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Influence of Growth Medium on Hydrogen Peroxide and Bacteriocin Production of *Lactobacillus* Strains**

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Summary

This study was conducted to investigate the inhibitory effect of bacteriocin and the production of hydrogen peroxide by four non-starter lactic acid bacteria, *Lactobacillus plantarum* 2142, *Lactobacillus curvatus* 2770, *Lactobacillus curvatus* 2775, *Lactobacillus casei* subsp. *pseudoplantarum* 2750 and the probiotic strain *Lactobacillus casei* Shirota, propagated in de Man Rogosa Sharpe (MRS) and tomato juice (TJ) broth. The methods were a commonly used agar diffusion technique and a microtiter assay method. The best peroxide-producing *Lactobacillus* strain was selected for screening the inhibitory activity against *Listeria monocytogenes*, *Bacillus cereus*, *Escherichia coli* and the activity of bacteriocins against *Lactobacillus sakei* and *Candida glabrata*. All of the investigated lactic acid bacteria (LAB) strains grown in MRS broth produced the highest concentration of hydrogen peroxide ranging from 2–6 μg/mL after 72 h of storage. *L. plantarum* 2142 produced enough hydrogen peroxide already after 24 h at 5 °C in phosphate buffer to inhibit the growth of *L. monocytogenes* and *B. cereus*. Crude bacteriocin suspension from the investigated LAB inhibited only slightly the growth of *L. sakei*, however, the same suspension from MRS completely inhibited the 6-fold diluted yeast suspension. The concentrated bacteriocin suspensions from the both broths inhibited the growth of *L. sakei* completely. Among the strains, *L. plantarum* 2142 seemed to be the best peroxide and bacteriocin producer, and the antimicrobial metabolite production was better in MRS than in TJ broth.

Key words: lactic acid bacteria, hydrogen peroxide, bacteriocin, broth

Introduction

The ability of lactic acid bacteria (LAB) to produce enough hydrogen peroxide (H_2O_2) without growing at refrigeration temperature should enable them to extend the shelf-life of some refrigerated foods without altering the acidity of the food (*1*). The synthesized H_2O_2 can inhibit the growth of psychrotrophic and pathogenic microorganisms at refrigeration temperature (*2*). *Lactobacillus delbrueckii* subsp. *lactis* have been shown to produce higher amounts of H_2O_2 at low temperatures and to inhibit pathogens during refrigerated storage. This study indicated the potential use of *Lactobacillus delbrueckii* subsp. *lactis* as a biopreservative in other refrigerated foods. Hydrogen peroxide-producing *Lactobacillus* strains, which were found to be inhibitory to *Bacillus*, *Proteus* and *Pseudomonas* species, were isolated (*3*). The bactericidal effect of H_2O_2 has been attributed to its strong oxidizing effect on the bacterial cells and to the destruction of basic molecular structures of cell proteins. Therefore it is of a gaining importance to select and propagate lactobacilli with high inhibitory activity.

Bacteriocins from LAB are proteinaceous compounds, which have inhibitory effect against closely related species and other Gram-positive bacteria. Several studies

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have been engaged in bacteriocins and their effect on Gram-positive bacteria (*4–8*). Bacteriocins form the pores in the membrane of sensitive cells and deplete the transmembrane potential and/or the pH gradient, resulting in the leakage of cellular materials (*9,10*). The inhibitory effect of bacteriocin is influenced by the phospholipid composition of the target strains and the pH of the environment (*11*). Several bacteriocins have effect against spoilage and pathogenic microorganisms, *e.g. Listeria monocytogenes*, *Clostridium botulinum* and *Bacillus cereus* (*12,13*). The bacteriocin producer strains can be used as natural food preservative starter cultures (*14–16*). Nowadays, interest has developed into biopreservatives because the customers wish to consume less artificial compounds. Natural bacteriocins may have good prospects in the preservation of food. Earlier we investigated some *Lactobacillus* strains in fermentation of vegetables (*17*) and these strains showed inhibitory effect not only against other lactobacilli, but also against certain yeasts (*18*).

