Bacterial linguistic communication and social intelligence

Eshel Ben Jacob[1], Israela Becker[1,2], Yoash Shapira[1] and Herbert Levine[3]

[1]School of Physics and Astronomy, The Raymond and Beverly Sackler Faculty of Exact Sciences, Tel-Aviv University, Tel-Aviv 69978, Israel
[2]Tel-Aviv Academic College of Engineering, 218 Bney Efraim Rd., Tel-Aviv 69107, Israel
[3]Center for Theoretical Biological Physics, University of California at San Diego, La Jolla, CA 92093-0319, USA
Corresponding author: Eshel Ben Jacob (eshel@tamar.tau.ac.il).

Bacteria have developed intricate communication capabilities (e.g. quorum-sensing, chemotactic signaling and plasmid exchange) to cooperatively self-organize into highly structured colonies with elevated environmental adaptability. We propose that bacteria use their intracellular flexibility, involving signal transduction networks and genomic plasticity, to collectively maintain linguistic communication: self and shared interpretations of chemical cues, exchange of chemical messages (semantic) and dialogues (pragmatic). Meaning-based communication permits colonial identity, intentional behavior (e.g. pheromone-based courtship for mating), purposeful alteration of colony structure (e.g. formation of fruiting bodies), decision-making (e.g. to sporulate) and the recognition and identification of other colonies – features we might begin to associate with a bacterial social intelligence. Such a social intelligence, should it exist, would require going beyond communication to encompass unknown additional intracellular processes to generate inheritable colonial memory and commonly shared genomic context.

Eons before we came into existence, bacteria had already invented many of the features that we immediately think of when asked 'what is life?' Back in the early 1940s, guided by this question, Schrödinger [1] proposed that organisms cannot simply feed on energy as man-made machines do. They must feed upon 'negative entropy' – absorb low entropy organic substances produced by lower organisms and exude high entropy waste products [1].

Bacteria, being the first form of life on Earth [2,3], had to devise ways to convert inorganic substance into living matter. This is not a solitary endeavor for the bacteria; under natural conditions, they use chemical communication to form hierarchically structured colonies, consisting of 10^9–10^{13} bacteria each [4–9]. By acting jointly, they can make use of any available source of energy and imbalances in the environment to reverse the spontaneous course of entropy production and synthesize life-sustaining organic molecules for themselves and parenthetically in the service of all other organisms.

As we are discovering, bacterial communication-based cooperation encompasses colony morphogenesis, which includes coordinated gene expression, regulated cell differentiation and division of tasks [4–9]. Collectively, bacteria can glean information from the environment and from other organisms, interpret the information in a 'meaningful' way, develop common knowledge and learn from past experience. The colony behaves much like a multicellular organism [5,6] or a social community [10–15], with elevated complexity and plasticity that afford better adaptability to whatever growth conditions might be encountered [9].

To achieve the proper balance of individuality and sociality, bacteria communicate using a broad repertoire of biochemical agents [16–19]. Some specific signals are described in Box 1, however, no doubt many more remain to be discovered. Each bacterium also has intricate intracellular genes and signaling mechanisms involving signal transduction networks [20] and gene language [21]. These are used to generate intrinsic meaning for contextual interpretations of the chemical messages and for formulating appropriate responses [9]. Biochemical messages are also used in bacterial cell–cell talk to exchange meaningful information across colonies of different species, and also with other organisms [19].

To come to grips with this phenomenology, we turn to the field of linguistics (Box 2), the metaphors of which have already begun to penetrate the description of bacterial communication. Usually, these metaphors refer to the structural (lexical and syntactic) linguistic motifs [22,23]. Here, we reason that bacterial chemical conversations also include assignment of contextual meaning to words and sentences (semantic) and conduction of dialogue (pragmatic) – the fundamental aspects of linguistic communication [24–28].

Using these advanced linguistic capabilities, bacteria can lead rich social lives for the group benefit. They can develop collective memory, use and generate common knowledge, develop group identity, recognize the identity of other colonies, learn from experience to improve themselves, and engage in group decision-making, an additional surprising social conduct that amounts to what should most appropriately be dubbed as social intelligence. This last term, originally coined to describe special mental skills that only humans use to conduct successful social lives [29,30], has been used more recently to describe linguistic, communication-based group behavior of other organisms, from primates to birds and insects [31–34].

