A field guide to bacterial swarming motility

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Abstract | How bacteria regulate, assemble and rotate flagella to swim in liquid media is reasonably well understood. Much less is known about how some bacteria use flagella to move over the tops of solid surfaces in a form of movement called swarming. The focus of bacteriology is changing from planktonic to surface environments, and so interest in swarming motility is on the rise. Here, I review the requirements that define swarming motility in diverse bacterial model systems, including an increase in the number of flagella per cell, the secretion of a surfactant to reduce surface tension and allow spreading, and movement in multicellular groups rather than as individuals.

Planktonic
Of bacteria: growing as dispersed cells in a liquid environment.

Flagellum
A complex molecular machine, assembled from over 40 different proteins, that is the motor for swimming and swarming motility. Rotation of a membrane-anchored basal body rotates a long, extracellular, corkscrew-shaped filament that acts like a propeller to generate force.

Type IV pilus
A proteinaceous pilus that extends from one pole of the cell, attaches to a surface and retracts, thus acting as the motor for twitching motility. Retraction causes the cell body to move towards the anchor point of the pilus.

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doi:10.1038/nrmicro2405
Published online
9 August 2010

Bacteria have traditionally been viewed as unicellular organisms that grow as dispersed individuals in a planktonic environment. Recently, this view has begun to change as we have gained an increasing awareness of the role of biofilms, which are communities of sessile organisms that secrete an extracellular matrix and aggregate as multicellular groups. Surface-associated bacteria have another option besides sessile aggregation: sometimes, these bacteria can become highly motile and migrate over the substrate in a process known as swarming. Biofilm research has renewed our interest in bacterial swarming motility, which is often oppositely regulated and antagonistic to biofilm formation[1].

Swarming motility is operationally defined as a rapid multicellular movement of bacteria across a surface, powered by rotating flagella[2] (FIG. 1). Although simple, accurate and mechanistically meaningful, this definition does not do justice to the wide array of phenotypes that are associated with swarming motility, nor does it emphasize all that remains unknown about this behaviour. Furthermore, it is important to acknowledge the common field-specific misnomers (BOX 1) and to distinguish swarming from behaviours such as swimming, twitching, gliding and sliding, which can also occur within or on top of solid surfaces[3] (FIG. 1).

Swimming motility is a mode of bacterial movement that is also powered by rotating flagella but, unlike swarming motility, swimming takes place as individual cells moving in liquid environments. Twitching motility is a surface motility powered by the extension and retraction of type IV pili, which confers slow cell movement, often with a jerky or 'twitchy' appearance[4]. Gliding motility is a catch-all definition for active surface movement that occurs along the long axis of the cell without the aid of flagella or pili. Gliding seems to have evolved independently in multiple lineages but generally involves the cell body moving through the use of focal-adhesion complexes that bind to a surface substrate[5]. Sliding motility is a passive form of surface spreading that does not require an active motor[2] but instead relies on surfactants to reduce surface tension, enabling the colony to spread away from the origin, driven by the outward pressure of cell growth. Furthermore, sliding is easily mistaken for swarming motility and can occur when the flagella are disrupted in bacteria that would normally swarm[6–10].

This Review introduces the phenomenon of swarming motility from a practical standpoint, then describes the cellular requirements and phenotypes that are associated with swarming from diverse model organisms and, finally, discusses some of the mysteries and controversies associated with this type of bacterial motility.

Studying swarming motility in the laboratory

Swarming motility seems to be narrowly conserved in the bacterial domain and is currently restricted to three families (FIG. 2). The reported number of swarming species is almost certainly an underestimate, because swarming motility is often inhibited by standard laboratory media and genetically abolished during the domestication of commonly-used laboratory strains[11–14]. The selection against swarming in these strains may be due to evolutionary forces that act when surface motility provides no advantage, for example in unstructured laboratory environments[15]. Alternatively, bacteria that spread promiscuously over plates are rarely welcomed

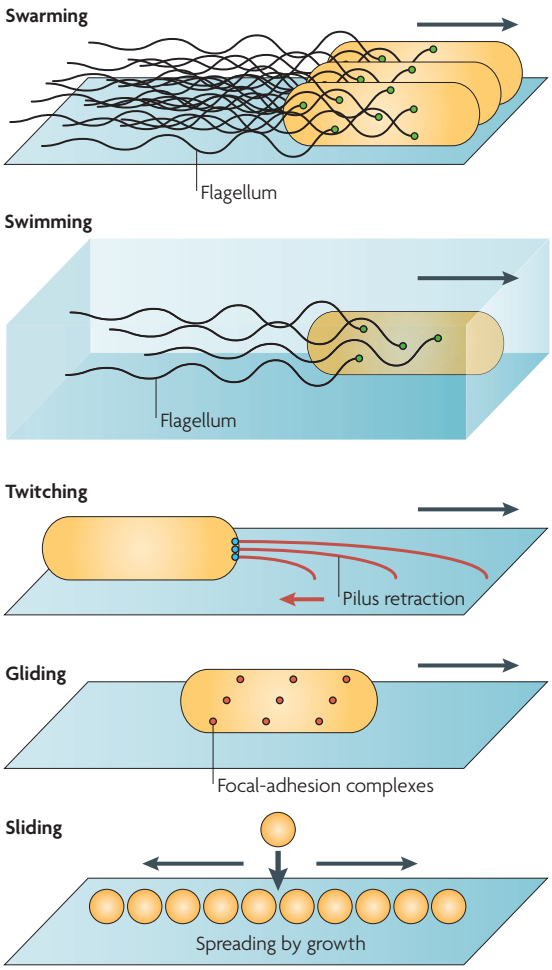

Figure 1 | **Bacteria move by a range of mechanisms.** Swarming is the multicellular movement of bacteria across a surface and is powered by rotating helical flagella. Swimming is the movement of individual bacteria in liquid, also powered by rotating flagella. Twitching is surface movement of bacteria that is powered by the extension of pili, which then attach to the surface and subsequently retract, pulling the cell closer to the site of attachment. Gliding is active surface movement that does not require flagella or pili and involves focal-adhesion complexes. Sliding is passive surface translocation that is powered by growth and facilitated by a surfactant. The direction of cell movement is indicated by black arrows, and the motors that power the movement are indicated by coloured circles.

by geneticists, and selection against swarming may be artificial in favour of small, compact colonies.

