Bulgarian Journal of Agricultural Science, 28 (No 5) 2022, 949–955

Antimicrobial potential of eleven *Lacticaseibacillus paracasei* strains isolated from mountain anthills

Toncho Dinev[1*], Nikolina Rusenova[2], Katya Velichkova[3] and Georgi Beev[1]

[1]*Trakia University, Faculty of Agriculture, Department of Biochemistry, Microbiology and Physics, 6000 Stara Zagora, Bulgaria*
[2]*Trakia University, Faculty of Veterinary Medicine, Department of Veterinary Microbiology, Infectious and Parasitic Diseases, 6000 Stara Zagora, Bulgaria*
[3]*Trakia University, Faculty of Agriculture, Department of Biology and Aquaculture, 6000 Stara Zagora, Bulgaria*
[*]*Corresponding author:* dinev_sz@mail.bg

Abstract

Dinev, T., Rusenova, N., Velichkova, K. & Beev, G. (2022). Antimicrobial potential of eleven *Lacticaseibacillus paracasei* strains isolated from mountain anthills. *Bulg. J. Agric. Sci., 28 (5)*, 949–955

Probiotics with antimicrobial activity are important alternative to antibiotics, which are ever more restricted because of the developing microbial resistance and some adverse effects following frequent application. The aim of the present study is to determine the antibacterial and antifungal activity of supernatants of eleven *Lacticaseibacillus paracasei* strains (FR1-11) isolated from mountain anthills and identified by Amplified Ribosomal DNA Restriction Analysis (ARDRA). Antimicrobial activity was determined against reference strains of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa, Penicillium chrysogenum, Aspergillus niger, Aspergillus carbonarius, Aspergillus ochraceus, Aspergillus parasiticus, Fusarium oxysporum, Fusarium graminearum* and clinical isolates of *Bacillus cereus*, *Listeria monocytogenes* and *Salmonella enteritidis* using agar well diffusion method. All strains of *Lacticaseibacillus paracasei* inhibited the growth of *Staphylococcus aureus, Listeria monocytogenes, Salmonella enteritidis* and *Penicillium chrysogenum,* 10 strains – *Pseudomonas aeruginosa,* 9 strains – *Aspergillus carbonarius*, 6 strains – *Fusarium oxysporum*, 5 strains – *Escherichia coli*, and 3 strains – *Bacillus cereus*. There are no active strains against *Aspergillus niger, Aspergillus ochraceus, Aspergillus parasiticus* and *Fusarium graminearum.* The highest antibacterial activity was measured against *Pseudomonas aeruginosa* with 14.7 mm inhibition zone of FR3 strain. The largest zones of inhibition against fungal strains were 12 mm, determined by the activity of FR2 and FR4 strains against *Penicillium chrysogenum*. As a whole, the supernatants of *Lacticaseibacillus paracasei* strains showed higher activity against bacterial strains compared to fungal strains.

Keywords: antibacterial; antifungal; ARDRA; *Lacticaseibacillus paracasei*

Introduction

Probiotics are live microorganisms, which when administered in proper amounts confer a health benefit on the host (Nemska et al., 2021). One of the most important properties of probiotic bacteria, and lactic acid bacteria (LAB) in particular, is their antimicrobial activity against pathogenic and food spoilage microorganisms, which could be useful as alternative to antibiotics and to control the speed of propagation of potentially harmful bacteria and fungi in target organisms, food and feed (Sirakov et al., 2016; Dinev et al., 2018). For example, it is reported that disturbance of the healthy balance of the microbial ecosystem in urogenital and gastrointestinal tract could be overcome by probiotic appli-

cation – alone or as a supplementation to standard antibiotic therapy (Petrova et al., 2009a; Iseppi et al., 2019). Furthermore, consumer's demand for chemical preservative-free processed foods has increased the research LAB with high antibacterial and antifungal potential in order to be used as biopreservatives (Cosentino et al., 2018).

LAB are well known probiotics which exert their beneficial effects through production of bacteriocins, organic acids, ethanol, CO_2, H_2O_2, 3-hydroxy fatty acids, cyclic dipeptides, etc., as well as immune-stimulation, competition for nutrients and binding sites with the intestinal pathogens (Petrova et al., 2009b; Chen et al., 2019; Nemska et al., 2019; Dinev et al., 2020). *Lacticaseibacillus paracasei* (*L. paracasei*) is a widespread species of *Lactobacillus* genus found in yogurt and milk (Mirzaei et al., 2018), koumiss (Danova et al., 2005; Zhang et al., 2011), cheese (Radulović et al., 2010; Smetanková et al., 2014; Georgieva et al. 2015; Mangia et al., 2019), fruits, flower inflorescences (Benavides et al., 2016), honey (Lashani et al., 2018), sourdough bread (Hassan & Bullerman, 2008), fish intestinal tract (Wei et al., 2019), etc. Since the probiotic characteristics are known to be strain-specific, there is a continuous research for new sources of probiotics with health benefits, including antimicrobial activity against pathogens and food spoilage microorganisms (Naeem et al., 2012; Benavides et al., 2016; Dinev et al., 2018; Staykov et al., 2018). To the best of our knowledge, this study is the only one in which *L. paracasei* strains have been isolated from anthills.

