Transient Surface Hydration Impacts Biogeography and Intercellular Interactions of Nonmotile Bacteria

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ABSTRACT There are many hydrated surface niches that are neither static nor continuously flowing that are colonized by microbes such as bacteria. Such periodic hydrodynamic regimes are distinct from aquatic systems where microbial dissemination is reasonably predicted by assuming continuous flow or static systems where motile microbes largely control their own fate. Here, we show how nonmotile bacteria exhibit rapid, dispersive bursts of movement over surfaces using transient confluent hydration from the environment, which we term “surface hydrodispersion,” in which cells traverse thousands of cell lengths within minutes. The fraction of the population disseminated by surface hydrodispersion is small—on the order of 1 cell per million. Thus, surface hydrodispersion can promote isolated distribution of single cells, which is unlike other characterized active and passive surface motilities. We describe this translocation using a continuous-time random-walk modeling approach and found in computational simulations that transient fluid accumulation, dilution, and gravitational pull are the contributing factors. Surface hydrodispersion, consistent with advection, is unlike simple colony expansion, as it dramatically alters spatial relationships, shown in this study with *Staphylococcus aureus*, which becomes increasingly virulent when isolated from *Corynebacterium striatum*. Surface hydrodispersion of nonmotile bacteria exploiting transient fluid availability and gravity is a mechanism that can result in sporadic and sudden shifts in microbial community behavior. To better understand how this movement can impact biogeography on the millimeter scale, this work describes a system for study of primary factors behind this movement as well as a stochastic model describing this dispersal.

IMPORTANCE Understanding the dynamics within microbiome communities is a challenge. Knowledge of phylogeny and spatial arrangement has led to increased understanding of numerous polymicrobial communities, yet these snapshots do not convey the dynamics of populations over time. The actual biogeography of any microbiome controls the potential interactions, governing any possible antagonistic or synergistic behavior. Accordingly, a shift in biogeography can enable new behavior. Little is known about the movement mechanisms of “nonmotile” microbes. Here, we characterize a universal means of movement we term hydrodispersion in which nonmotile bacteria are transported thousands of cell lengths in minutes. We show that only a small fraction of the population is translocated by hydrodispersion and describe this movement further using a random-walk mathematical model approach *in silico*. We demonstrate the importance of hydrodispersion by showing that *Staphylococcus aureus* can separate from a co-culture inoculation with *Corynebacterium striatum*, thus permitting transition to a more virulent state.

KEYWORDS dispersion, stochastic modelling, *Staphylococcus aureus*, *Corynebacterium striatum*, motility, polymicrobial, stochastic modeling

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Microbial communities play a critical role in our world, contributing to environmental sustainability (1), regulating metabolic health (2), protecting against virulent microbes (3, 4), and even influencing our moods (5). Understanding the dynamic nature of these microbial communities within two-dimensional (2D) and 3D space is a challenge. Knowledge of the species present and their spatial arrangement has brought tremendous insight into the role of microbiomes (6, 7). How dispersal impacts microbial populations remains a complex issue, but it is clear that dispersal impacts biogeography (8). Biogeography (specifically microbiogeography [7]) sways the interactions within a population, governing individual, combined, and synergistic behaviors (7). Shifts in biogeography can shift biochemistry and promote new behaviors and could mean the difference between carrying commensal *Staphylococcus aureus* in the nasal cavity and having a sinus infection (9). The proximity of different microbes to each other can substantially affect their function and subsequent activities, such as pathogenesis (10) or geochemical cycling (11). Thus, describing and differentiating microbial dispersal and subsequent dispersal patterns has great importance. This work describes bacterial dispersal by transient fluid flow, which was found to differ from simple linear flow and provides a statistical model that differentiates this translocation from well-characterized bacterial spreading.

In static liquid environments or on surfaces, bacteria are known to move by active or passive means. Active motility requires energy expenditure and relies on one or more appendages, specialized proteins, and surfactants (12–14). Passive motility uses only biological agents that help cells slide, such as secreted factors or modified cell surfaces (15–17). For example, mycobacteria and *S. aureus* produce surfactants and slide away from the colony center, powered by cell division (18–21). Despite the perceived advantage of microbial motility in colonization, many known microbes have not been characterized as encoding regulatory mechanisms for active or passive motility.

