INFECTION AND IMMUNITY, Jan. 1986, p. 294–301
0019-9567/86/010294-08$02.00/0

Vol. 51, No. 1

Cell Surface Characteristics of Coagulase-Negative Staphylococci and Their Adherence to Fluorinated Poly(Ethylenepropylene)

ANDRÉ H. HOGT,[1] JACOB DANKERT,[2]* C. E. HULSTAERT,[3] AND JAN FEIJEN[1]

Section of Biomaterials, Department of Chemical Technology, Twente University of Technology, 7500 AE Enschede,[1] Laboratory for Medical Microbiology, Department of Hospital Epidemiology, University Hospital,[2] and Center for Medical Electron Microscopy, University of Groningen,[3] 9713 EZ Groningen, The Netherlands

Received 6 May 1985/Accepted 1 October 1985

The ability of 21 nonencapsulated and 15 encapsulated coagulase-negative staphylococci (CNS) to adhere to xylene in xylene-water emulsions and to fluorinated poly(ethylenepropylene) (FEP) films revealed remarkable differences. Nonencapsulated CNS strains adhered well to FEP, whereas their adherence to xylene ranged widely. Encapsulated strains with low adherence to xylene showed slight adherence to FEP. Encapsulated strains which adhered well to xylene ranged widely in their adherence to FEP. It was concluded that results obtained from the xylene adherence test were not predictive of the adherence of CNS to the hydrophobic FEP surface. The number of nonwashed, slime-producing CNS strains adhering to FEP was similar to that of washed bacteria of the same strains. Bacterial adherence to FEP was decreased when FEP films were exposed to a solution containing extracellular products (EP) obtained from a slime-producing CNS strain. Bacterial adherence to xylene also decreased when the bacterial suspensions contained EP. Apparently, initial adherence of CNS to FEP and xylene is hampered by EP. Nonencapsulated and encapsulated CNS pretreated with proteolytic enyzmes failed to adhere to xylene and FEP, indicating that intact surface proteins or constituents associated with surface proteins mediated their adherence to xylene and FEP. Freeze-etch replicas of a CNS strain adhering to FEP showed a smooth, flattened area on the bacterial surface at the contact site of the bacteria with the FEP, indicating that an external layer was present at the bacterial surface.

Coagulase-negative staphylococci (CNS) have been recognized as important pathogens in hospitalized patients (17, 29, 35, 44). CNS are especially involved in infections associated with prosthetic implants (4, 8, 26) and medical devices (9, 36, 37). It has been reported that slime-producing CNS cause intravascular catheter-associated bacteremia (9) and ventriculoatrial shunt infections (5).

The initial step in the pathogenesis of implant- and medical device-associated infections due to CNS has been considered to be bacterial adherence to artificial surfaces (18, 28, 34). However, it is not known which bacterial surface structures or components of CNS are involved in the initial adherence stage. It has been suggested that bacterial adherence to artificial surfaces is mediated by hydrophobic bonding (15, 20, 30). CNS show a wide range of cell surface hydrophobicity (21, 22), but the relevance of this surface property in relation to the ability of CNS to adhere to surfaces of implants and medical devices has not been determined. Capsular surface structures of CNS may also be relevant for adherence. The presence of encapsulated CNS has been demonstrated (2, 21, 47). It was shown that the majority of encapsulated CNS were hydrophobic, although a strain with a definite hydrophilic capsule was also encountered (21). Slime production of CNS is thought to be a critical factor in the colonization of medical devices by CNS (11, 33), but the role of extracellular products (EP) in the adherence of CNS has not been elucidated. In a recent report, the importance of slime as a virulence factor of CNS in experimental endocarditis in rats could not be established (3).

In this study, we investigated the adherence of various CNS strains to fluorinated poly(ethylenepropylene) (FEP), a hydrophobic biomaterial used in various biomedical applications. The CNS strains tested belonged to different species and included nonencapsulated, encapsulated, and slime-producing strains. The morphology of bacteria adhering to FEP was examined by transmission electron microscopy (TEM) of freeze-etch replicas.

* Corresponding author.

MATERIALS AND METHODS

Bacterial strains and growth conditions. Thirty-six CNS strains were used in this study. Slide and tube tests with rabbit plasma (Sylvana, Grand Island, N.Y.) were performed to determine the absence of ability to clot plasma. CNS strains were classified according to the scheme of Kloos and Schleifer (27) with the Staph gallery (API Systems S.A., Montalieu Vercieu, France). Eight National Collection of Type Cultures strains were kindly donated by R. R. Marples, Central Public Health Laboratory, London, England. These strains had been isolated from six patients with prosthetic valve endocarditis. Twelve strains were isolated from blood samples taken from patients with bacteremia due to CNS (two or more separate blood cultures were positive). Seven and nine strains were isolated from the skin flora of open-heart patients and laboratory staff members, respectively. Bacteria stored in skim milk at −20°C were grown on sheep blood agar plates (Oxoid Ltd., London, England) and maintained on nutrient agar (Oxoid) slants at 4°C for 1 month.

Bacteria were cultured in Trypticase soy broth (TSB; BBL Microbiology Systems, Cockeysville, Md.) for 24 h at 37°C. Exponential-phase cells were obtained by culturing 2 ml of overnight broth in 100 ml of fresh TSB for 5 h at 37°C in a rotary shaker-incubator (New Brunswick Scientific Co., Inc., Edison, N.J., at 90 rpm. The bacteria were harvested by centrifugation in a J2-21 centrifuge (10,000 × *g*, 4°C, 10 min; Beckman Instruments, Inc., Palo Alto, Calif.), washed three times with phosphate-buffered saline (PBS [8.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 140 mM NaCl, 3 mM KCl]) (pH

7.2). After washing in PBS, standard bacterial suspensions prepared from cultures grown for 5 or 24 h in TSB were aspirated and ejected twice through a sterile steel needle attached to a 60-ml syringe, then filtered through an 8-μm (pore size) membrane filter (type SC; Millipore Corp., Bedford, Mass.) to remove bacterial clusters.

Bacteria were also grown in glucose-free TSB (GF-TSB; BBL) to reduce production of extracellular slime (10). To enhance production of slime, one strain (*Staphylococcus epidermidis* SL76) was grown for 24 h at 37°C in modified *Staphylococcus* 110 broth (46) additionally modified as described by Caputy and Costerton (7). Glucose (1% [wt/vol]) was used instead of mannitol.