The present study was conducted to determine if MRS or TJ broth are suitable media for lactobacilli to accumulate H_2O_2 and whether the produced hydrogen peroxide can inhibit the growth of undesirable organisms, such as *Listeria monocytogenes*, *Bacillus cereus* and *Escherichia coli*. Moreover, we investigated the influence of media on the effectiveness of crude and concentrated bacteriocin suspensions from several *Lactobacillus* strains.

Materials and Methods

Bacterial strains

Lactobacillus plantarum 2142, *Lactobacillus curvatus* 2770, *Lactobacillus curvatus* 2775, *Lactobacillus casei* subsp. *pseudoplantarum* 2750 and *Lactobacillus casei* Shirota were used in the study. *L. plantarum* 2142, *L. curvatus* 2770, *L. curvatus* 2775, *L. casei* subsp. *pseudoplantarum* 2750 strains (obtained from Dairy Institute of the Agricultural Faculty of Perugia) are known as non-starter lactobacilli in the cheese manufacturing. *L. casei* Shirota is a well-known probiotic strain. The strains were maintained at 5 °C in milk broth and were transferred in MRS broth (Merck, Darmstadt) using 1 % inocula; the four non-starter LAB were incubated at 30 °C and the probiotic strain at 37 °C for 24 h.

Listeria monocytogenes Ralovich Hab No. 10, *Bacillus cereus* T and *Escherichia coli* NCCAIM B 01111 were obtained from the Central Food Research Institute, Department of Microbiology. *Lactobacillus sakei*, generally used as indicator strain, was obtained from Dairy Institute of the Agricultural Faculty of Perugia and the yeast strain *Candida glabrata* CBS 138 from Microbiology and Biotechnology Department of Szent Istvan University of Budapest. A few colonies of *L. monocytogenes* from brain heart infusion (BHI) slant agar were transferred in BHI broth (Fluka Chemie, Buchs) and were incubated at 37 °C for 18 h. They were subcultured at least twice using 1 % inocula before the experiments. A few colonies of *B. cereus* and *E. coli* were subcultured in nutrient broth (Scharlau Chemie, Barcelona) from nutrient slant agar and were incubated at 37 °C for 18 h before the experiments. *L. sakei* was maintained and grown under the same conditions as the investigated *Lactobacillus* strains and the yeast was maintained on tryptone-glucose-yeast extract (TGE) agar (tryptone 0.5 %, yeast extract powder 0.25 %, dextrose 0.1 %, agar 0.9 %), and grown in TGE broth at 30 °C for 3 days.

Culture media

MRS and TJ broth (Fluka Chemie, Buchs) media were prepared according to the manufacturer's instruction. After autoclaving, the TJ broth was filtered through a 0.22-μm pore size filter to avoid the slurry caused by autoclaving. The slurry remained at the bottom of the bottle and to keep it sterile, we used sterile syringes and the filter. When TJ broth clogged the system, the filter was changed.

Hydrogen peroxide production

Hydrogen peroxide production by the four LAB strains grown in MRS and TJ broth was determined in sodium phosphate buffer. Each of the media was inoculated with a suspension of lactobacilli and incubated for 24 h at 30 or 37 °C, depending on the strains. The cells were then harvested by centrifugation at 6000 × *g* at 4 °C for 15 min. The cells were washed twice with 10 mL of cold, sterile phosphate buffer solution (1 M, pH=6.5). A mass of 1 g of the cell pellet was resuspended in 10 mL of cold, sterile sodium phosphate buffer (1 M, pH=6.5) and then for the preparation of the final buffer solutions, the initial dilution was further diluted (1:10) in phosphate buffer. In the final buffer solutions approx. 10^8 CFU/mL of *Lactobacillus* population were present. The cells were then incubated at 5 °C for a period of 3 days. After 0, 2, 24, 48 and 72 h of incubation, the cells were removed by centrifugation at 6000 × *g* at 4 °C for 15 min and the supernatants were assayed for hydrogen peroxide according to the methods of Villegas and Gilliland (*19*). The supernatant was also used for evaluation of inhibitory activity against pathogens by agar diffusion method. Each value presented here represents the mean value of two determinations.

