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Physicochemical Characterization of *Escherichia coli*

A Comparison with Gram-Positive Bacteria

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ABSTRACT

Eight *Escherichia coli* strains were characterized by determining their adhesion to xylene, surface free energy, zeta potential, relative surface charge, and their chemical composition. The latter was done by applying X-ray photoelectron spectroscopy (XPS) and infrared spectroscopy (IR). No relationship between the adhesion to xylene and the water contact angles of these strains was found. Three strains had significantly lower surface free energies than the other strains. Surface free energies were either obtained from polar and dispersion parts or from Lifshitz-van der Waals and acid/base parts of the surface free energy. A correlation ($r = 0.97$) between the polar parts and the electron-donor contributions to the acid/base part of the surface free energy was found. The zeta potentials of all strains, measured as a function of pH

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(2–11), were negative. Depending on the zeta potential as a function of pH, three groups were recognized among the strains tested. A relationship (r = 0.84) was found between the acid/base component of the surface free energy and the zeta potential measured at pH = 7.4. There was no correlation between results of XPS and IR studies. Data from the literature of XPS and IR studies of the gram-positive staphylococci and streptococci were compared with data from the gram-negative *E. coli* used in this study. It appeared that in these three groups of bacteria, the polysaccharide content detected by IR corresponded well with the oxygen-to-carbon ratio detected by XPS.

Index Entries: *E. coli*; infrared spectroscopy; X-ray photoelectron spectroscopy; zeta potential; contact angle; surface free energy.

INTRODUCTION

Urinary catheters are the most commonly used devices in hospitals (*1*) and urinary catheter-associated bacteriuria is the most frequently encountered hospital infection (*2*). This infection is often because of *Escherichia coli* (*2–5*), derived from the patient's gastrointestinal flora (*2*). Adhesion of *E. coli* onto the catheter is thought to be a relevant step in the pathogenesis of this infection (*2*). Other foreign-body material-associated infections are often owing to staphylococci (*2*). The adhesion process will be influenced by various bacterial surface properties (*2*). Extensive determination of surface free energies, zeta potentials, and chemical surface composition of a series of staphylococci and streptococci has yielded a comprehensive characterization of these microorganisms (*6,7*). Both staphylococci and streptococci are gram-positive bacteria, whereas *E. coli* is a gram-negative organism. It is of interest how the complex cell wall structure of this gram-negative bacterium with a much lower peptidoglycan content than the gram-positive bacteria will be reflected in the physicochemical properties of the cell surfaces. The aim of this study is to characterize the surfaces of eight *E. coli* strains by determining their hydrophobicity, surface free energy, zeta potential, relative surface charge, and chemical (elemental and molecular) composition, and to investigate whether these parameters can be related. In addition, the results were compared with similar data from literature for staphylococci and streptococci.

METHODS

Bacterial Strains

Eight *E. coli* strains isolated from patients with either catheter-associated or non catheter-associated urinary tract infections (Table 1) were biotyped (*8*) and serotyped according to Ørskov (*9*). The strains were grown in Brain Heart

Table 1
Relative Surface Charge (ARR) (%) and Relative Hydrophobicity (MATH) (%) of Eight Uropathogenic *E. coli* Strains

Strain and code	Catheter associated[a]	ARR, %[b]	MATH, %[c]
1. O2K2	+	66 ± 9	5 ± 5
2. O2K7	+	49 ± 6	5 ± 5
3. O8K(A)28	+	0 ± 0	1 ± 2
4. O39K1	+	71 ± 2	7 ± 1
5. O83K?	+	75 ± 4	3 ± 2
6. O111K58	–	0 ± 0	42 ± 1
7. O157K-	–	5 ± 8	33 ± 5
8. O161K-	+	–[d]	0 ± 0

[a]Isolated from patients with either catheter-associated (+) or noncatheter-associated (–) urinary tract infection.
[b]Percentage of bacteria bound to an anion-exchange resin in the anion-exchange resin retention (ARR) test; mean values ± S.D. (n = 3).
[c]Percentage of bacteria adhering to xylene in the microbial adhesion to hydrocarbon (MATH) test; mean values ± S.D. (n = 3).
[d]The value for this strain could not be determined in an accurate way.

