ANTIMICROBIAL POTENTIAL OF PROBIOTICS IN COMBINATION WITH STARTER LACTIC ACID BACTERIA*

T. Mančušková, A. Medveďová, Ľ. Valík

Slovak University of Technology in Bratislava, Faculty of Chemical and Food Technology, Department of Nutrition and Food Quality Assessment, Bratislava, Slovak Republic

The symbiotic interrelationship of probiotic bacteria and starter lactic acid bacteria is of fundamental importance in many fields of industrial microbiology and it is also a great interest of food technologists. This study deals with the antimicrobial potential of cell-free supernatants of *Lactobacillus acidophilus* NCFM, *L. rhamnosus* GG and Fresco culture when cultivated alone or together against 5 strains of *Escherichia coli* and 5 strains of *Staphylococcus aureus*. While the effect on *E. coli* was not proven (average change of final density compared to the control was only $\Delta N_{EC,24h}$ = 0.12 log CFU ml^{-1}), the decrease of *S. aureus* final density in the presence of nisin and cell-free supernatant of *L. acidophilus* NCFM and Fresco culture was considerable ($\Delta N_{SA,24h,nis10}$ = –1.95 log CFU ml^{-1} and $\Delta N_{SA,24h,NCFM+Fr24}$ = –0.69 log CFU ml^{-1}, respectively).

Lactobacillus, NCFM, LGG, antibacterial potential

DE GRUYTER OPEN

doi: 10.1515/sab-2017-0028
Received for publication on February 24, 2017
Accepted for publication on May 25, 2017

INTRODUCTION

The practice of using microbial cultures for the production and preservation of foods is ancient, although the understanding of the microbiology behind it is only recent. There are two basic approaches. Firstly, the use of microbiota to ferment raw materials, by means of which undesirable microorganisms are suppressed and a new product is created. Secondly, microbial cultures can be used as bioprotectants to inhibit undesirable microorganisms without substantially altering the product itself. In food production, lactic acid bacteria (LAB) are used most frequently and some of them may have undefined probiotic properties. Thus, a more recent approach is to use known probiotic bacteria as starter cultures or bioprotectants, thereby exploiting both their preservative function and health-benefitting properties.

The symbiotic interrelationships among microbiota are of fundamental importance in nature and in many fields of industrial microbiology. LAB have a great ability to enter associative relationships in mixed cultures. The growth and metabolism of some LAB strains may be stimulated by another LAB member, even in environmental conditions, where one or both bacteria are not able to grow by themselves (Furukawa et al., 2013; Sasaki et al., 2014; Wang et al., 2016).

Lactobacillus acidophilus NCFM and *Lactobacillus rhamnosus* GG are well-known probiotic LAB whose positive effects on consumers' health have been confirmed in many researches (Andreasen, 2010; Hojsak et al., 2010; Ringel-Kulka et al., 2011, Smith et al., 2013).

As in other probiotics, their probiotic properties relate to their abilities of adherence to epithelial cells, production of antimicrobial substances, inhibition of

* Supported by the Slovak Research and Development Agency, Project No. APVV-15-0006.

Table 1: Summary of tested antimicrobial substances

Label	Antimicrobial substance	Duration of cultivation
A	CFS of *Lb. acidophilus* NCFM	24 h
B	CFS of *Lb. rhamnosus* GG	
C	CFS of *Lb. acidophilus* NCFM co-cultivated with Fresco	
D	CFS of *Lb. rhamnosus* GG co-cultivated with Fresco	
E	CFS of Fresco	
F	CFS of *Lb. acidophilus* NCFM	48 h
G	CFS of *Lb. rhamnosus* GG	
H	CFS of *Lb. acidophilus* NCFM co-cultivated with Fresco	
I	CFS of *Lb. rhamnosus* GG co-cultivated with Fresco	
J	CFS of Fresco	
K	nisin, c = 10 mg.l^{-1}	-
L	nisin, c = 100 mg.l^{-1}	
X	control	-

bacterial translocation, competition for nutrients and immunomodulation. The mechanism of probiotics action is described in detail in Mancuskova et al. (2017).

Although there is a lot of information about probiotic potential of *L. acidophilus* NCFM and *L. rhamnosus* GG, data about their direct antimicrobial activity against pathogenic microorganisms is missing. Moreover, selection of suitable strains to ensure the desired properties of the final product, its microbial safety and beneficial effect for consumer is a great interest of food microbiologists and food technologists all over the world. For above reasons, this work deals with antimicrobial effect of cell-free supernatants (CFS) of probiotic strains *L. acidophilus* NCFM Howaru Dophilus (NCFM strain) and *L. rhamnosus* GG (LGG strain) cultivated with and without Fresco starter culture against pathogenic bacteria *Staphylococcus aureus* and *Escherichia coli*.

