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Pseudomonas fluorescens Adhesion and Transport through Porous Media Are Affected by Lipopolysaccharide Composition

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The objectives of this work were (i) to use transposon mutagenesis to produce mutants of *Pseudomonas fluorescens* that were altered in adhesion ability and transport through porous media and (ii) to identify the alterations in surface characteristics that were responsible for the changes in attachment. Mutants of *P. fluorescens* were generated with Tn*pho*A, which enabled identification of mutants that were altered in surface proteins. Transposon mutants were screened for alterations in adhesion ability by attachment assays on hydrophobic polystyrene and water-wettable polystyrene. Four Tn*pho*A mutants with increased adhesion to the hydrophobic surface and decreased adhesion to the water-wettable surface were obtained. Transport of the strains through porous media was evaluated by passing suspensions of each mutant and the parent through columns containing quartz sand and determining the number of cells retained in the columns. The mutants all demonstrated increased adhesion and retention in the columns. Southern analysis demonstrated two types of mutants with separate transposon insertion sites. Polyacrylamide gel electrophoresis of the strains demonstrated that the O antigen on the lipopolysaccharide was either attenuated or absent. Lack of this polysaccharide, and the consequent increased exposure of the lipid moiety of the lipopolysaccharide, is probably responsible for the increase in adhesion to the hydrophobic substrata and retention in the sand column. This work combined with previous studies of attachment of *P. fluorescens* demonstrates that more than one type of polymer can mediate the adhesion of this organism to nonbiological surfaces.

Movement of bacteria through porous media, such as sediments and aquifers, is largely controlled by the opposing processes of convective transport and the attachment of bacteria to surfaces. Most investigations of the transport and adhesive properties of subsurface bacteria have been conducted in the laboratory. Factors that have been identified as likely to affect transport in situ include sediment porosity and bacterial cell size (14, 15), matrix heterogeneity (20, 32), ionic strength of pore water (14, 16), and the specific bacterium used for experimentation (35). However, little is known about the properties of bacterial surfaces or adhesives that result in retention on particle surfaces.

Both polysaccharides and proteins appear to play some type of role in adhesion. Extracellular polysaccharides are synthesized by attached bacteria and strengthen their binding to surfaces (5), but experimental evidence indicates that proteins, such as outer membrane proteins, fimbriae, flagella, and enzymes, are required for the early stages of adhesion to surfaces (7, 13, 25). Thus, a fundamental question that remains to be answered is whether there are relatively few types of molecules that can act as bacterial adhesives, or whether macromolecules at the bacterial surface act in concert, through synergistic or antagonistic interactions, to determine the overall adhesiveness of the cell. Furthermore, the specific polymers and their characteristics (e.g., composition, size, configuration, charge, and nonpolar interactions) that are responsible for bacterial adhesiveness are yet to be determined.

Numerous workers have used parameters that are evaluated more easily than surface biochemistry and are an average expression of cell surface chemistry, e.g., surface hydrophobicity (15, 29), surface free energy (or related parameters) (3), and surface charge (15, 18). There is some indication that attachment to soil or sediment particles may be favored by microbial surface hydrophobicity (33). However, these gross measurements of surface properties do not consistently correlate with transport through porous media (15). Thus far, measurements of hydrophobicity and surface charge have been insufficient to account for and to predict the adhesive properties of bacteria, and further knowledge of specific adhesive polymers is required to understand transport phenomena.

The objectives of this work were (i) to produce mutants of a common adhesive organism that were altered in their attachment and transport characteristics and (ii) to identify the alterations in surface characteristics that were responsible for the changes in attachment. Our primary aim is to determine which polymers or structures at the cell surface are involved in attachment and their characteristics that determine adhesiveness. An ultimate goal is to establish the relationship between adhesiveness and transport through porous media.