Detection of bacterial capsules and production of slime. To demonstrate the presence of capsules, bacteria grown in TSB or GF-TSB for 5 and 24 h at 37°C were washed with PBS and stained by the India ink wet-film method (12). The ability of strains to produce slime was tested by the method of Christensen et al. (10). Briefly, bacteria were inoculated into glass culture tubes containing TSB or GF-TSB. After incubation for 24 h at 37°C, the tubes were emptied and then filled with an aqueous solution of alcian blue (0.1% [wt/vol]; ICI, Macclesfield, Cheshire, England) or safranin (0.1% [wt/vol]; Brocades, Maarssen, The Netherlands) to stain the adherent slime layer.

A high-molecular-weight EP was isolated from strain SL76 grown for 24 h in 100 ml of modified *Staphylococcus* 110 broth by the method described by Ekstedt and Bernhard (13). The bacteria were separated from the extrapolymer layer by mixing the culture in a Waring blender for 15 min at 4°C. The bacteria were sedimented, and the supernatant was concentrated 20-fold with immersible ultrafilters (type CX10; nominal molecular weight limit, 10,000; Millipore). The concentrate was then washed with PBS (60 ml) by the ultrafilter system to remove low-molecular-weight compounds. Protein content in the concentrate was determined by the micro-biuret method (25), and uronic acid content was determined as described by Blumenkrantz and Asboe-Hansen (6) with D-galacturonic acid (BDH, Poole, England) as a standard. The concentrate contained 2.4 mg of protein and 0.84 μmol of uronic acid per ml of PBS. This preparation, designated as EP concentrate, was stored at −20°C.

Pretreatment of bacteria with proteolytic enzymes. To study the effect of pretreatment of bacteria grown for 5 h in TSB with proteolytic enzymes on bacterial adherence, we suspended bacteria for 1.5 h at 37°C in 0.2 M citrate buffer (pH 3.0) containing 0.65% (wt/vol) NaCl and 0.1% (wt/vol) pepsin in PBS (pH 7.2) containing 0.1% (wt/vol) pronase (protease type XIV) or in PBS (0.2 M phosphate, pH 8.0) containing 0.1% (wt/vol) trypsin, α-chymotrypsin, or subtilisin (protease type VII). The suspensions were rapidly chilled and centrifuged (10,000 × *g*, 4°C, 10 min), and the bacteria harvested were washed with ice-cold PBS. All enzymes were purchased from Sigma Chemical Co., St. Louis, Mo. Bacterial cells suspended in enzyme-free buffer solutions were used as controls.

Bacterial adherence to xylene. Bacterial adherence to xylene was determined in a xylene-water system as described by Rosenberg et al. (41). A volume of 0.25 ml of analytical-grade *p*-xylene (pro analisi; E. Merck AG, Darmstadt, Federal Republic of Germany) was added to disposable glass tubes (inside diameter, 10 mm) containing late-exponential- or stationary-growth-phase bacterial cells in 3 ml of PBS adjusted to an optical density at 540 nm (OD_{540}) of 1.0 (model 24 spectrophotometer; Beckman). The tube contents were mixed on a mixer for 60 s, and after phase separation occurred, the OD_{540} of the aqueous phase was measured. The percentage of bacteria that adhered to xylene was calculated by dividing the difference between the OD_{540} values of the suspensions before and after mixing with xylene by the original OD_{540} value before mixing and multiplying by 100%.

Bacterial adherence to FEP. Disks (34 mm diameter) of an FEP [poly(tetrafluoroethylenehexafluoropropylene)] sheet (type 500 A; E. I. Du Pont de Nemours & Co., Inc., Wilmington, Del.) were ultrasonically cleaned (50 kHz; Bransonic 221; Branson Sonic Power Co., Danbury, Conn.) in a 1% (vol/vol) detergent solution (RBS 25; Hicol, Rotterdam, The Netherlands) for 30 min, followed by extensive rinsing with distilled water-ethanol-diethyl ether. Disks were tested for the presence of contaminating lipopolysaccharides by the *Limulus* lysate test (Mallinckrodt, Inc., St. Louis, Mo.) (20). The disks, sterilized by dry heat at 175°C for 3 h, were placed into wells of polystyrene culture dishes (six wells, 35-mm inner diameter; Costar, Cambridge, Mass.) and fixed with dry-heat-sterilized glass rings. Suspensions for testing were adjusted with PBS to an OD_{540} of 0.5 and contained 5×10^8 CFU/ml as determined by plate counting.

The FEP disks were incubated with the bacterial suspensions (3 ml) in a rotary shaker-incubator (90 rpm) at 37°C for 2.5 h and subsequently rinsed eight times with 3-ml amounts of PBS. Adhering bacteria were fixed with 4% (vol/vol) glutaraldehyde in PBS, and the number of adhering bacteria was counted by examination of six 0.05-mm^2 areas per disk by light microscopy (BHB/PM-10; Olympus Optical Co., Tokyo, Japan). If bacteria adhered to FEP films in clusters, the average number of cells per cluster was counted by high-magnification light microscopy or scanning electron microscopy (SEM). Samples of films with adhering bacteria fixed with 2% (vol/vol) glutaraldehyde in PBS and washed with water were dehydrated in a water-ethanol series, mounted on aluminum stubs, gold coated with a sputtering device (Balzers Union, Ltd., Principality of Liechtenstein), and examined with a JSM-35CF SEM (Jeol, Ltd., Tokyo, Japan).

Adherence of slime-producing CNS and the effects of EP concentrate on bacterial adherence. Extracellular slime of bacteria can be removed by washing (12). The adherence of nonwashed bacteria grown in TSB or GF-TB to xylene and FEP was compared with that of washed cells.

To study the effect of the presence of EP on bacterial adherence to xylene and FEP, we added EP concentrate (0.1 to 1.0% [vol/vol]) to suspensions containing washed bacteria immediately before performing adherence tests. We also preexposed bacteria or FEP films to EP concentrate before performing adherence tests. Bacteria were preexposed to a solution of EP concentrate in PBS (0.6% [vol/vol]) for 15 min at 37°C, sedimented, washed once, and suspended in PBS. FEP films were preexposed to PBS containing various amounts of EP concentrate ranging from 0.1 to 1.0% (vol/vol) for 1 h at 37°C and subsequently rinsed eight times with 3-ml amounts of PBS.

