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Electrophoretic Mobility and Hydrophobicity as a Measure To Predict the Initial Steps of Bacterial Adhesion

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The relationship between physicochemical surface parameters and adhesion of bacterial cells to negatively charged polystyrene was studied. Cell surface hydrophobicity and electrokinetic potential were determined by contact angle measurement and electrophoresis, respectively. Both parameters influence cell adhesion. The effect of the electrokinetic potential increases with decreasing hydrophobicity. Cell surface characteristics determining adhesion are influenced by growth conditions. At high growth rates, bacterial cells tend to become more hydrophobic. This fact can be of ecological significance for controlling the spread of bacteria throughout the environment.

Bacterial adhesion has been interpreted in terms of hydrophobicity or surface free energy (1, 2, 15). Although some authors have indicated an influence of the electrical charges of bacteria and solid surfaces on adhesion (7, 8, 10, 13), the influence of electrostatic interactions is generally ignored.

Most natural solid surfaces, as well as bacteria, are negatively charged (11). In aquatic environments, these surface charges are counterbalanced by oppositely charged ions, some of which are bound to the surface whereas the rest are distributed in a diffuse layer (14). The thickness of this diffuse layer depends on the ionic strength of the solution and the valencies of the counterions. The electrical interactions between particles (including bacteria) in solution are governed by the extension of the diffuse layer; increasing salt concentration results in a decrease in electrical interactions between two particles charged alike.

In the absence of steric contributions due to polymers or polyelectrolytes, the total long-range interaction between two surfaces charged alike is composed of two additive terms: electrostatic repulsion and van der Waals attraction. Depending on the concentration, the valency, and to a lesser extent, the type of the counterions, the repulsion energy can, under certain conditions, be compensated by the van der Waals attraction. For more details on this so-called DLVO theory, see Rutter and Vincent (14).

There are different ways to obtain information about electrostatic interactions. A quantitative method is to determine the electrical potential at each surface. This is experimentally quite difficult. As a good indication of this electrical potential, determination of the electrokinetic (or zeta) potential is usually sufficient. Under a number of simplifying assumptions, the electrokinetic or zeta potential can be calculated from the electrophoretic mobility. For exact determination of the zeta potential of bacteria, their conductance needs to be known as well. By ignoring particle conductivity, erroneous results may be obtained which differ by a factor 0.3 to 0.6 from the real values (4). Einolf and Carstensen (4) found that the conductivity of bacteria is comparable to that of a 0.01 M NaCl solution. Because of difficulties in determining bacterial conductivity accurately, for this study we decided to use electrophoretic mobility as a measurement of the electrostatic state of a bacterium without converting mobilities into zeta potentials. This is a justified procedure for comparison of different bacteria because their conductivities are likely to be very similar.

In this paper we relate electrophoretic mobility to bacterial adhesion on negatively charged polystyrene. In addition, the influence of the cultivation conditions on the cell surface characteristics were investigated. Finally, the bacterial electrophoretic mobilities were combined with results from hydrophobicity measurements (15) to obtain quantitative information on the relative contributions of both factors to bacterial adhesion.

MATERIALS AND METHODS

Growth and preparation of bacterial suspensions. Bacteria and preparation of bacterial suspensions were the same as described elsewhere (15). For most of the experiments, bacteria were grown in batch cultures and harvested in the early stationary phase. The growth medium for continuous cultivation was identical to that used for batch experiments. The chemostat culture was operated at 25°C. For electrophoretic mobility measurements, bacterial cell suspensions were washed twice in an appropriate dilution of phosphate-buffered saline (PBS); the last suspension was made immediately before measurement to prevent interference of ions leaking from the cells.

Measurement of electrophoretic mobility. Electrophoretic mobility was measured by laser Doppler velocimetry with a Zeta Sizer (Malvern Instruments, Malvern, England). A glass capillary was used as the electrophoresis cell. Bacteria were suspended in different PBS concentrations.

RESULTS

For different bacteria, a great diversity of electrophoretic mobilities, and therefore of electrokinetic potentials, was measured (Table 1). If, as suggested by Einolf and Carstensen (4), conductivity is taken into account in the conversion of mobilities into zeta potentials, the latter range from −10 to

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TABLE 1. Electrophoretic mobilities of different bacteria measured in PBS with an ionic strength of 7.5×10^{-3} M

