APPLIED AND ENVIRONMENTAL MICROBIOLOGY, June 1996, p. 1958–1963 Vol. 62, No. 6
0099-2240/96/$04.00+0

Inhibition of Initial Adhesion of Uropathogenic *Enterococcus faecalis* by Biosurfactants from *Lactobacillus* Isolates

MARTINE M. C. VELRAEDS,[1] HENNY C. VAN DER MEI,[1] GREGOR REID,[2] AND HENK J. BUSSCHER[1,2]*

Laboratory for Materia Technica, University of Groningen, Groningen, The Netherlands,[1] and Department of Microbiology and Immunology, University of Western Ontario, London, Ontario, Canada[2]

Received 5 September 1995/Accepted 20 March 1996

In this study, 15 *Lactobacillus* isolates were found to produce biosurfactants in the mid-exponential and stationary growth phases. The stationary-phase biosurfactants from *Lactobacillus casei* subsp. *rhamnosus* 36 and ATCC 7469, *Lactobacillus fermentum* B54, and *Lactobacillus acidophilus* RC14 were investigated further to determine their capacity to inhibit the initial adhesion of uropathogenic *Enterococcus faecalis* 1131 to glass in a parallel-plate flow chamber. The initial deposition rate of *E. faecalis* to glass with an adsorbed biosurfactant layer from *L. acidophilus* RC14 or *L. fermentum* B54 was significantly decreased by approximately 70%, while the number of adhering enterococci after 4 h of adhesion was reduced by an average of 77%. The surface activity of the biosurfactants and their activity inhibiting the initial adhesion of *E. faecalis* 1131 were retained after dialysis (molecular weight cutoff, 6,000 to 8,000) and freeze-drying. Fourier transform infrared spectroscopy and X-ray photoelectron spectroscopy revealed that the freeze-dried biosurfactants from *L. acidophilus* RC14 and *L. fermentum* B54 were richest in protein, while those from *L. casei* subsp. *rhamnosus* 36 and ATCC 7469 had relatively high polysaccharide and phosphate contents.

The normal healthy microflora of the female urogenital tract is dominated by lactobacilli. Facultative lactobacilli make up 50 to 90% of the aerobic vaginal microflora in women (31) and are also abundant in the aerobic urethral flora of healthy women of reproductive age, accounting for 38% of the aerobic flora (18). Females with recurrent urinary tract infections, however, have a disturbed ecological balance, and the flora consists almost entirely of uropathogens (17, 19, 28). In vitro and clinical studies have provided evidence which supports the notion that lactobacilli may protect their hosts and keep them from acquiring urinary tract infections (3, 32).

Lactobacilli are believed to interfere with pathogens by different mechanisms. First, it has been shown that certain *Lactobacillus* isolates attach well to human uroepithelial cells in vitro (5, 32) and that *Lactobacillus* whole cells (viable and nonviable) and cell wall fragments are able to inhibit uropathogenic bacteria from adhering to these uroepithelial cells by competitive exclusion (5, 6, 32). The affinity of a lactobacillus for uroepithelial cells appears to be more important than its size in this competitive exclusion effect (32). Second, lactobacilli are able to coaggregate with uropathogenic bacteria (35, 36); if this coaggregation is accompanied by inhibitor production by the lactobacilli (20), it may result in elimination of the uropathogens. Lactobacilli have also been shown to produce antibacterial compounds, such as lactic acid, hydrogen peroxide, bacteriocin-like substances (2, 11, 15, 20, 21, 35), and possibly biosurfactants.

Biosurfactants are compounds released by microorganisms with a distinct tendency to accumulate at interfaces, most notably liquid-air interfaces. Dairy *Streptococcus thermophilus* strains, for example, can produce biosurfactants which cause their own desorption (4), and oral *Streptococcus mitis* strains produce biosurfactants that inhibit adhesion of *Streptococcus mutans* (29). *Lactobacillus* and *Streptococcus* species have been shown to be able to displace adhering uropathogenic *Enterococcus faecalis* strains from hydrophobic and hydrophilic substrata in a parallel-plate flow chamber, possibly through biosurfactant production (22).

A rapid technique for measuring microbial biosurfactant production has been proposed by van der Vegt et al. (40); this technique is based on a shape analysis of axisymmetric droplets of a microbial suspension on a hydrophobic substratum. Since biosurfactants produced by the suspended microorganisms accumulate at the liquid-vapor interface, the resulting decrease in surface tension is immediately obvious from a flattening of the droplets.

