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Bifidobacterium strains inhabiting the gastrointestinal tract of rat as potential probiotics for animals*

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ABSTRACT

The aim of the study was to evaluate *in vitro* the potential probiotic functionality in the gut of animal-originated bifidobacteria strains. Eleven *B. animalis* and five *B. pseudolongum* strains isolated from Wistar rats were characterized for their tolerance to low pH and bile, and seven *B. animalis* and four *B. pseudolongum* strains for their ability to adhere to HT-29 cells and mucus. The strains showed high resistance to three-hour exposure to pH 3 (34-100% of the population survived) and six-hour exposure to the presence of 3% bile (28-93%), as well as differentiated adhesive abilities. The number of bacterial cells attached to 100 HT-29 cells ranged from 9 to 33 and from 17 to 645 for *B. pseudolongum* and *B. animalis* strains, respectively, whereas corresponding levels of adherence per square centimetre of polystyrene-immobilized mucin ranged from 3×10^5 to 4×10^5 and from 5×10^5 to 889×10^5 bacterial cells. Three *B. animalis* strains (KSP4, PS46 and PS11) resistant to low pH and bile, well-adhering to epithelial cells and mucus were selected as potential probiotics for animals and will be evaluated in future *in vivo* studies.

KEY WORDS: rat, probiotics, gut microflora, acid tolerance, bile resistance, adhesion, *Bifidobacterium*

INTRODUCTION

Probiotics are live microorganisms that, when administered through the digestive tract, positively affect the host's health. A variety of microbial species

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have been used as probiotics in human and animal nutrition, among which *Lactobacillus* and *Bifidobacterium* strains are most frequently used for humans, whereas *Bacillus, Enterococcus, Lactobacillus, Pediococcus, Streptococcus* and *Saccharomyces* yeast are applied as feed additives for livestock (Tannock, 1999; Dunne, 2001; Simon et al., 2001; Griggs and Jacob, 2005; Anadon et al., 2006; Taras et al., 2006). Only a few reports exist concerning the use of bifidobacteria in animal feeding (Mengheri et al., 1991; Abe et al., 1995; Meng et al., 1998; Griggs and Jacob, 2005).

Bifidobacteria are Gram-positive, rod-shaped, anaerobic bacteria that inhabit the gastrointestinal tract of humans and animals, where they constitute a part of the dominant anaerobic flora beneficially affecting host health (Bezkorovainy and Miller-Catchpole, 1989). For years, generally recognized as safe (GRAS), bifidobacteria have been used as active ingredients in functional food products. Studies on the role of bifidobacteria in the maintenance of host health have revealed that probiotic strains not only counteracted proliferation of opportunistic and harmful bacteria and reduced diarrhoea incidence, but also positively influenced digestion, host metabolism and immunity (Tannock, 1999; Dunne, 2001). The use of bifidobacteria in animal feeding could significantly support animal breeding.

Much knowledge about the function of probiotic bifidobacteria is based, however, upon work done in humans, whereas the results might not always be the same in animals. Furthermore, it is generally accepted that probiotic effectiveness is only achieved with a sufficient dose of living cells. Surveys of probiotic products on the market have revealed deficiencies in the viability of probiotic strains, especially in those containing bifidobacteria (Fasoli et al., 2003; Masco et al., 2005). Since probiotic cultures may encounter acidic conditions both in technological processes and during gastric transit, tolerance to low pH is a critical factor influencing probiotic functionality in the gut. Natural bacterial habitats are settled by different species, thus, it is also of paramount importance that the candidates for probiotics should be selected from the host species, especially if colonization is essential to gain the ultimate goal of application of the probiotic (Fuller, 1992). Therefore, studies on the selection of new strains highly resistant to gastrointestinal and technological conditions able to colonize a host's intestine seem to be of great importance.

