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Review

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Antibacterial Activity of Selected Standard Strains of Lactic Acid Bacteria Producing Bacteriocins – Pilot Study*

Aktywność antybakteryjna wybranych szczepów wzorcowych bakterii kwasu mlekowego produkujących bakteriocyny – badania wstępne

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Summary

Introduction: In this paper, an attempt was made to evaluate the antibacterial potential of standard strains of lactic acid bacteria (LAB) producing bacteriocins of various classes, thus demonstrating various mechanisms of cell membrane damages against the *Streptococcus agalactiae* strains (Group B Streptococcus, GBS), depending on surface polysaccharides and surface alpha-like protein genes.

Materials/Methods: Antimicrobial property of the strains of *L. plantarum* C 11, *L. sakei* DSMZ 6333, and *L. lactis* ATCC 11454 producing bacteriocins: JK and EF plantaricins, sakacin and nisin, respectively, against the GBS strains was evaluated. The chosen to the study GBS strains were represented by serotypes Ia, Ib, II, III, V and they had *bca*, *epsilon*, *rib*, *alp2* or *alp3* alpha-like protein genes. The experiment was conducted by means of suspension culture and the bacteria count was determined using the serial dilution method.

Results: A great ability of *L. plantarum* C 11 strain was proven to inhibit the GBS growth. The strain of *L. sakei* DSMZ 6333 did not demonstrate any ability to inhibit the growth of GBS, whereas *L. lactis* ATCC 11454 inhibited the growth of *S. agalactiae* indicator strains to a minor extent. Statistically significant differences were demonstrated between the GBS strains representing various serotypes against the antimicrobial activity of model LAB strains. The least sensitive to the activity of bacteriocins were the strains representing serotypes Ib and III, whereas the strains representing serotype II were the most sensitive. The sensitivity of the GBS strains to the antimicrobial activity of LAB was not dependent on alpha-like protein genes.

Discussion: Among the LAB standard strains producing bacteriocins, the strongest antimicrobial property was observed in the strain of *L. plantarum* C 11. Because of the generally known and verified strong antagonistic property of the strains of *L. plantarum* species against indicator bacteria, it is necessary to further pursue the research presented in this paper.

Key words: **Bacteriocins • plantaricins • antimicrobial activity • Lactic Acid Bacteria (LAB) • Group B Streptococcus (GBS)**

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Streszczenie

Wstęp: W pracy podjęto próbę oceny antybakteryjnego potencjału szczepów wzorcowych bakterii kwasu mlekowego (lactic acid bacteria – LAB) wytwarzających bakteriocyny różnych klas, a tym samym wykazujących różne mechanizmy uszkodzenia błony komórkowej wobec szczepów *Streptococcus agalactiae* (Group B Streptococcus – GBS). Wrażliwość *Streptococcus agalactiae* na działanie antybakteryjne szczepów wzorcowych LAB oceniono względem przynależności szczepów GBS do serotypów oraz obecności genów białek z rodziny alfa-podobnych.

Materiały/Metody: Oceniono właściwości przeciwdrobnoustrojowe szczepów *L. plantarum* C 11, *L. sakei* DSMZ 6333, *L. lactis* ATCC 11454 będących producentami bakteriocyn odpowiednio plantarycyn JK i EF, sakacyny oraz nizyny wobec szczepów GBS. Wybrane do eksperymentu szczepy GBS należały do serotypów Ia, Ib, II, III, V oraz zawierały geny białek *bca*, *epsilon*, *rib*, *alp2*, *alp3* z rodziny alfapodobnych. Eksperyment przeprowadzono metodą hodowli w zawiesinie, oznaczając liczbę drobnoustrojów metodą seryjnych rozcieńczeń.

Wyniki: Wykazano znaczną aktywność przeciwdrobnoustrojową szczepu *L. plantarum* C 11 wobec szczepów GBS. Szczep *L. lactis* ATCC 11454 hamował wzrost *S. agalactiae* w niewielkim stopniu, a szczep *L. sakei* DSMZ 6333 był pozbawiony takiej aktywności. Wykazano różnice istotne statystycznie we wrażliwości szczepów GBS reprezentujących różne serotypy wobec działania przeciwdrobnoustrojowego szczepów wzorcowych LAB. Najmniej wrażliwe na działanie bakteriocyn były szczepy należące do serotypów Ib oraz III, najbardziej zaś szczepy z serotypu II. Wrażliwość szczepów *S. agalactiae* na działanie przeciwdrobnoustrojowe LAB nie była zależna od genów białek z rodziny alfapodobnych.

Dyskusja: Spośród szczepów wzorcowych bakterii kwasu mlekowego wytwarzających bakteriocyny najsilniejsze właściwości antybakteryjne wobec paciorkowców z grupy serologicznej B wykazywał szczep *L. plantarum* C 11. Ze względu na powszechnie znane i potwierdzone silne właściwości antagonistyczne szczepów bakterii z gatunku *L. plantarum* wobec bakterii wskaźnikowych konieczna jest kontynuacja przedstawionych w pracy badań.

Słowa kluczowe: **bakteriocyny • plantarycyny • działanie przeciwdrobnoustrojowe • bakterie kwasu mlekowego • *Streptococcus agalactiae***

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Introduction

Antimicrobial peptides (AMP's) are produced and excreted by bacteria, fungi, plants, insects and vertebrata. AMP's posses an amphipathic structure and contain in their molecules from 12 to 120 amino acids. AMP's are known to have an inhibitory effect directed against enveloped viruses, bacteria, fungi, parasites as well as cancer cells [32].