The aims of this study were to screen a number of *Lactobacillus* strains for biosurfactant production by axisymmetric drop shape analysis and to study the chemical compositions of the biosurfactants produced by different strains by using Fourier transform infrared spectroscopy and X-ray photoelectron spectroscopy. The abilities of the biosurfactants to inhibit the initial adhesion of uropathogenic *E. faecalis*, the second-most-common uropathogen isolated from hospitalized patients with urinary tract infections and certain community patients with urinary tract infections (16, 30), were also studied with our parallel-plate flow chamber.

MATERIALS AND METHODS

Strains. Several *Lactobacillus* strains were investigated in this study. *Lactobacillus acidophilus* RC14, *Lactobacillus casei* 70, *L. casei* subsp. *rhamnosus* GR-1, and *Lactobacillus plantarum* RC6 and RC20 were all obtained from the urogenital tracts of healthy women; *L. casei* subsp. *rhamnosus* 36 was isolated from a woman with a history of urogenital infections; *L. acidophilus* T13 and *Lactobacillus fermentum* B54 were poultry isolates; and *L. casei* subsp. *rhamnosus* 81 was a dairy strain. Most of these strains were isolated between 1982 and 1986 and were stored with minimal numbers of passages; the exceptions were *L. casei* subsp. *rhamnosus* 36 and *L. casei* 70, which were isolated in 1992 (33). The following strains were obtained from the American Type Culture Collection: *L. fermentum* ATCC 23271 and ATCC 14931 (type strain), *L. acidophilus* ATCC 4356 (type strain), *L. casei* subsp. *casei* ATCC 393 (type strain), *L. casei* subsp. *rhamnosus* ATCC 7469 (type strain), and *L. plantarum* ATCC 14917 (type strain). Several studies have shown that the vaginal *Lactobacillus* flora is dominated by *L. acidophilus* and *L. fermentum*, but *L. casei*, *L. plantarum*, and *Lactobacillus jensenii*, among many other species, have also been isolated (25, 32, 38).

* Corresponding author. Mailing address: Laboratory for Materia Technica, University of Groningen, Bloemsingel 10, 9712 KZ Groningen, The Netherlands. Phone: (31) 50 3633140. Fax: (31) 50 3633159.

All strains were stored in MRS broth (the *Lactobacillus* broth described by J. C. De Man, M. Rogosa, and M. E. Sharpe; Merck, Darmstadt, Germany] [9]) containing 7% (vol/vol) dimethyl sulfoxide at −60°C. For each experiment, the bacteria were thawed, streaked onto MRS agar plates, and incubated at 37°C in an atmosphere containing 5% CO_2 for further culturing.

The uropathogen *E. faecalis* 1131 was stored in brain heart infusion broth (Oxoid, Basingstoke, United Kingdom) containing 7% (vol/vol) dimethyl sulfoxide at −60°C. Blood agar plates were inoculated by using frozen stock cultures and were incubated at 37°C to obtain cultures. For the adhesion assays, 200 ml of brain heart infusion broth was incubated overnight (for 18 h) with 10 ml of a 24-h subculture. The stationary cells were harvested by centrifugation at 10,000 × *g* for 5 min at 10°C and washed twice in demineralized water. After the bacteria were sonicated twice for 10 s at 30 W on ice with a Vibra Cell model 375 apparatus (Sonics and Materials, Inc., Danbury, Conn.) to break the aggregates and chains, the cells were counted in a Bürker-Türk counting chamber and diluted to a final concentration of 3×10^8 cells ml^{-1} in 250 ml of phosphate-buffered saline (PBS) with the pH adjusted to 7.0.

Growth curves. Growth curves for the *Lactobacillus* strains were determined because biosurfactant production may be influenced by the growth phase of the organisms (10). A 20-ml portion of MRS broth was incubated with 0.5 ml of an overnight *Lactobacillus* subculture, and growth was measured by determining the optical density at 600 nm at different times up to 30 h.

ADSA-P. Droplets of liquids with low surface tensions are more apt to deviate from a perfectly spherical shape than droplets of liquids with high surface tensions. The axisymmetric drop shape analysis by profile (ADSA-P) technique was developed to calculate the liquid surface tension and contact angle of an axisymmetric droplet from its shape on the basis of the classical Laplace equation of capillarity (37) which describes the shape of a droplet. In this study, the ADSA-P was performed as described by Noordmans and Busscher (26). An objective function, expressing the difference between a measured droplet profile and a theoretical Laplacian profile, was numerically minimized and made use of gravity, density, the three-phase (solid-liquid-vapor) line, the droplet profile coordinates, and *x* and *y* magnification factors as input parameters. The droplet profiles were digitized with a Supcon model EC 90 contour monitor. Exact *x* and *y* magnification factors were calculated from the profiles of perfectly spherical stainless steel balls. In order to use ADSA-P as a screening method for bacterial biosurfactant production, 100 μl of a bacterial suspension was put on fluoroethylenepropylene-Teflon (Fluorplast, Raamsdonkveer, The Netherlands) that was placed in an enclosed chamber to prevent evaporation. The surface tension of the suspension droplet was calculated from its shape as a function of time for 2 h at room temperature.