In aquatic environments, the dissemination and fate of bacteria and other microbes are most greatly influenced by flow patterns. Our current understanding of microbial dispersion on surfaces is primarily drawn from assuming scalable hydrodynamic dispersion of a homogeneous microbial population: neither of these basic assumptions is correct. Dispersal by ocean currents and dendritic river flow is well documented (22, 23). However, in unsaturated systems this is more complex. The porous-surface model allows for study of microbial movement in controlled, static liquid films to simulate unsaturated conditions (24). Other research has assessed the distribution of microbes in unsaturated zones in comparison with the mobility of organic matter (25, 26). When considering microbial environments on a timescale of hours to days, there are many hydrated surface niches that are neither static nor continuously flowing. Near-subsurface soils subject to periodic precipitation, tide pools, bathrooms, and the human nasopharynx and skin all exhibit surfaces that harbor bacterial growth for which confluent hydration is transient and periodic. The impact of small, transient volumes of fluid in such environments is sparsely studied.

Exploiting the transient hydration of a soft agar bacterial plate for dispersal and subsequent growth of bacteria, we observed rapid translocation carried out by three representative “nonmotile” and coculturable bacterial species identified from prosthetic joint infections, *Staphylococcus aureus*, *Enterococcus faecalis*, and *Corynebacterium striatum*. “Surface hydrodispersion” described here is a passive mechanism of microbial movement, consistent with advection but not well characterized in the current literature. It is notable because of its high velocity, sporadic nature, lack of required factors, and ability to dilute, isolate, and redistribute cells. Of course, such movement of nonmotile cells over great distances should also transport cells to unfavorable habitats (in contrast to directed active transport [27]). We showed that hydrodispersion is reproducible and predictable by controlling water availability in plate assays, and we demonstrated an ability to recapitulate this movement *in silico* by simulating the population fractions subject to gravitational pull and diffusion. We found that hydrodispersion significantly restructures biogeography on a scale several orders of magnitude larger than that of single cells.

It is now clear that microbes exhibit biogeography, but how phenomena at different scales contribute to influencing population heterogeneity and shaping range limitations remains poorly understood (28, 29). Our reductionist approach sheds light on environmental forces, timing, and bacterial dispersal patterns that characterize the surface hydrodispersion of nonmotile bacteria in transiently hydrated environments to better understand how these microbes contribute to microbial community formation. We believe that this movement impacts a wide variety of populations, ranging from polymicrobial infections to household environments and the rhizosphere, that experience gravitational pull coupled to hydration fluctuations over time.

RESULTS

Rapid and directed movement of nonmotile bacteria. In this study, we examined the movement of nonmotile bacteria on transiently hydrated surface environments. Prosthetic joint infection clinical isolates of *S. aureus*, *E. faecalis*, and *C. striatum* (30) were grown on (0.45%) soft agar, used to study motile bacteria. We were surprised to see this diverse group of nonmotile bacteria produce a trail of colonies at the agar/plate interface, while surface cells spread only slightly around the inoculation site (Fig. 1A to D). Colonies at the plate/agar interface sometimes formed in circular regions around the inoculation site, as would be expected from a sliding motility, but more often formed radial lines of colonies to the plate edge within hours. Time-lapse photography shows colonies, of approximately the same size, trailing to the plate edge and becoming visible simultaneously around 10 h of incubation (Fig. 1E and Movie S1). The even colony size along the radial growth observed supports an interpretation of swift, rather than gradual, cell movement. Additionally, these dispersed cells were substantially diluted from the initial inoculum, which is notably different from sliding motility. Needle inoculation deposited millions to hundreds of millions of cells (Table S1), while the dispersed fraction was on the order of tens to hundreds of CFU, many of which were spatially isolated from their neighbors. Thus, roughly one in one million cells was disseminated with spacing more than sufficient to observe the resultant CFU that grew on these plates after 24 h without dilution.

Separately, we found that oxygen availability has no impact on this dispersive movement by studying the anaerobe *Propionibacterium acnes*, which also exhibits this rapid movement at the agar/plate interface (Fig. S1).

The bacteria exhibiting this movement lack both flagella and pili, appendages used for active motility. However, bacteria without motility appendages have been shown to exhibit “sliding” motility, which is aided by production of surfactants (31). There are not reports in the literature that *C. striatum* or *E. faecalis* produces surfactants. *S. aureus* does produce phenol-soluble modulins (PSMs), which have been demonstrated to exhibit surfactant properties (32, 33). Therefore, we investigated the possible contribution of surfactant production to our dispersion phenotype by testing an *S. aureus* quorum sensing mutant (*agr* mutant) strain that does not make PSMs. We found that the *agr* mutant strain spread equivalently to wild-type *S. aureus* and conclude the translocation we observed does not require surfactants.

We subsequently became interested in describing the factors driving this rapid, dispersive movement of nonmotile bacteria, and we provide a model to predict how this movement could alter bacterial interactions.

Driving forces, speed, and duration of cell movement. We hypothesized that the dispersive movement of these nonmotile bacteria should be subject to gravity. When incubated with a 20° vertical tilt, cells moved with gravity in all cases, confirming the important role of gravitational pull in this directed cell movement (Fig. 2A). Interestingly, initial assays were not deliberately tilted but often exhibited directed movement. We attribute this result to subtle undulations in our incubator shelves. (We subsequently chose regions in our incubator shelves for assay incubation that were the most flat using a bubble level.) This outcome suggests that even small gravitational effects are sufficient to promote cell distribution over great distances.

