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PRELIMINARY CHARACTERISTICS OF *LACTOBACILLUS* AND *BIFIDOBACTERIUM* STRAINS AS PROBIOTIC CANDIDATES

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Microflora inhabiting the gastrointestinal tract of humans is there in dynamic balance that can be disrupted, which in turn leads to diseases. One of the methods applied to restore this balance could be administration of probiotics. Seven *Lactobacillus* and two *Bifidobacterium* strains, isolated from the gastrointestinal tract and bio-yoghurts, were characterised. Two of them – *L. acidophilus* BS and *L. salivarius* AWH revealed good survival at low pH (100% and 90%, respectively) and in the presence of a high concentration of bile salts (89% and 74%, respectively). In addition, they revealed high antagonistic activity against *Salmonella* and coaggregated with this pathogen. The observed autoaggregation and hydrophobic properties of their cell wall suggest their ability to adhere to epithelial cells. The results proved *L. acidophilus* BS and *L. salivarius* AWH strains to be candidates for new probiotics, however, it needs further confirmation under *in vivo* experiments.

INTRODUCTION

The gastrointestinal tract of humans is inhabited by over 500 bacterial species, staying in dynamic balance. Total count of bacteria inhabiting a human's body ($\sim 10^{14}$) is about ten times higher than total counts of cells building the body ($\sim 10^{13}$) [Holzapfel *et al*., 1998]. Intestinal microflora counts about 10^{10} – 10^{11} colony forming units per gram of intestinal digesta, including bacteria beneficial to the host, as *Bifidobacterium* and *Lactobacillus*, opportunistic ones, as for example *E. coli*, and harmful ones, as *Clostridium difficile*. Various factors can influence this balance (antibiotic therapy, radiotherapy, diseases, age, stress or diet), leading to increased counts of potentially harmful bacteria. One of the ways to restore bacterial balance is application of probiotics, defined as live microorganisms which when administered in adequate amounts confer a health benefit on the host [FAO/WHO Report, 2001]. Up-to-date experimental results proved a positive impact of probiotics on gut microflora balance, furthermore, prevention or alleviation of diarrhoea, lactose intolerance and inflammatory bowel disease. Probiotics have also been reported to stimulate the immunological system, counteract allergies, and decrease cholesterol level [Gill & Guarner, 2004]. Most of applied probiotic strains belong to *Lactobacillus* and *Bifidobacterium* genera.

Strains being candidates for probiotics should fulfil criteria specified in Joint FAO/WHO Working Group Report [2002]. They should reveal resistance to gastric acidity and bile salts, adhere to mucus and/or human epithelial cells, display antimicrobial activity against potentially pathogenic bacteria and ability to reduce pathogen adhesion to gut epithelium, and additionally reveal bile salt hydrolase activity.

The main aim of this experiment was to select and characterise the newly isolated strains as probiotic candidates.

MATERIALS AND METHODS

Bacterial strains. *Lactobacillus salivarius* AWH (isolated from chicken crop), *L. acidophilus* BS, *L. helveticus* b9, *L. acidophilus* La5, *L. casei* LcY, *L. plantarum* W42, *L. rhamnosus* K, *Bifidobacterium animalis* 30 (all from commercial bio-yoghurts) and *Bifidobacterium longum* KNA1 (from infant faeces), previously isolated and identified phenotypically as well as genotypically by Bielecka *et al*. [2003] and Wasilewska *et al*. [2003], were used in the study.

The bacteria were stored in skim milk supplemented with 10% glycerol at -70°C. They were multiplied in MRS (lactobacilli) (BTL, Lodz, Poland) under facultatively anaerobic conditions or in modified Garche's broth (bifidobacteria) [Rasic [1990]; g/L: meat peptone – 20, yeast extract – 2, L-cysteine hydrochloride – 0.4, lactose – 10, CH_3COONa – 6, $MgSO_4 \times 12H_2O$ – 0.12; $Na_2HPO_4 \times 12\ H_2O$ – 2.5, K_2HPO_4 – 2, pH 6.5]; under anaerobic conditions (GasPak Anaerobic system BBL, USA) before used in experiments.

