

Collective Motion of Surfactant-Producing Bacteria Imparts Superdiffusivity to Their Upper Surface

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ABSTRACT Swarming bacteria move on agar surfaces in groups, using flagella as motive organelles. Motility depends critically on surface wetness, which is enabled by osmotic agents and surfactants secreted by the bacteria. In a recent study, the upper surface of an *Escherichia coli* swarm was found to be stationary, as determined from the motion of MgO particles deposited on the swarm. This led to the remarkable conclusion that the bacteria move between two stationary surfaces—the agar gel below and the liquid/air interface above. That study suggested that secreted surfactants may contribute to immobilizing the upper surface of a swarm. Here, we test this proposition using two robust surfactant-producing bacteria. We find antithetically that the upper surfaces of both these swarms are mobile, showing a superdiffusive behavior in swarms with stronger surfactant activity. Superdiffusive behavior was not observed on the surface of a drop of bacterial culture, on bacteria-free culture supernatant, or on nonswarming surfactant-producer colonies, which suggests that superdiffusion is an emergent property resulting from the interaction of the collective motion of the bacteria within the swarm with the surfactant layer above. Swarming not only allows bacteria to forage for food, but also confers protective advantages against antimicrobial agents. Our results are therefore relevant to superdiffusive strategies in biological foraging and survival.

INTRODUCTION

Swarming motility is a group phenomenon in which flagellated bacteria migrate rapidly over agar surfaces, acquire more territory, and display increased resistance to antimicrobials (1–8). Why swarming motion is collective and not seen in isolated individuals is not completely understood. It is surmised that osmotic agents secreted by the colony attract water needed for the rotary flagella to generate the thrust necessary to propel the bacteria. Secreted surfactants reduce surface tension, allowing the water (and hence the colony) to expand readily. A swarming colony is dense and multilayered in the interior, and generally monolayered at its edges. Large-scale swirling and streaming motion of the bacteria is observed in the interior of the colony, whereas cells at the edge tend to be less motile (9–11). Despite their low motility, cells at the edge are thought to pump fluid outward by flagella motion, thus wetting the virgin agar surface ahead and promoting continual invasion of new space (11,12).

Agar is a solid gel, so the agar/swarm interface is stationary. Very little is known about the upper surface of the swarm, which is the liquid/air interface. Recently, this interface was reported to also be stationary in *Escherichia coli* swarms (13). In that study, MgO particles were deposited on the surface of the *E. coli* swarm near its advancing edge. The particles were observed to remain immobile (diffusing only a few micrometers), apparently unperturbed by the rapid movement of swarming cells underneath. The authors conjectured that the liquid/air interface may be

covered by a monolayer of surfactant that spreads until it reaches the edges of the plate, which prevents it from moving farther, thus pinning the upper swarm layer to the agar substrate. This conjecture was reported to be consistent with the observation that normal swarming rates were maintained under an oxygen-permeable sheet of polydimethylsiloxane (PDMS) placed on top of the swarm (14). However, *E. coli* is not known to secrete surfactants such as lipopeptides and glycolipids made by other swarming bacteria (15–19). We therefore decided to test the role of surfactants in immobilizing the upper swarm surface in two known surfactant producers—*Serratia marcescens* and *Bacillus subtilis*. By using the same method as in the Zhang et al. study (13), we show here that the upper surface of both *S. marcescens* and *B. subtilis* swarms is mobile, and that that of high surfactant-producing swarms is superdiffusive. Mobility of the upper surface was dependent on collective motion of the swarming bacteria. We did observe immobile MgO particles in *E. coli* swarms, as well as in *S. marcescens* swarms defective in surfactant production; however, these particles were trapped in the agar beneath the bacteria.

MATERIALS AND METHODS

Bacteria and swarm plates

The strains used in this study are listed in Table 1, along with their relevant characteristics. The two wild-type (WT) *S. marcescens* strains differ in surfactant production, as described in the text. Of the three *B. subtilis* motility mutants, DS1677 is nonmotile, because it lacks the flagellar filament gene *hag*; DS90 is motile and swarming-proficient but its flagellar motors are counterclockwise (CCW)-biased; and DS73 is motile but swarming-defective because its motors are clockwise (CW)-biased (18).

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TABLE 1 Strains used in this study

Strain	Relevant characteristic(s)		Source
	Swarming	Surfactant*	
<i>S. marcescens</i> 274	WT <i>S. marcescens</i>	+	ATCC [†]
RH1041	Mini-Mu Kan mutant of <i>S. marcescens</i> 274	+	(31) [‡]
<i>S. mar</i> A	WT <i>S. marcescens</i>	+	UT Austin [§]
<i>B. subtilis</i> 3610	Wild-type <i>Bacillus subtilis</i>	+	Daniel Kearns [¶]
DS1677	<i>B. subtilis</i> 3610 Δ hag	—	Daniel Kearns [¶]
DS73	<i>B. subtilis</i> 3610 <i>cheA::tet</i>	—	Daniel Kearns [¶]
DS90	<i>B. subtilis</i> 3610 <i>cheB::tet</i>	+	Daniel Kearns [¶]
AW405	Motile <i>E. coli</i> K12	+	Julius Adler ^{**}
RP437	Motile <i>E. coli</i> K12	+	John S. Parkinson ^{††}

*The surfactants made by *Serratia* and *Bacillus* strains are serrawettin and surfactin, respectively.