Preparation of freeze-etch replicas for TEM. FEP films exposed for 2.5 h at 37°C to a suspension of *S. epidermidis* SEP1 in PBS containing 10^9 CFU/ml were washed eight times with 3-ml volumes of PBS. From these films, samples (4 by 4 mm) were taken and prepared for examination of the contact surfaces of adhering bacteria by the freeze-fracturing and -etching technique (45). The samples were mounted in a Messing double-replica holder and transferred into melting nitrogen (−210°C). Fractures along the interface between

bacteria and polymer film were made at −170°C and 10^{-6} torr (133.322 × 10^{-6} Pa). Fracture faces were etched for 2 min at −100°C in a modified Polaron device. The etched surfaces were shadowed with platinum-carbon. Replicas were cleaned overnight in 40% (wt/vol) chromic acid and then in a 0.2% (wt/vol) sodium hypochloride solution. Subsequently, the replicas were washed in distilled water, mounted on 400-mesh copper grids, and examined in a Philips EM 300.

Statistical methods. The Student *t* test (14) was used to determine significant differences in bacterial adherence of nonencapsulated and encapsulated CNS strains to FEP films. Correlation coefficients (*r*) of linear regression analyses of data were determined by standard methods (14).

RESULTS

Identification, capsules, and slime production of CNS. Identification of the 36 strains showed that seven different species were present. Identified were 21 strains of *S. epidermidis*, 8 of *S. saprophyticus*, 2 of *S. hominis*, 1 of *S. cohnii*, 2 of *S. haemolyticus*, 1 of *S. warneri*, and 1 of *S. capitis*. Capsules were seen in 1 *S. epidermidis* strain and in all of the 15 strains not identified as *S. epidermidis*, except for one *S. saprophyticus* strain. Encapsulation was demonstrated in both exponential- and stationary-growth-phase cells. Slime production was observed in stationary-growth-phase cells of six strains by the alcian blue glass tube staining test (10). The slime-producing CNS strains were identified as four *S. epidermidis* (nonencapsulated), one *S. saprophyticus* (encapsulated), and one *S. hominis* (encapsulated).

Adherence of CNS to xylene and FEP films. The adherence to xylene of nonencapsulated (Fig. 1A) and encapsulated (Fig. 1B) CNS strains ranged from 7 to 95 and 6 to 93%, respectively. Washed bacteria of the four slime-producing, nonencapsulated strains showed different affinities toward xylene. The two slime-producing, encapsulated strains had a high affinity toward xylene. Adherence of exponential-phase cells to xylene was similar to that of stationary-growth-phase cells (data not shown).

The mean numbers of nonencapsulated CNS adhering to FEP, ranging from 43 × 10^3 to 87 × 10^3 cells per mm^2 of film (Fig. 1A), were significantly higher ($P < 0.01$) than those of encapsulated strains, which ranged from 1.6 × 10^3 to 84 × 10^3 per mm^2 of film (Fig. 1B). Some encapsulated strains with a low affinity toward xylene showed minimal adherence to FEP. However, no correlation between adherence to xylene and to FEP was observed ($r = 0.58$). Also, no correlation between the adherence of nonencapsulated strains to xylene and to FEP was found ($r = 0.18$).

Effect of pretreatment of CNS with proteolytic enzymes on adherence to xylene and FEP. After pretreatment of *S. epidermidis* and *S. saprophyticus* strains with pepsin, their adherence to xylene was negligible (Table 1). Strains pretreated with pronase and subtilisin also showed slight adherence to xylene. Pretreatment of nonencapsulated *S. epidermidis* SEP1 with trypsin had no effect, whereas pretreatment of encapsulated *S. epidermidis* NCTC 100892 and nonencapsulated *S. saprophyticus* PRE40 with trypsin decreased their adherence to xylene and FEP. The adherence of all CNS strains pretreated with pepsin to FEP was significantly reduced ($P < 0.001$). Subtilisin pretreatment also resulted in reduced adherence to FEP. Pretreatment of nonencapsulated *S. epidermidis* SEP1 with pronase did not affect adherence to FEP. In contrast, the adherence of *S. epidermidis* NCTC 100892 and *S. saprophyticus* PRE40 to FEP decreased after pretreatment with pronase. α-Chymotrypsin-pretreated *S. epidermidis* SEP1 showed unchanged adherence to xylene but slightly decreased adherence to FEP.

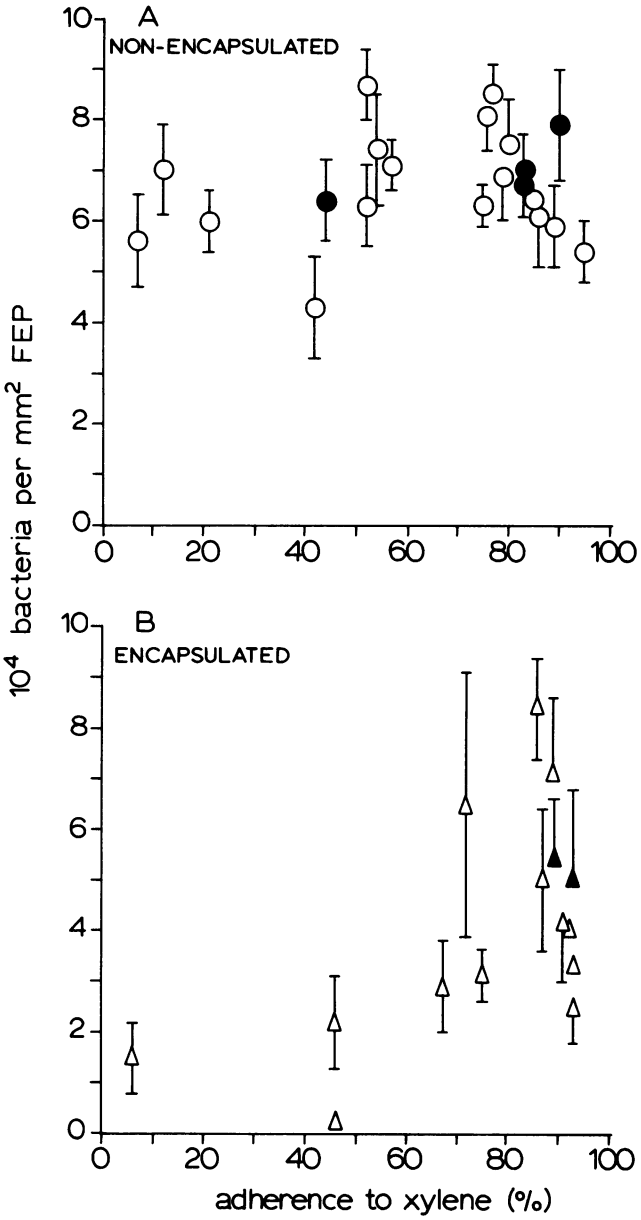

FIG. 1. Adherence of 36 CNS to FEP films as a function of adherence to xylene. Panel A: nonencapsulated, slime-producing (●) and non-slime-producing (○) strains. Panel B: encapsulated, slime-producing (▲) and non-slime-producing (△) strains.