In the available literature there are some studies on the antibacterial activity of *L. paracasei* strains, but experiments on the antifungal potential on this LAB species are rather scarce. The aim of the present study is to determine the antibacterial and antifungal activity of supernatants of eleven *L. paracasei* strains (FR1-11) isolated from mountain anthills which could serve as a basis for their use in this direction and more detailed future research.

Material and Methods

Isolation and identification of L. paracasei

Samples

The isolation of LAB from mountain anthills was performed by wooden sticks placed into them. The anthills, populated by red wood ants (*Formica rufa* L.), were located in Sinite Kamani National Park, Bulgaria.

The wooden sticks were placed into sterile containers with skimmed milk for bacteriological purposes. The samples were cooled and transported to the laboratory and then incubated at 37°C for 24 h. From the samples with visual coagulation one milliliter was transferred in 9 ml of sterile saline (0.85% NaCl, w/v), supplemented with peptone (0.1%, w/v; Oxoid, UK) and then serial dilutions from homogenates were prepared. One ml aliquot of the 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7}dilutions were pour-plated in MRS agar (Oxoid, UK) for isolation of *Lactobacillus* strains (each sample was plated in duplicate). After incubation at 37°C for 48 h, the morphology of the cells was observed by light microscopy after Gram staining. The strains were tested for the absence of catalase by direct application of 3% H_2O_2 to the colonies. The Gram-positive and catalase-negative rods were streaked three times on MRS agar (Oxoid, UK) in order to obtain pure cultures.

The bacterial isolates that were defined as *Lactobacillus* spp. on the basis of the test results were further classified by using ARDRA technique (Beev et al., 2021).

DNA extraction

For DNA studies, the *Lactobacillus* isolates were grown in MRS broth for 18 h at 37°C, and the genomic DNA was isolated using Genomic DNA Purification Kit (Fermentas, Spain), following the manufacturer's instructions.

Molecular identification

DNA from the reference strains *Lactobacillus helveticus* DSM 20075; *L. plantarum* DSM 20174; *L. casei* DSM 20011; *L. delbrueckii* ssp. *bulgaricus* DSM 20081 and *L. delbrueckii* ssp. *lactis* DSM 20072 was used as a template for PCR amplification using universal primers corresponding to the 5'-end fD1 (5'-AGAGTTTGATCCTGGCTCAG-3') and 3'-end rD1 (5'-TAAGGAGGTGATCCAGGC-3') of the 16S rRNA gene (Weisburg et al., 1991). The PCR product from 16S rDNA amplification was digested with endonucleases *Eco*RI and *Hae*III (NZYTech, Portugal). The restriction fragments were separated electrophoretically in 2% agarose gel (Cleaver Scientific Ltd, Hungary) and visualized by staining with fluorescent nucleic acid dye GelRed® (Biotium, USA). Restriction patterns identical to the references led to the lack of identification of the corresponding species. After that the species-specific PCR with particular primer sets was performed as follows: *L. paracasei* LMG13087 (5`-CCCACTGCTGCCTCCCGTAGGAGT-3` and 5`-CACCGAGATTCAACATGG-3`) (Roy et al., 2000) and *L. rhamnosus* LMG6400 (5`-CAGACTGAAAGTCTGACGG-3` and 5`-GCGATGCGAATTTCTATTATT-3`) (Walter et al., 2000) (Figure 1).