Most of the studies on probiotics have focused on the selection of probiotic strains designed for humans. There are only a few reports suggesting that animal-originated bifidobacteria such as *B. animalis* and *B. pseudolongum* strains may play a role in intestinal mucosal defence (Bezkorovainy and Miller-Catchpole, 1989; Mengheri et al., 1991; Meng et al., 1998). Likewise, to the best of our knowledge, there is no previous publication encompassing a larger number of animal-originated strains in terms of their sensitivity to gastrointestinal conditions

or adhesive abilities. Some authors have described the resistance to unfavourable gastric conditions of *B. animalis* subsp. *lactis* strains, closely related to *B. animalis* subsp. *animalis* species inhabiting animal intestines (Masco et al., 2004, 2005, 2007). Our previous investigations revealed that the rat's colon harbours bifidobacteria from the species of *B. animalis* and *B. pseudolongum,* with the first being predominant (Wasilewska et al., 2003).

The aim of the present study was to characterize strains of *B. animalis* and *B. pseudolongum* for their sensitivity to an acidic environment and presence of bile as well as their ability to adhere to HT-29 epithelial-like cells and mucin. Although these assays are not fully sufficient to predict the functionality of strains in the gut, they are considered as the most important prerequisites in the selection of *Bifidobacterium* strains to be used as probiotics.

MATERIAL AND METHODS

Bacterial strains and culture conditions

All bifidobacteria strains tested (see Table 1) were isolated in our laboratory from Wistar rats and identified to species using phenotypic and molecular methods (Wasilewska et al., 2003).

Strains were maintained frozen at -70°C in reconstituted skim milk (5% dry wt.) supplemented with sucrose (10%) at a ratio of 1:2. Before every experiment, strains from frozen stocks were subcultured twice in Garche's broth (Teraguchi et al., 1982), containing in grams per litre: bacto peptone 20.0, yeast extract 2.0, L-cysteine hydrochloride 0.4, lactose 10.0, CH_3COONa 6.0, $MgSO_4 \times 7\ H_2O$ 0.12, $Na_2HPO_4 \times 12\ H_2O$ 2.5, KH_2PO_4 2.0, and agar 1.0; pH after sterilization and cooling, 6.4. The broth was inoculated with 10^6-10^7 bifidobacterial cells (3% inoculum) and incubated in anaerobic jars (Gas Pak anaerobic system $H_2 + CO_2$, Oxoid, UK) at 37°C until the stationary phase was achieved (pH ~4.4).

Tolerance to low pH

Active cultures were thoroughly mixed and transferred quantitatively into two test tubes, in the first, the pH was adjusted to 3.0 with sterile 1 N HCl, whereas into the second (control) an equivalent volume of sterile distilled water was added. Both tubes were incubated in a tissue culture incubator (Cytoperm 8088, Hereus Instruments GmbH, Austria) in an atmosphere with lowered oxygen content (5% O_2 and 22% CO_2) at 37°C for 3 h. Numbers of live bacterial cells were counted as colony forming units (CFU) per ml on Garche's agar. The cultures were neutralized

with 1 N NaOH before plating. The plates were incubated anaerobically (Gas Pak anaerobic system) at 37°C for 48 h. Survival was calculated as the percentage of surviving population relative to the control population.

Resistance to bile salts

The procedure was similar as described above, however, the pH of the cultures was adjusted to 6 with 1 N NaOH before transferring into tubes and then a 15% solution of ox bile (Sigma) was added to a final concentration of 3%. The exposure to bile lasted for 6 h.

Adhesion to HT-29 cells

Seven *B. animalis* and four *B. pseudolongum* strains listed in Table 3 were tested for their adhesion to epithelial cells. Epithelium-like HT-29 cell line was purchased from Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ). HT-29 cells were grown routinely in Dulbecco's modified Eagle's minimal essential medium (DMEM) (Gibco BRL) supplemented with 2 mmol/l of L-glutamine, 1% (v/v) of non-essential amino acids and 10% (v/v) of inactivated (30 min, 56°C) foetal calf serum (Gibco BRL). For all experiments, cells were cultured in a tissue culture incubator (Cytoperm 8088, Hereus Instruments GmbH, Austria) at 37°C in a 5% CO_2 - 95% air atmosphere and 80% humidity.

For the bacterial adhesion assay, HT-29 cells were inoculated at a concentration of 2×10^4 cells per cm^2 of glass coverslips placed in six-well tissue-culture plates (Nunc, Germany). The culture medium was changed every other day and well differentiated monolayers were used at a post-confluence phase after 18-20 days of incubation.