[†]American Type Culture Collection.

[‡]This strain is a transposon (mini-Mu Kan) mutant (listed in the article (31) as Smu13a).

[§]Stocked in the Microbiology teaching laboratory.

[¶]Indiana University, Bloomington, Indiana.

^{||}Double colon (::) denotes a substitution: deletion of the gene preceding the symbol and insertion of the *tet* gene.

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Polystyrene Petri plates (100 × 15 mm) were filled with 25 ml molten swarm agar (0.5% agar (either Eiken (Tokyo, Japan) or Difco (Franklin Lakes, NJ)) and 2.5% Luria broth (LB) as nutrients (Sigma, St. Louis, MO) was used for *B. subtilis* and *S. marcescens*, whereas 0.45% Eiken agar and 2.5% LB with an additional 0.5% glucose was used for *E. coli*). Swarm plates were cooled at room conditions (23°C and 45% humidity) for 24 h for *B. subtilis* and *S. marcescens* and for 15 min for *E. coli*, as described by Zhang et al. (13). The plates were inoculated at the center with 5- μ l drops of cells grown to saturation in LB broth (Sigma) at 30°C for 18 h, reaching an optical density of OD₆₅₀ = 0.50 ± 0.05 (*B. subtilis*), OD₆₅₀ = 1.00 ± 0.05 (*S. marcescens*), and OD₆₅₀ = 1.00 ± 0.05 (*E. coli*). The plates were dried for another 5 min, covered, and incubated for 4 h at 30°C in a humid incubator.

MgO particles on swarm surface

We followed the protocol of Zhang et al., using hydrophobic MgO particles as tracers (13). Particles generated by burning a magnesium ribbon (magnesium ribbon roll, 12.5 g, The Science Company, Denver, CO) were collected in a beaker in accordance with the methods of Zhang et al. The open face of the beaker was placed on top of the swarm plates for 5 min to allow small particles to settle slowly on the swarm. For *E. coli* swarms, the plates were placed in a water bath during particle deposition, as in the Zhang et al. study. Particles in the range 0.2–1.0 μ m in diameter were tracked. Through the various experiments described in this article, the qualitative behavior of particles was observed to be independent of size, although particle speed varied with size, i.e., the smaller the particle, the faster it moved. Particles 0.2 μ m in size moved too fast and left the field of view too soon, preventing reliable statistics for the mean-squared displacement (MSD). Although 0.2- μ m particles could be easily tracked and analyzed, quantitative results are presented for the 0.5- μ m particles only.

MgO particles on drops

Bacteria-free culture supernatants were obtained from overnight cultures of WT *S. marcescens* and *B. subtilis* by passing them through a 0.2- μ m pore size polyethersulfone filter (Corning, Corning, NY). Using the technique described above, MgO particles were deposited on 5- μ l drops of either water or culture supernatants placed on glass slides. Most glass surfaces

disturb accurate measurements of the diffusion coefficient of particles due to the spreading of drops. To avoid this, we used special polytetrafluoroethylene-printed glass slides (63429-04, Electron Microscopy Sciences, Hatfield, PA) coated with hydrophobic hollow rings. Drops were placed in the circle and the rings stopped them from spreading, thus preventing drifts.

Imaging and particle tracking

A phase-contrast microscope (Olympus IX50 with an LD 60 \times phase-contrast PH2 objective) was used to observe the particles. To minimize air drafts, the microscope was housed in a closed space. The position of particles was judged by adjusting the focus. A CCD camera captured the particle motion at a rate of 60 frames/s with a spatial resolution of 1004 × 997 pixels over a field of view of 120 × 120 μ m². Images were directly streamed to a hard disk for 60-s periods, corresponding to 3600 images. Particle positions were tracked manually using a Tracker.exe web program (<http://www.cabrillo.edu/~dbrown/tracker/>).

Contact angle measurement

Water or bacterial culture supernatant drops 5 μ l in volume, prepared as described above, were placed on glass and plastic surfaces. We used premium 25 × 75 × 1-mm glass microscope slides (125442; Fisher Scientific, Waltham, MA), and the inner side of the bottom part of 100 × 15-mm plastic Petri dishes (0875712; Fisher Scientific). The glass slides were washed with ethanol and dried with N₂. For small angles of contact, where sideview images are not accurate, the diameter of each drop was determined by taking a close-up, top-view picture of the drop using a 10-megapixel camera (D200, Nikon, Tokyo, Japan) with a 60-mm lens. Pictures were taken 10 min after drop deposition. The angle of contact, θ (°), between drop and surface was determined using a spherical-cap-shaped approximation for small angles ($\theta < 30^\circ$) through

$$\theta = \frac{720 \cdot V}{\pi^2 \cdot a^3}, \quad (1)$$

where V is the volume of the drop (5 μ l), and a is the radius (mm) of the circle formed by the drop base. For large angles, sideview pictures were taken with the same camera and lens.

