Synbiotic is a combination of probiotic and prebiotic, which can synergistically promote the growth of beneficial bacteria or newly added species in the colon (Macfarlane et al., 2008). During the development of new synbiotic products it is very important to ascertain prebiotic and probiotic interactions and influence of prebiotic on probiotic growth and antibacterial activity. Prebiotics are described as non-digestible poly-or oligosaccharides that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of beneficial bacteria in the colon (Gibson and Roberfroid, 1995). Prebiotics themselves have many health promoting properties. Moreover, there is evidence that they can help modulate the growth of gut microbiota and stimulate bacteriocin production (Kunova et al., 2011; Patel and Goyal, 2012).

Inulin, palatinose and α-cyclodextrin used in this work are generally recognized as safe (GRAS) food additives (Wagner et al., 2006; Holub et al., 2010). Inulin is a linear polymer consisting of β-(2→1)-fructosyl-fructose linkages (Roberfroid, 2007). Palatinose is a reducing sugar, composed of glucose and fructose moieties, joined with α-1,6-glycosidic bonds. Commericially palatinose is made from sucrose by enzymatically reorganizing glycosyl bonds (Holub et al., 2010). Cyclodextrins (CDs) are circular oligosaccharides, composed of glucose residues linked by α-1,4-glycosidic bonds. The hydrophilic part of the molecule is faced to the outside of the ring, and the hydrophobic part is inside the ring (Szejtli, 2004). Commercially CDs are produced from a starch. These molecules are widely used in pharmacy, chemistry and food industry due to their ability to form inclusion complexes (Szejtli, 2004). Although βCD is more industrially used due to its lower price, αCD is characterized by better solubility in water (140 mg/ml) than βCD (18 mg/ml, 25°C) (Szejtli, 2004).

Inulin, palatinose and α-cyclodextrin can act in many health promoting ways, like stimulating immune system, reducing the amounts of pathogenic (Bacillus sp., Escherichia coli, Campylobacter jejuni, Salmonella et al., 2006; Patel and Goyal, 2012). Inulin is a liner polymer consisting of β-(2→1)-fructosyl-fructose linkages (Roberfroid, 2007). Palatinose is a reducing sugar, composed of glucose and fructose moieties, joined with α-1,6-glycosidic bonds. Commericially palatinose is made from sucrose by enzymatically reorganizing glycosyl bonds (Holub et al., 2010). Cyclodextrins (CDs) are circular oligosaccharides, composed of glucose residues linked by α-1,4-glycosidic bonds. The hydrophilic part of the molecule is faced to the outside of the ring, and the hydrophobic part is inside the ring (Szejtli, 2004). Commercially CDs are produced from a starch. These molecules are widely used in pharmacy, chemistry and food industry due to their ability to form inclusion complexes (Szejtli, 2004). Although βCD is more industrially used due to its lower price, αCD is characterized by better solubility in water (140 mg/ml) than βCD (18 mg/ml, 25°C) (Szejtli, 2004).

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Lactobacillus delbrueckii, L. curvatus, and many other OS (Broadbent et al., 2012). Although these OS also have sweetness due to their indigestibility they are characterized by low glycaemic index (for example, palatinose has about half the sweetness of sucrose, but its glycaemic index is only 32 (Holub et al., 2010)). This makes them even more favorable food additives for diabetic patients.

Like most prebiotics, inulin, palatinose and α-cyclodextrin are not digested in the upper gastrointestinal tract (except palatinose), which makes them available and longer lasting carbon sources for beneficial colon bacteria, like bifidobacteria (Bosscher et al., 2006; Goderska et al., 2008) and lactobacilli (Kunova et al., 2011). However, most investigations are focused on the influence of these OS on bifidobacteria and little is known about how they affect lactobacilli and other beneficial bacteria from other genera, like lactoccci, widely used in the food industry (Pan et al., 2008; Gänzle and Follador, 2012). Most of them produce various antibacterially active compounds and bacteriocins among them (Chen et al., 2006). Not much research is done in this area, but there is evidence that bacteriocins may have various effects on the gut microbiota, like facilitating the introduction of the producing strain into the gastrointestinal tract, inhibiting the invasion of competing and pathogenic strains, modulating the composition of the gut microbiota and influencing the host immune system (Dobson et al., 2012). While bacteriocin production is often growth-associated and is dependent on carbon availability, slow digestion of prebiotics is very important as they are much longer-lasting carbon sources and mostly fermented only in the colon (Macfarlane et al., 2008). In addition to this, it is important to clarify the effect of prebiotics on the growth and antibacterial activity of probiotic bacteria (Chen et al., 2006). It is shown that inulin stimulates the secretion of bacteriocins by Lactobacillus paracasei CMGB16 strain (Vamanu and Vamanu, 2010). However, little is known about the influence of aCD and palatinose on probiotic bacteria, but it has been shown that αCD can maintain the growth of Lactobacillus casei and increase the amounts of bifidobacteria (Jo et al., 2007). The analysis of L. casei supragenome has revealed that the bacterial strains of this species should be able to ferment inulin, palatinose and many other OS (Broadbent et al., 2012), which could be useful parts of symbiotics for diabetic patients. However, not many investigations of these properties have been made in vitro.