Infusion (BHI) broth (Difco Laboratories, Detroit, MI) for 18 h at 37°C without shaking. Bacteria in the stationary phase were harvested by centrifugation at 5000 × g for 10 min at 4°C and subsequently washed three times in demineralized water. All experiments were carried out in duplicate with two separate bacterial cultures.

Anion-Exchange Resin Retention (ARR)

The relative surface charge of the bacteria was determined by measuring their retention to an anion-exchange resin according to a modified procedure of Pedersen *(10,11)*. Pasteur pipets (inner diameter 5 mm) containing 0.5 g of anion-exchange resin Dowex 1 × 8 mesh size 100/200 (80–150 μm) (Serva, Heidelberg, FRG) were rinsed with 3 mL of phosphate-buffered saline (PBS, 0.4*M* NaCl, 8.1 m*M* Na_2HPO_4, 1.5 m*M* KH_2PO_4, pH = 7.4). Then 1 mL of a bacterial suspension in PBS with an absorbance at 540 nm of 1.0 (1-cm cuvet) was applied to the column. The column was eluted with PBS, and the absorbance of the eluate was measured. The relative surface charge of the bacteria was expressed as the percentage of the bacteria bound to the resin.

Microbial Adhesion to Hydrocarbon (MATH)

The relative hydrophobicity of the bacteria was measured according to Rosenberg *(12)*. Washed bacteria were suspended in PBS (113 m*M* NaCl, 8.1 m*M* Na_2HPO_4, 1.5 m*M* KH_2PO_4, pH = 7.4) to an absorbance of 1.0 at 540 nm. Three milliliters of this suspension were mixed for 60 s with 0.25 mL p-xylene (Merck,

Darmstadt, FRG). After phase separation, the absorbance of the aqueous phase at 540 nm was measured. The relative hydrophobicity of the bacteria was expressed as the percentage of the bacteria removed from the aqueous phase.

Contact Angles and Surface Free Energies

Bacteria were deposited on membrane filters (Millipore, HA, 0.45 μm) in order to produce a lawn of 50–100 stacked cell layers, suitable for contact angle measurements *(13)*. After a standard drying time of 20 min, plateau contact angles were determined in triplicate at 25°C using sessile droplets of water, formamide, diiodomethane, α-bromonaphthalene, and a series of water/*n*-propanol mixtures. Surface free energies were calculated according to two different approaches. These two approaches have been used by various authors to characterize bacterial surfaces and to predict bacterial adhesion at solid surfaces *(13–15)*. Unfortunately, conflicting results were reported by these authors.

In the first approach, the water, water/*n*-propanol, and α-bromonaphthalene contact angles were least-square fitted to *(13)*:

$$(\cos\theta + 1) \cdot \gamma_l = 2\,(\gamma_b^d \cdot \gamma_l^d)^{1/2} + 2\,(\gamma_b^p \cdot \gamma_l^p)^{1/2} - \pi_e \qquad [1]$$

in which d and p denote the dispersion and polar surface free energy components of the bacteria and the liquid employed, respectively. The equilibrium spreading pressure (π_e) is supposed to be zero for α-bromonaphthalene and constant for water and water/propanol mixtures. Parameters of the liquids were measured according to Busscher et al. *(13)*. The surface free energy of the bacterial surface (γ_b) is equal to the sum of the dispersion part (γ_b^d) and the polar part (γ_b^p). This approach has proven to be valid for low energy surfaces and for high energy surfaces on which spreading pressures may become significant *(16)*.

The second approach has been developed by Van Oss et al. *(17,18)* who extended Eq. 1 in an attempt to obtain data from contact angles that would give more information about the polar groups at a surface. In this approach, spreading pressures are neglected. The contact angles obtained with the pure liquids are inserted into the following equation:

$$(\cos\theta + 1) \cdot \gamma_l = 2\,(\gamma_{bv}^{LW} \cdot \gamma_l^{LW})^{1/2} + 2\,(\gamma_{bv}^{+} \cdot \gamma_l^{-})^{1/2} + 2\,(\gamma_{bv}^{-} \cdot \gamma_l^{+})^{1/2} \qquad [2]$$

in which LW denotes the Lifshitz-van der Waals surface free energy component, and + and – denote the electron-accepting and the electron-donating parameters of the acid/base surface free energy component, respectively, according to:

$$\gamma_{bv}^{AB} = 2\,(\gamma_{bv}^{+} \cdot \gamma_{bv}^{-})^{1/2} \qquad [3]$$

In this approach, the surface free energy of the bacterial surface (γ_{bv}) is equal to the sum of γ_{bv}^{LW} and γ_{bv}^{AB}. Parameters of the liquids were measured according to van Oss et al. *(17,18)*.