MATERIALS AND METHODS

Organisms and growth conditions. The test organism was *Pseudomonas fluorescens* H2, a river isolate (27). *Escherichia coli* S-17 (10) was the donor for transposon mutagenesis. *P. fluorescens* was cultured in medium containing 0.1% peptone, 0.07% yeast extract, pH 7.4 (PYE), with shaking (125 rpm) at 20°C. For antibiotic selection, filter-sterilized kanamycin (40 μg/ml) and streptomycin (125 μg/ml) were added after autoclaving of PYE. Spontaneous rifampin-resistant mutants of *P. fluorescens* were selected by plating a stationary-phase culture on PYE plates containing 15 g of agar per liter and 150 μg of rifampin per ml; a Rif^r strain designated R15 was selected for further work. The growth curves of strain R15 in PYE were similar to those of the parent strain. *P. fluorescens* H2 is also resistant to streptomycin. *E. coli* was cultured overnight in LB broth at 37°C with ampicillin (100 μg/ml) and kanamycin (40 μg/ml).

Mutagenesis with Tn*pho* A. We used the mini-Tn*pho*A constructed by de Lorenzo et al. (10), carried on a suicide delivery system by using plasmid pUT, a derivative of pGP704 (24). Donor *E. coli* strain S-17 was mated with *P.*

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fluorescens R15 (1), generating ca. 9,000 mutants per mating. Colonies were then picked and transferred to master plates with kanamycin, rifampin, and streptomycin, and resistant colonies were replica plated twice serially on PYE medium without antibiotics, followed by replica plating on PYE medium with kanamycin, rifampin, and streptomycin to ensure maintenance of kanamycin resistance (which was presumptive evidence of transposon insertion). These antibiotic-resistant colonies were then replica plated onto medium with 5-bromo-4-chloro-3-indolylphosphate (40 μg/ml) as described by Long et al. (19), so that bacteria with *pho*A fusions in periplasmic, membrane-associated, or secreted proteins produced blue colonies. Colonies were also replica plated on EMB medium to ensure that no *E. coli* were present.

Selection for adhesion mutants. Approximately 200 Tn*pho*A mutants that produced alkaline phosphatase were screened for altered adhesion properties. Eighteen-hour (early stationary phase) broth cultures of each Tn*pho*A mutant were adjusted to the same optical density (ca. 0.80 at A_{590}) after washing and resuspension in 0.1 M potassium phosphate buffer. These suspensions were exposed for 2 h to the test surfaces, which were 5-cm-diameter polystyrene petri dishes (PD) (Corning Glassworks, Corning, N.Y.) and 5-cm-diameter polystyrene tissue culture dishes (TCD) (Corning). PD are hydrophobic, with a water contact angle of 90°, whereas TCD are more hydrophilic, with a water contact angle of ca. 66° (26). After exposure to the bacteria, the substrata were rinsed, fixed, and stained with crystal violet (6). The numbers of attached cells were indirectly assessed by measuring the absorption of crystal violet-stained cells at A_{590} in a spectrophotometer. Four randomly selected areas were read on two duplicate dishes ($n = 8$). Tn*pho*A mutants whose adhesion values differed from those of R15 by at least 25% were assayed at least two additional, separate times to verify the change in adhesiveness.

Southern hybridizations. DNA was isolated from each mutant (1) and enzymatically cut with *Eco*RI. The resulting DNA fragments were separated on a 1% agarose gel and transferred to a nylon membrane (Genescreen; Dupont, Boston, Mass.) (1). To identify transposon insertion sites on the chromosomal DNA, plasmid pUT containing mini-Tn*5pho*A (10) was digested with *Eco*RI (Promega, Madison, Wis.) and run on an agarose gel, and the transposon fragment (ca. 2.5 kb) was excised and cleaned (Geneclean Kit; Bio 101, Vista, Calif.). The fragment was labeled with ^{32}P by random priming (Multi-Prime Labeling Kit; Amersham, Arlington Heights, Ill.) and used as a hybridization probe. DNA fragments hybridizing to the probe were visualized by autoradiography. A separate gel was also probed with pGP704, the plasmid from which the vector was derived, to ensure that the mutants did not contain residual plasmid.

Outer membrane proteins. Outer membrane proteins were isolated and analyzed by one-dimensional polyacrylamide gel electrophoresis (PAGE), as previously described (22).

LPS. For visualization of lipopolysaccharide (LPS) by PAGE, whole-cell lysates were digested with proteinase K by the method of Hitchcock and Brown (17). Samples were analyzed by PAGE as above except that no sodium dodecyl sulfate (SDS) was added to the gels and stained with an LPS-specific silver-staining procedure (17).