**Materials and Methods**

**Strain isolation from yogurts.** The sources of Lactic Acid Bacteria (LAB) were probiotic yogurts: “Actimel” and “Bifi”. For the isolation of strains, 1 g of each yogurt were added to 99 ml of 0.85 % sterile saline solution. Tenfold serial dilutions of the samples were made and appropriate dilutions were streaked on de Man, Ragosa and Sharpe (MRS) agar plates (Kormin et al., 2001). The plates were incubated for two days under anaerobic conditions at 37°C by placing a gas pack in the anaerobic jar (Merck).

**Bacterial strains and growth conditions.** Six Lactobacillus sp. and two Lactococcus sp. type strains were obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ): Lactobacillus acidophilus DSM 20079 (LA), Lactobacillus delbrueckii subsp. bulgaricus DSM 20081 (LD), Lactobacillus curvatus DSM 20010 (LC), Lactobacillus sakei subsp. sakei DSM 20017 (LS), Lactococcus lactis subsp. lactis DSM 20481 (LL) and L. lactis subsp. lactis DSM 20729 (LL2) (abbreviations of strains used further in the work are given in the brackets). Isolated and type strains of Lactobacillus sp. were cultured in MRS broth (Merck) or basal MRS (Saminathan et al., 2010). Type strains of Lactococcus sp. were grown in broth No. 92 (DSMZ culture medium list), which consisted of Tryptic Soy Broth (TSB, Merck), supplemented with 0.3% Yeast Extract (YE, Difco). Isolated strains were cultured aerobically or anaerobically, with (100 rpm) or without agitation, at 30°C. Type strains of L. acidophilus and L. delbrueckii subsp. bulgaricus were grown under anaerobic conditions, at 37°C. Other type strains were cultured aerobically, at 30°C.

**Oligosaccharide substrates.** Three different commercially available oligosaccharides were used in this study: inulin (Inl) (Alfa Aesar), palatinose hydrate (Pal) (TCI) and α-cyclodextrin (Ctd, αCD) (Merck). Stock solutions of these oligosaccharides were prepared in sterile distilled water and filter sterilized with 0.22 μm filters (Roht). The sterile oligosaccharide solutions were added to autoclaved basal MRS medium to obtain final oligosaccharide concentration of 1% (w/v). Glucose (Merck), which was a favourable carbohydrate source for all used LAB strains, was used as control (Saminathan et al., 2010).

**Partial identification of isolated strains.** Genomic DNA of isolated strains was extracted using GeneJet DNA Extraction Kit (Thermo Fisher Scientific). Universal bacterial 16S rDNA primers (27F and 1492R, Thermo Fisher Scientific) were used for PCR reactions. Reaction products were sequenced at the Sequencing Center of the Institute of Biotechnology, *In silico* data analysis was performed using the NCBI Blast program (http://blast.ncbi.nlm.nih.gov/Blast.cgi).
In order to determine the variety of strains isolated from yogurts, we performed PCR reaction with all strains genomic DNA and universal BOX primers. Results were analyzed by agarose gel electrophoresis (Sambrook and Russell, 2001).

**Antibacterial activity assay.** The antibacterial activity of strains was determined using the spot-on-lawn method (Kormin et al., 2001) with modifications. The tested strain was grown overnight in the middle of Petri dish with an appropriate agar medium. Then the layer of agar medium with inoculated other presumably sensitive strain was poured on top of the grown culture. The agar media with inoculated strain was prepared by adding 20% of 1.2 OD culture inoculum into an appropriate agar media. Isolated and type strains were tested against each other.

