Pol. J. Environ. Stud. Vol. 21, No. 5 (2012), 1399-1403

Original Research

Antagonistic Effect of Lactic Acid Bacteria on *Salmonella* Senftenberg in Mixed Cultures

Beata Szala[1]*, Zbigniew Paluszak[1], Ilona Motyl[2]

[1]Department of Microbiology, University of Technology and Life Sciences,
Bernardyńska 6/8, 85-029 Bydgoszcz, Poland
[2]Institute of Fermentation Technology and Microbiology, Technical University of Lodz, Poland

Received: 8 April 2011
Accepted: 20 April 2012

Abstract

Our study aimed to indicate the possibility of the application of 5 strains of *Lactobacillus plantarum* and 1 strain of *Lactobacillus brevis* isolated from plant material for reducing the count of *Salmonella* Senftenberg *W775* in mixed culture. All the tested strains completely inactivated growth of *Salmonella* Senftenberg rods during 48 h of common culture. The strongest antagonistic activity was shown by strain Nos. 11, 13, and 15, which after 24 h of co-culture totally eliminated the pathogen cells. No effect of the pathogen on the growth dynamics of lactic acid bacteria was observed.

Keywords: *Lactobacillus*, *Salmonella*, antagonism, mixed cultures

Introduction

Essential applications of lactic acid bacteria include not only probiotic food, but also environmental protection against pathogens [1-3]. Antagonistic properties of these bacteria can be used in various technologies. They aim to eliminate dangerous pathogens occurring in natural fertilizers of animal origin or sewage sludge that often contain a large load of pathogenic bacteria [4, 5]. Lactic acid bacteria play an essential role in food production, contributing to the inhibition of pathogenic bacteria growth. They may also slow down the process of the decomposition of organic matter in food [6-9].

Antagonistic activity of lactic acid bacteria is connected with the inhibiting effect of compounds they produce. Metabolites with inhibiting properties include lactic acid, hydrogen peroxide, and bacteriocins. The activity of lactic bacteria also is based on lowering the pH value. These mechanisms may result in a decrease of survival time of pathogen bacteria during, for example, the hygienization process of organic waste. Growing attention has been devoted to bacteriocins, which play a considerable role in regulation of pathogenic microorganism numbers in the environment and food [10]. The aim of this study was to estimate the mutual effect of 6 strains of lactic fermentation bacteria of the genus *Lactobacillus* isolated from plant material and *Salmonella* Senftenberg *W775* under conditions of pure and mixed culture.

Material and Methods

For our experiment we used 5 strains of *Lactobacillus plantarum* (No. 6,11,12,13,15) and 1 strain of *Lactobacillus brevis* (No. 8) isolated from plant material (sauerkraut) subjected to the process of fermentation. All of them were G-positive, catalase-negative rods. The strains were subjected to identification using API 50 CHL test (program APIWEB V5.1, API System, Bio Mèrieux) in the Institute of Fermentation Technology and Microbiology of the Technical University of Łódź. The tested strain *Salmonella* Senftenberg *W775* came from the

*e-mail: szabea@utp.edu.pl

Table 1. The number of *Salmonella* Senftenberg in monoculture and mixed cultures (mix 6, 8, 15) during 48h incubaction (cfu·ml^{-1}).

Condition of incubation	Incubation time (h)						
	0	8	16	24	32	40	48
Mix 8	3.45×10^{8}a	4.1×10^{7}a	9.5×10^{5}a	4.16×10^{4}a	nd a	nd a	nd a
Mix 6	2.99×10^{8}a	2.9×10^{7}a	3.0×10^{7}ab	1.46×10^{5}a	nd a	nd a	nd a
Mix 15	1.72×10^{8}a	2.33×10^{7}a	3.14×10^{6}ac	nd b	nd a	nd a	nd a
monoculture	5.5×10^{8}a	3.2×10^{8}b	2.39×10^{8}d	4.0×10^{8}c	2.4×10^{8}b	4.2×10^{7}b	3.17×10^{7}b

a, b, c, d – statistically significant differences between groups, p≤0.05; nd – no detected

