Review

Living on a surface: swarming and biofilm formation

Natalie Verstraeten[1], Kristien Braeken[1], Bachaspatimayum Debkumari[1], Maarten Fauvart[1], Jan Fransaer[2], Jan Vermant[3] and Jan Michiels[1]

[1] Centre of Microbial and Plant Genetics, Katholieke Universiteit Leuven, Kasteelpark Arenberg 20, B-3001 Heverlee, Belgium
[2] Department of Metallurgy and Materials Engineering, Katholieke Universiteit Leuven, Kasteelpark Arenberg 44, B-3001 Heverlee, Belgium
[3] Department of Chemical Engineering, Katholieke Universiteit Leuven, W. de Croylaan 46, B-3001 Heverlee, Belgium

Swarming is the fastest known bacterial mode of surface translocation and enables the rapid colonization of a nutrient-rich environment and host tissues. This complex multicellular behavior requires the integration of chemical and physical signals, which leads to the physiological and morphological differentiation of the bacteria into swarmer cells. Here, we provide a review of recent advances in the study of the regulatory pathways that lead to swarming behavior of different model bacteria. It has now become clear that many of these pathways also affect the formation of biofilms, surface-attached bacterial colonies. Decision-making between rapidly colonizing a surface and biofilm formation is central to bacterial survival among competitors. In the second part of this article, we review recent developments in the understanding of the transition between motile and sessile lifestyles of bacteria.

Flagella-mediated movement on a surface

Bacteria often thrive in surface-associated multicellular communities that have advantages over individual cells, such as protection against unfavorable environmental conditions (including predation, the presence of antimicrobials and the host immune response). Biofilms are sessile communities with microorganisms embedded within a matrix and attached to a surface. However, motile populations, such as swarming bacteria, can rapidly reach novel niches, which they can colonize; this provides ecological advantages to the bacteria [1,2]. The choice between sessile and motile lifestyles is clearly an important decision to be made by microorganisms that live in varying habitats and requires the integration of many environmental cues.

Swarming motility is a process by which bacteria can rapidly (several $\mu m\ s^{-1}$) advance on moist surfaces in a coordinated manner. It requires functional flagella and is coupled to the production of a viscous slime layer. The slime layer is thought to extract water from the agar and keeps the cells in a moist environment. Swarming is a group behavior that requires the cells to reach a certain cell number before the process is initiated. Furthermore, swarmers are often elongated as a result of the suppression of cell division.

Swarming is widespread in many genera of Gram-negative and Gram-positive flagellated bacteria and is typically assayed on a solidified medium, containing 0.5–2% agar, from which the bacteria are thought to extract water and nutrients. Species such as *Proteus mirabilis* and *Vibrio parahaemolyticus,* which are capable of vigorous swarming even on high-agar medium, often possess pronounced swarmer cell morphology with high numbers of flagella and prominent elongation (5- to 20-fold for *Vibrio* [3] and 10- to 40-fold for *Proteus* [2]). Swarming has been studied extensively in *P. mirabilis*, in which elongated, multinucleated and hyper-flagellated swarmer cells can spread as multicellular rafts across surfaces [2]. In *P. mirabilis* multicellular rafts, flagellar filaments from adjacent swarmer cells are interwoven in phase and form helical connections between the cells [4]. Periodically, the cells revert to the undifferentiated vegetative state; this reversion to the undifferentiated vegetative state is termed consolidation. Repeated alternation between both modes results in the appearance of characteristic terraced colonies. Swarming patterns of concentric zones are also found with *V. parahaemolyticus*. However, in contrast to the complex swarmers *P. mirabilis* and *V. parahaemolyticus*, many other species such as *Pseudomonas aeruginosa*, *Rhizobium etli*, *Serratia liquefaciens*, *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) and *Escherichia coli* move continuously and do not produce pronounced terraced colonies (Figure 1). Complex patterns of swarming have also been reported for Gram-positive bacteria such as *Bacillus subtilis* [5].

This review covers recent advances in swarming and the link between swarming motility and biofilm formation in several well-studied Gram-negative model systems. For several excellent reviews focused more extensively on swarming, see Refs [2,3,6–9].

Morphological differentiation and stimuli of swarming

Flagella enable bacteria to move towards favorable environments during swimming and contribute to the virulence of pathogens through adhesion and biofilm formation on host surfaces. The transcription of flagellar genes proceeds in a hierarchical, highly regulated manner with master regulatory genes (such as *flhDC* in Enterobacteriaceae, *fleQ* in *P. aeruginosa* and *flrA* in *Vibrio* spp.) integrating multiple environmental signals. The number of flagella is generally upregulated in swarmer cells. Swimming and swarming are similarly controlled at the level of

Corresponding author: Michiels, J. (jan.michiels@biw.kuleuven.be).

0966-842X/$ – see front matter doi:10.1016/j.tim.2008.07.004 Available online 3 September 2008

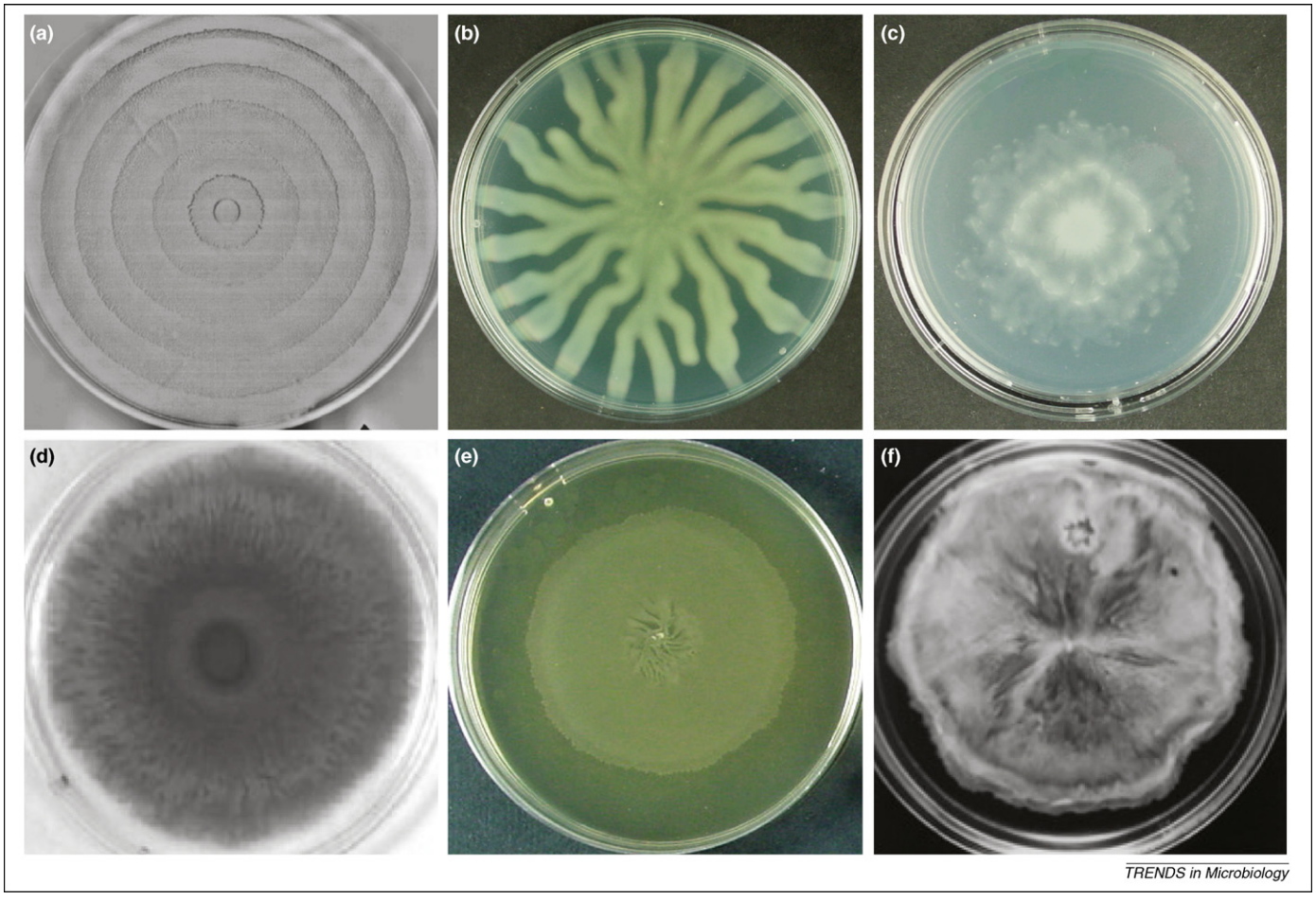

Figure 1. Pattern formation of swarming colonies. **(a)** *P. mirabilis*, **(b)** *P. aeruginosa*, **(c)** *R. etli*, **(d)** *S. marcescens*, **(e)** *S.* Typhimurium and **(f)** *E. coli*. Part (a) reproduced, with permission, from Ref. [88] [© (2001) American Physical Society (http://arxiv.org/abs/physics/0007087)]; part (d) reproduced, with permission, from Ref. [34] [© (2005) American Society for Microbiology (http://www.asm.org)]; and part (f) reproduced, with permission, from Ref. [89] [© (1998) National Academy of Sciences (http://www.nasonline.org)].

flagella production and function. However, as detailed below, specific sensing and regulatory mechanisms might control the increased flagellation of swarmers as well.