**Agar well diffusion assay.** 0.5 cm diameter wells were made, using sterile plastic pipette tips, in the agar media with inoculated sensitive strain, prepared as described above. Culture samples were taken every two hours from the beginning of culture cultivation in liquid media. Samples were centrifuged and the serial twofold dilutions of the cell free supernatants were made. The amount of 100 µl of every dilution was poured into the prepared wells and plates were incubated overnight at the temperature appropriate for the used indicator strain. Clear zones round the wells after incubation indicate the inhibitory activity, which was expressed quantitatively as arbitrary units per milliliter (AU/ml). The arbitrary unit (AU) was defined as the reciprocal of the highest dilution able to produce a clear zone of growth inhibition of the indicator strain (Vera Pingitore et al., 2007).

**Disintegration of cells.** Samples (5 ml) of the grown cultures were taken every 6 h from the beginning of growth. Samples were centrifuged, cells were suspended in 5 ml of phosphate buffer (pH 6.0) and then disrupted with the ultrasound disintegrator. Obtained cell disintegration was filtered through 0.22 µm filter (Roth) or centrifuged (5 min, 14 000 × g). In order to determine the antibacterial activity, 100 µl of the filtered, centrifuged and untreated disintegrates were tested using agar well-diffusion assay using A20 strain as indicator.

**Identification of bacteriocins.** Primers (20 pairs in total) used in this work were described in scientific publications or constructed using typical well known bacteriocin encoding gene sequences from the Genbank database (http://www.ncbi.nlm.nih.gov/Genbank/index.html), are listed in Table I.

**Results**

**Isolation of bacteria from yogurts.** Since the morphology of bacteria grown on the MRS medium did not have any differences, 9 strains of *Lactobacillus* sp. isolated were randomly selected: 4 strains from “Actimel” yogurt (“Danone”) (A isolates) and 4 from “Bifi” yogurt (UAB “Rokiškio pienas”) (B isolates). The amount of *Lactobacillus* sp. bacteria in examined yogurts was estimated at 10⁶ cells in 1 gram of yogurt. These data coincide with the information given by the producers. For a more detailed identification of the isolates 16S rDNA analysis was made. Results have shown that the isolated strains are most similar to *L. casei* and *L. paracasei* subsp. *tolerans* species (reliability of BLAST analysis results is 100%). Implementation of BOX-PCR reaction, used to estimate the variety of bacterial strains among isolates, has revealed distribution of strains in the profiles, fully complying with strains origin from different yogurts (data not shown).

**Antagonistic activity assay of isolated strains.** 3 strains from group A and 3 strains from group B were selected for the antagonistic activity assay. Using spot-on-lawn method it was determined that all isolated lactobacilli strains were able to produce clear zones inhibiting the growth of the strains, used as indicator (isolated strains were tested against each other). Results, given in Table II, reflect the antagonistic spectrum of the tested strains. The obtained results allow us to assert that strains belonging to groups A and B produce antibacterially active compounds. The size of the formed clear zones (Table II.) shows that the antibacterial compound of B group strains is more active or a larger amount of it is produced.

**Antibacterial activity of isolated and type strains.** Antibacterial activity of 3 isolated (A11, B13, A20) and 6 type strains against each other was determined and phenotypically evaluated (Table III.). Results have shown that LL2 strain possesses the highest antibacterial activity among type strains. The antibacterial spectrum and ability to inhibit the growth of tested strains of this strain did not differ from antibacterial spectrum and activity of isolated lactobacilli strains A11, A20 and B13. However, only LD strain had no antibacterial activity against any of the tested strains and was sensitive to all antibacterial compounds secreted by the tested strains.

**Identification of bacteriocins produced by isolated and type strains.** One of the possibilities to identify secreted bacteriocins is a search of bacteriocin encoding genes in the genomes of analyzed strains. For this purpose we used 20 pairs of primers constructed in accordance with genes of well known bacteriocins (GeneBank Database) or selected from scientific publications (Table I.). All primers were used for PCR reactions with genomic DNA of 15 strains (9 isolated and 6 type strains). The results of the size and sequence analysis of the obtained PCR products can be divided into five groups: 1) Two PCR products compatible with genes encoding nisin by their size and results of
BLAST analysis (primers 3 and W, genomic DNAs of LL2 and LL strains respectively, Table I). 2) PCR products, obtained with 5 primers constructed for detection of new bacteriocins of *L. casei* ATCC334 (63, 86, 93, 405, 406) (Kuo *et al.*, 2013) and genomic DNAs of isolated strains. Sequences of the products had homology with the sequences of new bacteriocins, mentioned before. 3) PCR products obtained with primers constructed according to prebacteriocin genes found in the genome of *L. casei* BDII strain (Genbank Database, GI: 385821700) (primers 92, 94 and 111 (Table I.), genomic DNAs of isolated strains), which were compatible with