collection of microorganisms of Hohenheim University in Stuttgart. Examined microorganisms were inoculated on LAPTg liquid medium [11] and incubated for 24 h (*Salmonella*) and 48 h (*Lactobacillus*) at 37ºC to obtain monocultures. In order to estimate the antagonistic effects of lactic acid bacteria on the strain *Salmonella* Senftenberg, mixed cultures of a volume of 20 ml were prepared. To obtain these cultures, 10 ml of the monoculture of one of lactic acid bacteria isolates and 10 ml of the monoculture of the pathogen were transferred to sterile flasks. Mixed cultures obtained were marked as mix 6, mix 8, mix 15, mix 11, mix 12, and mix 13, and incubated at 37ºC. Determination of antagonistic properties of the tested isolates toward the pathogen was conducted in two steps. Strains L6, L8, and L15 were tested in the first one, whereas in the second, L11, L12, and L13 in mixed cultures with *Salmonella* Senftenberg for 48 h incubation, making microbiological inoculations every 8h. To determine changes in the number of microorganisms in monocultures and mixed cultures, a series of 10-fold dilutions was prepared. Then 0.1 ml was transferred from each dilution onto the medium Rogoza Agar (Merck No. 5431) [12] for *Lactobacillus* rods and on the selective medium McConkey Agar (Merck No. 5465) [13] for *Salmonella*. Bacteria were incubated at 37ºC, for 24 h (*Salmonella*) and 48 h (*Lactobacillus*). Statistical calculations were carried out using the Statistica program. The results obtained were subjected to the analysis of variance and the significance of differences between the count of the tested microorganisms was determined at p=0.05.

Results

Statistical analysis of the results of the study presented in Table 1 proved significant differences between concentrations of *Salmonella* Senftenberg in mixed culture with lactic acid bacteria (mix 6, 8, 15), and monoculture of the pathogen already after 8 h of co-incubation. Statistical calculations indicate that mix 15 showed significantly lower values relative to the monoculture of enteric rods during 48 h mixed culture as compared with mix 6 and mix 8. Isolate No. 15 totally inhibited the growth of enteric bacteria cells after 24 h of the co-incubation. During two-day culture of *Salmonella* Senftenberg and *Lactobacillus plantarum* strains No. 6 and 15, and *L. brevis* No. 8, total inactivation of the pathogen was observed in all the tested mixed cultures (Table 1, Fig. 1). The largest decrease in the count of *Salmonella* Senftenberg rods in mixed culture No. 15 was observed between the 16th and 24th h of incubation, when the concentration of pathogen cells fell rapidly from values of 6 $\log_{10}$ to no detection level. *Lactobacillus* in mixed cultures No. 6 and 8, in turn, inactivated enteric rods after 32 h of co-culture. During the 48 h incubation of the monoculture of *Salmonella* Senftenberg, a high survival rate of those bacteria was observed. Pathogen cell concentrations in monoculture remained at the constant level of the order of 10^7-10^8 cfu·ml^{-1} throughout the experiment (Table 1, Fig. 1).

Significant differences in the pathogen count in mixed culture No. 11 related to the control occurred after 8 h incubation. Based on the statistical analysis, it was found that mixed cultures No. 12 and 13 exhibited considerably lower significant values in pathogen population count than the monoculture after 16 h incubation. Moreover, the results of the study show significant differences in concentration of pathogens between mixed cultures No. 11, 13, and 12 after 16 and 24 h of the experiment (Table 2, Fig. 2). All the tested strains had an antagonistic effect on bacteria of the genus *Salmonella*. Strains Nos. 11 and 13 totally inhibited the growth of pathogens between the 16th and 24th h of co-culture, when from a value of 5 $\log_{10}$ both isolates led to the total inactivation of *Salmonella*. In the case of the strain *Lactobacillus* in mixed culture No. 12, a large decrease in pathogen count was observed in the 32nd h of incubation (from the value 1.53×10^7 cfu·ml^{-1} to 1.0×10^2 cfu·ml^{-1}). A decrease in enteric bacteria count in monoculture from 9

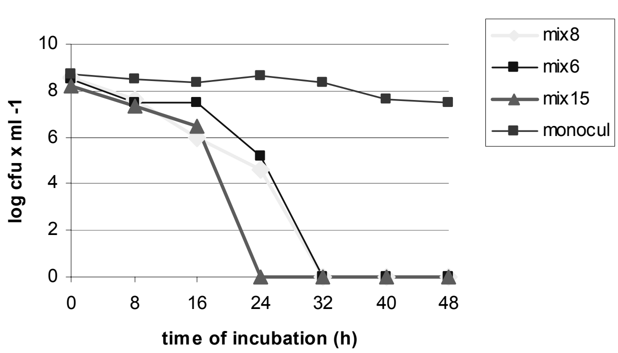

Fig. 1. Kinetics of *Salmonella* growth in monoculture (monocul) and mixed cultures (mix 6, 8, 15) with *Lactobacillus* strains during 48 h incubation.

Table 2. The number of *Salmonella* Senftenberg in monoculture and mixed cultures (mix 11, 12, 13) during 48 h incubation (cfu·ml^{-1}).

