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BIOTECHNOLOGY METHODS

Lactobacillus acidophilus La5 and *Bifidobacterium lactis* Bb12 cell surface hydrophobicity and survival of the cells under adverse environmental conditions

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Abstract Changes in the cell surface hydrophobicity (CSH) of probiotic bacteria *Lactobacillus acidophilus* La5 and *Bifidobacterium lactis* Bb12 and the survival of these cells were examined in response to varied cultivation conditions and adverse environmental conditions. An inverse linear relationship ($P < 0.01$) was detected between the CSH of intact *L. acidophilus* La5 and *B. lactis* Bb12 and survival of cells subjected to subsequent freezing/thawing, long-term storage or exposure to mineral and bile acids. The observed relationships were supported by significant correlations between the CSH and changes in composition of the cell envelopes (proteins, lipids and carbohydrates) of *L. acidophilus* La5 and *B. lactis* Bb12 examined using FT-IR spectroscopy and conventional biochemical analysis methods. The results also suggest that the estimates of hydrophobicity, being a generalized characteristic of cell surfaces, are important parameters to predict the ability of intact probiotic bacteria to endure extreme environments and therefore should be monitored during cultivation. A defined balance of cell components, which can be characterized by the reduced CSH values, apparently helps to ensure the resistance, improved viability and hence the overall probiotic properties of bacteria.

Keywords Hydrophobicity · *Lactobacillus acidophilus* · *Bifidobacterium lactis* · Survival · Adverse environmental conditions

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Introduction

Lactic acid bacteria *Lactobacillus acidophilus* La5 and probiotic bacteria *Bifidobacterium lactis* Bb12 are of considerable technological and commercial importance because of their role in the manufacturing and preservation of many fermented food products [6, 15, 31, 32]. These bacteria are widely used as probiotic strains, i.e. live microorganisms as a food supplement, which upon ingestion in certain numbers exert health benefits beyond their inherent basic nutrition [5, 20, 26]. However, beneficial effects depend on the ability of probiotic strains to survive under adverse environmental conditions (low pH of gastric juice and high concentration of bile salts in the proximal part of the small intestine), to adhere to epithelial surfaces and to multiply in the gastrointestinal tract [6, 15, 20, 23, 26]. Moreover, the strains selected for industrial purposes should tolerate adverse conditions encountered in industrial processes, either during starter handling and storage (freeze/thawing or dehydration/rehydration) or during food processing in which abiotic stresses such as heat, cold, acidity and high concentration of NaCl or ethanol are common [6, 15, 18].

The surface properties of bacteria are recognized as vital to the organism's survival because the various surface constituents mediate the contact and interaction of the bacterium with its environment [28]. In turn, the cell surface hydrophobicity (CSH) has been acknowledged as a physical measurable macroscopic characteristic of bacteria which generally reflects the proportion between hydrophilic and hydrophobic components of the cell envelope [9, 18]. In terms of its microbiological relevance, CSH is involved in diverse processes and interactions of physiological and technological importance [18, 25, 30, 40] e.g. cell-to-cell interaction, adherence of bacteria to solid

surfaces and host tissue, partitioning at liquid–liquid, solid–liquid or liquid–air interfaces, and resistance of cells to specific treatments by organic solvents or antibiotics. Recently we showed that the Gram-negative bacteria *Zymomonas mobilis* 113S CSH respond to varied environmental conditions (temperature and phase of growth, concentration or type of carbon source etc.); moreover, we observed a barrier function and survival decrease for more hydrophobic cells [34, 43].

Fourier transform infrared (FT-IR) spectroscopy is a non-destructive technique which allows one to obtain information on the overall chemical composition of a sample [5, 12, 29]. Over the years this technique has been used for chemical analysis and characterization of biological samples [11, 12, 28]. The macromolecular composition of microbial biomass is an integral characteristic of the organism's physiological state, reflecting the influence of cultivation conditions on cell regulatory mechanisms [11, 29, 42]. We recently reported that changes in the chemical composition of cells [34, 35, 43], as well as cell envelopes [36], are reflected by several indices of FT-IR spectra.

This study examines the ability of the probiotic bacteria *L. acidophilus* LA5 and *B. lactis* Bb12 to survive during exposure to mineral and bile acids as well as under conditions of freeze–thawing and long-term storage of intact cells; this survival ability is essentially reflected by the levels of CSH as well as the corresponding characteristics and chemical composition of the cell envelopes.

