Introduction

Bacterial colonization of myriad niches, both natural and man-made, begins with adhesion to surfaces (1, 2). The colonization of man-made surfaces (catheters, surgical implants, etc.) causes infection (3) because adhering biofilms resist physical and chemical assaults (4), and it contributes to the emergence of antimicrobial resistance (5). Understanding and minimizing bacterial adhesion is a cross-disciplinary “grand challenge” (6), in which it is key to understand the forces involved. Besides generic electrostatics and dispersion forces (7), there are also forces of biological origin associated with various adhesins and sticky organelles (for example, fimbrae) (6, 8–10).

Biophysical studies of bacterial adhesion typically fall into two categories (11): high-throughput, population-level work giving little single-cell information or low-throughput single-cell studies. We report a robust, generic, and high-throughput tracking and big-data analysis technique that reveals detailed single-cell information in a large population. Applying this technique to *Escherichia coli* on glass, we find substantial variability in the propensity for adhesion and the postadhesion dynamics. Some cells do not adhere despite repeated encounters, whereas others adhere rapidly upon contact. Among the adherers, a fraction pivot freely around their own attachment points and can be removed from the surface by gravity, whereas the remainder spend part of their time in a more strongly bound, rotationally constrained state. This variability remains in flagella- and fimbrae-deletion mutants.

From the biological perspective, such variability exemplifies phenotypic heterogeneity in a clonal population (12) and offers a new model for its study (13, 14). Given the variety of surfaces that bacteria may encounter, such adhesive phenotypic heterogeneity may be an instance of “bet hedging” (15), allowing survival when the environment changes unpredictably (for example, following fecal excretion for Enterobacteriaceae).

In soft matter terms, genetically monodisperse *E. coli* cells are adhesively polydisperse and behave as “living patchy colloids” bearing a variable number of sticky patches on their cell bodies. Thus, the considerable knowledge about patchy colloids accumulated over the past decade (16–18) can be deployed in the design of abiotic surfaces to minimize bacterial adhesion. Bacterial adhesion to engineered patchy soft surfaces has been studied before (19), but the possibility of patchiness on the bacteria themselves has not been considered in much detail.

We studied *E. coli* strain AB1157. Like all K-12 derivatives, it has mutations in the *rfb* gene cluster [here, in *rfbD* (20)] preventing O-antigen production (21). Thus, one major cause of variability and molecular roughness on the cell surface is absent. Our cells display a highly conserved layer of core oligosaccharides anchored to the outer membrane by lipid A (22), although the precise terminal sugars in AB1157 are unknown. Besides the wild-type (WT), to further simplify the surface, we also used a mutant (AD19 = AB1157 Δ*fimA*, Δ*fliF*; hereafter Δ*FF*) defective in producing type 1 fimbrae and flagella. In transmission electron micrographs (fig. S1), these cells appear smooth down to ≤ 10 nm. Any heterogeneities, or “patchiness,” on the surface of this mutant are likely due to membrane proteins.

Results

A high-throughput method reveals complex adhesion behavior

To monitor surface adhesion of *E. coli*, we loaded cell suspensions into 400-μm-high borosilicate glass capillaries at ~ 4.5 × 10⁷ cells/ml (WT) or ~ 1.5 × 10⁷ cells/ml (Δ*FF*) in phosphate motility buffer (MB) and observed them in an inverted microscope at ca. 22°C using a 60× phase-contrast objective (focal depth ~ 3.5 μm) (Fig. 1A). From measurements at the lower surface at the start of the experiment, we estimate an initial motile fraction between 25 and 35% [nonadhering motile cells swim at an average speed of ≲ 20 μm s⁻¹ (23)]; the remaining cells are nonmotile and diffuse as passive colloids. With 0.72 μM glucose included in the MB, the swimmers maintain their speed for at least 20 hours (23). Atomic force microscopy (AFM) measurements reveal that the glass surface is smooth, with a root-mean-squared roughness of 0.25 ± 0.003 nm (fig. S2).