Swarming motility generally requires an energy-rich, solid medium, but the specific conditions that support swarming depend on the organism concerned. Some bacteria, such as *Bacillus subtilis*, swarm on a wide range of energy-rich media, whereas other bacteria, such as *Salmonella enterica* and *Yersinia entercolitica*, require the presence of particular supplements (for example, glucose)[16–18]. Swarming is promoted by high growth rates, which may account for the requirement for energy-rich conditions[12,19,20]. Although some bacteria can swarm over almost any agar surface, most swarming bacteria require soft agar in a narrow range of concentrations. Media that are solidified, with agar concentrations above 0.3%, exclude swimming motility and force the bacteria to move, if possible, over the surface, and agar concentrations above 1% prohibit swarming of many bacterial species. It is conceivable that the standard 1.5% agar that is used to solidify media in the laboratory was specifically chosen for swarming inhibition.

When conducting swarming-motility assays, a defined set of conditions must be established and rigorously adhered to[21]. The water content of the medium is a crucial factor: too little water results in poor swarming, whereas too much water may permit swimming motility. To control the water content, swarm plates are poured to a standard thickness when the agar is relatively cool (~50 °C), thereby minimizing water loss from condensation on the plate lid. Finally, plates are dried briefly (for ~15 minutes), open-faced, in a laminar flow hood to remove surface water and minimize the contribution of swimming motility to surface movement[12,21].

Requirements for swarming motility

Flagella are the most important requirement for swarming motility, along with an increase in flagellar biosynthesis, but this type of movement also requires an increase in cell–cell interactions and the presence of a surfactant.

Flagella. Flagella may be observed by phase contrast microscopy using a simple crystal violet-based stain[22], by fluorescence microscopy using fluorescent dyes[23,24] or by electron microscopy[25,26]. The presence of flagellated cells at the front of a spreading colony is consistent with, but not conclusively demonstrative of, the mechanism of swarming motility. To confirm the mechanism of swarming, mutants with defects in flagella synthesis or function must be used to abolish colony spreading[27].

Most bacteria that swarm have a peritrichous arrangement of flagella, in which multiple flagella are distributed randomly on the cell surface[11,18,25,28–30]. Peritrichous flagella bundle together when rotated, to effectively increase flagellar stiffness and make force generation more efficient in viscous liquids, a property that may also explain their correlation with swarming[31–34]. Recently, *E. coli*, which is peritrichously flagellated, has been shown to swarm between two closely opposed fixed surfaces[24,35–37]. As a single flagellum requires minimal resource investment and is sufficient for swimming motility, it is tempting to speculate that the synthesis of multiple peritrichous flagella is a specific adapation to generate force in viscous environments and to swarm over and between surfaces.

The correlation between peritrichous flagella and swarming is not absolute, and some bacteria with flagella originating from a single cell pole can swarm. *Vibrio parahaemolyticus*, *Rhodosprillum centenum* and *Aeromonas* spp. each make a single polar flagellum that is sufficient to swim in liquids but must induce peritrichous flagella (also called lateral flagella) to swarm over surfaces[28,30,38–40]. The polar and lateral flagella are encoded by different genes, powered by separate motors and

Focal-adhesion complex
A putative cell surface-associated complex that anchors a bacterium to a substrate and might act as a motor for gliding motility. When coupled to an internal motor, the cell body moves relative to the focal-adhesion complex.

Box 1 | **Misnomers**

The term 'swarming motility' refers to the verb 'to swarm', meaning to move about in great numbers, because individual bacteria move rapidly in a larger group. However, the image of a swarm is appropriate for a range of bacterial phenomena, and use of the term 'swarm' in the broad sense has caused considerable confusion with respect to the formal definition of swarming motility.

Swarm assay of bacterial chemotaxis

A particularly unfortunate misnomer is found in the common vernacular of the chemotaxis of swimming bacteria. Bacteria inoculated into the centre of a nutrient-rich plate fortified with less than 0.3% agar will consume nutrients locally, generate a nutrient gradient and chemotax up the gradient through the pores in the agar[100]. Although bacteria technically swim through liquid-filled pores, the assay is called a 'swarm assay'. When reading the swarming literature, it is important to confirm that the agar concentration used in an experiment is greater than 0.3%, as this is the minimum agar concentration needed to exclude swimming and define swarming motility.

Swarmer cells of *Caulobacter crescentus*

Caulobacter crescentus is a bacterium that grows with a remarkable dimorphic life cycle[135]. Each round of cell division is asymmetric and gives rise to a non-motile 'stalked cell' that synthesizes a prosthecum with an adhesive holdfast at the tip, and a 'swarmer cell' that synthesizes a single flagellum and swims in liquid environments. *C. cresentus* swarmer cells have not been found to exhibit swarming motility on solid surfaces.

Swarms of *Myxococcus xanthus*

Myxococcus xanthus is a predatory, surface-associated bacterium that moves in large multicellular groups and secretes digestive enzymes to destroy and consume other bacteria in the environment[136]. Groups of *M. xanthus* are referred to as 'swarms', despite the fact that neither of the independent mechanisms by which they move over surfaces (twitching and gliding) requires flagella or constitutes swarming motility.

regulated differentially[30,39–42]. *Pseudomonas aeruginosa* is a short, rod-shaped bacterium that also makes a polar flagellum. During swarming, *P. aeruginosa* retains its polar flagella but synthesizes an alternative motor that is specifically required to propel movement on surfaces and through viscous enviroments[43,44]. Thus the expression of alternative motors is at least one way to facilitate swarming motility besides the use of peritrichous flagella.