RESULTS

MgO particles are superdiffusive on swarms of WT *S. marcescens*

Two WT strains of *S. marcescens* (274 and A) were used in this study. Both strains expanded rapidly on swarm agar and covered an entire Petri dish, inoculated in the center, within a few hours. A clear surfactant zone was seen preceding the leading edge of the swarm in both strains, but this zone was larger for strain A than for strain 274 (100 μm vs. 75 μm wide). Fig. 1 A shows a top view of an *S. marcescens* 274 colony. We have focused on the region of the colony close to the swarm edge (within $\sim 100 \mu\text{m}$), where bacteria were not very dense (Fig. 1 B).

MgO particles were gently deposited on the colony and their motion was analyzed as described in Materials and Methods (Fig. 1, C–F). Location of the particles within various regions of the swarm was determined by adjusting the focus. Particles that landed on the virgin agar, either did not move, or diffused within very small areas ($\sim 4 \mu\text{m}^2$), presumably trapped in pockets of liquid within the agar network (Movie S1 in Supporting Material). We refer to this motion as constrained diffusion. Particles that landed on the swarm showed two kinds of behavior. Most particles, regardless of size, penetrated the fluid layer and stuck to the agar, where they remained for the rest of the experiment. These particles were all below the swarm, and a majority of them showed constrained diffusion, similar to that seen with particles on virgin agar. Some particles did not stick and showed various trajectories, generally depending on their size. The larger the particle, the slower it moved. Phase-contrast microscopy revealed that particles that moved were all located either within the swarm or above it (Movie S2). Since no immobile particles or particles showing constrained diffusion were observed on top of the swarm, we concluded that the upper surface of the swarm is mobile.

Mobility of particles within the swarm is reasonable, because the particles constantly get hit by bacteria or follow bacterial streams and whirls. For long enough times ($>1 \text{ s}$) and distances ($>5 \mu\text{m}$), particle motion is diffusive, as shown by the MSD and trajectory of the particles (Fig. 2 A). On the other hand, one does not know a priori how particles might behave on the upper surface. Although it is reasonable to imagine that their movement would be influenced by the motion of the bacteria below, Zhang et al. found them to be immobile in *E. coli* swarms. We observed particles on the upper surface of *S. marcescens* swarms to move similarly to those within the swarm. MSDs and trajectories of these particles are shown in Fig. 2, B and C. Particles on the upper surface of the *S. marcescens* 274 swarm showed normal diffusion (Fig. 2 B), but those on the upper surface of *S. marcescens* A swarms showed superdiffusion, where the slope of the MSD in the log-log scale was >1 (Fig. 2 C). Superdiffusing particles left the field of vision rapidly, precluding the gathering of large enough data sets for step-length distributions.

The surface of WT *B. subtilis* swarms is also superdiffusive

The WT *B. subtilis* strain grew similarly to WT *S. marcescens* in that the swarm expanded rapidly and produced a visible surfactant zone ahead of the colony edge. However, the surfactant zone was wider ($\sim 150 \mu\text{m}$) in the *B. subtilis* swarm compared to *S. marcescens*, and the cells at the edge were sparser than in the *S. marcescens* swarms. Fig. 3 shows a top view of a WT *B. subtilis* swarming colony.

Despite the differences in swarm-colony morphologies between *S. marcescens* and *B. subtilis*, phase-contrast microscopy revealed the same results: particles that moved were all located within the swarm or above it, and those that did not move, or showed constrained diffusion, were all

