JOURNAL OF BACTERIOLOGY, Sept. 1996, p. 5472–5479 Vol. 178, No. 18
0021-9193/96/$04.00+0

Adhesion of the Positively Charged Bacterium *Stenotrophomonas* (*Xanthomonas*) *maltophilia* 70401 to Glass and Teflon

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Received 7 February 1996/Accepted 2 July 1996

Medical implants are often colonized by bacteria which may cause severe infections. The initial step in the colonization, the adhesion of bacteria to the artificial solid surface, is governed mainly by long-range van der Waals and electrostatic interactions between the solid surface and the bacterial cell. While van der Waals forces are generally attractive, the usually negative charge of bacteria and solid surfaces leads to electrostatic repulsion. We report here on the adhesion of a clinical isolate, *Stenotrophomonas maltophilia* 70401, which is, at physiological pH, positively charged. *S. maltophilia* has an electrophoretic mobility of $+0.3 \cdot 10^{-8}$ m^2 V^{-1} s^{-1} at pH 7 and an overall surface isoelectric point at pH 11. The positive charge probably originates from proteins located in the outer membrane. For this bacterium, both long-range forces involved in adhesion are attractive. Consequently, adhesion of *S. maltophilia* to negatively charged surfaces such as glass and Teflon is much favored compared with the negatively charged bacterium *Pseudomonas putida* mt2. While adhesion of negatively charged bacteria is impeded in media of low ionic strength because of a thick negatively charged diffuse layer, adhesion of *S. maltophilia* was particularly favored in dilute medium. The adhesion efficiencies of *S. maltophilia* at various ionic strengths could be explained in terms of calculated long-range interaction energies between *S. maltophilia* and glass or Teflon.

The colonization of medical implants by microorganisms can hardly be avoided when parts of these implants are exposed to the nonsterile exterior. Bacteria attach to the exterior parts of the implants, migrate along the surfaces into the interior of the body (14), and cause severe infections (5) or destroy the implant. Many such implants like urinary tract catheters and wound drainage and invasive vascular devices (for reviews, see references 6 and 8) have been studied with respect to the microbial populations living in biofilms associated with the surface (23, 28). In general, the initial step of biofilm formation is the adhesion of the microorganisms to surfaces by unspecific physicochemical interactions. These interactions are governed by the particular charges and hydrophobicities of both bacteria and the materials involved (24). Bacteria and most natural and artificial surfaces investigated so far are negatively charged (13, 21, 26, 30, 44). Therefore, adhesion will take place only when the resulting electrostatic repulsion is overcome by attractive forces like, e.g., van der Waals forces or hydrophobic interactions between bacterial surface polymers and the solid surface (24, 34). The deposition of colloidal particles on solid surfaces can be calculated by applying the Derjaguin, Landau, Verwey, and Overbeek (DLVO) theory of colloid stability (19, 36). Such calculation accounts for two forces, the van der Waals attraction and electrostatic interaction. This colloid-model calculation has already been applied successfully to the deposition of bacterial cells on solid surfaces (34).

The charge on the cell surface of a bacterium is usually inferred from its electrokinetic (zeta) potential ζ (32, 45), which, in turn, can be calculated from the mobility u of the bacterium in an electrical field. The charge results from anionic and cationic, e.g., acidic or basic, groups on the cell surface (32). Consequently, the effective charge is influenced by the specific adsorption of ions (32) and strongly depends on the pH and the ionic strength I of the medium. The dependence of u on I is due to the diffuse layer of counterions formed around charged particles. The higher I, the more ions are available to shield and neutralize the charge of the particle. The number of charged acidic or basic groups present in bacterial surface polymers depends on the pH of the medium. At pH values below the whole-cell isoelectric point (*iep*), the net charge of the bacterium is positive. The *iep* of most bacteria, reported so far, is in the range of pH 1.5 to 4.5 (32). An alternative method to investigate the charge of the cell surface is the titration of isolated cell walls or whole cells (43). The dissociation constants of titratable groups can be deduced from the amount of protons or hydroxide ions bound to the cells or cell walls.

Here we report on a clinical isolate, *Stenotrophomonas* (*Xanthomonas*) *maltophilia* 70401 (47), which is positively charged at physiological pH. The strain was isolated from a catheter specimen from a patient with a suspected urinary tract infection (47) and belongs to a genus which is known to be involved in human infections (31). We investigated pH and ionic strength dependencies of u. In order to identify the origin of the positive cell charge, we titrated the whole cells against acid and base to evaluate the equivalence points of titration of the functional groups on the cell surface. In adhesion experiments performed with two model surfaces (glass and Teflon), we showed that the positive charge of *S. maltophilia* considerably promoted its adhesion.

MATERIALS AND METHODS

Glossary. The following terms and abbreviations are used in this paper: A_b, area of a longitudinal cell section (in square meters); A_{col}, surface area present in the porous medium column (in square meters); B, blocking factor (dimensionless); c, effluent cell concentration (per milliliter); c_0, inflow cell concentration (per milliliter); $G_{DLVO}(h)$, DLVO interaction energy (in Boltzmann constant [joules per kelvin] · temperature [in kelvins]); h, distance between solid surface and bacterium (in meters); I, ionic strength of the medium (in moles per liter); *iep*, isoelectric point (in pH units); u, electrophoretic mobility (in square

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meters per volt per second); α_t, adhesion efficiency at time t (dimensionless); α_0, clean bed adhesion efficiency (dimensionless); Γ, number of deposited cells (per square meter); η_t, rate of particle deposition at time t (dimensionless); η_{trans}, particle transport rate (dimensionless); Θ, fraction of the collector surface covered with cells (in percent); θ_w, contact angle (in degrees); ζ, zeta potential of a particle (in volts).