Antimicrobial activity

In this study were included reference bacterial strains – *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and

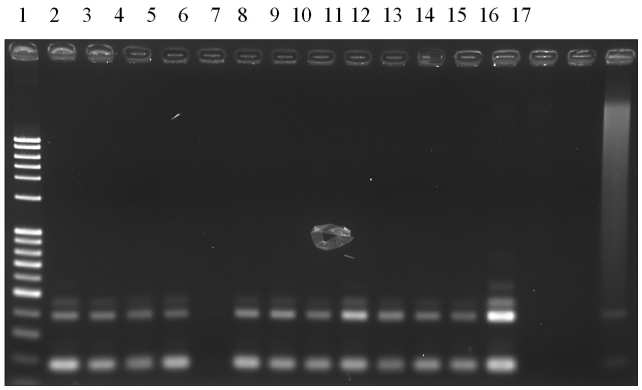

Fig. 1. Species-specific identification of *Lactobacillus* spp.: Lane 1 – molecular marker (100bp ladder, 1,5 kbp, 2 kbp); Lanes 2-5, 7-14: *L. paracasei* isolates; Lane 15 – positive control *L. casei* DSM20011; Lane 16 – positive control *L. rhamnosus* LMG6400; Lane 17 – positive control *L. paracasei* LMG13087

clinical isolates – *Bacillus cereus*, *Listeria monocytogenes* and *Salmonella enterica* subsp. *enterica* serovar *enteritidis*. The reference fungal strains used (*Aspergillus niger* NBIMCC 3252, *Aspergillus parasiticus* NBIMCC 2001, *Aspergillus carbonarius* NBIMCC 3391, *Aspergillus ochraceus* NBIMCC 2002, *Fusarium oxysporum* NBIMCC 125, *Fusarium graminearum* NBIMCC 2294 and *Penicillium chrysogenum* NBIMCC 129) were purchased from National Bank for Industrial Microorganisms and Cell Cultures (NBIMCC), Bulgaria. The strains were stored at 0-4°C.

L. paracasei isolates were grown in MRS broth at 37°C for 24 h and the supernatants were collected by centrifugation at 13 000 × *g* for 20 min, sterilized by using 0.22 μm filter, and their pH was adjusted to 7.0.

For measuring antibacterial activity, an agar well diffusion method was applied as previously described by Velichkova et al. (2018). Briefly, 18-20 h bacterial cultures grown on trypticase soy agar (TSA, Sigma-Aldrich, USA) supplemented with 5% defibrinated sheep blood were used to prepare inoculums in saline corresponding to 0.5 of the McFarland turbidity standard (1.5×10^8 CFU/mL) determined on Densilameter II (Erba Lachema, CZ). Cation-adjusted Mueller Hinton agar (Himedia, India) was poured in every *Petri* plate to achieve approximately 4 mm height of the layer. The agar surface was streaked three times with a sterile cotton swab preliminary dipped into the inoculum by rotating the plate three times to ensure equal distribution of the bacteria. Then, wells with a diameter of 6 mm were made by sterile cork borer and filled with 100 μL of the supernatants. Positive control with gentamicin at a concentration of 12.5 μg/mL and a negative one with MRS broth were carried out. The plates were incubated for 24 h at 37°C aerobically.

Antifungal activity of the supernatants was evaluated by agar well diffusion method described by Velichkova et al. (2018). In brief, 72 h old fungal cultures were grown on Potato dextrose agar (PDA, Biolife, Italy). 20 mL of PDA was poured in every *Petri* plate. After solidification, the agar surface was streaked three times with a sterile cotton swab preliminary dipped into the inoculum (1-2 x 10^4 CFU/mL) by rotating the plate three times to ensure equal distribution of the fungi. The wells were made by sterile cork borer of size 6.0 mm and were filled with 0.1 mL of the supernatants. Positive control with amphotericin B (Sigma-Aldrich, Germany) at a concentration of 25 μg/mL and negative one with MRS broth were performed. An incubation period of 3-5 days at 26-28°C was maintained.

Antimicrobial activity was evaluated by measuring inhibition zones (IZ) of microbial growth surrounding the supernatants in the wells. IZ were measured in millimeters and the diameter of the wells (6 mm) was included in the values presented. Antifungal activity was assumed in the presence of IZ ≥ 8.0 mm. The tests were performed in triplicate to determine the reproducibility of the results. The complete experiment was carried out under strict aseptic conditions.

Statistics

All analyses were carried out in triplicate and expressed as mean values ± standard deviation (SD). The data obtained was processed via Microsoft Excel 2010 using One-Way ANOVA.

Results and Discussion

Despite the numerous probiotic strains currently on the market, there is ongoing need for isolation of LAB strains with improved properties, including, among other things, high antimicrobial activity. Therefore, LAB isolated from their natural environment (e.g. mountain anthills) might possess better qualities compared to the well-known ones (Benavides et al., 2016). Furthermore, even though studies of LAB antimicrobial activity have been increasing over the last decades, there are not many experiments on antimicrobial activity of *L. paracasei* strains, especially with regard to the antifungal activity.

