Influence of growth conditions on hydrophobicity of *Lactobacillus acidophilus* and *Bifidobacterium lactis* cells and characteristics by FT-IR spectra

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Abstract. In this study we have found, that the values of cell surface hydrophobicity (CSH) of *L. acidophilus* LA5 and *B. lactis* Bb12 cells change in response to varied growth conditions – phase of growth, concentration or type of carbon source, presence of oxygen. An evaluation of FT-IR spectra using cluster and quantitative analyses revealed substantial changes of the chemical composition depending on the CSH level of *L. acidophilus* LA5 and *B. lactis* Bb12 cells. Decrease of the carbohydrate level was observed in proportion to the increased CSH values alongside with the elevated protein content of more hydrophobic cells of both cultures. The results of present study could help to specify the appropriate physiological state and environment for *L. acidophilus* LA5 and *B. lactis* Bb12 to ensure their probiotic properties.

Keywords: Hydrophobicity, *Lactobacillus acidophilus*, *Bifidobacterium lactis*, FT-IR spectroscopy, quantitative analysis

1. Introduction

Lactic acid bacteria (LAB), such as *Lactobacillus acidophilus* and *Bifidobacterium lactis* are of considerable technological and commercial importance because of their role in the manufacturing and preservation of many fermented food products, but they also have probiotic properties and shown beneficial effect on health [12]. Cell surface properties of bacteria are recognized as vital to the organism's survival since the various surface constituents mediate the contact and interaction of the bacterium with its environment [8]. 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 [5]. In the microbiological practice CSH appears to be concerned with diverse processes and interactions of physiological and technological importance [9,10,15].

FT-IR spectroscopy provides information about the unique chemical composition of bacteria, i.e., quantitative information about total biochemical composition of the cell wall components. By this tech-

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nique it is possible to characterize the cell surface and to obtain essential information about the chemical structure of bacterial surface [3,14]. A close relationship was established between the CSH and nitrogen-rich groups most notably protein content [6,11,16]. Changes in the surface properties of *Rhodococcus rhodochrous* were assessed by measuring the hydrophobicity and the FT-IR spectrum of enzyme treated and untreated bacteria. Measurement of the infrared spectrum revealed distinct changes in the spectrum associated with removal of macromolecules due to the enzymatic treatment [4].

A deeper understanding of the factors influencing the surface properties of LAB will definitely promote the selection and evaluation of strains having the desired characteristics for food processing and health benefits.

This study examines changes of the CSH in response to varied environmental conditions (carbon source and its concentration, phase of growth, the presence or absence of oxygen) during growth of bacteria *Lactobacillus acidophilus* LA5 and *Bifidobacterium lactis* Bb12 and their corresponding characteristics of chemical composition.

2. Materials and methods

2.1. Strain and culture conditions

L. acidophilus LA5 and *B. lactis* Bb12 were grown under anaerobic or aerobic conditions, at 37°C, in the medium consisting of ($\text{g} \cdot \text{l}^{-1}$) 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; $\text{MgSO}_4 \times 7\text{H}_2\text{O}$, 0.1; $\text{MnSO}_4 \times 5\text{H}_2\text{O}$, 0.05 and an appropriate carbon source (glucose or lactose) at 2 and 5% concentration, pH 6.5 for 18 or 44 h.

L. acidophilus LA5 and *B. lactis* Bb12 were grown under anaerobic or aerobic conditions at optimal growth conditions for 28 h to evaluate the invariability of strains.

2.2. Preparation of cells and cells surface hydrophobicity measurements

The cells from an appropriate phase of growth were recovered by centrifugation (5000g, 10 min, 20°C), washed twice with 50 mM phosphate buffer (pH 7.1), resuspended in the same buffer at standardized concentration of cells ($\text{OD}_{660} = 0.5$) 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. 3 ml of the respective cell suspension ($\text{OD}_{660} = 0.5 \pm 0.01$) 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 ($2 \text{ ml} \cdot \text{s}^{-1}$) 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).

2.3. FT-IR spectroscopy measurements

FT-IR absorption spectra were recorded with a HTS-XT microplate reader (Bruker, Germany). Samples of bacterial biomass (10–20 μl) were dried on a 96 place silicon plate at 50°C. Spectra were collected over a wavelength range 4000–600 cm^{-1} , with a resolution of 6 cm^{-1} . Each spectrum was produced by

coaddition of 32 scans, baseline corrected by rubber band method, CO₂ bands excluded and spectra evaluated by Opus 6.5 (Bruker, Germany). Quantitative analysis of bacterial biomass was performed using the previously determined molar extinction coefficients, and applying a system of Vierordt equations to evaluate the concentrations of proteins and carbohydrates [7].

