Rapid and directed group motility in *B. subtilis* does not rely on individual motility or chemotaxis

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Microbes routinely face the challenge of acquiring territory and resources on wet surfaces. Cells move in large groups inside thin, surface bound water layers, often achieving speeds of 30 µm/s within this environment, where viscous forces dominate over inertial forces (low Reynolds number). The canonical Gram-positive bacterium *Bacillus subtilis* is a model organism for the study of directed, collective migration over surfaces with groups exhibiting motility on length scales three orders of magnitude larger than themselves within a few doubling times. Genetic and chemical studies clearly show that the secretion of endogenous surfactants and availability of free surface water are required for this 'ultrafast' group motility. However, the relative importance of individual motility, chemosensing, and the presence of exogenous nutrient gradients in precipitating group surface motility are largely unknown. Here we demonstrate that, contrary to models, simulations and observations of surface motility in other bacterial species, (i) *B. subtilis* does not rely on chemotaxis to determine group motility direction, that (ii) the rate of dendritic expansion has only a weak dependence on motility and that rapid dendritic group motility is possible even with non-motile cells, and that (iii) water availability is likely a sensitive control parameter modulating an abiotic jamming transition that determines whether the group remains fluidized and therefore collectively motile. These data suggest that rapid surface motility does not result from individual motility and chemotaxis properties of the bacteria, but rather that a combination of biologically generated surface tension gradients and abiotic granular jamming regulate this ubiquitous ecological process.

Introduction

In their search for resources microbes contend with physically distinct environments ranging from soft surfaces to bulk Newtonian fluids and complex fluids like mucus. In bulk fluid environments, canonical microbes like *Escherichia coli* (1) and *Bacillus subtilis* (2, 3) ascend favorable chemical gradients via run-and-tumble motility (4). This mechanism of gradient ascent requires both flagellar-mediated motility and a complex system of phosphorylation-memory and chemical sensors on the bacterial surface, that together regulate the run-tumble transition frequency (5). In contrast, bacterial surface motility has different requirements and inputs, and different species have distinct modalities of surface transport. For instance, in

the predatory species *Myxococcus xanthus,* individual cells move in back-and-forth motions via so-called 'twitching' motility and assemble into larger motile groups that traverse surfaces as monolayers (6). Many other species, including the opportunistic pathogens *Serratia marcescens* (7, 8) and *Proteus mirabilis* (9), also form large groups of motile cells that are capable of rapidly expanding over surfaces, in some cases even against bulk fluid flow (10). Similarly, when present in sufficient numbers *Paenibacillus dendritiformis* exhibits intricate fractal-like pattern formation on soft agar surfaces in response to lateral chemical gradients (11, 12). Other cells respond to external gradients in non-chemical fields; for instance the cyanobacterium S*ynechocystis* is phototactic, responding to incident light by asymmetrically extending and retracting pili from its surface to create a biased random walk toward a light source (13). Crucial to its motion, *Synechocystis* modifies the local surface environment by secreting exopolysaccharides, and only when enough cells have participated in such surface modification can the group move toward the light source. These examples demonstrate that in response to various gradients, microbes have evolved distinct sensing capabilities and modalities of motion to acquire resources and respond to selective pressures on surfaces. Despite their differences, surface motility in all of these species appears to be a collective phenomenon, requiring the motion of and/or surface modifications by large numbers of cells. Therefore, understanding the physical forces that produce and guide microbial group motion on surfaces is integral to our understanding of microbial ecology in natural environments and will expand the suite of design tools for engineering microbial systems.

Efforts to model these systems (notably *P. dendritiformis*) have focused on reaction-diffusion partial differential equations, which reproduce, with high fidelity, many of the classes of bacterial surface patterning observed in experiments (14–19). These models rely on bacteria using chemotaxis to follow nutrient gradients that the colonies themselves produce through local consumption of nutrients (20). Growth and spreading of cells in dendritic patterns are modeled as a diffusion-limited conversion of nutrients into biomass at the growing tips of the dendrite; such models do not incorporate advective flow of cells nor material within the dendrite. Within a dense, actively swarming group whose velocity correlations rapidly decay on the length scale of a few cells (21), it is unclear how individual cells would be able to effectively modulate their tumble frequency and accumulate sufficiently long persistent runs to deliberately bias their random walks and hence execute run-and-tumble chemotaxis. Likewise, it is unclear how the chemotaxis of individual cells in a swarm could guide a group in the ascent of a nutrient gradient. Thus, the ability of large bacterial groups to traverse surfaces appears to involve the salient trio of motility, chemotaxis, and cell density, but the role that cell density plays and whether motility and chemotaxis are necessary features for surface motility are unknown.