Adherence of slime-producing and non-slime-producing CNS to xylene and FEP. To test the effect of slime on adherence, experiments were performed with three slime-producing strains (SL58, SL76, and PRE69) and one non-slime-producing strain (SEP1). When slime-producing strains were grown in GF-TSB, slime could not be demonstrated by the alcian-blue staining test (10) (Table 2). The adherence of washed bacteria grown in GF-TSB to xylene was similar to that of washed bacteria grown in TSB. Nonwashed bacteria of the slime-producing strains grown in TSB and GF-TSB were less able to adhere to xylene than

TABLE 1. Effect of pretreatment of CNS with proteolytic enzymes on adherence to xylene and FEP films

Staphylococcus strain	Capsule[a]	Pretreatment	Bacterial adherence to:	
			Xylene (%)[b]	FEP (cells [10^3] per mm^2)[b]
S. epidermidis SEP1	−	Trypsin	68 (79)	68 ± 8 (67 ± 5)
S. epidermidis SEP1	−	Chymotrypsin	65 (79)	47 ± 6 (67 ± 5)[d]
S. epidermidis SEP1	−	Pronase	20 (82)	76 ± 7 (66 ± 4)
S. epidermidis SEP1	−	Subtilisin	13 (80)	3 ± 3 (80 ± 13)[c]
S. epidermidis SEP1	−	Pepsin	0 (78)	1.4 ± 0.3 (68 ± 5)[c]
S. epidermidis SEP2	−	Pepsin	0 (61)	11 ± 7 (43 ± 5)[c]
S. epidermidis NCTC 100835	−	Pepsin	0 (0)	11 ± 5 (38 ± 4)[c]
S. epidermidis NCTC 100892	+	Trypsin	6 (28)	13 ± 11 (28 ± 4)[c]
S. epidermidis NCTC 100892	+	Pronase	0 (28)	7 ± 6 (28 ± 14)[d]
S. epidermidis NCTC 100892	+	Pepsin	0 (22)	1.4 ± 1.2 (32 ± 18)[d]
S. saprophyticus PRE40	−	Trypsin	0 (17)	30 ± 10 (90 ± 7)[c]
S. saprophyticus PRE40	−	Pronase	0 (17)	8 ± 3 (90 ± 7)[c]
S. saprophyticus PRE40	−	Pepsin	0 (0)	1.7 ± 0.6 (49 ± 11)[c]
S. saprophyticus A1	+	Pepsin	4 (96)	23 ± 10 (92 ± 36)[c]
S. saprophyticus SAP1	+	Pepsin	0 (0)	0.2 ± 0.2 (0.6 ± 0.3)

[a] +, Encapsulated; −, nonencapsulated.
[b] Mean or mean ± the standard deviation. Adherence values of controls (bacteria suspended in enzyme-free buffer solutions) are given in parentheses.
[c] Significant ($P \leq 0.005$) difference between treated and untreated bacteria by the Student t test.
[d] Significant difference ($P \leq 0.05$).

were washed bacteria (Table 2). The non-slime-producing strain showed a similar effect. Adherence of CNS strains grown in TSB to FEP films did not differ from that of strains grown in GF-TSB (Table 2). Differences between nonwashed and washed bacteria with respect to adherence to FEP also were not observed.

When EP concentrate was added to a suspension of washed bacteria of *S. epidermidis* SL76, the bacteria did not adhere to xylene and adhered slightly to FEP (Table 3). Presence of EP concentrate in the bacterial suspension at a final concentration of 0.2% (vol/vol) or higher resulted in a reduction of bacterial adherence to xylene (Fig. 2). Bacteria preexposed to the EP concentrate and then washed once showed similar adherence to xylene and FEP as the controls (Table 3). The number of bacteria adhering to FEP films preexposed to solutions containing a minimal amount of 0.2% (vol/vol) EP concentrate was significantly ($P < 0.001$) reduced (Table 4).

TEM of freeze-etch replicas. Freeze-fracturing of nonencapsulated *S. epidermidis* SEP1 adhering to FEP resulted in the separation of adhering bacteria from the FEP film surface. After fracturing, FEP films showed no bacterial remnants or surface defects.

Bacteria appeared to adhere to FEP via smooth, flattened contact areas with various diameters (Fig. 3A). Between the contact areas and the rough, irregular bacterial surface, a slightly elevated edge could be observed. Beyond this elevated edge on one part of the bacterial surface, concentric, circular structures representing the circular arrangement of peptidoglycan (1) were visible (Fig. 3B). Threadlike structures were seen at many sites, mostly extending from the edges of contact areas (Fig. 3A and B).

DISCUSSION

Bacterial adherence to xylene is generally used to estimate the hydrophobic or hydrophilic character of the bacterial cell surface (40). Cell surface hydrophobicity of nonencapsulated *S. epidermidis* strains ranged widely. For *Streptococcus mutans*, a similar wide range in hydrophobicity within one species was also reported (32).