E. coli and *S.* Typhimurium have 5–10 peritrichous flagella per cell, which is doubled during swarming [10]. The biosynthesis of flagella in these species is particularly well documented and involves >50 genes, which are expressed in three temporal classes. *flhDC* constitute the class I master operon that controls the expression of all class II and III flagellar genes. Its regulation is complexly controlled by a variety of input signals that act either negatively (e.g. the regulator OmpR inhibits expression at high osmolarity) or positively (e.g. by quorum sensing through the two-component system QseCB) at the level of *flhDC* expression. However, compared to swimmers, global gene expression analysis of swarmers did not reveal the upregulation of most *S.* Typhimurium flagellar genes including *flhDC,* the genes coding for the flagellar filament excepted [11]. It is not clear how this could lead to increased flagellation, but it has been suggested that post-transcriptional mechanisms are involved [11]. Wetness is clearly an important input signal controlling flagellar biosynthesis on a surface. It was demonstrated that the flagellum itself functions as a sensor of external hydration conditions [12]. The expression of class III genes, including those coding for the flagellar filament, is only activated upon completion of the flagellar basal body and hook after the export of the anti-σ factor FlgM, an inhibitor of class III transcription. The enhanced secretion of FlgM, mirroring favorable conditions for swarming motility, leads to increased flagellar filament numbers and, hence, swarmer cell differentiation. A mechanical function during swarming was attributed recently to FliL. A *S.* Typhimurium *fliL* mutant is completely swarming deficient but almost unaffected for swimming motility [13]. On swarm plates, this mutant releases flagella fractured within the rod. FliL is proposed to be part of the flagellar stator that exerts a stabilizing function on flagella exposed to increased torsional stress during swarming (Figure 2).

S. liquefaciens swarmers are elongated and hyperflagellated. Differentiation occurs as a result of surface contact and, although the molecular mechanisms are unknown, the signal is probably channeled through the *flhDC* operon, thereby constituting a major checkpoint for differentiation [14]. However, as in *S.* Typhimurium, no increase of *flhDC* transcription was observed upon induction [1].

P. aeruginosa swarmer cells are elongated and have two polar flagella instead of one. The signals leading to differentiation are currently unknown [15].

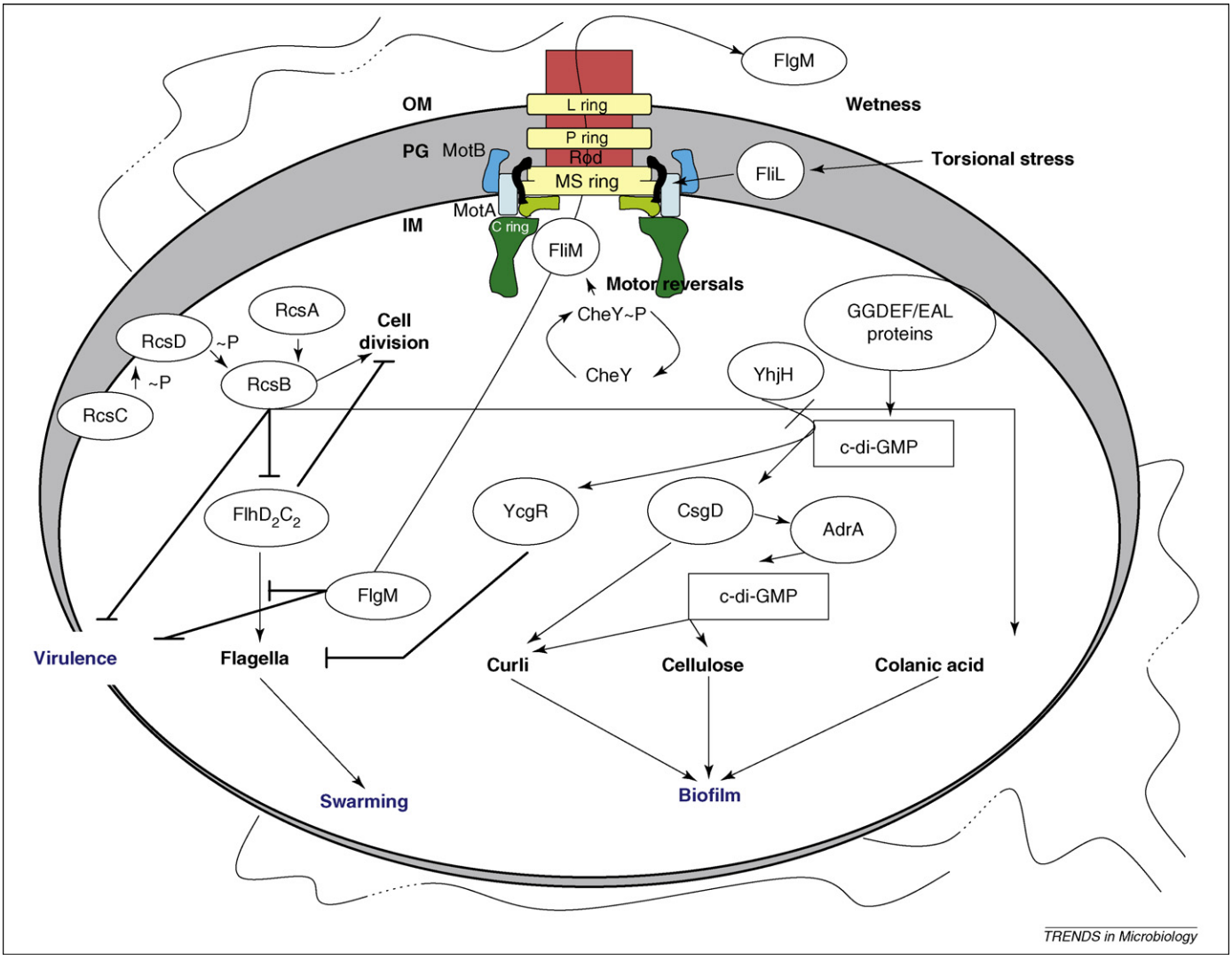

Figure 2. Regulatory networks that control swarming, biofilm formation and virulence gene expression. Schematic representations of these networks are shown for *E. coli* and *S.* Typhimurium. The flagellum is composed of the MS-, P- and L-ring components inserted into the inner membrane (IM), peptidoglycan (PG) and outer membrane (OM), respectively, and the rod (proximal and distal). The cytoplasmic C-ring constitutes the switch complex and is composed of FliM, FliG and FliN. In *S.* Typhimurium, the FlgM anti-σ factor is secreted through the rod when wetness is sufficiently high, thereby enabling the transcription of class III flagellar and virulence genes. FliL is suggested to reside around the MS ring between the MotAB stator, stabilizing the rod and sensing torque. A phosphorylated CheY (CheY∼P) promotes motor reversals, presumably through interacting with FliM. In the Rcs signal-transduction pathway, phosphate is transferred from the RcsC kinase, via RcsD, to RcsB. RcsB∼P activates cell division and colanic acid synthesis, and inhibits virulence and flagella-based motility, either alone or in conjunction with RcsA. At least six of 20 membrane-bound or cytoplasmic GGDEF- and EAL-domain proteins, which determine cyclic diguanosine monophosphate (c-di-GMP) production and degradation, affect CsgD expression in *S.* Typhimurium and integrate internal and external signals. CsgD activates the transcription of *adrA*, which codes for a diguanylate cyclase that activates cellulose post-transcriptionally and, partially, curli fimbriae expression. YcgR, in concert with c-di-GMP, inhibits flagellar assembly. This activity is antagonized by the phosphodiesterase protein YhjH. Symbols: arrowhead, positive regulation; bar head, negative regulation; wavy interrupted lines, flagella.

In *P. mirabilis*, swarming migration involves the differentiation of short, motile vegetative cells with a few peritrichous flagella into multinucleate aseptate swarmer cells of 20–40 times the vegetative cell length and with a more than 50-fold higher surface density of flagella [2]. Differentiation is induced upon contact with a surface and the inhibition of flagellar rotation [16]. The molecular mechanism transducing this information is currently unclear. Mutants in the *fliL* gene do not synthesize flagellin and are non-motile but are hyperelongated on agar medium [16]. FliL is hypothesized to sense the torque that is applied to the basal body and motor components when the flagellar motor stalls when faced with high-viscosity environments [16]. FliL, therefore, possibly functions as intermediate in the surface signal-transduction pathway and relays information, directly or indirectly, to FlhDC (Figure 3).