Materials and methods

Strain and culture conditions

L. acidophilus LA5 and *B. lactis* Bb12 (Chr. Hansen, Denmark) were grown under aerobic or anaerobic (BBL Gas Pak 150^{TM} System, USA) conditions, at 37 °C, in the MRS medium consisting of (g l^{-1}) peptone, 10.0; beef extract, 8.0; sodium acetate, 5.0; yeast extract (Sigma), 4.0; ammonium citrate, 2.0; KH_2PO_4, 2.0; Tween 80, 1.0; $MgSO_4{\cdot}7H_2O$, 0.1; $MnSO_4{\cdot}5H_2O$, 0.05 and an appropriate carbon source (glucose, lactose or sucrose) in the concentration range of 2–5 %, pH 6.5 for 18 or 48 h.

Preparation of cells and cells surface hydrophobicity measurements

The cells from an appropriate phase of growth were recovered by centrifugation (7,000×*g*, 10 min, 20 °C), washed twice with 50 mM phosphate buffer (pH 7.1), and resuspended in the same buffer at a standardized concentration of cells (OD_{660} = 0.5) to perform measurements of CSH.

A modified MATH (Microbial Adherence to Hydrocarbon) method [1] using *o*-xylene (Sigma) was basically performed throughout the study to assess the relative CSH. Thus, 3 ml of the respective cell suspension (OD_{660} = 0.5 ± 0.01) was mixed with 0.75 ml of *o*-xylene by vortexing for 2 min. After phase separation (40 min) the aqueous layer was extracted and contaminating xylene removed by bubbling air (2 ml s^{-1}) for 8 min, and the optical density of the aqueous phase was determined at 660 nm (OD_{660}). The results were expressed as percentage reductions in absorbance of the test suspensions (with *o*-xylene) as compared to the control suspensions (without *o*-xylene).

Freeze–thawing and long-term storage procedures of cells

Five or 10 ml of a suspension (8×10^{10} cells ml^{-1}) of cells in water was subjected to freezing (−20 °C) and subsequent thawing (30 °C, 0.5 h) or long-term storage (at −20 °C for 6 months) procedures. Counts of viable cells were determined using a standard serial dilution method and subsequent plating in triplicate onto the MRS agar following incubation at 37 °C for 48–72 h.

Cell low pH and bile acid tolerance measurements

The cells from a stationary phase of growth were recovered by centrifugation (7,000×*g*, 10 min, 20 °C), resuspended in an equal volume of MRS broth (devoid of yeast extract, pH 5.5) (cell concentration at OD_{660} = 0.5) and allowed to adapt for 30 min at 30 °C. Then cells were harvested by centrifugation and resuspended in an equal volume of MRS broth adjusted to pH 3.0, 2.5 and 2.0 using 1 N HCl or bile salts (Sigma), composed of sodium chlorate and sodium deoxycholate (1:1), were added to a final concentration of 3, 2 and 1 %. Counts of viable cells were determined after 30, 60 and 120 min using a standard serial dilution method (in peptone water) and subsequent plating in triplicate onto the MRS agar following incubation at 37 °C for 48–72 h. Bacterial counts were carried out as the log number of colony forming units (log CFU ml^{-1}). To measure the effects of stress exposure the log reduction (Δ log CFU) between the control sample and the test sample was compared according to the expression Δ log CFU = log control (CFU ml^{-1}) − log test sample (CFU ml^{-1}) [37].

Cell envelope extraction

The washed cells were suspended in water and sonicated for 20 min at 0.5 kHz in an ice-water bath. Unbroken cells were removed by centrifugation (6,000×*g*, 10 min, 4 °C). The envelope was pelleted by ultracentrifugation (25,000×*g*, 25 min, 4 °C), washed once with the phosphate buffer,

resuspended in water and centrifuged at 7,500×*g* for 20 min [13]. The cell envelope extraction quality was monitored by electron microscopy (Olympus BX51).

Determination of proteolytic activity

The proteolytic activity was determined according to the Kunitz method [19]. The reaction was stopped by the addition of trichloroacetic acid (TCA; 12 %). The samples were centrifuged (12,000×*g*, 4 °C, 5 min) followed by (after 30 min) measurement of the supernatant absorbance at 280 nm. One unit of proteinase activity was defined as the quantity of enzyme which is required to produce an increase of 0.01 in optical density at OD_{280} under test conditions [14].

Other analytical measurements

The concentration of total membrane proteins was assayed by the dye-binding Lowry (Folin–Ciocalteu's reagent, Fluka Chemie AG, Switzerland) procedure [21], using bovine serum albumin (Sigma) as a standard. The amount of phosphate was determined by the modified method of Bartlett [3]. The concentration of total neutral membrane carbohydrates was determined by means of Dreywood's anthrone reagent [24], using glucose as a standard.

