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The Adhesive Capability of Two *Lactobacillus* Strains and Physicochemical Properties of Their Synthesized Biosurfactants

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Summary

The aim of this study is to describe the adhesive capability of *Lactobacillus fermenti* 126 and *Lactobacillus rhamnosus* CCM 1825 as well as to isolate and evaluate the functional properties of their synthesized biosurfactants. Fourier transform infrared spectroscopy shows that both crude biosurfactants contain three components: protein, polysaccharide and phosphate in different ratio. The crude biosurfactants synthesized by *Lactobacillus fermenti* 126 and *Lactobacillus rhamnosus* CCM 1825 contain 8 and 9 fractions analyzed by capillary gel electrophoresis. *Lactobacillus fermenti* 126 and *Lactobacillus rhamnosus* CCM 1825 strains used in this study synthesize biosurfactants with low effectiveness, critical micelle concentration of 9.0 and 6.0 g/L, and surface tension of (45.1±0.1) and (43.6±0.6) mN/m, respectively. Biosurfactant synthesized by *Lactobacillus rhamnosus* CCM 1825 demonstrated higher emulsifying and froth-forming activity than that obtained from *Lactobacillus fermenti* 126, which resulted in better antiadhesive properties. The advantageous adhesive properties of these *Lactobacillus* strains were confirmed. A positive effect of the impregnation of polystyrene surface with an aqueous solution of biosurfactants on the inhibition of adhesion of *Escherichia coli* 22, *Klebsiella pneumoniae* 2 and *Pseudomonas aeruginosa* W2 to the impregnated surface was found.

Key words: *Lactobacillus*, adhesion, biosurfactants, *Enterobacteriaceae*

Introduction

Bacteria of genus *Lactobacillus* are part of the natural microflora of the human gastrointestinal and genitourinary tracts (*1*). It is common knowledge that the presence of bacteria in the gastrointestinal tract positively affects the functioning of a human body (*2*), which stems from various factors, *e.g.* adhesion of the bacteria to epithelium (*3*). Owing to these properties, bacteria of genus *Lactobacillus* hinder adhesion of pathogenic microflora to intestinal or genitourinary epithelium (*3*). The adhesive properties of *Lactobacillus* bacteria are one of the major criteria which determine their probiotic properties (*4*).

Metabolites produced by some lactic acid bacteria include biosurfactants (*5*,*6*). They are often used in medicine as components of therapeutic agents that control infections caused by various groups of microbes (*7*). There are well known examples of biosurfactant application as impregnating agents which hinder the adhesion of pathogens to the surface of medical equipment (*7*,*8*). The ability of *Lactobacillus* to synthesize surfactants makes the bacteria competitive with pathogenic microflora of the gastrointestinal tract (*3*,*9*).

The aim of this study is to characterize the adhesive capabilities of *Lactobacillus fermenti* 126 and *Lactobacillus rhamnosus* CCM 1825, as well as the properties of their

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synthesized biosurfactants. The chemical structure of crude biosurfactants, molecular mass, critical micelle concentration (CMC), emulsifying and froth-forming properties were determined. Furthermore, the antiadhesive effect of the biosurfactants adsorbed onto a polystyrene surface against *Enterobacteriaceae* pathogens: *Escherichia coli* 22, *Klebsiella pneumoniae* 2 and *Pseudomonas aeruginosa* W2 was also examined.

Materials and Methods

Microorganisms and culture conditions

The strains of lactic acid bacteria *L. fermenti* 126 and *L. rhamnosus* CCM 1825 were obtained from the Culture Collection of the Chair of Industrial and Food Microbiology (CCCIFM), University of Warmia and Mazury (UWM) in Olsztyn, Poland. The bacteria were kept frozen at –35 °C on MRS broth (Merck, Darmstadt, Germany) with the addition of glycerol (250 mL/L). Before each experiment, the bacteria were cultivated twice on MRS broth (Merck) at 37 °C for 18 h under anaerobic conditions.

Pathogenic *Enterobacteriaceae* of the CCCIFM, UWM in Olsztyn, Poland were used in the assays of the anti-adhesion properties. The strains of bacteria, namely *E. coli* 22, *K. pneumoniae* 2 and *P. aeruginosa* W2, were cultured on McConkey agar (Merck, Darmstadt, Germany) for 24 h at 37 °C.

Production of biosurfactants

The lactic acid bacteria were cultured in MRS broth (600 mL; Merck) and grown for 18 h under anaerobic conditions. An inoculum of 15 mL/L was used. Cells were harvested by centrifugation (10 000×*g*, 5 min, 10 °C), washed twice in demineralized water, and resuspended in 100 mL of phosphate buffered saline (PBS, 0.01 M KH_2PO_4/K_2HPO_4 and 0.15 M NaCl with pH adjusted to 7.0). The bacteria were left at room temperature for 2 h and stirred gently to release the biosurfactant. Subsequently, the bacteria were removed by centrifugation (10 000×*g*, 5 min, 10 °C) and the remaining supernatant was filtered through a 0.22-μm pore size filter (Millipore, Carrigtwohill Co, Cork, Ireland). The supernatant was dialyzed against demineralized water using dialysis membranes (molecular mass cut-off of 6.0–8.0 kDa, Spectra/Por® 1, Spectrum Laboratories, Inc, Rancho Dominguez, CA, USA), freeze-dried and stored at –20 °C, and the biosurfactant was used for further studies.

Fourier transform infrared spectroscopy (FTIR)

Aqueous solutions of biosurfactants (0.2 mL, 10 g/L) were adsorbed onto the surface of a ZnSe glass and dried (30 min, 50 °C) until a transparent film was obtained. Spectroscopic measurement of the sample was conducted with the diffusion method by measuring the absorbance at a wavelength range from 4000 to 500 cm^{-1} using an FTIR spectrometer (Spectrum One, Perkin Elmer, Inc, Waltham, MA, USA) equipped with a KBr beam separator and a DTGS (deuterated triglycerine sulphate) detector. Each sample was scanned 64 times at a resolution of 4.0 cm^{-1} with a scanner speed of 0.2 cm/s. The measurement of the background spectrum was conducted in the presence of an empty ZnSe glass.

Capillary gel electrophoresis

The molecular mass of the biosurfactant preparation was analyzed by means of a capillary electrophoresis system (BioFocus 3000, Bio-Rad Laboratories, Hercules, CA, USA) equipped with a UV-VIS detector and a ProteomeLab SDS-MW Analysis Kit (Beckman Coulter, Inc, Fullerton, CA, USA). In the experiment, standard mixture of proteins (SDS protein sizing standard, Beckman Coulter, Inc) with molecular mass of 10, 20, 35, 50, 100, 150 and 225 kDa was used. The analyzed solution of biosurfactants (50 μL, 1 g/L) was mixed with a buffer (50 μL, sample buffer, Beckman Coulter, Inc) and supplied with an internal standard (2 μL, Beckman Coulter, Inc) and 2-mercaptoethanol (5 μL, Sigma-Aldrich, St. Louis, MO, USA). The sample was heated for 5 min at 95–100 °C, cooled and stored at room temperature. The electrophoretic separation was conducted with the use of a silica capillary tube with a diameter of 50 μm and effective length of 24 cm. Before each separation, the capillary tube was stabilized by the application of the following six-stage rinsing: 0.1 M NaOH for 300 s, 0.1 M HCl for 120 s, H_2O for 120 s, separation buffer (SDS-MW gel buffer, Beckman Coulter, Inc) for 600 s and rinsed twice with H_2O. A sample of an aqueous solution of biosurfactants was injected electrophoretically onto the capillary tube (20 s, 15 kV) and separated from – to +, under the applied voltage of 17.5 kV, at a limit of current strength reaching 50 mA, at 25 °C, for 20 min. The qualitative composition of the biosurfactants was analyzed at a wavelength of 220 nm using a UV-VIS detector, based on the peak migration time, by means of BioFocus Integrator software (Bio-Rad Laboratories).