Production of crude bacteriocin suspensions

For the production of bacteriocin suspension LAB strains were grown in the two broths at 30 °C for 24 h. Supernatants were harvested by centrifugation at 6000 × *g* at 5 °C for 20 min, adjusted to pH=6.5, filtered through a 0.22-μm pore size filter and heat-treated at 80 °C for 10 min to inactivate proteases and kill the bacteria that might be present in the filtrates. We had observed earlier that the bacteriocin suspension did not lose its inhibitory effect after the heat treatment and other researchers also came to the same conclusion that the most bacteriocins from lactobacilli are heat stable (*20*).

Production of concentrated bacteriocin suspension

Crude bacteriocin suspensions were made and then freeze-dried and redissolved in sterile water to give three-fold concentration in comparison with crude bacteriocin suspension.

Agar diffusion method

To test whether the concentration of hydrogen peroxide and bacteriocin produced by LAB can inhibit the

growth of test microorganism, wells were made in the nutrient agar plate. The wells contained 450 μL (in three portions of 150 μL) of the culture supernatant with different concentrations of hydrogen peroxide, the crude and concentrated bacteriocin suspension and the plates were incubated at 50 °C for 30 min to accelerate the diffusion of the solutions into the agar. A volume of 7 mL of soft nutrient, BHI, MRS or TGE agar (agar 0.7 %) containing the test microorganism (700 μL added from the appropriately diluted bacterial suspension to soft agar and cooled down at 40 °C to obtain the bacterial concentration of 10^4 CFU/mL) was poured onto the agar plate. The plates were incubated at 30 or 37 °C for 18 h. The width of the inhibition zones was expressed in mm as difference in radius.

Microtiter assay method

In the microplate assay, 125 μL of the crude or concentrated bacteriocin in MRS or TGE broth (125 μL) were prepared in a 96-well microtiter plate, respectively. A volume of 25 μL of overnight culture of the indicator strains appropriately diluted (about 10^5 CFU/mL) was added to each well or the same quantity of water to the control. We used as control of cell growth the appropriate broth (MRS, TGE) with sterile, distilled water instead of bacteriocin solution, and it was inoculated with the test organisms, which showed the growth of *L. sakei* or *C. glabrata* when nothing influenced their growth (Control 1). We used broth-control: 125 μL of broth (usual or three-fold concentrated MRS and TJ broth) were added to 125-μL media of test organisms and it was inoculated with 25 μL of diluted test microorganisms. Thus we obtained the effect of different broths on test organisms and in this way we could predict the possibility of inhibitory effect of broths (mainly the concentrated broths) (Control 2). The plates were incubated at 30 °C for 24 or 40 h and the absorbance, beginning the 12th hour of incubation, was measured at 630 nm using a microtiter plate reader (Dynatech MR7000, Dynatech Laboratories Inc., Chantilly, USA). The absorbance was measured every hour. The absorbance of wells containing only broth and bacteriocin suspension without the indicator strain was subtracted from the absorbance of the wells that contained the indicator strain. Thus we obtained the absorbance of the cells. This absorbance is in direct proportion with the amount of cells, within certain limits.

Results and Discussion

Kinetics of hydrogen peroxide production

The difference was not significant ($p>0.05$) in the number of lactobacilli among the four strains. There was no increase in the number of lactic acid bacteria in the buffers during the 3-day storage period (data not shown). Hydrogen peroxide production by LAB cells suspended in phosphate buffer after the growth in different media is shown in Figs. 1 and 2. As Fig. 1 indicates MRS broth was better medium for propagation of lactic acid bacteria to produce hydrogen peroxide than TJ broth. We found a linear time-course induction of hydrogen peroxide in the case of all four lactobacilli strains. After 2 h of storage in phosphate buffer we detected the same concentration of hydrogen peroxide compared to the control bacteria cells (0 h). A linear increase could be observed after 24 h of storage. *L. plantarum* 2142 produced the highest concentration of peroxide. It reached the maximum level after 72 h of storage (5.45 μg/mL). *L. casei* Shirota seemed to be the worst peroxide producer (1.92 μg/mL) in MRS medium.