FT-IR spectroscopy measurements

FT-IR absorption spectra of *L. acidophilus* La5 and *B. lactis* Bb12 cell envelopes were registered on a microplate reader HTS-XT (Bruker) [10]. A 10- to 30-µl aliquot of each sample was dried on a 96-well silicon plate at ~50 °C and spectra were collected over the wavenumber range of 4,000–600 cm^{-1}, with 32 scans and a resolution of 4 cm^{-1}. In total 108 spectra from the series of three separate experiments were used for analyses. The data were processed with OPUS 6.5 (Bruker) software and baseline corrected by the rubber-band method. The quantitative calibration equations were performed with quantitative calibration program in OPUS 6.5 (Bruker), where *X* is the absorbance peak intensity (size) and *Y* is the component value (proteins, carbohydrates or lipids) calculated from absorbance intensities (at 1,660, 1,088 or 2,930 cm^{-1}, respectively).

Data processing and analysis

Hydrophilic and hydrophobic cell envelopes were obtained from 26 independent cultivations.

Freeze–thawing, long-term storage and acid tolerance procedures were performed in triplicate at least for cells of independent cultivation.

All analytical measurements for each sample were performed in triplicate at least.

The data were processed by correlation using the software Statgraphics ®Plus (Manugistics, Inc., US) and SPSS 11.0 for Windows (SPSS Inc. Ill., US) and included Pearson (parametric) correlation as well as non-parametric Spearman correlation testing.

At least 200 CFU for each viable cell count was employed to compute the log reduction (Δ log CFU).

The Fisher's *F* test for analysis of variance (ANOVA) was performed to evaluate the statistical significance of regression models and the Student's *t* test was employed to check the significance of regression coefficients. The *P* values less than 0.05 were considered to be statistically significant for both parametric and non-parametric tests.

Results

The CSH of the probiotic bacteria *L. acidophilus* La5 and *B. lactis* Bb12 substantially varied in response to changes of cultivation conditions (Table 1). Thus, the increase in proportion to the concentration of carbohydrate in the cultivation medium and the use of lactose as the sole carbon source resulted in higher values of CSH (Table 1).

It should be noted that the presence of prebiotic compound such as levan (*β*-2,6-polyfructan) in the cultivation medium markedly influenced the surface hydrophobicity of *L. acidophilus* La5. Thus, the addition of levan in the concentration range 1–4 % to the cultivation medium with lactose increases the CSH from 62 to 82 %. At the same time, the changes of cultivation temperature did not significantly affect the CSH of both cultures (data not shown).

The presence of oxygen during cultivation affected the CSH of both probiotic strains differently. Thus, under aerobic conditions the CSH values of *L. acidophilus* La5 and *B. lactis* Bb12 were found to increase or decrease, respectively. In turn, under anaerobic conditions a pronounced opposite effect on CSH was detected (Fig. 1).

The ability of *L. acidophilus* La5 and *B. lactis* Bb12 to survive under adverse conditions of freeze–thawing cycle as well as during long-term storage was found to correlate with the CSH values for intact bacterial cells. Inverse linear relationships (Fig. 2) were detected between the CSH and the survival of both bacteria stains exposed to freeze–thawing cycle and long-term storage.

Other physiological factors that may influence *L. acidophilus* La5 and *B. lactis* Bb12 cell viability are low external pH and an elevated concentration of bile salt. The survival of both strains decreases significantly after incubation at pH 3 and after only 30 min of treatment (Fig. 3). The highest survival reduction of *L. acidophilus* La5 and *B. lactis* Bb12 (0.3 and 0.8 Δ log CFU, respectively)

Table 1 Effect of varied growth conditions on the cell surface hydrophobicity of *Lactobacillus acidophilus* La5 and *Bifidobacterium lactis* Bb12

Strain	Growth conditions			Hydrophobicity, % ± SE
	Carbon source	Concentration, M	Presence of O_2	
La5	Glucose	0.13	+	57.40 ± 0.47
		0.26	+	62.90 ± 0.68
		0.39	+	66.87 ± 0.45
	Sucrose	0.13	+	60.40 ± 0.33
		0.26	+	64.30 ± 0.70
		0.39	+	67.90 ± 0.16
	Lactose	0.13	+	68.45 ± 0.61
		0.29	+	70.23 ± 0.61
		0.39	+	75.13 ± 0.61
Bb12	Glucose	0.13	–	60.27 ± 0.89
		0.26	–	64.56 ± 0.80
		0.39	–	70.40 ± 0.80
	Sucrose	0.13	–	62.27 ± 0.70
		0.26	–	66.40 ± 1.06
		0.39	–	72.75 ± 0.70
	Lactose	0.13	–	72.75 ± 0.60
		0.26	–	76.75 ± 0.65
		0.39	–	79.37 ± 1.00