BATH test. In the bacterial adherence to hexadecane (BATH) test, the relative hydrophobicity of strain R15 and the mutants was assessed by measuring their attachment to hexadecane, as described by Wiencek et al. (36), except that the suspending medium was 0.1 M KPO_4 buffer, pH 7.4. Hexadecane volumes of 0.1, 0.2, 0.6, and 1 ml were used with 3 ml of bacterial suspension. Both 18-h cultures and 48-h starved bacteria (below) were assayed.

Static adhesion trials. The attachment properties of the wild type and transposon mutants were quantified by the adhesion assay used to select adhesion mutants (above). Late log- or early-stationary-phase cells (18-h cultures) were assayed after their resuspension in 0.1 M potassium phosphate buffer and in artificial groundwater (AGW) (1.5×10^{-5} M KNO_3, 1.4×10^{-4} M $MgSO_4 \cdot 7H_2O$, 7.0×10^{-5} M $CaSO_4 \cdot 2H_2O$, 8.0×10^{-5} NaCl, 1.4×10^{-4} M $NaHCO_3$ [pH 6.8]) (31).

Bacteria that had been starved for 48 h in AGW, so that they more closely resembled organisms in nutrient-deficient groundwater, were also assayed in 0.1 M NaCl and in AGW. Each strain was cultured for 24 h in PYE, collected by centrifugation, resuspended in an equal volume of AGW, and incubated at 20°C for 48 h before being assayed for adhesion. There was no change in plate counts of bacteria after this period. Adhesion assays were performed at least three separate times for each strain.

Frequency distribution of clones. Attachment capability is not an all-or-none property; rather, most populations of bacteria comprise both bacteria that attach to available surfaces and bacteria that remain freely suspended. To evaluate the degree of heterogeneity within a population with respect to adhesion phenotype, we conducted static adhesion assays on clones derived from single colonies of three strains. *P. fluorescens* R15 (wild type) and transposon mutants VW55 and VW40 were each cultured in PYE for 18 h at 20°C. Serial dilutions were made in 0.1 M potassium phosphate buffer, plated on PYE agar, and incubated at 20°C. Thirty colonies of each strain were each transferred to a 50-ml aliquot of PYE, incubated for 18 h at 20°C, and assayed for attachment properties as described above.

Transport through silica sand-filled columns. Total transport of bacteria through sand-filled columns was determined by a method modeled on that described by Fontes et al. (14). AGW (11 ml) was placed in each glass chromatography column (inner diameter, 1.8 cm; Bio-Rad, Richmond, Calif.), and 48.2 g of autoclaved, dried quartz sand (0.45 to 0.25 mm; Accusand; Unimin, New Canaan, Conn.) was added slowly while the column was being tapped to facilitate even packing. This resulted in a column packed with sand and liquid just covering the packed sand.

Each strain was cultured for 24 h in PYE, collected by centrifugation, and resuspended in an equal volume of AGW. These suspensions were incubated at 20°C for 48 h to starve the cells. Two milliliters of this suspension was diluted with AGW to a final volume of 8 ml, placed on the top of a sand column, and run into the column by allowing 8 ml of solution to run out at the bottom, giving 10^{10} cells per column. The columns were allowed to stand at room temperature for 2 h, and then 3 pore volumes (33 ml) were passed through the column and collected for viable-cell counts. Column experiments were performed 6 times for strains VW12, VW37, VW40, and VW55 and 16 times for strain R15.

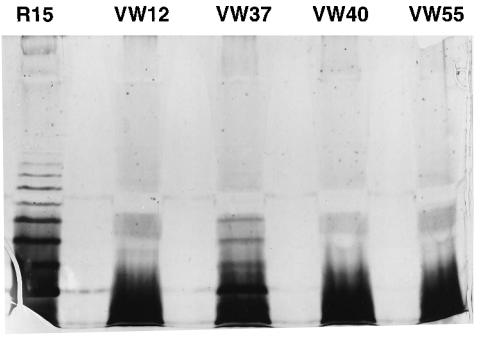

FIG. 1. Silver-stained polyacrylamide gel of LPSs from strains R15, VW37, VW12, VW40, and VW55.