Surface tension and critical micelle concentration measurements

The surface tension (ST) of biosurfactant solutions in phosphate buffered saline (PBS 0.01 M KH_2PO_4/K_2HPO_4 and 0.15 M NaCl with pH adjusted to 7.0) was measured by means of a digital K9 tensiometer (Krüss) with the ring method, using a platinum ring (De Noüy) 1.9 cm in diameter at a temperature of 22 °C (*7*,*8*). The measurement of the ST of each sample was conducted three times along with a control sample, *i.e.* a sterile solution of PBS. The presence of biosurfactants in the solution was confirmed based on a decrease in the value of surface tension of the isolated sample against the control sample.

All measurements were repeated three times and their average values were used. CMC was then determined from the break point of ST *vs.* its log of bulk concentration curve of biosurfactants in the range from 24.0 to 0.01172 g/L.

Oil displacement test

The experiment included measuring the diameters of lightened zones, formed due to the contact of the oil-water interface with the solution containing biosurfactants synthesized by *L. fermenti* 126 and *L. rhamnosus* CCM 1825, suspended in PBS buffer (*7*). The experiment was conducted on 9.0-cm Petri dishes filled with distilled water (25 mL) containing rapeseed oil (20 μL), and

aqueous solution of biosurfactants (10 μL, 2.5–12.5 g/L) poured onto its surface. PBS solution was used as control.

Determination of froth-forming properties

Aqueous solutions of biosurfactants synthesized by *L. fermenti* 126 and *L. rhamnosus* CCM 1825 strains were used in the study. The appropriate concentrations of biosurfactants were selected to achieve the value of the surface tension of 55, 60 and 65 mN/m. The biosurfactant solutions were homogenized with a homogenizer (Ultra-Turrax T-25, IKA®, Werke GmbH & Co. KG, Staufen, Germany) at 11 000 rpm for 5 min and the volume of the froth formed in the process was measured in a measuring cylinder (500 mL). The results were compared by determination of the frothing strength according to the formula:

$$Fp=V_1/V_2\times 100/\% \qquad /1/$$

where V_1 is the froth volume and V_2 is the volume of the analyzed solution.

Adhesive properties of Lactobacillus bacteria

Cells of *L. fermenti* 126 and *L. rhamnosus* CCM 1825 were multiplied in MRS broth (Merck) at 37 °C under stationary conditions for 18 h. The bacterial biomass, isolated from the culture by centrifugation (10 000×*g*, 5 min, 10 °C), was diluted with NaCl solution (0.9 %) to the population size of 10^6 CFU/mL and transferred to the wells (2.0 mL) of a polystyrene plate (6-well tissue culture plate, Sarstedt, Inc, Nümbrecht, Germany). The plates were incubated for 1, 3 and 5 h at 4 °C and washed twice with sterile PBS buffer (2.0 mL) in order to remove the nonadhered bacterial cells.

Adhered to the polystyrene surface, the lactic acid bacteria were stained with 4,6-diamidine-2-phenylindole (2.0 mL, Sigma-Aldrich, St. Louis, MO, USA) and washed twice with sterile distilled water (2.0 mL). The adhered cells were observed under a fluorescent microscope (BX 51, Olympus Europa GmbH, Hamburg, Germany) at a magnification of 200×, wavelength of 350 nm and counted in twenty randomly chosen fields of vision.

Antiadhesive properties of biosurfactants

A well of a sterile polystyrene plate (6-well tissue culture plate, Sarstedt, Inc) was impregnated with aqueous solutions of biosurfactant preparations (2.0 mL, 0.2 %), synthesized by *L. fermenti* 126 and *L. rhamnosus* CCM 1825. Following the plate incubation for 18 h at 4 °C, the wells were washed twice with sterile distilled water (2.0 mL).

Cells of *E. coli* 22, *K. pneumoniae* 2 or *P. aeruginosa* W2 were multiplied in McConkey medium (Merck) at 37 °C under aerobic conditions for 18 h. The bacterial biomass, isolated from the culture by centrifugation (10 000×*g*, 5 min, 10 °C), was diluted with NaCl solution (0.9 %) to the population size of 10^6 CFU/mL and transferred to wells (2.0 mL). Polystyrene plates were incubated for 1, 3 and 5 h at 4 °C; the wells were subsequently washed twice with sterile PBS buffer (2.0 mL) in order to remove the nonadhered bacterial cells. The antiadhesive properties of aqueous solutions of biosurfactants were determined with native, non-impregnated surface of polystyrene plate wells used as a reference sample.

The tested bacterial cells adhered to the native or impregnated surface were stained with 4,6-diamidine-2-phenylindole (2.0 mL, Sigma-Aldrich, St. Louis, MO, USA) and washed twice with sterile distilled water (2.0 mL). Adhered cells were observed under a fluorescent microscope (BX 51, Olympus Europa GmbH) with a magnification of 200×, wavelength of 350 nm, counted from 20 randomly chosen fields of vision and expressed as number of bacterial cell per cm^2 (N(cell)/cm^2).

Statistical analysis

Data analysis was carried out using data analysis software system STATISTICA v. 8.0 (StatSoft, Inc. Tulsa, OK, USA). A one-way analysis of variance (ANOVA) was used to study the significant difference between average values, at a significance level of α=0.05. Average comparisons were carried out using a Bonferroni test and Student's *t*-test. All data presented are average values±standard deviation of triplicates, obtained from two separate experiments unless stated otherwise.

Results

Description of biosurfactant preparations

Adhesion and the potential for competing with pathogens for intestinal epithelium surface are facilitated by the synthesis of biosurfactants. The productivity of biosurfactant synthesized by lactic acid bacteria ranges from 2 mg/g of glucose for *L. casei* ssp. *rhamnosus* 36 (*9*) to 90 mg/g of glucose for *L. pentosus* CECT-4023 (*10*).

A statistically significant difference ($p<0.05$, Student's *t*-test) was recorded between the productivities of biosurfactant synthesis on MRS medium by strains *L. fermenti* 126 and *L. rhamnosus* CCM 1825, which were used in this test; the productivity was, respectively, (111.5±3.5) and (146.5±5.2) mg/g of glucose. The productivity of biosurfactant synthesis measured against the microbial mass was also significantly different ($p<0.05$, Student's *t*-test): (383.16±12.02) and (515.85±17.61) mg/g of biomass, respectively for *L. fermenti* 126 and *L. rhamnosus* CCM 1825.

The analysis of the results obtained in this experiment showed that the average value of surface tension for the biosurfactant solution in PBS buffer following their extraction from the biomass of *L. fermenti* 126 was (50.17±0.15) mN/m and it was significantly higher ($p<0.05$, Student's *t*-test) than that recorded for the solution of biosurfactants ((46.47±0.21) mN/m) synthesized by *L. rhamnosus* CCM 1825.