A hallmark of swarmer cell differentiation in *P. mirabilis* is a sharp increase (30-fold) in the expression of *flhDC*. Several proteins involved in this have been identified including the leucine-responsive regulatory protein (Lrp), four Umo (upregulation of the master operon) proteins and a novel positive regulator of *flhDC* expression, WosA (wild-type onset with superswarming) [17]. When overexpressed, WosA results in a hyperswarming phenotype and constitutive swarmer cell differentiation in non-inducing conditions. However, the temporal control over swarming initiation is maintained. This might indicate that the signal needed for differentiation differs from that required for the initiation of swarming. The overexpression of *wosA* results in the upregulation of *flhDC* and *flaA* (class III) genes. Furthermore, *wosA* expression is upregulated under conditions that increase viscosity of the medium and

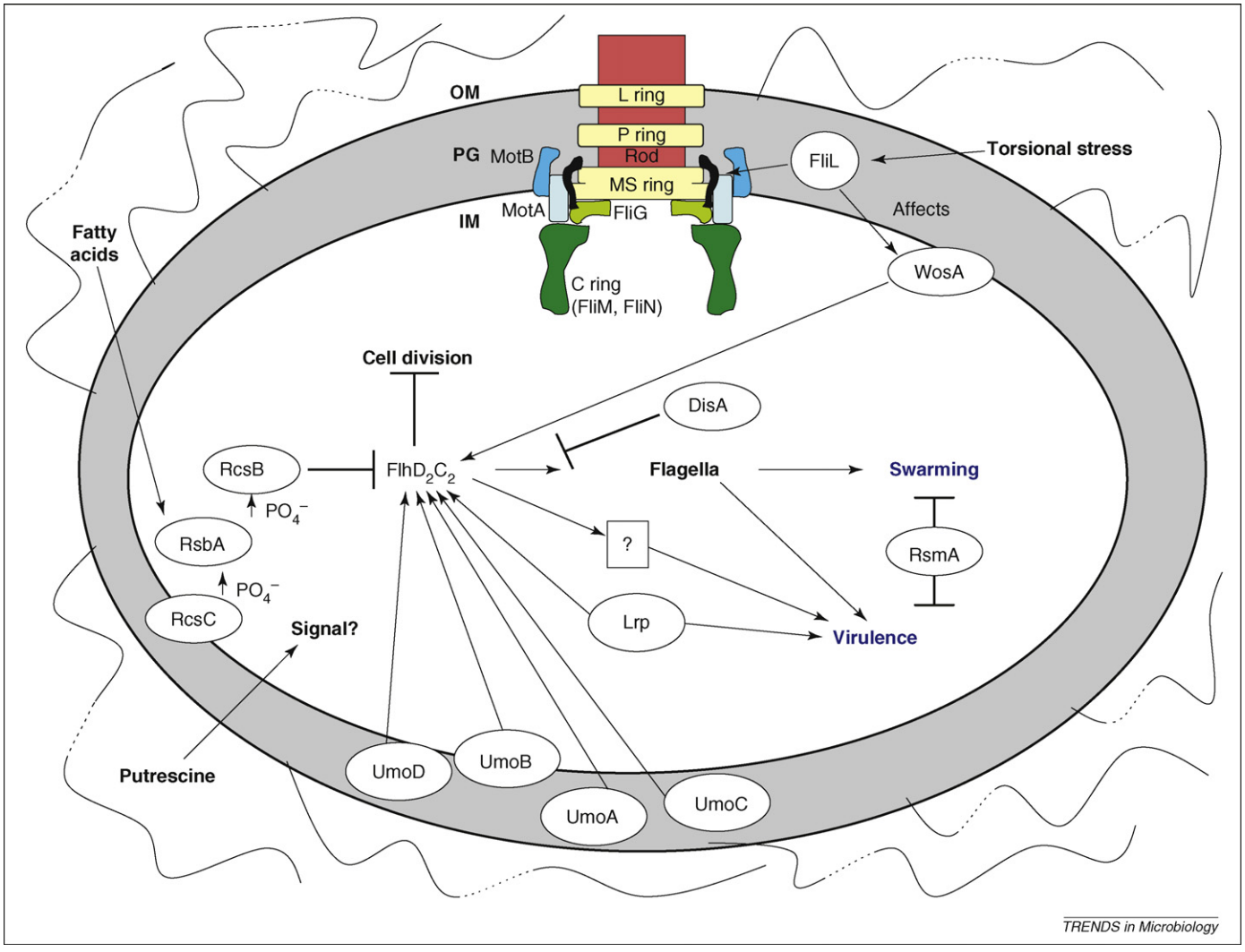

Figure 3. *P. mirabilis* regulatory networks of swarming and virulence. The flagellum is composed of the MS-, P- and L-ring components inserted into the inner membrane (IM), peptidoglycan (PG) and outer membrane (OM), respectively, and the rod (proximal and distal). The cytoplasmic C ring constitutes the switch complex and is composed of FliM, FliG and FliN. Expression or activity of $FlhD_2C_2$ in swarmers is controlled by Umo proteins, Lrp and RcsB. In the RcsC–RsbA–RcsB phosphorelay system, RsbA is involved in fatty acid sensing. Possibly, the predicted amino acid decarboxylase DisA inhibits activation of the $FlhD_2C_2$ heterotetramer or affects, via DNA binding, the transcription of class II and, hence, class III genes. Virulence is positively controlled by Lrp and $FlhD_2C_2$, either directly or indirectly (indicated by '?'). The small RNA-binding protein RsmA negatively controls swarming and virulence. Torsional stress is possibly sensed by FliL (which might be near the MS ring between the MotAB stator) and via the positive regulator WosA, which is transmitted at the level of increased *flhDC* expression. The signal-transduction pathway involved in putrescine sensing is unknown. Symbols: arrowhead, positive regulation; bar head, negative regulation; wavy interrupted lines, flagella.

in a *fliL* mutant. WosA, therefore, might be involved in the signaling cascade that senses solid surfaces, possibly via interaction with FliL resulting in increased *flhDC* expression.

In contrast to the enteric species that use only one flagellar system for both swimming and swarming, *V. parahaemolyticus* possesses two distinct flagellar systems. A single polar-sheathed flagellum is used for swimming motility and produced continuously, whereas peritrichous lateral flagella, encoded by *laf* genes, are expressed during swarming. The *laf* genes are induced by impairing polar flagellar rotation through increased viscosity or surface contact, as with *P. mirabilis*, and by iron limitation [7]. Therefore, it is proposed that the polar flagellum functions as a mechanosensor, which senses a decrease in flagellar rotation and activates the expression of lateral flagella. The flagellum-sensing signal-transduction pathway that leads to the activation of *laf* gene expression currently is unknown. Additionally, although it is not involved in the flagellum-sensing pathway, the *scr* (swarming and capsular polysaccharide regulation) system controls *laf* gene expression by modulating cellular cyclic diguanosine monophosphate (c-di-GMP) levels during surface colonization [18,19] (see later).

In conclusion, strong swarmers with clear morphological differentiation, such as *P. mirabilis* and *V. parahaemolyticus*, sense swarming conditions through the impairment of flagellar rotation, leading to cell differentiation. Instead of employing a mechanosensor, *S.* Typhimurium (a bacterium that does not swarm on high agar concentration) has developed an alternative sensing mechanism in which the flagellum is used to sense surface wetness, which is crucially important for swarming. In other bacterial species, the sensing mechanisms that lead to swarmer cell formation are still poorly understood, although components have been elucidated.

The chemotaxis system and modulation of flagellar function

Nutrient availability is crucial to sustain the energy-demanding process of swarming [7]. As a consequence, one would expect chemotaxis to have a role in the radial outgrowth of the swarm. The chemotaxis sensory system is important for swarming in bacteria that display a vigorous swarming pattern (i.e. *P. mirabilis* and *V. parahaemolyticus*) and in other swarming species, including *E. coli, S.* Typhimurium and *Serratia marcescens* [20,21]. However, chemotaxis is not necessarily required. The chemotaxis system, but not chemotaxis, is important for swarming behavior in *E. coli* and *S.* Typhimurium. Chemical gradients are, therefore, not a driving force for the outward movement of the swarming colony [7]. The chemotaxis system alters the switching between counterclockwise and clockwise rotation of the flagella. Counterclockwise rotation leads to bundled flagella and a smooth swimming mode, and clockwise rotation leads to tumbling. CheY is essential for generating clockwise motor rotation by interacting with the flagellar switch complex. An *S.* Typhimurium *cheY* mutant is swarming defective, and a constitutively active CheY∼P (phosphorylated chemotaxis response regulator) still enables switching of flagellar rotation, which supports swarming even in the absence of other chemotaxis proteins. This indicates that CheY is a crucial switch during swarming and reversing motor direction is essential in swarming migration [22]. Suppressor analysis of a smooth-swimming *cheY* mutant identified FliM, which is part of the switch complex. These suppressor strains restored the frequency of motor reversals and, consequently, rescued swarming. Mariconda *et al.* [22] postulated a mechanical role for the chemotaxis system whereby rotor reversals lead to flagella stirring surface moisture and possibly stripping lipopolysaccharide (LPS) from the surface of neighboring bacteria, which in turn promotes wetness. This wetting agent (possibly LPS) that is produced by swarming *S.* Typhimurium functions as an osmotic agent and is not a surfactant [23]. Alternatively, bacterial flagella might attach to the agar, thereby preventing any further movement. In this case, motor reversals could help the cells to detach from the agar surface [12].

The impact of motor reversals on swarming and biofilm formation has also been studied in *P. aeruginosa*. This bacterium encodes two flagellar stators called 'MotAB' and 'MotCD'. The *motY* gene, which is involved in motor function, seems to be required for *motAB* functioning [24]. Both MotAB and MotCD have a role in initial, reversible attachment through the cell pole (the first step in biofilm formation), and the MotAB stator also participates in the downstream irreversible attachment that occurs via the long axis of the cell body. Only MotCD is capable of supporting swarming, and mutations in either MotAB or MotCD render the strains defective in biofilm formation [25]. The defects in biofilm formation could be due in part to impacting flagellar reversal rates, as is the case in *E. coli*. *E. coli* locked in the tumbling chemotaxis mode by mutation have a reduced ability to attach to an abiotic surface compared to the wild type or mutants locked in the running mode [26]. During biofilm formation, SadB (surface attachment defective B) is essential for the transition from reversible attachment to stable surface interactions [27]. A *P. aeruginosa sadB* mutant can initiate surface attachment but fails to form microcolonies. A mutation in *sadB* also results in increased swarming (Figure 4). SadB inversely regulates swarming and biofilm formation via its ability to modulate flagellar reversals and by influencing the production of *pel* exopolysaccharides. By acting downstream of SadB, the chemotaxis-like cluster IV participates in the inverse regulation. An in-frame deletion of *pilJ*, a predicted methyl-accepting chemotaxis protein within cluster IV, results in increased flagellar reversals and causes hyperswarming and a biofilm-defective phenotype [28].