In the present study all eleven *L. paracasei* strains inhibited the growth of *Staphylococcus aureus* (*S. aureus*) with IZ of 9.7-12.6 mm (Table 1). These results were similar to the findings of Lozo et al. (2004), Iseppi et al. (2019), Mangia et al. (2019), and Amirkhanova et al. (2021) but lower than the data published by Radulović

Table 1. Antibacterial activity of selected strains of *L. paracasei* (mean±SD)

Strains	Diameter of inhibition zones, mm					
	S. aureus	*B. cereus*	*L. monocytogenes*	*E. coli*	*P. aeruginosa*	*S. enteritidis*
FR1	12.3±0.60[a]	-**	13.3±1.15[a]	-**	-**	11.7±0.57[a]
FR2	12.3±0.60[a]	-**	13.7±0.58[a]	-**	11±0	11.7±0.57[a]
FR3	10.3±0.33	11.3±0.58[a]	13.6±1.52[a]	11.3±0.58[a]	14.7±0.58[a]	11.7±1.52[a]
FR4	11±0	10.3±0.33	13.3±1.15[a]	10±1.00	11.7±1.52[a]	11±0
FR5	11±0	-**	13.3±1.15[a]	-**	12.0±0.33[a]	13.3±0.57[a]
FR6	12.6±1.52[a]	-**	12.3±0.60[a]	-**	10.3±0.33	13±0 [a]
FR7	11±0	-**	13.3±1.15[a]	10.7±1.15[a]	11.7±0.57[a]	11.3±1.53[a]
FR8	12.3±0.60[a]	-**	12.3±0.60[a]	11.7±0.57[a]	12.0±0.33[a]	11±0
FR9	10.3±0.33	-**	13.6±1.52[a]	-**	12.0±0.33[a]	12.7±0.57[a]
FR10	10.3±0.33	10.7±0.51[a]	13.6±1.52[a]	10.3±0.33	12.7±2.30[a]	11.7±0.57[a]
FR11	9.7±0.57	-**	14±1.73[a]	-**	12.3±0.60[a]	13.7±0.58[a]
Gentamicin	20±0 [a]	20.3±0.57[a]	25±0 [a]	15.7±0.60[a]	17.7±0.57[a]	-**
MRS	6±0[b]	6±0[b]	6±0[b]	6±0[b]	6±0[b]	6±0[b]

*Different letters in the columns denote significant differences between the inhibition zones *of L. paracasei* strains and negative control (MRS) values according to One-Way ANOVA ($p \leq 0.01$). ** no activity (6 mm diameter of the well).

et al. (2010), Bendali et al. (2011), Coman et al. (2014), Georgieva et al. (2015), Gutiérrez-Cortés et al. (2017) and Lashani et al. (2018) which found IZ up to 28 mm, that are higher that the IZ made by gentamicin in this study.

On the other hand, only 3 strains of *L. paracasei* were inhibitory against *Bacillus cereus* (*B. cereus*) with IZ of 10.3-11.3 mm. This activity was similar to the data of Lozo et al. (2004) but much lower than the results of Coman et al. (2014) and Georgieva et al. (2015) – over 20 mm IZ.

With regard to the antibacterial potential of *L. paracasei* strains against *Escherichia coli* (*E. coli*), only 5 strains inhibited the growth of this bacterium (IZ of 10.0-11.7 mm). The literature data regarding the activity of *L. paracasei* strains against *E. coli* is rather diverse. Zhang et al. (2011) and Iseppi et al. (2019) found no inhibition against *E. coli*, Mangia et al. (2019) – IZ=7 mm, Amirkhanova et al. (2021) – IZ=0-11 mm, Radulović et al. (2010) – IZ=0-25 mm, Georgieva et al. (2015) – IZ=22-23 mm, Gutiérrez-Cortés et al. (2017) – IZ=25 mm, and Wei et al. (2019) – IZ=25.93 mm. This again confirms the statement that LAB antimicrobial activity is strain-specific, rather than species-specific (Dinev et al., 2018).

All *L. paracasei* strains inhibited *Listeria monocytogenes* (*L. monocytogenes*) with large IZ of 12.3-14 mm. These strains of *L. paracasei* showed higher potential against *L. monocytogenes* than the strain studied by Mangia et al. (2019). As a whole, the results of this study coincide with the findings of Gutiérrez-Cortés et al. (2017), Iseppi et al. (2019) and Wei et al. (2019). The activity reported by Radulović et al. (2010) and Coman et al. (2014) in most cases is higher than in this experiment.