Molecular composition of the crude biosurfactants

An analysis of FTIR spectra of biosurfactants (Fig. 1) synthesized by *L. fermenti* 126 and *L. rhamnosus* CCM 1825 showed excitations at the wavelengths of 3285, 1653 and 1549 cm^{-1}, typical of stretching bonds >N-H, CO-N (AmI protein band) and N-H (AmII protein band) and indicates the presence of proteins in the sample. Excitation of biosurfactants synthesized by *L. rhamnosus* CCM 1825 was recorded at similar wavelengths, *i.e.* 3287, 1656 and 1547 cm^{-1}.

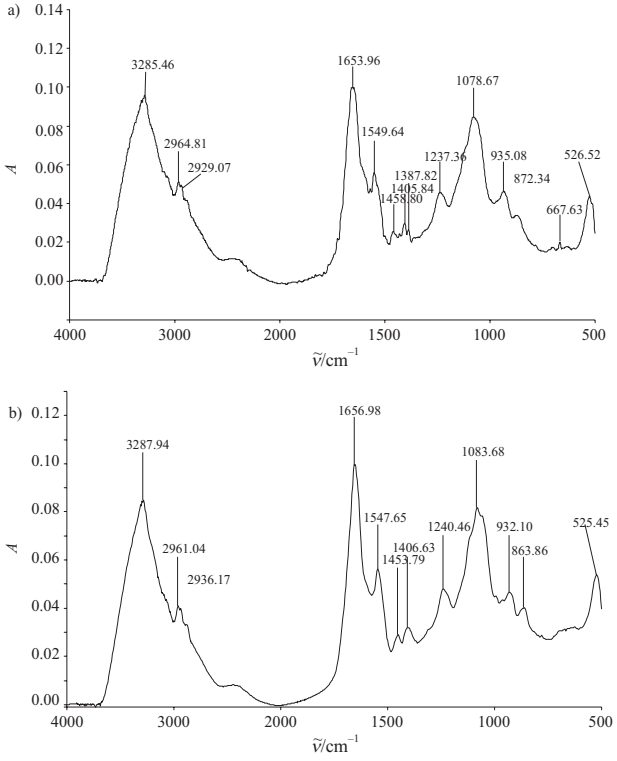

Fig. 1. FTIR spectrum of biosurfactants synthesised by: a) *L. fermenti* 126 and b) *L. rhamnosus* CCM 1825

The signal at the band of 2964, 2929 and 1458 cm^{-1}, as well as 2961, 2936 and 1453 cm^{-1} for biosurfactants obtained from *L. fermenti* 126 and *L. rhamnosus* CCM 1825, respectively, corresponds to C-H bonds of $-CH_3$, $-CH_2-$ and $>CH_2$ groups in aliphatic chains.

The band within the wavelength range from 1200 to 1000 cm^{-1} confirms the presence of a polysaccharide fraction in the both biosurfactants analyzed in the study. Excitation in the spectrum at 1078 and 1083 cm^{-1} (PII polysaccharide band) is typical of bond vibrations in the C-O-C group. The absorbance maxima in the FTIR spectrum of biosurfactants at 1237 and 1240 cm^{-1} (PI phosphate bond) and 935 and 932 cm^{-1} correspond to the stretching bonds formed by phosphorus and oxygen atoms (P-O-C) in aromatic and aliphatic molecules.

The absorbance coefficients for AmI, AmII, PI and PII bands, counted as the ratio to the C-H band for the biosurfactant isolated from *L. fermenti* 126 are equal to 2.0, 1.1, 0.9 and 1.7, respectively. *L. rhamnosus* CCM 1825 strains synthesize a biosurfactant for which the absorbance coefficients for bands AmI/CH, AMII/CH, PI/CH and PII/CH have greater values, equal to 2.5, 1.4, 1.2 and 2.1, respectively. A comparison of the value of absorbance coefficients suggests small differences in the chemical structure of biosurfactants. A larger fraction of proteins and phosphates has been shown to be present in biosurfactants isolated from *L. rhamnosus* CCM 1825 than in those obtained from *L. fermenti* 126.

Electrophoretic characterization of the crude biosurfactants

An analysis of molecular mass has shown that crude biosurfactants synthesized by *L. fermenti* 126 and *L. rhamnosus* CCM 1825 contain 8 and 9 fractions, respectively (Fig. 2). Cells of *L. fermenti* 126 synthesize surface-active compounds with molecular mass ranging from 14.9 to 54.2 kDa. It is noteworthy that among the crude biosurfactant fractions, there is one dominating, with the molecular mass of 37.5 kDa, which accounts for 22 % of all the proteins.

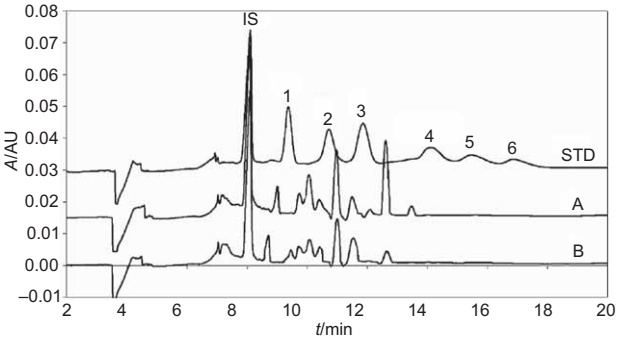

Fig. 2. Electropherogram of biosurfactants synthesized by *L. rhamnosus* CCM 1825 (A) and *L. fermenti* 126 (B) and the molecular mass standard (STD): 1 – 20 kDa, 2 – 35 kDa, 3 – 50 kDa, 4 – 100 kDa, 5 – 150 kDa and 6 – 225 kDa; IS=internal standard (10 kDa)

On the other hand, crude biosurfactants obtained from biomass of *L. rhamnosus* CCM 1825 have molecular mass ranging from 17.2 to 70.8 kDa. Two biosurfactant fractions with molecular mass of 31.4 and 53.4 kDa seem to dominate in the protein mixture. They account for 19.4 and 26.4 % of all the proteins, respectively. However, further purification of crude biosurfactants should be made to identify active fraction.

An analysis of biosurfactants of the same concentration by capillary gel electrophoresis has confirmed that the biosurfactants synthesized by *L. rhamnosus* CCM 1825 contain 44 % more protein fractions than those synthesized by *L. fermenti* 126.

Characterization of surface tension and critical micelle concentration of crude biosurfactants

Changes of ST *vs*. biosurfactant concentration in the PBS buffer are shown in Fig. 3. The results of the experiment indicate that at the same concentration (24.0 g/L), ST for the biosurfactants synthesized by *L. rham-*

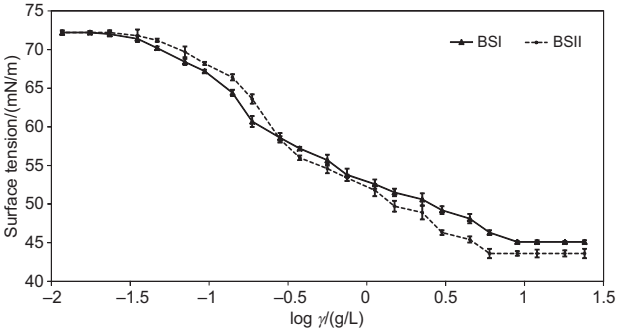

Fig. 3. Surface tension *vs*. concentration of crude biosurfactant synthesized by *L. fermenti* 126 (BSI) and *L. rhamnosus* CCM 1825 (BSII)

nosus CCM 1825 and *L. fermenti* 126 is equal to (43.6±0.6) and (45.1±0.2) mN/m, respectively.