Extracellular signals involved in swarming

Cell density is centrally important in swarming and a critical cell mass is necessary to initiate and sustain the swarming process. It is, therefore, not surprising that in many bacteria, swarming is coupled to quorum sensing [8]. In *P. aeruginosa* and *S. marcescens,* the production of the biosurfactants rhamnolipids and serrawetin is controlled by diffusible *N*-acyl-homoserine lactones (AHL). Rhamnolipids and their precursors are also suggested to have a signaling function and determine the pattern of the swarming colony (Box 1). Quorum-sensing control over *P. aeruginosa* swarming motility is nutritionally conditional. Quorum-sensing mutants are defective for swarming when grown on succinate but not on glutamate as their sole carbon source [29]. *P. aeruginosa* biofilm formation depends on quorum sensing in a conditional manner as well [30]. In *R. etli*, AHL molecules carrying a long-chain fatty acid moiety have a dual role in swarming [31]. The *cinIR* system is a quorum-sensing system that is involved in the production of AHL signals and is essential for swarming behavior, and it is autoregulated, which leads to high levels of expression and production of AHLs. Additionally, these AHLs have a direct function and might act as biosurfactants promoting surface translocation (Box 2). In *B. subtilis*, the production of the lipopeptide surfactant surfactin is also regulated in a cell-density-dependent manner, as in *P. aeruginosa, S. marcescens* and *R. etli* [7]. Peptides or amino acids are suggested to have a signaling function in *P. mirabilis* [6]. In addition to quorum sensing, cell density has also been proposed to account for the sufficient slime accumulation that is needed for spreading. In *V. parahaemolyticus,* OpaR, a repressor of swarming and homolog of the *Vibrio harveyi* LuxR transcriptional regulator, is involved in the opaque–translucent switching of colony morphology. This phase variation is mediated through the OpaR-dependent expression of the *cpsA* locus, which is involved in capsular polysaccharide production and responsible for the opaque phenotype. In addition, OpaR represses the expression of *laf* genes and consequently inhibits swarming behavior [32]. As OpaR is homologous to LuxR, it is probably involved in quorum sensing. Furthermore, OpaR enhances biofilm formation and cell–cell adhesion, and controls type-III secretion [33].

The presence of saturated or unsaturated fatty acids strongly affects swarming in *S. marcescens*, *P. mirabilis* and *P. aeruginosa*. *S. marcescens* swarming is inhibited by

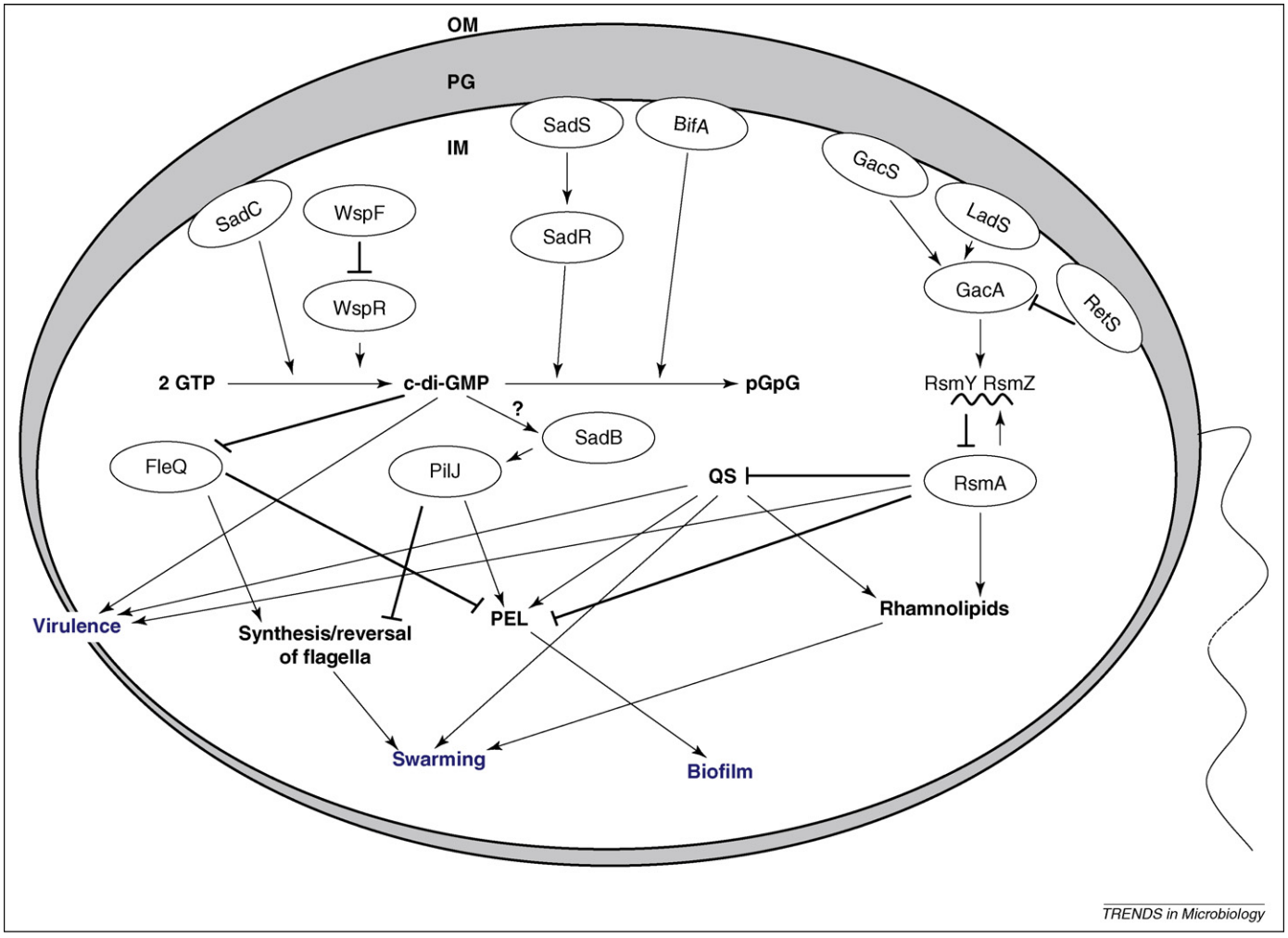

Figure 4. *P. aeruginosa* swarming, biofilm formation and virulence regulatory networks. Cyclic diguanosine monophosphate (c-di-GMP) pools are regulated by the diguanylate cyclases SadC and WspR and by the phosphodiesterases SadR and BifA. Other proteins involved are WspF and SadS. C-di-GMP is degraded to linear diguanylic acid (pGpG). The c-di-GMP signal is transmitted, possibly via SadB, to members of the CheIV chemotaxis-like cluster (depicted by the methyl-accepting chemotaxis protein PilJ), enhancing *pel* exopolysaccharide production and decreasing flagellar reversal rates. C-di-GMP also regulates the expression of virulence factors. FleQ, the master regulatory gene in flagellar synthesis, has been shown to be post-translationally regulated by c-di-GMP. Quorum sensing (QS) has a role in virulence, swarming and synthesis of the PEL polysaccharide and rhamnolipids. The post-transcriptional RNA-binding protein RsmA influences both biofilm formation and swarming by blocking the translation of *pel* and by enhancing rhamnolipid synthesis, respectively. Moreover, RsmA enhances virulence and negatively affects quorum sensing by inhibiting the translation of *rhlI* and possibly *lasI,* coding for *N*-acyl-homoserine lactone (AHL) synthases. The activity of RsmA is controlled by the small RNAs RsmY and RsmZ that are subject to negative autoregulation and are dependent on the response regulator GacA and sensor kinase GacS. Two sensor kinases (LadS and RetS) have been shown to participate in this cascade in *P. aeruginosa* PAK. Symbols: arrowhead, positive regulation; bar head, negative regulation; wavy interrupted line, flagellum.

certain saturated fatty acids and by elevated temperature and is mediated by the RssAB (regulation of *Serratia* swarming) two-component signal-transduction system. In addition to swarming at 37 °C, *rssAB* mutants also display a precocious swarming phenotype at 30 °C and increased hemolysin activity [34]. The inhibition of *flhDC* expression occurs directly through activated RssAB signaling [35]. Although structurally different, the *P. mirabilis* sensor kinase *rbsA*, which was identified initially in a screening for precocious swarming mutants, also mediates the saturated-fatty-acid-dependent inhibition of swarming [36]. The inhibitory effect of lauric acid, myristic acid and palmitic acid is exerted through an RsbA-dependent pathway, whereas the effect of stearic acid is independent of RsbA. In *P. aeruginosa*, fatty acids also affect swarming [37]. Because the cellular fatty acid profile is closely related to the swarming phenotype and possibly linked to the membrane fatty acid composition, Lai *et al.* [34] suggest a conserved mechanism of swarming in Gram-negative bacteria through the control of membrane fluidity. Alternatively, fatty acids might function as intercellular communication signals.