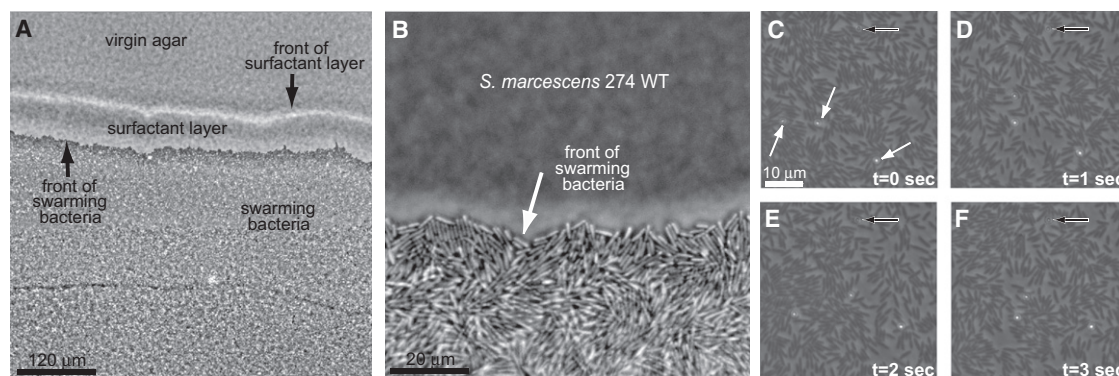


FIGURE 1 Top-view phase-contrast microscopy images of swarming *S. marcescens* 274 bacteria on an agar plate. (A) Low-resolution image showing the front of the swarm, the virgin agar, and the 75- μm -wide surfactant layer ahead of the swarm. (B) High-resolution image of the same colony as in A, showing the front of the swarm. (C–F) Four MgO particles (arrows), each $\sim 0.5 \mu\text{m}$ in diameter, deposited on the swarm. The three white particles (white arrows) are moving and are located on top of the swarm; note the contrast between these particles and the dark bacteria. The dark particle (black arrow) is located below the swarm and does not move. See Movie S2.

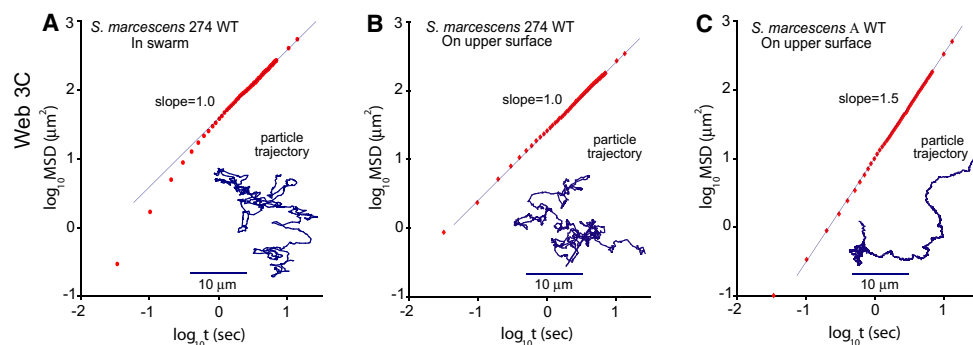


FIGURE 2 Diffusion of MgO particles deposited on *S. marcescens* swarms. The MSD as a function of time (every third data point (0.1 s)), were generated by averaging >10 similar particles, each ~0.5 μm in diameter. (Insets) Trajectories of a single particle for a period of 30 s. (A) Particles on an *S. marcescens* 274 swarm. The particles are located within the swarm, at the same height as the bacteria. Slope = 1, showing normal diffusion; $D \sim 5 \mu\text{m}^2/\text{s}$. (B) Particles on the upper surface of an *S. marcescens* 274 swarm. Slope = 1, showing normal diffusion; $D \sim 5 \mu\text{m}^2/\text{s}$. (C) Particles on the upper surface of an *S. marcescens* A swarm, which has a larger surfactant zone. Slope = 1.5, showing superdiffusion. The particle trajectory has more long, straight sections compared with those in A and B.

diffusion; $D \sim 5 \mu\text{m}^2/\text{s}$. (C) Particles on the upper surface of an *S. marcescens* A swarm, which has a larger surfactant zone. Slope = 1.5, showing superdiffusion. The particle trajectory has more long, straight sections compared with those in A and B.

below the swarm. Within the *B. subtilis* swarm, particles were found to move in the same way and with the same speeds as those within the *S. marcescens* swarm (Fig. 4 A). Particles at the upper surface of the swarm moved faster and formed superdiffusive trajectories, similar to particles in the upper surface of *S. marcescens* A swarms (Fig. 4 B and Movie S3). Particle behavior was similar in bacteria-free spaces, suggesting that their motion reflects the mobility of the upper surface and does not stem from direct collisions with moving bacteria (Fig. 4 C).

Contact-angle measurements to determine surfactant activity

Although the upper surfaces of all three bacteria examined were mobile, those of *B. subtilis* and *S. marcescens* A were superdiffusive. We were curious to test whether the difference in particle behavior was correlated with surfactant activity, since the surfactant zone in front of the swarming edge was wider in the colonies that showed superdiffusive particle behavior. To quantify surfactant activity, we performed the drop-collapse test, which is commonly used to determine wetting properties of a surfactant (20,21) (Fig. 5 A).

Supernatant drops (5 μl) prepared from overnight bacterial cultures and filtered free of bacteria were placed on clean surfaces (glass and plastic), and allowed to stand for 10 min,

as described under Materials and Methods (Fig. 5 B). We observed that the supernatant of *E. coli* strains showed wetting properties similar to those of water, confirming that *E. coli* is a poor surfactant producer. The wetting activity of *S. marcescens* 274 was better than that of *E. coli*, but that of *S. marcescens* A was even better. The results are quantified in Fig. 5 C. A mutant of *S. marcescens* 274 that is defective in surfactant (serrawettin) production showed smaller wetting than its parent strain, as expected. The best wetting was obtained with the *B. subtilis* supernatant. The drop-collapse data correlate very well with the width of the clear zone in front of the swarming edge, suggesting that this zone is indeed the surfactant layer. Taken together, these data suggest that the mobility of the upper surface of a swarm is related to the presence of surfactants, and that superdiffusive surface properties are related to superior surfactant activity.