Bacteriocins are a subgroup of AMP's. They are small peptides encoded by ribosomal DNA or plasmids characterized by antibacterial activity, most frequently against bacteria which are closely related phylogenetically (narrow spectrum of activity) or against other microbes (wide spectrum of activity) [18]. Bacteriocins are active against microbes already in picomolar or nanomolar concentrations.The mechanism of bacteriocins activity involves the perforation of cell membranes of microorganisms. Damage to bacterial membrane leads to the loss of ions, ATP and components which are indispensable for proper functioning and cell metabolism. Interestingly, bacteria producing a certain bacteriocin demonstrate immunity against the activity of the same [11]. Bacteriocins were divided into four classes taking into account their chemical structure, molecular mass, sensitivity to enzyme activity, contents of modified amino-acids and activity mechanism (Table 1). A substantial number of antimicrobial peptides are excreted by probiotic bacteria and subsumed into class I and II bacteriocins [30,31].

Many experiments were conducted which have proven the antibacterial effect of bacteriocins excreted by the genus of *Lactobacillus* on many species and indicator bacteria strains (Table 2).

Table 1. Classification and general characteristics of bacteriocins

Bactriocin classes	Bacteriocin subclasses	Molecular mass	Characteristics of class/subclass	Bacteriocin
Class I	**A**	<5 kDa	lantibiotics	nisin [16,43]
	B			marsacydin alametycin [16,42]
Class II	**IIa**	<10 kDa	pediocin-like bacteriocins	sakacin A, sakacin P [30]
	IIb		two-peptide bacteriocins	lactacin F [30]
	IIc		sec-dependent bactriocins	carno-bacteriocin A [30]
Class III		>30 kDa	heat-labile protein bacteriocins	lactococcin B [31]
Class IV		large protein	mixture of protein(s), lipid(s) and carbohydrate(s) in bacteriocin molecule	leucocin S, mesenterocin 52 [31]

Table. 2. Examples of bacteriocins from individual classes produced by LAB, place of LAB strains isolation and the extent of antimicrobial activity

Producer	Strain	Source	Bacteriocin	Class of bacteriocin	Action of bacteriocin
E. faecium	**H41B**	cheese	**enterocin A enterocin P**	**II**	antagonistic activity against *Listeria* spp. [35]
L. gasseri	**LA 39**	faeces of newborn	**gassericin A**	**IV**	antagonistic activity against *L. monocytogenes*, *S. ureus*, *B. cereus* [21]
L. acidophilus	**M 46**	faeces of newborn	**acidocin B**	**IV**	antagonistic activity against *L. monocytogenes*, *C. sporogenes*, *L. fermentum* and *Lactobacillus delbrueckii ssp. bulgaricus* [25]
L. plantarum	**C 11**	fermented cucumber	**plantaricin JK** (PlnJ, PlnK) **plantaricin EF** (PlnE, PlnF)	II b	antagonistic activity against indicator strains of *L. plantarum* 965, *L. sake* NCDO 2714, *L. viridescens* NCDO 1655, L. plantarum UI50 [2]
			plantaricin A (PlnA)	pheromone	synthesis induction of Pln J, PlnK, PlnE, PlnF; antibacterial activity [23]; cytotoxic activity against cancer cells [45]
L. sakei	**DSMZ 6333**	pork, vacuum-packaged	**sakacin A**	II a	anti- Listeria activity; antagonistic activity against *L. sake*, *L. curvatus*, *C. piscicola*, *E. faecalis*, *L. monocytogenes* [20,29]
L. lactis	**ATCC 11454**	meat	**nisin**	I (typ A)	a broad anti-microbial spectrum; is used as food preservative [42]

Probiotics are described as live microbes which, when administered in appropriate amounts, have a beneficial effect upon the health of host. With reference to this definition, many strains of lactic acid bacteria (LAB) fulfilling the criteria of probiotic bacteria have found application as dietary supplements [42]. In recent years, in connection with the increasing frequency of occurrence of pathogenic microbes resistance to antibiotics, there is a higher and higher interest in using probiotics or purified bacteriocins as alternative medical preparations. Research conducted by Millette at al. (2008) with using mouse models, where the inhibitory impact of post-culture liquids of *L. lactis* MM 19 and *P. acidilactici* MM 33 producing nisin and pediocin, respectively, against vancomycin-resistant *Enterococci*, or VRE was determined, demonstrated a reduction in the intestinal enterococcal population [27].

Then Kruszewska et al. (2004) showed antagonistic activity of mersacidin synthesized by *Bacillus* sp. against strains of *Staphylococcus aureus* (methicillin – resistant *S. aureus*, or MRSA) leading to the elimination of bacterial colonization in the mucous membrane of mouse nose [24]. Results Fernandez et al. (2008) demonstrated the efficacy of nisin in the medication of mastitis in women, which was caused by *Staphylococci* belonging to Gram-positive bacteria [13]. In order to form a modern preparation containing nisin, with

increased antibacterial efficacy, research on encapsulation of peptide in nanoliposomes is conducted [9]. Bacteriocins as medications would be entirely safe for patients and additionally their use would not result in the development of microbes resistance, due to their specific activity mechanism [36].