### Table I

<table>
<thead>
<tr>
<th>No</th>
<th>Primer (abbreviation)</th>
<th>Sequence</th>
<th>Tm, °C</th>
<th>Expected product (size, bp)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1BACTP (1)</td>
<td>F: 5’-TGA AGA TGT ATT TGG GTG CGT-3’&lt;br&gt;R: 5’-CAG GAG TGG TTT CTG GTG-3’</td>
<td>57</td>
<td>Lakticin 3147 (257)</td>
<td>(Digaitiene <em>et al.</em>, 2012)</td>
</tr>
<tr>
<td>2</td>
<td>2BACTP (2)</td>
<td>F: 5’-GGG CCT GCA GGA GGT CTT TCT TCT GAT GAT CAC-3’&lt;br&gt;R: 5’-GGG CCT TGA GGT AGT TGG TCG TTG-3’</td>
<td>75</td>
<td>Pedioicin PA (541)</td>
<td>(Digaitiene <em>et al.</em>, 2012)</td>
</tr>
<tr>
<td>3</td>
<td>3BACTP (3)</td>
<td>F: 5’-CTA TGT ACA CCC GGT GTG AA-3’&lt;br&gt;R: 5’-TTT ATG AGC TAG CGG AAT CA-3’</td>
<td>56</td>
<td>Nizin (590)</td>
<td>(Digaitiene <em>et al.</em>, 2012)</td>
</tr>
<tr>
<td>4</td>
<td>4BACTP (4)</td>
<td>F: 5’-ACA GGT GGA AAA TAT TAT GGT A-3’&lt;br&gt;R: 5’-TTT TGC TTA TTA TTA ATT CCA-3’</td>
<td>55</td>
<td>Sakacin P (150)</td>
<td>(Swetwiwathana <em>et al.</em>, 2009)</td>
</tr>
<tr>
<td>5</td>
<td>BACTA164-1NIZLL (A)</td>
<td>F: 5’-ATG ATG AGA GAA TTA ACT TTA ACT TGG ACT GAT TTG-3’</td>
<td>64</td>
<td>Nizin</td>
<td>(Kuo <em>et al.</em>, 2013)</td>
</tr>
<tr>
<td>6</td>
<td>BACTWNC-1NIZLL (W)</td>
<td>F: 5’-CTG GAA TTC ATA AGG AGG TCA AAA TG-3’&lt;br&gt;R: 5’-CGG GGT ACC TAC TCT TAC GTT ATT TGG-3’</td>
<td>69</td>
<td>Nizin A</td>
<td>(Millette <em>et al.</em>, 2008)</td>
</tr>
<tr>
<td>7</td>
<td>LacS (L)</td>
<td>F: 5’-ATG GAA TTR TTR CCR ACK GCY GCY GTY YTR TA-3’&lt;br&gt;R: 5’-ATG RTG RTG TTT RGC NSW RTA YTT-3’</td>
<td>72</td>
<td>Lakticin S</td>
<td>(Rodrigues <em>et al.</em>, 1995)</td>
</tr>
<tr>
<td>8</td>
<td>m2163 (63)</td>
<td>F: 5’-AAA CAT ATG AAA CGA AAG TGG CCC CCC AAA AC-3’&lt;br&gt;R: 5’-TTT GAA TTC GCG ACG ATC TCT TGA ACA TCA-3’</td>
<td>66</td>
<td>Class II bacteriocin (≤300)</td>
<td>(Kuo Y.-C. <em>et al.</em>, 2012)</td>
</tr>
<tr>
<td>9</td>
<td>m2386 (86)</td>
<td>F: 5’-ATT CAT ATG AGC ATC CTT CTT GCT GAT GGT ATT GAT TTG-3’</td>
<td>68</td>
<td>Class II bacteriocin (≤300)</td>
<td>(Kuo Y.-C. <em>et al.</em>, 2012)</td>
</tr>
<tr>
<td>10</td>
<td>m2393 (93)</td>
<td>F: 5’-GCT CAT ATG GAA AAC GGT GTG TTA TGG TCA-3’&lt;br&gt;R: 5’-AAA GAA TTC GGA ATC CCA GAA TGG CAG C-3’</td>
<td>69</td>
<td>Class II bacteriocin (≤300)</td>
<td>(Kuo Y.-C. <em>et al.</em>, 2012)</td>
</tr>
<tr>
<td>11</td>
<td>m2405 (5)</td>
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<td>63</td>
<td>Class II bacteriocin (≤300)</td>
<td>(Kuo Y.-C. <em>et al.</em>, 2012)</td>
</tr>
<tr>
<td>12</td>
<td>m2406 (6)</td>
<td>F: 5’-TCA CAT ATG AAA AAG AAA TTT GAT TGT GTC-3’&lt;br&gt;R: 5’-TAA GAA TTC GCC CAC TCT TAC-3’</td>