In this work we begin to clarify the relative contributions of motility, chemotaxis and cell density by examining the surface motility of the extensively-studied Gram-positive bacterium *B. subitilis* (5, 22–24). From a small central inoculum, wild-type cells can rapidly colonize an entire wet 10 cm agar plate in 1 - 2 hours via apparent swarming motility (25). Group motility over soft agar surfaces has been shown to depend on the secretion of 'surfactin', a bacterially produced bio-surfactant and wetting agent (20, 26–29). Functional knockouts for surfactin production (Δ*Srf*) result in a phenotype where individual cells are still motile and chemotactic in bulk fluid, but bacterial groups cannot move across surfaces (28). Localized secretion of surfactin is thought to generate a gradient in surface tension, and thus produce motion via the Marangoni force (23, 27, 30). Despite the recognized role of surfactin, the contributions of individual motility, chemotaxis, and surface density of cells toward group surface motility remain poorly understood. Thus, we employed a combination of high-resolution imaging and computational image processing, as well as genetic manipulations to determine to what extent motility and chemotaxis of individual cells are required for group level motility on surfaces. Surprisingly, we found that the movement of *B. subtilis* over soft agar surfaces in the direction of 'fresh' territory showed only weak dependence on local gradients in nutrient concentration, and that *neither* motility *nor* chemotaxis of individual cells was necessary for group motility over surfaces. Even in the extreme case where mutant *B. subtilis* were devoid of flagella (Δ*Hag*) groups of cells traversed the agar surface with patterns and rates similar to wild-type cells. Finally, our image analysis suggests that at high cell density, groups of cells are subject to a jamming-fluidization transition, that is, dense groups of cells behave like a two-dimensional, shear-thinning fluid (31). These data suggest that individual motility and group swarming have less to do with generating force for motion, and that rather, individual motility and subsequent swarming are mechanisms that maintain a fluidized state on which surface-tension gradient forces (Maragoni forces) can act to precipitate group motion over a surface. Thus, a jamming-like transition, like those found in other macroscopic granular systems (32, 33), may be a key regulator of bacterial surface transport.

Results

Bacillus subtilis is a model motile Gram-positive bacterium, capable of sensing and responding to its chemical environment via run-and-tumble chemotaxis (5). On wet surfaces wild-type *B. subtilis* rapidly move out from a central inoculum, apparently via collective swarming motility (25) that is known to require secretion of the endogenous bio-surfactant 'surfactin'. Mutants that lack the ability to produce surfactin (Δ*Srf*) do not expand from their central inoculum. We inoculated small, dense droplets of *B. subtilis* on soft (~0.5% w/v agarose) nutrient-rich agar surfaces. After a brief quiescent phase, groups of cells rapidly expanded over the surface in a dendritic pattern that reached the edge of the plate (~5 cm of travel) in less

than 6 hours. Dendrites robustly moved outward away from the original point of inoculation into fresh territory, at an average group motility rate of ~10 µm/s and up to ~30 µm/s (Fig. 1). Cells within the dendrites were highly motile, exhibiting swarming motility (28) with individual cells moving at rates of ~30 um/s but in highly circuitous paths. Notably, dendrites composed of individuals swimming in highly circuitous paths move directionally outward at peak rates comparable to what one would expect if all cells simply swam directly outward. Thus, we wanted to test the hypothesis that this rapid and directed movement is both related to the underlying motility of the bacteria and uses their innate chemotactic abilities to sense and direct movement along the outward-facing nutrient gradients created by the bacteria's own consumption of metabolites in the agar.

Surface motility does not require chemotaxis.

First, we wondered whether the surface motility of fully chemotactic wild-type cells followed local chemical gradients. We created agar plates with two distinct halves – one half with agar containing a rich defined medium (RDM), the other half containing NaCl buffer with agar, osmotically matched to the RDM to maintain the same osmotic potential (see Methods). An impermeable barrier separated the two halves, initially keeping the nutrients on one side. Once the agar set, we poured a thin (~ 1 mm) layer of osmotically matched NaCl buffer with agar, and thus linked the two regions into a contiguous surface that allowed cells to move freely between them. The nutrient-rich half also contained a red fluorescent tracer dye that allowed us to visualize chemical diffusion over the barrier between the two halves, as an approximate reporter of nutrient diffusion over the barrier. With or without cells, these plates showed diffusion of the dye from the nutrient rich side into the side devoid of nutrients, meaning that there was a negative concentration gradient on the surface from the nutrient rich to the nutrient poor side. We reasoned that if wild-type cells were responding to the outward-facing chemical gradients on a standard plate (i.e. isotropic with nutrients), they would sense the reverse nutrient gradient on these split plates and either avoid the nutrient poor region or show reduced motility toward it. Remarkably, wild-type cells inoculated on the nutrient rich side moved roughly evenly in all directions, moving down the concentration gradient into the zero-nutrient side of the plate at rates comparable to colony mates moving up the nutrient gradient on the other side of the inoculation point (Fig. 2). On the same plate a control wild-type inoculum was deposited onto the zero-nutrient region. In contrast to the nutrient-rich inoculum, these cells did not exhibit collective motility of any kind. These data suggest that while the directionality of motile groups is not particularly sensitive to nutrient gradients, cells need nutrients (at least initially) to be collectively motile, and that cells provided with nutrients initially can traverse large stretches of nutrient barren territory, while cells devoid of nutrients cannot.