The relatively even size of colonies that formed along these radial cell distributions was suggestive that the distribution of cells occurs within a small window of the overall

FIG 1 (A to D) *S. aureus* (A), an *S. aureus* *agr* quorum sensing mutant (B), *E. faecalis* (C), and *C. striatum* (D), all nonmotile, exhibited limited spreading on the agar surface but, more notably, exhibited directed movement in the interstitial space of the plate bottom, taking cells to the edge of a petri dish with overnight incubation. (E) Time-lapse imaging of the *S. aureus* *agr* mutant shows the synchronized appearance of colonies around 11 h of incubation.

overnight incubation time required to obtain visible colony growth. Again using a 20° vertical tilt, we examined plates that were horizontally rotated 60° every 10 min over 1 h (Fig. 2B). This timed shift changed the direction of cell movement to realign with the new gravitational pull, with the resultant pattern of colonies indicating the period(s) when cells moved. Movement within a 10-min window produced a single line of colonies (Fig. 3A), while movement over a 20-min period produced a wedge-shaped cluster of colonies or a bent line of cells, reflecting the shift in movement (Fig. 3B). All species exhibited a similar trend to move within the first 10 min and complete movement within 20 min of incubation (Fig. 3C) (i.e., no strain exhibited any movement at 30 min or longer). Such timing precludes the involvement of osmotic pressures as a driving force, as seen with *Bacillus subtilis*, where osmotic forces pull fluids from the surrounding environment—but this phenomenon requires a timescale of several hours (17, 34). The movement we observed in these experiments was likely driven entirely by fluid present at the time of inoculation and its subsequent flow directed by gravitational pull. Given the necessity for fluid and absence of active biological factors required, we assign the term hydrodispersion to the spreading we observed.

Fluid recovery of movement in drier environments. Repeating our assays under conditions with increased agar (1%) showed no movement of cells. This would be expected as the bound water is increased, limiting the formation of a confluent liquid layer. However, with addition of 100 μ l of sterile water to 1% agar plates, within

FIG 2 Role of gravitational pull and time in cell movement. (A) Movement of cells with gravity. A black line at the plate edge marks the lowest point of the plate during growth. (B) Visual representation of tilt and turn experiment to determine the duration of cell movement.

FIG 3 Plates were needle inoculated, incubated at an angle, and turned every 10 min. (A) Control plates, not turned, and those having movement within a 10-min window had 100% of motile cells travelling in one line with the force of gravity. (B) Spreading over a 20-min window resulted in a “pie piece” of colonies that followed one line in the first 10 min and then a new line after the plate was turned. (C) Analysis of 40 assays of 4 nonmotile species indicates that movement predominantly began within 10 min of inoculation and transpired over a 20-min window. Movement was seen to occur within 0 to 10, 10 to 20, or 20 to 30 min, a combination of these times, or not at all. (Some assays contributed to multiple categories, for example, having movement at both 0 to 10 and 10 to 20 min, yielding a sum of all percentages exceeding 100%.) (D) All samples showed gravity-influenced hydrodispersion. Plates with 1% Noble agar left untreated or with water added showed that interstitial cell movement is recovered with addition of water.

minutes of inoculation, 70 to 100% of movement was recovered over all species tested (Fig. 3D). This confirms fluid as the vector that dilutes, carries, and distributes cells. Furthermore, this result demonstrates that a transient increase in liquid is sufficient to shift a stationary condition to a dispersive condition.

Dissemination and isolation of species promote shifts in community interactions.

The ability to disperse and isolate cells in a mixed microbial population would allow the seeding of new and novel populations, potentially altering growth, antimicrobial resistance, and virulence. We performed experiments with cocultures of *C. striatum* and *S. aureus*, which are both known inhabitants of the nasopharynx (35). *C. striatum* is known to suppress the growth of *S. aureus* and promote commensalism when associated with this pathogenic microbe (9). When *S. aureus* shifts from comicrobial to mono-microbial growth its virulence increases, posing a health threat to the host. We inoculated single- and mixed-species cells onto hard agar and spotted with 10 μ l of sterile water. These conditions promoted hydrodispersion of cells both on the agar surface and in the interstitial space between the agar and petri dish. The distribution and isolation of hydrodispersed *S. aureus* cells can be seen in a combined image (Fig. 4A). Confocal scanning laser microscopy (CSLM) of dispersed cells from mixed inoculations showed the growth of colonies resembling the individual parent species. *S. aureus* formed larger colonies than *C. striatum* (Fig. 4B to D). Following their separation by surface hydrodispersion, these isolated *S. aureus* cells had the opportunity to proliferate and exhibit increased virulence. Typically, means of active and passive transport move cells as groups, but this experiment highlighted how surface hydrodispersion can disproportionately move individual cells and transform population interactions in a way that is uncharacteristic of other movement mechanisms.

