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Cell-cell recognition and social networking in bacteria

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SUMMARY

The ability to recognize self and to recognize partnering cells allows microorganisms to build social networks that perform functions beyond the capabilities of the individual. In bacteria, recognition typically involves genetic determinants that provide cell surface receptors or diffusible signaling chemicals to identify proximal cells at the molecular level that can participate in cooperative processes. Social networks also rely on discriminating mechanisms to exclude competing cells from joining and exploiting their groups. In addition to their appropriate genotypes, cell-cell recognition also requires compatible phenotypes, which vary according to environmental cues or exposures as well as stochastic processes that leads to heterogeneity and potential disharmony in the population. Understanding how bacteria identify their social partners and how they synchronize their behaviors to conduct multicellular functions is an expanding field of research. Here we review recent progress in the field and contrast the various strategies used in recognition and behavioral networking.

Keywords

Self-recognition; myxobacteria; type VI secretion system; cooperation; conflict

Introduction

Living in multifaceted natural communities, many species have developed the ability to identify other related individuals and form social bonds. Social recognition is a common trait across a broad range of species, including mammals (Penn & Frommen, 2010), plants (Chen *et al.*, 2012), insects (Leonhardt *et al.*, 2016) and single-celled organisms (Pathak *et al.*, 2013). Like higher organisms, microbes monitor and respond to their neighbors, including distinguishing between conspecific individuals and distinct microbial species (Strassmann *et al.*, 2011, Stubbendieck & Straight, 2016). Recognition of social partners allows microbes to conduct sophisticated group behaviors that increase their fitness. Notably, some unicellular species have taken steps toward multicellularity by assembling related individuals into tightly bound cooperative groups (Du *et al.*, 2015). A well-known example is the social amoeba *Dictyostelium discoideum*, a single-celled slime mold that uses an aggregation strategy to build spore-filled multicellular fruiting bodies. *D. discoideum* uses an allorecognition strategy whereby cells identify clonemates and close relatives through

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heterotypic interactions between polymorphic adhesion proteins (TgrB1 and TgrC1) (Hirose *et al.*, 2011). Capitalizing on the advantages of such unicellular systems, studies of the molecular and evolutionary basis of microbial social networks have proliferated over the past decade.

Bacteria are found within diverse growth niches in which different species are inevitably in a constant struggle for limited resources. To better compete, bacteria can form social groups in which the abilities of the many exceed those of the individual. Within natural microbial habitats, diversity of taxonomic units is high, and recent findings suggest that cell-cell recognition between microbes strongly influences their social outcomes (Wall, 2016). In this minireview we discuss different recognition strategies developed by social bacteria and their roles in establishing functional community-based networks. Along with genetic-based recognition systems, we also discuss how physiological or phenotypic variation between related individuals influences recognition and social networking. As this review underscores, single-celled bacterial species are experimentally robust systems for studying the molecular basis of recognition and its social consequences.

Genetic Recognition in Bacteria

Genetic recognition relies on the detection of perceptible cues, such as diffusible chemical signals or cell surface receptors. One function of recognition among closely related individuals is the synchronization of group responses**,** which leads to cooperative social functions (Papenfort & Bassler, 2016). The specificity of cell-cell recognition often limits the benefits of cooperation to kin and potentially avoids adverse interactions with non-kin. In other cases, inter-species recognition leads to cooperation. For example, bacterial colonization and biofilm development typically involve interactions between different species (Burmølle *et al.*, 2014). Taxonomic diversity can lead to benefits such as increased resistance to antibiotics and broader metabolic capabilities as compared with monoculture biofilms (Elias & Banin, 2012). In this section, we discuss examples of intra- and inter-species recognition and their social functions (Fig. 1).

1. Self-recognition and outer membrane exchange in myxobacteria

Myxobacteria are a group of soil-dwelling Gram-negative rods that exhibit complex social behaviors. During vegetative growth, cells form multicellular groups referred to as swarms that collectively move and forage for food. The sociality of myxobacteria is exemplified by their ability to aggregate into multicellular fruiting bodies analogous to social slime molds. Within myxobacterial groups, individual cells make frequent contact with each other by gliding motility (Kaiser, 2003). We recently identified a contact-dependent mechanism involving self-recognition that we named outer membrane exchange (OME) (Pathak *et al.*, 2013). During this process, copious amounts of outer membrane (OM) material are transferred between cells when they make physical contact (Nudleman *et al.*, 2005, Wei *et al.*, 2011). Cargo includes lipoproteins, OM phospholipids, lipopolysaccharide (LPS) and toxins (Cao *et al.*, 2015, Vassallo *et al.*, 2017). Because of the diversity of the cargo, OME serves as a platform for coordinating social functions. Participation in OME requires cells to make two key proteins, TraA and TraB, whereas individuals lacking either protein are