To determine whether chemotaxis of individual bacteria is required for groups of *B. subtilis* to traverse a soft agar surface we disrupted chemotaxis in two distinct ways, reasoning that each method may produce different outcomes in terms of group surface motility. First, we examined the surface motility of run-only (Δ*CheB*) mutants that were motile but lacked the ability to reorient via tumbling, and hence cannot perform run-and-tumble chemotaxis, regardless of which surface chemoreceptors were expressed. Second, we examined the surface motility of tumble-only (*ΔCheY*) mutants that have all of the flagellar machinery to be motile, but are permanently in the tumble state, and hence are both (effectively) non-motile and non-chemotactic (28). Despite that both strains cannot follow a chemical gradient of any kind, surprisingly, both strains exhibited dendritic expansion over agar surfaces with morphologies and speeds similar to the expansion of wild-type cells (Fig. 3A-B)

Together, both the split plate data and the run-only / tumble-only mutant data strongly suggest that group surface motility of *B. subtilis* neither requires chemotaxis nor relies on chemical gradients as a determinant of expansion direction.

Individual motility is not required for group motility

Given that tumble-only mutants, despite being motility deficient, exhibit surface expansion rates and morphologies similar to wild-type, we wondered if individual motility was in any way required for group surface motility. To be clear, we wanted to know if cells lacking the ability to move at all could expand across a plate at rates similar to wild-type. This is in contrast to expansion across plates through the replication of sessile cells at the leading edge of growth, as seen in *P. dentritiformis* (dendrite-like) or *E. coli* (growing circular colonies), both of which expand at much slower rates (34–36).

To assess surface motility in the absence of individual motility, we examined *B. subtilis* mutants lacking flagellar filaments (*ΔHag)* (28). These cells have intact chemotaxis machinery and sensors, and retain intact flagellar basal bodies (motors), but do not have flagella and hence cannot run, tumble, chemotax, or move in any way that requires the use of flagella. Even in the absence of any mechanism of individual propulsion, colonies inoculated at the center of a soft agar, nutrient-rich plate still expanded outward in a dendritic pattern (Fig. 2C). These data demonstrate that individual motility is not required for group motility, rather mechanisms other than individual motility and / or chemotaxis precipitate the emergent, colony-scale dendritic self-organization of *B. subtilis* on soft nutrient-rich surfaces.

Granular jamming transition may be a key regulator of surface motility

While the surface motility of *B. subtilis* was robust to genetic manipulations of chemotaxis and motility, it was very sensitive to agar gel stiffness as measured by percentage of agarose. Below ~ 0.45%

agarose by weight, the gel is sufficiently porous that bacteria can penetrate and swim within it, akin to canonical swim plates (25, 37), and above ~0.6% agarose, limited water availability hinders surface motility (25). Thus, across a relatively narrow range of agarose percentages, groups of *B. subtilis* exhibit three qualitatively distinct behaviors: swimming through agarose (below ~0.45%), rapid surface motility (between ~0.45% and ~0.6%), and slow growth by replication (above ~0.6%). Why does the transition from rapid surface motility to slow edge growth occur over such a narrow range of agarose stiffness, and correspondingly, water availability?

Past studies of *B. subtilis* characterized spreading and any attendant morphological behaviors at the length-scale of colonies (~1 cm) and on the timescale of bacterial replication, imaging colony morphology and spreading with minutes or hours between frames (14, 16, 25, 28). Indeed, much of what is known about spreading phenotypes and their genetic mechanisms was discovered through imaging at these scales. However, after determining that chemotaxis and individual motility were not required for group surface motility, we decided to perform high temporal and spatial resolution imaging of spreading colonies to illuminate processes that potentiate spreading. Specifically, we captured images at 30 or 60 frames per second with spatial resolution of 5 µm/pixel or 15 µm/pixel, respectively, both of which enabled us to see intensity variations produced by the movements of individual cells within the swarm (see SI movies). We wrote a custom image analysis script that examines the magnitude of swarm motion (i.e. a scalar measure of motion) as a function of position through time. Briefly, the algorithm characterizes the local intensity fluctuations at a position across a set of N (usually 5 – 7) frames and thus reports on the level of motile activity at each position through time. With this 'activity' filter, we were able to visualize which parts of the colony were actively swarming and in which parts cells were stationary (Fig. 4A-B).

This analysis revealed a number of salient features of surface motility. We observed that the rapid movement of dendrites and cellular groups was highly correlated with significant increases in the quantitative measure of activity from our image analysis, indicating that movement of individuals positively correlates with movement of the group. Conversely, regions whose constituent cells were stationary (low measure of activity) did not move or flow on the surface. However, the most striking feature was that we observed the formation of stark boundaries in activity level within contiguous regions of cells, indicating that within a genotypically and phenotypically identical subpopulation, the group could adopt two qualitatively distinct states of motion. We interpret the high activity state as the fluidized state in which cells are swarming, and the low activity state as the 'jammed' state where cells are stationary due to their packing density. Further, the phase boundary between these regions actively fluctuated in time, translating microns per second, which is faster than (potential) phenotypic switching in the local chemical environment (Fig. 4C). Next, we performed high resolution imaging of expanding tips that were subjected to evaporation, and hence reduced

water availability, which increased cellular packing fraction. These expanding tips showed a rapid and dynamic jamming transition as the meniscal boundary between the agar and the cellular suspension retracted (Fig. 5A-B). Then, to examine the reversibility of this transition, we took the same plates and expanding tips, resealed them to halt evaporation, and continued imaging while water from the gel rehydrated the cells. This allowed the colony to 'thaw' into domains of high activity cells, which then coalesced until the entire tip region regained fluidity and continued to expand. This strongly indicates that re-wetting and the corresponding reduction in packing fraction 'reverses' the jamming transition back to a fluidized and collectively motile state (Fig. 5C-D).