In *P. mirabilis*, the inactivation of the *speA* or *speB* genes, which are encoding proteins involved in putrescine biosynthesis, results in delayed swarmer cell differentiation. Exogenous putrescine abolishes this delay, indicating that it acts as an extracellular signal for swarming [38]. The *disA* gene, which is predicted to encode an amino acid decarboxylase, was initially identified in a screening for suppressor mutations restoring the *speA* swarming phenotype [39]. However, a *disA* mutation also increased swarming in a wild-type background. The inactivation of *disA* strongly affected flagellar class II and III gene expression. Based on tests with different decarboxylated amino acids, it is proposed that such a compound inhibits the assembly and/or activity of FlhDC.

Box 1. Functions of rhamnolipids in *P. aeruginosa* swarming colonies and biofilms

Swarming colonies often, but not always, produce biosurfactants or extracellular surface-active molecules that act as wetting agents. The only characterized surfactants produced by *P. aeruginosa* are amphiphilic glycolipids called 'rhamnolipids'. In liquid culture, *P. aeruginosa* produces a mixture of congeners that consists primarily of L-rhamnosyl-3-hydroxydecanoyl-3-hydroxydecanoate (monorhamnolipids) and L-rhamnosyl-L-rhamnosyl-3-hydroxydecanoyl-3-hydroxydecanoate (dirhamnolipids). RhlA is involved in the synthesis of the 3-(3-hydroxyalkanoyloxy) decanoic acid (HAA) precursor, and *rhlB* codes for a rhamnosyltransferase that catalyzes the transfer reaction of deoxythymidine diphosphate-L-rhamnose to a HAA molecule, resulting in the production of monorhamnolipids. Dirhamnolipids are produced by adding a second rhamnose moiety to a monorhamnolipid, a reaction that is catalyzed by RhlC [68].

The biosynthesis of rhamnolipids is regulated at multiple levels. *rhlAB* expression is dependent on the sigma factor σ^S. Moreover, the quorum-sensing regulators RhlR and LasR, complexed with their respective autoinducers *N*-butyryl-homoserine lactone and *N*-3-oxo-dodecanoyl homoserine lactone, control the transcription of both the *rhlAB* operon and the separately encoded *rhlC* gene. Furthermore, the production of rhamnolipids is positively controlled at a post-transcriptional level by the small RNA-binding protein RsmA and an antagonizing small regulatory RNA, RsmZ [69].

An *rhlA* mutation inhibits swarming and significantly reduces twitching motility but does not affect swimming [15,70]. Rhamnolipids are also involved in the formation of the fractal-like patterns that are formed by migrating *P. aeruginosa* colonies [71]. Dirhamnolipids promote tendril formation and migration, and serve as attractants for swarmers. Although HAAs possess surface-active properties, they inhibit tendril formation and migration, and serve as repellents. Monorhamnolipids neither attract nor repel migrating swarms but seem to act solely as wetting agents. These results contradict findings, possibly as a result of different degrees of purification of the rhamnolipids, that indicate that HAAs are the minimal surfactant required for swarming while rhamnolipids modulate the swarming pattern [72].

Temporal production of rhamnolipids also controls biofilm formation. Expression of the *rhlAB* operon is observed in microcolonies larger than 20 μm, and *rhlA* is preferentially expressed in the stalks. Rhamnolipids have been described (i) to promote microcolony formation [70]; (ii) to facilitate surface-associated bacterial migration and, thereby, the formation of mushroom-shaped structures [70]; (iii) to maintain the open channels in mature biofilms [73]; and (iv) to mediate the detachment of *P. aeruginosa* biofilms [48]. Moreover, expressing high levels of rhamnolipid production can impede biofilm formation [73].

Box 2. Physico-chemical aspects of bacterial swarming

Despite the often intricate genetic mechanisms that regulate swarming, there are also several ways in which physico-chemical phenomena could play a part in the dynamics of swarming and biofilm formation [74]. Possible parameters intervening in these are the heterogeneity of substrates, the surface-active nature of signaling molecules [31,70] and the dependence of viscosity on the concentration of bacteria and its effect on thin film hydrodynamics [75]. Likewise, the extracellular slime is a non-Newtonian fluid, the viscosity of which strongly depends on local deformation rates that will affect the spreading dynamics of the bacterial film [31]. Finally, at high concentrations of bacteria, large-scale coherent movements of bacteria with vortex-like motions might appear because of hydrodynamic coupling, in which the collective motion of bacteria through the viscous slime drives the fluid flow [76,77].

In particular, the striking 'fingering' patterns that are formed by some swarmer colonies on relatively soft subphases have attracted attention because they could be the signatures of instability. Two different approaches of treating the bacterial film as a continuum have been proposed to explain the pattern formation. A first approach starts from the experimentally observed sensitivity of bacterial swarming to the condition of the agar substrate. A nonlinear reaction–diffusion model has been proposed, in which the branching is due to the sensitivity of the system to local irregularities in the substrate [78]. This approach successfully reproduces the patterns, but a detailed comparison of growth kinetics has not been presented. Alternatively, a parallel has been drawn with the spreading of viscous drops under the influence of a surfactant, which leads to similar patterns [79]. Starting from the observation that several of the molecules, which are essential in swarming systems, are strong biosurfactants, the possibility of flows driven by gradients in surface tension has been proposed. Marangoni flows also lead to the observed fingering patterns. For *R. etli,* both the pattern formation and the spreading speed are consistent with those expected for Marangoni flows for surface pressures, thicknesses and viscosities found experimentally [31]. However, complications caused by the transport of oxygen [80] or signaling molecules could arise. Hence, further work describing the pattern-formation kinetics in quantitative terms is clearly warranted.

Extracellular matrix and swarming

The extracellular matrix that surrounds swarmers serves as a hydrated milieu and consists of polysaccharides, biosurfactants, peptides and proteins. Sufficient moisture needs to be trapped by this matrix to enable the proper functioning of the flagella and provide volume to the spreading colony. *P. mirabilis* produces an acidic capsular polysaccharide, named Cmf (colony migration factor), which stabilizes cell–cell contacts and acts as a lubricant, extracting water from the agar medium [40]. In this respect, the ratio of osmotic activity between the agar beneath the swarming colony and the slime matrix is important in creating the fluid environment that is needed for swarming.

The Rcs system, which is exclusively present in *Enterobacteriaceae*, was initially identified for its role in the regulation of the synthesis of the capsular polysaccharide colanic acid but has now been shown to have a more general role in sensing envelope stress and osmolarity. RcsCDB, a three-component His-Asp phosphorelay system, positively regulates the production of colanic acid and negatively controls swarming, swimming motility and virulence. Colanic acid contributes to the complex three-dimensional architecture of *E. coli* biofilms, and overproduction inhibits swarming in *S.* Typhimurium [41]. The inhibition of swarming is probably the consequence of RcsB negatively regulating expression of the *flhDC* operon [41]. Similarly, in *P. mirabilis*, a *rcsB* mutant displays a hyperswarming phenotype [42], in agreement with studies on the phenotype of *rsbA* (a homolog of *E. coli* RcsD) and *rcsC* mutants [43,44]. The signal(s) regulating the RscC–RsbA–RscB cascade is currently unknown. Given its role in colanic acid synthesis in *E. coli*, it might also affect swarming by influencing the amount and composition of the slime. Recently, a novel component of the extracellular matrix was described. The *S.* Typhimurium *flhE* gene, which belongs to the flagellar regulon, is not required for the production of flagella or swimming motility. However, it is essential for swarming and suggested to function as an extracellular matrix component that affects surface wettability [45].

Although not yet fully characterized in many swarming bacteria, it is becoming clear that the composition of the extracellular matrix is tightly controlled and determines

whether motility of the colony can occur or not. For example, colanic acid contributes to biofilm structure but inhibits swarming in *Enterobacteriaceae* species.

Inverse relation between swarming and biofilm formation

The link between motility and biofilm formation tends to be complex because both processes might involve similar components at certain stages and specific conditions. For example, the initiation of biofilm formation through reversible attachment often requires flagella, and motility on a surface can be crucial for biofilm architecture. However, motility is also involved in the release of bacteria from mature biofilms [46–48]. Nevertheless, bacteria might select between motility, such as swarming, and biofilm formation at certain stages.

It has become clear that the intracellular signaling molecule c-di-GMP, synthesized by diguanylate cyclases (GGDEF domain proteins) and degraded by specific phosphodiesterases (EAL- or HD-GYP domain proteins) functions as a second messenger in response to extracellular signals and regulates bacterial multicellular behavior, motility and virulence in many diverse bacteria. In general, high concentrations of c-di-GMP correlate with increased sessility and reduced motility and virulence. Many organisms have multiple proteins with GGDEF or EAL domains. For example, *S.* Typhimurium possesses 19 and *P. aeruginosa* has 38. Many of these proteins have a modular structure with periplasmic or cytoplasmic sensory units [49]. It is, therefore, to be expected that environmental and physiological signals might modulate the activity of these proteins and temporally determine global or local cellular c-di-GMP levels [50,51]. In this section, the role of c-di-GMP in decision-making between swarming and biofilm formation will be examined.