The bacterial flora of female reproductive tract consists for the most part of LAB but also of potentially pathogenic microbes like, for instance: *Streptococcus agalactiae*, *Escherichia coli*, *Enterococcus faecalis*, and fungi of the genus of *Candida* [6]. Using bacteriocins against potentially pathogenic microbes colonizing female reproductive tract would seem promising, especially in those patients in whom recurrent vaginitis are observed. An additional asset of bacteriocins is that they are excreted in minor concentrations into the vaginal milieu by some strains of the genus of *Lactobacillus* being the basic content of vaginal microflora [14,22,41]. Due to this fact the antimicrobial peptides have their share in keeping the physiologic balance of vaginal flora. Using preparations containing bacteriocins would be aimed at helping eradicating potentially pathogenic microbes from the vaginal milieu and thus preventing vaginitis in women. A special threat among the microbes colonizing the vagina pose the β-hemolytic *Streptococci agalactiae* subsumed into the Group B Streptococci (GBS). According to epidemiologic data there is a connection between colonization by GBS of anuses and vaginae in pregnant women and neonate infections caused by this bacteria. During a delivery there is a high risk of neonate colonization by GBS from the microflora of mother's vagina or the cervical canal vertically, with a probability of up to 70%. At present, the GBS infection prophylaxis in neonates consists in performing screening tests in pregnant women and in case of finding a GBS colonization in administration of a proper antibiotic to the parturient women prophylactically [17].

Unfortunately, in the recent years, GBS strains are more and more frequently found to be resistant to macrolides, lincosamides and streptogramin B. It constitutes a factor significantly decreasing the efficacy of the recommended prophylaxis [3,26]. That is why because of a low efficacy of antibiotic medication, especially in the case of carriage of GBS and adverse consequences of antibiotic administration, it is necessary to look for other, alternative ways to prevent GBS colonization of the reproductive tract.

In connection with this, this paper was aimed at the evaluation and comparison of antibacterial effect of the standard strains of LAB: *L. plantarum* C 11, *L. lactis* ATCC 11454 and *L. sakei* DSMZ 6333 which produce bacteriocins such as: JK and EF plantaricins, nisin, and sakacin, respectively, subsumed into various classes of antimicrobial peptides, thus having different bacterial cell membrane permeabilization mechanisms, on selected strains of *S. agalactiae* and additionally demonstration of relationships of sensitivity of selected GBS strains to GBS serotypes (Ia, Ib, II, III, or V) and the surface alpha-like protein genes (*bca*, *alp2*, *alp3*, *epsilon*, *rib*).

Materials and Methods

The strains of *S. agalactiae* came from the own collection of the Department of Bacteriology, Microbial Ecology and Parasitology, Chair of Microbiology, Jagiellonian University, Medical College. They were collected in the framework of projects to which funding was provided by the Ministry of Science and Higher Education No. 3 PO5E 084 25, No. 2 P05E 004 30 and No. NN 401 042337 and the research under the charter No. K/ZDS/000648, which was conducted with the permission of the Bioethics Committee of the Jagiellonian University Medical College No. KBET/267/B/2002 and No. KBET/143/B/2007. The strains of GBS (n=26) subjected to the research were isolated both from women with clinical symptoms of vaginatis, and from pregnant women routinely tested for GBS carriage, from neonates who, for various reasons, were admitted to the neonate pathology department as well as from neonates colonized with GBS with no symptoms of infection. Control was constituted by the model strains of *S. agalactiae* DSMZ 2134 (German Collection of Microorganisms and Cell Cultures, DSMZ), *S. agalactiae* ATCC 12403 and *S. agalactiae* ATCC BAA-611 (American Type Culture Collection, ATCC).

26 GBS strains representing the most frequently isolated serotypes Ia (n=8), Ib (n=3), II (n=4), III (n=9), V (n=2) to the study were selected. The GBS serotypes were determined by multiplex-PCR reaction according to procedures by Poyart et al. (2007) and Brzychczy-Włoch et al. (2011). Surface alpha-like protein genes (*bca* n=5, *epsilon* n=8, *rib* n=8, *alp2* n=4, *alp3* n=1) were detected with the application multiplex-PCR reaction according to the procedure that was described by Creti et al. (2004) [7,10,33].

GBS strains were cultured on the Columbia medium supplemented with 5% sheep blood (Columbia Blood Agar, BioCorp). One loopful with the volume of 1 µl of GBS culture on solid medium was transferred to 5 ml of TSB (Tryptic Soy Broth, Difco). The GBS culture which was prepared in such a way was incubated under oxygenous condition for 24 hours at 37°C (WS 140, Bolarus Cabinet). The density of the obtained bacterial suspension was assessed with the use of spectrophotometric measurement (Spectrophotometer UV/VIS V-550, Jasco) at wavelength of 600 mm within the range of 0.3–0.4 and it was 1×10^6 cfu/ml.

The LAB standard strains were obtained for the purposes of this experiment from university or commercial collections. The strain of *Lactobacillus plantarum* C 11 was obtained from the Department of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, As, Norway. The strain of *L. plantarum* C 11 excretes dipeptides with proven antibacterial property such as dipeptide plantaricins J and K (pln JK) and E and F (pln EF) and additionally the pheromone plantaricin A (pln A), a substance inducing the *L. plantarum* C 11 strain to produce the above-mentioned bacteriocins. Further strains of *Lactobacillus sakei* DSMZ 6333 and *Lactococcus lactis* ATCC 11454 came from the German Collection of Microorganisms and Cell Cultures (DSMZ) and the American Type Culture Collection (ATCC).