Only one strain of *L. paracasei* (FR1) was not inhibitory against *Pseudomonas aeruginosa* (*P. aeruginosa*). The other strains were active against this bacterium (IZ=10.3-14.7 mm). It should be mentioned that the activity of strain FR3 against *P. aeruginosa* (IZ=14.7 mm) was close to gentamicin (IZ=17.7 mm) which is a demonstration of good antibacterial potential. Antibacterial activity of the studied *L. paracasei* strains was consistent with the results of Lozo et al. (2004) and Radulović et al. (2010), but lower than the data reported by Coman et al. (2014).

All *L. paracasei* strains inhibited *Salmonella enterica* subsp. *enterica* serovar *enteritidis* (*S. enteritidis*) with IZ of 11-13.7 mm. This activity is within the range reported by Radulović et al. (2010) – IZ=0-26 mm. Other authors found different activities against *S. enterica* – Wei et al. (2019) reported no activity, while Coman et al. (2014) and Mangia et al. (2019) found similar potential to the one determined in the present experiment.

In conclusion, because the activity of *L. paracasei* strains usually is much lower than the antibiotic (gentamicin), this is indication that the active *L. paracasei* strains could be used as supportive therapy against sensitive bacteria along with other antimicrobials with higher antibacterial potential. As a whole, the strains examined showed high activity against *S. aureus*, *L. monocytogenes*, *P. aeruginosa* and *S. enteritidis*.

Mycotoxigenic fungi are possibly the most important pathogens of global significance in the context of food security and safety. They can decrease the quality and quantity of production, e.g. corn, rice, and peanuts, while pro-

ducing mycotoxin metabolites that could be carcinogenic in both damaged and apparently healthy products (Balendres et al., 2019). Therefore, finding LAB with antifungal activity against mycotoxigenic fungi is particularly useful and welcome because they could reduce the quantity of these micromycetes which simultaneously decrease mycotoxin contamination.

In this study, all strains of *L. paracasei* inhibited the growth of *Penicillium chrysogenum* (*P. chrysogenum*) with IZ of 7.2-12 mm (Table 2). The activity of strains FR5 and FR6, however, was very low (IZ=7.5 mm and 7.2 mm, respectively), demonstrating a lack of antifungal potential against *P. chrysogenum.* As a whole, the experimental data is within the range reported by Cosentino et al. (2018) who studied 6 strains of *L. paracasei* and found 2 strains with lack of activity against *P. chrysogenum* with IZ<3 mm (without the diameter of the culture spot), 2 strains with low activity (IZ=3-4 mm), as well as 2 strains with high activity (IZ≥8 mm).

There are 9 strains of *L. paracasei* which were inhibitory against *Aspergillus carbonarius* (*A. carbonarius*) with IZ of 8.5-10.7 mm. However, no strains of *L. paracasei* showed activity against *Aspergillus niger* (*A. niger*), *Aspergillus ochraceus* (*A. ochraceus*) and *Aspergillus parasiticus* (*A. parasiticus*). In the available literature only Roger et al. (2020) studied the antifungal activity of *L. paracasei* against *Aspergillus* spp. and found low to high activity (IZ=10-34 mm) but further comparison of this study results with comparable findings was prevented by the lack of literature data on the subject.

Only 6 strains of *L. paracasei* were active against *Fusarium oxysporum* (*F. oxysporum*) with IZ=10.8-11.5 mm. These findings are in line with the results of Cosentino et al. (2018) who studied 6 strains of *L. paracasei* and found 2 strains without activity against *P. chrysogenum* with IZ<3 mm (without the diameter of the culture spot), 2 strains with IZ of 3-4 mm, 1 strain with IZ of 5-7 mm and 1 strain with IZ≥8 mm. On the other hand, in this experiment was not found activity of *L. paracasei* strains against *Fusarium graminearum* (*F. graminearum*), whereas Hassan & Bullerman (2008) reported that *L. paracasei* strain inhibited the growth of *F. graminearum* in a liquid medium setting. However, further comparison on the antifungal activity of *L. paracasei* strains against *F. oxysporum* and *F. graminearum* was not possible because of the lack of relevant literature data.

Ultimately, *L. paracasei* strains were much more active against bacteria than fungi which is in line with the findings of Smetanková et al. (2014). However, as a whole, the activity of *L. paracasei* strains against bacteria was much lower than the positive control (gentamicin), whereas in some cases the studied *L. paracasei* strains were more active against fungi compared to the positive control (amphotericin B). The last shows their potential as biopreservatives. It is well-known that LAB exert their beneficial effects *in vitro* through production of bacteriocins, organic acids, ethanol, CO_2, H_2O_2, 3-hydroxy fatty acids, cyclic dipeptides, etc. (Dinev et al., 2020). The exact mechanism of action of *L. paracasei* strains examined in this study is a topic for future research.

