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Effects of Bacterial Cell Surface Structures and Hydrophobicity on Attachment to Activated Sludge Flocs

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We have shown that increased cell surface hydrophobicities of both well-characterized *Escherichia coli* strains and isolates from wastewater correlate well with increased adhesion to sludge flocs (Spearman rank correlation coefficient, 0.773; $P < 0.01$).

The activated sludge process of wastewater treatment depends on the formation of activated sludge flocs formed by microorganisms (mainly bacteria), inorganic particles, and exocellular polymers (4, 5, 16). During sedimentation of the flocs, dispersed material, such as bacterial cells and small flocs, attaches to the floc surface. The formation of activated sludge flocs and clarification by settling flocs are obviously based on bacterial aggregation and adhesion mechanisms.

Bacterial adhesion to surfaces can be seen as a two-step event (9, 11, 18), i.e., reversible adhesion due to long-range forces (3, 19) and, possibly, subsequent interactions mediating a direct contact between surfaces, such as hydrophobic interactions due to bacterial surface structures (1, 9).

Relatively few studies have addressed the roles of hydrophobic interactions in the flocculation process and in the adhesion of bacteria to flocs in wastewater. These investigations have shown that flocculation and sedimentation of flocs depend on the internal and external hydrophobicities of the flocs and of produced exopolymeric material in the flocs (16, 17). Also, the surface tension of the liquid influences the hydrophobicity of granular sludge from anaerobic sludge bed reactors (2). In the same system, adhesion of hydrophilic and hydrophobic cells is enhanced by low and high tension of liquid surfaces, respectively (15).

We investigated the roles of cell surface hydrophobicity and cell surface charge in attachment of bacteria to sludge flocs. The possible roles of specific surface structures on attachment were also studied. All adhesion experiments were performed on raw, activated sludge liquor taken from the aeration tanks at Ryaverket Wastewater Treatment Plant (WWTP) in Göteborg, Sweden. Bacterial attachment was therefore tested under natural conditions, including, e.g., with indigenous concentrations of bridging cations. By using well-characterized *Escherichia coli* probe strains, we were able to study the influences of cell surface hydrophobicity and charge and of cell surface structures on bacterial adhesion to sludge flocs. Strains isolated from the wastewater were used to verify results obtained with the *E. coli* strains.

The *E. coli* strains were grown in M9 medium (14) containing 5 μl of [^{3}H]leucine/ml (37 TBq/mmol; Amersham), harvested by centrifugation, suspended, kept in 0.01 M phosphate-buffered saline (PBS) overnight, and again harvested and resuspended in fresh PBS before use. The surface characteristics of the *E. coli* strains were determined by hydrophobic interaction chromatography (HIC) and electrostatic interaction chromatography. Columns were prepared as described by Hermansson et al. (6). Bacteria, approximately 10^9 cells, were added to the column and eluted with PBS. The radioactivities (disintegrations per minute) of the added samples and the eluate were determined by liquid scintillation chromatography. The overall hydrophobicities and charges of the cell surfaces were expressed as $(a - b)/a$, where a is the number of disintegrations per minute in the sample added to the column and b is the number of disintegrations per minute in the eluate.

Hydrophilic WWTP bacteria were isolated by passing effluent water through a column with hydrophobic glass beads (diameter, 0.5 mm) and plating the eluate on R2A agar (Difco), from which 13 randomly selected colonies were picked. For hydrophobic WWTP bacteria, sludge liquor was plated on R2A agar and colonies were screened for hydrophobicity by HIC. Eleven hydrophobic isolates were selected. All isolates were stored in glycerol at −70°C. WWTP bacteria were grown in 10% TGY broth (tryptone [0.5 g/liter], glucose [0.1 g/liter], yeast extract [0.25 g/liter]) and treated in the same way as the *E. coli* strains. The WWTP bacteria were tested for the expression of fimbriae (type 1) by yeast cell agglutination, motility in soft agar stabs, and capsule formation by microscopic examination of negative stained cells.

Bacterial attachment to sludge flocs was determined at room

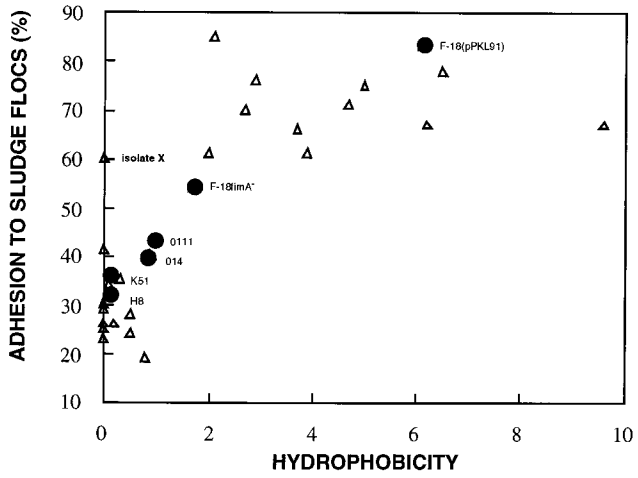

FIG. 1. Adhesion of *E. coli* strains and isolates from WWTP to activated sludge flocs plotted against hydrophobicities of the cell surfaces. Filled circles designate the *E. coli* strains, and open triangles designate the WWTP strains. The mean standard deviation for the adhesion was 1.6%.