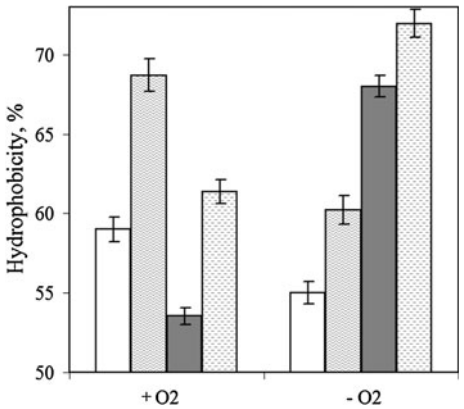

Fig. 1 Changes of the cell surface hydrophobicity of *L. acidophilus* La5 and *B. lactis* Bb12 upon the employed carbon source and the presence of oxygen: *white bar—L. acidophilus* La5 (0.13 M glucose); *wavy bar—L. acidophilus* La5 (0.13 M lactose); *shaded bar—B. lactis* Bb12 (0.13 M glucose); *dashed line bar—B. lactis* Bb12 (0.13 M lactose)

appeared after 120 min incubation at pH 2 (Fig. 4). At the same time, inverse linear relationships were detected between the CSH and survival of both bacteria strains exposed to low external pH values (Fig. 4) and 3 % bile acid (Fig. 5), thus confirming a substantially reduced ability of hydrophobic bacteria to survive under adverse environments.

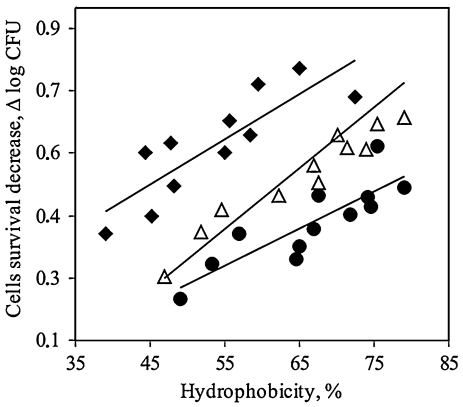

Fig. 2 Relationship between the cell surface hydrophobicity and survival decrease of intact *L. acidophilus* La5 cells exposed to freezing/thawing cycle (*filled diamond*) ($r^2 = 0.7208$, $r = 0.849$, $P < 0.01$) and *B. lactis* Bb12 cells exposed to freezing/thawing cycle (*filled circle*) ($r^2 = 0.73$, $r = 0.854$, $P < 0.01$) and storage (*open triangle*) ($r^2 = 0.7098$, $r = 0.843$, $P < 0.01$)

In addition, a decrease of CSH of *B. lactis* Bb12 was detected after incubation in the presence of mineral acid (HCl) or bile acid (Table 2). The CSH decrease was more pronounced in the presence of 3 % bile acid, especially in hydrophilic cells. The influence of mineral acid on CSH appeared to be similar, however less perceptible (Table 2).

It should be noted that proteolytic activity and the total amount of secreted protein in the medium during incubation of *L. acidophilus* La5 cells were markedly dependent on CSH values. Thus, the concentration of secreted protein and the activity of proteolytic enzyme were found to increase in direct proportion to the increased CSH values (Fig. 6).

Independent analytical measurements of the carbohydrate, protein and lipid content of *L. acidophilus* La5 and *B. lactis* Bb12 cell envelopes were performed. Concentrations of cell envelope basic components of both strains were found to correlate with the CSH values. Thus, the carbohydrate levels decreased in proportion to the increased CSH values of both strains alongside the elevated protein and lipid content in more hydrophobic cells (Fig. 7). At the same time, an examination of FT-IR spectra of cell envelopes for both strains revealed substantial changes of chemical composition depending on the CSH of bacteria. The CSH (41.1–75.9 %) of the cell envelopes was found to correlate with the ratio of absorbance intensities in the lipid (3,000–2,800 cm^{-1}), protein (1,700–1,500 cm^{-1}) and carbohydrate (1,200–900 cm^{-1}) regions (Table 3, Fig. 8). In addition to detected correlations, a significant multiple relationship between the CSH of *L. acidophilus* La5 and *B. lactis* Bb12 and protein and carbohydrate content in cell envelopes ($r = 0.967$, $P < 0.01$ and $r = 0.955$, $P < 0.01$, respectively), as well as the spectra absorbance intensities in the protein and carbohydrate