In *S.* Typhimurium, the multicellular rdar morphotype (red, dry and rough colony morphology on Congo red agar) is controlled by the LuxR-type master regulator CsgD. CsgD positively regulates the production of proteinaceous curli fimbriae and the extracellular polysaccharide cellulose, which are both matrix components of biofilms. Curli fimbriae promote initial cell–surface interactions and subsequently cell–cell interactions. CsgD probably directly activates transcription of the *csgBA* operon and the *adrA* gene [52]. *csgBA* are the structural genes of the fimbriae, whereas *adrA* codes for a GGDEF domain protein, stimulating cellulose biosynthesis and enhancing the expression of curli fimbriae, through the production of c-di-GMP [53,54]. CsgD expression itself is either positively or negatively controlled by at least six GGDEF and/or EAL proteins at the transcriptional and post-transcriptional levels [55]. Different GGDEF and/or EAL proteins can clearly have separate tasks. For example, the inactivation of the gene encoding the EAL domain protein YhjH reduces swarming and swimming but does not affect pellicle formation, and the inactivation of STM3375 strongly affects swarming but not the rdar morphotype [55]. The action of YhjH is antagonized by the PilZ domain protein YcgR, and both proteins are class III flagellar proteins [11,56,57]. Wolfe and Visick [51] proposed a model in which c-di-GMP levels are sensed by YcgR. Elevated levels then lead to the inhibition of proper flagellum assembly and, hence, interfere with motility. Spatial compartmentalization and specific protein–protein interactions have been proposed to account for the regulation of different targets by separate GGDEF- and EAL-domain proteins.

Two genetic loci, *scrABC* and *scrG*, were recently identified in *V. parahaemolyticus* through screening of overexpression cosmids conferring constitutive *laf* gene expression in liquid culture [18,58]. The overexpression of *scrABC* and *scrG* also decreased *cps* gene expression and resulted in altered cell adhesiveness and reduced biofilm formation but did not affect swimming motility. *scrA*, *scrB* and *scrC* code for a potential pyridoxal-phosphate-dependent enzyme, a putative periplasmic solute-binding protein and a membrane-bound GGDEF-EAL-protein, respectively. A model is proposed in which periplasmic ScrB senses a stimulus that is transmitted to ScrC via its periplasmic domain, possibly modulating the activity of cytoplasmic GGDEF and EAL domains. The *scrG* gene codes for a protein containing PAS, EAL and GGDEF domains [58]. *scrG* and *scrC* probably function in the same regulatory pathway and are probably involved in controlling the cellular level of c-di-GMP [18]. ScrC and ScrG might function primarily as phosphodiesterases in the cell. Decreased levels of c-di-GMP lead to the induction of *laf* gene expression and favor swarming, while inhibiting *cps* expression and biofilm formation. It is not clear how transduction of the c-di-GMP signal is accomplished at the level of gene expression. At least one regulator, CpsR, functions in the *scr* pathway that leads to *cps* expression [59].

SadC, BifA and SadB regulate aspects of motility and biofilm formation in *P. aeruginosa* PA14. The inner-membrane-localized diguanylate cyclase SadC produces c-di-GMP in response to an unknown signal, perhaps contact with a surface or changes in medium viscosity [60]. The amount of this signal molecule can be reduced by the phosphodiesterase BifA, providing a second control point by which c-di-GMP pools are regulated [61]. This c-di-GMP signal is then transmitted, perhaps via SadB, to the *pel* genes and/or members of the CheIV chemotaxis-like cluster, which results in the control of exopolysaccharide production and flagellar function. The *pel* and *psl* loci are involved in the production of the polysaccharide component of the matrix that is required for biofilm maturation in *P. aeruginosa*. As a consequence, c-di-GMP-mediated SadB could control biofilm-to-swarming transitions by modulating flagellar reversal rates. FleQ, the master regulator of flagella gene expression, has also been shown to repress the transcription of *pel* genes, and this repression is relieved by c-di-GMP. FleN, an antiactivator of FleQ, also participates in the control of *pel* expression [62].

Two environmentally responsive signal-transduction systems, the *wsp* and *rocS1RA1* (which is also designated *sadSRA*) loci, control the expression and/or activity of GGDEF and EAL domain proteins in *P. aeruginosa*. Mutations in *wspF* (which is part of a putative chemosensory signal-transduction operon) result in cell aggregation, altered colony morphology, decreased twitching motility and decreased swimming. The WspF protein is homologous to CheB, a methylesterase that is involved in adaptation to chemotactic stimuli. The WspF phenotypes depend on the

GGDEF domain response regulator WspR (a CheY homolog). The *wspF* mutation probably causes constitutive activation of WspR by phosphorylation [63]. A *wspF* deletion affects the expression levels of at least 560 genes, among which are the *psl* and *pel* operons with expression that is stimulated in a *wspF* mutant [63]. Available data indicate that this system functions analogously to chemotaxis-controlling systems, but instead of altered flagellar rotation, the output is c-di-GMP production. RocS1 (a sensor kinase) activates both RocA1 (a DNA-binding regulator that activates fimbrial *cup* genes) and RocR (which has a C-terminal EAL domain and functions as a negative regulator of *cup* expression). RocS1 interacts with receiver domains of RocA1 and RocR [64]. This three-component system, designated *sadARS*, is required for biofilm maturation [65]. The overexpression of *sadS* and/or *sadR* leads to a defect in biofilm maturation. The genes that are required for type-III secretion are among the downstream targets of SadARS. Taken together, the Wsp and Sad systems control cellular c-di-GMP levels and, as a result, motility, polysaccharide production and biofilm formation, thereby constituting an important switch between sessile and motile lifestyles.

GGDEF- and EAL-domain proteins are clearly important for bacteria to make choices between swarming and biofilm formation. Recently, the production of biosurfactant and swarming were shown to be controlled by a GGDEF and EAL protein in *Serratia* [66]. However, the fact that *P. mirabilis* contains only one such protein [67] could indicate that the mechanism that controls the choice between sessile and motile lifestyles might not be universally conserved.

Concluding remarks and future directions

Decision-making between swarming and biofilm formation might be crucial for the survival of bacterial colonies. Signals and signaling pathways that control this process in various bacteria are being uncovered. Swarmers need to adapt to a viscous and moist surface. Bacteria use flagella to sense these conditions by measuring torsion or by secreting repressors through the rod when humidity is sufficiently high. As a result, flagella production is enhanced and cells differentiate. Clearly, other inputs (such as cell density or temperature) are also required, affecting a multitude of global or specific regulatory pathways that regulate polysaccharide production, cell division, quorum sensing or virulence gene expression (Box 3). The intracellular second messenger c-di-GMP has a pivotal role in transmitting this information. Evidence is accumulating that it impacts the transition between sessile and motile lifestyles by enhancing the synthesis of fimbriae and capsules, and decreasing flagella synthesis and functioning. Many organisms, although they are genetically different, deal with the same problems and use similar tools to reach the same goal. Ultimately, control over these processes will be facilitated by the knowledge of crucial environmental and intracellular signals and the regulatory mechanisms that lead to sessile or motile bacterial behavior. The identification of these signals and mechanisms is an important challenge for future research.

Box 3. Coregulation of swarming with virulence determinants

Although hyperflagellation is likely to contribute to the rapid infection of host tissues, the link between swarming and virulence has not been unequivocally demonstrated for most swarming pathogenic species. However, in support of an increased virulence of swarmers, swarm cell differentiation is often accompanied by the expression of virulence determinants, which could benefit the bacteria in colonizing new environments.

Virulence proteins such as urease, metalloprotease, haemolysin and flagella are upregulated in swarming *P. mirabilis*, and phospholipase is induced in *S. liquefaciens* [6,16]. The genes encoding phospholipases are transcribed by the flagellar-specific sigma factor σ^{28} in *Yersinia enterocolitica* and *S. liquefaciens*. More recently, the expression of the *P. mirabilis* haemolysin operon was shown to be controlled by FlhDC, Lrp and UmoB [81]. In addition, defects in *fliL* also upregulate virulence gene expression [16].

Swarm cells of *S.* Typhimurium have altered global gene expression compared to swimmers and have been proposed to represent a distinct physiological state [11,82]. At the phenotypical level, *S.* Typhimurium swarmer cells exhibit an increased antibiotic resistance. In contrast to most flagellar genes in *S.* Typhimurium, genes implicated in LPS synthesis, virulence and iron acquisition are induced during swarming [11]. For example, SPI-1 (*Salmonella* pathogenicity island) genes are induced very early in a swarming colony. The regulation of expression of SPI-1 virulence genes is also coupled to the export of FlgM, indicating that external hydration serves as a signal for both swarming and virulence gene expression. Finally, RcsB dually regulates genes that are encoded by pathogenicity islands and other virulence genes in *S.* Typhimurium [41,83]. Therefore, the Rcs system is likely to suppress motility and virulence gene expression in a sessile state (see Figure 2 in the main text).

In *P. aeruginosa* swarmers, a large number of virulence-related genes are overexpressed (including the type-III secretion system and its effectors, extracellular proteases and iron transport). Moreover, two virulence genes (*lasB* and *pvdQ*) seem to be required for swarming. Swarming cells also exhibit adaptive antibiotic resistance [84]. The coregulation of swarming and virulence is also achieved through c-di-GMP signaling (see main text and Figure 4).

The post-transcriptional RNA-binding protein RsmA seems to be another important regulator. The overexpression of RsmA in *P. mirabilis* inhibited swarming and virulence factor expression [85]. However, in *P. aeruginosa*, RsmA positively affects both swarming and virulence while impairing biofilm formation by inhibiting the synthesis of the PEL polysaccharide and negatively influencing the quorum-sensing systems. The activity of RsmA is repressed by two small regulatory RNAs, RsmY and RsmZ, the synthesis of which is dependent on the sensor kinase GacS and the response regulator GacA. Two other sensor kinases involved in this regulatory pathway in *P. aeruginosa* PAK are RetS (regulator of exopolysaccharide and type-III secretion) and LadS (lost adherence sensor). RetS is required for the expression of the type-III secretion system and other virulence factors. A mutation in *retS* elevates *pel* and *psl* expression and results in enhanced biofilm formation while rendering the strain swarming deficient [86,87]. LadS and RetS signal-transduction pathways are truly antagonistic, and their reciprocal effects are accomplished, at least in part, through their opposite effect on the expression of small RNAs (see Figure 4 in the main text).