When cells transition from swimming to swarming, the number of flagella on the cell surface increases. Organisms with alternative flagellar systems become hyperflagellate in the transition from expression of a single polar flagellum to expression of multiple peritrichous flagella. Species with one flagellar system also seem to increase the number of flagella on the cell surface during swarming[6,18,20,25,29,45–47]. Even *P. aeruginosa*, which swims with a single polar flagellum, may produce two polar flagella when moving on a surface[48,49]. Mutations that reduce the expression of flagellar genes reduce flagellar number and reduce or abolish swarming[17,20,46,50–56]. Conversely, mutations that enhance the expression of flagellar genes increase flagellar number and enhance swarming[47,54,55,57–60]. The reason that swarming requires multiple flagella on the cell surface is unknown.

Rafting. Bacteria swim as individuals, but swarming bacteria move in side-by-side cell groups called rafts[11,17,20,24,26,29,36,49,61–63] (FIG. 3a). Raft formation is dynamic: cells recruited to a raft move with the group, whereas cells lost from a raft quickly become non-motile. The dynamism in cell recruitment and loss suggests that no substance or matrix maintains raft stability, except perhaps the flagella themselves. Indeed, scanning electron microscopy of a swarm of *Proteus mirabilis* revealed extensive rafting and, perhaps, intercellular bundling of flagella[26] (FIG. 3b). As with hyperflagellation, the reason that swarming motility requires raft formation is unclear at present.

Surfactant synthesis. Many swarming bacteria synthesize and secrete surfactants (short for 'surface-active agent'). Surfactants are amphipathic molecules that reduce tension between the substrate and the bacterial cell and, in doing so, can permit spreading over surfaces. Surfactants often manifest as a clear, watery layer that precedes the cells at the swarm front[11,29,45,49,64]. Some bacteria fail to make swarming surfactants and will only swarm on special agar with inherently low surface tension owing, perhaps, to the presence of a surfactant in the agar itself[9,18,35,40,65].

The presence or absence of secreted surfactant can be easily detected using a drop collapse assay[66,67]. When water is spotted onto a hydrophobic substrate (such as polystyrene), the surface tension of the water allows the drop to stay as a rounded bead. However, if a surfactant is also present, hydrophobic parts of the surfactant molecule associate with the surface of the hydrophobic substrate, whereas hydrophilic parts of the molecule associate with the water, causing both surfactant and water to spread further and causing the drop to 'collapse'. To test for surfactants, culture supernatants need only be spotted on a hydrophobic surface, and the degree to which the drop collapses is correlated with surfactant strength and concentration.

B. subtilis and *Serratia liquefaciens* secrete the potent lipopeptide surfactants surfactin and serrawettin, respectively[6,11,68–70] (FIG. 4). Both lipopeptides are made of a non-ribosomally assembled polypeptide that is closed into a ring by a fatty acid, and they are synthesized by homologous sets of enzymes[6,69–71]. Mutations that abolish surfactant production in these species also abolish swarming, and swarming can be rescued by exogenous addition of purified surfactant[11,16,68]. Initial characterization of *P. aeruginosa* implicated rhamnolipids as the swarming surfactants[48]. Di-rhamnolipid is composed of two rhamnose sugars attached to the complex fatty acid β-hydroxydecanoyl-β-hydroxydecanoate (HAA)[72–74] (FIG. 4). Subsequent investigation has shown that the di-rhamnolipid precursors HAA and mono-rhamnolipid also act as surfactants to promote swarm expansion[72,74,75]. The specific properties and potential antagonistic effects of HAA and rhamnolipid molecules during swarming continue to be investigated.

Surfactant production is commonly regulated by quorum sensing[68,76–78]. Surfactants are shared secreted resources and are effective only at high concentration. Therefore, quorum sensing may have evolved to regulate the production of surfactants to ensure that they are made only when there are sufficient bacteria present to make surfactants beneficial.

Both *E. coli* and *S. enterica* seem to swarm without surfactants. Lipopolysaccharide (LPS), a complex

Surfactant
A secreted molecule that associates with a surface and acts like a lubricant to reduce surface tension.

Hyperflagellate
Of a bacterium: with an increased number of flagella on the cell surface.

Quorum sensing
A strategy by which bacteria regulate gene expression in a manner that is dependent on high population density.