Conditions of incubation	Incubation time (h)						
	0	8	16	24	32	40	48
Mix 11	3.19×10^{8}a	2.43×10^{8}a	4.9×10^{5}a	nd a	nd a	nd a	nd a
Mix 12	5.03×10^{8}a	2.2×10^{8}ab	1.69×10^{7}b	1.53×10^{7}b	1.0×10^{2}a	nd a	nd a
Mix 13	1.73×10^{8}a	3.6×10^{8}ab	1.01×10^{5}a	nd b	nd a	nd a	nd a
monoculture	9.7×10^{8}a	1.0×10^{9}b	1.6×10^{8}c	2.6×10^{8}c	1.4×10^{8}b	6.0×10^{7}b	1.7×10^{5}b

a, b, c – statistically significant differences between groups, $p\leq0.05$; nd – not detected

$\log_{10}$ to 5 $\log_{10}$ during 48-hour incubation was also observed (Table 2, Fig. 2).

The study also indicated a high survival rate of the tested strains of *Lactobacillus* rods both in mixed culture and monoculture during 48 h of incubation. During co-culture of *Salmonella* Senftenberg and the tested strains of *Lactobacilllus,* no changes exceeding 1 $\log_{10}$ were observed in the population count of antagonistic bacteria. Concentrations of those microorganisms in mixed cultures remained constantly at a level of 10^{7}-10^{8} cfu·ml^{-1} (Table 3).

Discussion

Lactic acid bacteria have a broad spectrum of antagonistic activity. They have an inhibiting effect on the growth of both gram-positive and gram-negative bacteria, mainly through producing metabolites such as lactic acid, bacteriocins, and hydrogen peroxide [14, 15]. The present study aimed to indicate a possibility of the application of lactic acid bacteria for reducing the count of *Salmonella* Senftenberg *W775* in mixed culture. During two days of co-culture, the strongest antagonistic activity was shown by strain Nos. 11, 13, and 15, which led to the total inactivation of pathogen in mixed culture after 24 h. Strains 6 and 8, which inactivated the growth of enteric rods after 32 h of co-incubation, exhibited a weaker inhibition effect toward the pathogen. Strain 12 appeared to the isolate with the weakest antagonistic properties. The total inactivation of *Salmonella* Senftenberg was observed after 40 h of co-culture.

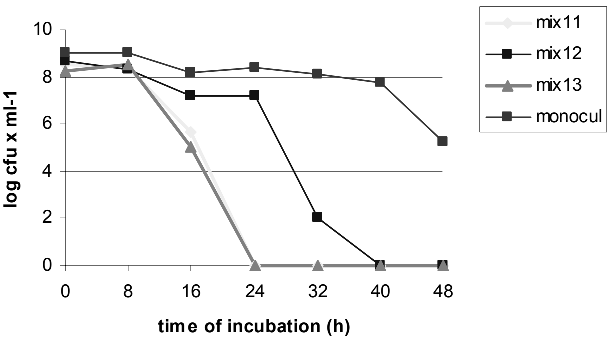

Fig. 2. Kinetics of *Salmonella* growth in monoculture (monocul) and mixed cultures (mix 11, 12, 13) with *Lactobacillus* strains during 48 h incubation.

Table 3. The number of *Lactobacillus* in monocultures and mixed cultures with *Salmonella* (mix 6, 8, 15, 11, 12, 13) during 48 h incubation (cfu·ml^{-1}).

Lactobacillus strain	Incubation time (h)		
	0	24	48
Monoculture (Lp6)	2.06×10^{7}a	1.06×10^{8}a	4.2×10^{7}a
Mix 6	1.51×10^{7}a	1.96×10^{8}a	1.5×10^{7}a
Monoculture (Lb8)	8.56×10^{7}a	4.66×10^{8}a	1.9×10^{8}a
Mix 8	4.36×10^{7}a	3.7×10^{8}a	1.53×10^{8}a
Monoculture (Lp15)	8.5×10^{8}a	1.2×10^{8}a	9.3×10^{7}a
Mix 15	1.14×10^{8}a	1.38×10^{8}a	1.7×10^{7}a
Monoculture (Lp11)	2.36×10^{8}a	1.43×10^{8}a	2.7×10^{7}a
Mix 11	7.8×10^{7}a	1.33×10^{8}a	1.2×10^{8}a
Monoculture (Lp12)	1.7×10^{7}a	1.01×10^{7}a	5.7×10^{7}a
Mix 12	5.3×10^{7}a	9.0×10^{6}b	6.3×10^{7}a
Monoculture (Lp13)	3.6×10^{8}a	1.86×10^{8}a	1.03×10^{8}a
Mix 13	2.03×10^{8}a	1.1×10^{8}a	1.12×10^{7}a

a – statistically significant differences between groups, $p\leq0.05$

Similar results were obtained by Bauza-Kaszewska [16], where significant differences between monoculture of *Salmonella* Senftenberg and the most effective strains of lactic acid bacteria were noted after 6 h of co-culture. The study by Bielecka [17] indicated a considerable decrease in the concentration of *Salmonella enteritidis* in mixed cultures with a strain of lactic acid bacteria of the genus *Bifidobacterium* animals between the 12th and 16th h of their co-culture.