Time-lapse images of the bottom and top glass surfaces were taken automatically at multiple locations to track arriving cells and their subsequent fate. We adapted algorithms used for colloidal rods (24)
Fig. 1. Tracking and classification of bacteria. (A) Schematic of adhering (blue), diffusing (olive), and swimming (red) bacteria in a capillary. (B) Trajectories tracing the most mobile point on a cell. Straight red lines are fits of the length axis, squares mark anchoring points, and lines mark the type: diffuser (D), swimmer (S), wobbler (W), pivoter (P), and active rotator (R). The image is black/white-inverted and thresholded to be clear in print. Inset: Adhering cell and schematic depicting the anchoring coordinate and most mobile coordinate c [−0.5, 0.5] on its length axis. (C) MSDs as a function of time interval t from trajectories of swimming (red), diffusing (olive), and adhering (blue) bacteria; thin lines show randomly selected individual trajectories and thick lines represent the average (R^2(A,t)) for each category. The insets show an example trajectory for each category; squares denote the position in the last frame. (D) Normalized distribution of the translational exponent k_T for adhering cells, showing three peaks for wobblers (W), pivoters (P), and active rotators (R). Data in in panels D and E for the nonflagellated ΔFF are shown in gray. (E) Two-dimensional histogram (logarithmic scaling) of the translational (k_T) and rotational (k_R) exponents for WT cells, showing three peaks for adherers (W, P, and R), one for diffusers (D), and one for swimmers (S). The distributions in panels D to F are based on 139,335 cells for WT and 10,582 cells for ΔFF, and data are weighted for trajectory durations.

to determine the position r, orientation θ, and projected length l_p of each cell (movie S1) and track these in time. Typical analyzed trajectories are shown in Fig. 1B and movie S2. Quantifying these trajectories allows us to classify cells' adhesive phenotypes.

Focusing first on translation, we identified for each cell the point of adhesive phenotypes. The initial analysis misidentified some diffusers as adherers and vice versa, for example, because of the width of the distributions and a few adherers that pivot around their poles and swing out of plane, showing up as rapid short-time motion. To resolve this issue, we further required that adherers be visible for at least 6 s. Trajectories with k_T ≤ 0.6 and shorter than 6 s were labeled as ambiguous. For trajectories greater than 6 s, we also calculated the translational exponents for the longer delay time of t = 4 s; we then used this exponent, k_T^4, as a cell's translational exponent if both k_T < 0.6 and k_T < k_T^4 and identified the trajectory on this basis.

Visual inspection of trajectories (movies S3 to S7) revealed correct automated classification of ≈ 95% of all cells using this algorithm. The shallow minimum in P(k_T) demarcating the D and S subpopulations coincides with the cutoff in P(k_T) measured for the ΔFF mutant (Fig. 1E, gray), which does not swim.

Similarly, we fitted the mean-squared orientational displacement (MSOD) = (θ(t + τ) − θ(t))^2 = C_0τ^k_R for τ < 0.4 s. The probability distribution of k_R, P(k_R), for WT adherers shows two peaks falling off to a long “tail” at k_R ~ 2 (Fig. 1E). We interpret k_R ~ 0 as corresponding to adhering cells that “wobble” (W) around a fixed orientation; k_R ~ 1 as corresponding to cells that “pivot” (P) around a fixed attachment point and undergo apparently free rotational diffusion; and k_R ~ 2 as corresponding to cells that “rotate ballistically” (R).