MATERIAL AND METHODS

Microorganisms source

In the present study, the following microorganisms were used: *Lactobacillus acidophilus* NCFM Howaru Dophilus (ATCC 700396) of Danisco A/S (Copenhagen, Denmark) provided by Rajo (Bratislava, Slovakia); *Lactobacillus rhamnosus* GG (ATCC 53103) provided by Dr. Ouwehand (University of Turku, Turku, Finland) through the mediation of Dr. Lauková (Institute of Animal Physiology, Slovak Academy of Sciences, Košice, Slovakia); Fresco DVS 1010 of Chr. Hansen A/S (Hørshom, Denmark) provided by Rajo; strains of *Staphylococcus aureus* 2064, *S. aureus* 2296, *S. aureus* 1759, *S. aureus* 14733 and strains of *Escherichia coli* BR, *E. coli* LJ, *E. coli* IM, *E. coli* IB from the collection of microorganisms of the Department of Nutrition and Food Quality Assessment (Slovak University of Technology in Bratislava, Slovakia); *S. aureus* CCM 3859 and *E. coli* CCM 3988 from the Czech Collection of Microorganisms (Brno, Czech Republic).

Bacteria preparation and storage

The strains of NCFM and LGG were stored in MRS broth (Biokar Diagnostics, Beauvais, France) at 5 ± 1°C, the strains of *S. aureus* and *E. coli* were stored in BHI broth (Sigma-Aldrich, St. Louis, USA) at 5 ± 1°C, and the deeply frozen Fresco culture was kept at –18 ± 1°C.

The overnight suspensions of the pathogens were prepared from a 24-hour-old culture of each strain grown aerobically in the BHI broth at 37 ± 0.5°C. The overnight suspensions of NCFM and LGG were prepared from a 24 h culture of every strain grown in the MRS broth at 37 ± 0.5°C, 5% CO_2. The overnight suspension of Fresco culture was prepared from a 24 h culture grown aerobically in the UHT milk (fat content 1.5%; Rajo) at 30 ± 0.5°C.

To prepare CFS, overnight LAB cultures were inoculated into UHT milk (initial concentration of overnight culture in milk 1%, v/v) and grown for 24 and 48 h by themselves; lactobacilli were grown for 24 and 48 h in the UHT milk also with Fresco culture.

Antimicrobial substances preparation

CFSs of LAB were prepared from cultures grown as summarized in Table 1. Cultures were centrifuged (3300 rpm, 10 min) and supernatants were collected. Their pH values were adjusted by NaOH solution (Lachema, Brno, Czech Republic; c = 1 mol l^{-1}) to pH 6.9 ± 0.1 to exclude an influence of reduced active acidity. The adjusted supernatant was filtered through syringe microfilter (Ø 0.22 µm; Sarstedt, Nümbrecht, Germany). Nisin solution (Jeneil BioProducts GmbH, Schechen, Germany) was prepared and sterilized using a syringe microfilter. The results were compared to the control experiments where the antimicrobial agent was replaced by sterile deionized water.

Pre-test of LAB antimicrobial activity

The agar diffusion test was used as a pre-test of antimicrobial activity of LAB against tested pathogen microorganisms as follows: 0.2 ml of test strains overnight cultures ($N_{0,EC}$, $N_{0,SA} \approx 9$ log CFU ml^{-1}) were spread on Plate Count Agar (PCA) (Biokar

Table 2: Agar diffusion pre-test for screening the antimicrobial activity of cell-free supernantants of lactic acid bacteria against *E. coli and S. aureus*

Antimicrobial agent	pH	*E. coli*					*S. aureus*				
		BR	LJ	IM	IB	CCM 3988	2064	2296	1759	14733	CCM 3859
A	3.90	+	+	+	++	++	++	+	+	+	++
	6.84	-	-	-	-	-	-	-	-	-	-
B	3.89	+	+	+	++	++	++	++	++	+	++
	6.83	-	-	-	-	-	-	-	-	+	-
C	3.87	+	+	+	++	++	++	+	+	+	++
	6.75	-	+	-	-	-	+	-	-	-	+
D	3.87	+	+	+	++	++	++	++	+	+	++
	6.89	-	+	-	-	-	-	-	+	-	-
E	4.27	+	+	+	++	++	++	+	+	+	++
	6.92	-	-	-	-	-	-	-	-	-	-
F	3.85	+	+	+	++	++	++	+	+	+	++
	6.92	-	-	-	-	-	-	-	-	-	-
G	3.81	+	+	+	++	++	++	++	++	++	++
	6.88	-	-	-	-	-	-	-	-	+	-
H	3.82	+	+	+	++	++	++	+	+	+	++
	6.95	-	+	-	-	-	+	-	-	-	+
I	3.85	+	+	+	++	++	++	++	+	+	++
	6.90	-	+	-	-	-	-	-	+	-	-
J	3.99	+	+	+	++	++	++	++	++	++	++
	6.96	-	-	-	-	-	-	-	-	-	-
K	6.86	-	-	-	-	-	+	++	+	-	-
L	6.86	-	-	-	-	-	++	++	++	+	++
X	6.80	-	-	-	-	-	-	-	-	-	-

- no zone after 24 h and 72 h cultivation

+ cloudy zone after 24 h cultivation, no or cloudy zone after 72 h cultivation

++ clear zone after 24 h and 72 h cultivation

Diagnostics). 50 µl of antimicrobial agents solutions were applied into wells (Ø 5 mm) cut in the agar. Nisin solutions of 10 mg l^{-1} and 100 mg l^{-1} concentration were used to compare the efficiency of CFSs. The inhibition zones were evaluated after 24 h and 72 h of aerobic incubation at 37 ± 0.5°C.