RESULTS

Mutagenesis. Approximately 3% of the Km^r Rif^r transposon mutants were alkaline phosphatase positive, indicating transposon insertion in a surface-expressed protein. Of these, 212 were assayed for alterations in adhesion properties, and 7 strains showed consistent, significant alterations in attachment to both test substrata. Initial Southern analysis indicated possibly four different insertion sites, and four representative mutants (VW12, VW37, VW40, and VW55) were selected for further analysis. Subsequent Southern hybridizations confirmed two different insertion sites; strains VW12, VW40, and VW55 had the same insertion site and were possibly siblings. Southern analysis also indicated that the plasmid vector was absent.

Cell surface properties. PAGE analysis of LPS demonstrated a marked difference between the parent strain R15 and the transposon mutants (Fig. 1). Strain R15 had the typical ladder pattern of the O antigen, whereas VW37 had only four bands in the O antigen region, and strains VW12, VW40, and VW55 lacked the ladder representing O antigen. PAGE analysis of outer membrane proteins demonstrated no differences in major protein bands.

Evaluation of relative bacterial surface hydrophobicity by the BATH test demonstrated no differences between the parent R15, the mutant strains, and bacterium-free controls. All assays showed <10% reduction in cell suspension optical density after mixing with hexadecane, indicating no detectable hydrophobicity for all strains. Results were similar for late-log-phase and starved suspensions.

Attachment assays of bacteria. For 18-h cultures suspended in phosphate buffer, strain R15 attached in higher numbers to the hydrophilic TCD than to the hydrophobic PD (Table 1). For all mutants, attachment to the hydrophobic PD was increased and that to the hydrophilic TCD was reduced. For strain VW37, the difference in the numbers of attached cells on the two substrata (A_{590} = 128.5 and 94.3, respectively) was considerably smaller than the differences for strains VW12,

TABLE 1. Attachment of parent (strain R15) and four adhesion mutants (VW12, VW40, VW55, and VW37) to hydrophobic polystyrene (PD) and hydrophilic polystyrene (TCD)[a]

Substratum	Strain	Mean 10^3 A_{590} (SE)			
		Log-phase bacteria		Starved bacteria	
		PB	AGW	NaCl	AGW
PD	R15	82.3 (1.6)	85.5 (2.3)	79.0 (2.0)	64.8 (1.9)
	VW37	128.5 (4.0)	206.3 (2.8)	107.7 (5.0)	260.0 (4.1)
	VW12	179.7 (8.20)	222.3 (3.9)	162.3 (7.4)	251.3 (4.0)
	VW40	232.2 (6.0)	242.0 (6.6)	155.5 (5.8)	254.2 (8.0)
	VW55	240.1 (8.4)	234.8 (4.2)	146.4 (4.0)	247.4 (3.7)
TCD	R15	189.4 (2.9)	136.2 (3.4)	177.1 (3.6)	148.0 (5.9)
	VW37	94.3 (3.8)	82.7 (2.2)	157.0 (4.4)	101.8 (4.1)
	VW12	89.3 (3.2)	83.1 (2.9)	128.5 (5.2)	91.1 (2.0)
	VW40	91.9 (2.8)	76.7 (2.2)	128.1 (6.2)	89.3 (2.8)
	VW55	109.1 (5.7)	77.0 (2.7)	119.3 (5.0)	89.0 (1.9)

[a] Attachment is expressed as mean 10^3 A_{590} of crystal violet-stained cells ± standard error ($n = 8$), which is an indirect measure of the number of attached cells. During the assay, bacteria were suspended in 0.1 M potassium phosphate buffer (PB), 0.1 M NaCl (NaCl), or AGW.

VW40, and VW55, which were more than twofold. For 18-h cultures suspended in AGW, attachment to PD generally increased (except for R15 and VW55) and attachment to TCD decreased compared with attachment by bacteria suspended in phosphate buffer.

For cultures that had been starved for 48 h in AGW, there was an increase in the number of cells attached to both types of surface compared with 18-h cultures (except for R15 on PD, for which there was a decrease). Starved bacteria were similar to unstarved bacteria in that they attached in greater numbers to the hydrophobic PD and in fewer numbers to the hydrophilic TCD compared with the parent strain R15 (the comparison is valid only for cells suspended in AGW). Strain VW37 suspended in NaCl did demonstrate a shift in substratum preference following starvation and attached in higher numbers on the hydrophilic TCD than on the hydrophobic PD.