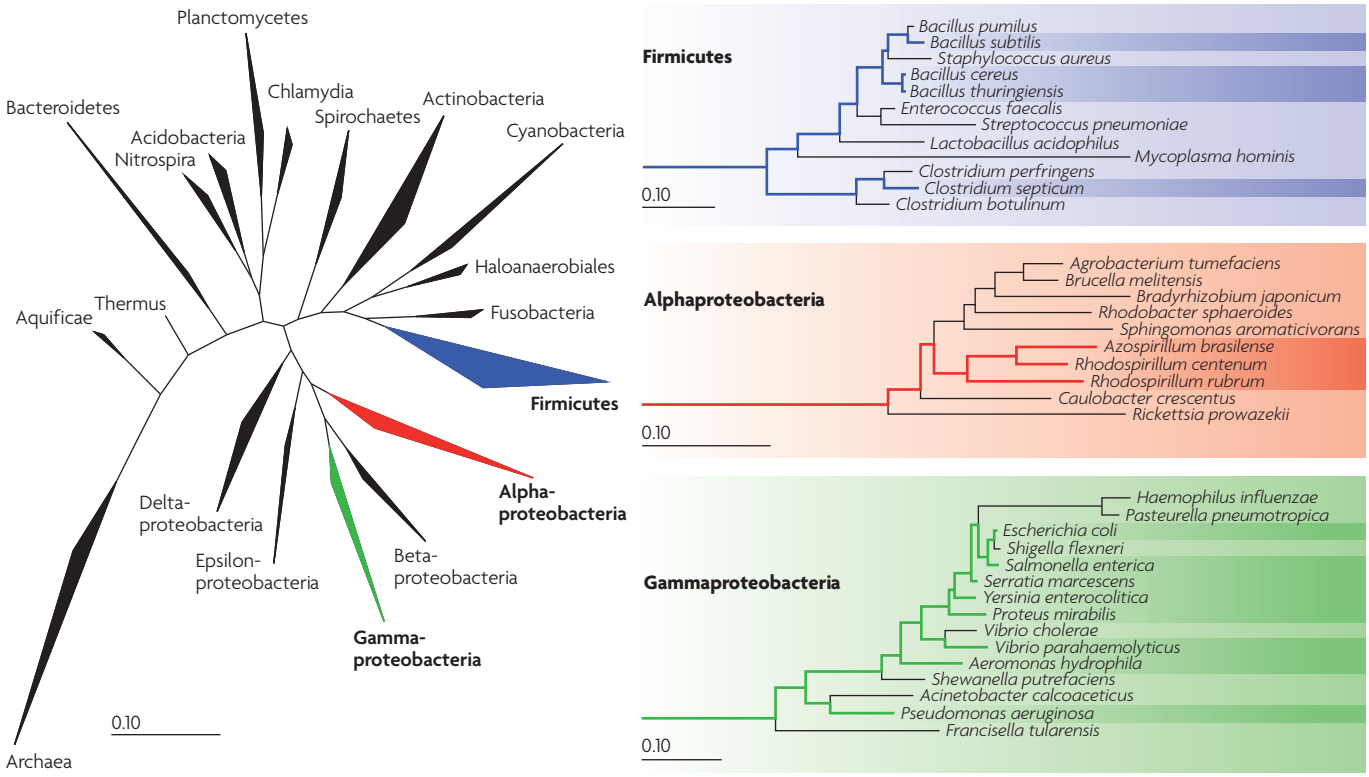

Figure 2 | **Phylogenetic distribution of swarming motility.** A bacterial phylogeny based on the 16S ribosomal RNA gene. Highlighted species names indicate the ability of the species to undergo swarming motility. Swarming motility has not yet been demonstrated for those species that are not highlighted. Trees were generated from 1,547 aligned positions using the neighbour-joining algorithm on distances determined under the HKY85+I+G substitution model in PAUP* v4.0b10. The scale bar represents a distance of 0.1 substitutions per site. Original trees constructed by D. Kysela, Indiana University, Bloomington, USA.

lipid–polysaccharide hybrid in the outer membranes of Gram-negative bacteria, was initially implicated as an important wetting agent, because mutations that abolished LPS also abolished swarming[65]. Consistent with a physical role for LPS, surface spreading could be restored to LPS-deficient mutants when they were introduced to a highly wettable surface or in the presence of exogenously provided surfactant[65]. Recently, swarming was restored to LPS-deficient *E. coli* mutants by secondary mutations in the Rcs (regulation of capsular synthesis) envelope stress response signal transduction pathway, which negatively regulates the expression of flagellar genes[63,79]. LPS-deficient *P. mirabilis* mutants are also unable to swarm, owing to reduced flagellar synthesis, and swarming can be similarly restored by mutations in the Rcs system[56]. Such studies showing genetic bypass indicate that LPS is dispensible for swarming, does not act as a wetting agent and is instead either directly or indirectly regulatory. The wetting agent that promotes *E. coli* swarming remains unknown.

Swarming-associated phenotypes

The swarming lag, cell elongation and colony pattern formation are all phenotypes that are associated with swarming motility but that can be abrogated or bypassed without loss of swarming behaviour.

The swarming lag. A lag period of non-motile behaviour precedes the initiation of swarming motility when bacteria are transferred from a liquid medium to a solid surface[11,61,80,81] (FIG. 5a). The swarming lag is constant for a particular set of conditions but may be shortened by increasing the inoculum density or abolished by using particular mutants[11,54,58,82–84]. The lag is poorly understood, but its occurence indicates that swimming cells must go through a change to become swarming proficient.

There seem to be at least three requirements to exit the swarming lag in *B. subtilis*. The first requirement is for high cell density, to induce surfactin production. Surfactin does not determine the minimum lag duration, however, because the lag is not reduced when cells are inoculated on agar that is preconditioned with surfactant[11]. The second requirement for exiting the swarming lag seems to be hyperflagellation, because the lag is abolished in cells that are artificially upregulated for flagellar synthesis[54]. The third requirement is poorly understood and inferred from the fact that the lag is abolished when actively swarming cells are harvested from a plate and reinoculated at high density on fresh swarming medium (FIG. 5a). A cell density-dependent lag period will occur, however, when surface-harvested swarming cells are diluted and reinoculated in the presence of surfactant (FIG. 5b). Thus, the third requirement may be a critical density of cells that is necessary to enable the

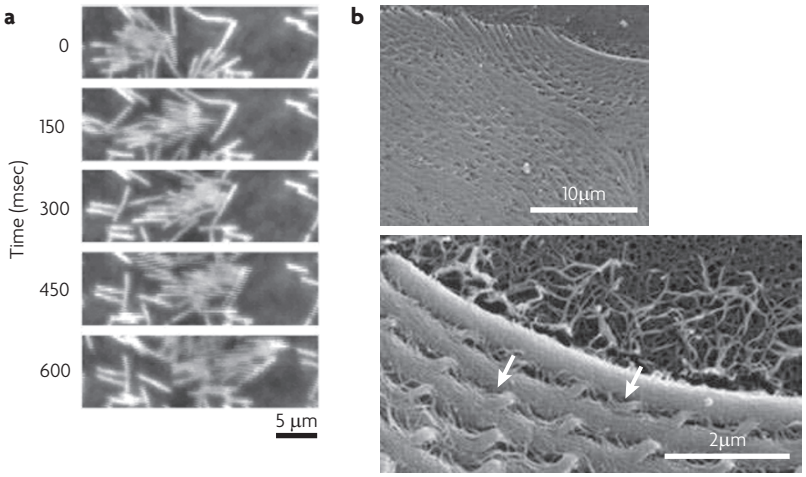

Figure 3 | **Rafting. a** | A time-lapse series of images of a *Bacillus subtilis* raft moving from left to right in a swarming monolayer. **b** | Images of elongated *Proteus mirabilis* cells swarming as a large raft in a catheter. Arrows indicate flagellar bundles. Part **a** images modified, with permission, from REF. 11 © (2003) Blackwell Scientific Publications. Part **b** images reproduced, with permission, from *Spinal Cord* REF. 137 © (2010) Macmillan Publishers Ltd. All rights reserved.

formation of nucleation centres for the dynamic multicellular rafts — reminiscent of both the critical protein concentration that is required for the assembly of tubulin and the dynamic instability of this protein[85,86].