MgO particles penetrate the surface of surfactant-minus *S. marcescens* and *E. coli* swarms

The surfactant-minus *S. marcescens* strain expanded much more slowly than its WT parent and did not show the typical monolayered edge of a WT swarm. MgO particles deposited on such colonies were difficult to track because they did not remain on the upper surface but went in and out of focus while moving along the z axis. This suggested that surfactant is needed to hold the small particles on top of the moving bacteria. We were surprised that a control experiment with *E. coli* swarms, which do not appear to make surfactant (see Fig. 5 C), failed to show MgO particles on the upper surface. All visible particles were either within the swarm and showed normal diffusion or appeared to be trapped in the agar below and were either immobile or showed constrained diffusion (Movie S4).

MgO particles show normal diffusion on culture supernatants

If surfactant is needed to hold the particles on the upper surface, or if its activity level influences particle diffusion,

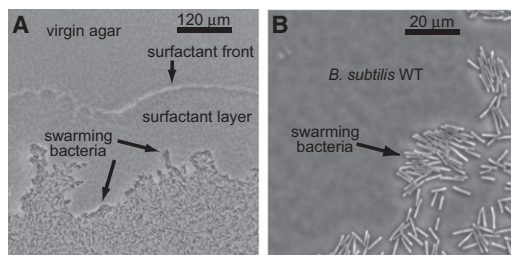


FIGURE 3 Phase-contrast microscopy images of swarming *B. subtilis* 3610 bacteria on an agar plate. (A) Same as in swarming *S. Marcescens* 274 (Fig. 1 A), except that the width of the swarm (150 μm) is larger. (B) Same as in swarming *S. Marcescens* 274 (Fig. 1 B), except that the bacteria are much sparser.

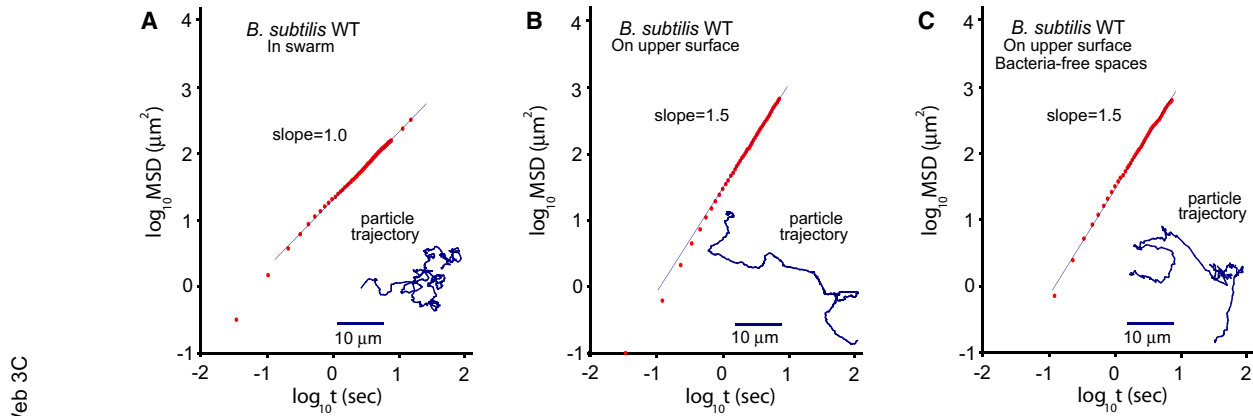


FIGURE 4 Diffusion of MgO particles deposited on *B. subtilis* swarms. The MSDs were calculated as in Fig. 2. (A) Particles are located within the swarm, as described in Fig. 2 A. Slope = 1; $D = 5 \mu\text{m}^2/\text{s}$, similar to that seen for *S. marcescens* 274 in Fig. 2 A. (B) Particles on the upper surface of a *B. subtilis* swarm. Slope = 1.5, showing superdiffusion. (Inset) Trajectory of a single particle for a period of 15 s. (C) Particles in bacteria-free spaces on the upper surface of a *B. subtilis* swarm. Slope = 1.5, showing superdiffusion. (Inset) Trajectory of a single particle for a period of 8 s.