Only adherers show three rotational modes. Diffusing and swimming cells both appear only as a single mode in k_R. This pattern is clear in the two-dimensional heat map of [k_T, k_R] (Fig. 1F), which shows three features for adherers (W, P, and R), one for diffusers (D), and one for swimmers (S). Of the three different adhering modes, the active rotators
observed for WT strains were not found in ΔFF cells, which is reflected by the missing tail at $k_R > 1.3$ for ΔFF (gray area in Fig. 1E). We attribute ballistic rotation to the presence of active flagella (27), which we further verified using a strain modified to permit flagella staining (movie S8) (28). We observed that most rotating cells were found adhering with a point on or close to the body. From the movies, it appears that active rotation originated from direct adhesion by a rotating motor or short filament or, indirectly, by freely moving flagella rotating an otherwise adhering cell. We also observed cells “tethered” by a flagellum with the cell body not attached to the surface, but these occurrences were rare. Flagella-mediated adhesion is implicated in biofilm formation and pathogenicity (29, 30). It may be electrostatically mediated (31–33), so that its rarity ($\leq 6\%$ of cells; cf. Fig. 1E) under our high-ionicity conditions (Debye screening length $k_D^{-1} = 1\, \text{nm}$) is perhaps unsurprising. Hereafter, we mostly neglect active rotators and focus on wobblers and pivoters.

The pivoting state is particularly intriguing: The diffusive angular motion implies that the cell is attached at a single, freely rotating locus. We observed many cells pivoting more than $2\pi$ within a single movie. Virtually no pivoters were observed in the adhesion of sterically stabilized synthetic bacterium-shaped hollow silica particles (fig. S1) (34), so that such free rotation is not a generic feature of colloidal rods attached to surfaces. Neither is it due to surface organelles, because pivoters were well represented in WT and ΔFF strains and in further mutants ΔFH and ΔFFH, which additionally lacked the hook protein (fig. S4). We later examine this pivoting behavior in more detail.

In sum, tracking the translational and rotational motion of E. coli at a borosilicate glass surface has revealed a variety of adhesive behavior: Some cells adhere, others do not; of the adherers, a small number rotate ballistically, whereas most either wobble or undergo pivoted rotational diffusion. The question naturally arises: Is this variety due to cells switching between different modes of behavior within our observational time scale or due to the presence of different kinds of cells? In statistical physics terms, is the “disorder” (presence of multiple modes of behavior) annealed or quenched? To answer this question, we turn our attention to the adhesion dynamics.

**Adhesive propensity is phenotypically heterogeneous**

We followed the arrival of cells on the lower capillary surface from the bulk (shown schematically in Fig. 2A) and observed the buildup of the different subpopulations to a steady state (Fig. 2B). The time between sealing the capillary and beginning observations ($\leq 10\, \text{min}$) was long enough for the near-surface swimmers to achieve dynamic equilibrium with the bulk (35), so that we observed a roughly constant number of near-surface swimmers. Meanwhile, the number of adherers and diffusers both increased steadily before saturating after $\sim 2\, \text{hours}$, which is consistent with nonswimmers sedimenting through the $h = 400\, \mu\text{m}$ capillary at an independently measured speed of $v_s = 0.06 \pm 0.01\, \mu\text{m} \, \text{s}^{-1}$. There was no detectable delay between the arrival of the first diffusers and the first adherers (Fig. 2B), so that adhesion is rapid on the scale of 5 min. Repeating with ΔFF mutants gave qualitatively similar dynamics except for the absence of swimmers and rotators (Fig. 2C). From this, we infer that flagella and fimbriae are not essential to the observed WT phenomenology.

Figure 2B shows that more than half of the population does not adhere to the surface and that this fraction stays approximately constant over 14 hours. We will argue that this is primarily due to inherent, phenotypic heterogeneity between the cells. However, we must first exclude the alternative possibility that the incomplete adhesion is maintained by a dynamic equilibrium between surface binding and