Overnight bifidobacterial cultures (24 h-old) were harvested by centrifugation at 5000 *g* for 10 min, washed in sterile phosphate buffer saline (PBS, pH 7.2) and resuspended in a mixture of modified Garche's broth (specially prepared according to a tissue culture procedure) and Dulbecco's MEM medium (at a ratio of 1:1) to a final concentration of 1×10^9 CFU per ml (OD_{550}=1.2). The bacterial suspensions were added to the monolayers washed twice with PBS buffer in the amount of about 1×10^8 bacterial cells per cm^2 of a well, and incubated for 1 h under conditions described for HT-29 cells. After incubation, the monolayers were washed five times with sterile PBS to remove non-adherent bacteria, preserved in graded methanol series: 25, 50, 75 and 96% (v/v) by immersing for 5 min in each solution, next Gram stained and analysed microscopically. Adherence was evaluated in 20 random microscopic fields and the number of bacterial cells attached per 100 epithelial cells was calculated. Each experiment was conducted in duplicate.

Binding to mucus

The strains tested towards adhesion to mucus are listed in Table 3. Adhesion to mucus was determined according to the method described by Cohen and Laux (1995) with some modification. In brief, 1 mg/ml of porcine mucin (Sigma; M2378) was diluted in HEPES (*N-2*-hydroxy-ethyl-piperazine-N'-2-ethanesulfonic acid)-Hank's buffer (HH; 10 mM HEPES; pH 7.4) and immobilized with 250 µl on polystyrene microtitre 24-well plates (Nunc) by overnight incubation at 4°C.

Freshly multiplied bacterial strains were harvested by centrifugation (5000 *g* for 10 min), washed twice with HH buffer and resuspended in HH. The optical density of bacterial suspensions at 600 nm was adjusted to 0.5 ± 0.02 in order to standardize the number of bacteria (1×10^8-2.5×10^8 CFU per ml).

The wells with immobilized mucus were washed twice with 500 µl HH to remove excess mucus. Afterwards, a suspension of 250 µl of each strain was added to two parallel wells. After 1 h incubation at 37°C, the wells were washed twice with 500 µl HH to remove unattached cells and thereafter the number of bacterial cells attached to mucus was determined. To this end, 1 ml of Triton X-100 (1% solution in HH buffer) was added to each well, next the wells were left for 5 min at room temperature and thoroughly scrapped with a sterile pipette tip in order to release all the attached bacterial cells to the suspension. Since it was impossible to scoop out the whole suspension inside the well, and also in order to correct for intra-assay variation, 0.5 ml of the bacterial suspension from two parallel wells were mixed together and analysed as a one trial. The number of bifidobacteria attached per well was counted as CFU using the pour plate method. Incubation of the plates was carried out at 37°C for 48 h under anaerobic conditions. Each adhesion assay was performed in duplicate. The final results were expressed as the number of bacterial cells attached per cm^2 of the surface covered with mucin.

Scanning electron microscopy

Monolayers of the epithelial cells on glass coverslips with the attached bacterial cells were used for scanning electron microscopic (SEM) studies. After the adhesion assay the monolayers were fixed with 2.5% glutaraldehyde for 1 h at room temperature, washed twice in PBS and postfixed in 2% OsO_4 for 30 min. Afterwards, the samples were washed three times in PBS and dehydrated in a graded acetone series: 25, 50, 75, 90 and three times in 100% (v/v) by immersing for 5 min in each solution, and then dried at the critical point using liquid CO_2 as the transition medium. Dehydrated samples were coated with gold in a vacuum evaporator (JOEL, JEE-4X) and examined with a JEOL 1200EX TEMSCAN electron microscope equipped with an EM-ASID10 scanning device, at 80 kV.

Statistical analysis

All results are presented as the average of at least two independent experiments with standard deviation. Each experiment was performed with two parallels to correct for an intra-assay variation. A simple correlation coefficient was calculated to measure the correlation between the features tested.

RESULTS

Eleven *B. animalis* and five *B. pseudolongum* strains isolated from rat faeces were tested for their sensitivity to low pH or the presence of bile. The results showing the behaviour of the strains in the presence of stress factors are reported in Tables 1 and 2. After 3-h exposure to pH 3, the survival of all the *Bifidobacterium* strains ranged from 34 to 102% of the population (Table 1). A comparison of inhibitory effects of bile salts on the strains yielded similar results. After 6-h exposure to the presence of 3% of ox bile in the medium, the number of live cells decreased about 1.1 to 3.6-times, which means 28-93% survivability of bacterial cells (Table 2). No significant correlation was found between the two tested features (r=-0.17).