it would be interesting to track particle behavior on a liquid/air interface where there was either no surfactant and/or no bacterial motion. MgO particles were therefore deposited on water and on bacteria-free supernatant drops placed on special glass slides that constrained the drop and prevented drift (see Materials and Methods). MgO particles showed normal diffusion on the liquid/air surfaces of both water and bacterial supernatant drops (Fig. 6, A and B). The diffusion coefficients, D (calculated from the MSD slopes, where $\text{MSD} = 4Dt$), of same-sized particles were similar in both

water and bacterial supernatant drops ($0.5 \mu\text{m}^2/\text{s}$) but much smaller than the diffusion coefficient measured for the motion of similar particles on moving swarms ($5 \mu\text{m}^2/\text{s}$). MgO particles deposited on *B. subtilis* culture drops (unfiltered) showed the same diffusion behavior as those on filtered supernatants or water (Fig. 6 C). This is a result of the energy source: on the bacteria-free drops, the particles get their energy from the Brownian motion of the liquid molecules, whereas on the swarm they get their energy mostly from the collectively moving bacteria below (22).

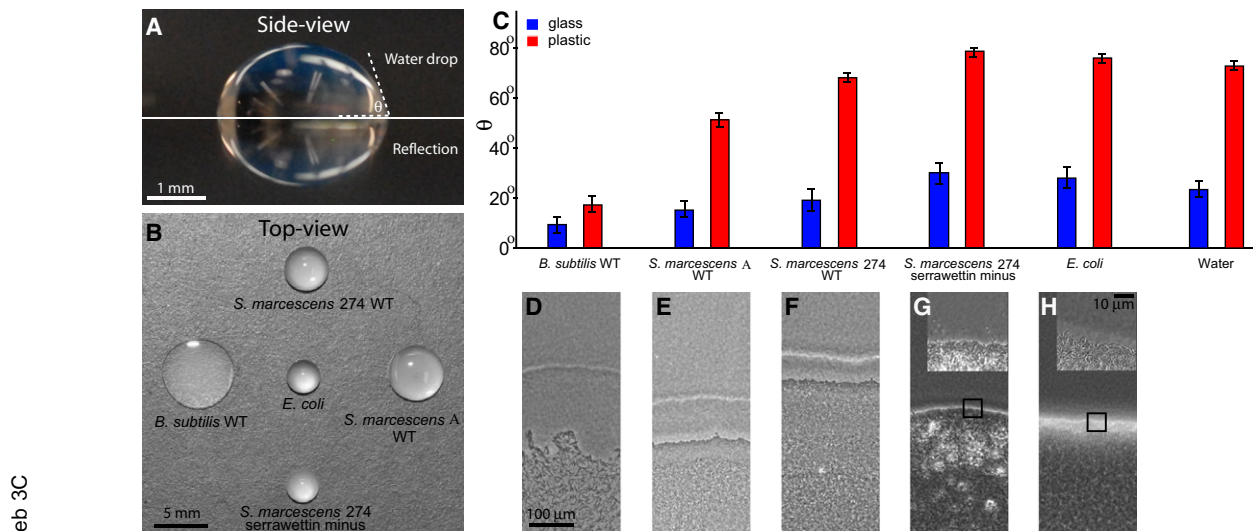


FIGURE 5 Drop-collapse experiment. (A) A side view of a $10\text{-}\mu\text{l}$ water drop deposited on a plastic surface. For such large contact angles, the angle was measured directly from the image. (B) A top-view image of various $10\text{-}\mu\text{l}$ bacteria-free supernatant drops deposited on a plastic surface. The larger the diameter of the drop base, the smaller the angle of contact and the larger the wetting properties. (C) A summary of measurements of the angle of contact of $10\text{-}\mu\text{l}$ drops on both plastic and glass. Strains used were *B. subtilis* (3610), *S. marcescens* A, *S. marcescens* 274, *S. marcescens* 274 serrawettin minus (RH 1041), and *E. coli* (AW405). For each strain, the average was calculated from 10 different drops. (D–H) Surfactant zones at the leading fronts of colonies grown on the agar for the strains shown in C. Note the correlation between the size of the zone and the collapse of the drop. For the *S. marcescens* 274 serrawettin minus strain (G), no surfactant zone was observed (see also inset of higher magnification). For *E. coli* (H) a small surfactant zone of $10 \mu\text{m}$ was observed. (Insets) Magnification of the outlined areas.