LAB were cultured on the MRS solid medium (Oxoid, Poland) and incubated for 24 hours at 37°C under anaerobic condition (Anaerobic culture chamber, dw Scientific). Then, they were (their amount being one loopful with the volume of 1 µl) spread over 5 ml of MRS liquid medium

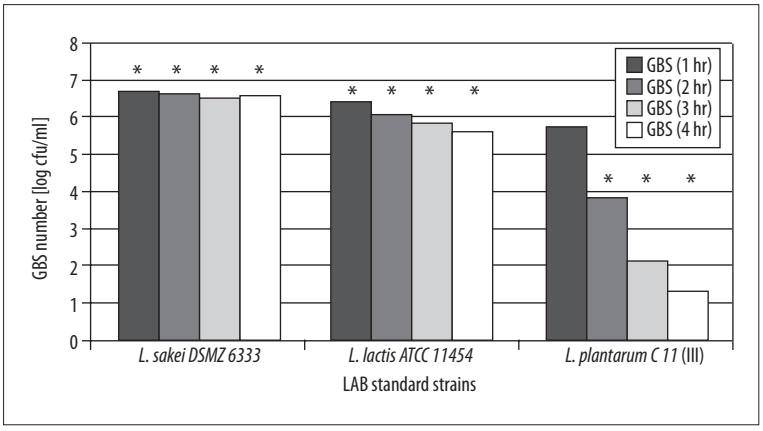

Fig. 1. Relation of the GBS number (log cfu/ml) after the first four full hours of the experiment (1 hr, 2 hrs, 3 hrs, 4 hrs) since the start with the antibacterial activity of the model LAB strains. Results described the logs of GBS number, which were not inhibited by investigated model of LAB strains. There was no statistical relationship between antimicrobial activity of *L. lactis* ATCC 11454 and *L. plantarum* C11 culture (variant III) after the first hour of the experiment. Statistically significant relationship was confirmed in activity of each of checked LAB strains against GBS (log cfu/ml) after following hours of experiment. * statistically significant relation

(Oxoid). The strains of *L. lactis* ATCC 11454 and *L. sakei* DSMZ 6333 were incubated at 32°C and 20°C, respectively, under anaerobic condition for 24 hours in MRS liquid medium [1,8]. In order to prepare the culture of *L. plantarum* C 11 for the experiment, bacteria were initially incubated for 8 hours at 30°C under anaerobic condition. Then, a pheromone pln A was added to the culture, the volume of which was just enough to obtain the concentration of pln A in the *L. plantarum* C 11 culture reading 1 µl/ml. The culture of this strain was continued for 6 hours, up to the moment, when the production of plantaricins J and K as well as E and F reached the maximum level [15]. In the experiment were used: 24-hour cultures of *L. lactis* ATCC 11454 and *L. sakei* DSMZ 6333 and the cultures of *L. plantarum* C 11 in 3 variants: the 1st 14-hour culture with no pln A added constituting a negative control; the 2nd 8-hour culture with pln A added, with its volume on the outset of the experiment being just enough to obtain the concentration of the pheromone of 1 µl/ml in the culture; the 3rd 14-hour culture containing produced plantaricins under the influence of pln A incubation added in the 8th hour. The optical density of the standard LAB strains liquid cultures used in the experiment for the wavelength of 600 nm was 0.6–0.7. The positive controls constituted the chemical solutions of pure nisin in Phosphate Buffered Saline (PBS, Sigma Aldrich), the concentrations of which were 1 µg/ml and 0.5 µg/ml.

The suspensions of the standard LAB cultures and the suspensions of selected GBS strains were mixed in the ratio of 9:1 and incubated under microaerophil conditions (incubator CLN ECO, POL-ECO Aparatura) at temperatures proper for individual model LAB strains. The mixtures of the LAB: GBS cultures were spread over Columbia solid medium supplemented with 5% sheep blood, the dilutions being 0, –2, –4, and –6, respectively within 10 min., 1 hr, 2 hrs, 3 hrs and 4 hrs after the moment they had been mixed. The spread plates with media were incubated for 48 hrs under microaerophil conditions at 37°C. Then, the grown GBS and LAB colonies were summed up, the number of which has been given in cfu/ml-units.

The following techniques of inductive statistics were used to analyze the results obtained: the analysis of variance (ANOVA) and as a supplementary analysis the Tukey-Kramer post-hoc test. If the distribution of the numerical variable significantly diverged from the Gauss curve, non-parametric alternatives of the above tests were used: Kruskal-Wallis test as the alternative to ANOVA and Steel test instead of Tukey-Kramer test [12,39]. Statistical procedures were calculated using the JMP 7.01 package (SAS Institute Inc.) [40].

Results

The activity of LAB producing bacteriocins (*L. plantarum* C 11, *L. sakei* DSMZ 6333 and *L. lactis* ATCC 11454) was evaluated against 26 GBS strains and model strains of *S. agalactiae* DSMZ 2134, *S. agalactiae* ATCC 12403 and *S. agalactiae* ATCC BAA-611. Results obtained after 1 hr, 2 hrs, 3 hrs and 4 hrs of the duration of experiment were selected as data for statistical analysis and they were related to the initial GBS number.

Among the three selected standard LAB strains producing bacteriocins the strain of *L. plantarum* C 11 demonstrated the strongest GBS growth inhibiting property. The growth inhibiting activity against selected GBS strains was checked in 3 *L. plantarum* C 11 culture variants (I, II, III) in mixtures of LAB-GBS. In the course of the experiment the GBS number changed comparably, irrespective of the *L. plantarum* C 11 culture variant. After 2 hours since the start of procedure the GBS number decreased by ca. 2–3 logarithms. Because of reaching the highest concentration of plantaricins (pln EF and pln JK) by *L. plantarum* C 11 culture variant III, it was chosen to comparative analysis of antimicrobial activity model LAB strains (Fig. 1). Throughout the experiment no significant reduction in the GBS number was noted under the influence of the activity of bacterial suspensions of *L. sakei* DSMZ 6333 and *L. lactis* ATCC 11454. Only in the presence of *L. lactis* ATCC 11454 the GBS number decreased by 1 logarithm throughout the experiment. Interestingly, nisin in concentrations of 1 µg/ml and 0.5 µg/ml in the GBS cultures demonstrated no antibacterial property against these microbes.