Frequency distribution of clones. Figure 2 illustrates the broad range of attachment values obtained with 30 cultures derived from one parent. The broadest ranges (R15 attached to the hydrophilic TCD and VW40 and VW55 attached to the hydrophobic PD) were obtained with the surfaces with the highest attachment numbers and were not related to the type of substratum.

Column experiments. For all strains tested (R15, VW12, VW37, VW40, and VW55), there were high levels of retention in the columns (Fig. 3). There was considerable variability in these replicate values for the parent R15, ranging between 43.1 and 91.7%. In contrast, all mutant strains were consistently retained in the columns, with replicate values ranging between 91.9 and 98.1%; all values were significantly different from those for the parent at $P < 0.01$ by Student's t test. Furthermore, the mean percent retention of strain VW37 (97.1%) was significantly different from the mean values for strains VW12 (94.8%), VW40 (95.0%), and VW55 (95.5%), at $P < 0.05$.

DISCUSSION

It is likely that different polymers mediate attachment depending on different substratum chemistries and medium compositions (13, 25). For example, polymers with nonpolar sites, e.g., pili and LPS, may dominate in binding to hydrophobic surfaces, whereas polymers capable of hydrogen bonding or

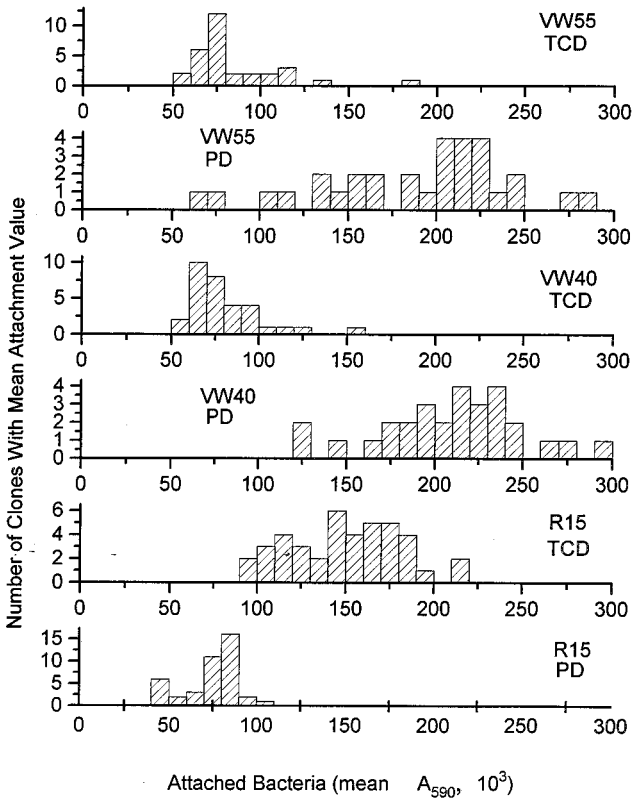

FIG. 2. Frequency distribution of mean attachment values for 30 clones derived from single colonies of strains R15, VW55, and VW40.

electrostatic interactions, e.g., polysaccharides, may function with hydrophilic surfaces. Moreover, different polymer types may act cooperatively in binding to the surface to stabilize the adhesive interaction (11). Also important are bacterial surface polymers that tend to prevent adhesion by binding water or

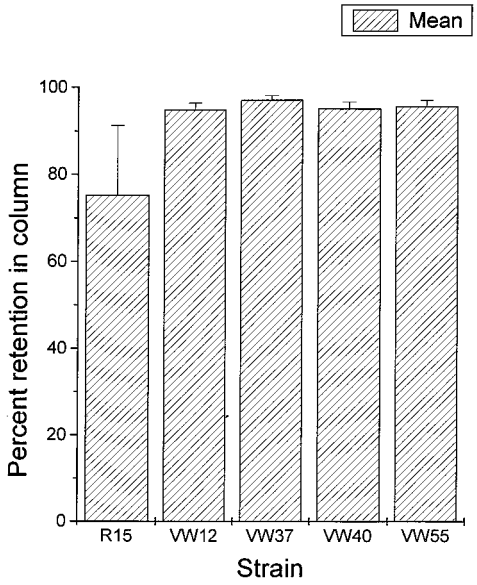

FIG. 3. Retention of bacteria in sand columns, expressed as a percentage of the number of cells introduced.

through other steric effects (27, 28) and thus may mask adhesive polymers. For example, a nonadhesive variant of the isolate used for these studies, *P. fluorescens* H2M, was found to produce an alginate extracellular polysaccharide, composed of mannuronic and guluronic acids, which enhanced the affinity of the bacterial surface for water and prevented adhesion to surfaces (27).