excluded from OME (Pathak *et al.*, 2012). TraA is a cell surface receptor, and it forms a functional adhesion with TraB (Cao & Wall, 2017) (Fig. 1). Overexpression of TraA/B leads to tight cell-cell binding (Vassallo *et al.*, 2015). Recognition specificity occurs by an N-terminal variable domain within TraA that governs homotypic interactions between receptors (Pathak *et al.*, 2013). TraA from different environmental isolates is highly polymorphic within this domain, whereas other regions of TraA are conserved within conspecific isolates. TraA polymorphisms restrict OME such that it occurs between clonemates and close relatives that produce identical or nearly identical TraA receptors.

The high level of sequence variation in environmental TraA receptors suggests that there are diverse recognition groups in nature. To date we have experimentally tested TraA receptors from 16 *Myxococcus xanthus* environmental isolates and found that they belong to six recognition groups (Pathak *et al.*, 2013) (Fig. 2A). Notably, TraA polymorphisms facilitate the formation of distinct social groups (Cao & Wall, 2017). For example, isogenic cells overproducing different TraA receptors form distinct cell clusters (aggregates) during growth in liquid medium. By chimeric allele analysis and site-directed mutagenesis, we discovered the malleable nature of TraA specificity, which provides a molecular explanation for how diversity of recognition arose in natural populations (Cao & Wall, 2017). For example, we found a conserved single residue switch within the TraA variable domain (Fig. 2B), and substitutions at this position, A205P or P205A, can alter the specificity of TraA recognition (Fig. 2C). By creating the corresponding substitutions in receptors that belong to six defined recognition groups, we created a unique allele that recognizes only itself; it does not recognize its parental allele or any other TraA receptor (Fig. 2C). The malleable nature of TraA likely allows it to tolerate many spontaneous sequence changes within its variable domain during evolution, which in turn can lead to changes in specificity and the diversification of myxobacterial social groups.

TraA serves as the first layer of recognition for determining OME partners. A second layer of recognition further distinguishes true clonemates for cooperation (Dey *et al.*, 2016, Vassallo *et al.*, 2017). This second layer involves the exchange of polymorphic toxins that reside in the OM. Such toxins, encoded within a highly variable prophage-like region of the genome, lead to the death of recipient cells that lack the cognate immunities. This dual recognition strategy ensures high selectivity in the sharing of OM components that can happen only between individuals with compatible TraA receptors and cognate toxin-immunity pairs. This form of kin discrimination also results in visible boundaries between two approaching swarm populations that express compatible TraA receptors but non-cognate toxin-immunity pairs, whereas swarms composed of identical cells freely merge together.

Recognition among clonemates and close siblings leads to beneficial interactions through the sharing of cellular goods. For example, OME can repair damaged cells by diluting defective materials in unhealthy cells and replenishing missing components from healthy cells (Vassallo *et al.*, 2015). Such behaviors increase the fitness of the population as a whole, which requires a minimum number of cooperative healthy cells to carry out multicellular functions (Vassallo & Wall, 2016). In contrast, different environmental isolates that produce compatible TraA receptors are likely to antagonize each other through the delivery of polymorphic toxins. This discrimination strategy likely plays a key role in regulating social

interactions and ensuring related individuals form uniform and cohesive groups to conduct sophisticated social functions.

2. Contact-dependent inhibition

Similar to OME, contact-dependent inhibition (CDI) is a bacterial recognition system that involves physical interactions between cells through surface-embedded receptors (Fig. 1). It was initially discovered in *Escherichia coli* isolate EC93, where it was shown to specifically inhibit the growth of other *E. coli* strains, i.e., not other species, by a mechanism that involves toxin delivery (Aoki *et al.*, 2005, Jones *et al.*, 2017). In Gram-negative bacteria, the core of a CDI system consists of three components: CdiA, CdiB and CdiI. CdiA/B are two-partner secretion proteins. CdiA is a long filamentous protein that harbors a C-terminal toxin domain (CT), and the transport of CdiA across the OM relies on the OM β-barrel transporter CdiB. Upon binding to receptors on neighboring cells through its receptor-binding region (RBR), CdiA releases its toxin domain, which is subsequently delivered into the cytoplasm of target cells. The presence of a cognate immunity protein, CdiI, which binds and inactivates the toxin, protects producer cells from self-intoxication (Aoki *et al.*, 2010). Growth of related non-self target cells lacking the cognate antitoxin is inhibited, usually through the disruption of the membrane proton-motive force or nucleic acid degradation. Specificity in this system occurs because the CdiA-CT and CdiI proteins are highly polymorphic across different bacterial isolates (Aoki *et al.*, 2010). Cells that contain a CDI system have a competitive advantage over cells lacking immunity by inhibiting their growth. As described below, recent findings have also uncovered cooperative roles for CDI (Jones *et al.*, 2017).