LAB antimicrobial activity quantification

Antimicrobial agents (Table 1) were added into 9 ml of pre-tempered BHI broth as follows: every CFS of single or mixed LAB cultures to final concentration of 10% (v/v; experiments A–J), nisin solution to final concentrations of 10 mg l^{-1} and 100 mg l^{-1} (experiments K, L), and sterile deionized water to final concentration of 10% (v/v; control X). Overnight cultures of the *S. aureus* and *E. coli* strains were inoculated into BHI broth to achieve the initial concentration of $N_0 \approx 3$ log CFU ml^{-1} according to Valik et al. (2008). Static incubation of the cultures was performed at 37 ± 0.5°C under aerobic conditions. Required amounts of media with added antimicrobial agents containing *S. aureus* and *E. coli* were taken in the 0th, 12th, and 24th hour of incubation to determine the actual microorganism density on the PCA agar according to EN ISO 4833-1 (2013).

Statistical analysis

Each experiment was performed in three separate trials. Results are presented as means of values with their standard deviations. Statistical analyses were carried out using MS Excel 2013. Parametric data were treated by independent two-sample Student's *t*-test (unequal variances) and confirmed by ANOVA test.

RESULTS

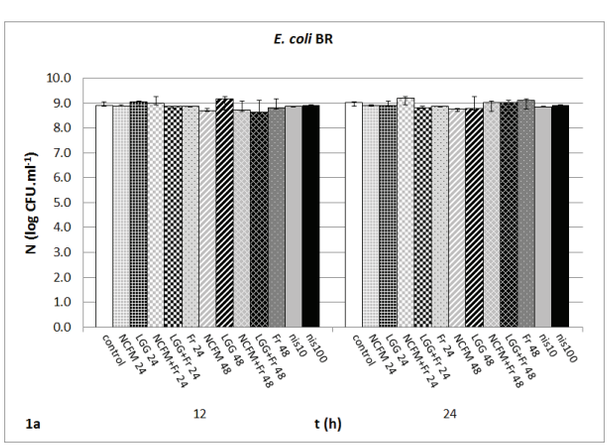
Agar diffusion pre-test of LAB antimicrobial activity

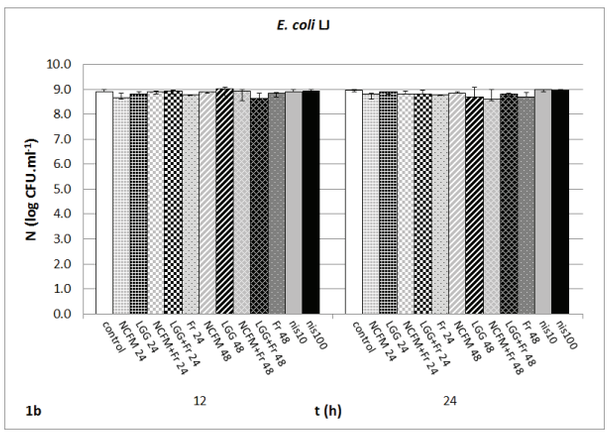
Solutions of antimicrobial substances with native and adjusted pH (to value 6.9 ± 0.1) were used in agar diffusion pre-test. Non-adjusted CFSs obtained

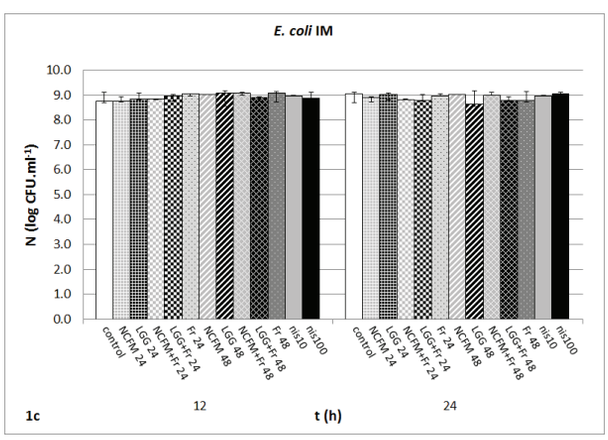
by cultivation of probiotics with or without Fresco culture for 24 h or 48 h reached final pHs under value 3.9 (pH_{Lb} = 3.86 ± 0.03), while final pH values of CFSs obtained by mono-cultivation of Fresco culture were slightly higher ($pH_{Fr,24h}$ = 4.27, $pH_{Fr,48h}$ = 3.99). Hazy or clear zones around the spot of application of antimicrobial solutions were observed after incubation of Petri dishes. Hazy zones were investigated and proven to be the plaques of tested pathogens according to methods published by Valik et al. (2016).