Salmonella Enteritidis 491 (isolated from hen), *S.* Enteritidis 458 (from ill patient), and *S.* Typhimurium 35 (from calf) obtained from the Veterinary Hygiene Department and the Sanitary and Epidemiology Station in Olsztyn (Poland), were used as the test strains. *S.* Enteritidis KOS64 was obtained from the Collection of *Salmonella* Microorganisms of the National Salmonella Centre, Institute of Maritime and Tropical Medicine, Gdynia (Poland). *Salmonella* strains were multiplied under aerobic conditions in hydrolysed milk supplemented with 0.3% yeast extract and 0.5% NaCl [Bielecka *et*

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al., 1998]. Their number was determined on MacConkey agar (BTL, Lodz, Poland). All strains were subcultured twice prior to the experiments.

Survival of bacteria at pH 3 and in the presence of bile salts. Survival of the strains at low pH was determined by adjusting acidity of the culture in the stationary phase of their growth to pH 3. In the case of bile salts, at first pH of culture was neutralised to pH 6, then bile salts (Difco) were added till 3% end concentration was obtained. Live cells of the cultures were determined as colony forming units (cfu) using pour plate method. *Lactobac illus* cultures were counted using double layer of MRS agar [Vanderzant & Splittstoesser, 1992]. Garche's agar and incubation in anaerobic conditions (GasPak Anaerobic system BBL, USA) were used for *Bifidobacterium* enumeration. The determinations were performed after 0, 40 and 180 min (survival at low pH) or 0, 1, 3 and 6 h (survival in the presence of bile salts). Survival of the investigated strains was expressed in the percentage calculated from log cfu number/mL in comparison with the control.

Growth in the presence of lysozyme. MRS and Garche's media containing 300 μg/mL of lysozyme were inoculated with 10^6 cfu/mL of *Lactobacillus* and *Bifidobacterium*, respectively. The cultures were incubated for 24 h under facultatively anaerobic (*Lactobacillus*) or anaerobic (pirogalol stoppers) (*Bifidobacterium*) conditions. The growth of strains in the presence of lysozyme was determined by measuring changes of absorbance (OD_{600}) every 3 h.

Antagonistic activity against *Salmonella*. The antagonistic activity against pathogens was studied in the associated cultures. Both the associated cultures and single strains as controls were cultivated in hydrolysed milk (Difco) supplemented with 0.3% yeast extract and 0.5% NaCl. The inoculum of *Lactobacillus* and *Bifidobacterium* was 10^6 cfu/mL, *Salmonella* – 10^5 cfu/mL. The cultures were incubated at 37°C, *Lactobacillus* + *Salmonella* under facultatively anaerobic conditions and *Bifidobacterium* + *Salmonella* under anaerobic one (pirogalol stoppers). After 0, 24 and 48 h counts of live *Salmonella* cells were determined on MacConkey agar (BTL, Lodz, Poland) (incubation – 37°C/24 h under aerobic conditions), *Lactobacillus* – on MRS agar (BTL, Lodz, Poland) with double layer (incubation 37°C/48 h) and *Bifidobacterium* – on Garche's agar (incubation – 37°C/48 h under anaerobic conditions). The antagonistic activity of the investigated strains was also determined in the associated cultures with stable pH at a level of 6.4±0.3.

Autoaggregation assay. Ability of the investigated strains to autoaggregation was determined according to the procedure described by Del Re *et al*. [2000]. Active cultures of *Lactobacillus* were multiplied in MRS medium, *Bifidobacterium* in Garche's medium until the stationary phase of growth was reached.

Then, the cells were harvested by centrifugation at 5000 *g* for 15 min, washed twice and resuspended in phosphate buffered saline (PBS) to get viable counts of approximately 10^8 cfu/mL. Cell suspensions (4 mL) were mixed by vortexing for 10 s and autoaggregation was determined during 5 h of incubation at room temperature. Every hour, 0.1 mL of the upper suspension was transferred to another tube with 3.9 mL of PBS and the absorbance (A) was measured at 600 nm. The per cent of aggregated cells was expressed as:

$$\text{Autoaggregation, (\%)} = [1 - (A_t - A_0)] \times 100,$$

where A_t – absorbance at time t, A_0 – absorbance at time 0.

Coaggregation assay. The method for preparing the cell suspensions for coaggregation was the same as that for autoaggregation assay. Test *Salmonella* strains (*S*. Enteritidis 491, *S*. Enteritidis 458 and *S*. Enteritidis KOS64) were multiplied in supplemented hydrolysed milk until the stationary phase of growth. Equal volumes (2 mL) of each cell suspension were mixed together. Control tubes were set up at the same time, containing 4 mL of each bacterial suspension on its own. The absorbance (A) at 600 nm of the suspensions was measured after mixing and after 5 h of incubation at room temperature. Samples were taken in the same way as in the autoaggregation assay. The coaggregation percentage was expressed as:

$$\text{Coaggregation, (\%)} = [((Ax + Ay)/2 - A(x+y))/((Ax + Ay)/2)] \times 100,$$

where x and y represents single strains, and (x+y) mixed strains.