Organism	Electrophoretic mobility (10^{-8} m · V^{-1} · s^{-1})[a]
1 *Pseudomonas fluorescens*	−2.36
2 *Pseudomonas aeruginosa*	−1.07
3 *Pseudomonas putida*	−1.60
4 *Pseudomonas* sp. strain 26-3	−0.29
5 *Pseudomonas* sp. strain 52	−2.67
6 *Pseudomonas* sp. strain 80	−1.74
7 *Escherichia coli* NCTC 9002	−0.42
8 *Escherichia coli* K-12	−1.38
9 *Arthrobacter globiformis*	−1.84
10 *Arthrobacter simplex*	−1.08
11 *Arthrobacter* sp. strain 177	−3.24
12 *Arthrobacter* sp. strain 127	−1.37
13 *Micrococcus luteus*	−1.62
14 *Acinetobacter* sp. strain 210A	−1.99
15 *Thiobacillus versutus*	−2.97
16 *Alcaligenes* sp. strain 175	−2.57
17 *Rhodopseudomonas palustris*	−2.68
18 *Agrobacterium radiobacter*	−1.48
19 *Bacillus licheniformis*	−2.40
20 *Corynebacter* sp. strain 125	−3.07
21 *Azotobacter vinelandii*	−2.45
22 *Rhizobium leguminosarum*	−2.10
23 *Mycobacter phlei*	−3.09

[a] Average standard deviation, ±0.15 × 10^{-8} m · V^{-1} · s^{-1}.

−90 mV. Electrophoretic mobility was measured as a function of salt concentration (Fig. 1). Normally, electrophoretic mobility increases with decreasing salt concentration. However, bacteria conduct part of the current, which leads to a reduction of mobility, particularly when the conductivity of the solution is low. As a result, maxima can occur in the mobility-log concentration diagram.

The electrophoretic mobilities of bacteria were combined with the adhesion behavior of bacteria to sulfated polystyrene as reported earlier (15) (Fig. 2). At the electrolyte strength (0.1 M PBS) used in the adhesion experiments, the electrostatic interactions between bacteria and surface are

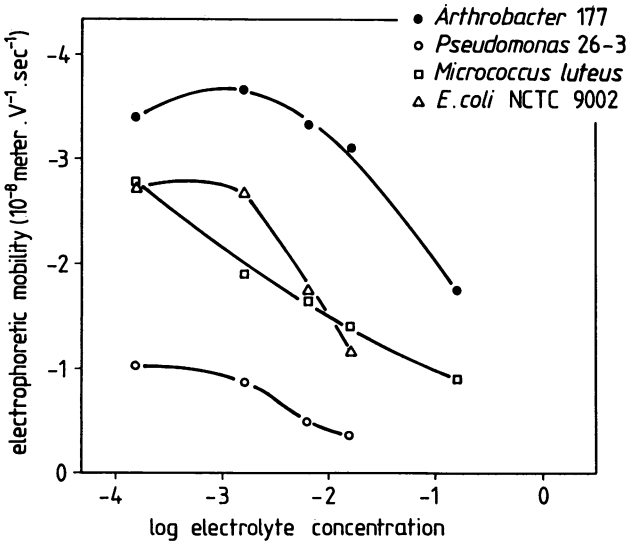

FIG. 1. Relationship between electrophoretic mobility and electrolyte concentration for four bacterial strains.

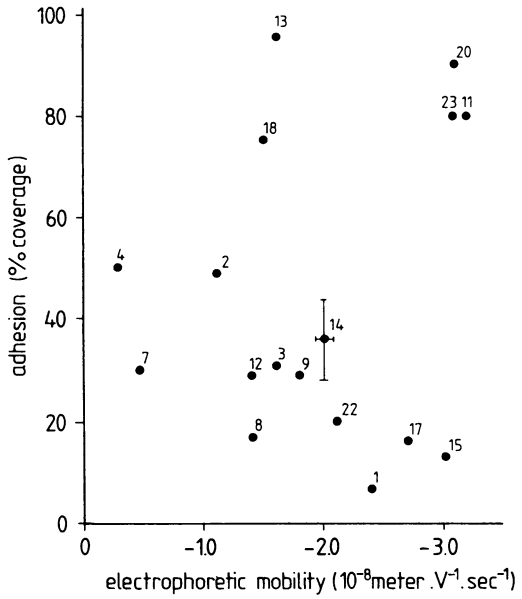

FIG. 2. Relationship between the electrophoretic mobilities of different bacteria (in 0.0075 M PBS) and their adhesion to negatively charged polystyrene (in 0.1 M PBS). The numbers refer to the different bacteria in Table 1. The bars indicate the average standard deviation.

strongly reduced. A full comparison was not possible because the adhesion measurements were done in 0.1 M PBS, whereas, because of practical limitations, electrophoresis had to be performed in 0.0075 M PBS. This fact, however, had no significant influence on the results shown in Fig. 2 since the relative range of mobilities remained approximately the same in 0.1 M PBS. A brief comparison between electrophoretic mobilities with 0.0075 and 0.05 M PBS showed no significant differences between the relative ranges of bacterial cell electrophoretic mobility (data not shown).