Cell elongation. It is commonly thought that swarming cells suppress cell division and that cell elongation is either a requirement for or an indicator of swarming motility. The connection between filamentation and swarming motility originates with *P. mirabilis*, which makes short rods when grown in broth and long filaments with multiple nucleoids when grown on surfaces[25,81,87] (FIG. 3b). Other bacteria were later found to have subpopulations of long cells enriched at the leading edge of a swarm[18,29,45,88,89]. To date, it is unclear whether elongated cells are required for swarming or whether they simply accumulate at the swarm edge. Despite the importance of elongation in the dogma of swarming motility, no mechanistic or regulatory connection has been elucidated at the molecular level for the control of cell division during swarming. Furthermore, substantial cell elongation is neither a requirement for nor co-regulated with swarming motility in many bacteria[11,16,39,40,48,49,90,91].

Other than the original observations in *P. mirabilis*, few studies have confirmed that the elongated cells observed during swarming are, in fact, filamentous. 'Filamentous' describes a defect in cell division such that cells continue to grow in the absence of septation. Cells that seem to be elongated can also arise through the failure of cell separation following successful division, resulting in cells that are linked end-to-end in long chains. Chains and filaments can be difficult to distinguish by phase contrast microscopy, but they can be differentiated by fluorescence microscopy coupled with membrane staining (FIG. 6). Before declaring that a cell is filamentous, one should determine whether or not septa are present.

Colony pattern formation. Swarming bacteria form macroscopic colony patterns on solid media. The patterns may take different shapes but the relevance of any particular pattern is unclear. Furthermore, it seems likely that all swarming bacteria can produce a range of patterns depending on the environmental conditions[92,93]. Therefore, pattern formation may be less of a commentary on swarming regulation and more of an indicator of environmental factors.

Featureless swarms are made when cells spread evenly and continuously outward from the point of inoculation, as a monolayer. The monolayer is transparent but may be seen when incident light is reflected off the surface or when oblique light is transmitted through the agar. Cell density in the monolayer is high and approximately uniform throughout the swarm, increasing slightly at the advancing edge[36]. When the monolayer reaches the boundaries of the plate, the colony grows into a featureless mat[11,20] (FIG. 7a).

The most famous irregular swarming pattern is the characteristic bull's eye formed by *P. mirabilis* that results from cyclic and synchronous waves of motility followed by regular periods of swarming cessation[81,82,94] (FIG. 7b). Each cycle produces a macroscopic 'zone of consolidation' or 'terrace'. In *P. mirabilis*, terraces are thought to arise owing to differentiation of sessile bacteria into swarming, filamentous cells followed by periodic and synchronous de-differentiation into non-swarming short cells[81]. By contrast, the terraces of *Proteus vulgaris* formed in spite of the fact that the cells remained constitutively elongated[95,96]. *Serratia marcescens* and certain *B. subtilis* mutants also form terraces, but the relationship of terracing to cell shape has not been studied in these cases[52,62].

Dendrites (also known as tendrils or deep branches) are long, thin regions of colonization emanating from a central origin (FIG. 7c). Dendrite formation in *P. aeruginosa* depends on secretion of multiple surfactants[72,74,75]. Rhamnolipid derivatives contribute to colony structure differently, as the precursor HAA acts as a repellent, and fully synthesized di-rhamnolipid acts as an attractant[75]. It is thought that dendrites of *P. aeruginosa* will expand and repel each other as a result of the complicated interplay between the two secreted molecules[72,75]. *B. subtilis* swarms as dendrites under some media conditions, and it is thought that dendrites might arise when the local rates of motility exceed the rate of bulk population growth[12,64]. Dendrites also commonly arise from sliding motility[6–10].

Some bacteria form spiraling vortices as they travel across the surface of the plate[2,62,90,97] (FIG. 7d). These vortices are large, localized groups of cells travelling in a common circular path and have also been referred to as 'wandering colonies' (REF. 2). In the case of *Paenibacillus vortex*, swarming motility combined with inherently curved cell morphology may produce the vortex pattern[90]. Consistent with an influence of cell shape, *B. subtilis* does not normally make vortices during swarming but will do so when mutations result in long, aseptate filamentous cells[98]. Therefore, the vortices may simply be the consequence of constraining swarming to conform to aberrant cell morphology.

