, R. Barrangou & J. L. Steele, 2012. Analysis of the *Lactobacillus casei* supragenome and its influence in species evolution and lifestyle adaptation. *BMC Genomic*s, **13**, 533.

Casas, I. A. & W. J. Dobrogosz, 2000. Validation of the probiotic concept: *Lactobacillus reuteri* confers broad-spectrum protection against disease in humans and animals. *Microbial Ecology in Health and Disease*, **12**, 247–285.

Cebeci, A. & C. Gürakan, 2003. Properties of potential probiotic *Lactobacillus plantarum* strains. *Food Microbiology*, **20**, 511.

Charteris, W. P., P. M. Kelly, L. Morelli & J. K. Collins, 1998. Antibiotic susceptibility of potentially probiotic *Lactobacillus* species. *Journal of Food Protection*, **61**, 1636–1643.

CLSI, 2006. Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved standard, 9th edm, CLSI document M2-A9. 26:1. Clinical Laboratory Standards Institute, Wayne, PA.

Condon, S., 1983. Aerobic metabolism of lactic acid bacteria. *Iranian Journal of. Food Science and Technology*, **7**, 15–25.

Danielsen, M. & A. Wind, 2003. Susceptibility of *Lactobacillus* spp. to antimicrobial agents. *International Journal of Food Microbiology,* **82**, 1–11.

Danova, S. & N. Georgieva, 2013. Probiotic Textbook. HTMU, p. 194 (BG).

Das, D. J., A. Shankar, J. B. Johnson & S. Thomas, 2019. Critical insights into antibiotic resistance transferability in probiotic Lactobacillus. *Nutrition*, **69**, 110–567.

European Food Safety Authority (EFSA), 2012a. Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance. *EFSA Journal*, **10**, 2740–2750.

European Food Safety Authority, 2012b. The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in the European Union in 2010. *EFSA Journal*, **10**, 2598.

Ezekiel, D. H. & J. E. Hutchins, 1968. Mutations affecting RNA polymerase associated with rifampicin resistance in *Escherichia coli*. *Nature*, **164**, 276–277.

Flórez, B. A., S. Delgado & B. Mayo, 2005. Antimicrobial susceptibility of lactic acid bacteria isolated from a cheese environment. *Canadian Journal of Microbiology*, **51**, 51–58.

Fons, M., T. Hege, M. Ladire, P. Raibaud, R. Ducluzeau, E. Maguin, 1997. Isolation and characterization of a plasmid from *Lactobacillus fermentum* conferring erythromycin resistance. *Plasmid,* **37**, 199–203.

Herrero, M., B. Mayo, B. Ganzales & J. E. Suarez, 1996. Evaluation of technologically important traits in lactic acid bacteria isolated from spontaneous fermentations. *Journal of Applied Bacteriology,* **81**, 565–570.

Hill, C., F. Guarner, G. Reid, G. R. Gibson, D. J. Merenstein, B. Pot, L. Morelli, R. B. Canani, H. J. Flint, S. Salminen, P. C. Calder & M. E. Sanders, 2014. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nature reviews. Gastroenterology & Hepatology*, **11**, 506–514.

Holliman, R. E. & G. P. Bone, 1988. Vancomycin resistance of clinical isolates of lactobacilli. *Journal of Infection*, **16**, 279–283.

Horowitz, B. J., P. A. Mardh, E. Nagy & E. L. Rank, 1994. Vaginal lactobacillosis. *The American Journal of Obstetrics and Gynecology*, **170**, 857–861.

Hummel, A. S., C. Hertel, W. H. Holzapfel & C. M. Franz, 2007. Antibiotic resistances of starter and probiotic strains of lactic acid bacteria. *Applied and Environmental Microbiology,* **73**, 730–739.

Kim, K. S., J. O. Morrison & A. S. Bayer, 1982. Deficient autolytic enzyme activity in antibiotic-tolerant lactobacilli. *Infection and Immunity*, **36**, 582–585.

Lapsiri, W., S. Nitisinprasert & P. Wanchaitanawong, 2011. *Lactobacillus plantarum* strains from fermented vegetables as potential probiotics. *Kasetsart Journal (Natural Science)*, **45**, 1071–1082.

McCormick, A. W., C. G. Whitney, M. M. Farley, R. Lynfield, L. H. Harrison, N. M. Bennett, W. Schaffner, A. Reingold, J. Hadler, P. Cieslak, M. H. Samore & M. Lipsitch, 2003. Geographic diversity and temporal trends of antimicrobial resistance in *Streptococcus pneumoniae* in the United States. *Nature Medicine*, **9**, 424–430.