TABLE 1. Survival of *Lactobacillus* and *Bifidobacterium* strains at pH 3.

Strain	Count of bacteria (log cfu/mL)				Survival after 180 min
	before decreasing pH	at pH 3			
	0	0	40 min	180 min	%
L. salivarius AWH	9.00±0.18	8.72±0.37	8.30±0.60	8.07±0.34	90
L. acidophilus BS	9.06±0.01	9.04±0.01	9.11±0.10	9.11±0.12	100
L. helveticus b_9	8.99±0.55	8.69±0.04	8.69±0.06	8.57±0.05	95
L. acidophilus La5	8.87±0.09	8.81±0.04	8.70±0.07	8.42±0.15*	95
L. casei LcY	9.36±0.07	9.35±0.02	9.28±0.05	9.28±0.04	99
L. plantarum W42	8.94±0.21	9.07±0.08	9.06±0.13	8.97±0.18	100
L. rhamnosus K	9.10±0.05	9.02±0.04	8.51±0.54	8.98±0.06	99
B. longum KNA1	8.47±0.15	8.47±0.17	7.86±0.10	7.86±0.09	93
B. animalis 30	9.18±0.03	9.17±0.06	9.19±0.04	9.29±0.06	100

Significance level: * $p \le 0.05$, $n=3$

TABLE 2. Survival of *Lactobacillus* and *Bifidobacterium* strains in the presence of 3% bile salts.

Strain	Count of bacteria (log cfu/mL)					Survival after 6 h
	before addition of bile salts	after addition of bile salts				
	0	0 h	1 h	3 h	6 h	%
L. salivarius AWH	9.07±0.04	8.25±0.00***	7.30±0.02***	7.16±0.01***	6.74±0.06 ***	74
L. acidophilus BS	8.77±0.14	8.43±0.22	8.15±0.08 **	7.83±0.09 **	7.81±0.10 **	89
L. helveticus b9	8.50±0.23	7.41±0.02 **	2.55±0.13***	2.12±0.14***	0.00±0.00 ***	0
L. acidophilus La5	8.76±0.06	8.70±0.06	8.68±0.05	8.59±0.07	8.57±0.04*	98
L. casei LcY	9.13±0.08	9.28±0.03	6.61±0.25***	6.62±0.10***	6.53±0.10***	72
L. plantarum W42	9.01±0.03	8.90±0.07	8.68±0.02***	8.27±0.26*	8.02±0.09***	89
L. rhamnosus K	9.15±0.03	9.13±0.04	7.07±0.18***	4.97±0.31***	4.93±0.07***	54
B. longum KNA1	8.80±0.08	7.00±0.44	6.14±0.50*	5.04±0.48**	4.48±0.54*	51
B. animalis 30	9.07±0.18	9.10±0.18	9.00±0.23	8.89±0.23	8.51±0.21	94

Significance level: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, $n=3$

Measurement of bacterial hydrophobicity. Hydrophobicity of the investigated bacteria was determined due to microbial adhesion to hydrocarbons. The cells were harvested by centrifugation at 5000 *g* for 15 min, washed twice in phosphate buffered saline and diluted in the same buffer to absorbance (A) 0.5 at 600 nm. A 0.6-mL portion of *n*-hexadecane (Sigma) was added to 3 mL of the bacteria. The tube was vortexed for 1 min. After 1-h incubation at 37°C, the OD_{600} of the water phase was determined. The hydrophobicity percentage was expressed as:

$$\text{Hydrophobicity, } (\%) = [(A_0 - A)/A_0] \times 100,$$

where: A_0 – absorbance before adding *n*-hexadecane, A – absorbance after 1 h of incubation.

Statistical analysis. The results were prepared in at least 3 replicates, expressed as log colony forming units (cfu) number per milliliter and subjected to statistical analysis using *t*-Student test (Microsoft Excel).