**Fig. 2. Capillary inversion assay.** (A) Schematic of adhering (blue), diffusing (olive), and swimming (red) cells in the capillary at loading (0 hours) and 3 hours after, when nonmotile cells have sedimented, and motile cells have reached the top and bottom surfaces. (B) Number of adherers, diffusers, and swimmers on the lower surface as a function of time for WT and (C) for ΔFF. (D) Schematic immediately (0 hours) and 3 hours after capillary inversion. (E) Number of cells on the lower surface after inversion for WT and (F) for ΔFF, where the number of adhering cells on the upper surface is also given. Small points are average values for single movies, and large points are weighted averages over groups of multiple movies, with vertical bars as the SE and horizontal bars as the time window for each point. The lines in panels B, C, E, and F are results from simulations with a minimal kinetic model.
unbinding of otherwise identical cells. For this, we use an inversion assay (36), in which we inverted the capillary and observed that nonadhering diffusing cells sediment toward the new lower surface, which, at this point, is virtually empty (Fig. 2D, left panel). When the sedimenting cells reach the new lower surface around 1 to 2 hours after inversion (Fig. 2, E and F), almost none of them adhere. This is inconsistent with a dynamic equilibrium between otherwise identical cells, for which we would expect the same ratio of diffusers to adherers on the lower surface before and after inversion. Therefore, we reason that there are differences between the adhesive properties of individual cells. In statistical physics terms, the disorder in adhesive propensity is quenched.

We next focus on adhering cells and distinguish between wobblers and pivoters on the basis of their MSOD. We report data for ∆FF mutants, for which there are no active rotators. As cells arrived on the lower capillary surface, the number of wobblers and pivoters increased linearly before saturating, showing a constant P/W ratio of 3:1 throughout (Fig. 3A). After capillary inversion, the dynamics of these two subpopulations diverged: Pivoters left the (new) upper surface at least an order of magnitude faster than wobblers, whose number remained almost constant (Fig. 3B). Thus, wobblers appear more tightly bound.

Over longer time scales, there is a slight increase in the number of adhering cells on the new lower surface for both ∆FF and WT cells. This increase is due to previously adhering cells detaching slowly from the new upper surface and sedimenting downward and reattaching to the new lower surface. We can show this by monitoring the decay in adherer numbers remaining on the (new) upper surface (green squares in Fig. 2F). Fitting the adherer number N to N(t) = N(0)e−γt returns a detachment rate of γ ≈ 0.032 hour⁻¹. After the time it takes for detached adherers to sediment through the capillary (less than 2 hours), all of these cells appear at and re-adhere to the (new) lower surface, with the rate of this attachment being approximately equal to the detachment rate, as one would expect from our picture. This shows that the adhesion properties of individual cells are preserved in time: Cells that were previously adhering on the (new) upper surface, detach, and sediment downward are capable of reattaching on the (new) lower surface.

**Postadhesion dynamics are also phenotypically heterogeneous**

We next focus on adhering cells and distinguish between wobblers and pivoters on the basis of their MSOD. We report data for ∆FF mutants, for which there are no active rotators. As cells arrived on the lower capillary surface, the number of wobblers and pivoters increased linearly before saturating, showing a constant P/W ratio of 3:1 throughout (Fig. 3A). After capillary inversion, the dynamics of these two subpopulations diverged: Pivoters left the (new) upper surface at least an order of magnitude faster than wobblers, whose number remained almost constant (Fig. 3B). Thus, wobblers appear more tightly bound.

The rate of arrival of pivoters on the (new) lower surface (Fig. 3C) equaled the rate of their disappearance from the (new) upper surface (Fig. 3B), with a delay in arrival consistent with sedimenting through 400 μm. The buildup of wobblers on the (new) lower surface commenced ≳ 1 hour after the arrival of the first pivoters, suggesting that pivoters may become wobblers. To confirm this, we monitored adherers on the lower surface before inversion over 8 hours in the steady state and measured the fraction of time Fw, each cell spent as a wobbler (Fig. 3D): ~ 70% of cells were always pivoting, whereas the remainder switched between wobbling and pivoting.

The data in Fig. 3D are not consistent with a single population of adherers stochastically switching between wobbling and pivoting states, for which we expect a broad distribution peaked around some intermediate Fw. The sharp peak at Fw = 0 (always pivoting) implies at least two populations: a stochastically switching fraction and a pure pivoting fraction.