Table 1. Viability of the tested *Bifidobacterium* strains after exposure to low pH[1]

Strain	Origin[2]	Number of *Bifidobacterium* cells, CFU/ml, $\times 10^7$		Survival[3] %
		in control	after exposure to low pH	
B. animalis PS11	A/duodenum	69.2 ± 6.8[4]	70.8 ± 4.6	102
B. animalis PS37	A/caecum	44.7 ± 7.3	45.7 ± 0.1	102
B. animalis PS46	A/caecum	55.0 ± 3.6	56.3 ± 3.7	102
B. animalis KSP4	B/faeces	55.7 ± 4.5	26.6 ± 4.5	48
B. animalis KSP5	B/faeces	61.7 ± 1.0	61.7 ± 0.4	100
B. animalis KSP6	B/faeces	35.5 ± 2.1	25.1 ± 1.3	71
B. animalis KS7	C faeces	41.7 ± 2.7	33.1 ± 2.2	79
B. animalis KS1b2	D/faeces	20.4 ± 3.4	6.9 ± 0.7	34
B. animalis KS7d3	D/faeces	40.7 ± 2.4	31.6 ± 0.6	78
B. animalis KS20a1	D/faeces	38.9 ± 2.5	20.4 ± 1.9	52
B. animalis KS29a3	D/faeces	63.2 ± 4.1	63.2 ± 4.1	100
B. pseudolongum PS14	A/colon	76.0 ± 7.4	26.5 ± 4.3	35
B. pseudolongum PS34	A/caecum	28.8 ± 1.1	30.9 ± 1.6	107
B. pseudolongum PS36	A/caecum	48.1 ± 6.2	28.0 ± 1.1	58
B. pseudolongum PS85	A/jejunum	38.5 ± 1.9	39.4 ± 1.9	102
B. pseudolongum KSI-9	E/faeces	133.6 ± 10.9	47.0 ± 6.1	35

[1] the strains were exposed to pH 3 for 3 h; [2] the capital letter denotes the source rat; [3] survival is shown as percentage of surviving population relative to population in control tube; [4] mean ± standard deviation

Table 2. Viability of the tested *Bifidobacterium* strains after exposure to bile salts[1]

Strain	Number of *Bifidobacterium* cells, CFU/ml, $\times 10^7$		Survival[2]
	in control	after exposure to bile salts	%
B. animalis PS11	43.3 ± 4.9[3]	34.0 ± 3.3	79
B. animalis PS37	26.0 ± 5.9	16.0 ± 5.6	62
B. animalis PS46	17.5 ± 3.4	14.7 ± 3.8	84
B. animalis KSP4	45.4 ± 6.6	37.2 ± 3.6	82
B. animalis KSP5	38.9 ± 1.3	25.1± 3.0	65
B. animalis KSP6	66.1 ± 2.3	50.1 ± 3.0	76
B. animalis KS7	63.2 ± 4.1	47.0 ± 6.1	74
B. animalis KS1b2	17.8 ± 4.0	16.6 ± 2.0	93
B. animalis KS7d3	23.4 ± 3.7	15.8 ± 2.6	68
B. animalis KS20a1	38.1 ± 3.7	26.3 ± 0.9	69
B. animalis KS29a3	23.9 ± 6.9	8.6 ± 1.4	36
B. pseudolongum PS14	21.7 ± 8.3	12.4 ± 1.6	57
B. pseudolongum PS34	24.0 ± 5.7	15.8 ± 2.6	66
B. pseudolongum PS36	19.5 ± 1.3	14.0 ± 1.6	72
B. pseudolongum PS85	13.9 ± 5.5	3.9 ± 2.7	28
B. pseudolongum KSI-9	76.0 ± 7.4	35.6 ± 4.6	47

[1] the strains were exposed to 3% ox bile for 6 h; [2] survival is shown as percentage of surviving population relative to population in control tube; [3] mean ± standard deviation

Epithelial-like HT-29 cells were used to assess the ability of the *Bifidobacterium* strains to adhere to epithelial cells.The adherence of the tested strains was differentiated and the number of bacterial cells attached to 100 HT-29 cells amounted from 9 to 645 (Table 3).