Mutagenesis has been an invaluable approach for identifying bacterial surface components involved in adhesive interactions with animal or plant hosts, as these usually involve a protein ligand on the bacterial surface. Bacterial adhesion to nonbiological surfaces, however, has been more difficult to address with molecular tools because a variety of surface polymers, including carbohydrates and lipids, appear to be involved (11, 13, 25). Furthermore, attachment to nonbiological surfaces is not a null phenotype but is better described by a continuum of adhesive phenotypes ranging from strongly adhesive to nonadhesive. A single population of bacteria may contain cells with apparently different adhesive tendencies, as was illustrated by the frequency distribution assays (Fig. 2). Confirmation of mutations in adhesion properties requires large alterations in adhesiveness, multiple replicate adhesion assays, and statistical comparisons. Thus, selective screening for mutants with altered adhesion characteristics is difficult and time-consuming. Screening can be streamlined somewhat by focusing on adhesion-deficient mutants (9), the use of microtiter plates as attachment substrata (6), or, as in this study, the use of a reporter system that allows screening of only those organisms with mutations in surface-associated proteins.

We used the Tn*5* derivative mini-Tn*pho*A (10), which enabled identification of mutants that are altered in genes encoding periplasmic, membrane-associated, or secreted proteins (21, 24). *pho*A codes for alkaline phosphatase, and subsequent to mutagenesis with Tn*pho*A, *pho*A is directly fused to the protein encoded by the target gene. Alkaline phosphatase is active only after transport across the cytoplasmic membrane, and its expression is detected by replica plating mutants on medium containing 5-bromo-4-chloro-3-indolylphosphate (a substrate for alkaline phosphatase), on which alkaline phosphatase-positive colonies (mini-Tn*pho*A mutants) turn blue. Thus, by using Tn*pho*A, we focused on only those mutants that were modified in proteins expressed at the cell surface and hence more likely to be involved in adhesion to particles (either as adhesins or by being involved in polymer synthesis). In this way, considerable effort was saved, since the large majority of mutants produced by ordinary transposon mutagenesis, e.g., Tn*5*, are likely to be modified in properties quite unrelated to attachment. Four adhesion mutants were identified. Although Southern analysis indicated that three of these (VW12, VW40, and VW55) were siblings, all were included in the adhesion and cell surface analyses to provide independent evaluations of the relationships between cell surface, adhesion, and transport properties.

The attachment surfaces were PD and TCD. TCD are commercially available and have been treated so that they have a somewhat higher negative charge and surface free energy, and thus water wettability, than untreated PD. For bacteria that adhere primarily via hydrophobic interactions (presumably through a high level of involvement of pili, lipid moieties of LPS, and/or nonpolar sites on proteins or acetylated polysaccharide), the greatest attachment levels would be expected on PD, the more hydrophobic surface. Conversely, for bacteria that adhere via electrostatic or polar interactions, including hydrogen bonding, greater attachment levels would be expected on surfaces with higher substratum surface free energy (which reflects an increase in surface charge and polarity), provided that electrostatic repulsion was minimized by a sufficient electrolyte concentration (30). Thus, the use of two substratum types in these assays allowed detection of alterations in attachment that involve either nonpolar or polar interactions. We routinely use these substrata in our laboratory and have successfully characterized a wide range of organisms (2, 23, 26).

In this study, all mutants showed increased attachment to hydrophobic surfaces and decreased attachment to the more hydrophilic substratum. These results indicate that hydrophobic interactions play an important role in attachment of the mutants but are less significant in attachment of the wild-type R15. Starvation of the bacteria did not alter the relative differences between parent and mutants, but starvation did result in increased attachment of all strains except R15 attaching to PD. Growth conditions, including starvation (4), have been shown to influence adhesion (12, 23), presumably by influencing cell surface polymers. Since the number of attached bacteria increased on both types of surfaces with starvation, there may have been a decrease in a surface polymer that facilitated hydration and suspension of the cells.