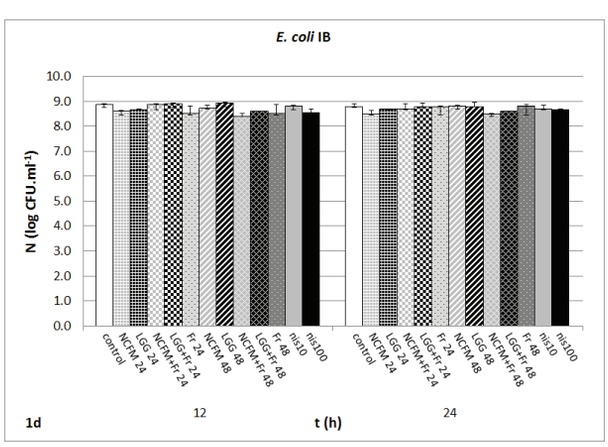
The agar diffusion pre-test proved the antimicrobial potential of organic acids produced by LAB. All the supernatants with the native pH inhibited growth of the tested pathogens. This was reflected in the formation of clear or hazy zones around the spot of CFS application. As can be seen in Table 2, all non-adjusted CFSs inhibited growth of *S. aureus* 2064 and CCM 3859 completely after 24 h of incubation. Antimicrobial efficiency of non-adjusted CFSs applied on *S. aureus* 2296, 1759, and 14733 depended on the type of CFS and formation of hazy zones was observed after 24 h of incubation (Table 2). Effect of CFSs with native pH was more explicit in the testing of *E. coli* strains. Formation of clear zones after a 24-hour cultivation was observed only in experiments with *E. coli* LB and CCM 3988. *E. coli* BR, MF, and LJ formed hazy zones around the spot of CFS application.

To exclude the antimicrobial activity of organic acids in CFSs of LAB, their active acidity was adjusted by NaOH to pH 6.8–7.0. As expected, antimicrobial potential of pH-adjusted CFSs was generally lower compared to the non-adjusted CFSs. No inhibition zones were observed in experiments where NCFM and Fresco CFSs were used, regardless of the duration of LAB cultivation. On the other hand, the highest antimicrobial potential was noted in experiments where CFSs from co-cultivation of Fresco and *L. acidophilus* NCFM (C, H) were applied. *S. aureus* 2064, *S. aureus* CCM 3859, and *E. coli* LJ were observed









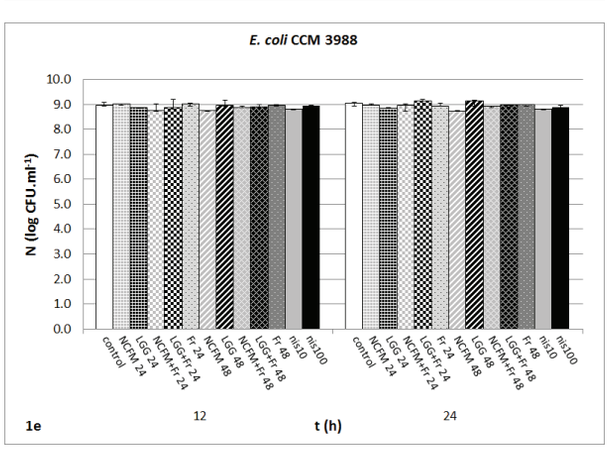
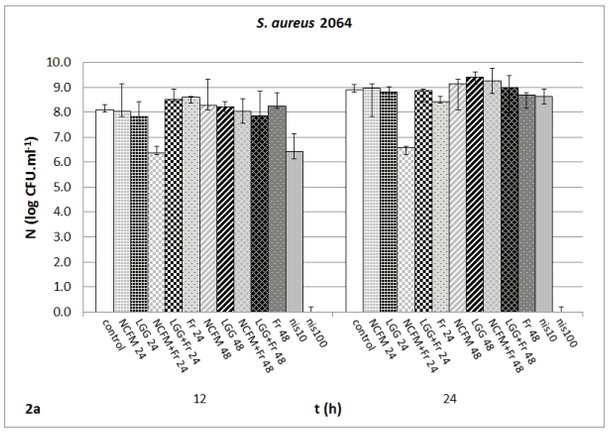
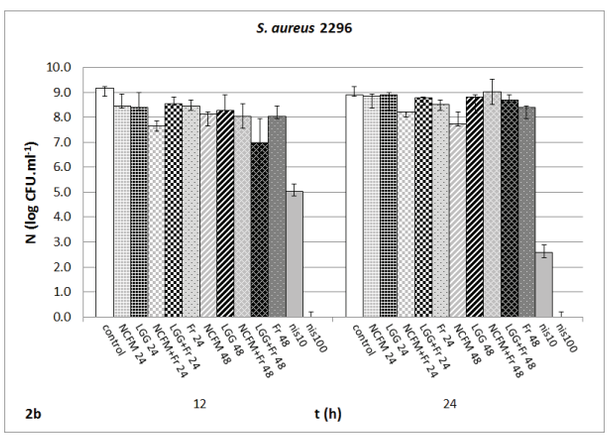
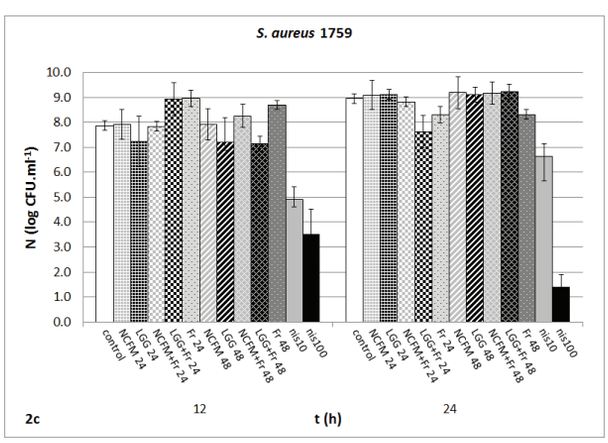