Taking into consideration the distinguishability of GBS strains as far as serotypes are concerned, significant differences were statistically proven in the antibacterial activity of the model LAB strains producing bacteriocins after 2 hours since the start of the experiment ($p=0.0155$). The least sensitive to the antagonistic activity of LAB turned out to be the strains representing serotypes Ib and III, and the most sensitive the strains representing serotype II. The difference in the sensitivity to the antibacterial activity of LAB between these serotypes was statistically significant ($p<0.05$) (Fig. 2).

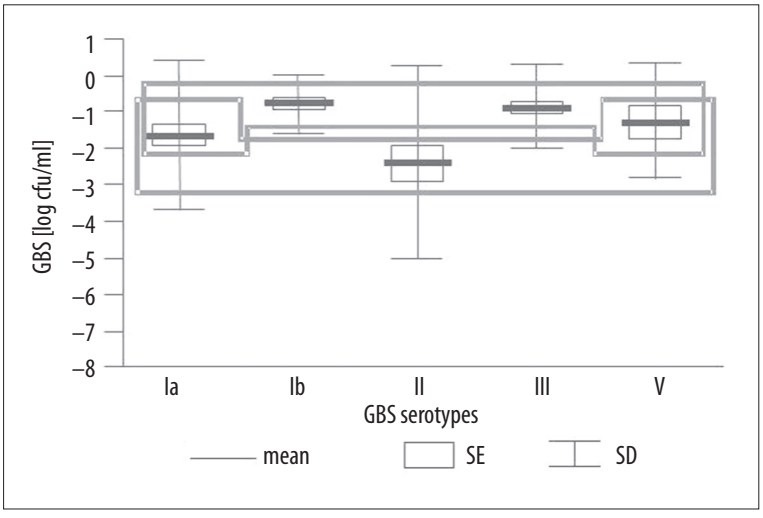

Fig. 2. Comparison of reduction in GBS number in individual serotypes (log cfu/ml) after 2 hours' duration of the experiment. Results expressed as log of number of bacteria, by which the GBS population was decreased with reference to the initial number of the population after 2 hours' duration of the experiment. The correspondence between the GBS serotypes and various sets denotes statistically significant relations between them; **mean** – mean formula, **SE** – standard estimation, **SD** – standard deviation

Subjecting to an analysis the sensitivity of GBS strains, depending on genes coding Alp family proteins they had, to the antagonistic activity of LAB it was demonstrated that after 2 hours since the start of the experiment the differences observed were nearly statistically significant (p=0.0683). The lowest sensitivity to the inhibiting activity of LAB was demonstrated by GBS strains with *alp2* gene (decrease in the GBS number by 1 logarithm), whereas the highest one by GBS isolates with *alp3* gene (decrease in the GBS number by 3 logarithms).

Because of the existing correlation of occurrence of surface saccharides and Alp family proteins and their genes, an additional statistical analysis was made. It encompassed the antimicrobial property of model LAB strains against GBS based on two variables. GBS strains representing serotypes III and Ib with surface protein genes *alp2*, *bca* and *epsilon* demonstrated a lower sensitivity to effects on the side of bacilli from the genus *Lactobacillus* in contradistinction to the strains representing serotype II with *bca* and *rib* genes.

Discussion

The basis of the experiment that was planned and encompassed by this paper were promising results of many pieces of scientific research demonstrating effective antimicrobial activity of LAB strains producing bacteriocins or of their products, purified bacteriocins, against strains of bacteria, also against the antibiotic-resistant ones [13,24,27]. The evaluation conducted regarding the antimicrobial property of the standard LAB strains producing bacteriocins (*Lactobacillus sakei* DSMZ 6333, *Lactococcus lactis* ATCC 11454 and *Lactobacillus plantarum* C 11) against GBS also constituted the continuation of research into the sensitivity of selected GBS strains to the antagonistic activity of selected LAB strains isolated in the vagina, their genera being *L. gasseri*, *L. plantarum*, *L. fermentum* and *L. rhamnosus* [5].

From among the standard LAB strains producing peptides demonstrating antibacterial property, the strongest inhibiting property against GBS was demonstrated by the strain of *L. plantarum* C11 which was isolated from pickled cucumbers. No sensitivity of the indicator GBS strains was demonstrated to the antibacterial activity of the strain of *L. sakei* DSMZ 6333 and a minor sensitivity to the inhibiting activity of *L. lactis* ATCC 11454, i.e. strains also isolated from foodstuffs. In the evaluation of the antagonism of LAB isolated from the vagina against GBS, a very strong antagonistic activity of the lactic acid bacteria was demonstrated compared with the standard LAB strains producing bacteriocins. Already within 2 hours' time after the experiment was started, in the milieu of several *Lactobacillus strains* isolated from the vagina a decrease in the number of *S. agalactiae* by several logarithms or a complete reduction of the GBS population was observed [5]. The selected LAB strains isolated from the vagina, their genera being *L. plantarum*, *L. gasseri*, *L. rhamnosus* and *L. fermentum*, like the standard LAB strains, their genera being *L. plantarum*, *L. sakei* oraz *L. lactis* demonstrated diversified antagonistic activity against GBS. From this it appears that the antimicrobial activity of LAB is not a attribute of the species, but it depends on the strain. Further, Awaisheh et al. (2009) while comparing the antibacterial property of LAB isolated from both humans, foodstuffs and fermented vegetables did observe the ability to inhibit the growth of indicator bacteria by all the analyzed LAB strains. The LAB, however, which were isolated from humans had the strongest antagonistic property [4].