These values indicate that they are a function of concentration for all the tested biosurfactant preparations. Surface-active substances synthesized by *L. rhamnosus* CCM 1825 and *L. fermenti* 126 strains reduced surface tension within the concentration range from 24.0 g/L to 0.02344 mg/L and from 24.0 mg/L to 0.01758 mg/L, respectively.

An analysis of the results for CMC shows that the lowest concentration for which the largest decrease of ST was recorded lies within the range from 6.0 to 4.5 g/L for biosurfactants synthesized by *L. rhamnosus* CCM 1825. The values of ST at those concentrations are equal to (43.6±0.6) and (45.4±0.4) mN/m, respectively. The largest decrease in ST for the biosurfactants synthesized by *L. fermenti* 126 lies within the concentration range from 9.0 to 6.0 g/L, in which the surface tension ranges from (45.1±0.1) to (46.3±0.3) mN/m, respectively.

Oil displacement measurements

Determination of the diameters of lightened zones formed as a result of contact of oil-water interface surfaces with the solution containing biosurfactants is an intermediate method of measurement of the sample activity towards hydrocarbons. The larger the lightened zone, the higher the activity of the biosurfactant samples.

Biosurfactants added at concentrations ranging from 2.5 to 12.5 g/L brought about statistically significant ($p<0.05$, ANOVA, Bonferroni test) changes in the size of lightened zones from (7±0.5) to (25±0.7) mm for *L. fermenti* 126 and from (14±0.7) to (30±0.5) mm for *L. rhamnosus* CCM 1825 (Table 1). It should be noted that the abilities of biosurfactants synthesized by *L. rhamnosus* CCM 1825 to change the interfacial tension were significantly ($p<0.05$, ANOVA, Bonferroni test) higher than the surface-active substances obtained from *L. fermenti* 126, regardless of the concentrations used.

An analysis of correlation has shown a strong linear relationship between the size of the lightened zones and the concentration of surface-active substances synthesized by the two strains of lactic acid bacteria. Pearson's linear correlation coefficient ($\alpha=0.05$) for the analyzed values was $r_{XY}=0.9932$ and $r_{XY}=0.9937$ for the biosurfactants synthesized by *L. fermenti* 126 and *L. rhamnosus* CCM 1825, respectively.

Froth-forming properties of crude biosurfactants

Surface tensions of 55, 60 and 65 mN/m were obtained for the concentrations of biosurfactants of 1.41, 0.39 and 0.12 g/L for *L. fermenti* 126 and 0.81, 0.22 and 0.12 g/L for *L. rhamnosus* CCM 1825.

An increase in the ST values of solutions of surface-active substances synthesized by *L. fermenti* 126 was accompanied by a statistically significant ($p<0.05$, ANOVA, Bonferroni test) decrease in their froth-forming properties, equal to (400±10), (280±5) and (130±5) % (Table 2). The findings also confirm a significant decrease ($p<0.05$, ANOVA, Bonferroni test) in the abilities of biosurfactants obtained from *L. rhamnosus* CCM 1825 with a decrease of their concentration in an aqueous solution. It was also found that at the same values of surface tension, the froth-forming abilities of biosurfactants obtained from *L. rhamnosus* CCM 1825 were stronger ($p<0.05$, ANOVA, Bonferroni test) than those of the surface-active substances obtained from *L. fermenti* 126.

Table 2. Froth-forming properties of biosurfactants synthesized by lactic acid bacteria

Sample	Foaming power/%			
	Surface tension/(mN/m)			
	PBS control	60	55	50
BS I	$(0\pm0.0)^{aA}$	$(130\pm5)^{aB}$	$(280\pm5)^{aC}$	$(400\pm10)^{aD}$
BS II	$(0\pm0.0)^{aA}$	$(150\pm5)^{bB}$	$(310\pm10)^{bC}$	$(460\pm10)^{bD}$

BS I – biosurfactant synthesized by *L. fermenti* 126
BS II – biosurfactant synthesized by *L. rhamnosus* CCM 1825
Results of three replicates are presented as average values± standard deviation. Average values in the same column followed by different lowercase letters are significantly different ($p<0.05$, ANOVA, Bonferroni test); average values in the same row followed by different uppercase letters are significantly different ($p<0.05$, ANOVA, Bonferroni test)

An analysis of the data shows that froth-forming properties depend on surface tension, with the relationship being stronger when the surface tension of the analyzed aqueous solutions of biosurfactants is lower. This regularity applies to all of the tested biosurfactant preparations. Pearson's linear correlation coefficient ($\alpha=0.05$) for the solutions was equal to $r_{XY}=-0.9378$ and $r_{XY}=-0.9492$ for the biosurfactants synthesized by *L. fermenti* 126 and *L. rhamnosus* CCM 1825, respectively.

Table 1. Diameters of the clearing zones on the oil surface obtained from oil displacement assay with several concentrations of crude biosurfactant

Sample	Diameter of the clearing zone/mm					
	Biosurfactant concentration/(g/L)					
	PBS control	2.5	5.0	7.5	10.0	12.5
BS I	$(0\pm0.0)^{aA}$	$(7\pm0.5)^{aB}$	$(11\pm0.6)^{aC}$	$(16\pm0.5)^{aD}$	$(22\pm0.6)^{aE}$	$(25\pm0.7)^{aF}$
BS II	$(0\pm0.0)^{aA}$	$(14\pm0.7)^{bB}$	$(18\pm0.6)^{bC}$	$(22\pm0.7)^{bD}$	$(25\pm0.5)^{bE}$	$(30\pm0.5)^{bF}$

BS I – biosurfactant synthesized by *L. fermenti* 126
BS II – biosurfactant synthesized by *L. rhamnosus* CCM 1825
Results of three replicates are presented as average values±standard deviation. Average values in the same column followed by different lowercase letters are significantly different ($p<0.05$, ANOVA, Bonferroni test); average values in the same row followed by different uppercase letters are significantly different ($p<0.05$, ANOVA, Bonferroni test)

Characterization of adhesive properties of Lactobacillus bacteria

The study indicates the existence of positive adhesive properties of *Lactobacillus* cells to a polystyrene surface. The size of *L. fermenti* 126 and *L. rhamnosus* CCM 1825 populations (Table 3) that adhered to the surface statistically increased with time ($p<0.05$, ANOVA, Bonferroni test). The number of *L. rhamnosus* CCM 1825 cells bound to the polystyrene surface increased from (687±223) N(cell)/cm^2 after 1 h to (1781±240) and (4344±298) N(cell)/cm^2 after 3 and 5 h, respectively. It should be noted that the adhesion to polystyrene was significantly greater ($p<0.05$, ANOVA, Bonferroni test) for *L. fermenti* 126 than for *L. rhamnosus* CCM 1825 at each time interval. After 5 h of contact of cells with the surface used in the test, the number of bacterial cells which adhered to the surface was equal to (5563±311) and (4344±298) N(cell)/cm^2 for *L. fermenti* 126 and *L. rhamnosus* CCM 1825, respectively.