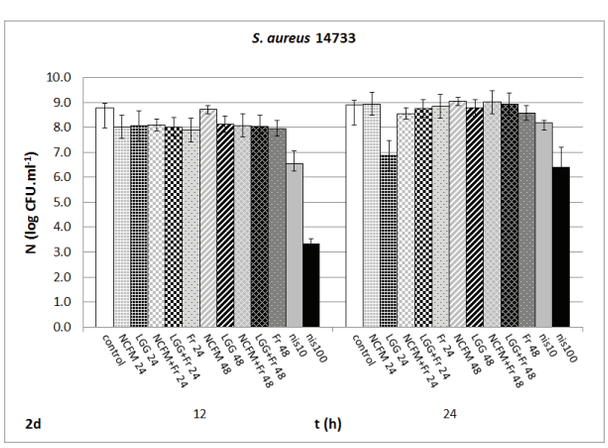
Fig. 1. Growth of *E. coli* in the presence of antimicrobial substances

means and error bars of the actual density (*N*) of *E. coli* were obtained from cultivation experiments carried out in three separate trials









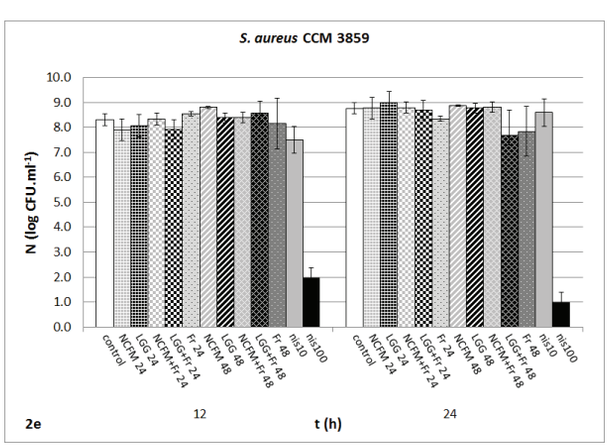


Fig. 2. Growth of S. aureus in the presence of antimicrobial substances

means and error bars of the actual density (N) of S. aureus were obtained from cultivation experiments carried out in three separate trials

to be sensitive to metabolic products formed in this co-cultivation (Table 2).

The application of nisin in the concentration of either 10 or 100 mg l^{-1} resulted in the formation of inhibition zones in experiments with staphylococci only. The inhibition of *E. coli* growth was not observed. Nisin in the concentration of 100 mg l^{-1} inhibited all tested staphylococci. Clear zones were formed in all staphylococci experiments but for the test with *S. aureus* 14733, where a hazy zone was formed. On the other hand, nisin solution in the concentration of 10 mg l^{-1} inhibited growth only in three out of the five tested staphylococci (*S. aureus* 2064, 2296, and 1759). This concentration is also the maximal concentration of nisin permitted in foods (Commission Directive 2010/69/EU).

LAB antimicrobial activity quantification

To quantify the antimicrobial effect of the LAB CFS against the *S. aureus* and *E. coli* strains, the experiments in BHI broth were carried out. The actual density (*N*) of pathogens was determined in the 12th and 24th hour of cultivation with the antimicrobial substances and compared to their growth without the presence of CFS (Figs. 1, 2).

As can be seen in Fig. 1a–e, pH-adjusted CFSs of lactic acid bacteria and the solutions of nisin had no significant effect on the growth of the tested *E. coli* strains and the average density in the 24th hour fell in a narrow range of values (N = 8.85 ± 0.03 log CFU ml^{-1}). This is mostly in agreement with the agar diffusion pre-test described above. Although *E. coli* LJ showed a sensitivity to CFSs obtained by co-cultivation of *L. acidophilus* NCFM with Fresco culture (antimicrobial substances C, H) and *L. rhamnosus* GG with Fresco (antimicrobial substances D, I) in pre-test (Table 2), experiments in BHI broth

did not reveal any significant change in microbial counts compared to the control sample ($P > 0.05$; Fig. 1b). The average actual densities of the strain in the 12th and 24th hour of cultivation with antimicrobial substances C, D, I or H reached $N_{12h,LJ} = 8.84 \pm 0.13$ and $N_{24h,LJ} = 8.78 \pm 0.08$ log CFU ml^{-1}, respectively. These values were at the same level as the results in control experiments ($N_{12h,LJ,X} = 8.91 \pm 0.01$ and $N_{24h,LJ,X} = 8.97 \pm 0.01$ log CFU ml^{-1}).

Weak or no antimicrobial activity against Gram-negative rods had been expected and no significant changes in the actual density of *E. coli* were noted in experiments with nisin as well ($P > 0.05$).