Of 36 CNS strains tested, 15 had capsules. The majority of the encapsulated CNS strains were hydrophobic independent of growth phase. Capsules were present around both exponential- and stationary-growth-phase cells (22). In contrast to our findings, it has been reported that various *Streptococcus pyogenes* and *Acinetobacter calcoaceticus* strains possessing capsules in the early-exponential growth phase lost their capsules in the late-exponential growth

TABLE 2. Effect of glucose in the growth medium and of washing the bacteria on the adherence of CNS to xylene and FEP films

S. epidermidis strain	Slime production when grown in[a]:		Adherence of bacteria grown in[b]:							
			TSB to:				GF-TSB to:			
			Xylene (%)		FEP (no. of cells [10^3] per mm^2)		Xylene (%)		FEP (no. of cells [10^3] per mm^2)	
	TSB	GF-TSB	Nonwashed	Washed	Nonwashed	Washed	Nonwashed	Washed	Nonwashed	Washed
SL58	(+)	−	73 ± 1	82 ± 1	37 ± 4	35 ± 4	52 ± 1	79 ± 1	34 ± 3	39 ± 9
SL76	(+)	−	32 ± 20	48 ± 5	55 ± 12	58 ± 5	37 ± 3	44 ± 3	48 ± 6	46 ± 7
PRE69	(+)	−	53 ± 15	80 ± 8	71 ± 4	68 ± 3	49 ± 10	84 ± 2	52 ± 6	54 ± 5
SEP1	(−)	−	55 ± 7	78 ± 8	67 ± 10	73 ± 9	42 ± 11	84 ± 4	56 ± 8	65 ± 5

[a] Slime production of CNS grown in TSB or in GF-TSB was determined by the alcian-blue glass culture tube staining test (10). +, Slime produced; −, no slime produced.
[b] Mean ± the standard deviation of at least duplicate experiments.

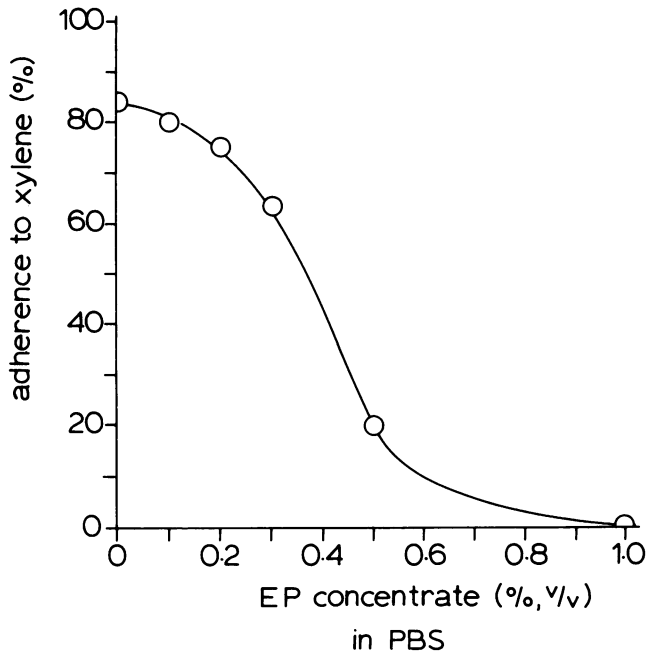

FIG. 2. Effect of EP preparation on the adherence of *S. epidermidis* SL58 to xylene. To study the effect of EP in suspensions of nonwashed bacteria on bacterial adherence, we added various volumes of EP preparation to suspensions of washed bacteria in PBS. The EP preparation contained 2.4 mg of protein and 0.84 μmol of uronic acid per ml of PBS.

phase, coinciding with an increase in their hydrophobicity (31, 42).

Slime production was demonstrated in 6 of the 36 CNS strains tested. Washed bacteria of slime-producing strains showed higher adherence to xylene than did nonwashed bacteria, suggesting that slime present on the bacteria decreased bacterial hydrophobicity. However, nonwashed bacteria of a non-slime-producing strain also showed lower hydrophobicity than did washed bacteria. In addition, washed and nonwashed CNS strains grown in GF-TSB (10) showed a similar difference in hydrophobicity.

It was previously found that washed bacteria suspended in PBS supplemented with small amounts of TSB showed a reduction in adherence to xylene (21). Therefore, the presence of residual amounts of TSB in nonwashed bacterial suspensions may explain the reduced adherence to xylene, probably because of coating of the xylene droplets by proteins (31) from the growth medium. The apparent reduction of the adherence of bacteria to xylene in suspensions containing EP concentrate may be caused by the same phenomenon.

All nonencapsulated CNS strains adhered well to FEP. This suggests that these strains had hydrophobic sites at the cell surface, which allowed the bacteria to adhere by hydrophobic bonding. From these results, it was expected that the strains would show a hydrophobic character as determined by the xylene adherence test. However, in this test the bacterial surface character of the strains ranged from hydrophilic to hydrophobic. Schulman and Leja (43) demonstrated that the stabilization of benzene-water emulsions by fatty acid- and alkyl sulfate-coated barium sulfate particles was related to the structure of the coating on the particles. It has to be realized that the xylene adherence test also provides information about the ability of bacteria to

TABLE 3. Effect of EP concentrate on the adherence of *S. epidermidis* SL76 to xylene and FEP

Bacterial suspension contained:	Bacteria adhering to:	
	Xylene (%)[a]	FEP (no. of cells [10^3] per mm^2)[a,b]
Washed bacteria (control)	46	87 ± 11
Washed bacteria and EP concentrate	0	6 ± 5[c]
Washed bacteria preexposed to EP[d]	48	93 ± 12

[a] The bacterial suspension (10^9 cells per ml) in the xylene adherence assay contained 0.6% (vol/vol) EP concentrate of *S. epidermidis* SL76. The bacterial suspension (0.5×10^9 cells per ml) used for the FEP adherence assay contained 0.3% (vol/vol) EP concentrate.

[b] Mean ± the standard deviation.

[c] Significantly different ($P \leq 0.001$) adherence values compared with controls.

[d] Bacteria were suspended in PBS containing 0.6% (vol/vol) EP concentrate. After incubation for 15 min at 37°C, the bacteria were sedimented, washed once, and suspended in PBS.

stabilize xylene-water emulsions. Therefore, the molecular organization and domain structure of hydrophobic and hydrophilic sites at the cell surface of the different strains determine their ability to stabilize xylene-water emulsions. This implies that nonencapsulated CNS strains, which are hydrophilic according to the xylene adherence test, have sufficient hydrophobic sites at the cell surface to allow their adherence to FEP.

Encapsulated CNS strains showed a wide range in their adherence to FEP, indicating that the number or size or both of hydrophobic sites at the capsular surface of these strains differ. It is remarkable that encapsulated strains with low adherence to FEP showed a high degree of partitioning between the xylene and water phases. These phenomena may be understood by assuming that xylene interacts with the capsular layer, causing reorientation of capsular constituents, resulting in the exposure of hydrophobic sites at the surface. Since xylene-induced reorientation of hydrophobic sites is less likely to occur at the surface of nonencapsulated strains, it may explain the differences in partitioning to xylene and adherence to FEP between nonencapsulated and encapsulated CNS. From our results it can be concluded that data obtained from the xylene adherence test are not necessarily predictive of bacterial ability to adhere to hydrophobic solid surfaces. This is also illustrated by the finding that pronase-pretreated, nonencapsulated *S. epidermidis* SEP1 showed strongly reduced adherence to xylene but unchanged adherence to FEP.