<td>63</td>
<td>Class II bacteriocin (≤300)</td>
<td>(Kuo Y.-C. <em>et al.</em>, 2012)</td>
</tr>
<tr>
<td>13</td>
<td>AcdT (AT)</td>
<td>F: 5’-ATG ATT TCA TCT CAT CA A AA ACG-3’&lt;br&gt;R: 5’-CTA AAA ACC GTC ATG ATA CCA ACG AGC GC-3’</td>
<td>57</td>
<td>Acidocin 8120 (141)</td>
<td>This work</td>
</tr>
<tr>
<td>14</td>
<td>CurA (CA)</td>
<td>F: 5’-GGG CAG GAA TGA TTT TTT GC ATC GC-3’&lt;br&gt;R: 5’-GGT CTT CTT CTT CAA TAT TAG ACC CTC-3’</td>
<td>65</td>
<td>Curvacin A (179)</td>
<td>This work</td>
</tr>
<tr>
<td>15</td>
<td>CurA2 (CA2)</td>
<td>F: 5’-GGG CAG GAA TGA TTT TTT GC ATC GC-3’&lt;br&gt;R: 5’-TGG CAT ATG GGG TCT GC-3’</td>
<td>65</td>
<td>Curvacin A and immunity gene (837)</td>
<td>This work</td>
</tr>
<tr>
<td>16</td>
<td>SakA (SA)</td>
<td>F: 5’-GGG CAG AGG AGA TTA TTA AGG-3’&lt;br&gt;R: 5’-CAT TCC AGC TAA ACC ACT AGC CC-3’</td>
<td>64</td>
<td>Sakacin A (197)</td>
<td>This work</td>
</tr>
<tr>
<td>17</td>
<td>SakP (SP)</td>
<td>F: 5’-GCA GAA GTA ACA GCA ATC CGT GG-3’&lt;br&gt;R: 5’-GTG TAG ATG TTT CGG CTT GG-3’</td>
<td>66</td>
<td>Sakacin P (622)</td>
<td>This work</td>
</tr>
<tr>
<td>18</td>
<td>92</td>
<td>F: 5’-ATG AAA AAG TTA CGA ACA CAA CAA GTT GTG-3’&lt;br&gt;R: 5’-TCA TCT AAA AAT CGT AGT CAA TCC CC-3’</td>
<td>65</td>
<td>Prebacteriocin (186)</td>
<td>This work</td>
</tr>
<tr>
<td>19</td>
<td>94</td>
<td>F: 5’-TGG GTG GAA AAG CCC ACA CCA AAG CAA GCT CAA GCC CAC-3’</td>
<td>72</td>
<td>Prebacteriocin (192)</td>
<td>This work</td>
</tr>
<tr>
<td>20</td>
<td>111</td>
<td>F: 5’-ATG ACA GAC AAA CGT GAAAC TTA ATG TCG-3’&lt;br&gt;R: 5’-GTA ATG CCC CCA AAC ACT GAG ATG-3’</td>
<td>67</td>
<td>Prebacteriocin (333)</td>
<td>This work</td>
</tr>
</tbody>
</table>
the prebacteriocin genes by their size and sequence. However, more detailed in silico analysis of these products, using Bactibase database (http://bactibase.pfba-lab-tun.org/physicochem), has shown that, most likely, these genes do not encode bacteriocins. 4) PCR products, obtained with primers for well known bacteriocin genes and genomic DNAs of isolated and some of the type strains (primer for nisin (3) with genomic DNA of isolated, LL and LA strains; primer for lactacin 3147 (1) with genomic DNA of isolated and LD strains; primer for curvacin A (CA2) with genomic DNA of isolated and LD strains), which were not compatible with these bacteriocin genes by their size and sequence analysis, but were identified as hypothetical or unrelated function proteins. 5) No PCR products were obtained with 7 primers for the well known bacteriocin genes (pediocin PA (2), sakacin P (4 and SP), sakacin A (SA), lactacin S (L), acidocin 8120 (AT), curvacin A (CA) (Table I)) and genomic DNAs of all strains.