Table 2. Antifungal activity of selected strains of *L. paracasei* (mean±SD)

Strains	Diameter of inhibition zones, mm						
	P. chrys.	*A. niger*	*A. carbon.*	*A. ochr.*	*A. paras.*	*F. oxysp.*	*F. gram.*
FR1	9.3±0.57	-**	9.7±0.57	-**	-**	10.8±0.50[a]	-**
FR2	12.0±0.33[a]	-**	8.5±0.25	-**	-**	11.2±0.28[a]	-**
FR3	11.3±0.58[a]	-**	10±0.5	-**	-**	-**	-**
FR4	12.0±0.33[a]	-**	-**	-**	-**	-**	-**
FR5	7.5±0.50	-**	9.7±0.57	-**	-**	11±0	-**
FR6	7.2±0.29	-**	9.3±0.57	-**	-**	11.5±0.50[a]	-**
FR7	10±1.00	-**	8.5±0.25	-**	-**	-**	-**
FR8	11.3±0.58[a]	-**	10.7±0.51[a]	-**	-**	-**	-**
FR9	11.7±0.57[a]	-**	8.5±0.25	-**	-**	11.2±0.28[a]	-**
FR10	10.7±0.51[a]	-**	-**	-**	-**	-**	-**
FR11	10.7±0.51[a]	-**	10.2±0.28[a]	-**	-**	11±0	-**
Amphotericin B	-**	9±0	13.8±0.28[a]	-**	11±0[a]	6±0	-**
MRS	6±0.05[b]	6±0	6±0[b]	6±0	6±0	6±0[b]	6±0

Note: P. *chrys. – Penicillium chrysogenum; A. niger – Aspergillus niger; A. carbon. – Aspergillus carbonarius; A. ochr. – Aspergillus ochraceus; A. paras. – Aspergillus parasiticus; F. oxys. – Fusarium oxysporum; F. gram. – Fusarium graminearum.* *Different letters in the columns denote significant differences between the inhibition zones *of L. paracasei* strains and negative control (MRS) values according to One-Way ANOVA ($p \leq 0.01$). ** no activity (6 mm diameter of the well).

Conclusions

All strains of *L. paracasei* inhibited the growth of *S. aureus, L. monocytogenes, S. enteritidis* and *P. chrysogenum,* 10 strains – *P. aeruginosa,* 9 strains – *A. carbonarius*, 6 strains – *F. oxysporum*, 5 strains – *E. coli*, and 3 strains – *B. cereus*. There are no active strains against *A. niger, A. ochraceus, A. parasiticus* and *F. graminearum.* The highest antibacterial activity was measured against *P. aeruginosa* with 14.7 mm inhibition zone of FR3 strain, while the largest zones of inhibition against fungal strains were 12 mm, determined by the activity of FR2 and FR4 strains against *P. chrysogenum*. The supernatants of *L. paracasei* strains showed higher activity against bacterial strains compared to fungal strains. Nevertheless, usually the activity of *L. paracasei* strains against bacteria was much lower than the positive control (gentamicin), whereas sometimes the studied *L. paracasei* strains were more active against fungi than the positive control (amphotericin B). In conclusion, the studied *L. paracasei* strains have good potential as supportive agents during therapy with antibiotics (or other antimicrobials), as well as biopreservatives. Their further application is a topic for future research.

Acknowledgements

This work was supported by the Bulgarian Ministry of Education and Science under the National Research Programme "Healthy Foods for a Strong Bio-Economy and Quality of Life" approved by DCM #577/17.08.2018.

References

Amirkhanova, Z., Akhmetova, S., Kozhakhmetov, S., Kushugulova, A., Bodeeva, R., Issina, Z. & Tusbayev, M. (2021). Screening of antimicrobial and adhesive activity of lactobacilli isolated from the national food products from different districts of the Karaganda region (Kazakhstan). *Open Access Macedonian Journal of Medical Sciences, 9(A),* 827-832.

Balendres, M., Karlovsky, P. & Cumagun, C. (2019). Mycotoxigenic fungi and mycotoxins in agricultural crop commodities in the Philippines: a review. *Foods*, *8,* 249.

Beev, G., Michaylova, M., Dinev, T., Naydenova, N., Tzanova, M. & Urshev, Z. (2021). ARDRA Analysis on biodiversity of lactobacilli isolated from Bulgarian raw buffalo milk. *Acta Microbiologica Bulgarica, 37(1),* 22-26.