Chemicals and buffers. All chemicals were purchased from Fluka Chemie AG, Buchs, Switzerland. Aqueous media were made with deionized water (NANOpure Cartridge System, SKAN AG, Basel, Switzerland). Phosphate-buffered saline (PBS) solutions of various I values were prepared by diluting a stock solution (I = 1 M) containing 49.3 g of NaCl, 2.9 g of KH_2PO_4, and 11.9 g of K_2HPO_4 liter^{-1}. Solutions of various pHs were prepared by mixing appropriate volumes of 1 M HNO_3, 1 M KNO_3, and 1 M KOH solutions and then diluting with deionized water and verifying the correct pHs. Titrants for proton titrations were prepared by diluting Titrisol (Merck, Darmstadt, Germany) to 0.1 or 1 M HNO_3 or KOH, respectively.

Bacteria and culture conditions. *S. maltophilia* 70401, a gift from A. Winn (University of Hull, Hull, United Kingdom), was cultivated on nutrient broth (Labo-Life, Pully, Switzerland) (8 g liter^{-1}) as described in reference 47. *Pseudomonas putida* mt2 (46) was cultivated in the mineral medium described by Schraa et al. (37), but without addition of yeast extract. Ethanol (50 mM) was the sole source of carbon and energy (33). The cultures were incubated in 300-ml Erlenmeyer flasks containing 100 ml of medium on a rotary shaker at 30°C, harvested in the late exponential phase, and washed three times either in cold (4°C) 10 mM PBS or in 10 mM KNO_3 solution. Finally, the cells were resuspended in 5 ml of PBS or KNO_3 solution, stored on ice, and used within 4 h. The cells for proton titration experiments were grown in 5-liter flasks containing 3 liters of medium and inoculated with 30 ml of preculture. Aeration was achieved by magnetic stirring at 1,000 rpm. The cells were grown at 30°C until late exponential phase, harvested, and washed three times in distilled water. Finally, they were suspended in KNO_3 solutions of the desired ionic strengths. The dry weight of a cell suspension was determined by weighing after freeze-drying.

Physicochemical characterization of bacteria. The electrophoretic mobility and the hydrophobicities of the bacterial cells were determined according to the method of van Loosdrecht et al. (44, 45). The electrophoretic mobility u was determined by using a Doppler electrophoretic light scattering analyzer (Zetamaster; Malvern Instruments Ltd., Malvern, Worcestershire, United Kingdom) with an application of 100 V. Measurements were performed either in 10 mM KNO_3 solutions of various pHs or in PBS of various I at pH 7.2. The zeta potential ζ was calculated from the electrophoretic mobility according to the method of Helmholtz-Smoluchowski (17). The bacterial surfaces for measurements of cell surface hydrophobicities were prepared by suspending the cells in 10 mM PBS and collecting them on 0.45-μm-pore-size Micropore filters (Schleicher & Schuell, Dassel, Germany). The filters were mounted on glass slides and dried for 2 h at room temperature. The cell surface hydrophobicities were inferred from the contact angles θ_w of drops of water on the lawns of bacteria. Contact angle measurements were done with a microscope equipped with a goniometric eye piece (Krüss GmbH, Hamburg, Germany) (44). The effective radii of the bacteria were calculated from the average cell width and length determined for about 100 cells under a light microscope with a video camera. The area of a longitudinal cell section was assumed to be equal to the area of the solid surface covered by one attached cell. The number of cells per milliliter per optical density unit at 280 nm was determined by counting them under the light microscope in a Thoma counting chamber. The determination of the elemental surface composition of bacteria by X-ray photoelectron spectroscopy (XPS) was performed by Henny Van der Mei with an X-ray photoelectron spectrometer (S-Probe; Surface Science Instruments, Mountain View, Calif.) (as described previously [41]). The cell surfaces of *S. maltophilia* and *P. putida* were studied by scanning electron microscopy. Samples were prepared by a deep-etching technique.

Extraction of lipopolysaccharides. Lipopolysaccharides were extracted with a method described by Leive, which has been found to yield 30 to 50% of the lipopolysaccharides of an *Escherichia coli* strain (20). Freshly harvested and washed cells were incubated in 50 ml of Tris buffer (200 mM, pH 8.0) containing 1 mmol of EDTA · liter^{-1} at 4°C. After 30 min, the extract was centrifuged and the supernatant was dialyzed against deionized water and lyophilized. The lipopolysaccharide content was quantified by a carbocyanin dye assay (9). Protein impurities were quantified with a protein assay kit (Bio-Rad, Glattbrugg, Switzerland). The lipopolysaccharide extract of *S. maltophilia* contained less than 2% (wt/wt) protein.

Potentiometric proton titration of bacteria. The bacteria were titrated according to the method of Van der Wal et al. (43). Freshly harvested cells (0.2 to 0.4 g [dry weight]) were suspended in 25 ml of KNO_3 of various ionic strengths. The titrations were performed at 25°C with an automatic titration system (Metrohm AG, Herisau, Switzerland) and a pH glass electrode (Metrohm AG) calibrated in the pH range of 2 to 12. Samples were flushed with nitrogen. As controls, titrations without cells were performed under the same conditions. The amounts of protons and hydroxide ions binding to the bacteria at a given pH were calculated by subtracting the amounts of titrant needed for the control titrations. Calculations were corrected for volume changes. For titrations at $I \geq 0.1$ M, 1 M HNO_3 or 1 M KOH was used as a titrant, whereas at $I \leq 0.01$ M, 0.1 M HNO_3 and 0.1 M KOH were used. The titrations were performed between pH 2 and 12 or pH 3 and 11 at high and low I, respectively, or until the cells began to lyse. To restrict the titration to mainly the outermost parts of the cell walls, the titrations were performed within 15 min, with a change of about 1 pH unit in 2 to 3 min (43). The penetration of protons into the cell interior of gram-positive cells during fast proton titrations of about 2 μmol min^{-1} · g^{-1} (dry weight) is negligible on the time scale of fast proton titrations (43). To study the influence of the penetration depth, titrations were also performed in 5, 20, and 90 min at I = 0.001 mol · liter^{-1}. The volume of titrant added was less than 10% of the total volume.