Table 3. Adhesion of the *Bifidobacterium* strains to HT29 cells and to mucus

Strain	Adhesion	
	to HT29 cells	to immobilized mucin
	bacterial cell number/100 HT29 cells[1]	CFU number/cm^2, $\times 10^5$
B. animalis PS11	155 ± 21	495 ± 300
B. animalis PS37	17 ± 3	664 ± 134
B. animalis PS46	645 ± 41	360 ± 60
B. animalis KSP4	150 ± 19	889 ± 209
B. animalis KS7	39 ± 10	5 ± 1
B. animalis KS20a1	67 ± 24	5 ± 1
B. animalis KS29a3	45 ± 12	21 ± 8
B. pseudolongum PS14	13 ± 5	3 ± 0
B. pseudolongum PS36	17 ± 8	3 ± 1
B. pseudolongum PS85	33 ± 9	4 ± 1
B. pseudolongum KSI-9	9 ± 3	3 ± 1

[1] the number of bacterial cells attached to 100 epithelial HT29 cells in monolayer; mean ± standard deviation; [2] the number of bacterial cells attached to 1 cm^2 of the surface covered with mucin

The highest number of attached cells was observed for *B. animalis* PS46. Two other *B. animalis* strains, PS11 and KSP4, also adhered well (~150 cells), whereas the four remaining *B. animalis* strains and all *B. pseudolongum* strains tested adhered weakly (9-67 cells). Bacterial cells adhered to the monolayers of epithelial-like HT-29 cells in a diffuse way - as dispersed single cells, or in a cluster (Figures 1 and 2).

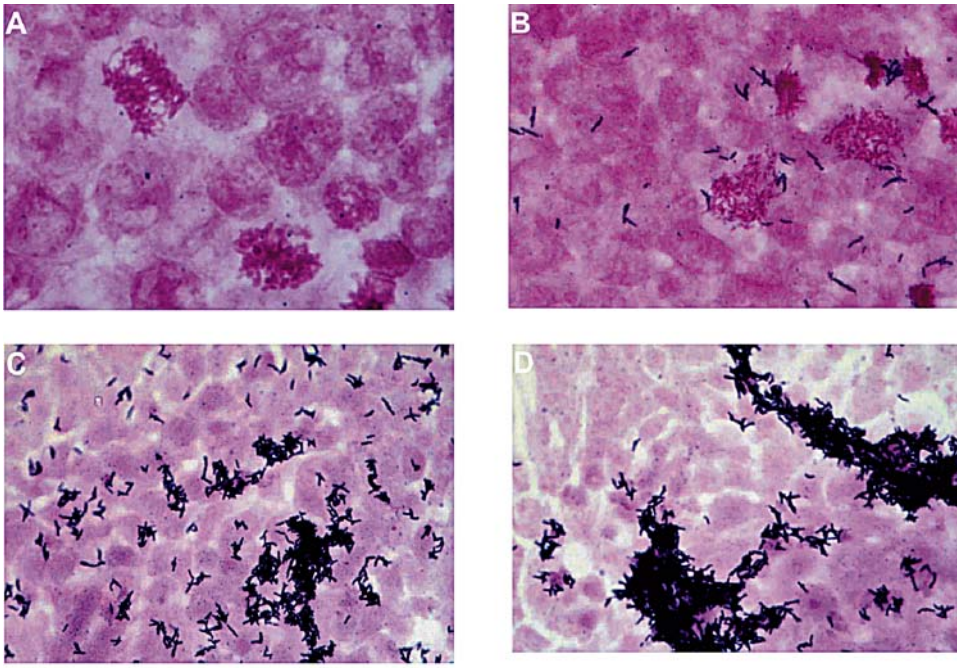

Figure 1. Light micrographs of *B. pseudolongum* PS14 showing no adherence (A, magnification ×1000) and *B. animalis* strain PS37 showing diffuse adherence (B, ×1000) and strain PS46 adhering diffusively and in clusters (C, D, ×750) to HT29 cells. Methanol fixed, Gram stained