Self-recognition in CDI was revealed by elucidating the binding interactions between CdiA and cell surface receptors (Ruhe *et al.*, 2013). BamA, a key component of the OM β-barrel assembly machine (BAM), was first identified as a receptor for CdiA from *E. coli* EC93. Although BamA is highly conserved among the Gram-negative bacteria, the sequence variations within its surface-exposed loops restrict $CdiA^{EC93}$ binding to *E. coli* strains. CdiA proteins from other *E. coli* isolates can bind different receptors, but again they bind receptors only from related bacteria. For example, the heterotrimeric complex of osmoporins OmpF and OmpC serves as a receptor for CdiA from *E. coli* EC536 (Beck *et al.*, 2016). Sequence variation within the surface-exposed residues in OmpC among different isolates prevents the binding and intoxication of distant strains. Therefore, CDI is suggested to serve as a tool for recognizing close relatives, and the subsequent toxin delivery step discriminates social partners.

CDI also promotes cooperative interactions. For example, CDI systems facilitate auto-aggregation and biofilm formation (Ruhe *et al.*, 2015, Anderson *et al.*, 2014). The adhesion properties of CdiA allow social interactions between sibling cells, whereby the specific recognition between CdiA and receptors excludes unrelated cells from engaging in CDI-dependent cell-cell adhesion (Jones *et al.*, 2017). In addition, recent work suggests that the exchange of toxin modules between siblings leads to cell-cell signaling and changes in gene expression that contribute to biofilm formation (Garcia *et al.*, 2016). Thus, as our knowledge

about the CDI system has increased, it has become apparent that this mechanism encompasses more than simply inhibitory processes.

3. Territoriality in *Proteus mirabilis*

Another cell contact-dependent mechanism that involves recognition has been described in *Proteus mirabilis*, a Gram-negative bacterium that collectively swarms over surfaces and forms a visible boundary (Dienes line) between colonies of different identities. Boundary formation in *P. mirabilis* represents an example of territoriality behavior, which is also observed in other bacteria such as *Bacillus subtilis* (Stefanic et al., 2015) and *M. xanthus* (Vassallo et al., 2017). In *P. mirabilis*, a functional T6SS is required for Dienes line formation (Alteri *et al.*, 2013). Different models underlying *Proteus* territoriality have been proposed (Fig. 1). One model invokes the T6SS as a weapon to deliver toxin effectors to non-self *P. mirabilis* cells that lack cognate immunity (Alteri *et al.*, 2013). Thus, when swarming colonies meet, T6SS-mediated attack leads to cell death and the formation of an inter-strain boundary. In a second model, T6SS delivers polymorphic Ids (Identification of self) proteins between swarming cells, which in turn govern *Proteus* territorial behaviors (Gibbs *et al.*, 2008, Saak & Gibbs, 2016). For example, when IdsD is delivered into neighboring cells, it specifically interacts with a cognate IdsE protein. Swarming populations expressing matching D–E pairs have the same identity and merge, whereas populations producing divergent D–E proteins are not recognized as self and form a boundary between swarms. The exchange of Ids proteins between cells does not have fatal consequences (Saak & Gibbs, 2016). Instead, the non-cognate D–E pairs result in negative swarm regulation. Therefore, IdsD is suggested to act as a regulatory factor for multicellular swarming, and D–E recognition allows cells to communicate during collective movements and facilitates swarm expansion of kin cells. Importantly, the recognition and antagonism in *Proteus* territoriality helps to exclude non-siblings from swarms and leads to a dominant strain within a niche(Gibbs & Greenberg, 2011). This model is consistent with clinical findings that a single genotype of *P. mirabilis* usually dominates during urinary tract infections (Gibbs & Greenberg, 2011).

4. Quorum sensing

Besides cell contact-dependent mechanisms, bacteria are capable of using diffusible factors to sense their surroundings and recognize self. Quorum sensing (QS) is a well-known bacterial cell-cell communication process, during which individuals process extracellular signals to synchronize group behaviors (Miller & Bassler, 2001) (Fig. 1). QS systems are found in diverse bacterial species. The signaling molecules synthesized by cells are termed autoinducers (AIs), and their concentration within an environmental niche reflects the population density of AI-producing cells. Once a population reaches a threshold density (i.e., a quorum), the signaling cells collectively change gene expression in a QS-regulated manner to generate a group response. QS promotes the fitness of bacteria by assembling individual cells into cohesive units that display various group activities, such as bioluminescence, virulence factor secretion, antibiotic production, social motility, sporulation and biofilm formation (Papenfort & Bassler, 2016). QS ensures that individuals do not frivolously undertake an activity when only a limited number of cells are present. For example, bioluminescence production is energetically costly and will not generate a fitness