Zeta Potentials

Washed bacteria were suspended in 0.01*M* KCl buffered with 8×10^{-4} Na_2PO_4 and 2×10^{-4} KH_2PO_4 at a concentration of 10^7 cells/mL and adjusted with 1*M* aqueous solutions of potassium hydroxide (KOH) or hydrogen chloride (HCl) to a pH varying from 2 to 11. The specific conductance of the buffer was 1.6 mS · cm^{-1}. Zeta potentials were measured with a precision of ±2 mV, employing a Lazer Zee meter 501 (PenKem, Bedford Hills, NY), by which scattering of incident laser light is used to enable detection of the bacteria at relatively low magnification. Measurements were carried out at 20°C with two different cultures, each in duplicate. The method is based on the application of the Smoluchowski equation.

X-Ray Photoelectron Spectroscopy (XPS)

XPS measurements were performed as described by Amory et al. *(19)*. After centrifugation of the bacterial suspension the pellet was transferred to a stainless-steel cup, immediately frozen in liquid nitrogen, and lyophilized at –5°C in a Lyovac GT1 (Leybold Heraeus, Inc., East Syracuse, NY). After lyophilization, the bacterial powder was pressed in stainless-steel troughs. These troughs were inserted in the chamber of the spectrometer, which was a Vacuum Generators ESCA 3 Mk II (Vacuum Generators Scientific Limited, East Grinstead, Sussex, UK) instrument equipped with a Tracor Northern TN 1710 signal averager for signal-to-noise ratio enhancement. A magnesium anode was used for X-ray production (14 kV, 20 mA). The pass energy was 50 eV. After a scan of the overall spectrum, peaks were recorded in the following order: C_{1s}, O_{1s}, N_{1s}, P_{2p}, and C_{1s} again. The area under each peak after linear background subtraction was used for calculation of the peak intensities, yielding O/C, N/C, and P/C ratios at the surface employing sensitivity factors determined by Wagner et al. *(20)*.

Fourier Transform Infrared Spectroscopy (FTIR)

After lyophilization of the bacterial pellet, 1–5 mg of the bacterial powder were mixed with KBr (1:50 by weight), ground for 1 min, and pressed to a pellet. Transmission infrared absorption spectra were recorded on a Fourier transform infrared spectrometer MX-S from Nicolet Instruments (Madison, WI). The effective spectral resolution and wave number accuracy were 4 cm^{-1} and 0.01 cm^{-1}, respectively. Five hundred scans were measured and averaged for each sample, using a KBr-pellet as reference. The most important absorption bands of bacteria were located at 2930 cm^{-1} (CH_2–CH_3 band, owing to C–H stretching), 1654 cm^{-1} (amide I band, owing to C=O stretching), 1542 cm^{-1} (amide II band, owing to N–H bending), and two bands at 1237 cm^{-1} and 1070 cm^{-1}, originating from phosphate (PI) and polysaccharide (PII) groups, respectively *(7,21)*. The area under the peaks of the absorption bands was determined by integration after linear background subtraction and normalized with respect to the area of the C–H stretching absorption bands around 2930 cm^{-1}.

Table 2
Contact Angles (θ) (°) and Surface Free Energies (γ) (mJ m^{-2}) of Eight *E. coli* Strains[a]