Table 3. Adhesive properties of lactic acid bacteria to polystyrene surface

Strain	Bacterial population/(N(cell)/cm^2)		
	Time/h		
	1	3	5
L. fermenti 126	$(1844\pm240)^{aA}$	$(2531\pm279)^{aA}$	$(5563\pm311)^{aB}$
L. rhamnosus CCM 1825	$(687\pm223)^{bA}$	$(1781\pm240)^{bB}$	$(4355\pm298)^{bC}$

Results of three replicates are presented as average values± standard deviation. Average values in the same column followed by different lowercase letters are significantly different ($p<0.05$, ANOVA, Bonferroni test); average values in the same row followed by different uppercase letters are significantly different ($p<0.05$, ANOVA, Bonferroni test)

Antiadhesiveness of biosurfactants

The results presented in Table 4 indicate the occurrence of adhesion of the cells of pathogenic strains of *E. coli* 22, *K. pneumoniae* 2 and *P. aeruginosa* W2 to polystyrene surfaces and to such surfaces impregnated with aqueous solution of biosurfactants synthesized by *L. fermenti* 126 and *L. rhamnosus* CCM 1825. The surface tension values for the biosurfactant solutions used in the study were significantly lower ($p<0.05$, Student's t-test): (48.5±0.5) and (48.1±0.4) mN/m as compared to the PBS solution, for which the ST was (72.0±0.2) mN/m.

The size of *Enterobacteriaceae* population per unit of polystyrene surface significantly increased ($p<0.05$, ANOVA, Bonferroni test) with increasing adhesion time, reaching the values of (3688±187), (4875±400) and (4313±187) N(cell)/cm^2, respectively for *E. coli* 22, *K. pneumoniae* 2 and *P. aeruginosa* W2, after 5 h of adhesion. The tested pathogenic strains were shown to be significantly different ($p<0.05$, ANOVA, Bonferroni test) in terms of the ability to adhere to polystyrene. *E. coli* 22 strain demonstrated the weakest adhesion properties regardless of the time of contact with the surface.

The antiadhesive properties of biosurfactants have been shown to depend ($p<0.05$, ANOVA, Bonferroni test) on the time of contact of *Enterobacteriaceae* with the impregnated surface. The size of the tested population of bacteria after 1 h of incubation on the polystyrene surface impregnated with the biosurfactants obtained from *L. fermenti* 126 and from *L. rhamnosus* CCM 1825 did not change significantly ($p<0.05$, ANOVA, Bonferroni test).

Extending the time of contact of bacteria with the surfaces to 3 h resulted in significant ($p<0.05$, ANOVA, Bonferroni test) changes in the population of adhering bacteria of only two strains – *K. pneumoniae* 2 (Fig. 4) and *P. aeruginosa* W2. It should be noted that the strongest, statistically significant ($p<0.05$, ANOVA, Bonferroni

Table 4. Antiadhesive properties of the biosurfactant synthesized by lactic acid bacteria

Strain	Bacterial population/(N(cell)/cm^2)		
	Time/h		
	1	3	5
	Polystyrene surface		
E. coli 22	$(1813\pm223)^{aA(a)}$	$(2438\pm272)^{aB(a)}$	$(3688\pm187)^{aC(a)}$
K. pneumoniae 2	$(2438\pm187)^{bA(a)}$	$(3688\pm136)^{bB(a)}$	$(4875\pm400)^{bC(a)}$
P. aeruginosa W2	$(2406\pm250)^{abA(a)}$	$(3656\pm400)^{bB(a)}$	$(4313\pm187)^{abC(a)}$
	Polystyrene surface impregnated with biosurfactant synthesized by *L. fermentii* 126		
E. coli 22	$(1438\pm223)^{aA(a)}$	$(2438\pm187)^{aB(a)}$	$(3156\pm136)^{aC(b)}$
K. pneumoniae 2	$(2000\pm250)^{abA(a)}$	$(3031\pm223)^{bB(b)}$	$(4313\pm187)^{bC(ab)}$
P. aeruginosa W2	$(2406\pm223)^{bA(a)}$	$(3063\pm187)^{bB(ab)}$	$(3719\pm136)^{cC(b)}$
	Polystyrene surface impregnated with biosurfactant synthesized by *L. rhamnosus* CCM 1825		
E. coli 22	$(1375\pm250)^{aA(a)}$	$(2375\pm223)^{abB(a)}$	$(3063\pm187)^{aC(b)}$
K. pneumoniae 2	$(1938\pm187)^{abA(a)}$	$(1939\pm187)^{aA(c)}$	$(4313\pm187)^{bB(b)}$
P. aeruginosa W2	$(2313\pm250)^{bA(a)}$	$(2594\pm223)^{bA(b)}$	$(3688\pm187)^{bB(b)}$

Results of three replicates are presented as average values±standard deviation. Significantly different populations of *Enterobacteriaceae* bacteria dependent on: the same surfaces are expressed as the average values in the same column followed by different lowercase letters ($p<0.05$, ANOVA, Bonferroni test); different surfaces are expressed as the average values in the same column followed by different lowercase letters in the bracket ($p<0.05$, ANOVA, Bonferroni test); time of adhesion in the same row followed by different uppercase letters ($p<0.05$, ANOVA, Bonferroni test)

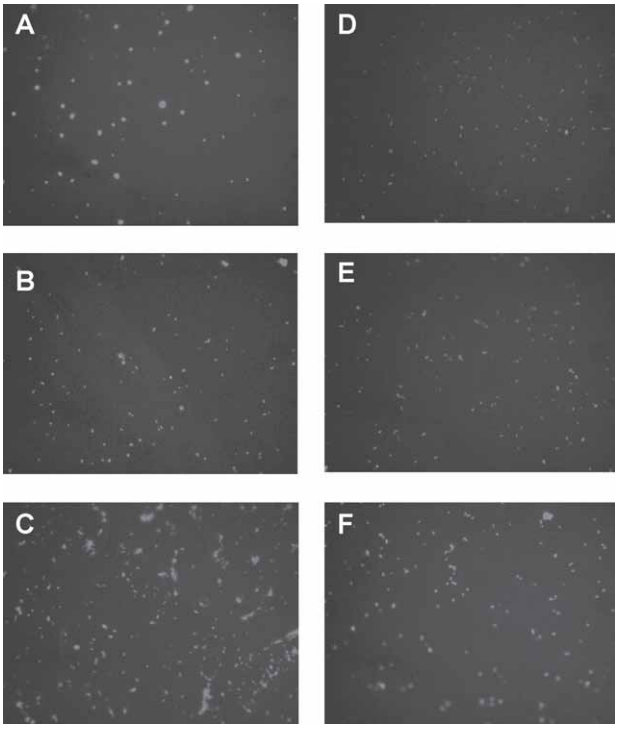

Fig. 4. Microscopic images of *K. pneumoniae* 2 adhering to native polystyrene surfaces after (A) 1 h, (B) 3 h and (C) 5 h and on polystyrene surfaces impregnated with crude biosurfactants synthesized by *L. rhamnosus* CCM 1825 after (D) 1 h, (E) 3 h and (F) 5 h

test), antiadhesive properties after 3 h of incubation were recorded for the biosurfactant synthesized by *L. rhamnosus* CCM 1825. The size of the population of *K. pneumoniae* 2 and *P. aeruginosa* W2, following impregnation with the biosurfactant, significantly ($p<0.05$, ANOVA, Bonferroni test) decreased by 47.5 and 29.0 %, respectively. Coating of the polystyrene surface with the surface-active substances produced by *L. fermenti* 126 significantly reduced ($p<0.05$, ANOVA, Bonferroni test) only the population of *K. pneumoniae* 2 by 17.8 %. The antiadhesive properties of *E. coli* 22 remained unchanged after 3 h of incubation, regardless of the type of surfaces used in the test.