Acknowledgements

We apologize to those authors whose work could only be referred to by citing recent work or review articles. We thank Sigrid De Keersmaecker for providing a picture of *S.* Typhimurium swarming. We also thank the anonymous reviewers of this article for their valuable comments. N.V. received a fellowship from the Fund for Scientific Research – Flanders (FWO). The work was supported by grants from the K.U.Leuven Research Council (IDO/05/012 and GOA/2003/09) and the FWO (G.0637.06).

References

1 Eberl, L. *et al.* (1999) Surface motility of *Serratia liquefaciens* MG1. *J. Bacteriol.* 181, 1703–1712
2 Rather, P.N. (2005) Swarmer cell differentiation in *Proteus mirabilis*. *Environ. Microbiol.* 7, 1065–1073
3 McCarter, L. and Silverman, M. (1990) Surface-induced swarmer cell differentiation of *Vibrio parahaemolyticus*. *Mol. Microbiol.* 4, 1057–1062
4 Jones, B.V. *et al.* (2004) Ultrastructure of *Proteus mirabilis* swarmer cell rafts and role of swarming in catheter-associated urinary tract infection. *Infect. Immun.* 72, 3941–3950
5 Kearns, D.B. and Losick, R. (2003) Swarming motility in undomesticated *Bacillus subtilis*. *Mol. Microbiol.* 49, 581–590
6 Fraser, G.M. and Hughes, C. (1999) Swarming motility. *Curr. Opin. Microbiol.* 2, 630–635
7 Harshey, R.M. (2003) Bacterial motility on a surface: many ways to a common goal. *Annu. Rev. Microbiol.* 57, 249–273
8 Daniels, R. *et al.* (2004) Quorum sensing and swarming migration in bacteria. *FEMS Microbiol. Rev.* 28, 261–289
9 Kaiser, D. (2007) Bacterial swarming: a re-examination of cell-movement patterns. *Curr. Biol.* 17, R561–R570
10 Harshey, R.M. and Matsuyama, T. (1994) Dimorphic transition in *Escherichia coli* and *Salmonella typhimurium*: surface-induced differentiation into hyperflagellate swarmer cells. *Proc. Natl. Acad. Sci. U. S. A.* 91, 8631–8635
11 Wang, Q. *et al.* (2004) Gene expression patterns during swarming in *Salmonella typhimurium*: genes specific to surface growth and putative new motility and pathogenicity genes. *Mol. Microbiol.* 52, 169–187
12 Wang, Q. *et al.* (2005) Sensing wetness: a new role for the bacterial flagellum. *EMBO J.* 24, 2034–2042
13 Attmannspacher, U. *et al.* (2008) FliL is essential for swarming: motor rotation in absence of FliL fractures the flagellar rod in swarmer cells of *Salmonella enterica*. *Mol. Microbiol.* 68, 328–341
14 Eberl, L. *et al.* (1996) Differentiation of *Serratia liquefaciens* into swarm cells is controlled by the expression of the *flhD* master operon. *J. Bacteriol.* 178, 554–559
15 Köhler, T. *et al.* (2000) Swarming of *Pseudomonas aeruginosa* is dependent on cell-to-cell signaling and requires flagella and pili. *J. Bacteriol.* 182, 5990–5996
16 Belas, R. and Suvanasuthi, R. (2005) The ability of *Proteus mirabilis* to sense surfaces and regulate virulence gene expression involves FliL, a flagellar basal body protein. *J. Bacteriol.* 187, 6789–6803
17 Hatt, J.K. and Rather, P.N. (2008) Characterization of a novel gene, *wosA*, regulating FlhDC expression in *Proteus mirabilis*. *J. Bacteriol.* 190, 1946–1955
18 Boles, B.R. and McCarter, L.L. (2002) *Vibrio parahaemolyticus scrABC*, a novel operon affecting swarming and capsular polysaccharide regulation. *J. Bacteriol.* 184, 5946–5954
19 Ferreira, R.B. *et al.* (2008) *Vibrio parahaemolyticus* ScrC modulates cyclic dimeric GMP regulation of gene expression relevant to growth on surfaces. *J. Bacteriol.* 190, 851–860
20 Sar, N. *et al.* (1990) Chemotactic control of the two flagellar systems of *Vibrio parahaemolyticus*. *J. Bacteriol.* 172, 334–341
21 Belas, R. *et al.* (1991) *Proteus mirabilis* mutants defective in swarmer cell differentiation and multicellular behavior. *J. Bacteriol.* 173, 6279–6288
22 Mariconda, S. *et al.* (2006) A mechanical role for the chemotaxis system in swarming motility. *Mol. Microbiol.* 60, 1590–1602
23 Chen, B.G. *et al.* (2007) The wetting agent required for swarming in *Salmonella enterica* serovar Typhimurium is not a surfactant. *J. Bacteriol.* 189, 8750–8753
24 Doyle, T.B. *et al.* (2004) The complex flagellar torque generator of *Pseudomonas aeruginosa*. *J. Bacteriol.* 186, 6341–6350
25 Toutain, C.M. *et al.* (2007) Roles for flagellar stators in biofilm formation by *Pseudomonas aeruginosa*. *Res. Microbiol.* 158, 471–477
26 McClaine, J.W. and Ford, R.M. (2002) Reversal of flagellar rotation is important in initial attachment of *Escherichia coli* to glass in a dynamic system with high- and low-ionic-strength buffers. *Appl. Environ. Microbiol.* 68, 1280–1289
27 Caiazza, N.C. and O'Toole, G.A. (2004) SadB is required for the transition from reversible to irreversible attachment during biofilm formation by *Pseudomonas aeruginosa* PA14. *J. Bacteriol.* 186, 4476–4485
28 Caiazza, N.C. *et al.* (2007) Inverse regulation of biofilm formation and swarming motility by *Pseudomonas aeruginosa* PA14. *J. Bacteriol.* 189, 3603–3612
29 Shrout, J.D. *et al.* (2006) The impact of quorum sensing and swarming motility on *Pseudomonas aeruginosa* biofilm formation is nutritionally conditional. *Mol. Microbiol.* 62, 1264–1277
30 Kirisits, M.J. and Parsek, M.R. (2006) Does *Pseudomonas aeruginosa* use intercellular signalling to build biofilm communities? *Cell. Microbiol.* 8, 1841–1849
31 Daniels, R. *et al.* (2006) Quorum signal molecules as biosurfactants affecting swarming in *Rhizobium etli*. *Proc. Natl. Acad. Sci. U. S. A.* 103, 14965–14970
32 Jaques, S. and McCarter, L.L. (2006) Three new regulators of swarming in *Vibrio parahaemolyticus*. *J. Bacteriol.* 188, 2625–2635
33 Henke, J.M. and Bassler, B.L. (2004) Quorum sensing regulates type III secretion in *Vibrio harveyi* and *Vibrio parahaemolyticus*. *J. Bacteriol.* 186, 3794–3805
34 Lai, H.C. *et al.* (2005) The RssAB two-component signal transduction system in *Serratia marcescens* regulates swarming motility and cell envelope architecture in response to exogenous saturated fatty acids. *J. Bacteriol.* 187, 3407–3414
35 Soo, P.C. *et al.* (2008) Regulation of swarming motility and $flhDC_{Sm}$ expression by RssAB signaling in *Serratia marcescens*. *J. Bacteriol.* 190, 2496–2504
36 Liaw, S.J. *et al.* (2004) Modulation of swarming and virulence by fatty acids through the RsbA protein in *Proteus mirabilis*. *Infect. Immun.* 72, 6836–6845
37 Inoue, T. *et al.* (2008) Inhibition of swarming motility of *Pseudomonas aeruginosa* by branched-chain fatty acids. *FEMS Microbiol. Lett.* 281, 81–86
38 Sturgill, G. and Rather, P.N. (2004) Evidence that putrescine acts as an extracellular signal required for swarming in *Proteus mirabilis*. *Mol. Microbiol.* 51, 437–446
39 Stevenson, L.G. and Rather, P.N. (2006) A novel gene involved in regulating the flagellar gene cascade in *Proteus mirabilis*. *J. Bacteriol.* 188, 7830–7839
40 Gygi, D. *et al.* (1995) A cell-surface polysaccharide that facilitates rapid population migration by differentiated swarm cells of *Proteus mirabilis*. *Mol. Microbiol.* 17, 1167–1175
41 Wang, Q. *et al.* (2007) The RcsCDB signaling system and swarming motility in *Salmonella enterica* serovar Typhimurium: dual regulation of flagellar and SPI-2 virulence genes. *J. Bacteriol.* 189, 8447–8457
42 Clemmer, K.M. and Rather, P.N. (2007) Regulation of *flhDC* expression in *Proteus mirabilis*. *Res. Microbiol.* 158, 295–302
43 Belas, R. *et al.* (1998) Characterization of *Proteus mirabilis* precocious swarming mutants: identification of *rsbA*, encoding a regulator of swarming behavior. *J. Bacteriol.* 180, 6126–6139
44 Takeda, S. *et al.* (2001) A novel feature of the multistep phosphorelay in *Escherichia coli*: a revised model of the RcsC → YojN → RcsB signalling pathway implicated in capsular synthesis and swarming behaviour. *Mol. Microbiol.* 40, 440–450
45 Stafford, G.P. and Hughes, C. (2007) *Salmonella typhimurium flhE*, a conserved flagellar regulon gene required for swarming. *Microbiology* 153, 541–547
46 O'Toole, G.A. and Kolter, R. (1998) Flagellar and twitching motility are necessary for *Pseudomonas aeruginosa* biofilm development. *Mol. Microbiol.* 30, 295–304
47 Klausen, M. *et al.* (2003) Involvement of bacterial migration in the development of complex multicellular structures in *Pseudomonas aeruginosa* biofilms. *Mol. Microbiol.* 50, 61–68
48 Boles, B.R. *et al.* (2005) Rhamnolipids mediate detachment of *Pseudomonas aeruginosa* from biofilms. *Mol. Microbiol.* 57, 1210–1223
49 Römling, U. (2005) Characterization of the rdar morphotype, a multicellular behaviour in *Enterobacteriaceae*. *Cell. Mol. Life Sci.* 62, 1234–1246
50 Jenal, U. and Malone, J. (2006) Mechanisms of cyclic-di-GMP signaling in bacteria. *Annu. Rev. Genet.* 40, 385–407
51 Wolfe, A.J. and Visick, K.L. (2008) Get the message out: cyclic-Di-GMP regulates multiple levels of flagellum-based motility. *J. Bacteriol.* 190, 463–475
52 Brombacher, E. *et al.* (2003) The curli biosynthesis regulator CsgD co-ordinates the expression of both positive and negative determinants for biofilm formation in *Escherichia coli*. *Microbiology* 149, 2847–2857