	E. coli strain							
	O2K2	O2K7	O8K(A)28	O39K1	O83K?	O111K58	O157K-	O161K-
θ_w	57 ± 2	29 ± 3	46 ± 8	21 ± 3	54 ± 1	18 ± 3	19 ± 1	26 ± 2
θ_f	30 ± 3	28 ± 6	29 ± 2	25 ± 3	36 ± 2	23 ± 4	17 ± 3	37 ± 13
θ_{di}	59 ± 4	60 ± 9	57 ± 5	54 ± 3	57 ± 5	60 ± 11	56 ± 20	57 ± 13
$\theta_{\alpha Br}$	35 ± 1	40 ± 6	36 ± 3	40 ± 2	39 ± 3	50 ± 8	46 ± 6	51 ± 6
γbd	37	35	36	35	35	30	32	29
γ_b^p	40	83	58	90	51	94	89	88
γ_b	77	118	94	125	86	124	121	117
γ_{bv}^+	4	2	2	2	2	3	3	2
γ_{bv}^-	16	49	29	55	22	56	52	61
γ_{bv}^{AB}	15	19	16	18	14	25	26	20
γ_{bv}^{LW}	33	32	33	33	33	30	31	31
γ_{bv}	48	51	49	51	47	55	57	51

[a]Contact angles of water (w), formamide (f), diiodomethane (di), and a-bromonaphthalene (aBr); mean values ± SD (n = 3); γ_bs are based on the concept of dispersion (d) and polar (p) surface free energy components while accounting for spreading pressures; γ_{bv}s are based on the concept of Lifshitz-van der Waals (LW) and acid/base (AB) surface free energy components; the AB component is subsequently separated in an electron-accepting (+) and an electron-donating (–) part.

Results

Anion-Exchange Resin Retention (ARR)

The relative surface charge of the strains obtained from patients with catheter-associated bacteriuria and from patients with bacteriuria not associated with catheters ranges from 0 to 75% (Table 1). Strains O8K(A)28, O111K58, and O157K- had a low relative surface charge compared to the other strains. Strain O161K- had an intermediate relative surface charge.

Microbial Adhesion to Hydrocarbon (MATH)

The majority of the *E. coli* strains showed a low affinity for the xylene/water interface. Only strains O111K58 and O157K- had a rather high affinity for the xylene/water interface (Table 1).

Contact Angles and Surface Free Energies

Table 2 shows the contact angles of the four liquids on the bacterial lawns. The water contact angles varied from 18 to 57°. Strains O2K2, O8K(A)28, and O83K? had higher contact angles than the other five strains. The formamide contact angles varied from 17 to 37°, and the α-bromonaphthalene contact angles from 35 to 51°, whereas a slight variation in diiodomethane contact

angles was noted. No relation between the contact angles of the last two liquids was observed. This was not expected, because the last two liquids were used to detect the dispersion (or Lifshitz-van der Waals) parts of the surface free energy. This might be explained by the fact that the two liquids have different surface tensions by which different macroscopic interactions (i.e., capillary action) with the bacterial lawn may occur. The surface free energy approach based on dispersion and polar components yielded lower polar parts (γ^p) for strain O2K2, O8K(A)28, and O83K? than for the other strains. Because the dispersion parts (γ^d) of these strains were almost equal, these strains showed rather low surface free energies. With the surface free energy approach based on Lifshitz-van der Waals and acid/base components, a relatively low electron donor contribution (γ_{bv}) for strains O2K2, O8(A)K28, and O83K? was obtained. This led to differences in the acid/base parts of the surface free energies and therefore also in the total surface free energies. The lower values of γ_{bv} compared with γ_b were owing to the fact that in the method of van Oss et al. *(17,18)*, the spreading pressure (π_e) is considered to be zero.

Zeta Potentials

The zeta potentials of the bacterial strains at different pHs are shown in Fig. 1. Zeta potentials of bacteria of two different overnight cultures coincided within 3 mV. According to the pH dependency of their zeta potentials, the eight bacterial strains could be divided into three different groups. The first group (Fig. 1A), containing strains O2K7, O8K(A)28, O39K1, and O161K-, exhibited major differences in the zeta potential when the pH was varied from 2 to 11. When the pH was decreased, the zeta potentials of these strains became less negative and showed a sharp increase in the pH range 4–5. Both strains in the second (O2K2, O83K?) and in the third group (O111K58, O157K-) (Fig. 1B), did not show much variation in zeta potential when the pH was varied. However, the zeta potentials of the second group of bacteria were clearly more negative than the zeta potentials of the third group.

Surface Composition (XPS)

The O/C ratio of the surface layer of the strains ranged from 0.28 to 0.43 as shown in Table 3. Strains O157K- and O161K- had a lower O/C ratio than the other strains. N/C ratios ranged from 0.026 to 0.085. Strain O8K(A)28 showed a lower N/C ratio than the other strains. The P/C ratio ranged from 0.009 to 0.020. Whereas the positions as well as the full widths at half maximum of the peaks were identical for both cultures, the atomic ratios of bacteria grown in separate cultures showed a 10% variation.