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TABLE 1. Cell surface hydrophobicities, surface charges, and cell surface characteristics of the different *E. coli* strains

E. coli strain	Mean hydrophobicity ± SD	Mean surface charge ± SD		Surface characteristics				Reference
		Negative	Positive	LPS[a]	Capsule	Flagella	Fimbriae[b]	
K51	0.1 ± 0.1	0.7 ± 0.1	0.1 ± 0.1	+	+	−	−	13
H8	0.1 ± 0.2	0.6 ± 0.4	0.1 ± 0.1	+	−	+	−	13
O14	0.9 ± 0.5	34 ± 30	0.6 ± 0.4	−	+	−	−	13
O111	1.0 ± 0.4	0.7 ± 0.2	0.6 ± 0.4	+	−	−	−	13
F-18*fimA*$^-$	1.7 ± 0.9	6.6 ± 1.7	0.4 ± 0.1	−	+	+	−	10
F-18(pPKL91)	6.1 ± 2.4	6.6 ± 0.8	4.9 ± 0.5	−	+	+	+	10

[a] LPS, lipopolysaccharide O side chain.
[b] −, negative for type 1, P, and CSA fimbriae. +, positive only for type 1 fimbriae (7a).

temperature (22°C). Bacteria were grown, harvested, kept in PBS overnight, and washed as described above. A ^{3}H-labelled cell suspension (50 μl) was mixed with 10 ml of raw, activated sludge liquor containing flocs and wastewater. The sample was gently mixed for a given time period, the sludge was allowed to settle for 10 min, and 2 ml of the supernatant was taken out. The amount of attachment is expressed as $(a - b)/a \times 100$, where a is the initial number of disintegrations per minute per milliliter and b is the number of disintegrations per minute per milliliter in the supernatant after sedimentation of the flocs. Adhesion of *E. coli* and WWTP isolates was tested with sludges of different characteristics, and representative data for attachment are shown in Fig. 1.

The hydrophobicities and charges, both negative and positive, and the surface structures of the tested *E. coli* strains are shown in Table 1. The *E. coli* strains showed a gradient of hydrophobic surface properties and various negative and positive cell surface charges.

Figure 1 shows representative results of the adhesion of the *E. coli* strains to activated sludge flocs after a contact time of 15 min before sedimentation. There was a strong correlation (Spearman rank correlation coefficient, 0.986; $P < 0.01$) between the cell surface hydrophobicities of the whole series of *E. coli* strains and adhesion to the sludge flocs (Fig. 1). For positive cell surface charges the correlation was weaker than for surface hydrophobicity (data not shown), and negative cell surface charges showed no correlation to adhesion (data not shown).

The 24 WWTP isolates also showed a clear correlation (Spearman rank correlation coefficient, 0.773; $P < 0.01$) between adhesion and surface hydrophobicity similar to that of the series of *E. coli* strains (Fig. 1). Hydrophilic isolates attached in low numbers to the sludge flocs, and with more hydrophobic properties, the attachment increased. At HIC values above approximately 3, adhesion seemed to be independent of hydrophobicity. The saturation value for attachment of hydrophobic isolates was approximately 60 to 80%. This upper limit of attachment may be due to an equilibrium between reversibly attached cells and free-living cells under these experimental conditions.

All the WWTP isolates were nonfimbriated (type 1 fimbriae). Two of the isolates were motile, indicating production of flagella. One bacterium produced exopolymers (isolate X in Fig. 1), which may have been involved in its rather strong adhesion. It is impossible to say if the WWTP isolates retain their in situ hydrophobicities, since bacteria often change their hydrophobicities rapidly due to nutrient fluctuations or culturing (7). However, there were no changes in hydrophobicity after repeated HIC measurements during storage of the isolates.

This is the first investigation of adhesion of well-characterized bacteria, with various surface hydrophobicities, to flocs in sludge liquor. It is shown that bacterial cell surface hydrophobicity is important for bacterial adhesion to activated sludge flocs. The absolute adhesion values for the *E. coli* strains varied for eight different sludges, sampled during a period of 2 years, but the general results and trends were the same. Positive cell surface charge seems to be important but does not show as clear a correlation to adhesion as hydrophobicity does. Differences in the levels of adhesion among the *E. coli* strains could not be attributed to a specific combination of surface structures but rather were attributed to overall hydrophobicity. However, for *E. coli* F-18(pPKL91) and *E. coli* F-18*fimA*$^-$, the presence of fimbriae is most likely the reason for the increased hydrophobicities, since the strains are identical except for the production of fimbriae. *E. coli* F-18(pPKL91) harbors an extra *fimB* gene on a *parB*-stabilized plasmid and is therefore phase locked "on" such that the cells express type 1 fimbriae. *E. coli* F-18*fimA*$^-$ has a defective *fimA* gene and is therefore nonfimbriated (10). Fimbriae in *E. coli* have been reported to increase cell surface hydrophobicity in several studies (6, 8, 12).

The adhesion of WWTP isolates to flocs was again found to be dependent on overall hydrophobicity in the same manner as for *E. coli* and could not be explained by the presence of a specific surface structure.

The results indicate that a low level of cell surface hydrophobicity may be the reason why free-living cells do not attach to flocs. These cells escape sedimentation in the treatment system and reduce the quality of the effluent.

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