Bacterial suspensions were prepared as follows. Lactobacilli were grown in 20 ml of MRS broth that had been inoculated with 0.5 ml of an overnight subculture. After 4 and 24 h, 10 ml of the culture was harvested by centrifugation at 10,000 × *g* for 5 min at 10°C and washed twice in fresh PBS (pH 7.0). The bacteria were counted as described above, resuspended in PBS to a final concentration of 5×10^9 cells ml^{-1}, and used immediately. *E. faecalis* 1131 was grown overnight in 10 ml of brain heart infusion broth (stationary phase) and prepared as described above. Bacteria were considered biosurfactant producers when the decrease in surface tension after 2 h was more than 8 mJ m^{-2} (40).

Biosurfactant production. For a select number of *Lactobacillus* strains, 600-ml cultures in MRS broth were grown overnight (for 18 h) to investigate the compounds produced. The cells were harvested by centrifugation at 10,000 × *g* for 5 min at 10°C, washed twice in demineralized water, and resuspended in 100 ml of PBS. The lactobacilli were incubated at room temperature for 2 h with gentle stirring for biosurfactant production. Subsequently, the bacteria were removed by centrifugation, and the remaining supernatant liquid was filtered through a 0.22-μm-pore-size filter (Millipore). A 10-ml portion of the supernatant was used immediately in the adhesion assay, and the remainder was dialyzed against demineralized water at 4°C in a Spectrapor membrane tube (molecular weight cutoff, 6,000 to 8,000; Spectrum Medical Industries, Inc.) and freeze-dried.

Adhesion assay. A parallel-plate flow chamber was used to study deposition of *E. faecalis* 1131 on glass and on glass with an adsorbed biosurfactant layer. In order to obtain laminar flow in the middle of the flow chamber, the depth and width of the inlet and outlet channels gradually decrease and increase, respectively. The flow chamber was mounted on the stage of an Olympus model BH-2 phase-contrast microscope equipped with an ×40 objective having an ultralong working distance (model ULWD-CD PLan 40 PL; Olympus). A charge-coupled device camera (CCD-MX High Technology, Eindhoven, The Netherlands) was linked to an image analyzer (TEA image manager; Difa, Breda, The Netherlands), which was installed in a 66-MHz 486 personal computer. This system allowed direct observation of bacterial adhesion over a field of view covering 0.011 mm^2.

The glass plates which were the top and bottom plates of the chamber (5.5 by 3.8 cm) and two Teflon spacers (thickness, 0.06 cm) were cleaned ultrasonically in a 2% RBS surfactant solution in water (Omnilabo International BV, The Netherlands) for 10 min, rinsed thoroughly with warm tap water, methanol, and demineralized water, and finally secured in the flow chamber. Glass plates prepared in this way were completely wettable by water (contact angle, 0°). The flow chamber was filled with 10 ml of freshly produced biosurfactant and left at room temperature for overnight adsorption. Subsequently, an *E. faecalis* suspension (3×10^8 cells ml^{-1} in 250 ml of PBS) was allowed to flow through the system at room temperature. A pulse-free flow (rate, 0.034 ml s^{-1}) was created by hydrostatic pressure, which produced a constant shear rate of 15 s^{-1}, and the suspension was recirculated by using a Multiperpex model 2115 peristaltic pump. Images were grabbed during the experiment and stored in the computer.

From the initial, linear increase in the number of adhering bacteria per unit area with time, the initial deposition rate was determined by a linear least-squares fitting procedure. After 4 h, the number of adhering bacteria was determined, and the suspension was drained from the system, which allowed an air-liquid interface to pass over the substratum (i.e., exposure to a high shear force occurred). After draining, the liquid in the system was changed to the cell-free buffer and a final image was taken. The numbers of adhering bacteria in the postdraining and predraining images were compared, which yielded the total number of bacteria retained after the passage of the air-liquid interface as an indication of the strength of adhesion.

Fourier transform infrared spectroscopy. Freeze-dried biosurfactants (2 mg) were ground with 100 mg of KBr and compressed at 7,500 kg for 3 min to obtain translucent pellets. Infrared absorption spectra were recorded with a model MX-S spectrometer obtained from Nicolet Instruments (the spectral resolution and wave number accuracy were 4 and 0.01 cm^{-1}, respectively). All measurements consisted of 500 scans, and a KBr pellet was used as the background reference. Quantitation of a spectral region of interest was determined by normalizing the area under the absorption bands with respect to the area of the CH absorption band around 2,932 cm^{-1}.