Inactivation rate of the tested Gram-negative bacteria in co-culture with LAB depends among other things on selection of the strain of lactic acid bacteria [18]. In the study by Bielecka [17] the total inactivation of cells of the tested pathogen under the influence of *Bifidobacterium* animals was observed after 48 h. Some strains of *Bifidobacterium* sp. showed a lower antagonistic activity toward the pathogen, and its amount in mixed cultures with less active isolates did not fall even after 72 h of the co-culture. In contrast, in the study of the effect of *Lactobacillus lactis* on

Salmonella tiphymurium and *S. enteritidis*, Brashears and Durre [19] and Drago [20] observed the total inactivation of the pathogens after 24 h. Guslis [21], in turn, observed a dynamic decrease in the amount of *S. gallinarum*, *S. enteritidis*, *S. tiphymurium* in co-culture with *L. fermentatum* as early as after 12 h in all the tested strains of *L. fermentatum*. A relationship between the selection of the strain and the antagonistic potential of lactic acid bacteria was also observed in the present study. As compared with strain Nos. 11, 13, and 15, the elimination of *Salmonella* Senftenberg affected by isolate No. 12 took place after 40 h of co-culture. In contrast, the most effective strains (Nos. 11, 13, 15) totally inhibited pathogen growth as early as after 24 h incubation.

All the tested strains of *Lactobacillus plantarum* and *Lactobacillus brevis* completely inactivated growth of *Salmonella* Senftenberg rods during 48 h of the common culture. Kraszewska [22] also showed high antagonistic activity of *L. plantarum* strains. The 6 tested strains inhibited the growth of indicator bacteria (both G+ and G-). The authors have concluded that this action is probably due to lactic acid production. Alakomi [23] also reports that organic acids produced by lactic acid bacteria are the agents that inhibited the growth of G- bacteria.

Research also indicated that the growth kinetics of lactic rods in monoculture and mixed cultures was comparable. During the co-culture of *Salmonella* Senftenberg *W775* and the tested strains of *Lactobacillus*, the concentrations of lactic rods remained constantly at a level of 10^7-10^8 cfu·ml^{-1}. The above results indicate the lack of the effect of bacteria from the genus *Salmonella* on the growth of *Lactobacillus* during the co-culture. The limited influence of pathogens on the number of lactic acid bacteria was also observed by Bielecka [24] and Drago [20]. Isolates of *Lactobacillus* with the largest antagonistic potential toward *Salmonella* Senftenberg may serve as material for further study concerning the sanitization of sewage sludge, composts, fertilizers of animal origin intended for agricultural purposes, and protection of food products against harmful microorganisms.

Conclusions

1. All the tested strains showed strong antagonistic activity against the pathogen. They totally inactivated growth of *Salmonella* Senftenberg rods during 48 h of the co-culture.
2. Among the tested *Lactobacillus* rods bacteria the strongest antagonistic activity was shown by strain Nos. 11, 13, and 15. They eliminated the pathogen after 24h of common culture.
3. *Lactobacillus* rods were resistant to the presence of the pathogen. No effect of the pathogen on the growth dynamics of lactic acid bacteria was observed.
4. The most active strain of *Lactobacillus* may serve as material for further study concerning the higienization of different environments such as sewage sludge, compost, or animal faeces applied for fertilizing.