Solid surfaces. Hydrophobic Teflon 350 (a copolymer of perfluoroalkoxyheptafluoropropylene and polytetrafluoroethylene) with a contact angle θ_w of 115 ± 2° and hydrophilic glass with a θ_w of 12 ± 2° were used as model surfaces to study bacterial adhesion. Teflon granules with an average diameter of 375 μm were obtained from Dolder AG (Basel, Switzerland). Glass beads with diameters of 450 ± 50 μm were purchased from Roth AG (Reinach, Switzerland). Both materials were cleaned by submerging them in chromosulfuric acid for 24 h at 60°C and rinsing first with 0.5 M KCl and then with deionized water. Finally, they were air dried and stored in glass containers until they were used.

Adhesion experiments. Adhesion experiments were performed in columns according to the method described by Rijnaarts et al. (33). Results of adhesion experiments conducted in columns have been shown to correspond to those adhesion results obtained in batch experiments (34). Glass columns (internal diameter, 1.0 cm; length, 9.5 cm) were filled with either glass or Teflon beads (33). The packings had a length of 9.3 ± 0.2 cm. The water-to-bed-volume ratios (porosities) of 0.34 ± 0.01 for glass and 0.33 ± 0.02 for Teflon were estimated from breakthrough curves, with chloride as a conservative tracer. Chloride concentrations were measured with a Microchlorocounter (Marius, Utrecht, The Netherlands). The pore volumes, which equal the volumes of liquid in the columns, were 2.54 and 2.46 ml per column for glass and Teflon columns, respectively. The influent was supplied to the vertical downflow columns by a peristaltic pump. The flow rate was kept constant for each column but varied from 70 to 80 cm h^{-1} between individual columns. Concentrated bacterial suspensions were diluted with PBS of the desired ionic strength to give concentrations c_0 of A_{280} = 0.6 ± 0.05, corresponding to about 2.0×10^8 cells of *P. putida* ml^{-1} and 1.1×10^8 cells of *S. maltophilia* ml^{-1}. The suspensions were applied to the columns for 90 min, during which the effluent concentration c was determined every 5 min. The influent concentration c_0 remained constant during the whole experiment. Experiments with each combination of strains and materials were performed simultaneously in triplicate at four ionic strengths (1 mM, 10 mM, 100 mM, and 1 M). The experiments at I = 1 M with *P. putida* were not feasible, since *P. putida* did not withstand high ionic strengths of 1 M over a longer time period as needed for transport experiments. *P. putida* obviously released cell material which affected transport experiments.

Calculation of adhesion efficiencies. The efficiency of adhesion of bacteria α_t in porous media upon collision (11) is the ratio of the dimensionless rate of particle deposition η_t and the dimensionless rate of particle transport (η_{trans}).

$$\alpha_t = \frac{\eta_t}{\eta_{trans}} \tag{1}$$

α_t is governed by chemical-colloidal forces like electrostatic and hydrophobic interactions and by interactions between the bacterial surface polymers and the solid surface. In the absence of either steric hindrance by surface polymers or electrostatic repulsion, $\eta_t = \eta_{trans}$ and α_t reaches unity. In the presence of repulsive forces, α_t falls below 1. η_t was obtained from experimental data by doing the mass balance over the cells retained by the column system and those eluted (10). η_{trans} was calculated according to the methods of Martin et al. (25) and Rijnaarts (32). Calculations included contributions of convection, diffusion, van der Waals attraction, sedimentation, and direct interception of cells.

Calculation of blocking factor *B*. An adhered cell may block a certain area of the free surface and prevent adhesion of approaching suspended cells (32). The blocking factor B is defined as the ratio of the blocked area around the attached cell to the cross-sectional area of that cell and was calculated by solving equation 2 for B (32):

$$\alpha_t = \alpha_0 (1 - B\Theta) \tag{2}$$

with $\Theta = \Gamma A_b / A_{col} \cdot \alpha_0$ is the initial adhesion efficiency at $\Theta = 0$.

Calculation of interaction forces between bacteria and solid surfaces. The interaction energies $G_{DLVO}(h)$ between the bacterial surfaces and the solid surfaces were calculated according to the DLVO theory of colloidal stability (19, 36). $G_{DLVO}(h)$ is the energy resulting from the sum of van der Waals and electrostatic interactions. The van der Waals attraction is proportional to the Hamaker constant for the interaction between the involved surfaces. Hamaker constants were taken from the work of Nir (29) and Rijnaarts et al. (34). The electrostatic interaction of negatively charged solid surfaces is repulsive with negatively charged bacteria and attractive with positively charged bacteria. Electrostatic interactions depend on the electrical potentials of the surfaces. For general estimations, the electrokinetic potentials ζ can be used instead of electric potentials (36). It was assumed that the plane of shear corresponds to a flat surface and that the charge is located on the plane of shear. It should be noted that the distance h between a flat solid surface and the plane of shear of a bacterial cell is a very approximate value, especially when $h < 10$ nm.