Collective decision-making

One example of the advantage of bacterial discourse is the starvation response of many species [35]. When growth conditions become too stressful, bacteria can transform themselves into inert enduring spores.

Sporulation is a process executed collectively and beginning only after 'consultation' and assessment of the colonial stress as a whole by the individual bacteria. Simply put, starved cells emit chemical messages to convey their stress. Each of the other bacteria uses the information for contextual interpretation of the state of the colony relative to its own situation. Accordingly, each of the cells decides to send a message for or against sporulation. Once all of the colony members have sent out their decisions and read all other messages, sporulation occurs if the 'majority vote' is in favor.

Fruiting bodies formation

The most illuminating example of bacterial social behavior is perhaps the predator Myxobacteria (e.g. *Myxococcus xanthus*) [10–15]. This organism exhibits the richest set of colonial behavior phenomena, including cooperative feeding on other bacteria, creation of rippling and streaming patterns, controlled cell differentiation and generation of colonial identity to signal out 'cheaters' (Box 3). Upon starvation, these bacteria do not simply sporulate. Instead, they cooperate to form fruiting bodies of various types, presumably for a more efficient dissemination of the spores by passing animals [36,37].

Cooperative hierarchical organization

Some bacterial strains organize their colonies by generating modules, each containing many bacteria, which are used as building blocks for the colony. This behavior is observed in, for example, *Paenibacillus vortex* [7–9], which form the bacterial vortices shown in Figure 1, and in other strains, such as *Bacillus circulans* [38] and *Paenibacillus alvi* [39]. Maintenance of the integrity of the vortex while serving as a higher-order building block of the colony requires advanced communication. Each cell in the vortex needs to be informed that its role is now more complex, being a member of both the specific vortex and the whole colony, so it can adjust its activities accordingly. This ongoing communication is particularly apparent when it comes to the birth of new vortices (Figure 1). New vortices emerge in the trail behind a vortex as a result of initiation signals that cause the bacteria within the trail to increase the production of lubricating fluid and to move rapidly as a turbulent 'biofluid' until an eddy forms and turns into a new vortex. The entire process appears to proceed as a continuous dialogue; a vortex grows and moves, producing a trail of bacteria, and is pushed forward by the very same bacteria left behind. At some point the process stalls, and this is the signal for the generation of a new vortex behind the original one, which then leaves home (the trail) as a new entity toward the colonization of new territory.

In Figure 2, we show two different colonial pattern responses to non-lethal stress of two different kinds of antibiotics: septrin, a suppressor of cell reproduction, which might enhance communication; and ampicillin, a distorter of cell wall structure, which might impair cell communication. In both cases, during a subsequent encounter with the same antibiotic the bacteria respond more efficiently; however, this effect is erased if they are exposed to neutral conditions (i.e. growth on plates in the absence of antibiotic or on LB media) in between stress encounters. It appears that the bacteria can generate an erasable collective memory, as if to learn from their experience [9,40].

Multi-colony communities

Once an entire colony becomes a new multi-cellular being with its own identity, it can be a building block for even more complex organizations of multiple colony communities or societies, such as species-rich biofilms [11,17,41]. To give one striking example, current estimates suggest that sub-gingival plaque contains 20 genera of bacteria representing hundreds of different species, each with its own colony of $\sim 10^{10}$ bacteria, altogether about a thousand times the human population on earth. The level of complexity of such a microbial system exceeds that of computer networks, electrical networks, transportation and all other man-made networks combined. To maintain social cooperation in such diverse societies, the bacteria need even more advanced linguistic skills, so that they can keep up their dialogue within the 'chattering' of the surrounding crowd.

Patterns harnessing the genome

Bacteria can cooperatively make drastic alterations to their internal genomic state and transform into different cells. For example, the *Paenibacillus dendritiformis* lubricating bacteria, when grown on poor substrates, have the freedom to select their identity from two distinct cell types that are available: the branching (B) and the chiral (C) morphotypes (Figure 3) [7–9]. On harder substrates, when greater densities of bacteria are required to produce sufficient amounts of lubricating fluid, the B morphotype is selected, leading to the formation of colonies with branching bush-like morphologies [42] that are reminiscent of the patterns generated by starved *Bacillus subtilis* bacteria [43]. The engineering skill of the *P. dendritiformis* bacteria is manifested during growth on softer substrates, when curly branches are formed. This special geometrical organization allows faster expansion while also using patches of food that have been left behind as the branches twist inward. For this to occur, the bacteria suppress cell division and elongate. Optical microscope observations during colony development have revealed the following: upon elongation, the cells alter their collective movement from the typical run-and-tumble of the short B cells to a coordinated forward-backward movement, which yields an organized twist of the branches with a specified handedness. It is now understood how the preferred handedness of the twist results from the cell–cell interaction and the inherent flagella handedness. [8]