Differences in the effects of the treatment of bacteria with

TABLE 4. Adherence of *S. epidermidis* SL76 to FEP films preexposed to EP concentrate

Amt of EP concentrate in PBS exposed to FEP films (% [vol/vol])[a]	Bacteria adhering to FEP (no. of cells [10^3] per mm^2)[b]
0 (control)	74 ± 9
0.1	34 ± 21
0.2	2.0 ± 1.5
0.3	0.8 ± 0.7
0.5	0.5 ± 0.4
1.0	0.3 ± 0.2

[a] FEP films were preexposed for 1 h at 37°C to 3 ml of PBS containing different amounts of EP concentrate prepared from *S. epidermidis* SL76 and subsequently rinsed eight times with PBS.

[b] Mean ± the standard deviation of at least three experiments.

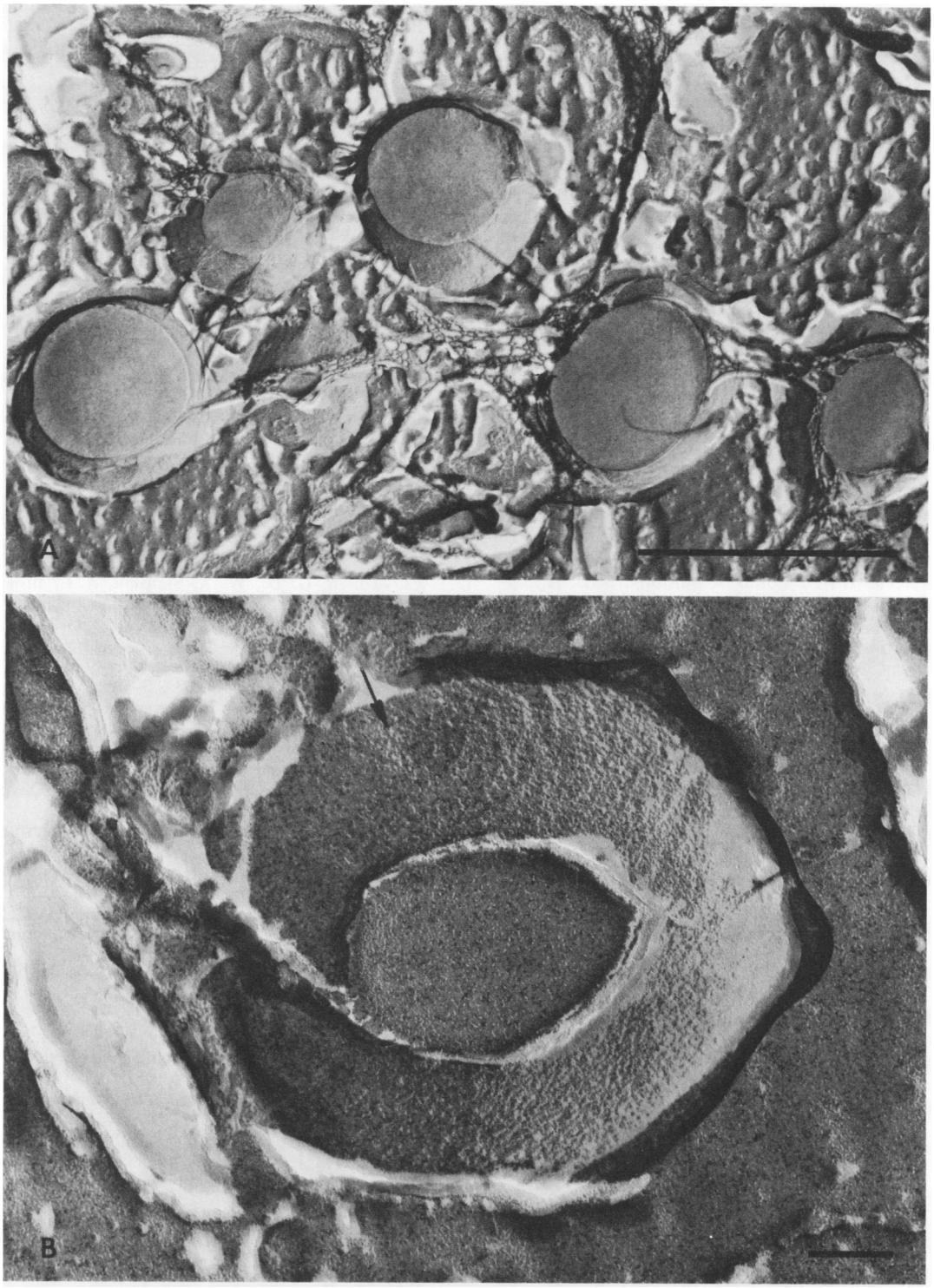

FIG. 3. Freeze-etch preparation of *S. epidermidis* SEP1 adhering to FEP film. (A) Overview of several adhering bacteria showing smooth, flattened contact areas and threads (magnification, ×39,200; bar, 1 μm). (B) The contact area is smooth, flat, and surrounded by an elevated edge. The arrow indicates concentric circular structures representing the circular arrangement of peptidoglycan covered by an external layer.

various proteolytic enzymes on their adherence to xylene and FEP reflect differences in the susceptibility of surface proteins to enzymatic action. The cell surface of *S. epidermidis* SEP1 differs from that of the other strains. Trypsin-pretreated *S. epidermidis* SEP1 showed adherence to xylene similar to that of untreated controls, whereas two other strains adhered less after treatment with this enzyme. Additionally, pretreatment of all CNS strains (*S. epidermidis* and *S. saprophyticus*) with pepsin significantly decreased bacterial adherence to xylene and FEP. *Streptococcus pyogenes* showed reduced adherence to hexadecane droplets after release of protein and lipoteichoic acid due to pepsin treat-

ment (31). It was concluded that lipophilic structures consisting of glycolipids complexed to surface proteins mediated the adherence of *Streptococcus pyogenes* to hexadecane. Our results indicate also that adherence of CNS to xylene and FEP is dependent on the presence of intact surface proteins or constituents associated with surface proteins. Proteinaceous residues not associated with mucopeptide have been demonstrated at the cell wall of *S. epidermidis* (19). In capsules of *S. epidermidis*, which are mainly composed of polysaccharides (23), proteins have also been observed as spikelike structures protruding from the cell wall (47).