IR-Spectroscopy of Bacteria

The AmI/CH (Table 3) ratio varied slightly among the different strains. Differences for the AmII/CH and the PI/CH ratios were minimal. The PII/CH ratio of strain O8K(A)28 was higher than for the other strains. The values

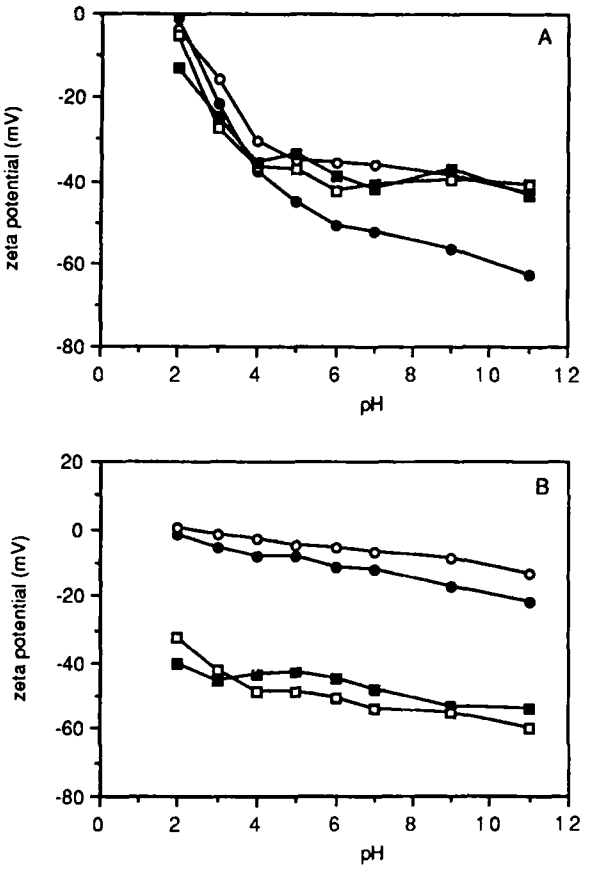

Fig. 1. Zeta potentials as function of the pH. Electrophoretic mobility was measured in 0.01*M* KCl buffered with 10^{-3}*M* phosphate. The pH was adjusted with 1*M* aqueous KOH and HCl solution in water. A: group one, containing strains O8K(A)28, O161K-, 02K7, and O39K1. B: group two, containing strains O2K2 and O83K?; group three, containing strains O111K58 and O157K-. A. —○— O8K(A)28; —●— O161K-; —□— O2K7; —■— O39K1. B. —○— O111K58; —●— O157K-; —□— O83K?; —■— O2K2.

of the ratios of the areas under the peaks of the absorption bands were reproducible within 10% when two overnight cultures using the same bacteria were compared. In contrast to findings by Nichols et al. *(22)*, the IR spectra of the *E. coli* strains in our study showed a very small shoulder at 1740 cm^{-1} owing to a C=O stretching band of the carboxylic acid groups. IR spectra of staphylococci had a clear shoulder at this wave number *(7)*, whereas streptococci did not give a shoulder at all *(21)*.

Discussion

Bacteria used for zeta-potential measurements are completely hydrated and will have the same surface characteristics as those used for adhesion experiments onto a solid surface if the same buffer is used. Bacteria that are used

Table 3
Characterization of *E. coli* strains with XPS and IR Spectroscopy

	E. coli strain							
	O2K2	O2K7	O8K(A)28	O39K1	O83K?	O111K58	O157K-	O161K-
O/C[a]	0.39	0.43	0.39	0.42	0.40	0.39	0.28	0.30
N/C	0.070	0.063	0.026	0.080	0.078	0.058	0.085	0.081
P/C	0.016	0.009	0.017	0.010	0.017	0.013	0.014	0.020
AmI/CH[b]	6.4	6.4	7.0	6.2	7.2	6.6	6.4	7.1
AmII/CH	2.4	2.3	2.4	2.3	2.5	2.4	2.5	2.6
PI/CH	1.3	1.1	1.3	1.1	1.3	1.3	1.0	1.2
PII/CH	3.7	3.2	4.5	3.2	3.4	3.5	3.3	3.0