The two possible morphotypes are inheritable and can coexist when encountering a range of growth conditions. However, when colonies of the B morphotype are grown on soft substrate, an intriguing phenomenon of spontaneous transition is observed; the majority of the grown colonies exhibit B $\rightarrow$ C transitions [7–9]. The reverse C $\rightarrow$ B morphotype transitions are observed

during growth on harder and richer substrates. In both cases, the newly selected pattern is the one that maximizes the rate of colony expansion, hinting that the colonial morphotype manipulation is applied to attain better adaptability. [9]

Here, again, there appears to be a semantic message-based dialogue that helps the cells collectively decide between the C and B patterns. For instance, Figure 4 shows colony growth that started from a prepared mixture containing more of the C morphotype and less of the B morphotype (in contrast to natural mixtures) under conditions that were favorable to C [7]. Naively, one would expect the colony to grow in a similar pattern to that observed for the C morphotype, but it doesn't. It starts out growing with a modified C pattern, switches to a B-type morphology, and only later synchronized transitions occur resulting in the normal C morphotype pattern. Apparently, it takes some time for the bacteria to sort out the conflicting situation arising from a colony that has commenced growth in this unnatural way where there are a majority of C morphotype cells.

It is clearly essential to figure out how the bacteria can obtain semantic meaning, so as to initiate, for example, the proper context-dependent transitions between different operating states of the genome. Drawing upon human linguistics (Box 1), to sustain a dialogue that is based on semantic messages the bacteria should also have pre-existing shared knowledge (collective memory) that is transferable upon cell replication. Undoubtedly, this will involve the dynamics of a transcription factor network that combines the incoming information with the internal state of the cell. In this scheme, cells have 'memorized' internal states of the genetic network that can be invoked by messages; similar to the actions that our brain takes to obtain the semantic meaning of sentences. Because these states will be similar but still vary in details from cell to cell, each bacterium has some freedom to assign its own meaning to chemical messages.

A metaphor or overlooked reality?

To sustain the observed bacterial behavior that we have dubbed social intelligence, the bacteria might need to use even more exotic genomic features. What we have in mind is a bacterial version of genome cybernetics, by which we mean the ability of the genome to perform information processing and alter itself accordingly [44,45].

To date, it has been shown that transposable elements and 'junk DNA' play a key role in genome cybernetics of higher organisms [46–48]. For example, specific strains of ciliates have two nuclei, one containing the coding parts of the DNA and the other composed of non-coding sequences with an abundance of transposable elements. Upon replication, the coding nucleus disintegrates and the non-coding nucleus is replicated. After replication, the non-coding nucleus uses its transposable elements to reconstruct a new coding nucleus. In yeast, transposable elements can effectively re-program the genome activity between replications. They are inserted into mRNA and give rise to new proteins without eliminating old ones. These findings illustrate that rather than wait for mutations to occur randomly, cells can apparently keep some genetic variation on tap and move them to 'hard disk' storage in the coding part of the DNA if they turn out to be beneficial over several life cycles.

Can bacteria use communication to collectively perform similar 'tricks'? As Francis Bacon said: 'It would be an unsound fancy and self-contradictory to expect that things which have never yet been done can be done except by means which never have yet been tried.' We will need to do experiments that specifically test for these conceptual questions and that correlate colonial patterns with intracellular changes and dynamics.

Epilogue

The life, death and well-being of each of our cells depends on a colony of hundreds to thousands of former bacteria it carries: the mitochondria, which have their own genetic code, collective self-identity and self-interests [49]. Could, then, our internal and external linguistic communication and social intelligence be traced back to bacteria – the simplest of all organisms? And if so, shouldn't we try to learn from bacteria about the immune and the central nervous systems, which we use to communicate with other organisms?