To investigate the influence of growth substrate and growth conditions on the hydrophobicity and electrophoretic mobility of bacteria, two complementary experiments were performed. In the former, the effect of the various substrates was measured. Cells were harvested in the early stationary phase. Only small influences of the growth substrate on the

TABLE 2. Contact angles and electrophoretic mobilities of different bacteria grown in batch cultures on various substrates

Growth substrate	Contact angle (°) of water[a] (electrophoretic mobility [10^{-8} m · V^{-1} · s]) with:			
	Pseudomonas sp. strain 26-3	*Arthrobacter* sp. strain 177	*Arthrobacter globiformis*	*Escherichia coli* (NCTC 9002)
Acetate	28 (−0.4)	62 (−3.2)	24 (−1.8)	NG[b]
Ethanol	21 (−0.3)	60 (−3.2)	23 (−1.8)	NG
Mannitol	21 (−0.4)	60 (−3.2)	23 (−1.8)	18 (−0.3)
Glucose	21 (−0.3)	64 (−3.2)	23 (−1.9)	19 (−0.5)
o-Xylene	NG[a]	61 (−3.1)	NG	NG

[a] Determined as described in reference 15.
[b] NG, No growth of these bacteria on this substrate.

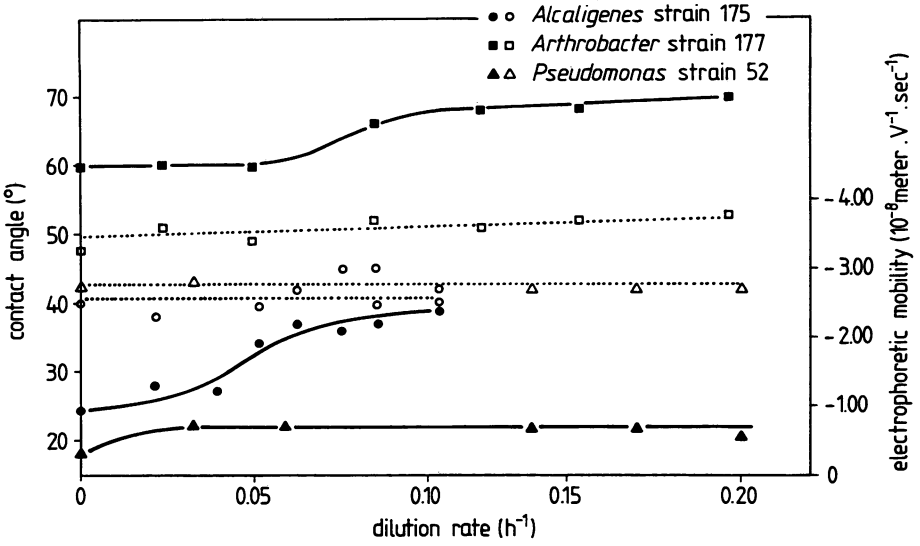

FIG. 3. Cell surface hydrophobicity (——), determined by the water contact angle method (15; standard deviation, ±1°), and cell electrophoretic mobility (. . . .) (standard deviation, ±0.15 × 10^{-8} m/Vs) as a function of dilution rate (in a chemostat).

surface properties were observed (Table 2). In the latter, the influence of bacterial growth rate on surface properties was measured in a chemostat (Fig. 3). Hydrophobicity increased with increasing dilution rate, whereas electrophoretic mobility did not change markedly. Similar results have been obtained with batch experiments in which the cell surface of the five strains tested increased during the exponential growth phase (15a).

DISCUSSION

Based on the data in Fig. 2, it can be concluded that no clear correlation between the electrophoretic mobility of bacteria and their adhesion to solid surfaces exists. However, when these data were combined with the results of contact angle measurements (15), the relative influence of electrokinetic potential became obvious (Fig. 4). Figure 4 was obtained by interpolating the data with a SAS/GRAPH computer program (SAS Institute Inc., Cary, N.C.). Surface hydrophobicity was the dominant characteristic (Fig. 4). At a high contact angle for water, complete adhesion is found, irrespective of mobility. However, at more hydrophilic cell surfaces electrokinetic potential became more influential. This means that bacteria may adhere in the so-called secondary minimum (14). In that case, it is impossible to calculate the Gibbs energy of adhesion from a balance of interfacial tensions (1, 2) because no phase boundaries are destroyed or formed.