In contrast, experiments performed with cocultures of *S. aureus* and *Pseudomonas aeruginosa*, which are both known inhabitants of the cystic fibrosis lung and burn

FIG 4 (A) *S. aureus* tagged with mCherry forms colonies that are dispersed and isolated by water on a surface. Scale bar = 1,000 μm . (B and C) Monocultures of *S. aureus* (B) and *C. striatum* (C) both exhibited dispersion on agar such that individual colonies were apparent. (D) Colonies resulting from a mixed inoculum of *S. aureus* and *C. striatum* were markedly distinct, indicating single cell isolation. Lower panel scale bars = 100 μm .

wounds (36, 37), showed a different result. To negate the influence of active motility mechanisms, we mixed *S. aureus* with a nonmotile *P. aeruginosa* mutant strain which is deficient for both flagellar and type IV pilus appendages and its production of the surfactant rhamnolipid that aids surface motility ($\Delta pilA \Delta flhC \Delta rhlAB$). (Thus, the three primary mechanisms of *P. aeruginosa* motility are nonfunctional in this strain.) We found that *P. aeruginosa* and *S. aureus* spread along the same path but not equally (Fig. 5). *P. aeruginosa* dominates the dispersed cell region. *P. aeruginosa* possesses several competitive mechanistic traits, including the type VI secretion system and quorum sensing-mediated production of phenazine and cyanide, which can suppress and kill competing microbes occupying the same space (38–40). At 18 h following dispersion, both colony types were present; however, *P. aeruginosa* grew over the top of *S. aureus* colonies. By 24 h, *S. aureus* colonies were notably reduced, and by 40 h, the engulfed *S. aureus* colonies had been eliminated.

Microbial dispersal patterns change with shifts in surface angle. We sought to quantify the cellular distribution that resulted from hydrodispersion. Not surprisingly, we found that the distribution pattern changed with the influence of gravity, which we tested by analyzing experiments conducted between 0° and 4° of plate tilt. Cells dispersed shorter distances in more radially uniform patterns at no or low degrees of tilt, while increasing the tilt shifted dispersal patterns to become more linear (Fig. 6). We quantified these changes in dispersion and shape of distribution by calculating the centroid deviation, roundness, and aspect ratio using ImageJ analysis software (34). The average roundness, $\frac{4(\text{area})}{\pi \times \text{major axis}^2}$, was highest with a 1° tilt and then diminished as the major axis lengthened with increasing tilt. The aspect ratio, $\frac{\text{major axis}}{\text{minor axis}}$, similarly reflected the changing shape of cellular distribution, with a notable shift seen at the highest (4°) tilt, as dispersal patterns both lengthened and narrowed. Samples with no tilt were more susceptible to nonuniform regions with increased spreading, likely due to agar assay irregularities; this yielded values for roundness and aspect ratio that reflected shapes with inconsistent radii (Fig. 6). More uniform spreading occurred with the introduction of small gravitational influences (i.e., 1° tilt). The trends of both the roundness and aspect ratio measurements serve to illustrate the shift in hydrodispersion distribution from circular to linear as the plate tilt increased from 1° to 4°. Lastly, we also measured the change in spread from the initial point of inoculation by calculating the Euclidean distance. This distance is a calculation of centroid deviance for the

FIG 5 (A and B) Photo (A) and fluorescence (B) imaging of hydrodispersion of *S. aureus* (red) and *P. aeruginosa* (green) coculture on agar after 18 h of incubation. (C to E) *P. aeruginosa* grew over the top of *S. aureus* by 18 h (C), dominating growth at the 24-h (D) and 40-h (E) time points.

estimated shape center from the known site of inoculation, thus reflecting collective movement of cells from beginning to end. This measurement was least influenced by variances that occurred with no tilt, and a clear pattern of increasing Euclidean distance with increasing tilt was observed.

We also quantified spreading of our nonmotile *P. aeruginosa* strain ($\Delta pilA \Delta fliC \Delta rhlAB$). While differences in the dispersal shapes measured were not considerably different from those exhibited by *S. aureus*, *P. aeruginosa* patterns showed greater variance than those of *S. aureus* and *P. aeruginosa* exhibited increased hydrodispersion coverage area compared to that of *S. aureus* at low angles of incline (Fig. 6). This suggests that nonmotile bacteria are more than plain particles that might serve as simple tracers of fluid flow. Their innate biological variations in cell surface and morphology influence their hydrodispersion.