Thus our data are consistent with a model in which the dense packing of surface bacteria act like a shear-thinning fluid that exhibits a jammed state if the non-uniform velocity field (internal shear) caused by motility decreases, and/or if water availability decreases due to either a stiffening of the agar surface or evaporation. In this model non-motile genotypes (such as *ΔHag*) can also exist in a surface-motile state if there is sufficient water availability to maintain a fluidized state, with the force and direction of group motility being produced by surface-tension gradients that result from the secretion and spreading of endogenous surfactin. Thus, an abiotic granular phase transition between qualitatively distinct jammed and fluidized states clarifies the interacting roles of individual bacterial motility, swarming motility, and water availability in the rapid group surface expansion of wild-type *B. subtilis.*

Discussion

Our data support a model of bacterial surface motility in which run-and-tumble chemotaxis is not the mechanism that determines the direction of migration, and individual motility does not play a crucial role in whether cells are collectively motile. Indeed, we found that cells that cannot move at all (*ΔHag*) still exhibit group surface motility. Rather, our data suggest that water availability – which can be viewed as a proxy for bacterial packing fraction – is a sensitive control parameter for an abiotic jamming transition in the granular material that is densely packed, expanding bacterial populations. We hypothesize that motility plays a contributory role, encouraging a fluidized state which is able to flow over the surface when driven by surface-tension gradients (Marangoni forces). Thus individual motility is not required for surface motility, but contributes to shear-thinning of the bacterial suspension, and expands the range of water availability / packing fractions over which bacterial groups can move via surface tension gradients.

The fact that bacterial groups rapidly expand over surfaces but do not exhibit bulk chemotaxis may have multiple underpinnings. First, it may be due to the infeasibility of individual chemotaxis in a dense, swarming mass of cells. Regardless of any local chemical signals, it is unclear how cells in such dense, turbulent swarms could generate persistent runs with punctuated random reorientations (tumbles), when

their available rotations are highly constrained by steric hindrance of neighboring cells and any persistent runs are reoriented by frequent collisions. Second, the magnitude of the nutrient gradient that cells create via consumption is reduced by the speed at which cells traverse the surface. At the leading edge, their rapid and persistent lateral motion into fresh territory might not significantly deplete the rich store of nutrients in the agar below. It remains an open question whether individual chemotactic activity – or other sets of individual motility rules – when enacted in a dense swarm could provide an emergent mechanism by which active, nematic actors influence their bulk directional flow. Other environmental contexts or species of bacteria may exhibit bulk chemotaxis, but in those situations it seems unlikely that the quintessential run-and-tumble algorithm would be effective for guidance.

The potential existence of an abiotic jamming transition that regulates group motility through the dense packing of nematic actors unifies a number of observed phenomena, including: (i) the fact that increasing agarose gel stiffness switches groups from rapid surface migration to slow steric growth, (ii) the necessity of secreting both surfactant and osmolytes to extract fluid from the substrate (25, 28), and (iii) the augmentation of rapid colony expansion by unbiased swarming motility or agitation via individual motility. We hypothesize that all of these reflect the relatively sharp transition between jammed states of densely packed bacteria that have low-shear properties akin to a 2D granular solid, and fluidized states that can flow over the surface. Colonies that are not able to achieve the fluidized state – for any of the above stated reasons – are constrained to expand on the order of 100 times slower over surfaces using the forces generated by cell wall growth and cell division (i.e. the mode of sessile growth one typically associates with single colonies growing on a petri dish).

Within the framework of an abiotic jamming transition, our data support multiple roles for surfactants (and specifically surfactin): (i) they reduce surface energy of the confining fluid envelope which permits a fixed osmolyte concentration to pull more fluid from the substrate, (ii) they reduce local surface tension and hence allow the 2D bacterial swarm to more easily extend its boundary, (iii) their localized secretion creates the surface energy gradient that drives group motion (independent of individual motility), and (iv) that same gradient points away from sources of secretion and therefore may be the director of motion away from previously colonized areas (as opposed to sensing and behavioral reactions to chemical gradients). This framework also provides a self-consistent explanation for the effects of exogenous surfactant addition. Experiments that added small concentrations of detergent to the gel (38, 39) showed increased surface coverage, presumably due to boundaries that expand more easily, and had less defined dendrites, presumably because there was more water available, less boundary energy, and less of a directed outward surface-energy gradient. In contrast, we hypothesize that the surface energy of aqueous media without surfactant provides enough downward force to pin individual cells against the soft gel, whereas groups of

cells secreting surfactants would only feel such downward forces at their meniscal boundaries. Finally, this framework also suggests that the transition to rapid surface motility is not directly related to the mechanical stiffness of the substrate (e.g. bulk modulus), rather stiffer polymer gels increase the osmo-mechanical work required to extract a unit volume of water from the gel. This also potentially explains why surface spreading assays are so sensitive to local humidity and gel drying times – all of these variables modulate water availability and hence modulate the effective state-variable that regulates the jamming transition. Whereas individual cells propel themselves and direct their motion according to the algorithms of chemotaxis, these data and previous work strongly suggest a fundamentally different set of rules governing group surface motility. Specifically, that collective secretion of surfactants drives and directs motion, while bacterial packing fraction – modulated through multiple mechanisms – is a sensitive control parameter that regulates transitions between fluidized (collectively motile) and jammed (immotile) states of the group. As the fields of bacterial ecology and engineering progress, we should thoughtfully consider how population dynamics are affected by mechanisms of group behavior, and their interactions with the physical environment, that are not fully described by genetics and biochemistry.