We found that the four LAB strains accumulated less hydrogen peroxide in the TJ broth compared to the MRS broth. A slight increase was observed in the peroxide production by lactobacilli prior to propagation in TJ broth (Fig. 2). During the whole storage period far lower levels of hydrogen peroxide could be detected. Although *L. plantarum* 2142 propagated in MRS broth accumulated the highest level of hydrogen peroxide, when propagated in TJ broth it synthesized only 0.74 μg/mL of H_2O_2 after 72 h of storage. *L. casei* subsp. *pseudoplantarum* 2750 grown in TJ broth seemed to be the best peroxide producer but the concentration of hydrogen peroxide was less than 2 μg/mL at the end of the storage period, although the same peroxide level produced by this strain grown in MRS broth could be detected already after 24 h. *L. casei* Shirota with probiotic properties grown in TJ broth was again not effective in the accumulation of peroxide.

Li *et al.* (*21*) found that the percentage of apoptotic cells induced with 400 μmol/L of hydrogen peroxide increased significantly 1 or 3 h after the stimulation and recovered rapidly, while the percentage of apoptotic cells induced with 4 mmol/L of hydrogen peroxide increased with time. In accordance with these changes observed mitochondrial function decreased in 400 μmol/L of H_2O_2-stimulated cells and in 4 mmol/L of H_2O_2-stimulated cells in 1 or 3 h. Swelling cristae and vacuole-like mitochondria were noted. These levels are twice higher than our measurements in the case of all four strains.

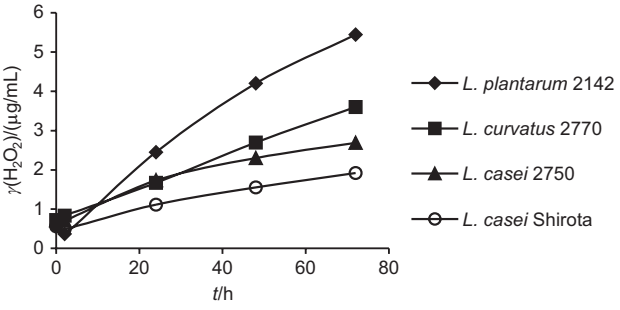

Fig. 1. Hydrogen peroxide production of lactobacilli propagated in MRS broth and suspended in phosphate buffer at 5 °C

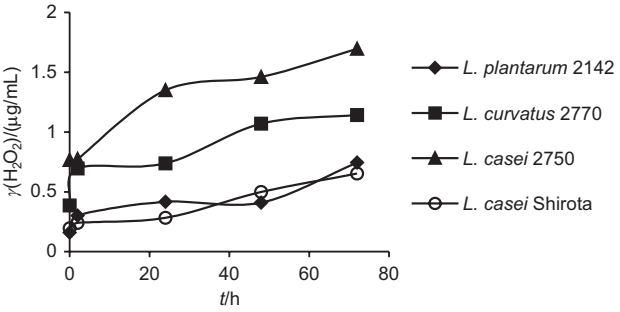

Fig. 2. Hydrogen peroxide production of lactobacilli propagated in TJ broth and suspended in phosphate buffer at 5 °C

Inhibitory activity

Actively growing lactic acid bacteria cells have been used in most works against undesirable microorganisms. The use of such methods would not be acceptable in many foods due to the increased acidity caused by the growing cultures. This suggests the use of non-growing cells of lactobacilli in some food products (*22*).

Some lactic acid bacteria produce sufficient amounts of hydrogen peroxide to inhibit many undesirable microorganisms without growing. Jaroni and Brashears (*23*) reported that suspension of 10^7 *L. delbrueckii* subsp. *lactis* I cells propagated in MRS medium showed the highest accumulation of H_2O_2 (7.5 μg/10^7 CFU) in phosphate buffer containing no glucose during the entire 5-day storage period. Ito *et al.* (*24*) showed that the cell-free filtrate of *L. casei lactis* subsp. *lactis* AI 62 cells containing 300–380 ppm of hydrogen peroxide had a strong bactericidal effect against psychrotropic food-borne pathogens such as *Listeria*, *Yersinia* and *Aeromonas* species and mesophiles such as *E. coli*.