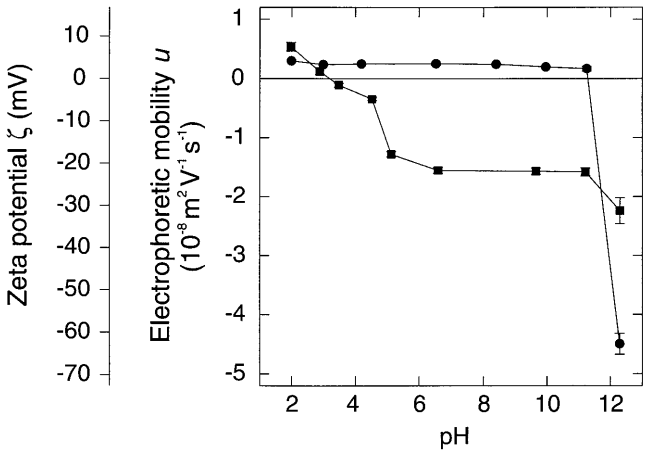

FIG. 1. Electrophoretic mobilities u and zeta potentials ζ of *S. maltophilia* (●) and *P. putida* (■) as a function of the pH. Error bars indicate standard deviations from the means of 10 measurements. (The errors are in most cases smaller than the extension of the datum points.)

RESULTS

Physicochemical characteristics of bacteria. The *iep* values of *P. putida* and *S. maltophilia* were at pH 3.2 and above pH 11, respectively. Positive ζ values of *S. maltophilia* were constant over a wide pH range and dropped to highly negative values above the *iep* (Fig. 1). ζ of *S. maltophilia* became negative at $I > 0.05$ M, whereas ζ of *P. putida* approached zero at high I (Fig. 2). θ_w of *S. maltophilia* and *P. putida* were $19 \pm 2°$ and $40 \pm 2°$, respectively. According to the classification of Bendinger et al. (1), *S. maltophilia* is hydrophilic, whereas *P. putida* is moderately hydrophobic.

Chemical and microscopic characterization of cell surfaces. The elemental surface composition as determined by XPS was N/C = 0.063, O/C = 0.235, and P/C = 0.005 for *S. maltophilia* and N/C = 0.028, O/C = 0.153, and P/C = 0.007 for *P. putida*. The higher N/C ratio may indicate a higher protein content in the cell surface of *S. maltophilia* compared with *P. putida*. Fast titrations of whole cells were performed to characterize acidic and basic cell surface constituents. pH regions in which such functional groups are titrated can be inferred from inflection points in the titration curves. The corresponding equivalence points appear as maxima in reciprocal differential titration curves (43) (Fig. 3). For *S. maltophilia* 70401, a maximum was found at pH 7.5 when I was 0.1 M, with a shift to pH 8.3 at I = 0.001 M (Fig. 3A). This shift is due to the increased surface charge at lower ionic strength affecting the equivalence point (43). The position of this maximum probably indicates the presence of proteins on the cell surface. Free amino groups with high pK_a values are present in lysin and arginin (pK_a 10.8 and 12.5, respectively) and terminal amino groups of proteins (pK_a 7.8) (38). No maxima or regions of high values for δ pH/δ millimole of H^+ were found in the acidic pH range, indicating the absence of carboxylic or phosphate groups (35). The appearance of a small maximum in the range of pH 11 was accompanied by the lysis of cells. In contrast, *P. putida* mt2 exhibited titratable groups in the range from pH 3 to 6, indicating the presence of acidic polysaccharides and/or phosphate (Fig. 3B). Groups with equivalence points above pH 7 may be due to the presence of either proteins or phosphate groups. The absence of distinct maxima can be explained by the presence of a multitude of different functional groups in the cell surface, which are continuously titrated over the pH range from 3 to 10. Scanning electron micrographs of the outermost cell surfaces of both strains showed no regular patterns, indicating the absence of protein surface layers (micrographs not shown).

Deposition of *S. maltophilia* 70401 and *P. putida* mt2 on glass and Teflon. The deposition of *S. maltophilia* onto glass and Teflon increased with decreasing I (Fig. 4A and B). Values of c/c_0 after initial breakthrough ranged from 0.4 (1 mM) to 0.9 (1 M) with glass (Fig. 4A), whereas c/c_0 values with Teflon were in the range of 0.02 at 1 mM $\leq I \leq$ 100 mM and 0.15 at I = 1 M, respectively (Fig. 4B). The increases of c/c_0 values during filtration at 1 mM $\leq I \leq$ 100 mM were ascribed to substratum blocking by attached cells. B of *S. maltophilia* on Teflon ranged from below 1 at $I \leq 0.01$ to 19 at I = 0.1 M. This indicates the absence of repulsion between attached cells and those approaching the collector surface at low I and considerable repulsion at high I. The rapid increase of c/c_0 at I = 1 M is more likely due to a progressive contamination of the collector surfaces, in particular those of hydrophobic Teflon, by surface active cell constituents released by cell lysis or as a reaction to the osmotic shock (2). Breakthrough curves of *P. putida* with both materials were affected in an opposite way by I (Fig. 4C and D). Values of c/c_0 ranged between 0.5 (100 mM) and 0.9 (1 mM) with glass and between 0.15 (0.1 M) and 0.33 (1 mM) with Teflon. B was below 1 at all conditions, indicating insignificant repulsion between cells. By using the theoretical rates η_{trans} of transport of both bacterial strains to the collector surfaces, the adhesion efficiencies could be calculated from the breakthrough curves. Values of α_0 of *S. maltophilia* with glass and of *P. putida* with both materials were below 1, indicating that only part of the bacteria actually adhered upon reaching the surfaces (Fig. 5). In contrast, α_0 of *S. maltophilia* on Teflon at $I \leq 0.1$ M reached unity. Values of α_0 up to 1.2 were considered acceptable because of possible inaccuracies in the calculation of η_{trans}.