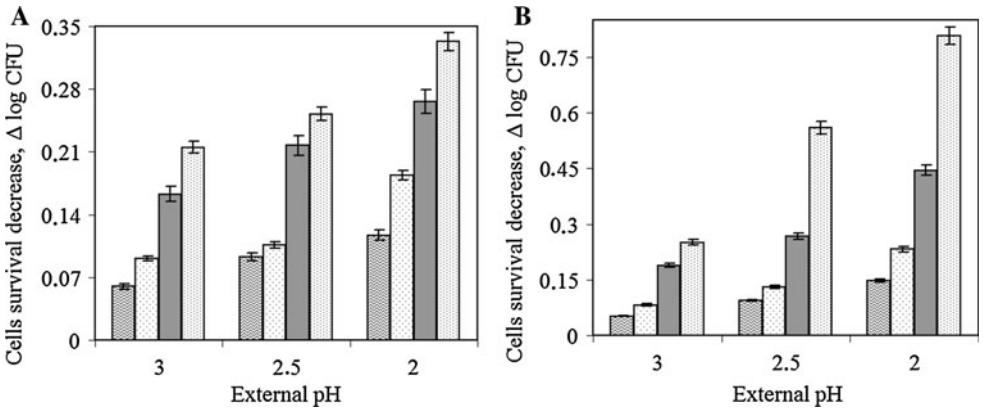

Fig. 3 Survival decrease of *L. acidophilus* La5 (**a**) (*wavy bar*—30 min, CSH = 35 %; *scattered dotted bar*—30 min, CSH = 54 %; *shaded bar*—120 min, CSH = 35 %; *dotted bar*—120 min, CSH = 54 %) and *B. lactis* Bb12 (**b**) (*wavy bar*—30 min, CSH = 52.7 %; *scattered dotted bar*—30 min, CSH = 69.5 %; *shaded bar*—120 min, CSH = 52.7 %; *dotted bar*—120 min, CSH = 69.5 %) exposed to mineral acid (HCl, pH 2)

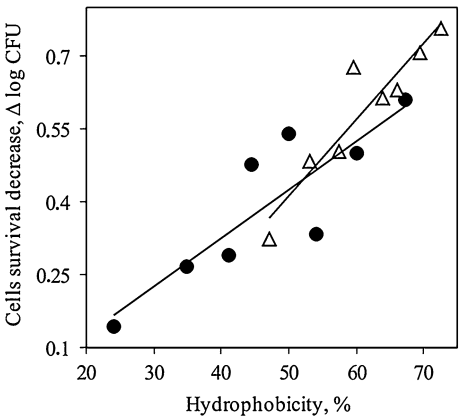

Fig. 4 Relationship between the cell surface hydrophobicity and survival decrease of *L. acidophilus* La5 (*filled circle*) ($r = 0.862$, $P < 0.01$)and *B. lactis* Bb12 (*open triangle*) ($r = 0.935$, $P < 0.01$) cells exposed to mineral acid (HCl, pH 2, 120 min)

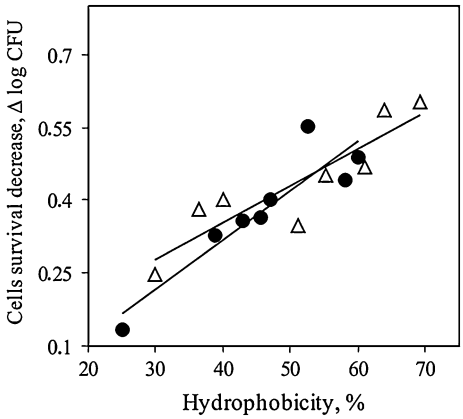

Fig. 5 Relationship between the cell surface hydrophobicity and survival decrease of *L. acidophilus* La5 (*filled circle*) ($r = 0.912$, $P < 0.01$) and *B. lactis* Bb12 (*open triangle*) ($r = 0.896$, $P < 0.01$) cells exposed to 3 % bile acid, 120 min

regions ($r = 0.912$, $P < 0.01$ and $r = 0.870$, $P < 0.01$, respectively) was found.

To examine the relationships between the concentration of proteins, carbohydrates and lipids of *L. acidophilus* La5 and *B. lactis* Bb12 cell envelopes and FT-IR spectral data the quantitative calibration was performed by means of the OPUS program (2nd order polynomial equations). The data obtained from FT-IR spectra quantitative analysis and biochemical determinations were found to be consistent (Table 4).