Benavides, A., Ulcuango, M., Yépez, L. & Tenea, G. (2016). Assessment of the *in vitro* bioactive properties of lactic acid bacteria isolated from native ecological niches of Ecuador. *Revista Argentina de Microbiología, 48(3),* 236-244.

Bendali, F., Madi, N. & Sadoun, D. (2011). Beneficial effects of a strain of *Lactobacillus paracasei* subsp. *paracasei* in *Staphylococcus aureus*-induced intestinal and colonic injury. *International Journal of Infectious Diseases, 15,* e787-e794.

Chen, C.-C., Lai, C.-C., Huang, H.-L., Huang, W.-Y., Toh, H.-S., Weng, T.-C., Chuang, Y.-C., Lu, Y.-C. & Tang, H.-J. (2019). Antimicrobial activity of *Lactobacillus* species against carbapenem-resistant *Enterobacteriaceae*. *Frontiers in Microbiology, 10*, 789.

Coman, M., Verdenelli, M., Cecchini, C., Silvi, S., Orpianesi, C., Boyko, N. & Cresci, A. (2014). *In vitro* evaluation of antimicrobial activity of *Lactobacillus rhamnosus* IMC 501®, *Lactobacillus paracasei* IMC 502® and SYNBIO® against pathogens. *Journal of Applied Microbiology, 117(2),* 518-527.

Cosentino, S., Viale, S., Deplano, M., Fadda, M. & Pisano, M. (2018). Application of autochthonous *Lactobacillus* strains as biopreservatives to control fungal spoilage in Caciotta cheese. *BioMed Research International,* 3915615.

Danova, S., Petrov, K., Pavlov, P. & Petrova, P. (2005). Isolation and characterization of *Lactobacillus* strains involved in koumiss fermentation. *International Journal of Dairy Technology, 58(2),* 100-105.

Dinev, T. (2020). Antimicrobial potential of probiotic lactic acid bacteria. *Dema Press,* Rousse (Bg).

Dinev, T., Beev, G., Tzanova, M., Denev, S., Dermendzhieva, D. & Stoyanova, A. (2018). Antimicrobial activity of *Lactobacillus plantarum* against pathogenic and food spoilage microorganism: a review. *Bulgarian Journal of Veterinary Medicine, 21(3),* 253-268.

Georgieva, R., Yocheva, L., Tserovska, L., Zhelezova, G., Stefanova, N., Atanasova, A., Danguleva, A., Ivanova, G., Karapetkov, N., Rumyan, N. & Karaivanova, E. (2015). Antimicrobial activity and antibiotic susceptibility of *Lactobacillus* and *Bifidobacterium* spp. intended for use as starter and probiotic cultures. *Biotechnology & Biotechnological Equipment, 29(1),* 84-91.

Gutiérrez-Cortés, C., Suárez, H., Buitrago, G. & Díaz-Moreno, C. (2017). Isolation and evaluation of the antagonist activity of lactic acid bacteria in raw cow milk. *Agronomía Colombiana, 35(3),* 357-364.

Hassan, Y. & Bullerman, L. (2008). Antifungal activity of *Lactobacillus paracasei* subsp. *tolerans* against *Fusarium proliferatum* and *Fusarium graminearum* in a liquid culture setting. *Journal of Food Protection, 71(11),* 2213-2116.

Iseppi, R., Messi, P., Camellini, S. & Sabia, C. (2019). Bacteriocin activity of *Lactobacillus brevis* and *Lactobacillus paracasei* ssp. *paracasei*. *Journal of Medical Microbiology, 68,* 1359-1366.

Lashani, E., Davoodabadi, A. & Soltan Dallal, M. (2018). Antimicrobial effects of *Lactobacillus plantarum* and *Lactobacillus paracasei* isolated from honey against *Staphylococcus aureus*. *Journal of Babol University of Medical Sciences, 20(3),* 44-49.

Lozo, J., Vukasinovic, M., Strahinic, I. & Topisirovic, L. (2004). Characterization and antimicrobial activity of bacteriocin 217 produced by natural isolate *Lactobacillus paracasei* subsp. *paracasei* BGBUK2-16. *Journal of Food Protection, 67(12),* 2727-2734.

Mangia, N. Saliba, L. & Deiana, P. (2019). Functional and safety characterization of autochthonous *Lactobacillus paracasei* FS103 isolated from sheep cheese and its survival in sheep and

cow fermented milks during cold storage. *Annals of Microbiology, 69,* 161-170.