Christensen et al. (10) suggested that slime-mediated adherence may be a critical factor in the pathogenesis of *S. epidermidis* infections on medical devices. Precoating of FEP films with EP prepared from *S. epidermidis* did not promote bacterial adherence but resulted in a significant reduction of the number of adhering bacteria. This finding is in agreement with that of Pringle et al. (38), who reported that nonspecific adherence of *Pseudomonas fluorescens* to hydrophobic solid surfaces was inhibited by extracellular bacterial slime. To study the effect of slime on bacterial adherence to FEP, we also studied the adherence of nonwashed, slime-producing strains grown in TSB and GF-TSB. No differences in the adherence of washed and nonwashed bacteria to FEP could be demonstrated. Apparently, the amount of slime present in suspensions of nonwashed bacteria was not sufficient to affect bacterial adherence to FEP, because addition of EP to bacterial suspensions resulted in a reduction of bacterial adherence.

Morphological studies by SEM and TEM may give insight into structures involved in bacterial adherence to biomaterials. However, classical preparation methods for SEM and TEM have disadvantages such as shrinking of biological structures due to fixation, dehydration, and embedding procedures (39). Foot processes of CNS adhering to intravascular catheters observed by Franson et al. (16) with SEM should be considered an artifact due to such procedures. Freeze-etch replicas of a nonencapsulated CNS strain adhering to FEP revealed a smooth, flattened contact area at the bacterial surface. This contact area may be the result of a partial rearrangement of an external, irregular proteinaceous layer at the bacterial surface (39) upon bacterial adherence to FEP. Such a layer probably covers peptidoglycan, which is partly arranged in concentric, circular structures in the cell walls of young staphylococci (1).

The mechanism of in vivo adherence of CNS to biomaterials has yet to be defined, taking protein adsorption from body fluids onto bacterial and biomaterial surfaces into account.

ACKNOWLEDGMENTS

Discussions with I. Molenaar and the technical assistance of F. Dijk (Center for Medical Electron Microscopy, University of Groningen, The Netherlands) are gratefully acknowledged.

This study was supported by a grant from the Netherlands Organization for the Advancement of Pure Research (Z.W.O.), the Hague.

LITERATURE CITED

1. **Amako, K., A. Umeda, and K. Murata.** 1982. Arrangement of peptidoglycan in the cell wall of *Staphylococcus* spp. J. Bacteriol. **150**:844–850.
2. **Anderson, J. C., and C. D. Wilson.** 1981. Encapsulated, coagulase-negative strain of *Staphylococcus simulans*. Infect. Immun. **33**:304–308.
3. **Baddour, L. M., G. D. Christensen, M. G. Hester, and A. L. Bisno.** 1984. Production of experimental endocarditis by coagulase-negative staphylococci: variability in species virulence. J. Infect. Dis. **150**:721–727.
4. **Bandyk, D. F., G. A. Bermi, B. L. Thiele, and J. B. Towne.** 1984. Aortofemoral graft infections due to *Staphylococcus epidermidis*. Arch. Surg. **119**:102–108.
5. **Bayston, R.** 1984. A model of catheter colonization *in vitro* and its relationship to clinical catheter infections. J. Infect. **9**:271–276.
6. **Blumenkrantz, N., and G. Asboe-Hansen.** 1973. New method for quantitative determination of uronic acids. Anal. Biochem. **54**:484–489.
7. **Caputy, G. G., and J. W. Costerton.** 1982. Morphological examination of the glycocalyces of *Staphylococcus aureus* strains Wiley and Smith. Infect. Immun. **36**:759–767.
8. **Choo, M. H., D. R. Holmes, B. J. Gersh, J. D. Maloney, J. Meredith, J. R. Pluth, and J. Trusty.** 1981. Permanent pacemaker infections: characterization and management. Am. J. Cardiol. **48**:559–564.
9. **Christensen, G. D., A. L. Bisno, J. T. Parisi, B. McLaughlin, M. B. Hester, and R. W. Luther.** 1982. Nosocomial septicemia due to multiply antibiotic-resistant *Staphylococcus epidermidis*. Ann. Intern. Med. **96**:1–10.
10. **Christensen, G. D., W. A. Simpson, A. L. Bisno, and E. H. Beachey.** 1982. Adherence of slime-producing strains of *Staphylococcus epidermidis* to smooth surfaces. Infect. Immun. **37**:318–326.
11. **Christensen, G. D., W. A. Simpson, A. L. Bisno, and E. H. Beachey.** 1983. Experimental foreign body infections in mice challenged with slime-producing *Staphylococcus epidermidis*. Infect. Immun. **40**:407–410.
12. **Duguid, J. P.** 1951. The demonstration of bacterial capsules and slime. J. Pathol. Bacteriol. **63**:673–685.
13. **Ekstedt, R. D., and J. M. Bernhard.** 1973. Preparation and characterization of a slime layer material produced by *Staphylococcus aureus*. Proc. Soc. Exp. Biol. Med. **142**:86–91.
14. **Fisher, R. A., and F. Yates.** 1957. Statistical methods for biological agricultural and medical research. Oliver and Boyd, Edinburgh.
15. **Fletcher, M.** 1980. Adherence of marine micro-organisms to smooth surfaces, p. 345–374. *In* E. H. Beachey (ed.), Bacterial adherence. Receptors and recognition, series B, vol. 6. Chapman & Hall, Ltd., London.
16. **Franson, T. R., N. K. Sheth, H. D. Rose, and P. G. Sohnle.** 1984. Scanning electron microscopy of bacteria adherent to intravascular catheters. J. Clin. Microbiol. **20**:500–505.
17. **Fulginity, V. A.** 1984. *Staphylococcus epidermidis* septicemia in children: an emerging and difficult problem. J. Am. Med. Assoc. **252**:1054.
18. **Gristina, A. G., and J. W. Costerton.** 1984. Bacterial adherence to biomaterials: the clinical significance of its role in sepsis. Biomaterials Trans. **7**:175.
19. **Grov, A., and S. M. Helgeland.** 1971. Immunochemical characterization of *Staphylococcus epidermidis* and *Micrococcus* cell walls. Acta Pathol. Microbiol. Scand. Sect. B **79**:812–818.
20. **Hogt, A. H., J. Dankert, J. A. de Vries, and J. Feijen.** 1983. Adhesion of coagulase-negative staphylococci to biomaterials. J. Gen. Microbiol. **129**:2959–2968.
21. **Hogt, A. H., J. Dankert, and J. Feijen.** 1983. Encapsulation, slime production and surface hydrophobicity of coagulase-negative staphylococci. FEMS Microbiol. Lett. **18**:211–215.
22. **Hogt, A. H., J. Dankert, and J. Feijen.** 1985. Adhesion of *Staphylococcus epidermidis* and *Staphylococcus saprophyticus* onto a hydrophobic biomaterial. J. Gen. Microbiol. **131**:2485–2491.
23. **Ichiman, Y., and K. Yoshida.** 1981. The relationship of capsular-type of *Staphylococcus epidermidis* to virulence and induction of resistance in the mouse. J. Appl. Bacteriol. **51**:229–241.
24. **Inman, R. D., K. V. Gallegos, B. D. Brause, P. B. Redecha, and C. L. Christensen.** 1984. Clinical and microbial features of prosthetic joint infection. Am. J. Med. **77**:47–53.
25. **Itzhaki, R. F., and D. M. Gill.** 1964. A micro-biuret method for