The findings indicate that after 5 h of impregnation of the surfaces with biosurfactants, the number of all the pathogenic microbes was significantly reduced ($p<0.05$, ANOVA, Bonferroni test) as compared to the native surface. The surface-active substances produced by *L. fermenti* 126 demonstrated antiadhesive properties towards *E. coli* 22 and *P. aeruginosa* W2. The size of populations of these bacteria decreased ($p<0.05$, ANOVA, Bonferroni test) from (3688±187) to (3156±136) N(cell)/cm^2 and from (4313±187) to (3719±136) N(cell)/cm^2, respectively. Polystyrene impregnation with an aqueous solution of biosurfactants synthesized by *L. rhamnosus* CCM 1825 resulted in hindering the adhesion of all the *Enterobacteriaceae*. The number of *E. coli* 22, *K. pneumoniae* 2 and *P. aeruginosa* W2 cells which adhered to the surface was lower ($p<0.05$, ANOVA, Bonferroni test) by 16.9, 23.7 and 14.5 %, respectively, as compared to the native polystyrene surface.

Extending the contact time of pathogens with the surfaces coated with the surface-active substance to 5 h revealed that the biosurfactants used in the test have similar antiadhesive properties ($p<0.05$, ANOVA, Bonferroni test). Similar properties were recorded for the biosurfactants produced by *L. casei* 8/4 (*11*).

Discussion

Lactobacillus bacteria, which are part of a group referred to as lactic acid bacteria, are known to have health-promoting properties commonly used in food industry (*1*). Administered in the appropriate numbers, lactic acid rods may have a beneficial effect on the human body. Probiotics usually denote microbes which form a protective barrier against pathogenic flora, which stimulate the immune system, improve the nutritional quality of food, enhance lactose hydrolysis and reduce the cholesterol level (*12–14*).

The antimicrobial properties of the bacteria stem from their ability to synthesize specific metabolites, *e.g.* bacteriocins and lactic acid. One of the criteria for including bacteria in a group of probiotics are their adhesive properties which facilitate their populating the gastrointestinal tract (mainly the intestines) and the genitourinary tract, thereby providing an effective barrier against pathogenic microflora (*15*). Adhesion of lactic acid bacteria cells to the intestine is important in maintaining the healthy composition of gastrointestinal microflora, but it also enhances the activity of enzymes and stabilizes the intestine permeability (*16*). Mechanisms of microbial cell adhesion to the intestinal epithelium may be non-specific biochemically or specific, resulting from the synthesis of certain compounds on bacterial cell surface, which then enter into interactions with receptors on epithelial cells (*17*). Biosurfactants are among the metabolites which participate in the process of specific adherence to epithelium (*18*).

Biosurfactants synthesized by LAB are increasingly applied in medicine as components of therapeutic agents, which play an important role in the prevention and control of infections caused by pathogens from various groups of microbes (*3,19*). Due to their importance in the adhesion of microbial cells to various surfaces – polystyrene, glass or silicon rubber – they are applied as impregnates of the surfaces of medical equipment (*3,18,20*).

Most known biosurfactants synthesized by lactic acid bacteria consist of protein and polysaccharide components (*11,21,22*). Studies conducted by Busscher *et al.* (*23*) showed that *Streptococcus thermophilus* B strain synthesizes a mixture of biosurfactants, with their glycolipidic fraction demonstrating the highest surface activity. Glycolipids, which reduce surface tension in liquid-liquid systems, have been isolated from the bacteria of *S. thermophilus* A strain and from some *Lactobacillus* strains (*9,22*). Among the biosurfactants synthesized by lactic acid bacteria, there are also glycosyldiglycerides, isolated from *Lactobacillus* spp. (*9*).

It has been shown with the use of Fourier transform infrared spectroscopy that *L. fermenti* 126 and *L. rhamnosus* CCM 1825 bacteria synthesize biosurfactants which are mixtures of several compounds. This preliminary

study showed that the FTIR spectra of both crude biosurfactants contain three components: a protein, polysaccharide and phosphate. Furthermore, their purification should allow identification of active fraction.

A comparison of the value of absorbance coefficients for AmI, AmII, PI and PII bands counted as the ratio to the C-H band suggests small differences in the chemical structure of biosurfactants. *L. rhamnosus* CCM 1825 synthesizes a biosurfactant with larger fraction of proteins and phosphates than in those obtained from *L. fermenti* 126. Literature data showed that *L. lactis* 53 released similar crude biosurfactant characterized with absorption band ratios of AmI, AmII, PI and PII with respect to the C-H band of 1.3, 1.0, 0.9 and 2.1, respectively (*7*). The FTIR analysis showed that biosurfactant fraction obtained from *L. lactis* 53 by hydrophobic interaction chromatography (HIC) exhibited a similar protein content but lower polysaccharide and phosphate contents as compared to the crude biosurfactant. However, fractionated and crude biosurfactant had similar CMC of 14 g/L, suggesting an important role of protein compound in decreasing surface tension (*7*).

On the contrary, the most surface-active biosurfactant fraction produced by *S. thermophilus* A obtained by hydrophobic interaction chromatography exhibits CMC of 20 g/L, similar to the crude biosurfactant. The FTIR spectra showed that this crude biosurfactant consists of a mixture of protein, phosphates and polysaccharides. Nevertheless, purified surface-active biosurfactant fraction had no absorption bands AmI and AmII characteristic for protein compounds, indicating that other compounds were responsible for decreasing surface tension (*22*).

Surface-active substances with similar structures have been described by Velraeds *et al.* (*9*) and termed surfactins. They showed that lactic acid bacteria can synthesize biosurfactants composed of the same components, but in various proportions. The chemical structure of biosurfactants, mainly the proportions of their components, seems to significantly affect their antiadhesive properties. Biosurfactants that contain a higher fraction of proteins and which are synthesized by *L. acidophilus* RC14 and *L. fermentum* B54 are more effective in hindering the adhesion of *E. faecalis* 1131 to glass than those in which high polysaccharide and phosphate fractions have been found (*21*).

Surfactants synthesized by microbes are more easily biodegradable and less toxic than those obtained by chemical synthesis; their chemical structure is also more varied. They also demonstrate very good ability to lower surface tension. An example could be surfactin synthesized by *Bacillus subtilis*, which lowers the surface tension of water to 27 mN/m at the CMC of 0.011 mg/mL, whereas synthetic sodium dodecyl sulphate (SDS) lowers the surface tension of water to 37 mN/m at CMC of 2.0–2.9 mg/mL. The effectiveness of surfactants is described by their ability to lower surface tension. A good surfactant can lower ST of water from 72 to 35 mN/m (*24*). *L. fermenti* 126 and *L. rhamnosus* CCM 1825 strains used in this study synthesize biosurfactants with low effectiveness, with CMC of 9.0 and 6.0 g/L and ST of (45.1±0.1) and (43.6±0.6) mN/m, respectively. It should be noted that these values relate to the nonpurified preparations of biosurfactants. The process of biosurfactant purification alters their properties, *e.g.* fraction A, isolated from the biosurfactant synthesized by *Lactococcus lactis* 53, has a characteristic value of CMC of 14 g/L and ST of 35 mN/m, whereas such values for the nonpurified surfactant are CMC of 14 g/L and ST of 40 mN/m (*7*).