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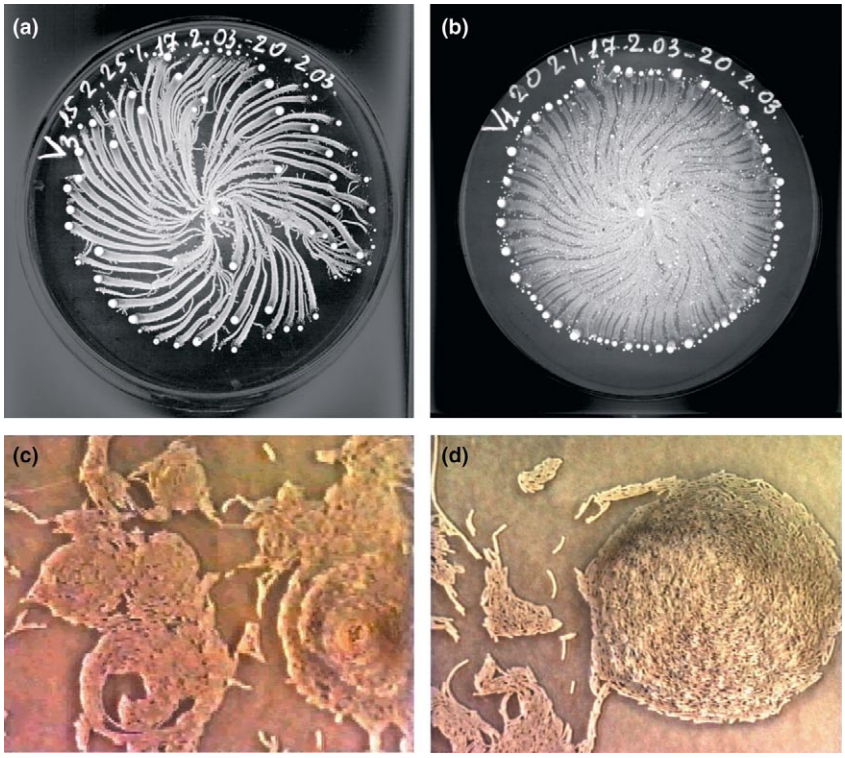

Figure 1. Cooperative hierarchical organization. Complex patterns developed after three-day growth of the *Paenibacillus vortex* bacteria (taken from the same culture) on 8.8 cm diameter plates, with (a) 15 gl^{-1} peptone and 2.25% agar; and (b) 20 gl^{-1} peptone and 2.0% agar (the substrate was richer and softer). The bacterial population of these colonies is greater than the human population on earth, however, they coordinate their behavior. Each vortex (bright dot) is composed of many cells that swarm collectively around their common center at ~10 micron sec^{-1}. The vortices vary in size from tens to millions of bacteria, according to their location in the colony. Both clockwise and anticlockwise rotating vortices are observed, although the majority has the same handedness. The cells in the vortex replicate, and the vortex expands in size and moves outward as a unit, leaving behind a trail of motile but usually non-replicating cells – the vortex branch. The twist of the vortex branch is determined by the handedness of its rotation. The dynamics of the vortices are quite complicated and include attraction, repulsion, merging and splitting of vortices. However, from this complex, seemingly chaotic movement, a colony with complex but non-arbitrary organization develops, as shown in the top pictures. (c,d) Snapshots from a video recording taken during formation of new vortices are shown (magnification x500, the pictures are ~100 microns wide; the bars represent the individual bacteria). (c) The dynamics within the branch are shown. An embryonic vortex, similar to the one on the right side of (c), organizes its structure, grows and consequently leaves the branch – as occurs in (d).