RESULTS AND DISCUSSION

Survival of *Lactobacillus* and *Bifidobacterium* strains during passage through the upper part of the gastrointestinal tract is essential for their probiotic action. The most negative physiological factors affecting bacterial cells are low pH of gastric juice and high concentration of bile salts in the proximal part of the small intestine. All examined strains survived at 90–100% during 3 h of exposure to pH 3 (Table 1). *B. animalis* 30, *L. acidophilus* BS and *L. plantarum* W42 were characterised by the highest survival, and *L. salivarius* AWH – by the lowest. Three per cent of bile salts were a crucial factor for determination of survival of investigated strains. They survived at 0-98% (Table 2). *L.* acidophilus La5 and *B. animalis* 30 proved to be the best surviving, *L. acidophilus* BS and *L. plantarum* W42 survived at 89%, and the most sensitive one was *L. helveticus* b9. The population number of majority of the investigated strains decreased gradually during exposure to bile salts, except for *L. helveticus* b9 whose live cell number was reduced to the greatest extent during the first hour of exposition. Growth of majority of the investigated strains was not affected by lysozyme, however *B. longum* KNA1 and *L. rhamnosus* K were slightly or highly inhibited, respectively, and *L. helveticus* b9 and *B. animalis* 30 were completely inhibited (Figure 1). The results obtained so far suggest that majority of the newly isolated strains are resistant to low pH, bile salts and lysozyme, so that they could survive passage through the upper part of the gastrointestinal tract and reveal their potential probiotic action on host organism.

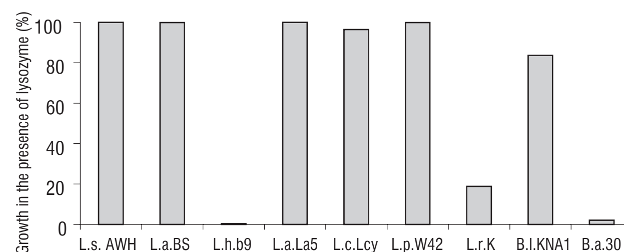

FIGURE 1. Bacterial growth in the presence of 300 μg/mL of lysozyme.

All the strains examined revealed antagonistic activity against *Salmonella* strains, inhibiting the growth and reducing the number of pathogen population in the associated cultures (Tables 3–5). After 48 h, the population of *S*. Enteritidis 491, *S*. Enteritidis 458 and *S*. Typhimurium 35 in the monocultures reached 9.10±0.04, 9.06±0.01 and 9.00±0.19 log cfu/mL, respectively. In the associated cultures with *L. salivarius* AWH, *L. acidophilus* BS or *L. helveticus* b9, all *Salmonella* populations were completely inactivated during 24 h. Complete inactivation of each *Salmonella* strain by *B. longum* KNA1 was achieved after 48 h of incubation. *B. animalis* 30 and *L. plantarum* W42 revealed the weakest antagonistic activities, however, each of them caused a sudden decrease in pathogen live cell counts. All the investigated strains showed antibacterial activity, but bactericidal activity was proved only for few of them. The main factors causing pathogen growth inhibition were probably organic acids

TABLE 3. Reduction of *Salmonella* Enteritidis 491 population in the associated cultures with *Lactobacillus* and *Bifidobacterium.*

Strain	Inoculum	*S.* Enteritidis 491				pH	
		Count (log cfu/mL)		Degree of reduction (%)			
	0 h	24 h	48 h	24 h	48 h	24 h	48 h
S. Enteritidis 491	5.44±0.12	9.09±0.13	9.10±0.14			6.16	6.15
L. salivarius AWH	6.78±0.00	0.00±0.00	0.00±0.00	100	100	4.13	4.09
L. acidophilus BS	6.45±0.01	0.00±0.00	0.00±0.00	100	100	3.62	3.48
L. helveticus b9	6.08 ±0.15	0.00±0.00	0.00±0.00	100	100	3.58	3.43
L. acidophilus La5	6.33±0.01	3.48±0.00	0.00±0.00	62	100	3.62	3.48
L. casei LcY	6.70±0.20	1.94±0.00	0.00±0.00	79	100	4.20	3.87
L. plantarum W42	5.90±0.60	5.59±0.00	0.23±0.00	39	79	4.37	3.91
L. rhamnosus K	6.73±0.10	5.08±0.00	0.00±0.00	44	100	4.25	3.83
B. longum KNA1	6.39 ±0.09	1.39±0.26	0.00±0.00	85	100	4.26	4.18
B. animalis 30	6.09±0.13	5.20±0.24	1.99±0.09	43	78	4.64	4.28

n=3

TABLE 4. Reduction of *Salmonella* Enteritidis 458 population in the associated cultures with *Lactobacillus* and *Bifidobacterium*