Modeling microbial surface hydrodispersion as gravity-directed movement replaces Brownian motion. We further investigated surface hydrodispersion *in silico* where cells were represented in a continuous time random-walk model in which a fraction of inoculated cells experience a random dispersive-like movement reflecting a combination of a Brownian motion and a linear movement directed by gravity. We chose this stochastic approach because we desired not only to capture the resultant pattern of dispersed cells but also to account for the fact that only a small fraction of cells actually leave the point of inoculation. With an increasing pull of gravity (i.e., tilt angle), the fraction of cells dominated by gravity increases and the microbial dispersal pattern transitions from circularly symmetric and diffused to an increasingly long and narrow line of cells. As noted above, since movement occurs early after inoculation, this movement is independent of cell growth. In the presence of transient hydration, we found that gravitational pull is the predominant force shaping cell movement patterns.

Following a continuous time random-walk philosophy, the motion of bacteria during the hydration period is governed by the following Langevin equation:

$$x_{i+1} = x_i + (1 - M)\xi + M\eta$$

$$t_{i+1} = t_i + \tau$$

where x_i is the location vector of a cell at step i ; $1 - M$ is the fraction of the jump made up of a Brownian motion and, correspondingly, M is the fraction attributed to the gravitational jump; ξ is a 2-dimensional vector whose components are normally distributed random variables with zero mean and variance reflecting diffusive jumps, which is parameterized with the case where no gravitationally oriented hydration is observed; η is a vector whose components align with the direction of gravity and whose values are

FIG 6 Variation in 2D geometric patterns for dispersed cells of *S. aureus* USA300 and *P. aeruginosa* $\Delta pilA \Delta fliC \Delta rhlAB$ with increasing plate tilt. (a) Roundness; (b) aspect ratio; (c) centroid deviance; (d) area.

chosen from a uniformly distributed positive definite random variable; and τ is a random waiting time, which for most particles is much longer than the hydration period. In our experiments, the hydration period was held constant to 10 min. Any particle whose waiting time is longer than the hydration period does not move, meaning that only a small fraction of bacteria actually move. A uniform distribution of jump sizes is chosen for parsimony and a lack of sufficient statistics to parameterize the distribution comprehensively, but any appropriate positive definite distribution should reproduce similar movements, and as data become more available and detailed mechanisms are understood, improved distributions may become attainable (e.g., by Bayesian updating). The range of the uniform waiting time distribution is chosen such that statistically during any given realization, a reasonably small fraction of particles (~ 1 in 10^6) move.

Representative images of cell distributions from *in silico* experiments conducted using this model were generated over a range of M values (Fig. 7E to H and Fig. S2). Again, M is equivalent to the cell fraction subject to gravitational pull, and we found that all patterns exhibited our plate assay experiments associated with M values of < 0.2 . Roundness, aspect ratio, and centroid deviance were measured as a function of M and compared to experimental plate tilt assay results (Fig. 8). Assessment of these *in silico* results revealed that roundness and centroid deviance changed most dramatically when M was low and few cells jumped to the gravity-influenced state. The aspect ratio changed little at the lowest gravitational influences ($M < 0.05$) but then increased

FIG 7 (A to D) Resultant cell dispersal patterns of *S. aureus* USA300 with increasing plate tilt and (E to H) correlated images of *in silico* experiments using a random-walk model for increasing values of M (the gravity-influenced state).

gradually with increased values of M (>0.2), due to the continued narrowing of cells as gravitational forces increased.

DISCUSSION

The movement of nonmotile bacteria on transiently hydrated surfaces is poorly understood, with gaps in our current understanding of microbial dispersal mechanisms and subsequent bacterial community formation. Here, we characterize the rapid, fluid-driven dispersal mechanism of nonmotile bacteria that is relevant to surfaces subject to periodic changes in hydration. During the relatively short periods of confluent hydration in our experiments, we found that surface hydrodispersion is explained by a mechanism of gravity advection that has been used to describe distribution of bacteria in large water bodies (41). Strikingly, our laboratory and simulation experiments highlighted how this dispersal differs greatly from colony expansion (i.e., growth), where cell-cell contact and community interactions are generally maintained over time. Surface hydrodispersion promotes the isolation of individual cells in newly colonized areas, which more radically and stochastically affects biogeography. Such periodic and transient hydration events are remarkably common in a variety of environments, including intertidal zones, upper vadose soil layers, bathrooms, and the human nasopharynx, armpit, and lung.