The attachment assay results suggest an increase in relative hydrophobicity of mutant surfaces compared with the parent. Furthermore, characterization of cell surfaces of the mutants demonstrated a major alteration in LPS structure consistent with loss (VW12, VW40, and VW55) or attenuation (VW37) of the O antigen on the LPS. These modifications were observed in both log-phase and starved cells. This result is consistent with the observed increase in attachment to hydrophobic surfaces, as the lipid moiety would be more exposed in the mutants, making their surfaces more hydrophobic. Although the BATH test did not indicate changes in hydrophobicity in the mutants, all strains were too hydrophilic to be evaluated by this relatively gross measure of relative hydrophobicity (34).

Outer membrane protein analysis showed no changes in major outer membrane proteins. This contrasts with the work of DeFlaun et al. (8), in which modifications in the quantity of flagellin (either deletion or overexpression of flagella) resulted in a decrease in adhesion of *P. fluorescens* Pf0-1. Clearly, attachment can be determined by more than one type of polymer, even in the same species. For *P. fluorescens* alone, protein (9), extracellular polysaccharide (26), and now LPS have been shown to strongly influence attachment.

In the column transport experiments, retention of the mutants in the columns was greater and more consistent in replicate trials than retention of strain R15. These experiments more closely paralleled results from attachment assays with hydrophobic surfaces than assays with hydrophilic substrata. As silica sand should be negatively charged and thus hydrophilic, the reverse result might be expected. However, these data suggest that relatively nonpolar sites do occur on the sand and that adhesion to sand might be strongly influenced by hydrophobic interactions. This is consistent with the work of Stenström (33), who observed a positive correlation between bacterial surface hydrophobicity and attachment to quartz and magnetite. Furthermore, in those studies, the bacterial hydrophobicity of some strains was related to attenuation of LPS. Alternatively, there is no connection between increased adhesion of mutants to hydrophobic PD and to quartz sand, and different adhesion mechanisms are involved with the two types of surface.

Starvation resulted in moderate increases in attachment of all strains to both PD and TCD except for R15 attaching to PD (suspension in AGW [Table 1]). This result cannot be explained at this stage, as PAGE analysis of LPS showed no changes with starvation. However, PAGE demonstrates only gross differences in LPS structure, and more detailed analysis could reveal modifications that affect adhesiveness.

Changes in the suspending solution altered the numbers of attached cells. For mutants attaching to PD, suspension in AGW with very low ionic strength resulted in an increase in the number of attached cells compared with suspension in 0.1 M NaCl or phosphate buffer (except for VW55 in log phase). In contrast, for all strains attaching to TCD, suspension in AGW resulted in a decrease in the number of attached bacteria. This could be explained by an increase in repulsion between like-charged surfaces and is consistent with the results of Fontes et al. (14), in which a 10-fold increase in ionic strength reduced recovery of bacteria passed through silica sand columns to ~25%, depending upon sand particle size. Similarly, Gannon et al. found that with a *Pseudomonas* sp. suspended in distilled water, almost all the cells passed through saturated test columns of aquifer sand, whereas when suspended in 0.1 M NaCl, most bacteria were retained in the column (16). This contrasts with the work of DeFlaun et al. (9), in which alteration of the ionic strength of the suspending medium did not affect adhesion; in that case, however, attachment was related to the presence of flagella. Conceivably, ionic strength is relatively insignificant when protein acts as the adhesion but becomes important when charged polysaccharides, e.g., LPS or extracellular polysaccharide, are present as adhesives or even as dispersants.

In summary, this work further demonstrates that multiple polymers are involved in determining the cell adhesiveness of a given bacterial species. Evidence is provided that LPS composition affects the adhesive properties of *P. fluorescens*, whereas previous studies have demonstrated the significance of protein (9) and extracellular polysaccharide (27). The presence of multiple adhesives helps to explain the considerable variability and flexibility in attachment properties that have been observed with different substrata (13, 25) and environmental conditions (12).

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