Calculation of long-range physicochemical forces between bacteria and either glass or Teflon. The physicochemical characteristics of both bacterial strains were used to calculate G_{DL} as a function of the distance between the bacteria and the solid surfaces and the ionic strength. As an example, Fig. 6 shows the results for I = 10 mM. $G_{DLVO}(h)$ values for *S. maltophilia*

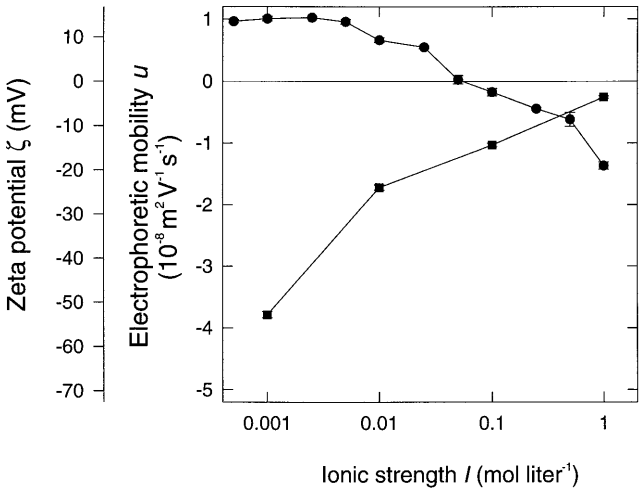

FIG. 2. Electrophoretic mobilities u and zeta potentials ζ of *S. maltophilia* (●) and *P. putida* (■) at various ionic strengths at pH 7.0. Standard deviations are indicated by error bars. (The errors are in most cases smaller than the extension of the datum points.)

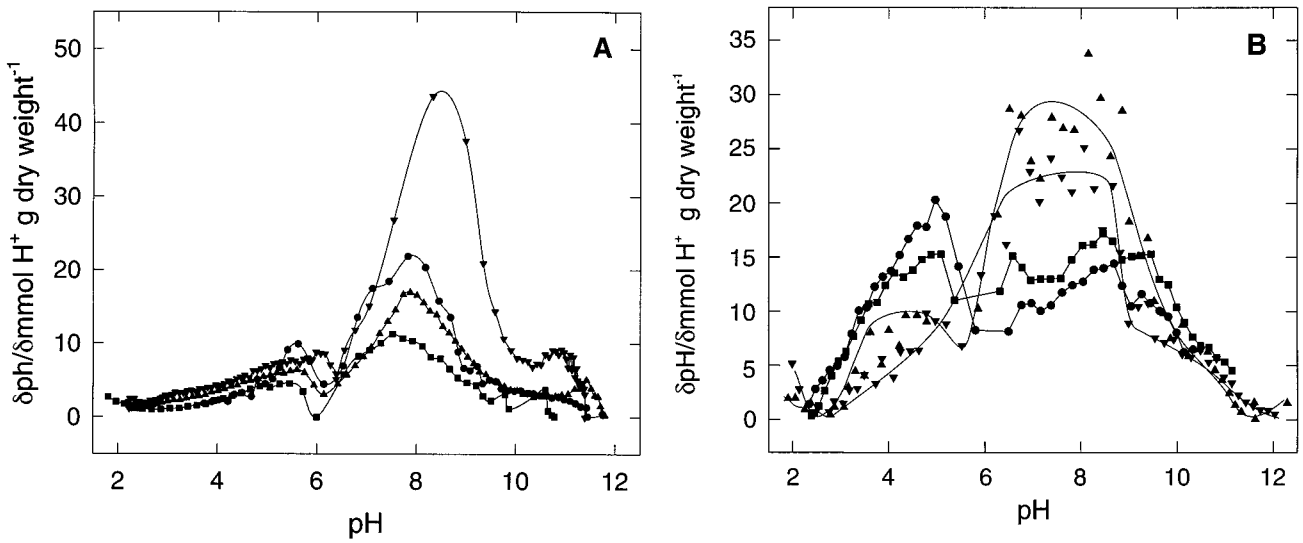

FIG. 3. First derivative of titrations conducted at ionic strengths of 1 (●), 0.1 (■), 0.01 (▲), and 0.001 M (▼). Experiments were performed with *S. maltophilia* (A) and *P. putida* (B).

and both surfaces were negative, and the resulting forces were attractive at all *h* (Fig. 6A). This is due to the electrostatic attraction resulting from the positive charge of *S. maltophilia*. The attractive forces generally ranged about 5 nm (glass) and 25 nm (Teflon) from the surface into the liquid. *P. putida* was repulsed by glass and Teflon (Fig. 6B). With glass, a secondary energy minimum was present at a distance of 7 nm from the surface. The energy barrier of 232 *kT* (*k* is Boltzmann constant [in joules per kelvin] and *T* is temperature [in kelvins]) between the primary and the secondary minimum is too high to be overcome by the cell and thus prevents the direct contact between the bacterium and the solid surface (34).

DISCUSSION

The *iep* of *S. maltophilia* 70401 is around pH 11. The cells are positively charged at physiological pH. The high *iep* of *S. maltophilia* differs from bacterial *iep* values found so far, which are usually below pH 5 (35). Proton titrations show that titratable groups with equivalence points above 7 were present on the cell surface, whereas no distinct titratable groups were detected below pH 7. This indicates that proteins were responsible for the positive charge of *S. maltophilia*. In contrast, the negatively charged *P. putida* mt2 possessed titratable groups over the entire pH range. The composition of the repeating unit of the polysaccharide residue of lipopolysaccharides from *S. maltophilia* had been elucidated previously (47):