Small quantities of free fluid combined with gravitational forces promote a transition from radial to directional transport, which has the capacity to move bacteria thousands of times their cell length in minutes. We found that movement in the environment tested occurred within a 20-min window and then ceased. Cells in marginally drier environments lost this movement but regained it upon addition of fluid. Hence, changes in a microbial landscape from surface hydrodispersion would be rapid, vary in symmetry, and occur at irregular intervals, and cells may traverse relatively long distances. Long distance and rapid displacement without chemotaxis may promote the transport of these cells through unfavorable environments to new regions in a way that is not possible for microbes employing active motility.

FIG 8 Measurements of distribution shape and movement from *S. aureus* USA300 experiments as a function of increasing tilt and increasing *M* (the gravity-influenced state) from *in silico* data. (A) Centroid deviance from initial point of inoculation; (B) distribution roundness; (C) aspect ratio. Gray circles show measured values from individual tilt experiments with *S. aureus* USA300, and black squares show measurements from *in silico* experiments.

Patterns of hydrodispersed cells differ from those resulting from other active and passive surface motilities, such as swimming, swarming, and sliding (Table 1). These previously described active and passive surface motility mechanisms all depend upon one or more genetic determinants and a cascade of signal transduction events. Surface hydrodispersion, however, occurs without quorum sensing, surfactants, appendages, or special proteins. In fact, surface hydrodispersion resulted in isolated distribution of single cells, which is unlike other described translocation mechanisms. Hydrodispersion occurs in the absence of cooperation, or symbiosis, with a motile partner (42). We have additionally demonstrated that surface hydrodispersion occurs with anaerobic nonmotile bacteria. As the primary requirement is transient fluid flow, we believe that this mechanism of movement and dispersion of cells is quite common, yet it is not fully understood.

TABLE 1 Comparison of hydrodispersions with active and passive motility types

Movement type	Motilities included	Pattern of growth in homogeneous environment (shading represents microbial dispersal)	Distribution patterns	Required factors	Cellular interactions	Timescale to travel centimeter distances
Active transport	Swimming twitching swarming gliding		Generally radial, but can be shaped by chemotaxis, fluid availability or cell death in a heterogeneous environment	Flagella, pili, surfactant	Colony expansion that maintains interactions, but separation based on capacity for motility	Minutes to hours
Passive Transport	sliding		Radial expansion without chemotaxis	Surfactant, specialized surface proteins, cell division	Colony expansion that maintains cellular contact and interactions	Hours to days
Surface hydrodispersion	n/a		Dispersed cells, radial to linear as gravitational forces increase, no chemotaxis	Transient fluid flow, interstitial space, gravity	Random isolation of cells, dispersal through unfavorable environments	Minutes

Another distinction between this dispersal method and other surface motilities involves cellular contact and interactions. We show how this movement dilutes and isolates cells. In a mixed population, such movement can alter interactions that dictate population behaviors. Both active and passive surface motilities maintain relatively close cellular interactions as communities expand. Passive motility pushes cells of the population into new regions, powered by cell division. Active motility may separate cell types based on motility speeds, but individual cell isolation, as seen with surface hydrodispersion, is not achieved through this movement. The appearance of isolated colonies supports a mechanism of community reorganization, unique to this movement, among other surface motilities. Dissimilarity seen in the microbiogeography of communities formed by dispersion of comicrobial inoculations of *S. aureus* and *C. striatum* versus *S. aureus* with a nonmotile mutant of *P. aeruginosa* serves to illustrate a range of transitory influences by hydrodispersion upon biogeography. The notoriously hostile *P. aeruginosa* spreads more efficiently and dominates newly colonized spaces over *S. aureus* under these conditions, while *S. aureus* and *C. striatum* effectively coexist in close proximity following dispersion.

The surface hydrodispersion we describe differs from bacterial movement and dissemination attributable to simple fluid flow in that only a small fraction of cells is translocated in punctuated bursts. We note that this small fraction of dispersed cells was frequently translocated long distances. We recapitulated the general attributes of this dispersal using a continuous-time random-walk approach. Namely, we matched the small fraction of cells ultimately dispersed during a hydration event and coarsely matched the resultant patterns exhibited by hydrodispersed cells. Our stochastic model approach reflects both the probability of cells that move from the larger population and resulting patterns of the fraction of cells that disperse. We found that assigning this fraction of the larger population to be influenced by gravitational pull, in the presence of transient fluid motion, captured the cell dispersal, symmetry of growth, and resultant biogeographical patterning and community formation observed in our experiments.

Limitations, conclusions, and future perspectives. In this study, we have used a stochastic random-walk model approach to characterize hydrodispersion because we do not yet know the parameters that should be included in a mechanistic model. The bacterial rearrangement of roughly 1 in 10^6 cells we have described here occurs on a length scale of many millimeters within a thin liquid film height of just a few micrometers. Because we

used agar assays in our experiments, it is possible that some inoculated bacteria were confined within the agar semisolid and not subject to hydrodispersion.