As *L. plantarum* 2142 propagated in MRS broth produced the highest concentration of hydrogen peroxide from the four lactobacillus strains, we chose this strain to further investigate the inhibitory effect of the produced peroxide against the food spoilage bacteria using the agar diffusion method.

We tested the effect of different hydrogen peroxide concentrations produced by *L. plantarum* 2142 in phosphate buffer after 0, 2, 24, 48 and 72 h of storage at 5 °C against *L. monocytogenes*, *B. cereus* and *E. coli*, and measured the width of the inhibition zones.

As Table 1 and Figs. 3 and 4 indicate, the growth of *L. monocytogenes* and *B. cereus* was influenced by *L. plantarum* 2142. The growth of *L. monocytogenes* was inhibited by the hydrogen peroxide accumulated by *L. plantarum* 2142, especially after 24 and 48 h of storage in phosphate buffer. These seemed to be the ideal storage times for lactobacilli to inhibit the growth of *L. monocytogenes*, although *L. plantarum* 2142 accumulated the highest concentration of hydrogen peroxide after 72 h. The hydrogen peroxide present in the buffer after 48 and 72 h also inhibited the growth of *B. cereus*. We did not observe any inhibition zones in the case of *E. coli.*

Table 1. Inhibitory activity of *L. plantarum* 2142 caused by the produced hydrogen peroxide

Target bacteria	Incubation period of *L. plantarum* cells in phosphate buffer at 5 °C				
	0 h	2 h	24 h	48 h	72 h
L. monocytogenes	±	±	+	+	±
B. cereus	–	±	±	+	+
E. coli	–	–	–	–	–

+ strong inhibition zone (2 mm), ± weak inhibition zone (0–2 mm), – no inhibition zone

Effect of bacteriocin

Table 2 shows the effect of crude bacteriocin suspensions (CBS) from MRS broth. Out of the three investigated strains, the bacteriocin from *L. plantarum* 2142 and *L. curvatus* 2775 strains showed inhibition. Also the CBS of these two strains from TJ broth had inhibitory effect, but the effect of strain *L. curvatus* 2775 was greater (Table 2). The broths did not inhibit the multiplication of *L. sakei*. The inhibitory effects of bacteriocin suspension of *L. plantarum* 2142 from the two broths were equivalent and significant. The inhibitory effects of bacteriocin suspension of *L. curvatus* 2775 in the investigated broths were different, but the growth of test organism was lower in comparison with Control 1. The CBS of *L. curvatus* 2775 from TJ broth showed significant inhibition. Only the CBS of strain *Lactobacillus casei* subsp. *pseudoplantarum* 2750 from MRS had inhibitory effect, but it was smaller than the effect of bacteriocin from the other strains.

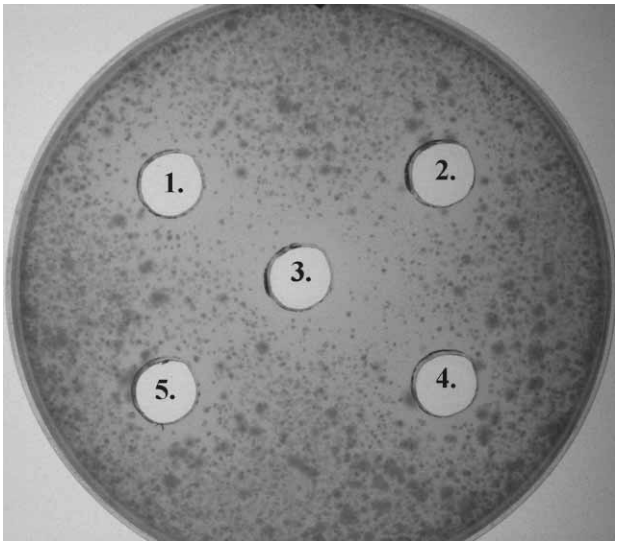

Fig. 3. Inhibition of *Listeria monocytogenes* growth by hydrogen peroxide produced by *L. plantarum* 2142
1. $\gamma(H_2O_2)/(\mu g/mL)=0.55$; **2.** $\gamma(H_2O_2)/(\mu g/mL)=0.37$; **3.** $\gamma(H_2O_2)/(\mu g/mL)=2.45$; **4.** $\gamma(H_2O_2)/(\mu g/mL)=4.20$; **5.** $\gamma(H_2O_2)/(\mu g/mL)=5.45$