The standard LAB strains producing bacteriocins, such as *L. sakei* DSMZ 6333 and *L. lactis* ATCC 11454 in contradistinction to the LAB originating from the vagina did not demonstrate any antibacterial activity against GBS, or only a minor one. The lack of inhibiting property of *L. sakei* DSMZ 6333 producing sakacin could have been undoubtedly caused by the very narrow spectrum of this peptide activity, limited mainly to LAB. For instance, the research conducted by Schillinger et al. (1989) to evaluate the antagonistic property of the strain of *L. sake* Lb 706 excreting sakacin demonstrated that this strain inhibited the growth of among others: *L. sake*, *L. curvatus*, *L. paramesenteroides*, *E. faecalis* and *Listeria monocytogenes*. The strain of *L. sake* Lb 706, however, was not active against other Gram-positive and Gram-negative bacteria, e.g. against *Staphylococcus aureus* or against *Salmonella typhimurium* [37].

Then, the model strain of *L. lactis* 11454 excreting nisin reduced the population of several GBS strains researched into by 1 logarithm throughout the experiment, whereas the solution of chemically pure nisin had no such property.

It could be connected with factors present in the culture of *L. lactis* ATCC 11454, having an antibacterial effect on GBS, i.e. factors other than nisin or ones acting synergistically in relation to nisin [34].

From among the selected standard LAB strains, the strongest antagonistic activity against GBS, comparable with the activity of the strains of bacteria from the genus of *L. plantarum* isolated from the vagina was demonstrated by the strain of *L. plantarum* C 11. Results of the research by Huett et al. (2006) and Xu et al. (2008) have proven that the activity of the genus *L. plantarum* strongly inhibits the growth of *C. albicans*, *E. coli*, *S. aureus* and *P. aeruginosa* [19,44]. Anderssen et al. (1998) demonstrated that plantaricins J and K as well as E and F excreted by the strain of *L. plantarum* C11 are able to inhibit the growth of indicator strains, among others of *L. plantarum*, *Pediococcus pentosaceus*, *Lactobacillus viridescens*. Also, plantaricin A demonstrates antagonistic property against microbes. That is why both the interaction between plantaricins J and K as well as E and F, and also between plantaricins J, K and plantaricin A and plantaricins E, F and plantaricin A is of a synergistic nature intensifying their antibacterial activity [2,28].

In the experiment described in this paper, in the 24-hour liquid cultures of selected LAB strains, products of bacterial metabolism were accumulated. Moreover, throughout the experiment metabolites of antibacterial nature were synthesized and excreted. The strong property inhibiting the growth of GBS already after 2 hours after the start of experiment may be explained by the presence of *L. plantarum* C 11 metabolites (bacteriocins, short fatty acids, hydrogen peroxide). However, the preservation of non-oxygenic condition of the culture no doubt influenced the limitation of production by the LAB strains of hydrogen peroxide or of lactic acid [37].

The sensitivity of the GBS representing individual serotypes to the activity inhibiting LAB was no doubt connected with differences in the structure of polysaccharide bacterial capsule. The GBS strains representing the serotype III are for the most part isolated from cases of carriage in pregnant women and causing infections in neonates [7]. It may also be connected with the occurrence of specific mechanisms of evasion and resistance to the antibacterial activity of strains such as among others LAB colonizing the same environmental niches of the human body. Furthermore, promising are the results indicating a considerable sensitivity of the GBS strains representing the serotype V which is more and more frequently described as a factor of infections, especially in the group of adult patients, to the antagonistic activity of LAB originating from the vagina [38].

The results of the presented experiment indicate that the strains of *S. agalactiae* are sensitive to the antimicrobial activity of *L. plantarum* C 11 strain producing dipeptides JK and EF. Because of a small pool of the analyzed GBS strains, it would be advisable to include a larger pool of strains into research. Additionally, it would be interesting to obtain results of evaluation of the influence on GBS of mixtures of LAB strains producing bacteriocins presenting various classes. The obtained data would reveal synergistic influence, if any, of LAB strains on pathogenic microbes. It would also be useful to check the influence of the model LAB strains obtained from cultures along with purified bacteriocins as well as their mixtures. The obtained results could be useful in the future in order to research into new probiotic preparations used to control and prevent inflammatory conditions of female reproductive tract.

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References

[1] Aasen I.M., Moretro T., Katla T., Axelsson L., Storro I.: Influence of complex nutrients, temperature and pH on bacteriocin production by *Lactobacillus sakei* CCUG 42687. Appl. Microbiol. Biotechnol., 2000; 53: 159–166

[2] Anderssen E.L., Diep D.B., Nes I.F., Eijsink V.G., Nissen-Meyer J.: Antagonistic activity of *Lactobacillus plantarum* C11: two new two-peptide bacteriocins, plantaricins EF and JK, and the induction factor plantaricin A. Appl. Environ. Microbiol., 1998; 64: 2269–2272

[3] Andrews J.I., Diekema D.J., Hunter S.K., Rhomberg P.R., Pfaller M.A., Jones R.N., Doern G.V.: Group B streptococci causing neonatal bloodstream infection: antimicrobial susceptibility and serotyping results from SENTRY centers in the Western Hemisphere. Am. J. Obstet. Gynecol., 2000; 183: 859–862