Strain	Inoculum	*S.* Enteritidis 458				pH	
		Count (log cfu/mL)		Degree of reduction (%)			
	0 h	24 h	48 h	24 h	48 h	24 h	48 h
S. Enteritidis 458	5.43±0.09	8.93±0.13	9.06±0.01			6.14	6.20
L. salivarius AWH	6.63±0.08	0.00±0.00	0.00±0.00	100	100	4.26	4.25
L. acidophilus BS	6.48±0.06	0.00±0.00	0.00±0.00	100	100	3.70	3.55
L. helveticus b9	5.92±0.43	0.00±0.00	0.00±0.00	100	100	3.62	3.52
L. acidophilus La5	6.40±0.06	2.92±2.71	2.44±2.33	67	73	3.93	3.82
L. casei LcY	6.60±0.05	5.59±0.07	1.43±1.24	37	84	4.23	3.90
L. plantarum W42	6.67±0.07	6.29±0.37	0.98±1.69	30	89	4.44	3.95
L. rhamnosus K	6.41±0.36	6.10±0.64	1.74±1.98	32	81	4.42	3.88
B. longum KNA1	6.39±0.09	2.40±0.02	0.00±0.00	73	100	4.37	4.20
B. animalis 30	6.09±0.13	3.60±0.02	1.99±0.00	60	78	4.65	4.28

n=3

TABLE 5. Reduction of *Salmonella* Typhimurium 35 population in the associated cultures with *Lactobacillus* and *Bifidobacterium*.

Strain	Inoculum	*S.* Typhimurium 35				pH	
		Count (log cfu/mL)		Degree of reduction (%)			
	0 h	24 h	48 h	24 h	48 h	24 h	48 h
S. Typhimurium 35	5.34±0.29	8.81±0.27	9.00±0.19			5.92	5.90
L. salivarius AWH	6.67±0.25	0.00±0.00	0.00±0.00	100	100	4.20	4.16
L. acidophilus BS	6.65±0.05	0.00±0.00	0.00±0.00	100	100	3.68	3.54
L. helveticus b9	5.84±0.00	0.00±0.00	0.00±0.00	100	100	3.61	3.47
L. acidophilus La5	6.37±0.07	2.72±2.47	1.39±2.41	69	85	3.94	3.76
L. casei LcY	6.88±0.03	4.93±0.77	3.07±2.66	44	66	4.27	3.91
L. plantarum W42	6.52±0.07	5.85±0.43	4.81±0.69	34	47	4.49	4.02
L. rhamnosus K	6.76±0.00	5.91±0.09	0.97±1.95	33	89	4.35	3.88
B. longum KNA1	6.39±0.09	2.45±0.19	0.00±0.00	72	100	4.38	4.21
B. animalis 30	6.09±013	7.72±0.10	3.35±0.11	15	63	4.70	4.37

n=3

produced by lactic acid bacteria, leading to a decrease in pH of the culture. Biedrzycka & Bielecka [2002] proved that the dynamics and rate of medium acidification were the significant factors determining the degree of *Salmonella* reduction

TABLE 6. Reduction of *Salmonella* Enteritidis 491 population in the associated cultures with *Lactobacillus* or *Bifidobacterium* under neutralization (pH 6.4).

Strain	Count (log cfu/mL)			
	inoculum *S/P*	24 h	48 h	72 h
Salmonella Enteritidis 491	5.40±0.06	8.97±0.04	9.15±0.05	9.08±0.01
L. salivarius AWH	6.66±0.12	8.76±0.16	9.14±0.01	9.13±0.06
L. acidophilus BS	6.42±0.02	8.60±0.16	9.07±0.09	9.22±0.07*
L. helveticus b9	6.50±0.01	8.78±0.03**	9.13±0.02	8.99±0.07
L. acidophilus La5	6.46±0.04	8.62±0.01***	8.92±0.01**	9.14±0.01*
L. casei LcY	6.64±0.17	8.40±0.10***	8.78±0.19*	9.01±0.08
L. plantarum W42	6.52±0.03	8.60±0.02***	8.96±0.02**	9.03±0.01*
L. rhamnosus K	6.59±0.01	8.72±0.02***	8.77±0.03***	9.10±0.02
B. longum KNA1	6.54±0.04	8.98±0.03	9.00±0.04*	8.99±0.02**
B. animalis 30	6.56±0.01	9.07±0.04	9.09±0.08	9.03±0.06