When the data in Table 1 were compared with those on bacterial hydrophobicity reported before (15), the trend emerged that relatively hydrophobic cells also had high negative electrokinetic potentials. The combination of high surface potential and a hydrophobic surface seems to be contradictory, but the charged groups only occupy a minor fraction of the total surface area. Assuming that all charge is caused by carboxyl groups on the outer surface at a relatively high surface charge of 100 mC · m^{-2}, not more than

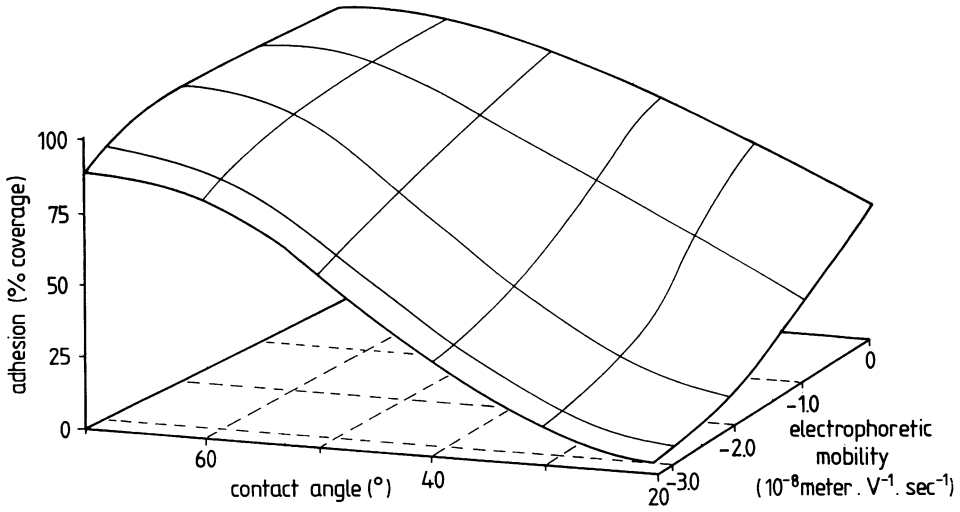

FIG. 4. Relationship between bacterial adhesion and cell surface characteristics as determined by electrophoretic mobility and contact angle measurements (interpolation of the data in Fig. 2 in this communication and those in Fig. 5 in reference 15).

8% of the surface would consist of charged groups. This is probably already an overestimation because the surface potential results only partly from charged groups at the outer surface. The rest originates from charged groups situated in deeper layers of the cell wall. The finding that none of the hydrophobic bacteria had low electrophoretic mobility might be because isolation of a hydrophobic organism with a low electrokinetic potential from a natural sample would be very difficult. These kinds of bacteria would adhere very strongly to surfaces and to each other. Detachment of a single bacterium from other cells or particles is essential in at least one step during the isolation procedure. Therefore, hydrophobic bacteria with low electrokinetic potential could have escaped classical microbiological isolation techniques. Another explanation for the difficulty in finding such bacteria could be that hydrophobicity combined with low electrical charge is of considerable ecological disadvantage for an organism since these characteristics prevent spreading and thus colonization of new habitats. Such a competitive handicap could be detrimental for a nonmotile microorganism.

The observation that bacteria become more hydrophobic during the exponential growth phase (15a) or at high growth rates in a chemostat (Fig. 3) agrees with the experience of many bacteriologists that, during continuous cultivation at high dilution rates, many bacteria tend to form flocs or stick to surfaces present in the culture vessel. Although studying changes in bacterial adhesion behavior under different conditions may help to explain the role of surfaces in microbial physiology and ecology, only few experiments related to this subject have been published. Fattom and Shilo (5) observed benthic cyanobacteria to become more hydrophobic and adhere to solids under optimal growth conditions. Also, Malmqvist (12) found an increase in cell hydrophobicity during exponential growth. Wrangstadh et al. (16) showed that production of an extracellular polysaccharide under starvation conditions induced a decrease in cell surface hydrophobicity and thus in the number of adherent cells. Better adhesion of log-phase cells was observed by Fletcher (6), Marshall et al. (13), and Zvyagintsev et al. (17). Similar results were reported by T. L. Sie (Ph.D. dissertation, University of Hamburg, Federal Republic of Germany, 1985), who measured better adhesion of microorganisms to air bubbles during the exponential growth phase. On the other hand, Kjelleberg and Hermansson (9) reported an increase in hydrophobicity with four of seven marine isolates upon starvation, and Dawson et al. (3) found a marine *Vibrio* sp. to become more adherent during starvation. Only in this last case was adhesion found to be stimulated by the formation of polymeric fibrils.

From the few observations which have been reported up to now, the following hypothesis may put forward. Most terrestial, lacustrine, and near-shore microorganisms tend to adhere under optimal growth conditions, whereas some open-ocean microorganisms adhere during starvation. Although these findings seem contradictory, both behaviors may favor spreading of microorganisms under unfavorable conditions. Detachment of bacteria in soil or sediments during starvation allows on organism to be transported with the pore water, whereas attachment to particles in an aquatic environment increases the vertical transport velocity of a microorganism. In both cases, detachment or attachment increases the chance of reaching environments richer in nutrients elsewhere in the soil or in deeper waters and sediments.

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