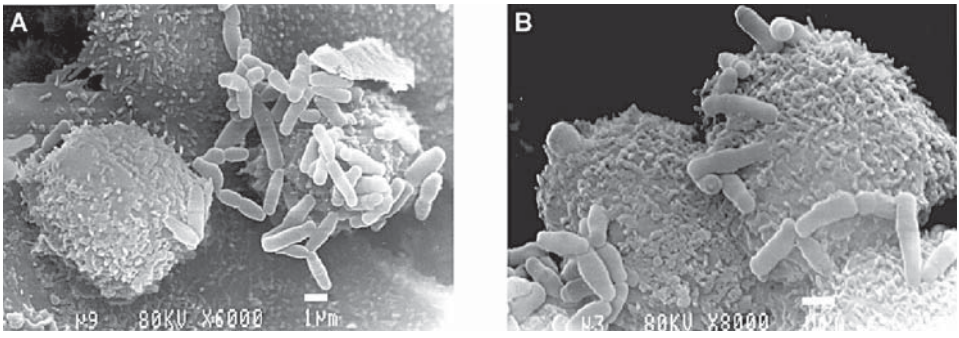

Figure 2. Scanning electron micrographs of *B. animalis* PS46 showing adherence in clusters (A) and diffuse adherence onto the brush border of HT29 cells (B)

The tested bifidobacterial strains were also able to bind to mucus, and their adherence to mucin was as much differentiated as their adhesion to epithelial cells (Table 3). The highest binding rates were observed for *B. animalis* PS11, PS37, PS46 and KSP4 strains (360-889×10^5 bacterial cells attached per cm^2 of the surface covered with mucin). The other strains, except for well-adhering *B. animalis* KS29a3 (21×10^5 cells) showed weak binding abilities to mucin, below 5.5×10^5 cells per cm^2 (Table 3). An insignificant positive linear correlation (r=0.32) was observed between adhesion of the tested strains to epithelial cells and to mucin. In general, the strains adhering well to epithelial cells also adhered well to mucin. However, *B. animalis* PS37 cells adhered weakly to HT-29 cells and strongly to mucin.

DISCUSSION

Resistance to gastric acidity and bile salts as well as attachment to mucus or epithelial cells are the most important prerequisites for the effectiveness of probiotics in the host gut (Charteris et al., 1998). So far, most of the studies on probiotics have been focused on the selection of probiotic strains designed for humans and originating from humans, therefore animal-originated bifidobacteria have been studied very rarely. Resistance to unfavourable gastric conditions is a rare property among human-originated bifidobacterial species, such as *B. bifidum, B. infantis, B. longum, B. breve, B. adolescentis* or *B. catenulatum*, a great majority of the strains belonging to those species appeared to be sensitive to low pH or bile (Clark and Martin, 1994; Lankaputhra and Shah, 1995; Zavaglia et al., 1998). The performed studies revealed that the tested animal-originated strains survived the 3-h exposure to pH 3 well. In all cases, 0 to merely 2.8-fold reduction of live cells was observed, whereas survival at the level of >10^6 CFU/ml is sufficient for probiotic effectiveness in the gut (Charteris et al., 1998; Collado and Sanz, 2006). Resistance to low pH and bile of single animal-originated strains of *B. animalis* and *B. pseudolongum* (originating from type or industrial collections) was observed by Lankaputhra and Shah (1995) and Zavaglia et al. (1998). Among the other tested bifidobacteria, strains of *B. animalis* subsp. *lactis* seem to show the highest tolerance to physiological and technological stress conditions and, as a consequence, have been preferentially included in probiotic products in the food industry, although many researchers stress the importance of host specificity of probiotic strains (Jayamanne and Adams, 2006; Masco et al., 2007). The *B. lactis* strains were isolated from fermented milk and, as they are closely related to *B. animalis* species, are classified at a subspecies level of *B. animalis* (Meile et al., 1997; Masco et al., 2004). The observed resistance of *B. animalis* strains isolated

from rats may be a species-specific feature; however, more studies encompassing a greater number of *B. animalis* strains originating from different animal species should be performed. Due to high tolerance to low pH, *B. animalis* strains can also be more suitable for technological processing than other bifidobacteria. Our previous research on the survival of *B. animalis* strains in commercial bioyoghurts with pH 4.5-4.7 during 4 weeks of storage under refrigeration revealed merely a 2-4-fold reduction of live bifidobacterial cells (Bielecka et al., 2000).