Significance level: * p≤0.05, ** p≤0.01, *** p≤0.001, n=3

in the associated cultures. No dramatic decrease in counts of live pathogen cells was observed in the associated cultures upon neutralisation (Table 6). Although, there were significant differences between counts of *Salmonella* in the control and the associated cultures after 24 h, they were significantly diminished after 48 and 72 h. The mechanism of antibacterial action of probiotic strains has not been completely elucidated yet, however, its multiplex character guarantees efficiency [Bielecka *et al.*, 1998; Tannock, 2002].

The ability of strains to autoaggregation seems to be an essential prerequisite for the adhesion of bacterial cells to intestinal epithelium, whereas their coaggregation abilities with pathogens enable forming the effective barrier that prevents colonisation of epithelium by harmful bacteria [Boris *et al.*, 1997; Del Re *et al.*, 2000]. Autoaggregation and coaggregation abilities increase a chance of bacterial maintenance in the gastrointestinal tract. Among the investigated strains, *L. helveticus* b9 and *L. acidophilus* BS revealed the highest autoaggregating properties (80.29±1.67% and 77.58±1.46%, respectively), and *B. animalis* 30 – the lowest ones (Figure 2). Differences in the aggregation abilities between species belonging to the same genera or strains belonging to the same species were reported by others authors. Garriga *et al.* [1998] observed autoaggregation properties amongst 12 out of 28 examined *Lactobacillus* strains isolated from chicken crop and 1 out of 7 strains isolated from intestinal digesta. Ehrmann *et al.* [2002] studied aggregation properties of bacteria isolated from ducks' gastrointestinal tract. The screening of 112 strains of lactic acid bacteria, isolated from the crop and caecal digesta of 12 ducks, showed that 31 strains were characterised by significant autoaggregation properties. Bacteria belonging to *Lactobacillus* genera revealed strong autoaggregation properties, whereas *Enterococcus*, *Weissella* and *Pediococcus* – negligible ones or no autoaggregation.

Considerable differences in the coaggregation abilities were observed between the investigated strains. *L. acidophilus* BS strain was characterised by the best coaggregation properties – the strain coaggregated with all the tested *Salmonella* strains. *L. salivarius* AWH coaggregated with two *Salmonella* strains (491 and 458), whereas *B. animalis* 30 only with *S*. Enteritidis 458 (Table 7). The abilities of *Lactobacillus* and *Bifidobacterium* to coaggregate with pathogens were

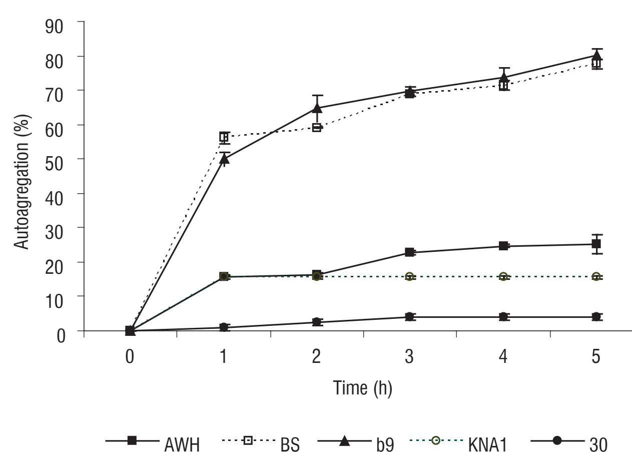

FIGURE 2. Aggregation ability of the investigated strains.
Abbreviations: AWH – *L. salivarius* AWH, BS – *L. acidophilus* BS, b9 - *L. helveticus* b9, KNA1 – *B.* longum KNA1, 30 – *B. animalis* 30, error bars represent standard deviations (SD), *n*=3.

observed by other authors. Bujnakova *et al.* [2004] observed coaggregation between 4 out of 6 investigated autoaggregating *Lactobacillus* strains and 3 out of 5 autoaggregating *Bifidobacterium* strains and enterohaemorrhagic *E. coli* O157: H7. The results suggest that abilities of bacteria to coaggregation and their antimicrobial properties are a strain-dependent feature.