[a]Ratios of elements at the surface as detected by XPS.
[b]Molecular composition as detected by IR; 2930 cm^{-1}, CH2–CH3 band (C–H stretching); 1654 cm^{1}, AmI: Amide I band (C=O stretching); 1542 cm^{-1}, AmII: Amide II band (N–H bending); 1237 cm^{-1} PI: phosphate band; 1070 cm^{-1}, PII: polysaccharide band

for contact angle measurements and spectroscopic techniques as XPS and IR are, however, dried before these measurements. It has to be realized that surface characteristics of bacteria may be influenced significantly by their state of hydration and the composition of fluids in which bacteria are suspended.

Under physiological conditions, most bacterial surfaces carry a net negative charge *(23)*. This negative charge, owing to the presence of ionogenic groups at the surface, is counterbalanced by ions of opposite charge. With increasing distance from the surface, the counterion concentration decreases, and a diffuse double layer is formed. The thickness of this layer is determined by the ionic strength of the suspending medium and the valency of the counterions. The zeta potential of a bacterium is defined as the potential at the plane of shear, which is usually located in the diffuse double layer. The most important ionogenic groups at the bacterial surface are amino, carboxylic acid, and phosphate groups. Amory et al. *(24)* and Mozes et al. *(25)* found that the electrophoretic mobility of a series of yeasts and bacteria at pH = 4 was determined by the phosphorus content of the cell wall as determined by XPS. The electrophoretic mobility increased with increasing P/C ratios and became constant when the P/C ratio reached a certain value (P/C: 0.006 for bacteria). The leveling of the electrophoretic mobility beyond a certain P/C ratio was thought to be attributed to an increase of the apparent pK_a value of the acidic groups at the surface with increasing surface concentration of these groups *(26)*. Another reason for the leveling of the electrophoretic mobility might be an increased counterion condensation at the more negative surface *(27)*. However, for the *E. coli* strains used in our study, no relationship between zeta potentials and P/C ratios was found. It has to be realized that the role of carboxylic acid and amino groups in determining the surface charge of bacteria and yeasts cannot be ignored. For all strains tested, no positive zeta potentials were observed. This finding was not observed in studies with

Table 4
Ranges in XPS and IR Data of Different Bacterial Species

	Streptococci[a]	Staphylococci[b]	*E. coli*[c]
O/C	0.31–0.50	0.45–0.55	0.28–0.43
N/C	0.05–0.13	0.15–0.20	0.03–0.09
P/C	0.006–0.01	0.02–0.04	0.009–0.02
AmI/CH	5.6–7.1	7.2–8.7	6.2–7.2
AmII/CH	1.6–2.4	2.5–3.6	2.3–2.6
PI/CH	1.1–1.7	2.5–4.4	1.0–1.3
PII/CH	3.7–6.1	6.0–8.3	3.0–4.5

[a]Taken from refs. *21* and *28*.
[b]Taken from ref. *7*.
[c]This work.

staphylococci *(7)* and streptococci *(28)*, and indicates that relatively few amino groups are present at the surface of *E. coli*. The relatively low N/C ratios for these strains as compared with staphylococci and streptococci (Table 4) supports this observation. A correlation ($r = 0.81$) between the zeta potentials of the strains at pH = 7.4 and their relative surface charge values (ARR) (Table 1) at pH = 7.4 was observed. High ARR values correspond with high negative zeta potentials, indicating that strains with a high negative surface charge have a strong interaction with the anionic-exchange resin.

According to their water contact angles, strains O2K2, O8K(A)28, and O83K? were less hydrophilic than the other five strains. This was not confirmed by the MATH data. The lack of agreement between water contact angles and MATH data has also been reported by others *(29–31)*. Strains with a high ARR value, had a low MATH value whereas strains with a low ARR value had a relatively high MATH value, except for strain O8K(A)28.