In order to mechanistically describe hydrodispersion, it will be important to consider influences of surface chemistry, surface friction, surface tension, liquid evaporation, liquid film height, and cell morphology to describe hydrodispersion in future work by moving beyond the random-walk approach used in this study where the random-jump attributes applied were nonspecific. Perhaps most importantly, the fluid dynamics of the thin liquid film containing unattached bacteria in these experiments require definition. There is not a clear mathematical description of “general” spreading for particles (or bacteria) in thin liquid films. It will be important in future work to quantify the spatial and temporal flow regime forces in these transient hydration environments to allow understanding and prediction of bacterial fate (e.g., the settling velocity of individual bacteria). The small differences in spreading geometry between *P. aeruginosa* and *S. aureus* highlight a need to understand the relevant biophysical aspects of hydrodispersion that are distinct from comparable spreading of any micrometer-sized inert particle.

In our experiments, hydrated surfaces changed from a confluent hydration condition to a disparate unjoined hydration condition within 30 min of incubation. We suggest that surface hydrodispersion is a factor that should be considered when assessing control conditions of laboratory motility assays. Certainly, the periodic confluent hydration of many human cell and environmental surfaces will vary greatly, but the basic periodic aspects of confluent to disparate hydration back to confluent represent a hydration cycle fitting to numerous surfaces that promote bacterial growth.

Lastly, we found that surface hydrodispersion occurs on a timescale similar to, if not sometimes faster than, that of active motility (13, 43). As frequency of hydrodispersion would vary considerably from environment to environment, and is likely sporadic, studying the translocation of nonmotile bacteria over a long timescale would greatly underestimate the velocity of individual dispersal events. While studying isolated dispersion events is possible using laboratory methods, global variations in humidity (and thus evapotranspiration) will notably influence the period of hydrodispersion. In these experiments, we controlled incubator humidity but not the overall lab environment. While these results have shed light on the unique nature of this fleeting, selective movement, a more detailed assessment of the complexity of the dominant physical, chemical, and biological components will be required to understand how hydrodispersion contributes to the biogeography of real systems.

MATERIALS AND METHODS

Bacterial growth and strains. All strains and plasmids used for these experiments are listed in Table 2, and primers and gBlocks used for strain construction and validation are listed in Table S2. Strain construction details are included in the supplemental material.

Bacterial cultures were grown planktonically overnight in LB medium, needle inoculated onto plate assays containing Todd-Hewitt broth (Acumedia, Lansing, MI) or tryptone (Sigma Scientific, St. Louis, MO)/salt with 5% sheep's blood (Hardy Diagnostics, Santa Maria, CA) media containing either 0.45%, 1.0%, or 1.5% Noble agar (Sigma Scientific), as indicated. All inoculated assays were next dried with lids off for 5 to 15 min. Plates were then incubated overnight at 30°C and 85% humidity with the exception of those for time-lapse images, which did not have humidity control.

Time-lapse imaging. Strains were needle inoculated onto 0.45% agar Todd-Hewitt plates, inverted, and incubated in the dark at 30°C. (Images were captured every 30 min using an IPEVO Ziggi HD Plus CDVU-06IP document camera and IPEVO Presenter software (Amazon) connected to a personal computer (PC) via a 30-ft universal serial bus (USB) cable. Indirect backlit illumination was provided for a 2-min interval every 30 min using a programmable timer) to create a modified “bucket of light” (44) setup in which a VILTROX L116T RA CRI95 light-emitting diode (LED) light panel (Amazon) was arranged 5 cm below a dark center cover situated 3 cm below a Plexiglas platform that supported one plate assay.

Timing of cell movement. Plate assays (0.45% Noble agar) were inoculated as described above, incubated with a 20° tilt, and rotated 60° every 10 min for the first hour (Fig. 2B). Plates were then left for overnight incubation at 30°C. Motility time and duration were judged by the appearance of colonies extending from the inoculation site at least halfway to the plate edge.

Fluid requirement for cell movement. Plate assays containing 1% Noble agar were inoculated as described above. A 100- μ l aliquot of sterile water was added to plates at the site of inoculation. Plates were inverted and incubated overnight at 30°C with 85% humidity. Plates with cells at least half the distance to the plate edge were classified as showing cell motility.