Methods

Isogenic cultures of *B. subtilis* strains at 0.5 OD were mixed 50% in glycerol aliquots and individually snap frozen and stored at -80 C. For each experiment, 200 µL aliquots were removed and thawed, then diluted in 10 ml LB and grown in a 37 C shaking incubator for 4 hours until mid-log phase, then pelleted. The supernatent was removed, and the bacterial pellets were then resuspended in 200 µL LB, yielding a culture with an approximate OD of 10, then 1.5 µL of that culture was deposited on plates of Teknova EZ-RDM and 0.5% agarose, then dried 10 mins and sealed with parafilm. Depending on the type of experiment, plates were then either incubated for 1 hr then imaged or imaged immediately at 37 C.

Plates were created by mixing 100 mL Teknova EZ-RDM and 0.5% agarose by weight, autoclaving for 15 minutes, allowing the media to cool to 50 C in an incubator before being poured into 4 plates (25ml each) and cooled under flame in open air for 10, 15, or 30 minutes before immediate inoculation, creating a range of initial gel hydrations to examine the effects of water availability on colony morphology.

Brightfield microscopy was performed using a Nikon SMZ-25 stereo zoom microscope, with a P2-SHR Plan Apo 1x objective, and a Prior ES111 OptiScan stage. High speed images were taken using an Andor Zyla 5.5 CMOS camera. Phase contrast microscopy was performed using a Nikon Eclipse Ti-E inverted microscope, with a S Plan Fluor ELWD 20x Ph1 ADM objective. High speed images were taken using an Andor iXon Ultra 888 EMCCD camera.

Activity overlays were generated using custom Matlab scripts which calculated the per-pixel sum over the differences between 5 or 7 consecutive frames. Kymographs were generated using custom Matlab

scripts to identify and interpolate contours, then sum over cross-sectional slices of intensities along the contours over time. All scripts and Matlab code are available upon request.

Acknowledgements

We thank Prof. Daniel Kearns and his lab for their generous gift of the *B. subtilis* strains used in this study. We thank Hannah Bishop and Eric Corwin for critical reading of the manuscript. This work was supported by the University of Oregon.

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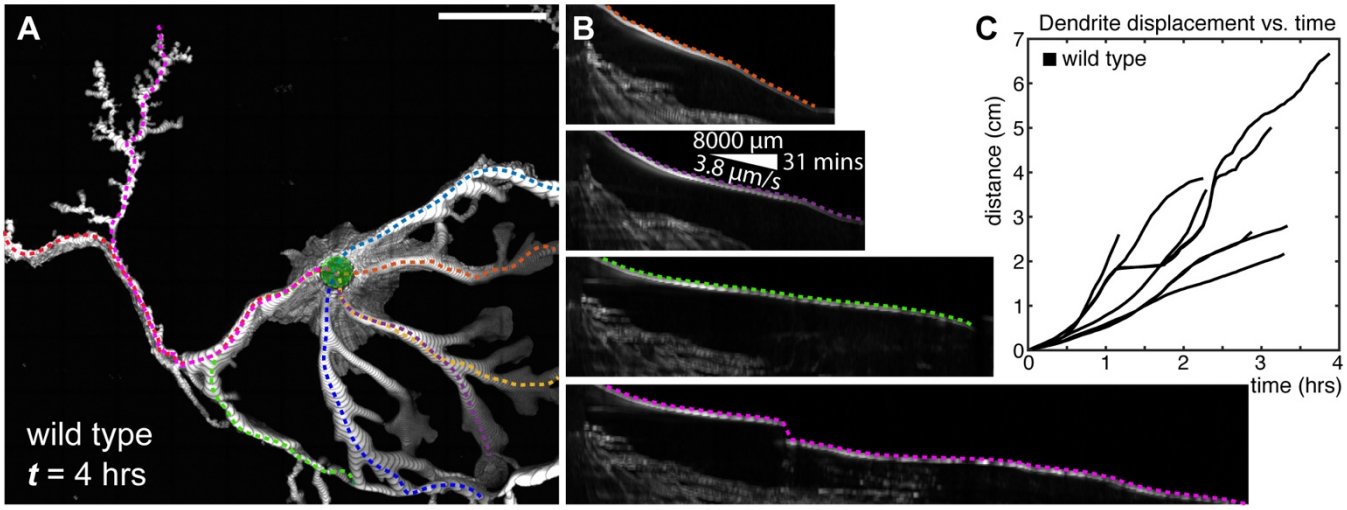

Figure 1. Expansion dynamics of wild-type *Bacillus subtilis*. **(A)** A maximum intensity projection of *B. subtilis 3610* (wt) spreading across nutrient rich plate with 0.5% agarose w/v. Colored contours (dashed lines) are drawn along selected dendrites. Original colony inoculation is shown as green circle. Scale bar is 1 cm. **(B)** Kymographs constructed from imaging data along specific contours (colors matched with (A)). The vertical dimension is time and the horizontal dimension is arc-length of the path of the dendrite. **(C)** Plot of dendrite displacement vs. time, showing rapid surface motility ranging from ~4 µm/s up to ~10 µm/s. See SI for the complete time series.