[4] Awaisheh S.S., Ibrahim S.A.: Screening of antibacterial activity of lactic acid bacteria against different pathogens found in vacuum-packaged meat products. Foodborne Pathog. Dis., 2009; 6: 1125–1132

[5] Bodaszewska M., Brzychczy-Włoch M., Gosiewski T., Adamski P., Strus M., Heczko P.B.: Ocena wrażliwości paciorkowców grupy serologicznej B na działanie bakterii z rodzaju *Lactobacillus*. Med. Dośw. Mikrobiol., 2010: 62: 153–161

[6] Boris S., Barbés C.: Role played by lactobacilli in controlling the population of vaginal pathogens. Microbes. Infect., 2000; 2: 543–546

[7] Brzychczy-Włoch M., Gosiewski T., Bodaszewska-Lubas M., Adamski P., Heczko P.B.: Molecular characterization of capsular polysaccharides and surface protein genes in relation to genetic similarity of group B streptococci isolated from Polish pregnant women. Epidemiol. Infect., 2012; 140: 329–336

[8] Buchman G.W., Banerjee S., Hansen J.N.: Structure, expression, and evolution of a gene encoding the precursor of nisin, a small protein antibiotic. J. Biol. Chem., 1988; 263: 16260–16266

[9] Colas J.C., Shi W., Rao V.S., Omri A., Mozafari M.R., Singh H.: Microscopical investigations of nisin-loaded nanoliposomes prepared by Mozafari method and their bacterial targeting. Micron, 2007; 38: 841–847

[10] Creti R., Fabretti F., Orefici G., von Hunolstein C.: Multiplex PCR assay for direct identification of group B streptococcal alpha-protein-like protein genes. J. Clin. Microbiol., 2004; 42: 1326–1329

[11] De Vuyst L., Leroy F.: Bacteriocins from lactic acid bacteria: production, purification, and food applications. J. Mol. Microbiol. Biotechnol., 2007; 13: 194–199

[12] Ferguson R., Takane Y.: Analiza statystyczna w psychologii i pedagogice. Wydawnictwo naukowe PWN, Warszawa 2009

[13] Fernandez G.A., Delgado S., Herrero H., Maldonado A., Rodriguez J.M.: The bacteriocin nisin, an effective agent for the treatement of staphylococcal mastitis during lactation. J. Hum. Lact., 2008; 24: 311–316

[14] Gupta S.M., Aranha C.C., Bellare J.R., Reddy K.V.: Interaction of contraceptive antimicrobial peptide nisin with target cell membranes: implications for use as vaginal microbicide. Contraception, 2009; 80: 299–307

[15] Hauge H.H., Mantzilas D., Moll G.N., Konings W.N., Driessen A.J., Eijsink V.G., Nissen-Meyer J.: Plantaricin A is an amphiphilic α-helical bacteriocin-like pheromone which exerts antimicrobial and pheromone activities through different mechanisms. Biochemistry, 1998; 37: 16026–16032

[16] Héchard Y., Sahl H.G.: Mode of action of modified and unmodified bacteriocins from Gram-positive bacteria. Biochimie, 2002; 84: 545–557

[17] Heczko P.B., Niemiec T., Lauterbach R., Przondo-Mordarska H., Strus M., Drzewiecki A., Brzychczy-Włoch M.: Zalecenia dotyczące wykrywania nosicielstwa paciorkowców grupy B (GBS) u kobiet w ciąży i zapobiegania zakażeniom u noworodków spowodowanym przez ten drobnoustrój. Zakażenia, 2008; 8: 87–96

[18] Heng N.C., Ragland N.L., Swe P.M., Baird H.J., Inglis M.A., Tagg J.R., Jack R.W.: Dysgalacticin: a novel, plasmid-encoded antimicrobial protein (bacteriocin) produced by *Streptococcus dysgalactiae* subsp. equisimilis. Microbiology, 2006; 152: 1991–2001

[19] Huett P., Shchepetova J., Loivukene K., Kullisaar T., Mikelsaar M.: Antagonistic activity of probiotic lactobacilli and bifidobacteria against entero- and uropathogens. J. Appl. Microbiol., 2006; 100: 1324–1332

[20] Jones R.J., Zagorec M., Brightwell G., Tagg J.R.: Inhibition by *Lactobacillus sakei* of other species in the flora of vacuum packaged raw meats during prolonged storage. Food Microbiol., 2009; 26: 876–881

[21] Kawai Y., Saito T., Toba T., Samant S.K., Itoh T.: Isolation and characterization of a highly hydrophobic new bacteriocin (gassericin A) from *Lactobacillus gasseri* LA 39. Biosci. Biotechnol. Biochem., 1994; 58: 1218–1221

[22] Kelly M.C., Mequio M.J., Pybus V.: Inhibition of vaginal lactobacilli by a bacteriocin-like inhibitor produced by *Enterococcus faecium* 62-6: potential significance for bacterial vaginosis. Infect. Dis. Obstet. Gynecol., 2003; 11: 147–156

[23] Kristiansen P.E., Fimland G., Mantzilas D., Nissen-Meyer J.: Structure and mode of action of the membrane-permeabilizing antimicrobial peptide pheromone plantaricin A. J. Biol. Chem., 2005; 280: 22945–22950

[24] Kruszewska D., Sahl H.G., Bierbaum G., Pag U., Hynes S.O., Ljungh A.: Mersacidin eradicates methicillin-resistant *Staphylococcus aureus* (MRSA) in a mouse rhinitis model. J. Antimicrob. Chemother., 2004; 54: 648–653