Resistance to stress factors of the tested animal-originated strains was confirmed during their exposition to bile. The applied 3% concentration of bile corresponds to the top physiological concentrations of bile in the human gut and makes up an equivalent of 1.5% of bile salts. Likewise, in an acidic environment, all tested *B. animalis* and *B. pseudolongum* strains survived the 6-h exposure to bile well, although in comparison with their survivability at pH 3, the numbers of viable cells of the strains tested were slightly reduced. Nevertheless, the applied high concentration of bile and the observed survival rate suggest that the tested strains may be good candidates for the elaboration of probiotic products.

Interaction of probiotic bacteria with the intestinal mucosa may enhance the possibility for colonization, immune system modulation, and defence against enteropathogens (Tannock, 1999; Dunne, 2001). Unlike sensitivity to unfavourable gastrointestinal conditions, the tested strains differed in their adhesion to both mucosal enterocytes and mucus. The experiments revealed a dissimilarity of the adhesive properties of both tested *Bifidobacterium* species, and also a strong diversity of the adhesive abilities among the *B. animalis* strains. All well-adhering strains belonged to *B. animalis* species and four out of the eleven strains tested strongly adhered to mucus, whereas only one strain showed strong adherence to epithelial cells. Crociani et al. (1995) described good adhesion of two *B. animals* and one *B. pseudolongum* strain to Caco-2 cells. Good adherence of some *B. animalis* subsp. *lactis* strains to mucus has also been documented in other recent papers (Kirjavainen et al., 1998; He et al., 2001; Rinkinen et al., 2003). However, all those strains originated from type or industrial culture collections.

An important function of beneficial microflora is to form a barrier against colonization of the gastrointestinal tract by pathogenic bacteria. Commensal bacteria colonizing the mucus layer adjacent to the epithelium block receptors and protect the intestine from pathogen invasion. Light and scanning electron microscopy studies of the adhesion to HT-29 cells showed that the tested bifidobacterial cells adhered in clusters, probably to mucus secreted by goblet cells, or in diffuse way-to a brush border of enterocytes (Figures 1 and 2). This suggests that the receptors for bacterial adhesion on mucus may be different, or present in different numbers than on enterocytes, but also that some bifidobacterial cells can be able to form biofilm on the mucosal layer and nearby goblet cells. Such a hypothesis is in

agreement with high adhesion of some strains to mucus, especially those isolated directly from the intestine. Our previous scanning electron microscopy studies of bacterial adhesion to rat colon revealed that bifidobacteria adhered to intestinal epithelium by filaments, also connecting bacterial cells and consequently forming characteristic microcolonies (Bielecka et al., 1998). Bernet et al. (1993) observed higher adhesion of some human-originated bifidobacterial strains to HT29-MTX (a homogenous subpopulation of goblet cells) than to Caco-2 cells. The ability of probiotic bacteria to attach to host mucus seems to be of a great importance as it ensures mutual interaction. Enterocytes are continuously exfoliated from the tips of the villi and replaced with new cells migrating out of the crypt to reach the villous apex. The strains adhering to both mucus and enterocytes seem to be well predisposed to intestinal colonization. Our previous *in vitro* research revealed that the *B. animalis* PS46 strain, well adhering to HT-29 cells and mucin, also strongly adhered to the intestinal wall of rat colon (Bielecka et al., 1998).

In conclusion, among the tested strains three *B. animalis* strains (PS11, PS46 and KSP4) adhered well to mucin and enterocytes and can be considered to be potential probiotic candidates. Although the ecology of animal-type bifidobacteria has been studied very rarely, it is known that both *B. animalis* and *B. pseudolongum* strains are natural inhabitants of a majority of animals (hogs, chickens, turkeys, cattle, calves, piglets, sheep, mice, rats, dogs) (Bezkorovainy and Miller-Catchpole, 1989). All of them can be potential probiotic recipients; nevertheless, *in vivo* studies on the evaluation of the effectiveness of selected strains in host gut are necessary.

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