Table 2 Changes of the cell surface hydrophobicity of *Bifidobacterium lactis* Bb12 exposed to acidic conditions

Treatment	Hydrophobicity, % ± SE		
	Control (intact cells)	After treatment (120 min)	Relative decrease
HCl (pH 2)	55.6 ± 0.17	51.1 ± 0.19	8.1
	57.3 ± 0.19	55.0 ± 0.16	4.0
Bile acid (3 %)	47.2 ± 0.18	20.0 ± 0.20	57.6
	61.2 ± 0.18	34.1 ± 0.19	44.3

Discussion

Gram-positive bacteria should be considered as more hydrophobic in comparison to Gram-negative organisms owing to markedly hydrophobic constituents of the cell envelope [1, 31, 32, 41]. The *L. acidophilus* La5 and *B. lactis* Bb12 possess a typical Gram-positive cell wall consisting mainly of peptidoglycans, (lipo)teichoic acids, proteins and polysaccharides; however, it remains to be investigated in more detail [8, 31, 32, 39]. It has been reported that CSH is highly strain-specific, i.e. variable over a wide range within genera, and therefore it is distinctly affected by changes of environmental conditions such as phase of cultivation, growth rate, medium composition and temperature [15, 17, 22, 27, 32, 35, 39]. In this respect the observed increase in the CSH values of *L. acidophilus* La5 and *B. lactis* Bb12 in response to elevated concentrations of carbon source (Table 1) are in

conformity with data from our preliminary study of *L. acidophilus* and *B. lactis* CSH [35]. At the same time, it was detected that the presence of lactose in the cultivation medium (in comparison to glucose or sucrose) causes the highest values of CSH for both probiotic strains. The similar effect of lactose was reported for *Lactococcus lactis* strain [16].

It is known that fructan-containing prebiotics (e.g. levan) influence the growth and survival of bacteria [23]. The observed positive effect of levan on the CSH of *L. acidophilus* LA5 shows that levan influences not only the productivity of probiotic bacteria [33], but also the CSH with increases (up to 82 %) of hydrophobicity in combination with lactose in the cultivation medium.

It was reported that the presence of oxygen influences the metabolism and biochemical properties of *Bifidobacterium* spp.; besides, it was shown that the *L. acidophilus* strain appears to be more oxygen-tolerant [38]. On the other hand, it was noted that *B. longum* exposed to oxygen underwent changes in its cellular fatty acid profiles [38]. The observed diverse effects of oxygen on the CSH of *L. acidophilus* La5 and *B. lactis* Bb12 (Fig. 1) indicate somewhat different structural responses and surface modifications (likely in membrane lipids) of these bacteria as a result of respective changes of cultivation conditions.

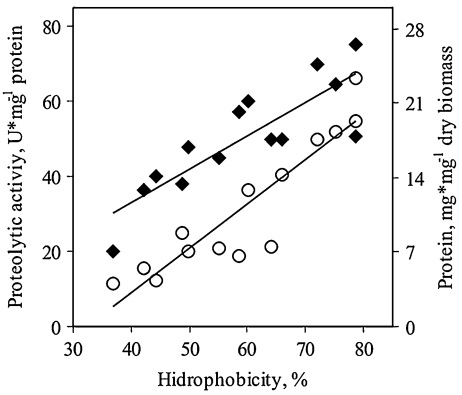

Fig. 6 Relationship between the cell surface hydrophobicity and the proteolytic activity (*filled diamond*) ($r = 0.846$, $P < 0.01$) and the total amount of secreted protein in the medium (*open circle*) ($r = 0.706$, $P < 0.01$) of *L. acidophilus* La5 during incubation

Freezing represents a complex of stress conditions in which microorganisms undergo severe physiological disturbances, including a reduction of membrane fluidity [6]. Besides, the membrane integrity and denaturation of macromolecules during the cryo-conservation of bacteria have been reported as the survival determinant factors [6]. Therefore, the observed CSH-dependent survival of *L. acidophilus* La5 and *B. lactis* Bb12 during the freeze–thaw cycle and long-term storage (Fig. 2) indicates that the freezing-dependent structural changes most likely occur in membrane lipids. It should be noted that the proportion of shorter and/or unsaturated fatty acids in membrane lipids of *L. delbrueckii* increases as the cultivation temperature decreases [2].

Acidic conditions are a powerful environmental stress to which lactic acid bacteria are subjected in the gastrointestinal tract [6, 15, 18, 20, 23, 26, 32]. It was reported that pH values strongly affected the survival of bacteria, and the presence of bile salts in the environment of bacteria cultures is more detrimental than the effect of pH 3 [6, 15, 26], because it can destabilize the membrane integrity of bacterial cells [26]. There are a lot of data indicating a similar decrease in survival of *Lactobacillus* and *Bifidobacteria* strains at low external pH or in the presence of bile acid [7, 26, 27]. The results of the present study (Figs. 3, 4, 5) show that the survival of *L. acidophilus* La5 and *B. lactis* Bb12 strongly depends on the CSH level, confirming that more hydrophilic cells have a greater potential to survive under adverse environments.