X-ray photoelectron spectroscopy. Freeze-dried biosurfactants were dissolved in demineralized water (3 mg ml^{-1}), and 50-μl droplets were put on gold-coated glass slides (1 by 1 cm). After air drying, the glass slides were inserted into the chamber of a spectrometer (S-probe; Surface Science Instruments). The residual pressure in the spectrometer was approximately 10^{-9} Pa. A magnesium anode was used for X-ray production (10 kV, 22 mA) with a spot size of 250 by 1,000 μm. After a scan of the overall spectrum in the binding energy range of 1 to 1,200 eV at a low resolution (150 eV pass energy), peaks over a 20-eV binding energy range were recorded at a high resolution (50 eV pass energy) in the following order: C_{1s}, O_{1s}, N_{1s}, P_{2p}, and C_{1s} again. This order was used to be able to account for contamination or deterioration of the samples caused by X-ray radiation. The areas under the peaks, after Shirley background subtraction and correction with instrument sensitivity factors, were used to calculate the elemental surface concentration ratio of N to C.

The C_{1s} peak was decomposed by a least-squares fitting program into four Gaussian components set at 284.8 eV (C1), 286.2 eV (C2), 288.0 eV (C3), and 289.2 eV (C4) by imposing a constant full width at half maximum of 1.35 eV; these four components were thought to be representative of the carbon involved in C—C and C—H bonds, C—O and C—N bonds, (C═O)—N and (C═O)—O bonds, and (C═O)—OH bonds, respectively.

RESULTS

Biosurfactant production. Growth curves were obtained for four *Lactobacillus* strains to determine their mid-exponential and stationary growth phases (data not shown). In order to compare bacterial biosurfactant production in the two phases, ADSA-P was performed with suspension droplets from 4-h (mid-exponential-phase) cultures and 24-h (stationary-phase) cultures. The liquid surface tension of the suspension droplets is plotted versus time in Fig. 1 for four *Lactobacillus* strains. As the decreases in surface tension exceeded 8 mJ m^{-2} (40), all four strains produced biosurfactants after both 4 and 24 h of growth. Since in general the 24-h cultures exhibited the largest and most rapid decreases in liquid surface tension, the 15 *Lactobacillus* strains selected for this study were examined for biosurfactant production in the stationary growth phase.

Table 1 summarizes the maximal decreases in the surface tensions of the bacterial suspensions measured after 2 h. The decreases in surface tension were calculated relative to the surface tension of PBS to correct for lower initial values as a result of instantaneous secretion of surface-active compounds by some *Lactobacillus* strains. On the basis of the data in Table 1, it was concluded that all of the *Lactobacillus* isolates tested produced biosurfactants.

E. faecalis 1131 did not produce any surface-active compounds.

Inhibition of initial adhesion of *E. faecalis*. The biosurfactants of three strongly biosurfactant-producing *Lactobacillus* strains, *L. fermentum* B54, *L. casei* subsp. *rhamnosus* ATCC 7469^T (T = type strain), and *L. acidophilus* RC14, as well as

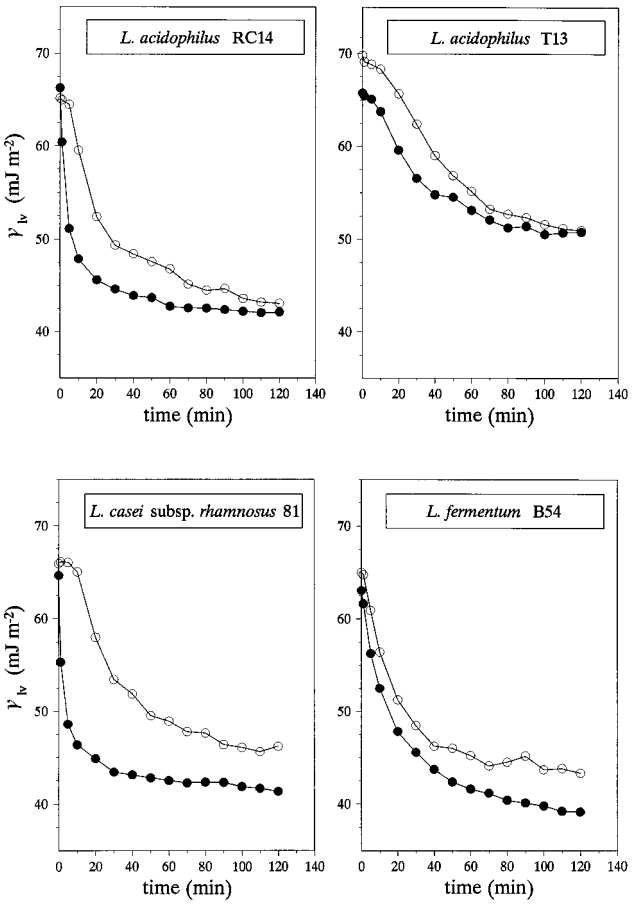

FIG. 1. Liquid surface tension (γ_{lv}) of *Lactobacillus* suspension droplets as a function of time as determined by ADSA-P. Lactobacilli were harvested in the mid-exponential growth phase (○) and the stationary growth phase (●).