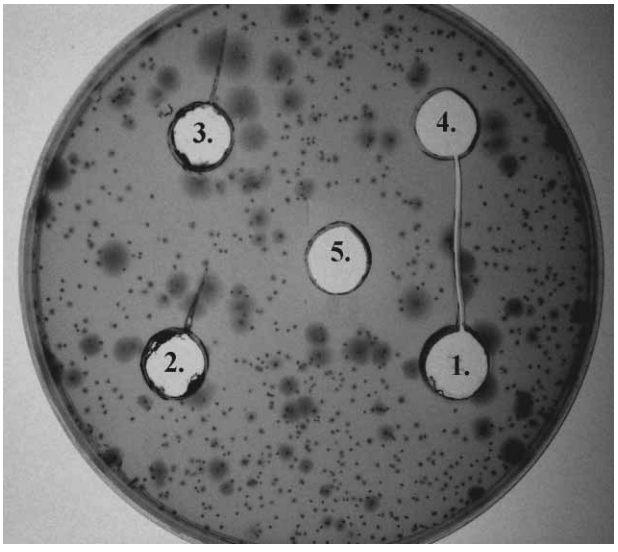

Fig. 4. Inhibition of *Bacillus cereus* growth by hydrogen peroxide produced by *L. plantarum* 2142
1. $\gamma(H_2O_2)/(\mu g/mL)=0.55$; **2.** $\gamma(H_2O_2)/(\mu g/mL)=0.37$; **3.** $\gamma(H_2O_2)/(\mu g/mL)=2.45$; **4.** $\gamma(H_2O_2)/(\mu g/mL)=4.20$; **5.** $\gamma(H_2O_2)/(\mu g/mL)=5.45$

Table 2. Inhibitory effect of crude bacteriocin suspension of the three investigated strains from MRS and TJ broth on *L. sakei* (*N*=3)

	A_{630nm}				
	Mean value	Standard deviation		Mean value	Standard deviation
Control 1	0.7723	0.0607	Control 1	0.7723	0.0607
Control 2	1.0070	0.0607	Control 2	0.8397	0.0672
MRS-2142	0.6643*	0.0321	TJ broth-2142	0.6833	0.0513
MRS-2750	0.8690*	0.0387	TJ broth-2750	0.9940*	0.0979
MRS-2775	0.6993*	0.0528	TJ broth-2775	0.5193*	0.1881

Control 1 – without inhibitory component, control 2 – investigated broth without bacteriocin, MRS/TJ broth-2142, 2750 or 2775 – crude bacteriocin suspension of investigated strains (marked by strain number) from MRS/TJ broth
*significantly different from control 1 group ($p<0.05$)

When we investigated the effect of concentrated bacteriocin suspension (ccBacter) from the both broths it was found that it inhibited the growth of *L. sakei* completely, but the growth of test organism was also retarded by the concentrated MRS (Table 3).

Table 3. Inhibitory effect of 3-fold concentrated bacteriocin suspension from strain *L. plantarum* 2142 on *L. sakei* (*N*=3)

	A_{630nm}	
	Mean value	Standard deviation
Control 1	0.92967	0.10372
Control 2 MRS	–0.00333*	0.00416
Control 2 TJ broth	0.65967*	0.00802
ccBacter MRS	0.00533*	0.00751
ccBacter TJ broth	0.01700*	0.00100

Control 1 – without inhibitory component, control 2 – investigated, concentrated broth without bacteriocin, ccBacter – concentrated bacteriocin suspensions of 2142 strain from the investigated broths
*significantly different from control 1 group ($p<0.05$)

Table 4 shows the effect of CBS from *L. plantarum* 2142 strain against *Candida glabrata.* The CBS from MRS had greater inhibitory effect, but the yeast grew less in the wells that contained also MRS. There was a significant difference between the TJ broth and the CBS from TJ broth, but the CBS from the TJ broth did not have such inhibitory effect as that from MRS. The crude bacteriocin solution from MRS inhibited completely the 6-fold diluted yeast suspension.