Comparing the effects of mineral and bile acid on the survival of both strains, we noted that the decrease of survival caused by bile salts was higher than that caused by low external pH values (Figs. 4, 5), particularly for hydrophobic cells. This is in agreement with the observed destructive action of bile salts on phospholipids and proteins in cell membranes which results in the lost of cell integrity [4].

It has been reported that the presence of bile salts causes a decrease in CSH [7, 17]. We noted that the decrease of

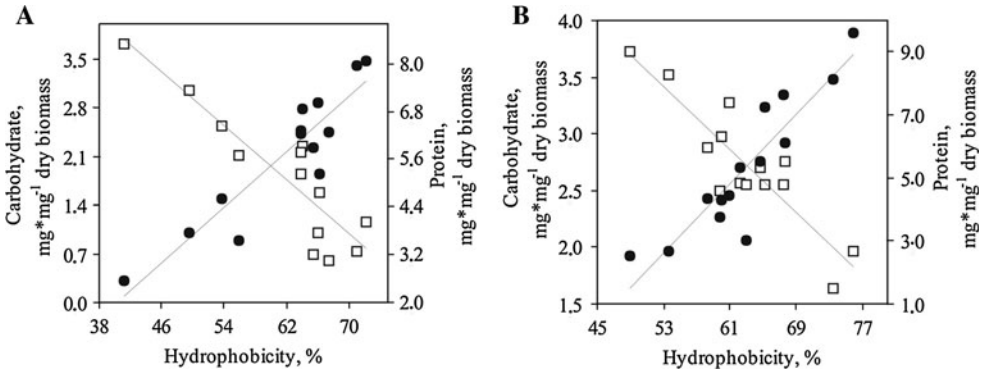

Fig. 7 Relationship between the cell surface hydrophobicity of *L. acidophilus* La5 (**a**) and *B. lactis* Bb12 (**b**) cell envelopes and the carbohydrate level (*open square*) ($r = -0.896$, $P < 0.01$ (**a**) and $r = -0.893$, $P < 0.01$ (**b**)), and protein content (*filled circle*) ($r = 0.921$, $P < 0.01$ (**a**) and $r = 0.906$, $P < 0.01$ (**b**))

CSH of *B. lactis* Bb12 after incubation in the presence of mineral acid (HCl) or bile acid (Table 2) also depends on CSH levels. It was noted that cell surfaces of lactobacilli may adapt their CSH in response to environmental changes, such as external pH values [22]. Therefore, a more pronounced decrease of CSH in the presence of 3 % bile acid, especially for hydrophilic cells (Table 2), most likely indicates the ability of bacteria to adapt to adverse environmental conditions towards increasing hydrophilicity of cell envelopes.

It should be mentioned that a leakage of intracellular proteins into the medium as well as an increase of proteolytic activity in direct proportion to growing CSH (Fig. 6) likely reflects the reduction of the permeability barrier in *L. acidophilus* La5 cells. Unlike the extracellular enzyme secretion in *Z. mobilis* [43], in *L. acidophilus* La5 proteolytic enzyme activity increases in parallel with the total amount of secreted protein in the medium along with the increase of CSH.

Table 3 Correlations between the cell surface hydrophobicity of *Lactobacillus acidophilus* La5 and *Bifidobacterium lactis* Bb12 and FT-IR absorbance (*A*) intensities

Parameters to be correlated	FT-IR indices	Correlation coefficient, Spearman's (ρ)	
		La5, $n = 13$	Bb12, $n = 13$
Absorbance intensities			
CSH	ν_{as} (CH_3) ~ 2,960 cm^{-1}	0.829**	0.705**
	ν_{as} (CH_2) ~ 2,930 cm^{-1}	0.898**	0.602*
	ν_{sym} (CH_3) ~ 2,880 cm^{-1}	0.853**	0.641*
	ν_{sym} (CH_2) ~ 2,850 cm^{-1}	0.909**	0.717**
	Amide I ~ 1,660 cm^{-1}	0.872**	0.691**
	>C=O str (esters) ~ 1,745 cm^{-1}	0.697**	0.662*

* Significant at the 0.05 level

** Significant at the 0.01 level

The observed relationships between the CSH of *L. acidophilus* La5 and *B. lactis* Bb12 and the content of principal cell envelope components (Fig. 7) appear in agreement with a common view on the hydrophobic nature of constituents in the polymorphic composition of cell envelopes from Gram-positive bacteria [13, 32]. Some studies of microbial cell surfaces reviled that the presence of (glyco-)proteinaceous material at the cell surface results in higher hydrophobicity, whereas hydrophilic surfaces are related to the presence of polysaccharides [17, 31, 32]. Our previous studies of *L. acidophilus* cells [35] and the analysis of FT-IR spectra indicated a decrease of carbohydrate levels and an elevated protein content in more hydrophobic cells. In the present investigation the analysis of FT-IR spectra of *L. acidophilus* La5 and *B. lactis* Bb12 cell envelopes as well as chemical analysis data confirmed substantial changes in protein and carbohydrate, as well as lipid content, depending on CSH (Figs. 7, 8; Table 3).