gain when undertaken by only a few cells. Notably, the mechanisms underlying QS systems can differ significantly from one species to another, and AIs synthesized by different organisms are also chemically diverse (Papenfort & Bassler, 2016). The QS receptors can exhibit extraordinary ligand-binding specificity, which allows cells to precisely identify AIs produced by related individuals within a heterogeneous population (Hawver *et al.*, 2016). In addition, QS can occur between divergent bacterial species. For example, AI-2, one of the most common and broadly recognized communication signals, serves as a means for inter-species interactions that facilitates the development of multispecies biofilms (Rickard *et al.*, 2008). QS also plays a role in interbacterial competition. In *Vibrio cholera*, QS controls the activation of type VI secretion system (T6SS) (Zheng *et al.*, 2010, Shao & Bassler, 2014), a mechanism that allows *V. cholera* cells to kill other bacterial species and create a favorable niche free of non-kin competitors (MacIntyre *et al.*, 2010).

5. Oral biofilm

Human oral biofilms represent one of the best-studied microbial ecosystems, within which various recognition mechanisms occur among cells either through direct physical contacts or through the exchange of diffusible chemicals. The oral cavity is a complex environment that consist of saliva (liquid), teeth (hard surface) and epithelial tissues (soft surface) and is exposed to a fluctuating amount of nutrients, changes in temperature and mechanical perturbations. Approximately 500 distinct species have been shown to reside within oral environments (Zaura *et al.*, 2009). To colonize and survive in this niche, different microbial species recognize and interact with each other to create multispecies biofilms (Kuramitsu *et al.*, 2007). For example, specific coaggregation between different microbial species plays a key role in the colonization, organization and growth of oral biofilms (Kolenbrander *et al.*, 2010) (Fig. 1). The initial colonizers first adhere to the oral surfaces, which then allows other species (early, middle or late colonizers) to subsequently bind to form multispecies communities. Within oral biofilms, different bacterial species communicate by cell-cell contact between surface receptors as well as by QS molecules (e.g., AI-2), which likely synchronizes gene expression and cell behaviors (Bassler *et al.*, 1997). In addition, the metabolism of different cells may influence other residents living within the same biofilm (Kuramitsu *et al.*, 2007). Such metabolic interactions can be either antagonistic or cooperative, depending on whether the metabolic products of one organism are adverse or suitable for the growth of others. Oral biofilms thus serve as an attractive and relatively well understood model for studying complex inter-species recognition and social interactions.

Physiological Heterogeneity and Recognition in Bacteria

Genetic diversity inherent to microbial life in natural environments is accompanied and complicated by physiological heterogeneity within clonal populations (Lidstrom & Konopka, 2010, Stewart & Franklin, 2008). Such physiological variability is widespread and results in the formation of subpopulations with qualitatively different phenotypes, and as described here it can influence cell-cell recognition and social interactions. We define physiological heterogeneity as cell-to-cell variations in measurable parameters between clonal cells that exhibit differences in, for example, morphology, growth rate, age, cellular damage loads and gene expression patterns (Lidstrom & Konopka, 2010). In general, these

variations are caused by differences in microenvironments (e.g., nutrient and oxygen gradients in biofilms) or emerge as a consequence of stochastic differences in gene expression or phase variation under relatively homogenous environments (Ackermann, 2015, Serra *et al.*, 2013, Stewart & Franklin, 2008). Stochastic variations are distributed in populations in the form of 'noise', e.g., random fluctuations in gene expression, and can take distinct forms. For instance, bistability or multistability occurs when gene expression patterns in clonal populations segregate into two or more, respectively, stable states or phenotypes (Veening *et al.*, 2008). These distinct phenotypes can be epigenetically inherited through several rounds of cell division (Casadesús & Low, 2013). Cellular differentiation leads to even greater physiological differences, whereby subpopulations of clonal cells acquire distinct morphologies and specialized functions (e.g., heterocysts in cyanobacteria and developmental differentiation in myxobacteria) (Wolk *et al.*, 1994, O'Connor & Zusman, 1991). One example of bimodality stabilized by a positive feedback loop is the development of competent cells in *B. subtilis*. When *B. subtilis* enters the phase of late exponential growth, QS initiates stabilization of the ComK transcription factor levels in cells, and in turn ComK activates a regulon responsible for competence and its own synthesis. Because ComK levels stochastically fluctuate between individual cells, only a fraction of cells (~10%) will reach the ComK threshold level needed to create a positive feedback loop, and the population will transiently bifurcate into competent and non-competent cells (Maamar & Dubnau, 2005). In addition to competent cells, *B. subtilis* forms other distinct morphotypes such as matrix producers, motile and non-motile cells, producers of extracellular proteases and cells that sporulate. Differentiation into these cell types is mutually exclusive, as regulatory mechanisms involved in gene expression control lead to particular phenotypes while simultaneously repressing genes governing other cell types(Lopez *et al.*, 2009).