TABLE 1. Maximal decreases in the liquid surface tension of *Lactobacillus* spp. and *E. faecalis* suspensions determined after 2 h by ADSA-P

Strain	$-\Delta\gamma_{lv}$ (mJ m^{-2})[a]
L. acidophilus strains	
ATCC 4356 (type strain)	27 ± 1
RC14	26 ± 0
T13	18 ± 3
L. casei 70	21 ± 0
L. casei subsp. *casei* ATCC 393 (type strain)	12 ± 4
L. casei subsp. *rhamnosus* strains	
ATCC 7469 (type strain)	27 ± 1
GR-1	19 ± 3
81	27 ± 1
36	19 ± 2
L. fermentum strains	
ATCC 14931 (type strain)	27 ± 4
ATCC 23271	20 ± 3
B54	29 ± 1
L. plantarum strains	
ATCC 14917 (type strain)	24 ± 2
RC6	23 ± 1
RC20	26 ± 4
E. faecalis 1131	4 ± 0

[a] The maximal decrease in liquid surface tension ($-\Delta\gamma_{lv}$) was determined relative to the surface tension of PBS (68 mJ m^{-2}). The results are expressed as means of duplicate experiments performed with separately grown cultures ± differences between the experiments.

the biosurfactant of *L. casei* subsp. *rhamnosus* 36 (which was used previously in displacement studies with *E. faecalis* [22]), were selected for further investigation. The influence of these biosurfactants on the initial adhesion of *E. faecalis* 1131 was studied in a parallel-plate flow chamber by using glass plates with and without an adsorbed biosurfactant layer. Figure 2 shows an example of the kinetics of the initial adhesion of *E. faecalis* 1131 to glass and to glass coated with *L. acidophilus* RC14 biosurfactant. Note that *E. faecalis* adhesion to the *L. acidophilus* RC14 biosurfactant layer was delayed by approximately 7,700 s (exact determinations of the delay times from the graphs are somewhat arbitrary). A similar delay period of highly reversible adherence was also observed for enterococcal adhesion to an *L. fermentum* B54 biosurfactant layer; in this case the delay period was approximately 7,000 s. From the initial linear trajectories shown in Fig. 2, initial deposition rates were calculated (Table 2). On the basis of the data in Table 2, it was concluded that the biosurfactants from *L. acidophilus* RC14 and *L. fermentum* B54 decreased the initial deposition rate of *E. faecalis* 1131 by 76 and 65%, respectively ($P < 0.01$; Student's *t* test); the numbers of adhering cells after 4 h were also reduced on these biosurfactant layers by 82 and 72%, respectively ($P < 0.01$; Student's *t* test).

The *L. casei* subsp. *rhamnosus* ATCC 7469^T biosurfactant slightly decreased the initial deposition rate of *E. faecalis* 1131, but the difference was not significant at $P < 0.1$ (Student's *t* test). However, the number of adhering cells after 4 h was comparable to the number on clean glass. The biosurfactant produced by *L. casei* subsp. *rhamnosus* 36 did not inhibit enterococcal adhesion at all.

None of the biosurfactants tested affected the retention of adhering enterococci after passage of an air-liquid interface after 4 h; under these conditions the level of retention was almost 100%.

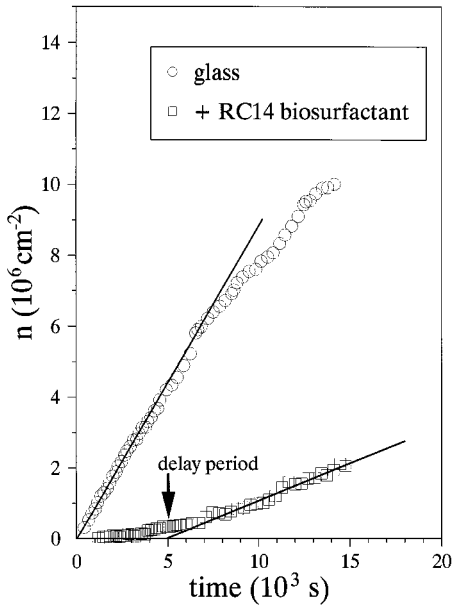

FIG. 2. Kinetics of initial adhesion of *E. faecalis* 1131 in PBS to glass and glass with an adsorbed *L. acidophilus* RC14 biosurfactant layer, as determined in a parallel-plate flow chamber at a shear rate of 15 s^{-1}. The lines represent the trajectories of the curves from which the initial deposition rates were calculated.