Surface free energies were calculated according to the methods of Busscher et al. *(13)* and Van Oss et al. *(17,18)*. There is a good linear relation ($r = 0.97$) between the polar component (γ^P) of the surface free energy according to Busscher et al. *(13)* and the electron-donor part (γ^-) of the acid/base component of the surface free energy according to van Oss et al. *(17,18)* (Fig. 2). High values of the polar components correspond with high values of the electron-donor parts. Apparently these surface free energy components correlate with corresponding properties of the surface. Plotting the acid/base component of the surface free energy against the zeta potential at pH = 7.4 (Fig. 3) yielded a linear correlation ($r = 0.84$). High values of acid/base components of the surface free energies correspond with small negative zeta potentials. A good explanation for this correlation cannot be given.

Nitrogen in the cell wall is present as amide bonds (protein and peptidoglycan), as amine groups (protein, peptidoglycan, and teichoic acid), and as inorganic salts. The N/C ratio for the strains used in this study ranged

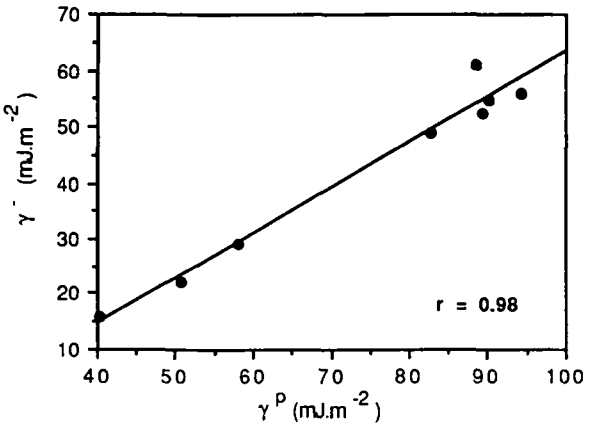

Fig. 2. Relation between the electron-donor parameter (γ^-) of the surface free energy of the eight *E. coli* strains based on the concept of Lifshitz-van der Waals and acid/base surface free energy components *(17,18)*, and the polar part (γ^P) of the surface free energy based on the concept of dispersion and polar surface free energy components *(13)*.

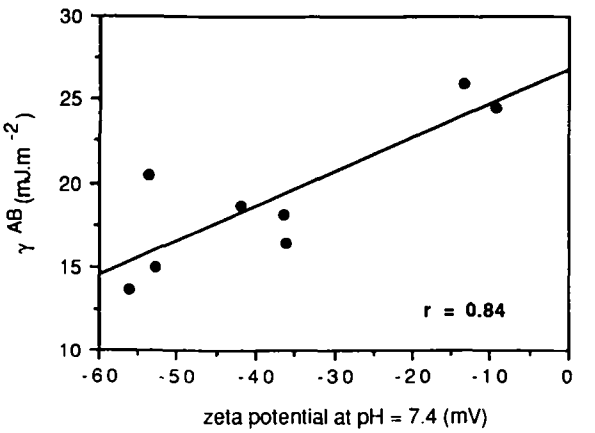

Fig. 3. Relation between the acid/base component of the surface free energy of the eight *E. coli* strains based on the concept of Lifshitz-van der Waals and acid/base surface free energy components *(17,18)*, and the zeta potentials at pH = 7.4.

from 0.026 to 0.085 (Table 4). The AmI/CH ratio as determined with IR varied more than the AmII/CH ratio possibly because of differences in the content of unsaturated aldehydes and ketones in the cell wall causing fluctuations in the AmI/CH ratio, but not in the AmII/CH ratio. Compared with staphylococci and oral streptococci, the *E. coli* strains had the lowest N/C ratios, although the difference between streptococci and *E. coli* was much less than

Table 5
Cell-Wall Characteristics of Gram-Positive and Gram-Negative Bacteria[a]

	Gram positive	Gram negative
Cell		
Cell wall (dry weight)	15–20%	5–10%
Cell wall		
Thickness	20–50 nm	10–15 nm
Aminosugar	10–30%	1–10%
Lipid	0–2%	10–20%
Protein	<12%	35–40%
Lipopolysaccharides	–	20–25%
Peptidoglycan	30–50%	<10%
Teichoic acid	<50%	–

[a]Taken from refs. *32* and *33*.

the difference between streptococci and staphylococci (Table 4). Staphylococci clearly had the highest N/C ratios. The higher N/C ratios of the gram-positive microorganisms might be explained by the higher peptidoglycan content in the cell wall of these microorganisms than in the cell wall of the gram-negative *E. coli* (Table 5). The nitrogen present in peptidoglycan is incorporated mainly in amide bonds. The AmII/CH ratio as detected with IR indicated that staphylococci possessed much more amide bonds compared with streptococci and *E. coli*, which had more or less equal concentrations of amide bonds. It has to be mentioned that the penetration depth of XPS (2–5 nm) is much smaller than that of IR (microns). Therefore, XPS provides information of the outer part of the cell wall, whereas IR probably gives information about the whole cell. However, the high N/C ratio for staphylococci as detected with XPS corresponds well with the high concentration of amide bonds as detected with IR *(7)*.