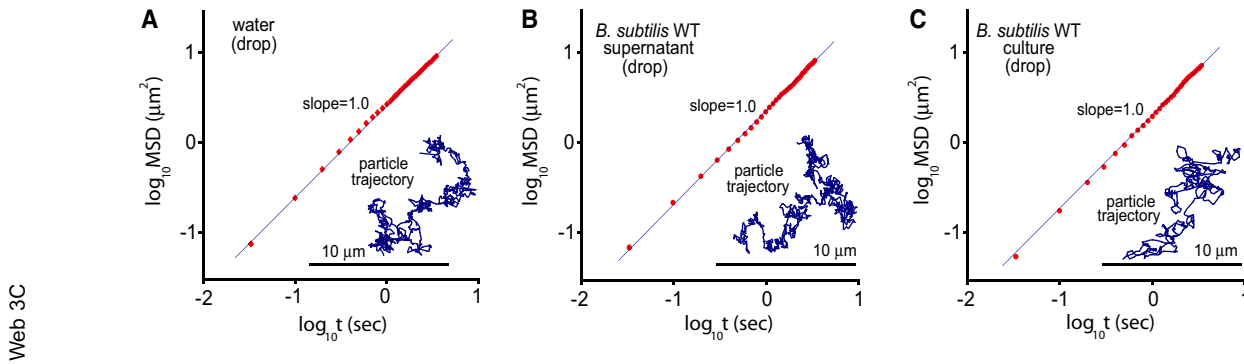


FIGURE 6 Diffusion of MgO particles on drops of (A) water, (B) supernatant of *B. subtilis*, and (C) culture of *B. subtilis*. All other descriptions are as in the legend to Fig. 2. All graphs show normal diffusion, with $D \sim 0.5 \mu\text{m}^2/\text{s}$ (10 times smaller than in the case of the wild-type *S. marcescens* 274 swarm).

The ability of particles to diffuse on a surfactant-laden drop shows that surfactants do not immobilize a surface. The absence of superdiffusive particle behavior on these drops shows that surfactants alone do not impart superdiffusive properties to the surface. Therefore, superdiffusion of the upper swarm surface is a property that emerges as a result of interaction between the collective bacterial motion below and the surfactant layer above.

MgO particle behavior on mutant *B. subtilis* swarms

To further test the idea that superdiffusion of the upper swarm surface is an emergent property of collective motion, three different *B. subtilis* motility mutants were tested for behavior of MgO particles on swarm agar. All of these mutants are derived from the surfactant-producing parent 3610 and should produce similar amounts of surfactin (Table 1); this was confirmed by the drop-collapse assay. The behavior of MgO particles on culture drops of the mutant strains was also similar to that of the parent strain (Fig. 6 C; data not shown). Two of these mutants were nonswarming, either because they had no flagella (DS1677) or because their flagellar motors were CW-biased (DS73), whereas the CCW-biased mutant (DS90) showed normal swarming (18). MgO particles deposited on the CCW mutant showed trajectories similar to that of the WT, both within and on top of the swarm. In particular, particles located on the upper surface showed superdiffusion, as found in the wild type (Fig. 7 A). The CW mutant colony expanded at a rate similar to that of wild type, but the collective packlike movement characteristic of WT cells was absent. Instead, these cells showed only a limited back-and-forth movement (Movie S5). MgO particles deposited on the surface of these colonies showed normal diffusion but had diffusion coefficients 100 times smaller than on a water drop (Fig. 7 B). The nonflagellate strain did not expand significantly. Remarkably, MgO particles on its surface were stationary (Movie S6).

The limited-to-nonexistent diffusive behavior of particles on the surface of the nonswarming mutants, when compared

to that of particles on the surface of their culture drops, suggests that these mutant colonies do not retain sufficient water, and that the surfactant layer by itself is nondiffusive. We note that a defect in water retention has been reported for nonswarming CW/CCW-biased mutants of *Salmonella* (23,24). On the other hand, superdiffusive particle behavior on the surface of CCW mutant swarms, whose swarming behavior was indistinguishable from that of the WT, strongly supports our conjecture that superdiffusion is a property imparted to the upper surfactant surface by the collective bacterial motion in the fluid below.

DISCUSSION

Swarming bacteria provide an excellent model for analyzing the general principles underlying collective motion in nature (25,26), because large numbers of them can be easily observed in a regulated environment, their dynamics can be accurately recorded, and their motility can be altered by genetic and environmental manipulation if desired. Such analytical studies are still in their infancy (10–12). A major open question in swarming is the dynamics of the upper surface of the swarm through which bacteria interact with the environment. A recent study, which reported that the upper surface of an expanding *E. coli* swarm was stationary, suggested that such an immobile surface might be created by secreted surfactants, and could reduce water loss and even promote tissue invasion (13). However, our study of swarms made by two known surfactant-producing bacterial species comes to an opposite conclusion. Using the technique of Zhang et al., where movement of MgO particles deposited on the upper swarm surface was recorded for several seconds, the upper surface was found to be mobile in our experiments. In addition, a striking observation was that upper surfaces of swarms with stronger surfactant activity were superdiffusive (27). The MgO particles on such surfaces migrated faster than mobile particles inside the same area of the swarm and faster than particles that migrated on the upper surface of strains with a lower surfactant activity. The diffusion coefficient of particles on