The interstate switching dynamics can be quantified (Fig. 3E) by a time-dependent transition matrix, P(at,Δt), giving the probability of a cell in state “a” at time t being in state “b” at time t + Δt, where [a] = {P, W} and [b] = {P, W, O}, with P, W, and O standing for pivoting, wobbling, and off, respectively, with the latter denoting a cell detaching

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**Fig. 3. Polydispersity in adhering cells for ∆FF.** (A) Number of wobbling and pivoting cells on the lower surface before capillary inversion and (B and C) on the upper and lower surface after capillary inversion. Solid lines in panels A to C are from simulations with a minimal kinetic model. Data correspond to the inversion experiments in Fig. 2 (C and F). (D) Histogram showing the fraction of time cells are identified as wobbling on the lower surface before inversion, as in experiments (from a total of 117 movies over six different positions recorded between 4 and 12 hours before capillary inversion) and simulations. (E) Probabilities for a cell in a wobbling (W) or pivoting (P) state at time t to be found wobbling, pivoting, or off the surface (O) at a later time t + Δt (lines are for the kinetic model). (F) Schematic of weakly and strongly adhering cells: filled red circle, bound adhesive patch; empty circles, unbound adhesive patch.
from the surface altogether. There is significant P ↔ W switching on a
time scale of several hours. However, some cells are mostly either
the P state or the W state: Only ~10% of originally P cells become
W cells and ~30% of originally W cells become P cells. The discernible
rate of W → O transitions explains the slow buildup of wobblers on the
(new) lower surface after inversion (Fig. 3C). Direct visual analysis of
the trajectories confirms that W → O transitions occurred via the se-
quence W ↔ P → O. This implies that wobbling cells detaching from
the upper surface first become pivoters before they detach and that
they reattach as pivoters before becoming wobblers. On the lower sur-
f ace before inversion, detachments and reattachments of cells oc-
curred at a steady rate over time scales of hours. Detached cells were
rarely observed to reattach at the same site but almost always at
alternative locations on the surface (fig. S5 and movie S9), which again
demonstrates that adhesion is not limited by a low number of binding
sites on the glass substrate.

In sum, we conclude that adhering cells are also phenotypically het-
erogeneous and can either be purely pivoting or switch between pivot-
ing and wobbling states. Moreover, a pivoting cell may relatively easily
be at any of these patches and appear as a wobbler, pivoter, or a free diffuser.

A patchy-colloid model explains *E. coli* adhesion dynamics

In a simple model consistent with our observations (Fig. 3), cells may
have 0, 1, or ≥2 adhesive patches available for simultaneous surface
binding. Cells without patches are permanent nonadherers. Cells with
1 patch are "pure pivoters"—such a cell bound to a surface using its
single adhesive patch may detach stochastically because of thermal
motion. Cells with ≥2 patches may be actually bound to a surface at ≥2, 1,
or 0 of these patches and appear as a wobbler, pivoter, or a free diffuser.

Note that our ΔFF mutant data show that these patches are not neces-
sarily (if at all) associated with flagella or fimbriae. Moreover, in our
model, cells with at least one available adhesive patch will eventually
encounter the surface with this patch because of rotational diffusion.
This mimics reality, where the rotational self-diffusion time in bulk
τ_{rot} \approx \frac{(2\pi D_{rot})^2}{4\pi D_{rot}} \approx 1 \text{ min for a free cell}
(assuming typical measured values for } D_{rot} \text{ for deflagellated *E. coli*} \text{ (37), so that it can explore many
different orientations during an experiment.}

The observed rate at which pivoters leave the upper capillary surface
after inversion (γ = 0.032 hour⁻¹; Fig. 3B) allows us to estimate the adhes-
ion strength of a single patch. A particle confined to a potential of
depth ΔU escapes at rate (38)

\[
γ = \left( \frac{k_B T}{2\pi k_B T} \right)^\frac{1}{2} \exp \left( -\frac{\Delta U}{k_B T} \right)
\]