Table 4. Inhibitory effect of crude bacteriocin suspensions of strain *L. plantarum* 2142 on *C. glabrata* (*N*=3)

	A_{630nm}	
	Mean value	Standard deviation
Control 1	0.08000	0.01249
Control 2 MRS	0.23600*	0.07113
Control 2 TJ broth	0.54733*	0.14692
Bacteriocin MRS	0.19967*	0.04822
Bacteriocin TJ broth	0.39833*	0.04557

Control 1 – without inhibitory component, control 2 – investigated broth without bacteriocin, bacteriocin – crude bacteriocin suspensions of *L. plantarum* 2142 strain from the investigated broths

Fig. 5 shows the inhibitory effect of concentrated bacteriocin suspension of *L. plantarum* 2142 strain from MRS (a) and TJ broth (b). The concentrated MRS broth

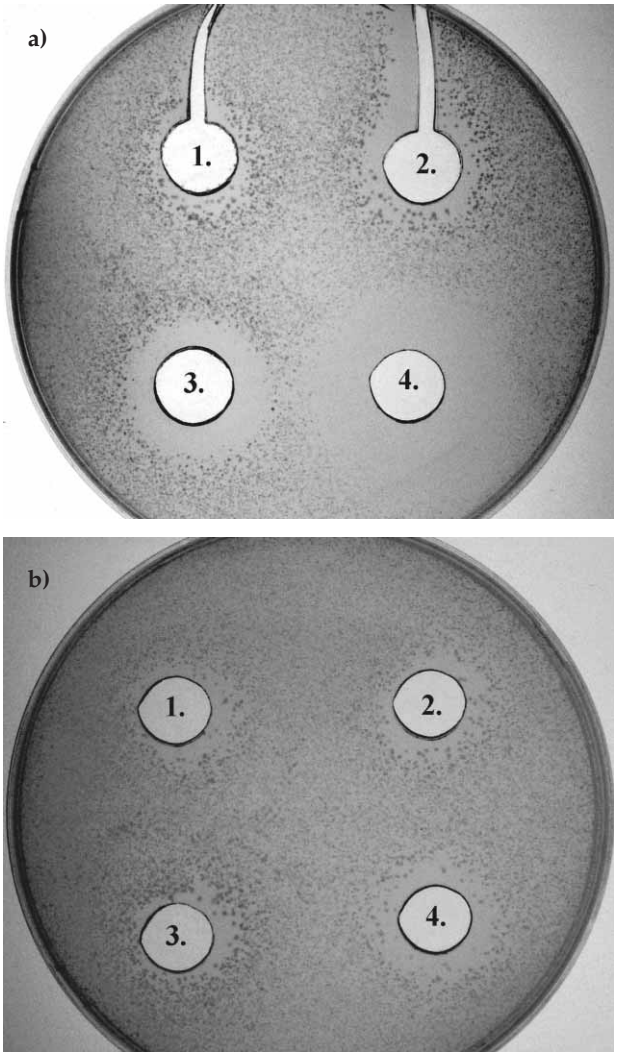

Fig. 5. Inhibitory effects of concentrated bacteriocin suspensions from *L. plantarum* 2142 against *C. glabrata*
a) from MRS broth: **1.** MRS broth, **2.** crude bacteriocin suspension from MRS broth, **3.** concentrated MRS broth, **4.** concentrated bacteriocin suspension from MRS broth
b) from TJ broth: **1.** TJ broth, **2.** crude bacteriocin suspension from TJ broth, **3.** concentrated TJ broth, **4.** concentrated bacteriocin suspension from TJ broth

also showed inhibition that might be explained by the glucose repression, but the bacteriocin suspension had significantly greater effect.

Conclusion

Until now no data has been available about the ability of non-starter lactic acid bacteria and probiotic bacteria grown in MRS or TJ broth to produce hydrogen peroxide in phosphate buffer at 5 °C. Furthermore, we also investigated the influence of the above mentioned growth media on the crude and concentrated bacteriocin suspension from the investigated LAB strains.