Mirzaei, E., Lashani, E. & Davoodabadi, A. (2018). Antimicrobial properties of lactic acid bacteria isolated from traditional yogurt and milk against *Shigella* strains. *GMS Hygiene and Infection Control, 13,* 1-5.

Naeem, M., Ilyas, M., Haider, S., Baig, S. & Saleem, M. (2012). Isolation, characterization and identification of lactic acid bacteria from fruit juices and their efficacy against antibiotics. *Pakistan Journal of Botany, 44,* 323-328.

Nemska, V., Karsheva, M.,. Danova, S. & Georgieva, N. (2021). Microbiological, physico-chemical and rheological evaluation of yoghurts, fermented with lactobacilli from traditional Bulgarian dairy products. *Journal of Chemical Technology and Metallurgy, 56(3),* 526-532.

Nemska, V., Logar, P., Rasheva, T., Sholeva, Z., Georgieva, N. & Danova, S. (2019). Functional characteristics of lactobacilli from traditional Bulgarian fermented milk. *Turkish Journal of Biology, 43,* 148-153.

Petrova, M., Georgieva, R., Dimitonova, S., Ivanovska, N., Hadjieva, N. & Danova, S. (2009a). Inhibitory activity of vaginal lactobacilli against human pathogens. *Biotechnology & Biotechnological Equipment, 23*(Suppl.1), 627-631.

Petrova, M., Georgieva, R., Dojchinovska, L., Kirilov, N., Iliev, I., Antonova, S., Hadjieva, N., Ivanova, I. & Danova, S. (2009b). Lactic acid bacteria against pathogenic microbes. *Trakia Journal of Sciences, 7(2),* 33-39.

Radulović, Z., Petrović, T., Nedović, V., Dimitrijević, S., Mirković, N., Petrušić, M. & Paunović, D. (2010). Characterization of autochthonous *Lactobacillus paracasei* strains on potential probiotic activity. *Mljekarstvo, 60(2),* 86-93, (Sr).

Roger, T., Edzili Awono, A., Youté Fanche, S. & Essia Ngang, J. (2020). Biopreservation against fungi contamination of traditional food (*Iassa*) produce by fermentation of *Hibiscus sabdariffa* seeds. *Journal of Microbiology Research, 10(3),* 71-79.

Roy, D., Ward, P., Vincent, D. & Mondou, F. (2000). Molecular identification of potentially probiotic lactobacilli. *Current Microbiology, 40(1),* 40–46.

Sirakov, I., Lutz, M., Graber, A., Mathis, A., Staykov, Y., Smits, T. & Junge, R. (2016). Potential for combined biocontrol activity against fungal fish and plant pathogens by bacterial isolates from a model aquaponic system. *Water*, *8(11),* 518.

Smetanková, J., Hladíková, Z., Zimanová, M., Greif, G. & Greifová, M. (2014). Lactobacilli isolated from lump sheep's cheeses and their antimicrobial properties. *Czech Journal of Food Sciences, 32(2),* 152-157.

Staykov, Y., Sirakov, I., Zhelyazkov, G. & Stoyanova, St. (2018). Mobile, modular type recirculation installation, for integrated and sustainable cultivation of hydrobionts and plants. *International Scientific Conference „Blue Economy and Blue Development"* 1-2 June 2018, Burgas, 217-223.

Velichkova, K., Sirakov, I., Rusenova, N., Beev, G., Denev, S., Valcheva, N. & Dinev, T. (2018). *In vitro* antimicrobial activity on *Lemna minuta*, *Chlorella vulgaris* and *Spirulina* sp. extracts. *Fresenius Environmental Bulletin, 27,* 5736-5741.

Walter, J., Tannock, G. W., Tilsala-Timisjarvi, A., Rodtong, S., Loach, D. M., Munro, K. & Alatossava, T. (2000). Detection and identification of gastrointestinal *Lactobacillus* species by using denaturing gradient gel electrophoresis and species-specific PCR primers. *Applied and Environmental Microbiology, 66(1),* 297 – 303.

Wei, X., Zhang, Y., Zhou, H., Tian, F. & Ni, Y. (2019). Antimicrobial activities and *in vitro* properties of cold-adapted *Lactobacillus* strains isolated from the intestinal tract of cold water fishes of high latitude water areas in Xinjiang, China. *BMC Microbiology, 19,* 247.

Weisburg, W. G., Barns, S. M., Pelletier, D. A. & Lane, D. J. (1991). 16S ribosomal DNA amplification for phylogenetic study. *Journal of Bacteriology, 173(2),* 697-703.

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.

Received: **July, 15, 2022**; *Approved*: **August, 18, 2022; *Published:*** **October, 2022**