Here, \( k_B \) is Boltzmann’s constant, \( T \) is the temperature, and \( D \) is the
particle’s diffusivity inside a potential well of stiffness \( k_B \). Dimensional
analysis suggests that \( k_B \) is ΔU/L², where \( L \) is the interaction range.
From AFM, we estimate 20 ≤ Λ ≤ 100 nm for bacteria adhering to a
variety of surfaces (39, 40). Separately, from the typical maximum MSD
of the anchoring coordinate of pivoting cells, we estimate \( L \approx 200 \text{ nm} \)
(fig. S6). ΔU is rather insensitive to the exact value used. Taking a con-
servatively broad range of 5 nm < \( L < 150 \text{ nm} \) and \( D = 0.4 \mu \text{m}^2 \text{s}^{-1} \) (41),
we obtain 15 k_B T < ΔU < 23 k_B T. This is well within the range of, for
example, electrostatic and hydration effects (42) and hydrophobic
interactions (43) between glass and proteins (44) or lipopolysaccharides
(45). We note that if electrostatic attractions were to play a role
in adhesion, these are likely very local because of the smallness of the
Debye screening length \( (k_D = 1 \text{ nm}) \) and the fact that the bacterial
surface bears a net negative charge (zeta potential = -16 mV measured
for our WT strain). The latter observation means that any electrostatic
attraction with a negative glass surface (33) must be due to localized
minority positive charges on the bacterium arising from, for example,
amine and hydroxyl groups at around neutral pH (9).

In a previous work where an inversion assay was used (36), it was
suggested that gravitational pull might contribute to the detachment of
cells from the upper surface (after inversion). The potential energy
difference due to gravity for displacing a cell over a distance comparable
to the interaction range, \( U_g \approx 0.05 k_B T \), is much smaller than our esti-
mated binding energy of an adhesive patch and therefore has a negligi-
ble effect on the detachment rate. Nonetheless, gravity causes a more
subtle difference between the adhesion dynamics on the lower and
upper surfaces. Once a cell on the upper surface detaches, it immedi-
ately starts sedimenting downward (away from the surface), making it highly
improbable that it will reattach. On the lower surface, detaching cells
remain close to the same surface and reattach to it eventually.

To deduce the likely maximum number of adhesive patches on our
cells available for simultaneous surface binding, we consider the sta-
tistics of the point of minimum translation ("anchoring point," \( A \) ≤ 0.5;
Fig. 1B, inset). The distribution of anchoring points for wobblers (≥2 patches)
is given by some weighted average over the individual
patch locations (see the Supplementary Materials) and so must be
narrower than that for a pivoter with a single patch. Figure 4 plots the
observed normalized distribution of the anchoring coordinate \( |A| \) for
pivoters (Fig. 4A) and wobblers (Fig. 4B), being narrower for the latter
(SDs 0.27 and 0.22, respectively). We modeled the anchor-point
distribution for adherers with \( n \) patches (Fig. 4B), assuming that these
patches are placed along the cell axis according to the P(|A|) for pivoters,
and that the anchoring coordinate for a wobbler is the average of the
coordinates for these patches (see the Supplementary Materials). Our
data suggest \( n = 2 \) or 3. This is as expected: Higher \( n \) would mean that
pivoters and nonadherers should be very rare and that wobblers would
almost never detach, contrary to observations.

Note that the experimental distribution of |A| for pivoters is slightly
peaked near the cell center (|A| ≈ 0) and toward the cell pole (|A| ≈ 0.3)
(Fig. 4A). The former is an artifact: A spherocylindrical cell adhering
at one pole will appear as a small circle with an anchoring point iden-
tified in the cell center, artificially shifting the distribution toward |A| = 0.
This can be removed by excluding cells with small projected lengths
(orange line), although this does not significantly modify the predicted
P(|A|) for wobblers. The other peak indicates that cells have a slight pref-
ference for polar adhesion, as previously found for other strains (36, 46).
The observation that the peak is not at the pole itself is again due to the
cell’s spherocylindrical shape.