Physiological Heterogeneity: the Good and the Bad

In fluctuating environments, the presence of cells with distinct physiological states often enables a clonal population to better adapt to sudden environmental changes. This strategy allows populations to spread risk or bet-hedge against changing conditions (Veening *et al.*, 2008). Persister cells, which are in a state of dormancy, are one example of such a mechanism. The advantage of dormancy is that those cells are resistant to certain stressors, such as antibiotics that act on metabolically active cells. After the insult has passed, persisters become metabolically active and re-populate the biofilm (Lewis, 2010, Maisonneuve & Gerdes, 2014). Although this strategy provides direct benefits to persisters under stressful conditions, it can also be viewed as a social trait influenced by kin selection, as suggested by mathematical modeling by Gardner et al. (Gardner *et al.*, 2007). Specifically, by being dormant, persisters decrease the level of competition within a population that faces resource exhaustion. Reduced reproductive output by persisters means reduced individual fitness and, as such, persistency can be viewed as an altruistic trait, with the benefits of it, according to the model, limited to close relatives. This behavior is in accordance with Hamilton's rule stating that altruistic traits will be favored by selection as long as the reproductive cost to the actor performing the behavior is outweighed by the reproductive benefit of the recipient multiplied by its relatedness to the actor. (Hamilton,

1963, Hamilton, 1964). Another example where physiological heterogeneity impacts antibiotic resistance was recently described in *E. coli*. Here, asymmetric distribution of the antibiotic efflux pump AcrAB-TolC during cell division leads to pumps clustering at the old pole, which in turn makes progeny cells with old poles more resistant to antibiotics compared to their daughter cells with newer poles (Bergmiller *et al.*, 2017).

Division of labor is another example of how physiological heterogeneity benefits bacterial populations. Here, subpopulations undertake the task of producing goods that are used by the whole community or a subpopulation. The expression of virulence factors in *Salmonella typhimurium* in a murine colitis model is a case where physiological heterogeneity benefits the population through both division of labor and bet-hedging. During infection, a fraction of the *S. typhimurium* population will express type 3 secretion system 1 (T3SS-1), a major virulence determinant, and use it to invade host intestinal mucosa evoking an inflammatory response. Products of this response, such as tetrathionate, help *S. typhimurium* outcompete commensal microbes found in the intestinal tract. These metabolites are used by a *S. typhimurium* subpopulation residing in the gut lumen but are not available to the T3SS-1–producing cells. Additionally, T3SS-1–expressing cells are slow growing and exhibit higher levels of antibiotic tolerance compared with their siblings that do not express T3SS-1, a trait that can be considered a bet-hedging strategy, similar to persisters.

In contrast, physiological heterogeneity can potentially be detrimental to the fitness of a population. Social bacteria, in particular, rely on their ability to synchronize cellular responses and engage in collective behaviors. As discussed above, QS is one of the means for controlling the physiological state of a population, where a behavioral change depends on population density and signaling In some instances, population fitness is compromised by the failure of cells to synchronize a response, thus blocking the formation of biofilms (Parsek & Greenberg, 2005), secretion of virulence factors (Smith & Iglewski, 2003) or survival during the stationary phase (Goo *et al.*, 2012). This failure to synchronize can result from either genetic (e.g. presence of social mutants) or physiological heterogeneity. Furthermore, the production of public goods—secreted factors shared by a community—is energetically costly, and an inability to develop a synchronized response hinders the fitness of a population. For example, *Vibrio fischeri* uses ~20% of its metabolic potential to produce luciferase which results in bioluminescence only if produced on a population-wide scale. Bioluminescence produced by *V. fischeri* is beneficial to its symbiont organism, marine bobtail squid, which in turn provides a niche and food for the bacterium (Ruby, 1996). If *V. fischeri* cells fail to produce a homogenous or synchronized response, insufficient levels of bioluminescence will be made and their symbiotic relationship and fitness will be compromised. In another example, developmental aggregation of myxobacteria may be inhibited by physiological heterogeneity. This multicellularity-by-aggregation strategy requires that cellular behaviors be synchronized, which is obtained through population density dependent intercellular signaling (Kaiser, 2004, Zhang *et al.*, 2012). Moreover, as indicated above, we suggest that the mixing of cellular components by OME facilitates the development of a homogeneous cell population that is better suited to conducting synchronized functions (Vassallo *et al.*, 2015).