**Ability of the isolated and type strains to assimilate OS.** Considering the results of BLAST and BOX-PCR analysis, only strains A11 and B13 were used for further experiments, as they represent different groups of BOX-PCR profiles and possess the highest antibacterial activity among the strains of their groups (Table II.).

Data from the graphs presented in Fig. 1. (A and B) suggest that in all cases both A11 and B13 strains most weakly assimilate palatinose hydrate. On the other hand, inulin is poorly assimilated only by strain A11 (Fig. 1A). The growth of strain B13 with inulin is equivalent to the growth of this strain with glucose, except that the exponential growth phase begins about 4 h later (Fig. 1B). The increase of OS concentration does not influence the growth of strains.

Analysing the influence of different carbon sources (glucose, inulin and palatinose) on the type strains of *Lactobacillus* sp. and *Lactococcus* sp., the following was determined:

1. In the medium with glucose as an only carbon source *L. lactis* subsp. *lactis* strains (LL and LL2) grew faster than strains LS and LA. LL and LL2 strains reached the stationary phase after 6 and 8h respectively (Fig. 1C and D), while strains LS and LA reached this phase of growth after 14 and 28h respectively (Fig. 1E and F). Evaluation of growth time and cell density at the beginning of the stationary phase suggests that strain LL2 grows fastest in the medium with glucose.

2. Strain LL assimilates palatinose best among the type strains (Fig. 1C). The culture reaches cell density of 1.2 OD in the beginning of stationary phase after 14h of growth, while assimilation of inulin is ineffective – maximum reached OD was 0.5.

3. The assimilation of inulin and palatinose by strain LS is completely ineffective (Fig. 1E).

4. Strain LL2 equally poorly assimilates both inulin and palatinose. Only 0.5 OD cell density is reached in the beginning of the stationary phase, after 5h of growth (Fig. 1D).

5. LA also ineffectively assimilates inulin and palatinose. The stationary phase with the cell density of 0.5 OD is reached after 12h in the medium with inulin and only after 24h when growing with palatinose (0.45 OD) (Fig. 1F).

The MRSb medium becomes opaque after addition of αCD and it becomes impossible to track the alterations of OD. Therefore, the influence αCD on the growth of strains was analysed only with strains LL and LL2. Results (Fig. 1C and D) have shown that αCD is actively assimilated by LL2 strain.

**Evaluation of antibacterial activity of isolated strains.** Antibacterial activity of culture supernatants was evaluated by the well-diffusion method during the growth (Vera Pingitore *et al.*, 2007). Strain A20 was used as indicator for the evaluation antibacterial activity of the isolated strains, while testing on agar medium by spot-on-lawn method it demonstrated the highest

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### Table II

Results of antibacterial spectrum of isolated strains.

<table>
<thead>
<tr>
<th>Secreting strain</th>
<th>Sensitive strain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A11</td>
</tr>
<tr>
<td>A11</td>
<td>++</td>
</tr>
<tr>
<td>A15</td>
<td>+</td>
</tr>
<tr>
<td>A20</td>
<td>+</td>
</tr>
<tr>
<td>B1</td>
<td>++</td>
</tr>
<tr>
<td>B4</td>
<td>++</td>
</tr>
<tr>
<td>B13</td>
<td>+++</td>
</tr>
</tbody>
</table>

*“+” – diameter of the clear zone around the secreting culture exceeds 1.5 mm; “++” – clear zone ≤ 2.5 mm, “+++” – zone > 2.5 mm.*

### Table III

Results of antibacterial activity of isolated and type strains.

<table>
<thead>
<tr>
<th>Sensitive strain</th>
<th>A11</th>
<th>B13</th>
<th>A20</th>
<th>LA</th>
<th>LD</th>
<th>LL</th>
<th>LL2</th>
<th>LC</th>
<th>LS</th>
</tr>
</thead>
<tbody>
<tr>
<td>A11</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
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*“+/−” – clear zone is visible only by the borders of the secreting culture; “+” – clear zone is hardly visible, ≤ 0.5 mm; “++” – clear zone ≤ 1.5 mm; “+++” – clear zone > 2.5 mm.*
sensitivity to the antibacterial compounds secreted by other isolated strains (Table II). However, no antibacterial activity was detected while testing supernatants from the cultures grown in the liquid medium. The negative results could be caused by:

- interaction between antibacterial compound and cell surface;
- accumulation of antibacterial compound inside the cell;
- low concentration of the secreted antibacterial compound.