estimating proteins. Anal. Biochem. **9**:401–410.
26. **Karchmer, A. W., G. L. Archer, and W. E. Dismukes.** 1983. *Staphylococcus epidermidis* causing prosthetic valve endocarditis: microbiology and clinical observations as guides to therapy. Ann. Intern. Med. **98**:447-455.
27. **Kloos, W. E., and K. H. Schleifer.** 1975. Simplified scheme for routine identification of human *Staphylococcus* species. J. Clin. Microbiol. **1**:82–88.
28. **Locci, R., G. Peters, and G. Pulverer.** 1981. Microbial colonization of prosthetic devices. III. Adhesion of staphylococci to lumina of intravenous catheters perfused with bacterial suspensions. Zentralbl. Bakteriol. Abt. I Orig. B Hyg. Krankenhaushyg. Betriebshyg. Praev. Med. **173**:300–307.
29. **Lowy, F. D., and S. M. Hammer.** 1983. Staphylococcus *epidermidis* infections. Ann. Intern. Med. **99**:834–839.
30. **Minagi, S., Y. Miyake, K. Inagaki, H. Tsuru, and H. Suginaka.** 1985. Hydrophobic interaction in *Candida albicans* and *Candida tropicalis* adherence to various denture base resin materials. Infect. Immun. **47**:11–14.
31. **Ofek, I., E. Whitnack, and E. H. Beachey.** 1983. Hydrophobic interactions of group A streptococci with hexadecane droplets. J. Bacteriol. **154**:139–145.
32. **Olsson, J., and G. Westergren.** 1982. Hydrophobic surface properties of oral streptococci. FEMS Microbiol. Lett. **15**:319–323.
33. **Peters, G., R. Locci, and G. Pulverer.** 1981. Microbial colonization of prosthetic devices II. Scanning electron microscopy of naturally infected intravenous catheters. Zentralbl. Bakteriol. Abt. 1 Orig. B Hyg. Krankenhaushyg. Betriebshyg. Praev. Med. **173**:293–299.
34. **Peters, G., R. Locci, and G. Pulverer.** 1982. Adherence and growth of coagulase-negative staphylococci on surfaces of intravenous catheters. J. Infect. Dis. **146**:479–482.
35. **Ponce de Leon, S., and R. P. Wenzel.** 1984. Hospital-acquired bloodstream infections with *Staphylococcus epidermidis*. Review of 100 cases. Am. J. Med. **77**:639–644.
36. **Press, O. W., P. G. Ramsey, E. B. Larson, A. Fefer, and R. O. Hickman.** 1984. Hickman catheter infections in patients with malignancies. Medicine **63**:189–200.
37. **Price, E. H.** 1984. *Staphylococcus epidermidis* infections of cerebrospinal fluid shunts. J. Hosp. Infect. **5**:7–17.
38. **Pringle, J. H., M. Fletcher, and D. C. Ellwood.** 1983. Selection of attachment mutants during the continuous culture of *Pseudomonas fluorescens* and relationship between attachment ability and surface composition. J. Gen. Microbiol. **129**:2557–2569.
39. **Reynolds, R. E.** 1982. The bacterial cell: major structures, p. 43–98. *In* J. Mandelstam, K. McQuillen, and I. Dawes (ed.), Biochemistry of bacterial growth, 3rd ed., Blackwell Scientific Publications, Ltd., Oxford.
40. **Rosenberg, M.** 1984. Bacterial adherence to hydrocarbons: a useful technique for studying cell surface hydrophobicity. FEMS Microbiol. Lett. **22**:289–295.
41. **Rosenberg, M., D. Gutnick, and E. Rosenberg.** 1980. Adherence of bacteria to hydrocarbons: a simple method for measuring cell surface hydrophobicity. FEMS Microbiol. Lett. **9**:29–33.
42. **Rosenberg, E., N. Kaplan, O. Pines, M. Rosenberg, and D. Gutnick.** 1983. Capsular polysaccharides interfere with adherence of *Acinetobacter calcoaceticus* to hydrocarbon. FEMS Microbiol. Lett. **17**:157–160.
43. **Schulman, J. H., and J. Leja.** 1954. Control of contact angles at the oil-water-solid interfaces. Emulsions stablilized by solid particles ($BaSO_4$). Trans. Faraday Soc. **50**:598–604.
44. **Wade, J. C., S. C. Schimpff, K. A. Newman, and P. H. Wiernik.** 1982. *Staphylococcus epidermidis*: an increasing cause of infection in patients with granulocytopenia. Ann. Intern. Med. **97**:503–508.
45. **Willison, J. H. M., and A. J. Rowe.** 1980. Freeze-fracturing and freeze-etching, p. 171–244. *In* A. M. Glauert (ed.), Practical methods in electron microscopy, vol. 8. Elsevier/North-Holland Publishing Co., Amsterdam.
46. **Yoshida, K., and R. D. Ekstedt.** 1968. Relation of mucoid growth of *Staphylococcus aureus* to clumping factor reaction, morphology in serum-soft agar, and virulence. J. Bacteriol. **96**:902–908.
47. **Yoshida, K., and Y. Minegishi.** 1979. Isolation of an encapsulated strain of *Staphylococcus epidermidis*. J. Appl. Bacteriol. **47**:299–301.