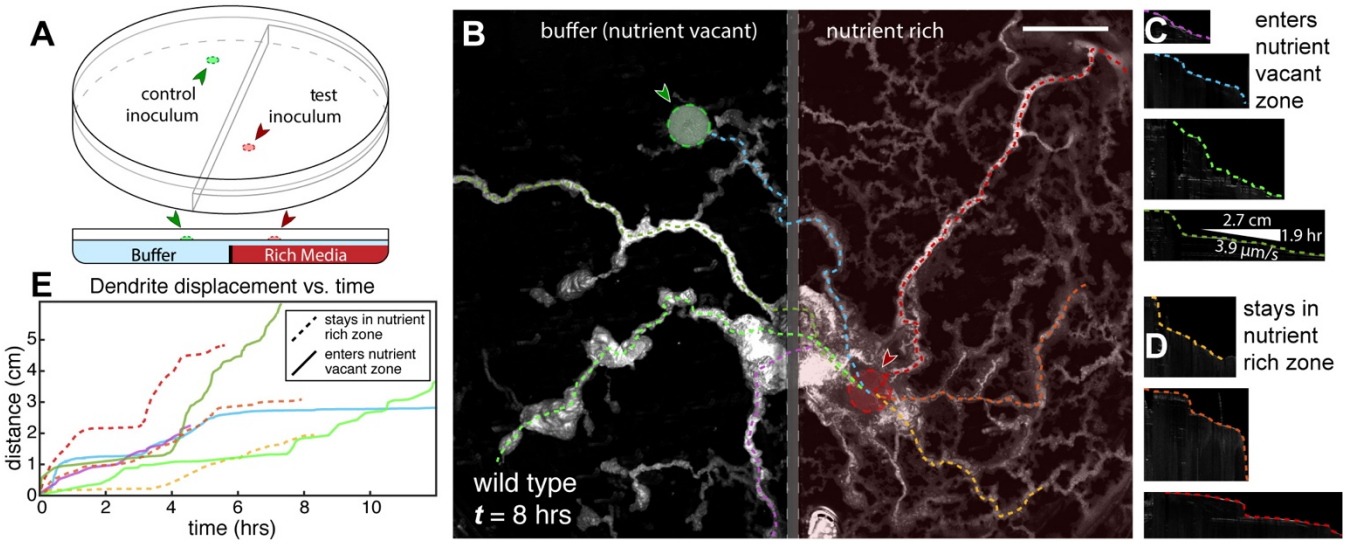

Figure 2. Surface motility in the presence of an exogenous nutrient gradient. **(A)** Low agarose plates were poured with a barrier between nutrient-rich media doped with a fluorescent tracer dye (rhodamine) on the right half of the plate and osmotically balanced zero-nutrient buffer on the left, with a top layer of balanced zero-nutrient buffer to create a contiguous surface. The spatial distribution of the dye is a proxy for nutrient diffusion, and demonstrates that the barrier limits diffusion into the zero-nutrient region. Two dense colonies (OD 5, 1.5μl) were deposited on either side of the barrier. The colony on the zero-nutrient side acts as a no-nutrient motility control. **(B)** Wild type *B. subtilis* inoculated on the nutrient-rich region (right, red dashed circle) spreads dendritically across the divided plate into the zero-nutrient region, effectively traversing along a negative nutrient gradient. An identical inoculum on the zero-nutrient region (green dashed circle) exhibits no collective motility. Scale bar is 1 cm. **(C,D)** Kymographs were generated along curves that trace the extending dendrites. **(C)** dendrite paths that cross the barrier into the zero-nutrient region. **(D)** dendrite paths that remain in the nutrient-rich region. Both types of dendrites exhibit similar rates and behaviors. **(E)** Dendrites extend across the plate from the colony on the nutrient rich side, both into the nutrient-rich region and across the barrier and down the steep negative nutrient gradient. Dashed lines correspond to dendrites which never encounter the barrier and solid lines correspond to those that cross the boundary.