The ability of these bacteria to synthesize biosurfactants makes them adhere better to various surfaces due to physicochemical changes of the surface (*25*). They also hinder adherence of pathogenic bacteria to epithelial cells, both in the gastrointestinal and genitourinary systems in humans. This increases the competitiveness of *Lactobacillus* in populating the ecological niche in those systems (*1,10*).

There are literature data which confirm the ability of other species of lactic acid bacteria to adhere to polystyrene surfaces. A study conducted by Boonaert *et al.* (*26*) could be used as an example; it involved evaluation of *L. lactis* adhesion to polystyrene surface. Adhesion of *L. lactis* was at the level of 10^6 CFU/cm^2, *i.e.* it was greater by three orders of magnitude than the values recorded in this study. Lower adherence of cells in our study may be the result of the inoculum applied with a density of 10^6 CFU/mL, compared to the inoculum of $5{\cdot}10^8$ CFU/mL, used in the experiment conducted by Boonaert *et al.* (*26*). Crude biosurfactants released by *Lactobacillus paracasei* exhibited a considerable antiadhesive activity against *Staphylococcus aureus*, *S. epidermidis* and *S. agalactiae*. However, low activity of biosurfactant at the same concentration of 25 mg/mL was observed against *P. aeruginosa* and *E. coli* (*27*).

The findings of the study conducted by Czaczyk *et al.* (*28*) showed that *L. casei* Shirota, *L. acidophilus* LC1 and *L. rhamnosus* GG strains do not synthesize substances which facilitate their adhesion to polystyrene surface. This indicates that an ability to adhere to specific surfaces is a strain-dependent feature.

Biosurfactants of microbial origin are known to affect both adhesion and desorption of microbes from various surfaces. Bacteria of *S. thermophilus* strain synthesize biosurfactants which make them desorb from glass, leaving an impregnated surface with antiadhesive properties (*29*). Strains of *Streptococcus mitis* secrete surface-active substances which hinder adhesion of *S. mutans* (*30*).

The findings of this study show that cells of *Lactobacillus* bacteria which synthesize biosurfactants adhere well to a polystyrene surface. Biosurfactants have also been found to demonstrate various effectiveness in hindering adhesion of pathogenic *E. coli* 22, *K. pneumoniae* 2 and *P. aeruginosa* W2 to the surface used in this study. Surface-active compounds synthesized by *L. rhamnosus* CCM 1825 were more effective between 0.8 and 56.3 % in hindering the adhesion of the tested pathogens than those secreted by *L. fermenti* 126. The good antiadhesive properties of these biosurfactants are confirmed by oil displacement assay and froth-forming properties. These properties determine the ability of biosurfactants to change the surface tension on the liquid-liquid and liquid-air interface, respectively. Biosurfactants synthesized by *L. rhamnosus* CCM 1825 demonstrated higher interfacial tension and froth-forming activity than those obtained from *L. fermenti* 126, which probably resulted in better antiadhesive properties.

Molecular description of biosurfactants synthesized by *L. fermenti* 126 and *L. rhamnosus* CCM 1825 by FTIR spectroscopy revealed differences in the structure of the two compounds. Moreover, gel capillary electrophoresis has also confirmed dissimilarities in the structure of both biosurfactants, which may be the cause of difference in their antiadhesive properties. A greater protein fraction was found in the biosurfactant obtained from *L. rhamnosus* CCM 1825 than in that from *L. fermenti* 126. Gel capillary electrophoresis performed on *Lactobacillus* biosurfactants showed that the present protein was not a pure single component protein. Literature data suggest that crude biosurfactants synthesized by some *Lactobacillus* strains are composed mainly of proteins with molecular mass from 14.4 to above 94.0 kDa (*9*). The molecular mass of crude biosurfactants synthesized by *L. rhamnosus* CCM 1825 and *L. fermenti* 126 varied between 14.9 and 70.8 kDa and was comparable to those synthesized by *L. casei* ssp. *rhamnosus* 36 and *L. acidophilus* RC14. Other microorganisms like *Pseudomonas aeruginosa* and *Torulopsis bombicola* released different forms of rhamnolipids and sophorolipids, respectively (*9*).

Better emulsifying and froth-forming properties of surface-active substances isolated from *Kluyveromyces fragilis*, containing greater portions of proteins than polysaccharides, was confirmed by del Carmen Vasallo *et al.* (*31*). Higher protein fraction in biosurfactants favours their activity in oil-water and air-water interfaces.

It should be noted that in our earlier studies cells of *Lactobacillus* strains used in the current experiment had been found to adhere well to intestinal epithelial cells cultured *in vitro* and represented by the Caco-2 cell line (*18*). Positive adhesive properties may be linked to the ability to synthesize surface-active substances.

Literature data suggest that biosurfactants synthesized by the cells of some *Lactobacillus* strains favour their adhesive properties. Hence, they are applied in medicine as impregnates of medical equipment surfaces (*20,22*). Biosurfactants synthesized by *L. acidophilus* ATCC 4356 and *L. fermentum* B54 effectively hinder the adhesion of producer cells to hydrophilic and hydrophobic surfaces (*32*).

The results presented in this study indicate the importance of biosurfactants in hindering the adhesion of pathogenic bacteria to polystyrene surfaces. Scientific reports have confirmed the effect of biosurfactants on hindering the adhesion of pathogenic bacteria to various surfaces. *L. acidophilus* NCFM and *L. fermentum* RC-14 cells synthesize biosurfactants, which significantly hinders the adhesion of *E. faecalis* 1131 to polystyrene surfaces (*1*). Synthesis and secretion of biosurfactants by lactic acid bacteria contribute to hindering the adhesion of pathogenic bacteria in model systems and under natural conditions (gastrointestinal and genitourinary tract) and may determine the competitiveness of *Lactobacillus* strains in populating ecological niches (*25*).

Conclusions

In conclusion, it may be claimed that the results of this study have confirmed the good adhesion of the *Lactobacillus* strains used in this study to polystyrene surfaces. The analyzed strains of lactic acid bacteria synthesize biosurfactants, whose molecules consist of proteins, polysaccharides and phosphates, and which demonstrate good antiadhesive properties against *Enterobacteriaceae*. Therefore, it may be suggested that *Lactobacillus fermenti* 126 and *Lactobacillus rhamnosus* CCM 1825 strains, which synthesize biosurfactants, can be used in the production of food, *e.g.* yoghurts, and can contribute to hindering the adhesion of pathogenic bacteria to epithelial cell receptors in humans. Confirmation of this hypothesis will be sought in further studies.