→3)-β-L-Rha*p*-(1→4)-α-L-Rha*p*-(1→3)-α-D-Fuc*p*-(1→
3
↑
α-D-Glc*p*1

The repeating unit lacks functional groups which could account for the positive cell charge. As a consequence, further positively charged components must be present in the cell envelope. We tried to identify the location of the positive charges by doing titrations at different rates to achieve various penetration depths of the protons (43). The faster the titration was performed, the more dominant the maximum of the first derivative of the titration curve above pH 7 was (data not shown). However, a lipopolysaccharide extract which comprises material from the outer monolayer of the outer membrane contained only traces of proteins. These observations indicate that the proteins responsible for the positive cell charge are probably located in the lipid bilayer of the outer membrane. The ratios of N/C and O/C of *S. maltophilia* and *P. putida* were relatively low compared with XPS data collected by Cowan et al. (7), which ranged from 0.05 to 0.14 for N/C and from 0.2 to 0.35 for the O/C ratio. This fact is in accordance with the low absolute charges of both strains. The relatively low N/C ratio of *S. maltophilia* indicates a low protein content of the cell surface. This is in agreement with the electron microscopic view that revealed the absence of a closed protein surface layer and the fact that only traces of lipopolysaccharide-associated proteins were found. It therefore seems that the positive charge of *S. maltophilia* is due mainly to the absence of negatively charged groups, rather than being a consequence of an uncommon accumulation of positive charges. Already, a low protein concentration in the outer membrane can thus be responsible for the slight positive charge. Makin and Beveridge (22) showed that charged lipopolysaccharides may shield charges present in deeper cell wall layers. The lipopolysaccharides from *S. maltophilia* are uncharged (47) and therefore not able to shield charges present in the outer membrane.

The structure of the polysaccharide repeating unit of *S. maltophilia* is similar to that of a polysaccharide that has been isolated from a *Rhodococcus* strain (27), which contained glucuronic acid, galactose, fucose, and rhamnose in a molar ratio of 1:1:1:2. Neu and Poralla (27) suspected the desoxymonosaccharides fucose and rhamnose of causing the considerable hydrophobicity of this strain. This is in contrast to the extreme hydrophilicity of *S. maltophilia* ($\theta_w = 19°$).

The positive charge of *S. maltophilia* favors its adhesion to negatively charged surfaces. In the case of Teflon, all *S. maltophilia* cells approaching the collector surface attached to it and adhesion was governed exclusively by the transport of the cells to the Teflon surface. When the electrostatic attraction was neutralized by high ion concentrations, considerably fewer cells adhered to the Teflon material. This is in agreement with the observation that high ion concentrations changed the cell charge to slightly negative (Fig. 2) and the fact that the van der

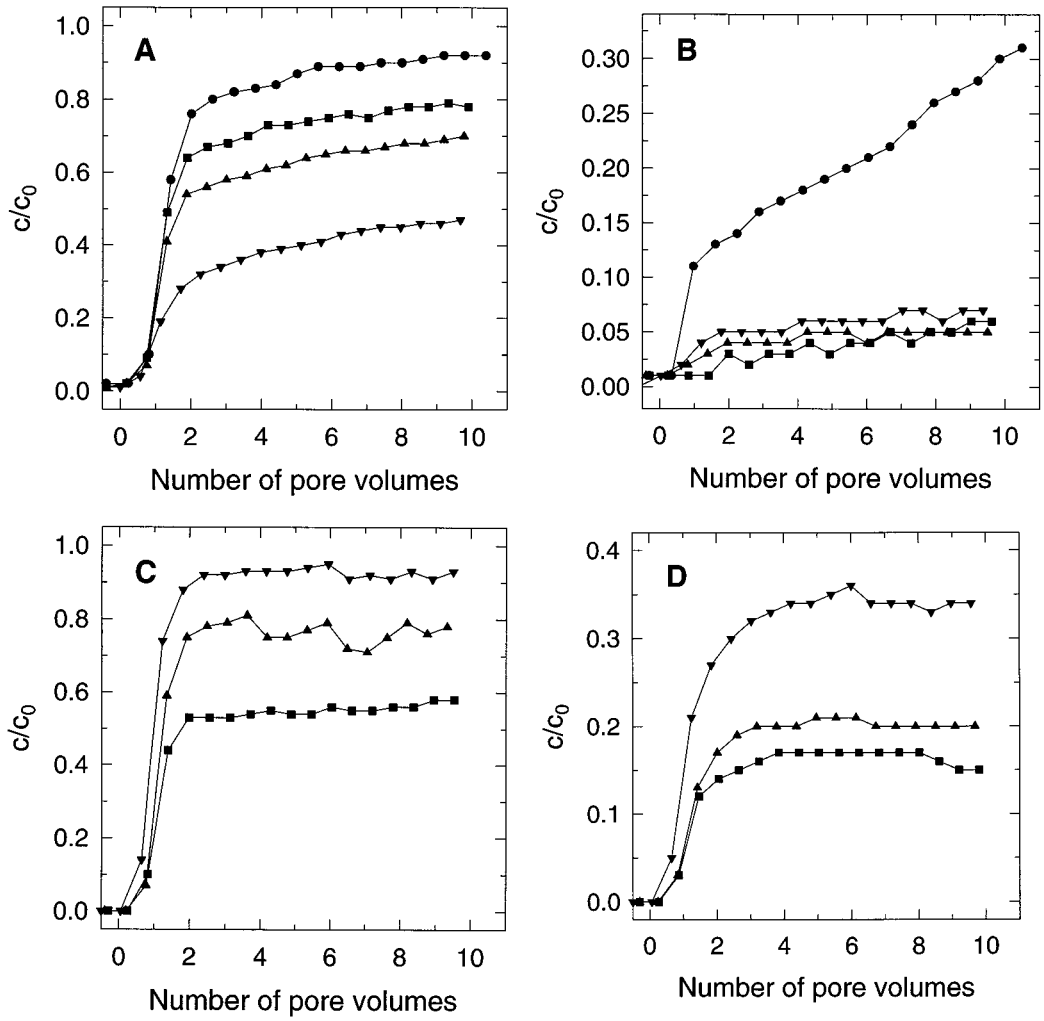

FIG. 4. Breakthrough curves of column experiments conducted at ionic strengths of 1 (●), 0.1 (■), 0.01 (▲), and 0.001 M (▼). Experiments were performed with *S. maltophilia* (A and B) and *P. putida* (C and D) on glass (A and C) and Teflon (B and D).