Physiological Recognition as a Function of Social Integration

Plants can carry out self/non-self recognition based on their physiological state rather than genetic identity (Gruntman & Novoplansky, 2004, Falik *et al.*, 2006). Here, genetically identical individuals are perceived as alien after a short period of separation during which their physiological state has changed (Gruntman & Novoplansky, 2004). In general, cells recognize each other through phenotypic properties shaped by their genotype and expression thereof, which is influenced by environmental and stochastic fluctuations. Although not as well studied as genotype recognition, the process of bacterial recognition and discrimination based on physiological states also influences social interactions. We recently described a mechanism that myxobacteria use to address physiological heterogeneity within a mixed population consisting of growing and starving cells. The question we asked was whether physiologically distinct siblings would cooperate, antagonize or maintain neutral interactions. To test this, we created auxotroph strains and mixed them with their parent strain. Notably, on minimal medium, where the auxotrophs were starving for a metabolite, they were antagonized (killed) by their prototroph siblings. In contrast, when strains were mixed and placed on minimal media with the missing auxotroph metabolite or on rich medium, the strains grew equally well and interacted harmoniously. Similarly, when auxotrophs and prototrophs were mixed on starvation agar they harmoniously interacted. These findings show that antagonism only occurs under conditions when strains are physiologically different; otherwise their interaction are cooperative. We further found that antagonism depends on T6SS and gliding motility, and we identified a novel effector-immunity pair, TsxEI, that mediates killing (V. Troselj, A. Treuner-Lange, L. Søgaard-Andersen, D. Wall, submitted). Antagonism is caused by decreased levels of a specific immunity protein (TsxI) in starving cells, which makes them susceptible to intoxication. In contrast, within a homogeneously starving population, T6SS-mediated killing is not detected, indicating that starvation downregulates T6SS function. We hypothesize that the biological purpose of this sibling antagonism is to recognize and eliminate less-fit cells from the population or to delay the onset of development by cannibalizing cells that enter the developmental program prematurely. Likewise, when a population develops a consensus response to starvation, the cells synchronize their behavior and commit to development.

Another example of sibling discrimination based on physiological states is seen in *B. subtilis* cannibalism. When exposed to nutrient limitation or stress, a subpopulation of *B. subtilis* accumulates phosphorylated Spo0A (Spo0A~P), the master regulator of sporulation. Spo0A~P controls both sporulation and matrix production, and its accumulation to a threshold level is regulated by multiple input signals. Whereas high levels of Spo0A~P initiate sporulation, lower levels trigger extracellular matrix production in cells and simultaneously initiate a pathway involved in cannibalism that is mediated by two toxins, Skf and Sdp, with co-expression of cognate antitoxins conferring immunity to the producer cells. As Spo0A levels vary stochastically among cells, a subpopulation of cells will not reach the threshold level of Spo0A~P for becoming matrix and toxin producers. These cells, therefore, remain susceptible to the Skf and Sdp toxins and are lysed and cannibalized by their siblings (López *et al.*, 2009, González-Pastor *et al.*, 2003, González-Pastor, 2011). Furthermore, cells that reach Spo0A~P levels high enough to initiate sporulation can also

cannibalize non-sporulating cells using the same mechanism. In both cases, cannibalism delays the onset of sporulation for toxin producers by providing nutrients for prolonged vegetative growth.

Monitoring or policing cell populations by identifying and eliminating individuals that pose a threat is a feature of eukaryotic organisms that use surveillance or immunity systems. However, eukaryotes are not alone in this ability, as bacteria have also evolved systems to monitor their populations. One example is how social mutants (cheaters) are dealt with among social bacteria. Cheaters are non-cooperative individuals that utilize public goods without contributing to their production. Consequently, these cooperative behaviors are vulnerable to exploitation, as cheaters do not pay the metabolic cost of producing the public goods and therefore have a fitness advantage over cells that do produce them. This means that cooperative individuals are at risk of being outcompeted unless the population has mechanisms that control and/or eliminate cheaters (Hibbing *et al.*, 2010, Wang *et al.*, 2015, Velicer *et al.*, 2000, Manhes & Velicer, 2011). This policing behavior helps address the problem of genotypic and physiological heterogeneity that impedes social cooperativity. QS is a trait that is vulnerable to exploitation. One example for how policing occurs involves *Pseudomonas aeruginosa* populations, in which QS cooperators produce cyanide, which inhibits the growth of QS mutants but not of the cooperators (Wang *et al.*, 2015). Similarly, in *Burkholderia thailandensis*, T6SS expression is induced by QS in cooperators, which renders QS mutants susceptible to T6SS-mediated poisoning (Majerczyk *et al.*, 2016). Whereas these mechanisms keep social mutants from exploiting public goods, they can also be used against siblings that are physiologically different. Namely, cells that are genetically equipped to engage in QS can fail to do so because of gene expression variability or microenvironment differences that renders them blind and/or unresponsive to the QS signal. In these cases, the non-cooperative cells are discriminated against and face the same fate as QS mutants.