We can simulate a minimal kinetic model based on the schematic in
Fig. 3F. Bacteria are modeled as noninteracting point particles in a box
with periodic boundary conditions in the horizontal \((x, y)\) plane and
impenetrable boundaries in the vertical \((z)\) direction. Cells diffuse and
sediment toward the lower surface. For ΔFF mutants, there are three
independent subpopulations: nonadherers that do not bind to the sur-
face; weak adherers that bind to the surface to become pivoters at rate
\( k_{adh} \) when within a distance \( \Delta x_{adh} \) and strong adherers that bind to the
surface as pivoters, also at \( k_{adh} \), and can switch stochastically to a
wobbling state or back, respectively, with rates \( k_{wpw} \) and \( k_{wpw} \). Both weak
and strong adherers can only detach from the surface, at rate \( k_{adw} \), when
in the pivoting state.
The model uses values of the diffusivity and sedimentation speed based on independent measurements, and $\Delta z_{\text{ab}} = 100 \text{ nm}$, in line with the typical interaction range. Fitting the on-surface switching dynamics (Fig. 3E) gives estimates for $k_{\text{PW}}, k_{\text{WP}},$ and the ratio of weak to strong adherers. Varying the remaining parameters, $k_{\text{adh}}, k_{\text{anot}}$ and the total numbers of adherers and nonadherers credibly reproduces the main features of all our observations (curves in relevant parts of Figs. 2 and 3). We model the WT cells in the same way, except that a fraction of cells can swim and a subfraction of those can adhere as active rotators. The cell numbers are fitted, but the swimming speed is measured independently. Full model details are given in the Supplementary Materials, including used cell numbers (table S1) and movies depicting the simulations (movies S10 and S11).

**DISCUSSION**

We investigated the adhesion of *E. coli* AB1157 in buffer solution to glass. Using high-throughput and big-data analysis methods, we found strong heterogeneities between individual cells within a clonal population that are not related to type 1 fimbriae or flagella. These heterogeneities appear in the propensity for adhesion as well as the postadhesion dynamics. At least two types of adherers exist. Freely pivoted weak adherers are rotationally unconstrained and detach relatively easily. Strong adherers switch between this pivoting state and a rotationally constrained wobbling motion, in which they are more firmly bound to the surface.

A model of cells with adhesive patches that bind and unbind stochastically to the surfaces can explain our results. In this model, what differentiates subpopulations is the number of accessible patches per cell. Nonadhering cells have no patches and are always freely diffusing, weak adherers have one patch, and strong adherers have multiple adhesive patches that can bind simultaneously. These patches are estimated to have an interaction range of $\lesssim 100 \text{ nm}$ and contact energy of 15 to 23 $k_{\text{B}}T$. A very recent direct measurement of two Gram-positive species of *Staphylococcus* bacteria (47) shows that their adhesion to surfaces is mediated by sticky patches of radii $\sim 100$ to 300 nm, from which an interaction range of $20 \lesssim L \lesssim 120$ nm is obtained. Thus, the physical mechanisms we have deduced from our experiments for *E. coli* may well be generic, even where specific details vary, for example, the interaction strengths measured in (47) are much larger, at $>10^7 k_{\text{B}}T$.

However, the properties of the bacterial surface depend on many variables. Thus, for example, the acid-base properties of the surfaces of *E. coli* K-12 and *Bacillus brevis* cells are dependent on species, growth phase, as well as the composition of the growth media (48). These properties also change upon adhesion (9). When initial adhesion leads to the formation of a full-blown biofilm, different adhesive proteins are involved in binding cells to the substrate or to each other (10, 49). Both population variations in adhesion strength (50) and the location on the cell surface of adhesive interactions (51) are of importance. The study of a variety of organisms under diverse conditions will therefore be needed to establish a full picture.

Our work raises important questions about the biomolecular details of adhesion, most obviously the nature of the adhesive patches. Their rotational freedom implicates membrane proteins, which can rotate freely in lipid bilayers (52), whereas the interaction range of $L \sim 100 \text{ nm}$ suggests the involvement of cell surface macromolecules, consistent with a recent suggestion based on more direct, AFM measurements (47). Such rotational freedom raises the further question of the possible translational freedom of these patches, because individual proteins can also translate diffusively in the bacterial outer membrane (53). Both motions are strongly dependent on temperature because of lipidic phase transitions. If future work finds these adhesive patches to have temperature-dependent mobility, then bacteria will constitute a new class of patchy colloids. The interaction of mobile adhesive patches with rough (54) and patchy (19) substrates offers intriguing possibilities.