3. Results

The CSH of bacteria *L. acidophilus* LA5 and *B. lactis* Bb12 substantially varied from 80.2 to 60.2% in response to changes of cultivation conditions (absence or presence of oxygen, concentration or type of carbon source, phase of growth). The CSH of *L. acidophilus* LA5 considerably increased in proportion to the concentration of carbohydrate in the cultivation medium and the use of lactose as the sole carbon source resulted in the higher values of CSH. Similar variation of CSH in response to changes of cultivation conditions were observed for bacteria *Zymomonas mobilis* 113S and recently reported [17]. In an analogous way the CSH of *L. acidophilus* LA5 responded to changes of cultivation time in the range of 18 (logarithmic growth phases) to 44 h (stationary phase). The same results were detected for bacteria *Lactobacillus rhamnosus* GG [2]. The lowest CSH indices were attained for *L. acidophilus* LA5 cells grown under anaerobic condition for 18 h at lower concentration of glucose in the medium.

The FT-IR spectra of bacterial cells were very simple. The bands between 3000 and 2800 cm⁻¹ are related to the alkyl-hydrocarbons groups, the so-called fatty acid region as the bands at 2956, 2934 and 2875 cm⁻¹ characterize asymmetric CH₃ stretching, the asymmetric CH₂ stretching and the symmetric CH₃ stretching, respectively. A protein region of bacteria occurs between 1655 cm⁻¹ ascribed to the carbonyl stretching of secondary amides (Amide I) and at 1545 cm⁻¹ to N–H bending of Amide II (N–H bending) both indicating protein in the cell wall. At frequencies of 980–1200 cm⁻¹ a wide and intensive carbohydrate and phosphate band is observed, with maximum at 1070 cm⁻¹ caused by valence C–O–C group vibrations in the cyclic structures.

Comparison of IR spectra of both cultures grown under optimal conditions showed that the protein content was higher in *L. acidophilus* LA5 biomass in aerobic environment – 59.56% DW, and 58.99% DW in *B. lactis* Bb12 after growth in anaerobic environment. The content of carbohydrates in biomass of *L. acidophilus* LA5 was 10.85–10.29% DW and 8.34–8.43% DW in *B. lactis* Bb12 grown in anaerobic or aerobic environment. These results show that the content of carbohydrates in cells was not noticeably affected by oxygen during the growth.

FT-IR spectra of cell biomass with different CSH qualitatively were very similar, while the intensities of carbohydrate, protein and lipid bands changed indicating to quantitative variations of the main biochemical cell components. Cluster analysis discriminated the spectra by strain peculiarities determined by environment, carbon source and growth time. The cluster of biomass spectra with CSH from 61.2 to 75.1% are shown in Fig. 1.

At the same time biochemical characteristics for cells of varied CSH displayed commensurable changes and, as a result, significant correlative relationships were ascertained between the data. Thus, the decrease of the carbohydrate level was observed of more hydrophobic *L. acidophilus* LA5 cells. On the contrary, the content of proteins graduated in direct proportion to the rise of *L. acidophilus* LA5 CSH level. For example in cell biomass with CSH from 61.2 to 75.1% the total carbohydrate content decreased from 20.93 to 10.50% DW and the protein content increased from 58.99 to 67.45% DW. Similar relationships were detected for bacteria *Z. mobilis* 113S and reported previously [13]. Observed

Fig. 1. Cluster of FT-IR spectra of *B. lactis* Bb12 and *L. acidophilus* LA5 with different CSH.

significant ($P < 0.01$) relationships therefore indicate that proportions of both hydrophilic (carbohydrates) and hydrophobic (proteins) constituents of the cell could be responsible for the overall CSH of *L. acidophilus* LA5 [12].

4. Conclusions

This study showed that the values CSH of *L. acidophilus* LA5 and *B. lactis* Bb12 cells are changed by environment and growth conditions. It was shown that the concentration of cell proteins is directly proportional to the CSH and the concentration of cell carbohydrates is inversely proportional to the CSH of *L. acidophilus* LA5 and *B. lactis* Bb12 bacterial cell biomass. The results of present study could help to specify the appropriate physiological state of *L. acidophilus* LA5 and *B. lactis* Bb12 to predict their probiotic properties. In this study FT-IR spectroscopy was used for analysis of whole cell biomass and quantitative differences in the carbohydrate and protein content were demonstrated. Since hydrophobicity is cell surface characteristics then further studies of cell envelopes is considered to be more informative.

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