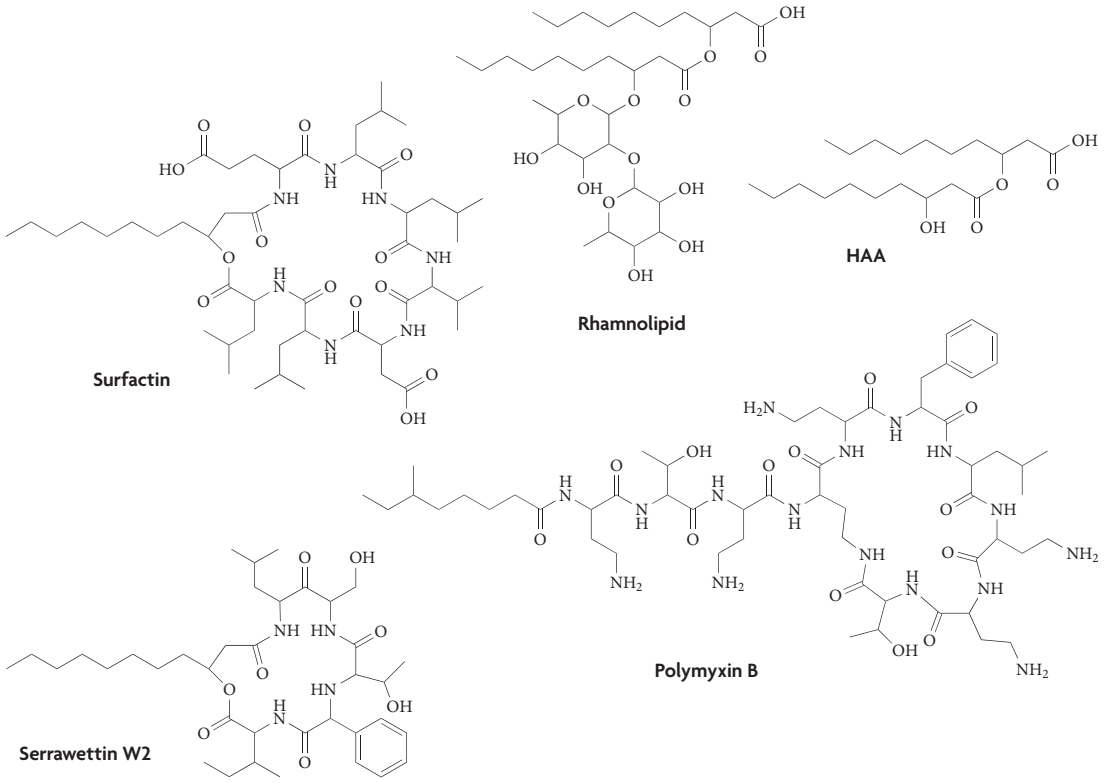

Figure 4 | **Surfactants.** Swarming bacteria use chemically distinct secreted surfactants to spread over solid surfaces, such as surfactin (produced by *Bacillus subtilis*) and serrawettin W2 (produced by *Serratia liquefaciens*). *Pseudomonas aeruginosa* synthesizes β-hydroxydecanoyl-β-hydroxydecanoate (HAA), a complex fatty acid that is a component of rhamnolipid; both HAA and rhamnolipid are thought to contribute to swarming motility. Polymyxin B is an antibiotic that is included here for comparison with the swarming surfactants.

Non-swarming cells that are unable to spread across the surface grow as a confined colony in the centre of the plate (FIG. 7e). On prolonged incubation, the colony diameter of a non-swarming strain may increase owing to the contribution of sliding motility. The selective pressure for suppressor mutations that restore motility to non-swarming strains is strong. Suppressor mutants segregate from the competition of the colony in asymmetric flares and exploit the uncolonized agar, giving them a massive growth advantage[52,54,83] (FIG. 7f). Putative suppressor mutants should be clonally isolated from flares and retested for swarming to determine whether or not they have genetically inherited the ability to swarm. Suppressor mutants may arise rapidly, so it is advantageous to characterize the swarming defect of a mutant over a limited time frame using a quantitative swarm assay rather than simply inoculating the centre of a plate and incubating overnight[11,52].

Swarming mysteries and controversies

The role of chemotaxis. Chemotaxis is the directed movement of an organism with respect to a chemical gradient. Bacteria mediate chemotaxis by biasing the duration spent in one of two behaviours, either running in a relatively straight line or tumbling erratically to acquire a new direction. Running and tumbling are controlled by the direction in which the flagella rotate. A series of chemotaxis signal transduction proteins detects stimuli in the environment, transduces the stimulus and controls the direction of flagellar rotation[99].

Swarming bacteria migrate rapidly away from the point of inoculation, and one might assume that swarming behaviour is chemotactically oriented, because the movement resembles the chemotactic behaviour of bacteria swimming through a loose agar substrate[100–102]. Furthermore, some swarming bacteria have been shown to be proficient for chemotaxis towards particular chemicals[103,104] and phototactic towards light[38,105]. Finally, some mutants that are defective in chemotaxis also lose the ability to swarm[11,18,102,104,105]. Despite these behavioural phenomena and genetic data, chemotaxis is unlikely to drive bulk swarm expansion, because cells in a swarm do not exhibit the running and tumbling behaviour that forms the basis of chemotactic orientation and are instead randomly reoriented by external collisions with other bacteria[36]. In addition, swarming is unaffected when chemotaxis is abolished by saturating receptor proteins with non-metabolizable ligand analogues[106], and some mutants that are severely defective in chemotaxis are not impaired for swarming[11,83,107].

The role of chemotaxis is further complicated by the fact that the chemotaxis signal transduction proteins are often required for swarming in ways that are seemingly unrelated to the control of directed movement. One

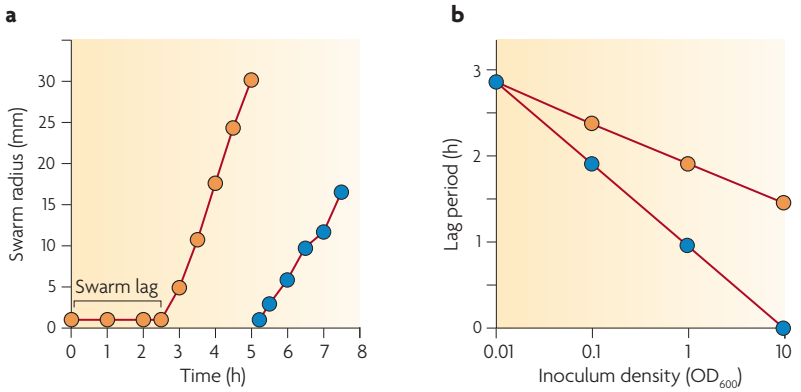

Figure 5 | **Swarming lag. a** | When *Bacillus subtilis* cells are transferred from broth culture to a solid surface, a lag precedes active swarming of the bacteria (orange circles). The lag is abolished if actively swarming cells are re-inoculated onto a fresh surface (blue circles). **b** | When saturating amounts of purified surfactant are added to the plates before inoculation, the lag period of *B. subtilis* decreases with increasing cell density of the inoculum, whether broth-grown bacteria (orange circles) or actively swarming bacteria (blue circles) are used as inocula. Part **a** data from REF. 11.

model suggests that the subset of chemotaxis mutants that cause excessive tumbling physically disrupt the ability of cells to form stable multicellular rafts[11,107]. Another model proposes that the chemotaxis system maintains the periodic switches in flagellar rotation that are necessary to somehow extract water from the substrate[91]. A third model invokes the idea that chemotaxis (Che) proteins have a second function involved in regulating the expression of flagellar genes and/or flagellar assembly[45,108]. Although Che proteins are sometimes required for swarming, the outward expansion of swarming bacteria seems to be a rapid, non-directed means of distributing a bacterial population over a surface.