TABLE 2. Initial adhesion of *E. faecalis* 1131 in PBS to glass with and without adsorbed biosurfactant layers from various *Lactobacillus* isolates in a parallel-plate flow chamber[a]

Source of biosurfactant	Delay period (10^3 s)	Initial deposition rate (cm^{-2} s^{-1})	Adhesion after 4 h (10^6 cm^{-2})	% Retention[b]
	0	889 ± 149	10.5 ± 1.8	99 ± 1
36	0	739 ± 115	8.8 ± 1.6	98 ± 0
ATCC 7469^T	0	652 ± 30	9.0 ± 1.4	98 ± 2
B54	7.0 ± 0.6	313 ± 64	3.1 ± 0.3	97 ± 3
RC14	7.7 ± 0.2	209 ± 82	1.9 ± 0.7	93 ± 7

[a] The results are expressed as means ± standard deviations of values from triplicate experiments in which separately grown cultures were used.
[b] The percentage of adhering bacteria retained after exposure to an air-liquid interface after a 4-h experiment.

Adhesion assays in which dialyzed (molecular weight cutoff, 6,000 to 8,000) and freeze-dried biosurfactants were used yielded values within the same range, as shown in Table 2, indicating that the active compounds were not lost during dialysis or freeze-drying. Similar results were obtained for the adhesion of *E. faecalis* to biosurfactant layers which had previously been subjected to a 2-h flow of cell-free buffer, indicating that adsorbed biosurfactants did not desorb under the influence of a fluid flow. In a separate experiment, the dose response for inhibition of *E. faecalis* adhesion was determined by studying adhesion to glass after adsorption of *L. acidophilus* RC14 biosurfactant from a 10-fold-diluted solution. Even a 10-fold-diluted solution reduced the initial deposition rate and level of adhesion after 4 h to the values obtained with the concentrated biosurfactant solution, albeit the delay period was only 4,000 s.

Fourier transform infrared spectroscopy. The molecular composition of the biosurfactants used in the adhesion assays was investigated by Fourier transform infrared spectroscopy. Figure 3 shows the spectra of the freeze-dried biosurfactants. All of the spectra had essentially the same absorption bands, and only the relative areas under the various absorption bands differed. The most important bands were located at 2,932 cm^{-1} (CH band: CH_2-CH_3 stretching), 1,652 cm^{-1} (AmI band: C═O stretching in proteins), 1,537 cm^{-1} (AmII band: N—H bending in proteins), 1,234 cm^{-1} (PI band: phosphates), and 1,066 cm^{-1} (PII band: polysaccharides). Table 3 shows the absorption band ratios of AmI, AmII, PI, and PII with respect to the CH band around 2,932 cm^{-1} for the biosurfactants. The biosurfactants from *L. acidophilus* RC14 and *L. fermentum* B54 appeared to contain more protein than the biosurfactants from *L. casei* subsp. *rhamnosus* 36 and ATCC 7469^T. The *L. casei* subsp. *rhamnosus* 36 and ATCC 7469^T biosurfactants, on the other hand, had relatively high polysaccharide and phosphate contents. Note that an extra band was observed at 1,750 cm^{-1} for the *L. casei* subsp. *rhamnosus* 36 and ATCC 7469^T and *L. fermentum* B54 biosurfactants.

X-ray photoelectron spectroscopy. X-ray photoelectron spectroscopy of the freeze-dried biosurfactants yielded the elemental surface concentration ratio of N to C and the components of the C_{1s} peak (Table 3). From the ratio of N to C and the C3 + C4 peak fractions, it was concluded that the biosurfactants from *L. acidophilus* RC14 and *L. fermentum* B54 were very rich in protein, while the biosurfactants from *L. casei* subsp. *rhamnosus* 36 and ATCC 7469^T had lower protein contents.