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References

1. G. Reid, *In vitro* testing of *Lactobacillus acidophilus* NCFM™ as a possible probiotic for the urogenital tract, *Int. Dairy J. 10* (2000) 415–419.
2. E. Isolauri, Role of probiotics in food hypersensitivity, *Curr. Opin. Allergy Clin. Immunol. 2* (2002) 263–271.
3. M.M.C. Velraeds, G. van de Belt-Gritter, H.J. Busscher, G. Reid, H.C. van der Mei, Inhibition of uropathogenic biofilm growth on silicone rubber in human urine by lactobacilli – A teleologic approach, *World J. Urol. 18* (2000) 422–426.
4. R. del Campo, D. Bravo, R. Cantón, P. Ruiz-Garbajosa, R. García–Albiach, A. Montesi-Libois *et al.*, Scarce evidence of yogurt lactic acid bacteria in human feces after daily yogurt consumption by healthy volunteers, *Appl. Environ. Microbiol. 71* (2005) 547–549.
5. C. Heinemann, J.E.T. van Hylckama Vlieg, D.B. Janssen, H.J. Busscher, H.C. van der Mei, G. Reid, Purification and characterization of a surface–binding protein from *Lactobacillus fermentum* RC–14 that inhibits adhesion of *Enterococcus faecalis* 1131, *FEMS Microbiol. Lett. 190* (2000) 177–180.
6. L. Rodrigues, I.M. Banat, J. Teixeira, R. Oliveira, Biosurfactants: Potential applications in medicine, *J. Antimicrob. Chemother. 57* (2006) 609–618.
7. L. Rodrigues, J.A. Teixeira, H.C. van der Mei, R. Oliveira, Physicochemical and functional characterization of a biosurfactant produced by *Lactococcus lactis* 53, *Colloids Surf. B: Biointerfaces, 49* (2006) 79–86.
8. P. Singh, S.S. Cameotra, Potential applications of microbial surfactants in biomedical sciences, *Trends Biotechnol. 22* (2004) 142–146.
9. M.M.C. Velraeds, H.C. van der Mei, G. Reid, H.J. Busscher, Physicochemical and biochemical characterization of biosurfactants released by *Lactobacillus* strains, *Colloids Surf. B: Biointerfaces, 8* (1996) 51–61.
10. L. Rodrigues, A. Moldes, J. Teixeira, R. Oliveira, Kinetic study of fermentative biosurfactant production by *Lactobacillus* strains, *Biochem. Eng. J. 28* (2006) 109–116.
11. P. Gołek, W. Bednarski, B. Brzozowski, B. Dziuba, The obtaining and properties of biosurfactants synthesized by bacteria of the genus *Lactobacillus*, *Ann. Microbiol. 59* (2009) 119–126.
12. R. Fuller, Probiotics in human medicine, *Gut, 32* (1991) 439–442.
13. S.E. Gilliland, Health and nutritional benefits from lactic acid bacteria, *FEMS Microbiol. Rev. 7* (1990) 175–188.
14. M. Zhang, X. Hang, X. Fan, D. Li, H. Yang, Characterization and selection of *Lactobacillus* strains for their effect

on bile tolerance, taurocholate deconjugation and cholesterol removal, *World J. Microbiol. Biotechnol. 24* (2008) 7–14.

15. W.A. Walker, L.C. Duffy, Diet and bacterial colonization: Role of probiotics and prebiotics, *J. Nutr. Biochem. 9* (1998) 668–675.
16. M. Katayama, D. Xu, R.D. Specian, E.A. Deitch, Role of bacterial adherence and the mucus barrier on bacterial translocation: Effects of protein malnutrition and endotoxin in rats, *Ann. Surg. 225* (1997) 317–326.
17. M.F. Bernet, D. Brassart, J.R. Neeser, A.L. Servin, Adhesion of human bifidobacterial strains to cultured human intestinal epithelial cells and inhibition of enteropathogen-cell interactions, *Appl. Environ. Microbiol. 59* (1993) 4121–4128.
18. P. Gołek, W. Bednarski, M. Lewandowska, Characteristics of the adhesive properties of *Lactobacillus* strains synthesizing biosurfactants, *Pol. J. Natur. Sci. 22* (2007) 333–342.
19. S.S. Cameotra, R.S. Makkar, Recent applications of biosurfactants as biological and immunological molecules, *Curr. Opin. Microbiol. 7* (2004) 262–266.
20. M.M. Velraeds, H.C. van der Mei, G. Reid, H.J. Busscher, Inhibition of initial adhesion of uropathogenic *Enterococcus faecalis* to solid substrata by an adsorbed biosurfactant layer from *Lactobacillus acidophilus*, *Urology, 49* (1997) 790–794.
21. M.M. Velraeds, H.C. van der Mei, G. Reid, H.J. Busscher, Inhibition of initial adhesion of uropathogenic *Enterococcus faecalis* by biosurfactants from *Lactobacillus* isolates, *Appl. Environ. Microbiol. 62* (1996) 1958–1963.
22. L.R. Rodrigues, J.A. Teixeira, H.C. van der Mei, R. Oliveira, Isolation and partial characterization of a biosurfactant produced by *Streptococcus thermophilus* A, *Colloids Surf. B: Biointerfaces, 53* (2006) 105–112.
23. H.J. Busscher, C.G. van Hoogmoed, G.I. Geertsema-Doornbusch, M. van der Kuijl-Booij, H.C. van der Mei, *Streptococcus thermophilus* and its biosurfactants inhibit adhesion by *Candida* spp. on silicone rubber, *Appl. Environ. Microbiol. 63* (1997) 3810–3817.
24. C. Mulligan, B. Gibbs: Factors Influencing the Economics of Biosurfactants. In: *Biosurfactants: Production, Properties, Applications*, *Vol. 48*, N. Kosaric (Ed.), Marcel Dekker Inc., New York, NY, USA (1993) pp. 329–371.
25. G. Reid, B.S. Gan, Y.M. She, W. Ens, S. Weinberger, J.C. Howard, Rapid identification of probiotic *Lactobacillus* biosurfactant proteins by ProteinChip tandem mass spectrometry tryptic peptide sequencing, *Appl. Environ. Microbiol. 68* (2002) 977–980.
26. C.J.P. Boonaert, Y.F. Dufrêne, S.R. Derclaye, P.G. Rouxhet, Adhesion of *Lactococcus lactis* to model substrata: Direct study of the interface, *Colloids Surf. B: Biointerfaces, 22* (2001) 171–182.
27. E.J. Gudiña, J.A. Teixeira, L.R. Rodrigues, Isolation and functional characterization of a biosurfactant produced by *Lactobacillus paracasei*, *Colloids Surf. B: Biointerfaces, 76* (2010) 298–304.
28. K. Czaczyk, A. Olejnik, P. Miężał, W. Grajek, Searching for simple models to study the adhesion of probiotic bacteria, *Żywność-Nauka Technologia Jakość, 1* (2005) 84–96 (in Polish).
29. H.J. Busscher, T.R. Neu, H.C. van der Mei, Biosurfactant production by thermophilic dairy streptococci, *Appl. Environ. Microbiol. 41* (1994) 4–7.
30. I.H. Pratt-Terpstra, A.H. Weerkamp, H.J. Busscher, Microbial factors in a thermodynamic approach of oral streptococcal adhesion to solid substrata, *J. Colloid Interface Sci. 129* (1989) 568–574.
31. M. del Carmen Vasallo, M.C. Puppo, G.G. Palazolo, M.A. Otero, L. Beressc, J.R. Wagner, Cell wall proteins of *Kluyveromyces fragilis*: Surface and emulsifying properties, *LWT – Food Sci. Technol. 39* (2006) 729–739.
32. P. Cadieux, J.D. Watterson, J. Denstedt, R.R. Harbottlee, J. Puskas, J. Howard *et al.*, Potential application of polyisobutylene-polystyrene and a *Lactobacillus* protein to reduce the risk of device-associated urinary tract infections, *Colloids Surf. B: Bionterfaces, 28* (2003) 95–105.