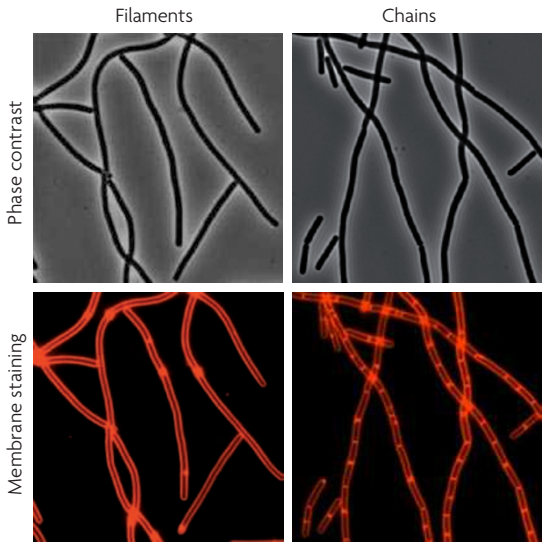

Figure 6 | **Cell filaments and cell chains.** *Bacillus subtilis* mutant cells are compared using phase contrast and fluorescence microscopy using the membrane dye FM4-64 (which has been false-coloured red). Chains of cells from a *swrA* (swarming-motility protein A gene) mutant[54] have regular septa, whereas filaments from a *minJ* mutant[98] do not.

The mechanism of surface sensing. Swarming motility requires contact with a solid substrate, and interaction with a surface may induce cells to become swarming proficient during the swarming lag. If surface contact is indeed an inducing stimulus, it stands to reason that the cells must contain a signal transduction system to transduce this information. Elucidating the mechanism of surface sensing, or determining the molecular basis for the bacterial sense of touch, is the 'holy grail' of swarming-motility research.

The sense of touch is poorly understood for all systems, but it is particularly problematic for bacteria. The plasma membrane contains signal transduction systems but is separated from the site of surface contact, either by the thick peptidoglycan of Gram-positive bacteria or the de-energized outer membrane of Gram-negative bacteria. Therefore, polymers that transit these layers may provide a conduit for signal transduction, and bacterial flagella are potential candidates for a surface sensor. In *V. parahaemolyticus*, the single polar flagellum has been implicated as a sensor, as inhibition of the polar flagellum (by contact with a surface or by various other means) activated expression of the lateral-flagella genes[28,80,109–111]. When flagellar rotation is impeded by contact with a surface, cells may sense changes in ion flux through the flagellar motor[110,111]. Alternatively, cells may sense torque stress on flagellar rotation, perhaps through a poorly understood flagellum-associated transmembrane protein called FliL[112–114].

The mechanism of force generation. During swimming motility, peritrichous flagella on one cell coalesce into a bundle and rotate to propel the bacterium in an approximately straight run. A swimming cell tumbles when one or several of these flagella change their direction of rotation. Swarming bacteria run but do not tumble, and they occasionally back up when all flagella in the cell reverse their direction of rotation, so that the cell moves backward through the flagellar bundle[37]. Furthermore, swarming occurs in multicellular groups, and it is not known why the same flagella that are sufficient for the propulsion of single cells in a liquid are not sufficient for the propulsion of single cells on surfaces. Perhaps rafting promotes flagellar bundling between cells. If so, how is flagellar rotation coordinated between cells to promote unidirectional movement and raft stability? How is flagellar rotation coordinated in cells to result in direction reversals? How are the many flagella rotated at high cell density without tangling or breaking? Advances in imaging the flagella of individual cells in a swarm will hopefully resolve these and other questions about the mechanism of group propulsion[24,37].

Swarming as a developmental state. Swarming motility is a behaviour. Occasionally, the description of swarming motility becomes entangled with the observation of long, hyperflagellated cells and suggests the existence of a developmental programme. Indeed, the long and short forms of *P. mirabilis* seem to be physiologically different[87,115,116]. Other bacteria experience transcriptional