DISCUSSION

In this study, 15 *Lactobacillus* isolates were screened for biosurfactant production by ADSA-P. All of the strains se-

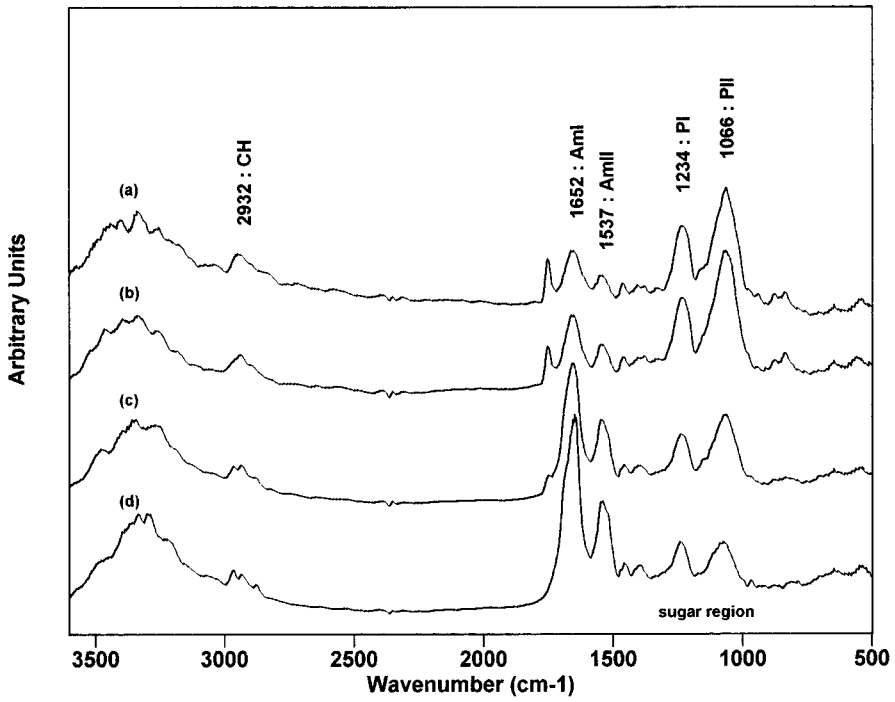

FIG. 3. Fourier transform infrared spectroscopy spectra of biosurfactants from *L. casei* subsp. *rhamnosus* 36 (spectrum a), *L. casei* subsp. *rhamnosus* ATCC 7469^T (spectrum b), *L. fermentum* B54 (spectrum c), and *L. acidophilus* RC14 (spectrum d). The absorption bands used for quantification are indicated.

TABLE 3. Chemical composition data for *Lactobacillus* biosurfactants expressed as infrared absorption band ratios as determined by Fourier transform infrared spectroscopy and N/C ratio and decomposition of the C_{1s} peak as determined by X-ray photoelectron spectroscopy, with data for reference compounds included for comparison

Strain or compound	Fourier transform infrared spectroscopy band ratios				X-ray photoelectron spectroscopy			
	AmI/CH	AmII/CH	PI/CH	PII/CH	N/C ratio	Decomposition of C_{1s} peak		
						C1	C2	C3 + C4
36	2.4	0.9	3.3	7.3	0.130	0.55	0.31	0.15
ATCC 7469^T	2.7	1.0	3.0	8.4	0.139	0.51	0.32	0.16
B54	7.8	3.2	1.9	4.9	0.192	0.48	0.31	0.21
RC14	11.2	4.6	2.4	3.6	0.197	0.44	0.30	0.26
Albumin[a]	8.6	4.5	0	0	0.240	0.50	0.29	0.21
Salivary glycoprotein[b]	ND[c]	ND	ND	ND	0.182	0.52	0.32	0.16
Dextran[d]	0	0	0	5.0	0	0.20	0.69	0.12
Lipoteichoic acid[e]	0	0	2.5	7.5	≤0.1	≤0.1	0.8–0.9	≤0.1

[a] Bovine albumin fraction V (Sigma). X-ray photoelectron spectroscopy data from reference 8.
[b] X-ray photoelectron spectroscopy data from reference 8.
[c] ND, not determined.
[d] Dextran 500 (molecular weight, 500 to 700; Serva). X-ray photoelectron spectroscopy data from reference 8.
[e] Lipoteichoic acid from *S. mutans* (Sigma). X-ray photoelectron spectroscopy data were calculated for teichoic acid by Mozes and Lortal (24).

creted surface-active compounds in PBS, and it appeared that the production per bacterial cell was generally greater and more rapid when the bacteria were harvested in the stationary phase than when they were harvested in the mid-exponential growth phase. In addition, the initial adhesion of uropathogenic *E. faecalis* 1131 to glass was significantly slowed by adsorbed biosurfactants from *L. acidophilus* RC14 and *L. fermentum* B54 (but not by adsorbed biosurfactants from *L. casei* subsp. *rhamnosus* 36 and ATCC 7469^T), while the number of adhering cells after 4 h of adhesion was also markedly decreased.