Waals forces arising from Teflon are negligible. The adhesion of *S. maltophilia* to glass was generally lower than that to Teflon and continuously decreased with increasing *I*. α_0 values were below 0.5 at all *I* (Fig. 5). The adhesion is thus much lower than could be expected from the calculated interaction curve in Fig. 6A. The reduced adhesion efficiency indicates a cell repulsion due to steric hindrance by bacterial surface polymers (35). *P. putida* efficiently adhered to Teflon although the long-range forces of interaction with Teflon were repulsive. The electrostatic repulsion might have been overcome by bacterial surface polymers bridging the distance between the bacteria and the surface (32). The low adhesion efficiency of *P. putida* with glass at $I \leq 0.01$ is due to high energy barriers arising from the electrostatic repulsion (Fig. 6B) probably in combination with unfavorable polymer binding to the glass surface. The latter assumption is supported by the limited adhesion even at high *I*, where the sum of both long-range forces was attractive.

Harkes et al. (16) investigated the adhesion of negatively charged *E. coli* strains on a positively charged copolymer of hydroxyethylmethacrylate and trimethylaminoethylmethacrylate-chloride (pHEMA–pTMAEMA-Cl). This combination of a positively charged solid surface and negatively charged cells also creates attractive electrostatic forces. Accordingly, a much higher adhesion of *E. coli* to the positively charged surface than to negatively charged surfaces was found (16). Hogt et al. (18) suggested that only diffusion, not the adhesion step itself, governed the overall rate of adhesion of negatively charged staphylococci to a positively charged surface (pHEMA–pTMAEMA-Cl). We found that the deposition of *S. maltophilia* on Teflon collectors was indeed exclusively diffusion limited. Although our transport calculations did not account for long-ranging electrostatic attraction, the error introduced by this is small, since the attraction never reached further out than 20 nm from the solid surface (Fig. 6). The influence of *I* on the adhesion of positively charged latex particles was investigated by Elimelech (10). His finding that adhesion was high at low ionic strength and decreased with increased ionic strength confirms our results with *S. maltophilia.*

Thus far, from a total of 156 strains (references 1, 3, 7, 12, 14–16, 33, 39, 40, 42, and 44 and unpublished data) only two positively charged bacterial strains at neutral pH have been described (Fig. 7). A nonencapsulated *Staphylococcus* sp. (39) showed the strange behavior of changing five times the sign of charge between pH 2 and 11. The charge of *Streptococcus thermophilus* (3), a hydrophilic strain ($\theta_w = 27°$), was positive

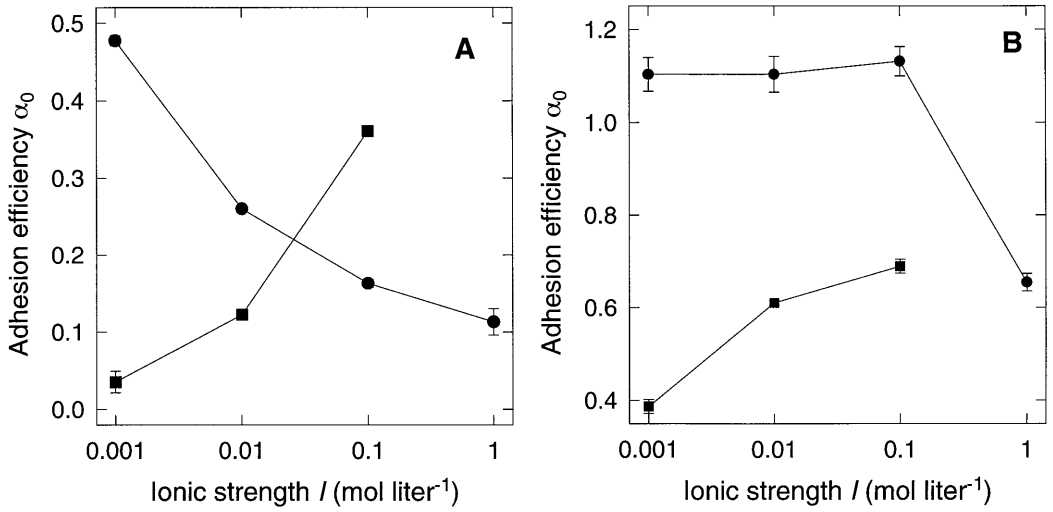

FIG. 5. Bacterial adhesion efficiencies α_0 of *S. maltophilia* (●) and *P. putida* mt2 (■) on glass (A) and Teflon (B) at various ionic strengths. Error bars indicate standard deviations of measurements performed as triplicates. (In some cases, the errors are smaller than the extension of the datum points.)

only in 10 mM potassium phosphate buffer and not in deionized water. This fact was attributed to the specific adsorption of potassium ions onto the cell surface. Moreover, the positive charge increased at higher pH, and no distinct isoelectric point was found. This strain adhered better to the hydrophobic surfaces fluoroethylenepropylene (θ_w = 106°), polypropylene (θ_w = 101°), and polymethylmethacrylate (θ_w = 69°) when suspended in potassium phosphate buffer than when suspended in deionized water. Deposition on glass (θ_w = 12°) was much higher than that on the hydrophobic surfaces, which is in contrast to our finding with *S. maltophilia*. Furthermore, the adhesion onto glass from deionized water, in which the strain was negatively charged, was about as high as that from potassium phosphate buffer, in which the strain was positively charged. This cannot be explained by electrostatic interactions and was attributed to physiological reactions of *S. thermophilus* to the osmotic stress in water (3). All other strains described were distributed over a range between $-4.5 < u < 0 \times 10^{-8}$ m^2 V^{-1} s^{-1} and 15° < θ_w < 120°. Interestingly, bacteria with a high negative charge are all clinical isolates, whereas no isolates from environmental samples like, e.g., soils or aqueous systems with $u < -3.5 \times 10^{-8}$ m^2 V^{-1} s^{-1} have been described yet. It may be that for strains of medical significance which are adapted to body fluids of relatively high ionic strength, usually in the range of 150 mM or even higher, the charge is not as critical as for strains which have their natural habitats in soils, aquifers, or freshwater environments. The ionic strength of solutions in all environmental systems except seawater is usually below 20 mM (32). In such environments, positively charged bacteria could be permanently bound to surfaces, whereas highly negatively charged bacteria could hardly attach to surfaces. These conclusions are based on only a few bacterial strains. However, it seems likely that for the clinical iso-