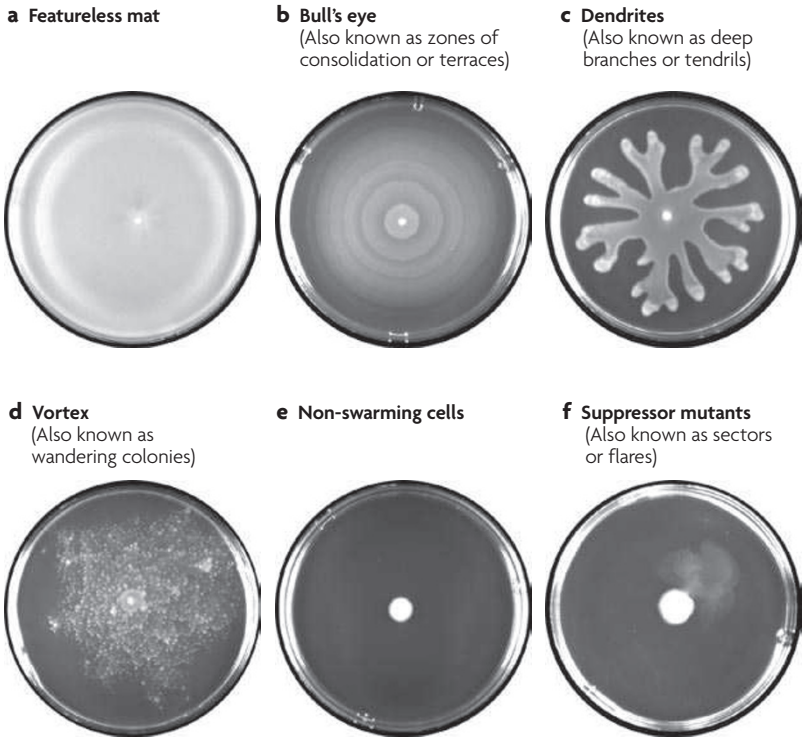

Figure 7 | **Colony pattern formation.** Various colony patterns formed by swarming bacteria. Uncolonized agar is black and bacterial biomass is white. **a** | A featureless swarm formed by *Bacillus subtilis* str. 3610. **b** | The bull's eye pattern formed by *Proteus mirabilis* str. PM7002. **c** | Dendrites formed by *Pseudomonas aeruginosa* str. PA14. **d** | A vortex formed by *Paenibacillus vortex* str. V. **e** | A non-swarming mutant isolate of *B. subtilis* str. 3610. **f** | A non-swarming mutant isolate of *B. subtilis* str. 3610 with a suppressor mutant flare that can be seen above-left of the inoculation site.

and proteomic changes when they come into contact with a surface, but these changes are mostly related to metabolism and stationary phase, and the expression of flagellar genes is unaffected[117–119]. Furthermore, cells do not seem to be developmentally 'committed' to the swarming state and tend to rapidly lose their swarming character when transferred to broth[80]. The swarm lag indicates that swimming cells must change in order to become swarming proficient, but it is not clear that swarm cells constitute a true developmental state.

Swimming in two dimensions? Researchers who study swarming are often asked: "How do you know that swarming is not simply swimming motility constrained in two dimensions?" The possibility that swarming is an artefact of swimming is difficult to dismiss, as both behaviours often require the same flagella, and there are exceptions to the swarming requirements discussed above. For example, it has been speculated that the apparent increase in number of flagellar per cell that occurs during swarming is an optical illusion in some bacteria[88,117]. Furthermore, rafting may be a consequence of, rather than a requirement for, swarming, because individual *E. coli* cells occasionally move independently of rafts, and rafts may arise passively when the movement of an individual cell is forced to conform to that of its neighbours[36]. Much of the recent swarming literature comes from studies of *E. coli* and *S. enterica*, which are powerful model systems for swimming motility but which have some of the most conditional swarming phenotypes. It will be important to determine how the swarming of *E. coli* and *S. enterica* relates to the swarming of other bacteria.

Future directions

For those who are convinced that swarming motility is a separate and distinct behaviour, many questions remain.What physiological changes take place during the swarming lag? Is surface contact a direct stimulus and, if so, how is it transduced? Is cell division coupled to swarming and, if so, what is the mechanistic connection? How is force generated and coordinated in multicellular rafts? How many bacterial species are swarming proficient, and how many times has swarming been bred out of laboratory isolates? Finally, what is the ecological relevance of swarming motility? Although the perfect surface of a carefully dried agar plate is never found in the environment, swarming may occur on nutrient-rich, soft substrates such as hydrated soils, plant roots and animal tissues, and swarming cells enjoy various advantages.

In addition to promoting swarming motility, surfactants are potent antimicrobials[120–122]. Therefore, swarming motility may be a take-and-hold strategy, in which the same surfactants used to spread across the surface of an object also simultaneously prevent colonization and growth by competing microorganisms. Surfactants also enhance bioavailability of molecules by increasing the solubility of hydrocarbons or the surface hydrophobicity of hydrocarbon consumers[123–125]. Hydrophobic compounds are often surface associated, and therefore surfactants and swarming may aid bacterial nutrition[123].

Bacterial movement over surfaces may enable pathogenic species to migrate over, adhere to and disperse from sites of infection[26,39,126,127]. Swarming may protect pathogens from macrophages, as swarm cells were shown to have enhanced resistance to engulfment[128]. In addition, toxin secretion is often co-regulated with swarming motility[126,129]. Furthermore, bacteria of diverse species seem to become resistant to a broad range of antibiotics when swarming[130,131]. The mechanism of generalized multidrug resistance seems to be unrelated to known active antibiotic-efflux systems and is instead likely to be a passive phenomenon resulting from rapid spreading of cells at high density[118,130,132]. Nonetheless, some bacteria have specialized systems to resist their own secreted surfactants[52,132,133]. Cationic peptides like polymyxin B have surfactant like structures, and bacteria may express some antibiotic-resistance systems to avoid autotoxicity during swarming[134] (FIG. 4).

The study of swarming motility promises to yield novel insights into the physiology of multicellular behaviour in bacteria. New swarming-specific genes await discovery and investigation. New biochemical mechanisms are needed to connect swarming

phenotypes to other, better-understood cell physiologies. Swarming offers cytological insight into how the number of flagella is controlled. It also provides biophysical models of how flagella function at a surface, as well as being a powerful evolutionary selection pressure. As microbiologists become more interested in life at a surface, bacterial swarming motility will surely move the field forwards.

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Acknowledgements
I am grateful to R. Belas, H. Berg, E. Déziel, R. Harshey, D. Kysela, L. McCarter, G. O'Toole, P. Rather, R. Rudner, J. Shrout and D. Weibel for thoughtful discussions about swarming motility and critical reading of the manuscript. I also thank P.R., G.O'T. and R.R. for donation of the bacterial strains used in figure 7b–d. Work in my laboratory is supported by the US National Institutes of Health (grant GM093030).

Competing interests statement
The author declares no competing financial interests.

DATABASES

Entrez Genome Project: http://www.ncbi.nlm.nih.gov/genomeprj
Bacillus subtilis | *Paenibacillus vortex* | *Proteus mirabilis* | *Pseudomonas aeruginosa* | *Rhodosprillum centenum* | *Salmonella enterica* | *Serratia marcescens* | *Vibrio parahaemolyticus* | *Yersinia entercolitica*

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