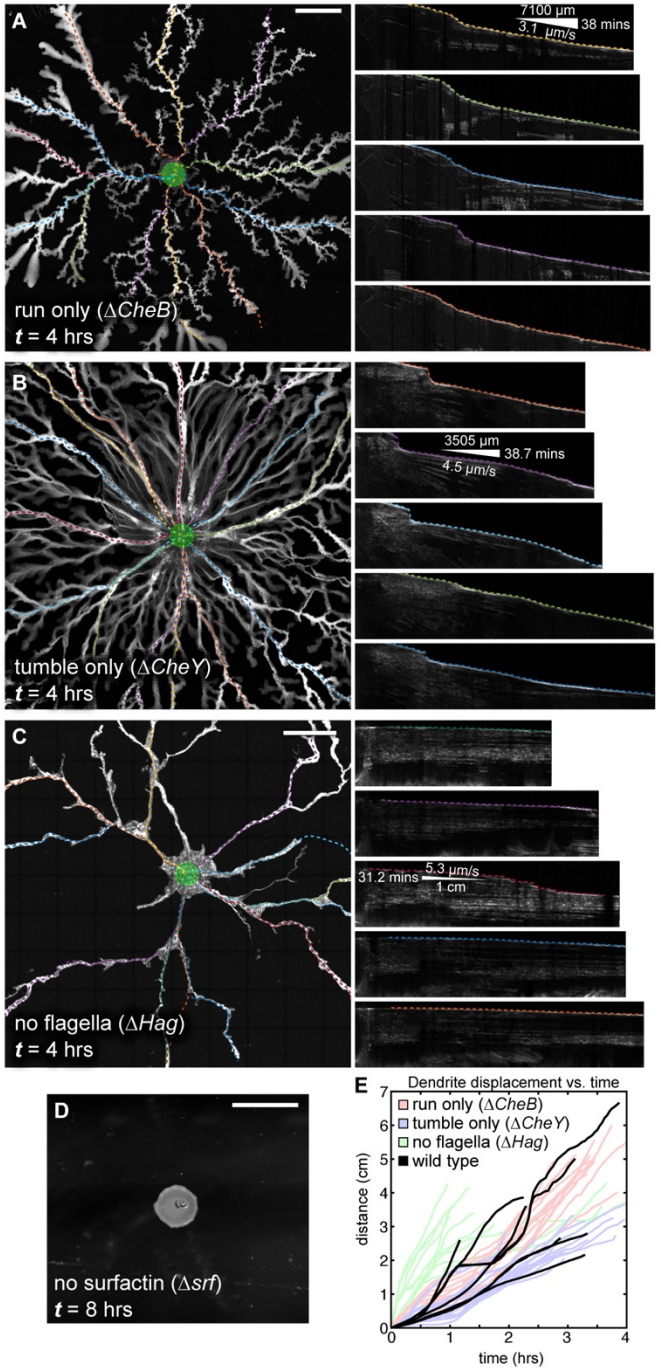

Figure 3. *B.*subtilis genotypes that lack the ability to chemotax or self-propel can still exhibit collective motility. Maximum intensity projections of dendrite dynamics for **(A)** run-only (Δ*CheB*), **(B)** tumble-only (Δ*CheY*), and **(C)** flagellar hook-deficient (Δ*Hag*) genotypes, with contours (dashed lines) overlaid on dendrites and associated kymographs (right) with leading edges indicated (dashed lines). **(D)** Surfactin deficient (Δ*srf*) mutants are not collectively motile. **(E)** Each of the motility mutants (A-C) exhibit similar dendritic morphologies and speeds of advancing dendrites, as illustrated in this plot of dendrite tip displacement vs. time. Note that some dendrites of the non-motile Δ*Hag* genotype outpace all other motile genotypes. These samples represent typical variation of advancing dendrites for their respective genotypes. All scale bars are 600µm.