Neut, C., S. Mahieux & L. J. Dubreuil, 2017. Antibiotic susceptibility of probiotic strains: Is it reasonable to combine probiotics with antibiotics? *Médecine et Maladies Infectieuses*, **47**, 477–483.

Nicas, T. I., C. T. Cole, D. A. Preston, A. A. Schabel & R. Nagaraj, 1989. Activity of glycopeptides against vancomycin-resistant gram-positive bacteria. *Antimicrobial Agents and Chemotherapy,* **33**, 1477–1481.

O'Brien, T. F., 2002. Emergence, spread, and environmental effect of antimicrobial resistance: How use of an antimicrobial anywhere can increase resistance to any anti- microbial anywhere else. *Clinical Infectious Diseases*, **34**, 78–84.

Pulido, P., R. Benomar, N. Abriouel, H. Lucas López, R. Martínez Cañamero & M. A. Gálvez, 2005. Microbiological study of lactic acid fermentation of caper berries by molecular and culture-dependent methods. *Applied and Environmental Microbiology*, **71**, 7872–7879.

Putman, M., H. W. van Veen, J. E. Degener & W. N. Konings, 2001. The lactococcal secondary multidrug transporter LmrP confers resistance to lincosamides, macrolides, streptogramins and tetracyclines. *Microbiology*, **147**, 2873–2880.

Raftis, E. J, B. M. Forde, M. J. Claesson & P. W. O'Toole, 2014. Unusual genome complexity in *Lactobacillus salivarius* JCM1046. *BMC Genomics,* **15**, 771.

Roberts, M. C. & S. L. Hillier, 1990. Genetic basis of tetracycline resistance in urogenital bacteria. *Antimicrobial Agents and Chemotherapy*, **34**, 261–264.

Salminen, S., A. von Wright, L. Morelli, P. Marteau, D. Brassart, W. M. de Vos, R. Fonden, M. Saxelin, K. Collins, G. Mogensen, S. E. Birkeland & T. Sandholm, 1998. Demonstration of safety of probiotics – a review. *International Journal of Food Microbiology*, **44**, 93–106.

Savadogo, A., C. A. T. Ouattara, A. J. Ilboudo, D. Karou & A. S. Traore, 2010. Isolation and susceptibility to antibiotics of bacterial strains from Burkina Faso fermented milk samples. *Advance Journal of Food Science and Technology*, **2**, 91–95.

Sharma, P., S. K. Tomar, V. Sangwan, P. Goswami & R. Singh, 2015. Antibiotic Resistance of *Lactobacillus* sp. Isolated from Commercial Probiotic Preparations. *Journal of Food Safety,* **36**, 10.

Snydman, D. R., 2008. The safety of probiotics. *Clinical Infectious Diseases*, **46**, 104–111.

Tannock, G. W., J. B. Luchansky, L. Miller, H. Connell, S. Thode-Andersen, A. A. Mercer & T. R. Klaenhammer, 1994. Molecular characterization of a plasmid-borne (pGT633) erythromycin resistance determinant (ermGT) from *Lactobacillus reuteri*. *Plasmid*, **31**, 60–71.

Teuber, M., L. Meile & F. Schwartz, 1999. Acquired antibiotic resistance in lactic acid bacteria from food. *Antonie van Leeuwenhoek*, **76**, 115–137.

Thumu, S. C. & P. M. Halami, 2012. Acquired resistance to macrolide–lincosamide–streptogramin antibiotics in lactic acid bacteria of food origin. *Indian Journal of Microbiology,* **52**, 530–537.

Yüksekdağ, Z. N. & Y. Beyatli, 2008. Antimicrobial activity and antibiotic susceptibility of some lactic acid bacteria isolated different dairy products. *Archiv für Lebensmittelhygiene*, **59**, 216–220.

Zarazaga, M., Y. Sainz, A. Portillo, C. Tenorio, R. F. Ruiz-Larrara, R. del Campo, F. Baquero & C. Torres, 1999. *In vitro* activities of ketolide HMR3647, macrolides, and other antibiotics against *Lactobacillus*, *Leuconostoc* and *Pediococcus* isolates. *Antimicrobial Agents Chemotherapy*, **43**, 3039–3041.

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Correspondence:

Svetla Danova
Department of General Microbiology, The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences,
26, Acad. G. Bonchev Str., 1
113, Sofia, Bulgaria,
e-mail: stdanova@yahoo.com