Social bacteria may oscillate between homogeneous and heterogeneous populations based on their temporal needs and environmental cues. As discussed above, both states have an adaptive value in fluctuating environments that likely depends on the species' lifestyle. Thus, social bacteria that engage in collective behaviors benefit from mechanisms that enable cell-cell signaling, recognition and synchronization of behaviors (Fig. 3). In contrast, populations can also successfully adapt by differentiating into different cell types (Fig. 3). Regardless of the final outcome, bacteria discriminate not only between self and non-self but also between their phenotypic or physiological states. Therefore, physiological differences also need to be taken into account to understand how bacteria recognize and cooperate with each other.

Conclusion

Genetic recognition enables bacteria to communicate and establish homogeneous populations with siblings in which cooperative behaviors are limited to close relatives. In social bacteria, kin recognition facilitates complex social behaviors that depend on the population density of like individuals. Additionally, molecular recognition systems can mediate inter-species relationships in diverse and stratified multispecies communities found

in natural environments or in eukaryotic hosts. Bacterial interactions and the ability of bacteria to form complex communities are also shaped by physiological heterogeneity and the ways in which it is recognized and managed within populations. Physiological heterogeneity is a layer of complexity in bacterial social networks that needs to be better addressed in future research to allow a complete understanding of bacterial behavior. This understanding may help us manipulate bacterial social behavior for medical, ecological or industrial purposes, given the application and development of tools that enable precise tracking of cell-to-cell differences in populations.

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Abbreviations

T6SS	type VI secretion system
OME	outer membrane exchange
CDI	contact dependent inhibition

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Originality Significance Statement

The authors confirm that this minireview is original and describes recent advances in recognition and social interactions in bacteria.

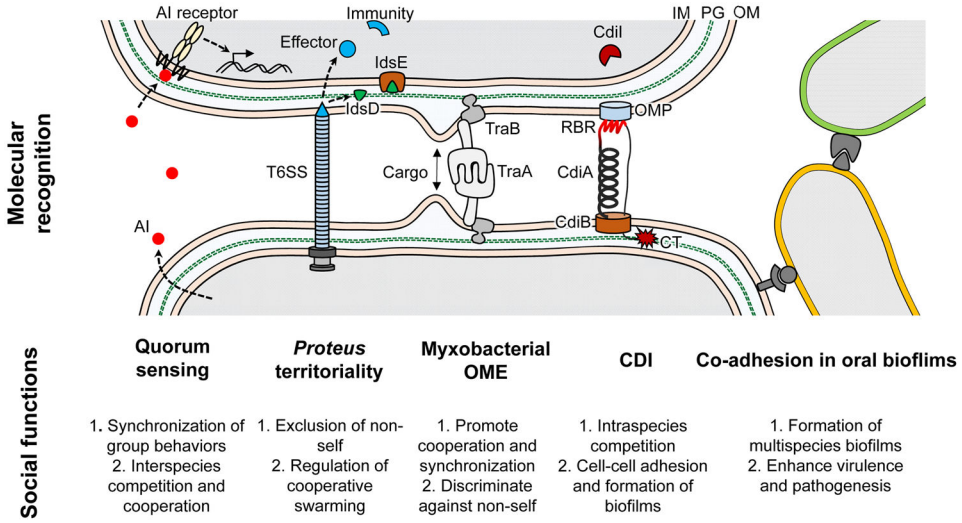

Fig. 1. Social recognition in bacteria

Schematics of representative recognition systems used by social bacteria. AI, autoinducer; OMP, outer membrane protein; IM, inner membrane; PG, peptidoglycan; OM, outer membrane; CT, C-terminal toxin; RBR, receptor binding region; T6SS, type VI secretion system. See text for details.