[25] Leer R.J., van der Vossen J.M., van Giezen M., van Noort J.M., Pouwels P.H.: Genetic analysis of acidocin B, a novel bacteriocin produced by *Lactobacillus acidophilus*. Microbiology, 1995; 141: 1629–1635

[26] Lin F.Y., Azimi P.H., Weisman L.E., Philips J.B. 3rd, Regan J., Clark P., Rhoads G.G., Clemens J., Troendle J., Pratt E., Brenner R.A., Gill V.: Antibiotic susceptibility profiles for group B streptococci isolated from neonates, 1995–1998. Clin. Infect. Dis., 2000; 31: 76–79

[27] Millette M., Cornut G., Dupont C., Shareck F., Archambault D., Lacroix M.: Capacity of human nisin- and pediocin-producing lactic acid bacteria to reduce intestinal colonization by vancomycin-resistant enterococci. Appl. Environ. Microbiol., 2008; 74: 1997–2003

[28] Moll G.N., van den Akker E., Hauge H.H., Nissen-Meyer J., Nes I.F., Konings W.N., Driessen A.J.: Complementary and overlapping selectivity of the two-peptide bacteriocins plantaricin EF and JK. J. Bacteriol., 1999; 181: 4848–4852

[29] Naghmouchi K., Belguesmia Y., Baah J., Teather R., Drider D.: Antibacterial activity of class I and IIa bacteriocins combined with polymyxin E against resistant variants of Listeria monocytogenes and *Escherichia coli*. Res. Microbiol., 2011; 162: 99–107

[30] Nissen-Meyer J., Rogne P., Oppegard C., Haugen H.S., Kristiansen P.E.: Structure-function relationships of the non-lanthionine-containing peptide (class II) bacteriocins produced by gram-positive bacteria. Curr. Pharm. Biotechnol., 2009; 10: 19–37

[31] Oppegard C., Rogne P., Emanuelsen L., Kristiansen P.E., Fimland G., Nissen-Meyer J.: The two-peptide class II bacteriocins: structure, production, and mode of action. J. Mol. Microbiol. Biotechnol., 2007; 13: 210–219

[32] Pálffy R., Gardlik R., Behuliak M., Kadasi L., Turna J., Celec P.: On the physiology and pathophysiology of antimicrobial peptides. Mol. Med., 2009; 15: 51–59

[33] Poyart C., Tazi A., Réglier-Poupet H., Billoët A., Tavares N., Raymond J., Trieu-Cout P.: Multiplex PCR assay for rapid and accurate capsular typing of group B streptococci. J. Clin. Microbiol., 2007; 45: 1985–1988

[34] Rattanachaikunsopon P., Phumkhachorn P.: Synergistic antimicrobial effect of nisin and p-cymene on *Salmonella enterica* serovar Typhi *in vitro* and on ready-to-eat food. Biosci. Biotechnol. Biochem., 2010; 74: 520–524

[35] Renye J.A. Jr., Somkuti G.A., Paul M., Van Hekken D.L.: Characterization of antilisterial bacteriocins produced by *Enterococcus faecium* and *Enterococcus durans* isolates from Hispanic-style cheeses. J. Ind. Microbiol. Biotechnol., 2009; 36: 261–268

[36] Rossi L.M., Rangasamy P., Zhang J., Qiu X.Q., Wu G.Y.: Research advances in the development of peptide antibiotics. J. Pharm. Sci., 2008; 97: 1060–1070

[37] Schillinger U., Lücke F.K.: Antibacter activity of *Lactobacillus sake* isolated from meat. Appl. Environ. Microbiol., 1989; 8: 1901–1906

[38] Skoff T.H., Farley M.M., Petit S., Craig A.S., Schaffner W., Gershman K., Harrison L.H., Lynfield R., Mohle-Boetani J., Zansky S., Albanese B.A., Stefonek K., Zell E.R., Jackson D., Thompson T., Schrag S.J.: Increasing burden of invasive group B streptococcal disease in nonpregnant adults, 1990–2007. Clin. Infect. Dis., 2009; 49: 85–92

[39] Sokal R.R., Rohlf F.J.: Biometry: the principles and practice of statistics in biological research. 3rd edition; W. H. Freeman and Co., New York 1995

[40] Stevens G.: A nonparametric multiple comparison test for differences in scale parameters. Metrika, 1989; 36: 91–106

[41] Vera Pingitore E., Hébert E.M., Nader-Macias M.E., Sesma F.: Characterization of salivaricin CRL 1328, a two-peptide bacteriocin produced by *Lactobacillus salivarius* CRL 1328 isolated from the human vagina. Res. Microbiol., 2009; 160: 401–408

[42] World Health Organization, Food and Agriculture Organization of the United Nations. Probiotics in food. Health and nutritional properties and guidelines for evaluation. FAO Food and Nutrition Paper 85, Rome 2006

[43] Xie L., van der Donk W.A.: Post-translational modifications during lantibiotic biosynthesis. Curr. Opin. Chem. Biol., 2004; 8: 498–507

[44] Xu H.Y., Tian W.H., Wan C.X., Jia L.J., Wang L.Y., Yuan J., Liu C.M., Zeng M., Wei H.: Antagonistic potential against pathogenic microorganisms and hydrogen peroxide production of indigenous *Lactobacilli* isolated from vagina of Chinese pregnant women. Biomed. Environ. Sci., 2008; 21: 365–371

[45] Zhao H., Sood R., Jutila A., Bose S., Fimland G., Nissen-Meyer J., Kinnunen P.K.: Interaction of the antimicrobial peptide pheromone Plantaricin A with model membranes: implications for a novel mechanism of action. Biochim. Biophys. Acta, 2006; 1758: 1461–1474

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