The dialyzed (molecular weight cutoff, 6,000 to 8,000) and freeze-dried biosurfactants isolated from *L. casei* subsp. *rhamnosus* 36 and ATCC 7469^T, *L. acidophilus* RC14, and *L. fermentum* B54 were examined by Fourier transform infrared spectroscopy and X-ray photoelectron spectroscopy and compared with the reference compounds albumin, salivary glycoprotein, dextran, and lipoteichoic acid (Table 3). On the basis of the data in Table 3, it was concluded that the biosurfactants from *L. acidophilus* RC14 and *L. fermentum* B54 were richer in protein and contained less polysaccharide and phosphate than the biosurfactants from *L. casei* subsp. *rhamnosus* 36 and ATCC 7469^T. In the Fourier transform infrared spectroscopy spectra of the *L. casei* subsp. *rhamnosus* 36 and ATCC 7469^T and *L. fermentum* B54 biosurfactants, an extra absorption band at 1,750 cm^{-1}, corresponding to the presence of ester carbonyl groups, was observed. As only the biosurfactants from *L. fermentum* B54 and *L. acidophilus* RC14 effectively inhibited the adhesion of *E. faecalis* to glass in our parallel-plate flow chamber, we concluded that the responsible substance in these biosurfactants is proteinlike.

Different classes of biosurfactants are distinguished on the basis of their chemical structures. The biosurfactants that are most often isolated and most extensively investigated are the glycolipids, including the rhamnolipids produced by *Pseudomonas aeruginosa* and *Pseudomonas fluorescens*. Other groups of biosurfactants are the lipopeptides and proteinlike substances, phospholipids, substituted fatty acids, and lipopolysaccharides (12, 14). Little is known about *Lactobacillus* biosurfactants, although much information concerning bacteriocins from lactobacilli and other lactic acid bacteria is available (1, 15, 23).

Recently, Lin et al. (18) isolated a biosurfactant from *Bacillus licheniformis* JF-2 and identified it as a lipopeptide on the basis of Fourier transform infrared spectroscopy data, a method used in this study. Many of the nondialyzable biosurfactants that have been isolated to date are glycoproteins or protein-(lipo)polysaccharide complexes. *Candida lipolytica*, for example, produces the surfactant liposan, a 27.6-kDa proteinlike complex that is composed of 83% carbohydrate (7), and the bacteriocin lactocin 27 was isolated from culture supernatants of *Lactobacillus helveticus* LP27 as a protein-lipopolysaccharide complex (molecular weight, >200,000) (15).

The main physiological role of biosurfactants is to facilitate the uptake of water-immiscible substrates by lowering the surface tension at the phase boundary, emulsification, and enabling the microbial cells to adhere to the organic compounds (12, 13). Biosurfactants can also exhibit antibiotic activity against various microbes. *Bacillus* species, for example, produce a broad spectrum of lipopeptides, and most of these compounds are known for their antibiotic characteristics (14). Involvement of biosurfactants in microbial adhesion, as demonstrated in this study, has been described previously. Dairy *S. thermophilus* strains, for example, produce biosurfactants that cause their own desorption (4), and oral *S. mitis* strains secrete biosurfactants that inhibit the adhesion of *S. mutans* (29). The low levels of biosurfactants produced have greatly hampered research on the role of biosurfactants. In addition, the amount of a biosurfactant that is required to exert a major effect in the microenvironment of a biofilm is extremely small. Therefore, the role of biosurfactants in adhesion phenomena may have been underestimated previously.

Although many whole-cell studies on the probiotic effect of lactobacilli have been performed (3, 22, 27), no information concerning the underlying mechanism of this *Lactobacillus* action has been obtained. The displacement of adhering *E. faecalis* 1131 from hydrophobic and hydrophilic substrate by *L. casei* subsp. *rhamnosus* 36 in a parallel-plate flow chamber, for example, has been attributed to possible biosurfactant production by the lactobacilli (22). Since in this study biosurfactant from *L. casei* subsp. *rhamnosus* 36 had only an extremely minor inhibitory effect, it is likely that in the two-strain displacement experiment of Millsap et al. (22) the displacement of adhering *E. faecalis* was mainly due to collisions between flowing lactobacilli and adhering enterococci (a "knock off" effect) rather than to biosurfactant production.

Many investigators have focused on the use of live *Lactobacillus* whole cells to restore the ecological balance in the intestinal flora and urogenital tract (3, 27, 39). Previously, oral supplementation, urogenital instillation, and coating of urinary catheters and diapers with lactobacilli have been tested to various extents (3, 27, 34). In this study, we found that biosurfactant layers of several *Lactobacillus* strains inhibited the adhesion of a uropathogenic *E. faecalis* strain to glass in a parallel-plate flow chamber for at least 4 h. At this point, it should be emphasized that only one pathogen was studied and that the several *Lactobacillus* strains investigated did not inhibit the uropathogen equally well. This indicates that there are different aspects of the adherence of the pathogen and that it should not be expected that the products of different *Lactobacillus* strains will produce equivalent results for any given pathogen.

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