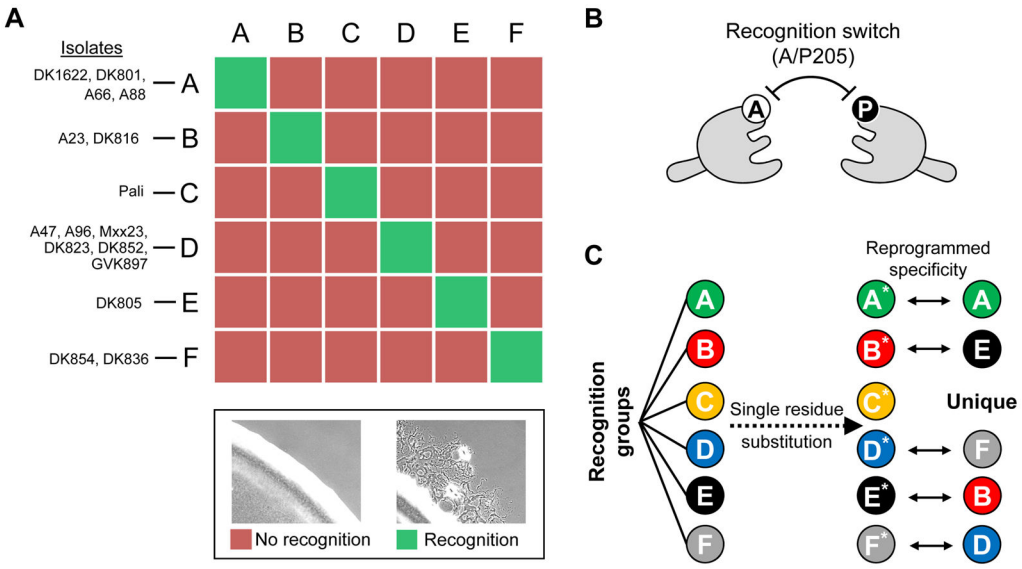

Fig. 2. TraA recognition in myxobacteria

A) 16 *M. xanthus* environmental isolates form six distinct recognition groups. Members of each group are shown. Representative micrographs of experimental assay that determines TraA recognition specificity is shown (bottom). In brief, TraA cell-cell recognition between two strains results in motility (flares) at the edge of a mixed colony(Pathak *et al.*, 2013). B) Single amino acid substitutions in a TraA (A/P205) switch leads to a change in homotypic recognition specificity between receptors (Cao & Wall, 2017). C) TraA homotypic recognition reprogrammed through single residue substitutions (A205P or P205A) from six recognition groups. TraA receptors after A/P substitutions are indicated with asterisks, and their recognition specificities are shown on the right.

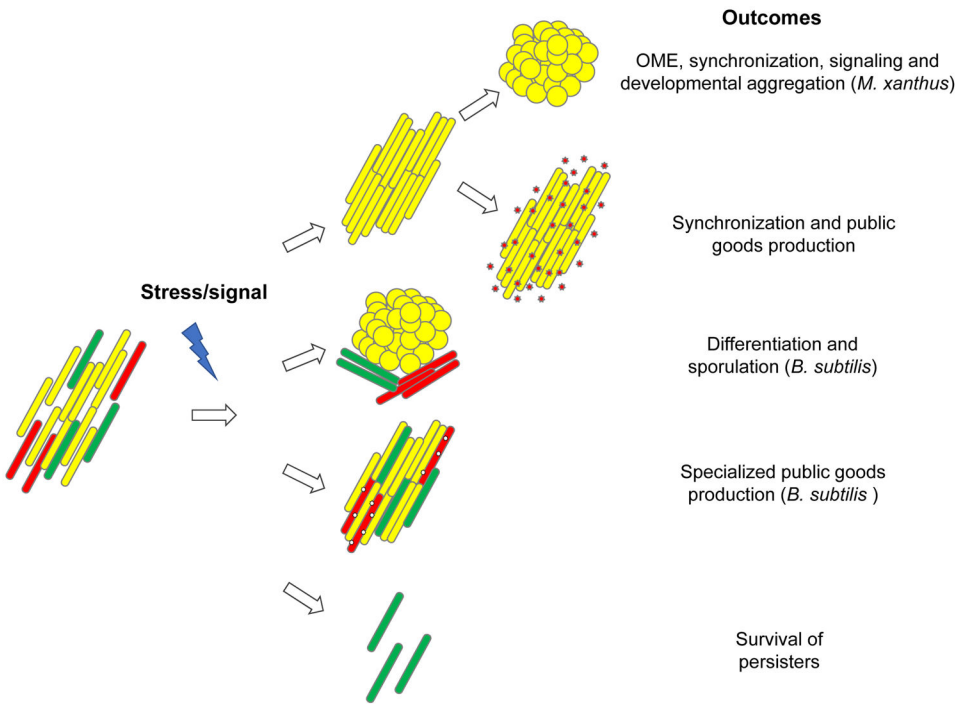

Fig. 3. Physiological heterogeneity and integration of social functions

An environmental cue (a stressor or a signal) induces a response in a physiologically heterogeneous bacterial population**,** as represented by the red, yellow and green rods. Depending on the species and the nature of the signal, the social responses vary and can lead to population synchronization or differentiation (top to bottom).