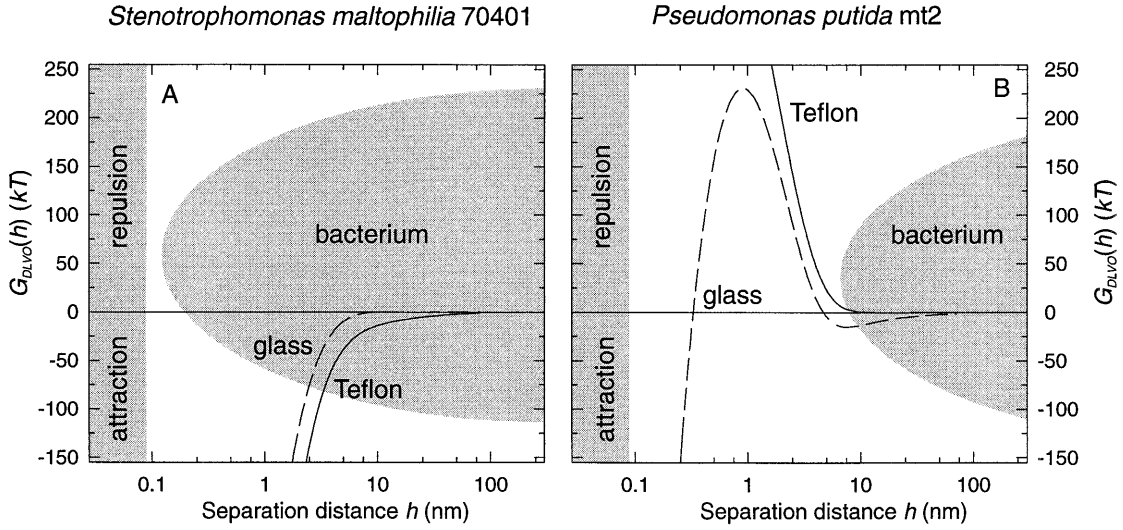

FIG. 6. DLVO interactions between *S. maltophilia* (A) and *P. putida* (B) and either glass (dashed line) or Teflon (solid line). The position of the bacterial cell is indicated schematically.

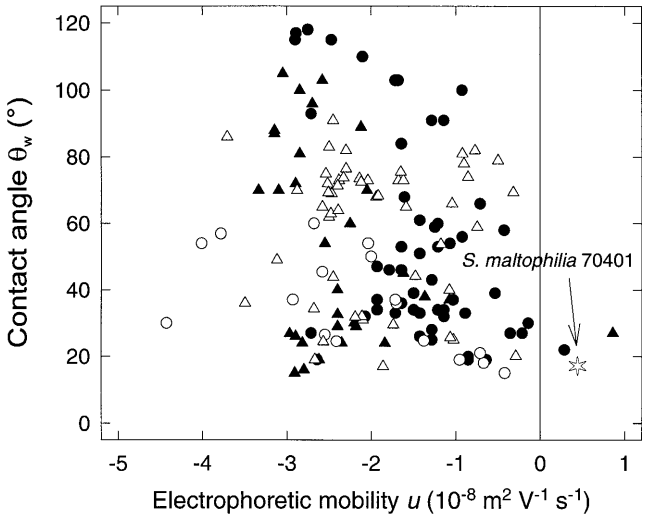

FIG. 7. Contact angles θ_w plotted against the electrophoretic mobilities u of gram-negative (triangles) and gram-positive (circles) clinical isolates (open symbols) and environmental samples (filled symbols). All values for u were measured at $I = 10$ mM (or 8.1 mM [39]) and pH 7.0 to 7.2. The contact angles were measured in either distilled water or at $I = 10$ mM. As buffer, either PBS or potassium phosphate buffer was used.

lates adhesion to body tissues by specific mechanisms is more important than unspecific adhesion.

The wide range of bacterial surface properties as summarized in Fig. 7 makes it difficult to design solid surfaces to which bacteria would not adhere. In addition, any surface will be coated by organic compounds. In nature, there is a vast variety of organic molecules. Body implants will probably be coated by serum or tissue proteins and glycosaminoglycans. Such coatings modify the surface properties and make the control of bacterial adhesion very difficult. It is advisable to accept the fact that colonization of solid surfaces cannot be completely prevented. Where uncontrolled bacterial colonization is detrimental, bactericidal coatings (toxic metals, organometal compounds, antibiotics) are unavoidable. Busscher et al. (4) recently recommended development of materials which establish weak bonding between their surface and the biofilm to achieve detachment of the biofilm at low shear forces. One could also think about coating nonbiological materials with organic layers which easily detach or dissolve. Such self-cleaning mechanisms would prevent the development of undesired, long-term stable biofilms.

ACKNOWLEDGMENTS

We thank Henny Van der Mei from the Laboratory for Materia Technica, University of Groningen, Groningen, The Netherlands, for carrying out the XPS measurements and Ernst Wehrli from the Institute for Microbiology, ETH Zurich, for preparing the electron micrographs.

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