TABLE 2 Strains and plasmids used in this study

Strain or plasmid	Relevant characteristic(s)	Reference(s) or source
Strains		
<i>S. aureus</i> PJI	PJI isolate	30
<i>S. aureus</i> USA300	Reference MRSA ^a wild-type strain	
<i>S. aureus</i> BB1929	<i>agr</i> mutant of USA300; does not produce PSM	Horswill Lab collection
<i>E. faecalis</i> PJI	PJI isolate	30
<i>C. striatum</i> PJI	PJI isolate	30
<i>P. acnes</i>	ATCC 11828	ATCC
<i>P. aeruginosa</i>		
JDS25	PAO1C (ATCC 15692) $\Delta pilA$; markerless	12, 50, 51
CSM10	JDS25 $\Delta flhC$; markerless	This study
CSM12	CSM10 $\Delta rhlAB$; markerless	This study
CSM13	CSM12::miniTn7 <i>gfp2</i> ; Cm ^r Gm ^r	This study
Plasmids		
pEX18Gm	Allelic replacement vector; Gm ^r	52
pRK600	Mobilization plasmid; Cm ^r	53
pUX-BF13	Conjugation helper plasmid; Ap ^r	54
pBK-miniTn7- <i>gfp2</i>	miniTn7 <i>gfp2</i> ; Cm ^r Ap ^r Gm ^r	55
pCSM103	<i>flhC</i> allelic replacement vector in pEX18Gm; Gm ^r	This study
pAEM1	<i>rhlAB</i> allelic replacement vector in pEX18Gm; Gm ^r	This study

^aMRSA, methicillin-resistant *S. aureus*.

Small-variation tilt angle assays. Todd-Hewitt plates containing 0.45% Noble agar were made as described above. *S. aureus* USA300 was grown overnight in LB medium and diluted to an optical density of 1.0. An inoculum of 0.5 μ l was injected into the interstitial space of inverted plates and set inverted at the designated angle at room temperature overnight. The desired angles of 1°, 2°, and 4° were obtained by resting the edge of the plate on thickness gauge measures of 0.5, 1.0, and 2.0 mm (Amazon). Plates were then incubated at 37°C for 24 h.

Confocal scanning laser microscopy (CSLM) of cell dispersal. *P. aeruginosa*, *C. striatum*, and *S. aureus* USA300 cells tagged with mCherry were grown overnight in LB, diluted 1:10 in LB, and needle inoculated onto tryptone/salt/erythromycin (10 μ g/ml) plates with 1.5% Noble agar. Ten microliters of sterile water was added at the inoculation site, and plates were incubated overnight at 30°C and 85% humidity with a 20° tilt. Images of cells were obtained using a 90i upright Nikon A1 confocal microscope equipped with a Nikon Plan Apo 2 $\times$ /0.10 objective, with excitation at 561.5 nm and capturing emission of 595 $\pm$ 50 nm.

Measurement of inoculation cell number. We assessed the cell population inoculated to our plate assay experiments by quantifying the number of cells transferred by our needle inoculation protocol. Cells from overnight planktonic LB cultures of *S. aureus* and *C. striatum* were needle inoculated into microtiter plate wells containing 50 μ l of LB medium to approximate the depth of medium in our agar-based assay plates. These 50- μ l volumes were then diluted 1:1,000 in 1 ml of LB medium, and 10 μ l was plated. Each inoculation was performed three times, and three separate aliquots were plated from each replicate. CFU were counted following overnight 37°C incubation.

In silico simulations using the random-walk model. A random-walk modeling approach is used to describe positions of cells over a series of time steps (45–48). Cells are considered particles with a location, x , where random “jumps” to the next particle position, $x + dx$, can be influenced by two processes: Brownian motion and gravity, with weight $1 - M$ and M , respectively. Particle diffusion attributable to Brownian motion is subject to spreading in two directions by the stochastic variable ξ . Particle spreading attributable to gravitational pull is subject to the stochastic parameter η , which aligns components with the direction of gravity. The position and time equations were implemented numerically in Matlab (release R2016b, 64 bit) using a standard MacBook Pro laptop (2.9 GHz, Intel iCore 5 with 16 GB random access memory [RAM]). In silico experiments conducted using this model were performed over a range of M values from 0 to 1. Each value of M was tested for an “inoculum” of 100 million particles. Results were visualized using inbuilt plotting software for Matlab.

Measurement of dispersal patterns. Resultant images of cell dispersal were acquired using a Nikon D3300 camera and analyzed using ImageJ (49). Briefly, using the wand, the edges of distribution zones in plate images and model-derived images were manually outlined as the area of interest and then measurements of shape, centroid, and fit ellipse were calculated. Outlying colonies were included within the distribution zones.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.4 MB.

SUPPLEMENTAL FILE 2, AVI file, 2.3 MB.

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We have no competing interests to declare.

A.A.W. carried out all laboratory experiments. D.B. designed the mathematical model and carried out simulation experiments. A.A.W., D.B., and J.D.S. conceived and designed the experiments and helped draft the manuscript. C.S.M., A.E.M., and N.M.-S. designed, constructed, and validated phenotypes of *P. aeruginosa* strains. A.A.W. and C.S.M. developed and implemented imaging protocols. All authors gave final approval for publication.

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