Oxygen is present in all molecules of the cell wall. The O/C ratio of polysaccharides is much higher than of peptidoglycan and protein. The O/C ratios of the *E. coli* strains as detected with XPS varied from 0.28 to 0.43 (Table 4) and are in the same order as those of oral streptococci, but lower than those of staphylococci (Table 4), indicating that the cell wall of the latter microorganisms contains a higher concentration of polysaccharides. This was confirmed by the higher PII/CH ratio as detected with IR found for staphylococci in comparison with streptococci and *E. coli* (Table 4 and Fig. 4). The PII/CH ratios of the eight E. coli strains ranged from 3.0 to 4.5, indicating that there are differences in polysaccharide contents between the eight strains. These data did not correspond with the O/C ratios of these *E. coli* strains, which can be explained by the difference in penetration depths when applying the two methods.

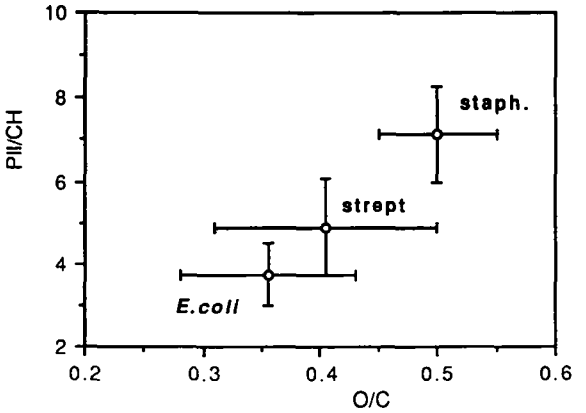

Fig. 4. The ratio of the areas under the peaks at 1070 cm^{-1} (polysaccharides) and 2930 cm^{-1} (C–H stretching), as a function of the O/C surface concentration ratios for staphylococci, streptococci, and *E. coli*. Error bars indicate regions in which the values of the three groups are located.

Phosphorus present in the cell wall is found in phospholipids, teichoic acid, and lipopolysaccharides. Differences in P-content in the surface components of the bacteria will only be detected by XPS, but not by IR owing to the higher penetration depth of IR. The PI/CH ratio among the eight *E. coli* strains did not vary much, whereas the P/C ratio did. The P/C ratios of the eight *E. coli* strains were higher than those of oral streptococci, but lower than those of staphylococci (Table 4). The rather high P/C ratios of *E. coli* might be explained by the relatively high content of phospholipids in the outer membrane of this gram-negative organism *(32)*. The difference in P/C ratios between the staphylococci and streptococci is in agreement with the high PI/CH ratio found for the staphylococci and the low PI/CH ratio in streptococci. The large difference in P/C ratio between the gram-positive organisms may be explained by the high amount of teichoic acid in staphylococci, whereas this cell wall component is not prominent in streptococci *(33)*.

Relations between XPS and IR data as found for staphylococci and oral streptococci were not found for the eight *E. coli* strains used in this study. This might be because of differences between the cell wall composition of gram-negative and gram-positive bacteria. The cell wall structure of *E. coli* is more complex and the thickness of the cell wall is less than the cell wall of gram-positive microorganisms. Because the penetration depth of IR is much higher than of XPS, it is possible that for bacteria with a relatively thick cell wall, such as gram-positive bacteria, the IR data allow a better characterization of the cell wall than for bacteria with a much thinner cell wall, such as gram-negative bacteria. Good relations between surface free energies and XPS or IR data as reported for streptococci and staphylococci *(7,21,28)* were

not found in this study. However, it can be concluded that differences between various species of bacteria (staphylococci, streptococci, and *E. coli*) as detected with XPS are often confirmed by IR data.

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