The present study indicated the suitability of MRS broth for growing lactic acid bacteria other than *L. delbrueckii* subsp. *lactis.* All of the investigated LAB strains grown in MRS broth produced the highest concentration of hydrogen peroxide ranging from 2–6 μg/mL in phosphate buffer at 5 °C after 72 h of storage. Among the strains, *L. plantarum* 2142 seemed to be the best peroxide producer.

L. plantarum 2142 produced enough hydrogen peroxide already after 24 h (2.45 μg/mL) at 5 °C in phosphate buffer to inhibit the growth of *L. monocytogenes* and *B. cereus*. Although the amount of the produced peroxide was efficient against the above mentioned psychrotropic bacteria, we had to optimize the growth conditions for lactobacilli because too high level of H_2O_2 can cause an oxidative stress in the intestinal epithelial cells, which can lead to the mutation of mitochondrial genes (*25*).

We can conclude that *L. plantarum* 2142 propagated in MRS and stored in phosphate buffer at 5 °C during 3 days produced enough hydrogen peroxide to inhibit the growth of *B. cereus* and *L. monocytogens*, which is an optimal concentration because it does not cause any mutation in mitochondrial genes.

Results showed that the bacteriocin suspensions of all *Lactobacillus* strains grown in MRS broth had the best inhibitory effect on both *L. sakei* and *C. glabrata*. The *L. plantarum* 2142 strain showed the best inhibitory effect of the investigated *Lactobacillus* strains. The concentrated bacteriocin suspensions showed great, complete inhibition against both test strains in all cases. The effectiveness of bacteriocins was influenced considerably by growth medium. The best inhibitory effect of hydrogen peroxide and bacteriocins from MRS might be explained by the fact that this medium is rich in organic compounds, peptides and salts that can be utilized by lactobacilli. The bacteriocins of *L. plantarum* 2142 strain from MRS showed good inhibitory effect, so after further investigation it can be used as starter culture and its bacteriocins as biopreservative against spoilage yeast and other lactic acid bacteria strains in the lacto-fermentation of vegetables.

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Utjecaj podloge na proizvodnju vodikova peroksida i bakteriocina u sojevima *Lactobacillus*

Sažetak

Ispitana je proizvodnja vodikova peroksida i inhibicijsko djelovanje bakteriocina sa četiri vrste bakterija mliječne kiseline, *Lactobacillus plantarum* 2142, *Lactobacillus curvatus* 2770, *Lactobacillus curvatus* 2775, *Lactobacillus casei* subs. *pseudoplantarum* 2750 i u probiotskom soju *Lactobacillus casei* Shirota, uzgojenih na podlozi de Man Rogosa Sharpe (MRS) i podlozi od soka rajčice (engl. tomato juice – TJ). Pri ispitivanju se koristio uobičajeni agar-difuzijski postupak i mikrotitarske ploče. Soj laktobacila koji je proizvodio najviše peroksida odabran je za ispitivanje inhibicijskog djelovanja na sojeve *Listeria monocytogenes*, *Bacillus cereus*, *Escherichia coli* te djelovanje bakteriocina na *Lactobacillus sakei* i *Candida glabrata*. Svi ispitani sojevi bakterija mliječne kiseline (engl. lactic acid bacteria – LAB), uzgojeni na MRS-podlozi, proizveli su najviše vodikova superoksida (2–6 μg/mL nakon 72 sata). Da bi inhibirao rast *L. monocytogenes* i *B. cereus, L. plantarum* 2142 proizveo je dovoljno vodikova superoksida već nakon 24 sata pri 5 °C u fosfatnom puferu. Nepročišćena suspenzija bakteriocina dobivena od bakterija mliječne kiseline općenito je slabo inhibirala rast *L. sakei*, dok je suspenzija iz MRS-podloge potpuno inhibirala 6 puta razrijeđenu suspenziju kvasca. Koncentrirane suspenzije bakteriocina iz obiju podloga potpuno su inhibirale rast *L. sakei*. Čini se da je soj *L. plantarum* 2142 najbolji proizvođač peroksida i bakteriocina, a MRS-podloga je bila bolja od TJ podloge.