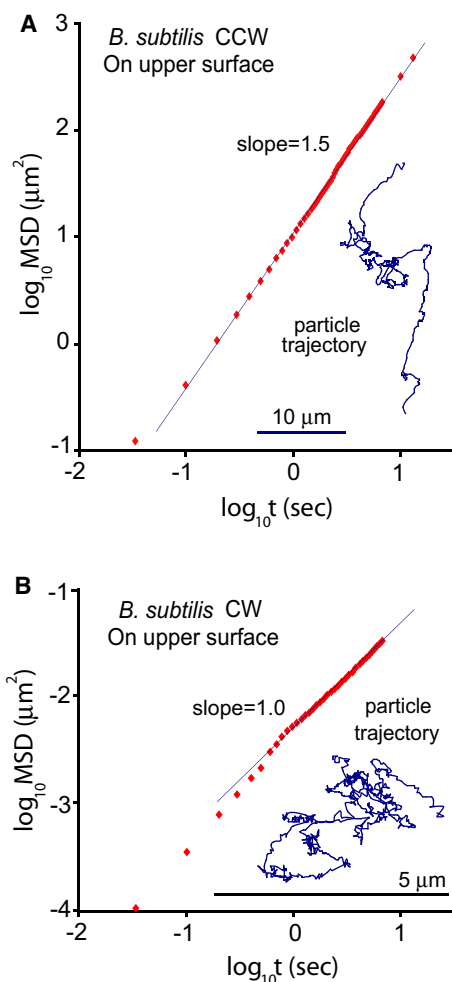


FIGURE 7 Diffusion of MgO particles deposited on motility mutants of *B. subtilis*. The MSDs were calculated as in Figs. 2 and 4. (A) Particles on the upper surface of a CCW-biased *B. subtilis* mutant swarm. Slope = 1.5, showing superdiffusion. (Inset) Trajectory of a single particle for a period of 15 s. (B) Particles on the upper surface of a CW-biased *B. subtilis* mutant swarm. Slope = 1.0, showing normal diffusion with very small diffusion coefficients ($0.005 \mu\text{m}^2/\text{s}$), 100 times smaller than in the case of particles on a water drop. (Inset) Trajectory of a single particle for a period of 30 s. Note the very short particle displacement compared with that observed for the CCW swarm (Fig. 7 A).

the swarms was an order of magnitude larger than that for similar particles on surfactant-laden, or surfactant-free (e.g., water), bacterial culture supernatant drops. The difference in particle behavior on these different surfaces shows that the superdiffusive motion of the upper surface of a swarm is powered by the collective motion of the bacteria and therefore arises from interactions between the collectively migrating bacteria and the surfactant-covered upper surface. We speculate that the mechanism underlying the superdiffusion phenomenon on the swarm surface might be related to both local gradients of surfactants being generated within the colony and continuously changing interactions between the agar and the advancing edge of the colony.

Our studies suggest that surfactant is needed to hold the small MgO particles on top of the swarming bacteria, because these particles failed to float on the upper surfaces of moving nonsurfactant strains (surfactant-minus *S. marcescens* and *E. coli*). It is interesting that when compared to the diffusion of particles on a surfactant-containing drop of bacterial culture, those on the surface of immotile surfactant-producing colonies (nonflagellated *B. subtilis*) were stationary, and those on the surface of a similar nonswarming strain that displayed limited bacterial motion on the agar surface (CW-biased *B. subtilis*) showed a diffusion coefficient two orders of magnitude smaller. These results suggest that these nonswarming mutants do not trap sufficient water within their colonies, and that the surfactant layer itself is immobile. That the direction of flagellar motor rotation influences the amount of water contained in a colony, a phenomenon not presently understood, has been reported for other swarming bacteria (23,24).

As suggested for other biological systems, superdiffusion (e.g., Lévy flight searches) may confer adaptive advantages in relation to Brownian strategies and provide a means for long-range communication (28). Examples of such possible advantages during swarming are circulation of nutrients encountered by the advancing edge of the swarm to inner or older regions of the colony, more efficient acquisition of oxygen from the atmosphere to reach bacteria under a multilayered pile, and better regulation of temperature. This last attribute would be especially important in surfactant-producing swarms that have large areas of monolayered cells at the advancing edge, where temperature losses due to radiation would be significant. Superdiffusion should also facilitate the transport of signaling molecules packaged within membrane vesicles, which are expected to float on the surface of surfactant-producing swarms of bacteria such as *Pseudomonas aeruginosa* (29).

A recent study by Zaid et al. (30) finds that superdiffusive properties in biological systems could result from fluid flow that occasionally accelerates colloidal tracers to relatively large velocities. Their model showed that non-Gaussian tails are generic and arise owing to a combination of truncated Lévy statistics for the velocity field and algebraically decaying time correlations in the fluid. Future studies in which better tracking devices are used to monitor superdiffusing particles and bacterial trajectories more extensively will help to build models that correlate bacterial motion within the swarm to mobility of the upper surface, increasing our understanding of emergent superdiffusive properties and their advantages for optimizing bacterial foraging, signaling, and survival strategies.

SUPPORTING MATERIAL

Six movies are available at [http://www.biophysj.org/biophysj/supplemental/S0006-3495\(11\)00877-0](http://www.biophysj.org/biophysj/supplemental/S0006-3495(11)00877-0).

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