Another key question is the origin of the phenotypic heterogeneity we observed. This may be an instance of phase variation—the reversible switching on and off of gene expression (55), often of surface structures (56). On the other hand, adhesion may trigger a process of diversification in gene expression analogous to the process discovered recently in biofilm formation (57). Elucidating these issues using modern tools for time-dependent gene and protein expression assays is now an urgent task.

Finally, our work has implications for the design of antiadhesion surfaces, which is a key part of the ongoing fight against bacterial infections. First, it appears that we must now think in terms of designing antiadhesion surfaces that account for the range of adhesive phenotypes. Second, designing antiadhesion technologies will involve computer simulations, which, however, will never be fine-grained enough to include all details. Our finding that bacteria are patchy colloids vis-à-vis surface adhesion can help to design viable coarse-graining strategies and...
provide the necessary parameters for the computational study of this important phenomenon.

**MATERIALS AND METHODS**

**Bacterial suspensions**

All bacterial cells (E. coli AB1157 WT and various mutants; see the Supplementary Materials) were initially grown on LB agar plates, then inoculated and transferred to 10 ml of liquid LB, and incubated for 16 hours at 30°C. A fresh culture was inoculated as 1:100 dilution of overnight grown cells in 35 ml of tryptone broth and grown for 4 hours (to late exponential phase). Cells were washed three times with MB [6.2 mM K2HPO4 (Sigma-Aldrich), 3.8 mM KH2PO4 (Fisher Chemical), 67 mM NaCl (Fisher Chemical), and 0.1 mM EDTA (Sigma-Aldrich)] by careful filtration and were resuspended in MB at an optical density of ≈ 0.03 (WT) and ≈ 0.01 (ΔFF) at 600 nm, corresponding to ≈ 4.5 × 107 (WT) and ≈ 1.5 × 107 (ΔFF) cells/ml (23). Just before starting the adhesion assays, glucose solution in MB was added to the dispersion (dispersion contains 0.72 mM glucose after addition). We measured the electrophoretic mobility of the bacteria μE using a Malvern Zetasizer Nano Z.

**Adhesion assays**

Cell suspensions were loaded into borosilicate glass capillaries (Vitrocom, 0.4 × 8.0 × 50 mm) subsequently sealed with Vaseline. We recorded movies (1040 × 1024 pixels, 30 frames/s) focused on lower or upper capillary surfaces using a Mikrotron MC1362 camera on an inverted Nikon Ti-U microscope with a 60x phase-contrast objective (Nikon Plan Fluor, Ph2; numerical aperture, 0.7). Custom LabVIEW software controls the camera, a motorized stage (H117P21N4 with ProScan III, Prior Scientific), and a piezo objective actuator (P-725.4CD with E-753, Physik Instrumente) to automatically record movies at multiple locations on the inner capillary surface in time (23).

**Analysis**

For each movie, we filtered out background signals and noise, after which we determined the positions, orientations, and projected lengths of bacterial cells (41). We determined the positions, orientations, and projected lengths of bacteria using an adapted algorithm specifically developed for rod-shaped colloids (24) and tracked bacteria in successive frames to obtain continuous trajectories. The suspension was so dilute that cells rarely collided, but those sections of trajectories crossing closer than 2.5 μm were omitted from the analysis. Other remaining misidentifications (for example, two neighboring cells recognized as one) were rare and did not affect the results significantly. Trajectories shorter than 0.8 s were not considered for classification. Trajectories displaying an adhering signature kT < 0.6 but shorter than 6 s were considered ambiguous.

**SUPPLEMENTARY MATERIALS**

Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/4/4/eaao1170/DC1

**REFERENCES AND NOTES**

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