Accordingly, the cultivation of strains A11 and B13 was repeated after addition of 0.5% and 1% of Tween-80, which should reduce the interaction between proteins and cell surface and also enlarge the amount of antibacterial compound in the culture supernatant (the initial concentration of Tween-80 in the MRS medium is 0.1%). However, no clear zones were detected after repeated evaluation of antibacterial activity of culture supernatants.

Culture samples (5 ml) were taken every 6 hours from the beginning of growth. The samples were centri-

fuged and cells were disintegrated. Antibacterial activity of filtered, centrifuged and untreated cell disintegrates was tested by the well-diffusion assay, but no antibacterial activity was detected.

Aiming to increase the amount of antibacterial compound, A11 and B13 strains were grown in the liquid medium till the end of exponential growth phase. 50 ml of every culture supernatants were salted out with 80% saturation of ammonium sulphate. Suspension of proteins was dialyzed in the phosphate buffer and concentrated up to 2 ml of volume. The antibacterial activity of the crude protein extracts was tested by the well-diffusion method, but no definite clear zones were detected.

Another factor that could influence the production of bacteriocins is a deficiency of certain minerals. So strains A11 and B13 were grown in medium supplemented with combination of minerals, which were optimized in our laboratory to the growth and bacteriocin production of bacteria from other genera. It was shown that addition of NaHSO₄ and MnSO₄ to the medium (MnSO₄ is an ingredient of MRSb medium) almost completely inhibits the growth of strain A11, but in the case of strain B13, these minerals only prolong the lag phase of growth. Other used combinations of minerals practically have no influence on the growth of the strains. However, culture supernatants did not possess any antibacterial activity either.

It is known that pH of the growth medium strongly influences to the bacteriocin expression. But no antibacterial activity in culture supernatants after changing the pH of the growth medium from 5.6 to 7.2 was detected.

**OS influence on bacteriocin secretion.** Strain A20 was replaced with LL as indicator strain (Table III.) and the antibacterial activity of A11 and B13 culture supernatants repeatedly grown with different carbon sources (glucose or 1% OS) was evaluated by well-diffusion assay. In this case about 2 mm of diameter unclear transparency zones around the wells with the culture supernatants after 12 and 14 h of growth were detected. The activity of secreted putative bacteriocins was minor – after diluting the supernatants only two times the activity was not detected. LL2, LS, LD and LC strains were also used as indicators but no clear zones had been detected around the wells with A11 and B13 strains grown in liquid medium culture supernatants either.

No antibacterial activity was detected in culture supernatants while analyzing the influence of OS on the type strains, although all type strains, sensitive to the grown strains respectively (Table III.), were used as sensitive. Positive results were obtained only with strains LL2 and LA while using LS and LC strains as indicators.

The antibacterial activity of strain LL2 culture supernatants was tested using LC strain as indicator, as it is characterized by the highest sensitivity to the bacteriocin secreted by LL2 strain (Table III). After 6 h of growth of this strain in the medium with glucose or α-cyclodextrin, the antibacterial activity of culture supernatants was two times higher (80 AU/ml) when grown with αCD, than with glucose (40 AU/ml), although the cell density of the culture grown with glucose at that moment was almost two times bigger, than grown with αCD (1.5 OD and 0.8 OD respectively) (Fig. 1G). These results show that αCD positively influences bacteriocin production or activity. While growing the strain in the medium with inulin or palatinose, the antibacterial activity was detected only after 8 h (20 AU/ml). That suggests that strain LL2 is not capable of assimilating palatinose and inulin and, therefore, the antibacterial activity of culture supernatants is very weak (Fig. 1G). It should be noted that culture supernatant, obtained after 10 h of growth with αCD, maintains the same activity as after 8 h of growth (320 AU/ml), and while growing in the medium with glucose, the activity of culture supernatant decreases two times (Fig. 1G).

Strain LS is characterized by high sensitivity to the antibacterial compound produced by strain LA (Table III). Due to this fact, this strain was used as indicator for evaluation of the antibacterial activity of strain LA grown in the liquid medium. Besides, both these strains can be cultivated in the MRS medium. Diverse influence of OS on the antibacterial activity of LA strain, grown with different carbon sources – glucose, palatinose and inulin was shown (Fig. 1H). During the growth of this strain, the antibacterial activity of culture supernatant occurred in the 8th hour of growth. Culture supernatants of 16, 20 and 24 hours possessed the highest activity (320 AU/ml) during the exponential growth phase of the investigated strain. Cell density of the LA culture after 8 h of growth in the medium with glucose or with palatinose practically does not differ. Although the antibacterial activity of the culture supernatant with palatinose is two times lower, the activities becomes equal after 28 h of growth, when the culture ends the exponential growth in the medium with glucose and is already in the stationary phase in the medium with palatinose (Fig. 1F and H). The cell density of the culture is equal (0.5 OD) after 12 h of growth in the medium with glucose and in the medium with inulin, but the antibacterial activity of the culture supernatant with inulin is 8 times lower. The antibacterial activity decreases two times after 16 h of growth in the medium with inulin and becomes undetectible after 20 h.