Although the inhibition effect of antimicrobial substances produced by lactic acid bacteria on the growth of *E. coli* strains was not proven in this study, experiments performed with *S. aureus* species gave encouraging results (Fig. 2a–e).

None of the tested staphylococci reacted on the presence of substances A and F (obtained by cultivation of the NCFM strain for 24 and 48 h, respectively) significantly ($P > 0.05$). The results showed that the average concentrations of *S. aureus* in BHI broth supplemented with NCFM CFSs reached $N_{12h,S.aureus,A,F} = 8.21 \pm 0.33$ log CFU ml^{-1} and $N_{24h,S.aureus,A,F} = 8.85 \pm 0.42$ log CFU ml^{-1}, respectively. The values lied within the interval of the standard deviations of control samples ($N_{12h,S.aureus,X} = 8.44 \pm 0.53$ log CFU ml^{-1} and $N_{24h,S.aureus,A,F} = 8.88 \pm 0.07$ log CFU ml^{-1}, respectively).

A similar reaction of pathogens was observed during their incubation with substances B and G, which were obtained by cultivation of the LGG strain for 24 and 48 h, respectively. An exception occurred in enterotoxigenic *S. aureus* 14733. The density of *S. aureus* 2064, 2296, 1759 and CCM 3859 during the cultivation with LGG CFSs achieved average values $N_{12h,S.aureus,B,G} = 7.89 \pm 0.49$ log CFU ml^{-1} and $N_{24h,S.aureus,B,G} = 8.95 \pm 0.13$ log CFU ml^{-1}, respectively. This is at the level of controls, where the actual densities in the 12th and 24th hour of cultivation were $N_{12h,S.aureus,X} = 8.36 \pm 0.57$ log CFU ml^{-1} and $N_{24h,S.aureus,A,F} = 8.87 \pm 0.07$ log CFU ml^{-1}, respectively. However, the density of viable cells of *S. aureus* 14733 after 24 h of cultivation with substance B (LGG, 24 h) was only $N_{24h,14733,B} = 6.88 \pm 0.12$ log CFU ml^{-1}, i.e. about 100 times lower than in the control ($N_{24h,14733,X} = 8.89 \pm 0.05$ log CFU ml^{-1}) (Fig. 2d).

As expected, pH-adjusted CFS of Fresco culture (antimicrobial substances E, J) did not exhibit any inhibition effect when cultivated with *S. aureus*, as well (Figs. 1, 2).

The symbiotic interrelationships among bacteria and their great ability to enter associative relationships in mixed cultures are very important in food industry. Therefore, antimicrobial potential of probiotics in co-culture with starter lactic acid bacteria was examined.

Despite the hopeful results of agar diffusion pre-test (Table 2), experiments in BHI broth showed that the CFS obtained by cultivation of the NCFM strain with Fresco culture for 48 h (antimicrobial substance H) or LGG with Fresco for 24 and 48 h (D and I, respectively) did not affect the actual density of the tested staphylococci. The average viable cells concentration of *S. aureus* in experiments with CFSs D, H, I after 24 h and 48 h of cultivation were comparable to the control experiments and they achieved $N_{12h,S.aureus,H,D,I} = 8.08 \pm 0.51$ log CFU ml^{-1} and $N_{24h,S.aureus,H,D,I} = 8.76 \pm 0.48$ log CFU ml^{-1}, respectively.

On the other hand, the cell-free supernatant of the NCFM strain co-cultivated with the Fresco culture for 24 h (antimicrobial substance C) inhibited the growth of *S. aureus* 2064, 2296, and 14733 significantly ($P < 0.05$). Lower actual densities of strains were observed compared to the control samples (X) and the samples where CFS from the NCFM mono-cultivation (A) was applied, as well (Fig. 2a, b, d). The strongest inhibition effect was recorded in the experiment with *S. aureus* 2064, where its density in the 24th hour of cultivation was by about 2.33 log CFU ml^{-1} lower than in the control.

Concurrently with testing of LAB CFSs, the antimicrobial effect of nisin was examined. As expected, the substance inhibited growth of Gram-positive cocci. A complete or a substantial inhibition (drop by more than 5 log CFU ml^{-1} compared to the control) was noted in experiments with nisin at the concentration of 100 mg l^{-1}. Ten-time lower amount of nisin caused a considerable inhibition of staphylococci, too. A decrease of the actual pathogens' density compared to the control was recorded after 12 h of cultivation with nisin (concentration 10 mg l^{-1}) and it ranged from 0.98 to 4.14 log CFU ml^{-1}. Based on the results, nisin is sufficiently effective against *S. aureus* in the concentration limited by Commission Directive 2010/69/EU, i.e. of 10 mg l^{-1}, and highly effective in the concentration 100 mg l^{-1}.

DISCUSSION

An ability of *E. coli* to produce organic acids as the products of heterofermentation, the resistance to short-chain organic acids and the metabolic features evolved in *Escherichia* genus can help this pathogen to survive in presence of lactic acid bacteria (Zhang et al., 2011). This is in agreement with the results of the agar-diffusion pre-test which showed that the strains of *E. coli* were less sensitive to the low pH value of CFSs compared to the strains of *S. aureus*. Inhibition of the growth rate is then proportional to the concentration of undissociated lactic acid (Presser et al., 1997).