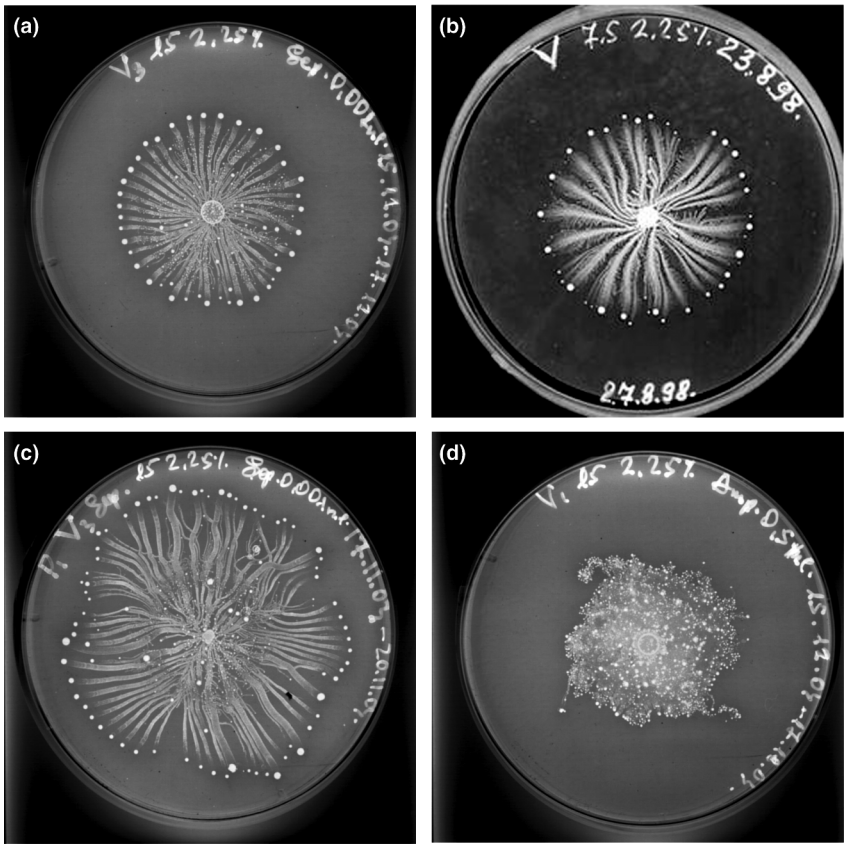

Figure 2. Colonial response to non-lethal stresses. (a) The response of a colony of *Paenibacillus vortex* to septrin (co-trimoxazole), which inhibits synthesis of folic acid and suppresses cell reproduction, is shown (15 gl^{-1} peptone and 2.25% agar with added antibiotic). On the basis of comparisons of model simulations with colonial patterns and microscope observations, it was proposed that, in response to septrin, bacteria enhance their cooperation by intensifying chemotactic attraction to form larger vortices; they also elevate repulsive chemotactic responses to signals emitted by the bacteria behind the vortices, which helps push the large vortices faster away from the stress they detect (not 'knowing' that there is antibiotic ahead as well) [9,40]. (b) Colonial development under metabolic stress due to nutrient deficiency is shown – no antibiotic, but half the level of nutrients. We emphasize the abundance of small vortices in this case and slower colonial expansion compared with (a). The pictures were taken after (a) 2 days and (b) 4 days of growth. These differences further support the idea of enhanced cooperation in the presence of septrin. (c) Growth was started from a cluster of bacteria taken from a colony grown in the presence of septrin. Comparison of (c) and (a) illustrates colonial memory and 'learning from experience' (growth conditions are the same in both). Memory can be erased by growing the bacteria on substrate with no antibiotic or on LB growth media. (d) Disorganized colonial development in response to ampicillin (which distorts cell wall structure) is shown. It might appear that ampicillin impairs communication-based coordination. However, colonial learning from experience can lead, under some conditions, to faster expansion in the presence of ampicillin.