It should be noted that the growth of the LA strain measuring the cell density of the cultures in the media with inulin and palatinose only slightly differs (1.25 times) (Fig. 1F). However, the antibacterial
activity of culture supernatants with palatinose at those hours, when it is possible to evaluate the antibacterial activity of the supernatants with inulin, is at least 8 times higher (Fig. 1H).

Discussion

Three oligosaccharides were used in this work: palatinose and inulin, characterized by low caloricity and prebiotic properties, and α-cyclodextrin – practically not investigated as a component of synbiotics. The possibility to use these prebiotics in the development of synbiotics for healthy and especially for diabetic people, may be an effective mean for enhancement of the immune system and prevention of colon infections by pathogenic microorganisms.

Probiotics are the second component of synbiotics. One of the ways by which probiotics may influence proliferation of pathogenic bacteria in the gastrointestinal tract are bacteriocins, secreted by them. In this work, it has been demonstrated that Lactobacillus sp. strains, isolated from "Activia" and "Bif" yogurts, are bacteriocin producers. In solid medium clear transparent zones, indicating growth inhibition of the sensitive strain, independently of whether glucose or OS were used as an only carbon source, were obtained. However, no antibacterial activity was detected in the liquid media. According to the literature, there was shown, that in many cases LAB strains exhibits antibacterial activity on solid but not in liquid media. However, further investigations showed that most of these bacteriocins could be produced also in liquid media, but only under optimized conditions (Maldonado-Barragán et al., 2009). We did not succeed to obtain crude extracts of these bacteriocins, although all methodical procedures usual for bacteriocin extraction were carried out. However, the obtained PCR products suggests that they may be the newly characterized bacteriocins of L. casei ATCC 334 (Kuo et al., 2013). Furthermore, analysis results of the ability to ferment the used carbon sources and influence of microelements on the growth of A11 and B13 strains confirm the distribution of isolated lactobacilli strains into two physiological groups, compatible with BOX-PCR profiles and isolation sources. These results suggest that B group lactobacilli have specific genetic systems enabling them to ferment inulin. However, palatinose is not assimilated by strains belonging to both A and B groups.

In the case of two type L. lactis and two type Lactobacillus strains it was determined that in the growth media with different OS as an only carbon source L. lactis subsp. lactis strains demonstrated good fermentation of α-cyclodextrin, almost equal to that of glucose. However, Lactococcus sp. strains differ in the efficiency of palatinose assimilation – LL stands out of all tested strains for the ability to ferment palatinose. It shows that this strain has specific enzymes, required for the transport and hydrolysis of this disaccharide.

LS strain is totally unable to assimilate inulin and palatinose. On the other hand, LA strain ferments these OS inefficiently. Analysis of the influence of glucose, palatinose, inulin and α-cyclodextrin on the bacteriocin expression of LL and LL2 strains shows that in the case of glucose and α-cyclodextrin bacteriocins appear in the medium in the late stationary phase, which suggests that these antibacterial substances, according to PCR results – possibly nisin, are released into the growth medium during the lysis of cells.

The analysis of isolated lactobacilli in the medium with inulin as an only carbon source show that this OS can be used as a prebiotic to create synbiotics with B group lactobacilli isolated from yogurts. According to the analysis of L. casei genomes, carried out by Broadbent and coworkers (2012), members of this species are able to ferment inulin. This and the results of 16S rDNA analysis suggest that our isolated strain B13 belongs to the L. casei species. Palatinose and LA strain could be used as synbiotics with the effective antibacterial activity. The same property would be characteristic of a synbiotic based on α-cyclodextrin and strain LL2.

The presented results offer the possibility to create synbiotics on the basis of probiotics – Lactobacillus sp. and Lactococcus sp. and prebiotics – palatinose, inulin and α-cyclodextrin, which could regulate not only the growth of different lactobacilli and lactococci, but also the efficiency of their antibacterial activity.

Acknowledgements

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Literature