To exclude the inhibition effect of organic acids produced by lactic acid bacteria, active acidity of CFSs

was treated and the pH of antimicrobial substances was adjusted to 6.9 ± 0.1.

Similar results as presented in our study were determined by Zhang et al. (2011) who tested antimicrobial activity of LGG strain against *E. coli* ATCC 25922. While the application of non-treated LGG CFS led to the formation of a growth-free inhibition zone, the absence of the inhibition zone was detected in an experiment with CFS adjusted to pH 6.5. In accordance with our study, no significant inhibition effect of the NCFM strain on *E. coli* was reported by Barefoot, Klaenhammer (1983) and Ahmed et al. (2010). Similarly, our experiments in BHI broth performed to quantify the antimicrobial activity of CFSs showed that the pH-adjusted antimicrobial substances had no significant effect on *E. coli* final density ($P > 0.05$).

Bacteriocins produced by lactic acid bacteria are usually able to inhibit only the growth of similar or closely related bacterial strains. Therefore, weak or none antimicrobial activity of nisin against Gram-negative rods had been expected and this assumption was proven by our experiments. Gram-negative bacteria, including *E. coli*, are resistant to nisin due to their protective outer membrane. This membrane forms the outermost layer of the cell envelope and functions as an efficient barrier against certain hydrophobic solutes and macromolecules (Olasupo et al., 2003).

Extensive studies on the antimicrobial potential of lactobacilli antimicrobial substances independent of their pH values have been conducted. Although the NCFM strain produces bacteriocin lactacin B and it has been proven to be active against the members of *Lactobacillaceae* family, a pH-independent inhibition of *S. aureus* has not been recorded in literature (Barefoot, Klaenhammer, 1983; Ahmed et al., 2010). Small bioactive peptides from *L. rhamnosus* GG and their bactericidal activity against Gram-negative and Gram-positive bacteria were studied by Lu et al. (2009). As the inhibition effect of peptide on *E. coli* and *Salmonella* Typhi was dose-dependent but considerable, the effect on *S. aureus* was only weak.

Arques et al. (2011) observed that 10 000 IU ml^{-1} of nisin (corresponding to 10 mg l^{-1}) was able to reduce *S. aureus* counts by 4.68 log CFU ml^{-1} in milk after 4 h of incubation at 37°C. Felicio et al. (2015) reported nisin to be an effective preservative during cheese manufacturing. According to their research the whey and curd treated with 500 IU ml^{-1} nisin showed by about 2 log CFU ml^{-1} lower *S. aureus* counts compared to untreated cheese.

The Fresco culture is used in dairy industry for its strong ability to suppress the growth of undesirable microbiota due to the rapid matrix acidification (Le Marc et al., 2009) but the production of other important antimicrobial metabolites has not been proven. This is in agreement with our study, where no inhibition effect on the growth of *E. coli* and *S. aureus* was observed.

CONCLUSION

Although the CFS of probiotic strains *L. acidophilus* NCFM and *L. rhamnosus* GG did not show the direct inhibition effect on *S. aureus* and *E. coli in vitro*, another probiotic features (ability of displacement of pathogens adhered to intestinal human mucus, competition for nutrients, inhibition of bacterial translocation, etc.) should be taken into account while considering the strains as potentially beneficial for human health. However, this study showed that the commensalism between lactic acid bacteria can play an important role in the development of probiotic products and in the health care. Among the spectra of the CFS, the most effective against *S. aureus* was the supernatant obtained by co-cultivation of the probiotic strain *L. acidophilus* NCFM and Fresco culture for 24 h. It was able to reduce *S. aureus* 2064 counts by about 2.33 log CFU ml^{-1} compared to its growth without the presence of any antimicrobial substance.

REFERENCES

Ahmed Z, Wang Y, Cheng Q, Imran M (2010): *Lactobacillus acidophilus* bacteriocin, from production to their application: an overview. African Journal of Biotechnology, 9, 2843–2850. doi: 10.5897/AJB2010.000-3111.

Andreasen AS, Larsen N, Pedersen-Skovsgaard T, Berg RMG, Moller K, Svendsen KD, Jakobsen M, Pedersen BK (2010): Effects of *Lactobacillus acidophilus* NCFM on insulin sensitivity and the systemic inflammatory response in human subjects. British Journal of Nutrition, 104, 1831–1838. doi: 10.1017/S0007114510002874.

Arques JL, Rodriguez E, Nunez M, Medina M (2011): Combined effect of reuterin and lactic acid bacteria bacteriocins on the inactivation of food-borne pathogens in milk. Food Control, 22, 457–461. doi: 10.1016/j.foodcont.2010.09.027.

Barefoot SF, Klaenhammer TR (1983): Detection and activity of lactacin B, a bacteriocin produced by *Lactobacillus acidophilus*. Applied and Environmental Microbiology, 45, 1808–1815.

Commission Directive 2010/69/EU of 22 October 2010 amending the Annexes to European Parliament and Council Directive 95/2/EC on food additives other than colours and sweeteners. Official Journal of the European Union, L 279, 22–31.