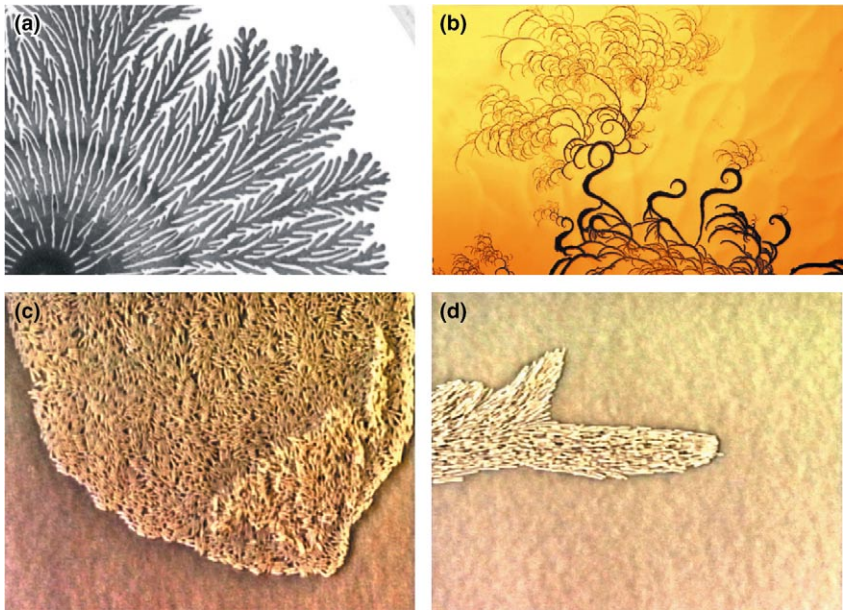

Figure 3. Engineered self-organization of branching and chiral morphotypes. Examples of the (a) branching and (b) chiral patterns of the B and C morphotypes of the lubricating *Paenibacillus dendritiformis* bacteria, formed by self-organization under nutrient limitation. The top photographs are macro-level (centimeter scale) views of parts of the branch organization. The bottom pictures are snapshots from video recordings during growth, showing bacterial organization

within the branches on the micro-level (magnification x500; each bar is an individual bacterium). The shorter B bacteria are randomly positioned and oriented – a reflection of their random swimming and tumbling movement within the branch. The well-defined boundaries of the branch are set by the collectively produced lubricating fluid within which they can move. The highly outbound structure of the branches in this example is due to repulsive chemotactic signaling from the pre-spore bacteria close to the colony center. The longer C bacteria have random positions but with specific orientation (analogous, for example, to some liquid crystals). Therefore, they can only rotate close to the branch tip. Owing to the inherent flagella handedness, tumbling is also with specific handedness, which, in turn, leads to the twisting (chiral) growth of the branches. The special geometrical organization (termed broken chiral symmetry) of these colonies is reminiscent of the patterns also developed by *Bacillus mycoides* [55].

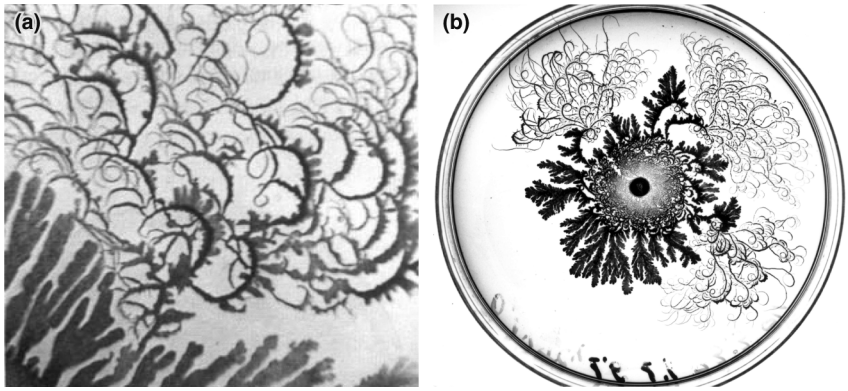

Figure 4. Communication-based morphotype transitions. The chiral morphotype is advantageous during colonial growth on softer substrates. On such substrates, colonies grown from a prepared culture of B bacteria first develop as a branching morphotype, and after approximately one day, spontaneous bursts of the preferred chiral morphotype are initiated and consequently outgrow (a) the original B colony. For this to happen, a sufficiently large group of C bacteria must be formed, by some synchronized and/or autocatalytic gene expression transition of many B bacteria, into the C morphotype. In addition, the newly formed C bacteria have to find their fellows within the large crowd of B cells and burst out as a group with new identity. (b) This process involves a special dialogue between C morphotypes within a population made up of a majority of B morphotypes. This growth starts from a culture largely made up of C morphotype bacteria; an artificial context that is the opposite of what bacteria encounter during natural spontaneous morphotype transitions. The outcome is an initially chiral pattern with a different geometry than that of a pure C colony due to the presence of the additional B cells that maintain their identity. Later, in a synchronized manner, the pattern switches to a mainly branching one with some handedness. Next, the C pattern bursts out in a manner similar to that observed when the growth starts from a pure culture of B morphotype bacteria.

Box 1. Molecular biology of bacterial communication

Communication capabilities can be inferred from observed multicellular behavior, but ultimately must be grounded in the interactions of specific biomolecules. Here, we briefly review some recent discoveries of these molecular underpinnings [16–19,50–52].

Many Gram-negative bacterial species use quorum-sensing molecules to turn on the expression of a variety of genetic suites (e.g. virulence genes) once the species density exceeds a threshold. A typical case arises in *Vibrio fischeri* where production of a membrane-permeable homoserine lactone by LUXI is sensed by the LUXR protein and turns on luminescence. In our terminology, the communication appears analogous to the lexical and synthetic levels of linguistic (see Box 2 in the main text).