Hydrophobicity of bacterial cell wall was determined on the basis of their adhesion to the hydrocarbon phase of solution containing *n*-hexadecane [Perez *et al.*, 1998; Vinderola *et al.*, 2004]. The highest hydrophobic properties were revealed by *L. salivarius* AWH (90% of cells adhered to hydrocarbon) and *L. acidophilus* BS (81%), whereas the lowest ones - by *L. helveticus* b9 (21%). These results are convergent with data obtained by Kmet & Lucchini [1997] due to which the adhesion of cells of 4 investigated *Lactobacillus* strains to *n*-hexadecane varied between 49 and 69%. Lee & Poung [2002] showed that commercial strains of *L. rhamnosus* GG and *L. casei* Shirota revealed high hydrophobicity along with 73.7% and 66.7% adhesion to *n*-hexadecane, respectively. Gusils *et al.* [1999] also observed significant differences

TABLE 7. Coaggregation of *Lactobacillus* and *Bifidobacterium* strains with *Salmonella* and cell surface hydrophobicity measured by microbial adhesion to hydrocarbons.

Strain	Coaggregation with *Salmonella* strain (%)			Hydrophobicity (%)
	S. Enteritidis 491	*S.* Enteritidis 458	*S.* Enteritidis KOS 64	
L. salivarius AWH	19±0.63	6±2.16	-	90±0.64
L. acidophilus BS	46±7.59	77±1.71	82±6.53	81±0.70
L. helveticus b9	-	-	-	21±0.51
B. longum KNA1	-	-	-	16±0.29
B. animalis 30	-	9±0.94	-	62±0.62

n=3

between hydrophobicity of *Lactobacillus* strains (*L. fermentum* – 23%, *L. animalis* – 50%, *L. fermentum* subsp. *cellobiosus* – 75%), suggesting a wide variability among strains belonging to the same species and between species of *Lactobacillus* genus.

Del Re *et al.* [2000] and Ehrmann *et al.* [2002] reported on the correlation between abilities of adhesion to epithelium as well as their autoaggregation and hydrophobicity, as measured by microbial adhesion to hydrocarbons (*n*-hexadecane). These last two bacterial properties could be used for preliminary screening to identify potentially adherent bacteria. The observed in the present work high hydrophobicity of *L. salivarius* AWH and *L. acidophilus* BS cells in connection with their autoaggregation abilities suggest their potential properties of adhesion to epithelial cells.

CONCLUSIONS

Good survival at low pH and in the presence of bile salts, high resistance to lysozyme, high autoaggregation, coaggregation with pathogen and hydrophobic properties of cell wall suggesting adhesive potential, which in connection with bactericidic properties against *Salmonella*, predestine *Lactobacillus acidophilus* BS and *L. salivarius* AWH strains as good probiotic candidates to be confirmed under further *in vivo* studies.

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WSTĘPNA CHARAKTERYSTYKA SZCZEPÓW *LACTOBACILLUS* I *BIFIDOBACTERIUM* JAKO KANDYDATÓW NA PROBIOTYKI

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Mikroflora zasiedlająca przewód pokarmowy człowieka znajduje się w dynamicznej równowadze, która może ulec zachwianiu pod wpływem różnych czynników, główne antybiotyków, infekcji, niewłaściwej diety czy stresów. Najlepszym ze znanych dotychczas sposobów przywrócenia tej równowagi jest zastosowanie odpowiednich pod względem jakościowym i ilościowym probiotyków. W niniejszej pracy scharakteryzowano wcześniej wyizolowane z przewodu pokarmowego oraz zidentyfikowane fenotypowo i genotypowo szczepy *Lactobacillus* i *Bifidobacterium*. *L. acidophilus* BS i *L. salivarius* AWH dobrze przeżywały (odpowiednio 100 i 90%) w pH 3, w obecności 3% soli żółciowych (odpowiednio 89 i 74%) oraz charakteryzowały się właściwościami bakteriobójczymi lub bakteriostatycznymi wobec *Salmonella* Enteritidis 491 w hodowlach wspólnych, jak również zdolnością do koagregacji z komórkami tego patogenu. Zdolność komórek badanych szczepów do autoagregacji i hydrofobowe właściwości ich ściany komórkowej sugerują możliwość adhezji do nabłonka jelitowego. Uzyskane wyniki wskazują, że szczepy *L. acidophilus* BS i *L. salivarius* AWH są potencjalnymi kandydatami na probiotyki. Wymagają one jednak potwierdzenia efektywności ich działania w warunkach *in vivo*.