EN ISO 4833-1 (2013): Microbiology of the food chain – Horizontal method for the enumeration of microorganisms – Part 1: Colony count at 30 degrees C by the pour plate technique. International Organization for Standardization, Geneva, Switzerland.

Felicio BA, Pinto MS, Oliveira FS, Lempk MW, Pires ACS, Lelis CA (2015): Effects of nisin on *Staphylococcus aureus* count and physicochemical properties of Minas Frescal cheese. Journal of Dairy Science, 98, 1364–4369. doi: 10.3168/jds.2015-9520.

Furukawa S, Watanabe T, Toyama H, Morinaga Y (2013): Significance of microbial symbiotic coexistence in traditional fermentation. Journal of Bioscience and Bioengineering, 116, 533–539. doi: 10.1016/j.jbiosc.2013.05.017.

Hojsak I, Snovak N, Abdovic S, Szajewska H, Misak Z, Kolacek S (2010): *Lactobacillus* GG in the prevention of gastrointestinal and respiratory tract infections in children who attend day care centers: a randomized, double-blind, placebo-controlled trial. Clinical Nutrition, 29, 312–316. doi: 10.1016/j.clnu.2009.09.008.

Le Marc Y, Valik L, Medvedova A (2009): Modelling the effect of the starter culture on the growth of *Staphylococcus aureus* in milk. International Journal of Food Microbiology, 129, 306–311. doi: 10.1016/j.ijfoodmicro.2008.12.015.

Lu R, Fasano S, Madayiputhiya N, Morin NP, Nataro J, Fasano A (2009): Isolation, identification, and characterization of small bioactive peptides from *Lactobacillus* GG conditional media that exert both anti-Gram-negative and Gram-positive bactericidal activity. Journal of Pediatric Gastroenterology and Nutrition, 49, 23–30. doi: 10.1097/MPG.0b013e3181924d1e.

Mancuskova T, Medvedova A, Ozbolt M, Valik L (2017): The medical functions of probiotics and their role in clinical nutrition. Current Nutrition and Food Science, 13, 1–18. doi: 10.2174/1573401313666170405152905.

Olasupo NA, Fitzgerald DJ, Gasson MJ, Narbad A (2003): Activity of natural antimicrobial compounds against *Escherichia coli* and *Salmonella enterica* serovar Typhimurium. Letters in Applied Microbiology, 37, 448–451. doi: 10.1046/j.1472-765X.2003.01427.x.

Presser KA, Ratkowsky DA, Ross T (1997): Modelling the growth rate of *Escherichia coli* as a function of pH and lactic acid concentration. Applied and Environmental Microbiology, 63, 2355–2360.

Ringel-Kulka T, Palsson OS, Maier D, Carroll I, Galanko JA, Leyer G, Ringel Y (2011): Probiotic bacteria *Lactobacillus acidophilus* NCFM and *Bifidobacterium lactis* Bi-07 versus placebo for the symptoms of bloating in patients with functional bowel disorders: a double-blind study. Journal of Clinical Gastroenterology, 45, 518–525. doi: 10.1097/MCG.0b013e31820ca4d6.

Sasaki Y, Horiuchi H, Kawashima H, Mukai T, Yamamoto Y (2014): NADH oxidase of *Streptococcus thermophilus* 1131 is required for the effective yogurt fermentation with *Lactobacillus delbrueckii* subsp. *bulgaricus* 2038. Bioscience of Microbiota, Food and Health, 33, 31–40. doi: 10.12938/bmfh.33.31.

Smith TJ, Rigassio-Radler D, Denmark R, Haley T, Touger-Decker R (2013): Effect of *Lactobacillus rhamnosus* LGG® and *Bifidobacterium animalis* ssp. lactis BB-12® on health-related quality of life in college students affected by upper respiratory infections. British Journal of Nutrition, 109, 1999–2007. doi: 10.1017/S0007114512004138.

Valik L, Medvedova A, Bajusova B, Liptakova D (2008): Variability of growth parameters of *Staphylococcus aureus* in milk. Journal of Food and Nutrition Research, 47, 18–22.

Valik L, Medvedova A, Liptakova D (2016): Laboratory Practicals in Food Microbiology (Laboratórium z mikrobiológie potravín). Nakladateľstvo STU, Bratislava. (in Slovak)

Wang T, Xu Z, Lu S, Xin M, Kong J (2016): Effects of glutathione on acid stress resistance and symbiosis between *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. International Dairy Journal, 61, 22–28. doi: 10.1016/j.idairyj.2016.03.012.

Zhang Y, Zhang L, Du M, Yi H, Guo C, Tuo Y, Han X, Li J, Zhang L, Yang L (2011): Antimicrobial activity against *Shigella sonnei* and probiotic properties of wild lactobacilli from fermented food. Microbiological Research, 167, 27–31. doi: 10.1016/j.micres.2011.02.006.

Corresponding Author:

prof. Ing. Ľubomír Valík, Slovak University of Technology in Bratislava, Faculty of Chemical and Food Technology, Department of Nutrition and Food Quality Assessment, Radlinského 9, SK-812 37 Bratislava, Slovak Republic, phone: +421 259 325 518, e-mail: lubomir.valik@stuba.sk