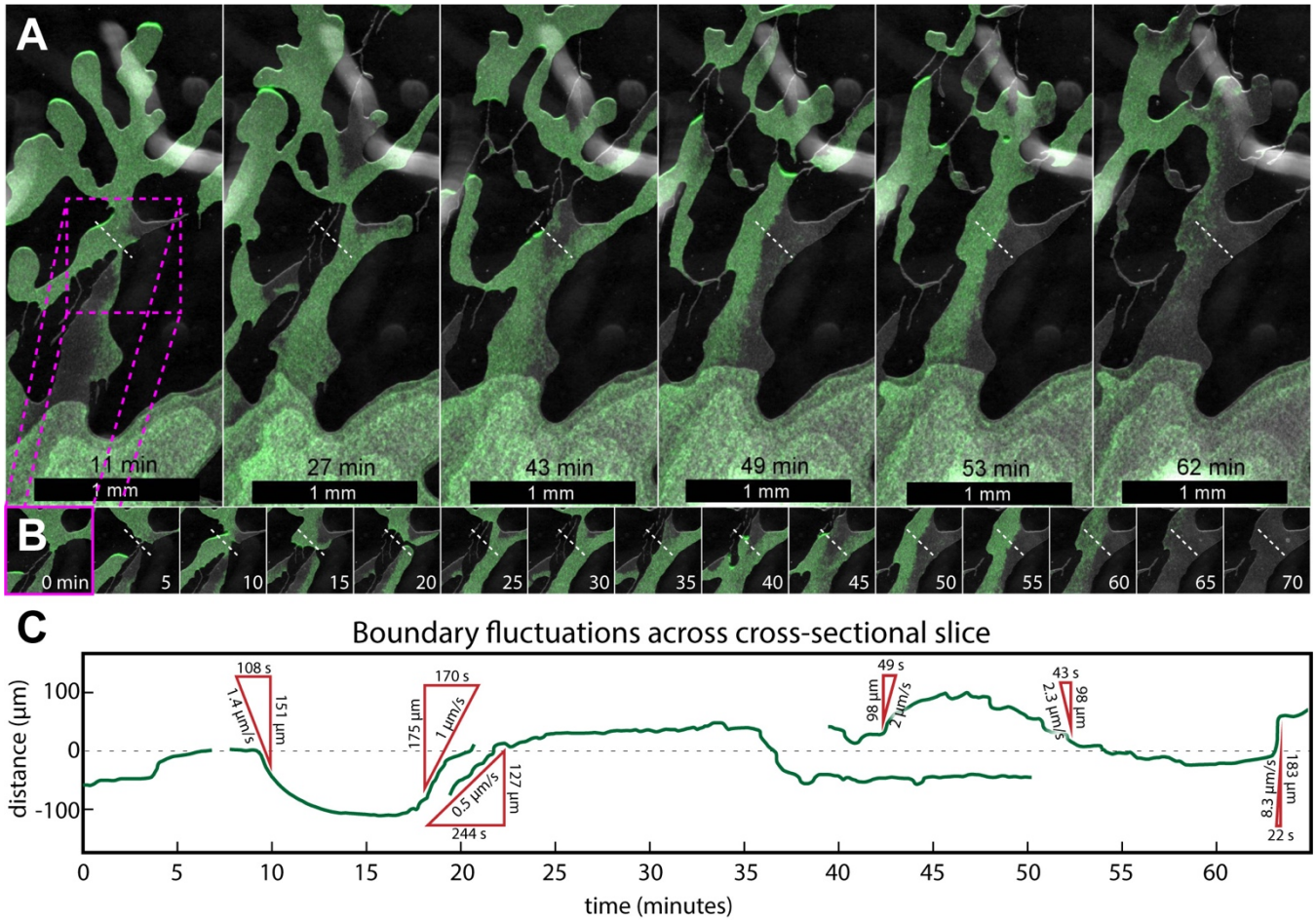

Figure 4. Cellular monolayers exist in fluidized, motile states and jammed, stationary states. **(A-B)** Time-lapse imaging of wild type *B. subtilis* spreading dendritically on a nutrient-rich low agarose surface. We applied a computational image filter that measures local intensity fluctuations over time, and then colors regions of high motile activity (green) and leaves stationary regions uncolored (gray). Transient active regions and fluctuating boundaries between active and stationary regions can be seen emerging and dissipating on timescales shorter than (potential) phenotypic switching (see SI video). These data are consistent with bacterial groups undergoing a dynamic jamming transition that halts collective motion and dendrite expansion. **(C)** A plot of the boundary positions between fluidized and jammed regions over time (across the white dashed line in (A)). Scale triangles indicate rates of boundary movement, with several boundary fluctuations moving on the order of 1 - 10 µm/s.

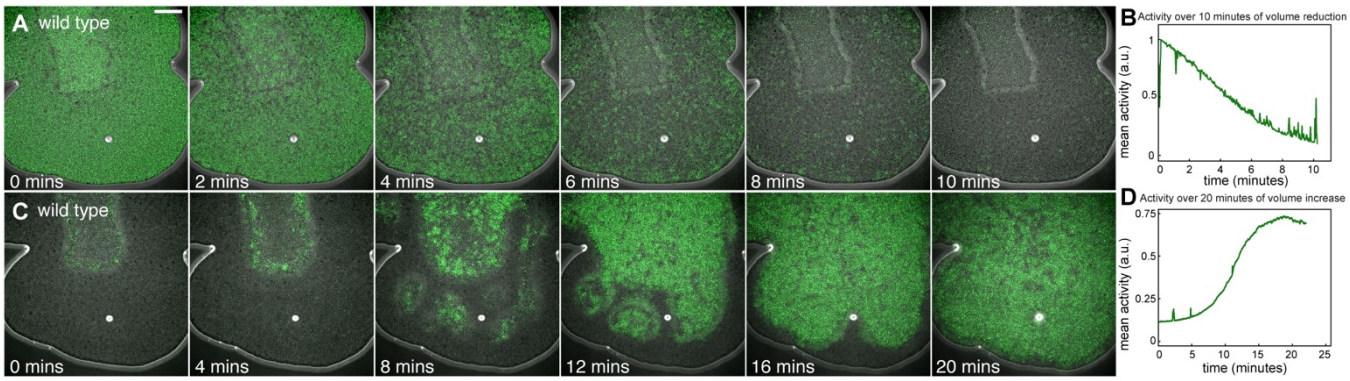

Figure 5. Water availability and cellular packing fraction control the jamming transition. **(A)** Time-lapse imaging of the tip of a wild type *B. subtilis* dendrite on a nutrient-rich low agarose plate. At $t = 0$, the plate was unsealed and its surface was exposed to air and imaged while being allowed to evaporate for 10 minutes. The overall effect was less water available at the agarose/air interface, and thus a slight, transient increase in cellular packing fraction that caused both collective motion (green color) and the computational measure of mean motile activity **(B)** to drop sharply as the cellular group entered the jammed state. Across the entire dendrite tip collective motility halted. **(C - D)** The same plate was then immediately resealed and imaged over 20 minutes. The sharp drop in evaporation rate allowed cells to retain more water from the gel and thus decreased their packing fraction. This ultimately allowed fluidized domains of motile cells to emerge and coalesce until the entire dendrite was, once again, collectively motile and had resumed expansion. Scale bar is 100μm.