The observed multiple correlations between the concentration of proteins and carbohydrates, as well as the spectra absorbance intensities in the protein and carbohydrate regions, and the CSH levels of both strains demonstrate that the chemical composition of *L. acidophilus* La5 and *B. lactis* Bb12 cell envelopes is closely linked to the CSH values, thereby reflecting the relations between their hydrophilic (carbohydrates) and hydrophobic (proteins) constituents.

The quite similar data for protein, carbohydrate and lipid concentrations of *L. acidophilus* La5 and *B. lactis* Bb12 cell envelopes obtained by quantitative analysis of FT-IR spectra and biochemical determinations (Table 4) strongly suggest the use of FT-IR spectroscopy as a convenient approach to qualitative and quantitative studies of bacterial cell envelopes.

The results also suggest that the estimates of hydrophobicity, being a generalized characteristic of cell surfaces, are important parameters to predict the ability of intact probiotic bacteria to endure extreme environments

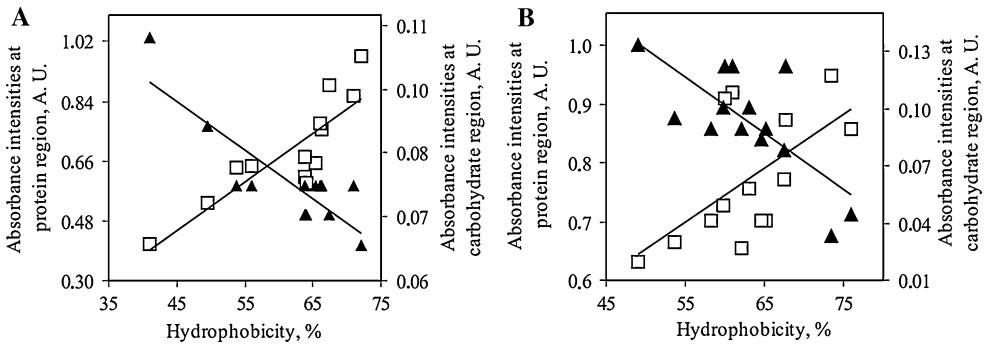

Fig. 8 Relationship between the cell surface hydrophobicity of *L. acidophilus* La5 (**a**) and *B. lactis* Bb12 (**b**) cell envelopes and the absorbance intensities in the protein (*open square*) (at 1,540 cm^{-1}, $r = 0.852$, $P < 0.01$ (**a**) and $r = 0.603$, $P < 0.05$ (**b**)) and carbohydrate (*filled triangle*) (at 985 cm^{-1}, $r = -0.814$, $P < 0.01$ (**a**) and $r = -0.735$, $P < 0.01$ (**b**)) regions

Table 4 Principal component content of *Lactobacillus acidophilus* La5 and *Bifidobacterium lactis* Bb12 cell envelopes, determined by chemical and FT-IR quantitative analyses (calibration equations)

Strain	Analytical chemical analysis of membrane			IR quantitative analysis of membrane[a]		
	Proteins mg/mg^{-1} dry biomass ± SE	Carbohydrates	Lipids	Proteins mg/mg^{-1} dry biomass ± SE	Carbohydrates	Lipids
La5	5.72 ± 0.47	1.80 ± 0.17	1.53 ± 0.12	5.69 ± 0.66	1.73 ± 0.27	1.56 ± 0.20
Bb12	5.41 ± 0.28	2.68 ± 0.15	1.05 ± 0.08	5.44 ± 1.01	2.77 ± 0.51	1.01 ± 0.12

[a] Estimates from the quantitative calibration equations, where X is the absorbance peak intensity, Y is the component value (proteins, carbohydrates or lipids) calculated from absorbance intensities (at 1,660, 1,088 and 2,930 cm^{-1}, respectively)

and should therefore be monitored during cultivation. A defined balance of cell components, which can be characterized by the reduced CSH values, apparently helps to ensure the resistance, improved viability and hence the overall probiotic properties of bacteria.

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