References

1. GODERSKA K., CZARNECKI Z. Characterization of selected strains from *Lactobacillus acidophilus* and *Bifidobacterium bifidum*. African Journal of Microbiology Research **1**, (6), 065, **2007**.
2. LIGOCKA A., PALUSZAK Z. Capability of lactic acid bacteria to inhibit pathogens in sewage sludge subjected to biotechnological processes. Bull Vet Inst Pulavy **49**, 23, **2005**.
3. VISSER R., HOLZAPFEL W., BEZUIDENHOUT J. KOTZÈ J. Antagonism of lactic acid bacteria against phytopathogenic bacteria. Appl Environ Microbiol **52**, 552, **1986**.
4. LAUKOVÁ A. *In vitro* treatment of different isolates from cattle dung and pig slurry by nisin. Acta Vet Brno **69**, 147, **2000**.
5. LIGOCKA A., PALUSZAK Z., HERMANN J. Influence of physico-chemical factors on the effectiveness of bacteriocins of lactic acid bacteria activity against the pathogens in sewage sludge. Med Wet **61**, 1413, **2005**.
6. FORESTIER CH., DE CHAMPS CH., VATOUX C., JOLY B. Probiotic activities of *Lactobacillus casei* rhamnosus: *in vitro* adherence to intestinal cells and antimicrobial properties. Res. Microbiol. **152**, 167, **2001**.
7. KRASZEWSKA J., WZOREK W., WOJTASIK I. Effect of culture conditions of *Lactobacillus plantarum* strains on their antagonistic activity. Żywność Nauka Technologia Jakość **2**, (47), 153, **2006**.
8. LEWUS C.B., KAISERA., MONTVILLE T.J. Inhibition of food-borne bacterial pathogens by bacteriocins from lactic acid bacteria isolated from meat. Appl Environ Microbiol **57**, (6), 1683, **1991**.
9. SCHILLINGER U., GEISEN R., HOLZAPFEL W. H. Potential antagonistic microorganisms and bacteriocins for the biological preservation of foods. Trends Food Sci Technol **7**, 158, **1996**.
10. MISHIRA C., LAMBERT J. Production of antimicrobial substances by probiotics. Asia Pacific J Clin Nutr **5**, 20, **1996**.
11. RAIBAUD P., CAULET M., GALPIN J. V., MOCQUOT G. Studies on the bacterial flora of the alimentary tract of pigs. II . Steptococci: selective enumeration and differentation of the dominant group. J. Appl. Bacteriol. **24**, 285, **1961**.
12. ROGOSA M, MITCHELL J. A., WISEMAN R.F. A selective medium for the isolation of oral and fecal lactobacilli. J. Bact. **62**, 132, **1951**.
13. MACCONKEY A. Lactose fermenting bacteria in faeces. J. Hyg. B. 333-379, **1905**.
14. SERVIN A.L. Antagonistic activities of lactobacilli and bifidobacteria against microbial pathogens. FEMS Microbiol Rev. **28**, 405, **2004**.
15. VUYST L., LEROY F. Bacteriocins from lactic acid bacteria. Production, purification and food applications. J Mon Microbiol Biotechnol **13**, 194, **2007**.
16. BAUZA-KASZEWSKA J., SZALA B., PALUSZAK Z. Antagonistic effect of lactic bacteria on *Salmonella* in co-cultures. Med Wet **62**, (11), 1313, **2006**.
17. BIELECKA M. Scientific basis for the use of probiotics. Post Mikrobiol **43**, 11, **2004**.
18. REID G., BURTON J. Use of *Lactobacillus* to prevent infection by pathogenic bacteria. Microbes and Infection, **4**, 319, **2002**.

19. BRASHEARS M.M., DURRE W.A. Antagonistic action of Lactobacillus *lactis* toward *Salmonella* sp. and *Escherichia coli* O157:H7 during growth and refrigerated storage. J Food Prot **62**, 1336, **1999**.
20. DRAGO L., GISMONDO R., LOMBARDI A., GOZZINI L. Inhibition of *in vitro* growth of enteropathogens by new *Lactobacillus* isolates of human intestinal origin. FEMS Microbiology Letters **153**, 455, **1997**.
21. GUSLIS C., ROSS R., DRAKSLER D., PEREZ C., TOUS M. Inhibitory effects of chick Lactobacilli on Enteropathogenic *Salmonella*. Journal of Animal and Veterinary Advances **5**, 126, **2006**.
22. KRASZEWSKA J., WZOREK W., SZTANDO E., RACZYŃSKA-CABAJ A. Antagonistic activity of lactic acid bacteria of the species *Lactobacillus plantarum*. Acta Sci. Pol. Technol. Aliment. **4**, (1), 39, **2005**.
23. ALAKOMI H., SKYTTA E., SAARELA M., MATTILA-SANDHOLM T., LATVA-KALA K., HELANDER I. Lactic acid permeabilizes Gram-negative bacteria by disrupting the outer membrane. Appl. Environ. Microbiol. **66**, 2001, **2000**.
24. BIELECKA M., BIEDRZYCKA EL., BIEDRZYCKA E., SMORAGIEWICZ W., ŚMIESZEK M. Interaction of *Bifidobacterium* and *Salmonella* during associated growth. Int. J Food Microbiol. **45**, 151, **1998**.