53 Simm, R. *et al.* (2004) GGDEF and EAL domains inversely regulate cyclic di-GMP levels and transition from sessility to motility. *Mol. Microbiol.* 53, 1123–1134
54 Kader, A. *et al.* (2006) Hierarchical involvement of various GGDEF domain proteins in rdar morphotype development of *Salmonella enterica* serovar Typhimurium. *Mol. Microbiol.* 60, 602–616
55 Simm, R. *et al.* (2007) Role of EAL-containing proteins in multicellular behavior of *Salmonella enterica* serovar Typhimurium. *J. Bacteriol.* 189, 3613–3623
56 Frye, J. *et al.* (2006) Identification of new flagellar genes of *Salmonella enterica* serovar Typhimurium. *J. Bacteriol.* 188, 2233–2243
57 Ryjenkov, D.A. *et al.* (2006) The PilZ domain is a receptor for the second messenger c-di-GMP: the PilZ domain protein YcgR controls motility in *Enterobacteria*. *J. Biol. Chem.* 281, 30310–30314
58 Kim, Y.K. and McCarter, L.L. (2007) ScrG, a GGDEF-EAL protein, participates in regulating swarming and sticking in *Vibrio parahaemolyticus*. *J. Bacteriol.* 189, 4094–4107
59 Güvener, Z.T. and McCarter, L.L. (2003) Multiple regulators control capsular polysaccharide production in *Vibrio parahaemolyticus*. *J. Bacteriol.* 185, 5431–5441
60 Merritt, J.H. *et al.* (2007) SadC reciprocally influences biofilm formation and swarming motility via modulation of exopolysaccharide production and flagellar function. *J. Bacteriol.* 189, 8154–8164
61 Kuchma, S.L. *et al.* (2007) BifA, a cyclic-Di-GMP phosphodiesterase, inversely regulates biofilm formation and swarming motility by *Pseudomonas aeruginosa* PA14. *J. Bacteriol.* 189, 8165–8178
62 Hickman, J.W. and Harwood, C.S. (2008) Identification of FleQ from *Pseudomonas aeruginosa* as a c-di-GMP-responsive transcription factor. *Mol. Microbiol.* 69, 376–389
63 Hickman, J.W. *et al.* (2005) A chemosensory system that regulates biofilm formation through modulation of cyclic diguanylate levels. *Proc. Natl. Acad. Sci. U. S. A.* 102, 14422–14427
64 Kulasekara, H.D. *et al.* (2005) A novel two-component system controls the expression of *Pseudomonas aeruginosa* fimbrial *cup* genes. *Mol. Microbiol.* 55, 368–380
65 Kuchma, S.L. *et al.* (2005) A three-component regulatory system regulates biofilm maturation and type III secretion in *Pseudomonas aeruginosa*. *J. Bacteriol.* 187, 1441–1454
66 Williamson, N.R. *et al.* (2008) Integrated regulation involving quorum sensing, a two-component system, a GGDEF/EAL domain protein and a post-transcriptional regulator controls swarming and RhlA-dependent surfactant biosynthesis in *Serratia*. *Environ. Microbiol.* 10, 1202–1217
67 Pearson, M.M. *et al.* (2008) Complete genome sequence of uropathogenic *Proteus mirabilis*, a master of both adherence and motility. *J. Bacteriol.* 190, 4027–4037
68 Soberón-Chávez, G. *et al.* (2005) Production of rhamnolipids by *Pseudomonas aeruginosa*. *Appl. Microbiol. Biotechnol.* 68, 718–725
69 Heurlier, K. *et al.* (2004) Positive control of swarming, rhamnolipid synthesis, and lipase production by the posttranscriptional RsmA/RsmZ system in *Pseudomonas aeruginosa* PAO1. *J. Bacteriol.* 186, 2936–2945
70 Pamp, S.J. and Tolker-Nielsen, T. (2007) Multiple roles of biosurfactants in structural biofilm development by *Pseudomonas aeruginosa*. *J. Bacteriol.* 189, 2531–2539
71 Tremblay, J. *et al.* (2007) Self-produced extracellular stimuli modulate the *Pseudomonas aeruginosa* swarming motility behaviour. *Environ. Microbiol.* 9, 2622–2630
72 Caiazza, N.C. *et al.* (2005) Rhamnolipids modulate swarming motility patterns of *Pseudomonas aeruginosa*. *J. Bacteriol.* 187, 7351–7361
73 Davey, M.E. *et al.* (2003) Rhamnolipid surfactant production affects biofilm architecture in *Pseudomonas aeruginosa* PAO1. *J. Bacteriol.* 185, 1027–1036
74 Matsuyama, T. and Matsushita, M. (1993) Fractal morphogenesis by a bacterial cell population. *Crit. Rev. Microbiol.* 19, 117–135
75 Bees, M.A. *et al.* (2000) The interaction of thin-film flow, bacterial swarming and cell differentiation in colonies of *Serratia liquefaciens*. *J. Math. Biol.* 40, 27–63
76 Hernandez-Ortiz, J.P. *et al.* (2005) Transport and collective dynamics in suspensions of confined swimming particles. *Phys. Rev. Lett.* 95, 204501
77 Narayan, V. *et al.* (2007) Long-lived giant number fluctuations in a swarming granular nematic. *Science* 317, 105–108
78 Zorzano, M.P. *et al.* (2005) Reaction-diffusion model for pattern formation in *E. coli* swarming colonies with slime. *Phys. Rev. E Stat. Nonlin. Soft Matter Phys.* 71, 031908
79 Matar, O.K. and Troian, S.M. (1999) The development of transient fingering patterns during the spreading of surfactant coated films. *Phys. Fluids* 11, 3232–3246
80 Tuval, I. *et al.* (2005) Bacterial swimming and oxygen transport near contact lines. *Proc. Natl. Acad. Sci. U. S. A.* 102, 2277–2282
81 Fraser, G.M. *et al.* (2002) Swarming-coupled expression of the *Proteus mirabilis hpmBA* haemolysin operon. *Microbiology* 148, 2191–2201
82 Kim, W. and Surette, M.G. (2004) Metabolic differentiation in actively swarming *Salmonella*. *Mol. Microbiol.* 54, 702–714
83 Arricau, N. *et al.* (1998) The RcsB-RcsC regulatory system of *Salmonella typhi* differentially modulates the expression of invasion proteins, flagellin and Vi antigen in response to osmolarity. *Mol. Microbiol.* 29, 835–850
84 Overhage, J. *et al.* (2008) Swarming of *Pseudomonas aeruginosa* is a complex adaptation leading to increased production of virulence factors and antibiotic resistance. *J. Bacteriol.* 190, 2671–2679
85 Liaw, S.J. *et al.* (2003) Role of RsmA in the regulation of swarming motility and virulence factor expression in *Proteus mirabilis*. *J. Med. Microbiol.* 52, 19–28
86 Goodman, A.L. *et al.* (2004) A signaling network reciprocally regulates genes associated with acute infection and chronic persistence in *Pseudomonas aeruginosa*. *Dev. Cell* 7, 745–754
87 Ventre, I. *et al.* (2006) Multiple sensors control reciprocal expression of *Pseudomonas aeruginosa* regulatory RNA and virulence genes. *Proc. Natl. Acad. Sci. U. S. A.* 103, 171–176
88 Czirók, A. *et al.* (2001) Theory of periodic swarming of bacteria: application to *Proteus mirabilis*. *Phys. Rev. E Stat. Nonlin. Soft Matter Phys.* 63, 031915
89 Burkart, M. *et al.* (1998) The chemotaxis system, but not chemotaxis, is essential for swarming motility in *Escherichia coli*. *Proc. Natl. Acad. Sci. U. S. A.* 95, 2568–2573