The small peptide signaling systems identified in Gram-positive cells appear more capable. A well-studied case involves the two peptides COMX and CSF (competence stimulating factor), which control the transition to DNA uptake competence in *Bacillus* via the COMK transcription factor, which is relieved by degradation of the peptides. The same system is involved in the alternative decision to sporulate. Apparently, cells interpret these factors in conjunction with internal state information so as to decide upon their fate – this is what we refer to as the semantic level of communication (see Box 2 in the main text). This decision must then be relayed to other cells so that they can act accordingly [19,50,51]. Evidence that there is such a decision-generated response comes from the fact that the percentage of competent cells is strictly controlled. In addition, it has been suggested that sporulating cells emit chemorepellent signals so as to direct the colony away from zones of nutrient depletion [42].

There are other examples. Myxobacteria use the C-factor – a surface exposed protein – to present outside information about their physiological status. This way the bacteria can assess the physiological status of each other to properly coordinate their motion during starvation. The recently identified autoinducer AI-2 in *Vibrio harveyi* [51,53] appears to be responsible for interspecies message-passing of the type that probably occurs quite regularly in multi-species biofilms; for instance, there might be some pheromone-based negotiation for the trade of genetic information (see Box 3 in the main text).

We fully expect that this is merely the tip of the iceberg. The bacterial world, no less than the eukaryotic one, is full of unpredictable variation and thus appropriate behavioral responses must be fashioned by cooperative information gathering, collective decision-making and multicellular action.

Box 2. Linguistic communication

The two discoveries in the 1950s, including the universal grammar and the structural code of DNA, later led to the linkage of linguistics and genetics. The first discovery suggested universal structural motifs and combinatorial principles (syntactic rules) at the core of all natural languages, and the second provided analogous universals for the genetic code of all living organisms. Chomsky's meaning-independent syntactic grammar approach, along with computational linguistic methods, is widely used now in biology, especially in bioinformatics and structural biology but increasingly also in system biology and ecology. The focus has been mainly on the structural

aspects used to exchange information, or the two levels of formal linguistics [21–23]: lexical – formation of words from their components (e.g. characters and phonemes); syntactic – organization of phrases and sentences in accordance with well-defined grammar rules.

We propose that bacterial signaling also involves linguistic communication: the term currently used to describe the meaning-exchange function of language [25,26]. It includes the semantic aspects of linguistics that are associated with the assignment of context-dependent meaning to words, sentences and paragraphs [24–28].

When reading a text, for example, one has semantic freedom to assign to it different meanings. Each reader has cognitive flexibility to assign his own meanings to the text, according to personal knowledge and specific expectations, or purpose in reading the text. The meaning of a text is often captured only after reading it several times. At each such iteration, words, sentences and paragraphs can assume different meanings in the reader's mind. Iterative reading is necessary because there is a hierarchical organization of contextual extraction of meaning. Namely, each word contributes in the reader's mind to the interpretation of the entire sentence that the word is part of. However, at the same time the generated whole meaning of the sentence can change the meaning assigned to each of the words that the sentence is composed of.

Beyond the individual semantic level of linguistics, some linguists identify a dialogue among conversers (discourse or goal-driven conversation), using shared semantic meanings as the pragmatic level of linguistics [24–28]. This higher level of linguistic communication requires the conversers to have common goal in conducting the dialogue, shared knowledge and mutual intensions and expectations (presupposition, implicature and attribution). The group usage of a dialogue can vary from activity coordination through collective decision-making to the emergence of a group self-identity.

Box 3. Cooperation and clashes of social intelligence

The term social intelligence refers to human mental skills beyond the mathematical and academic ones connected with analytical intelligence that are required to conduct a successful social life [29–31]. Therefore, it is generally associated with special cognitive capacities of humans, such as perceiving self and group identity, perceiving self and group goals, engaging in adaptive social interactions, and acting together for personal and group benefit.

We illustrated that, by using linguistic communication, bacteria show patterns of collective behavior that might reflect some fundamental aspects of social intelligence. Additional clues are provided by the variety of strategies Myxobacteria can use when their social intelligence is challenged by cheaters – opportunistic individuals who take advantage of the group's cooperative effort [12–15]. For example, they can single out defectors by collective alteration of their own identity into a new gene expression state. By doing so, the cooperators can generate a new 'dialect' that is hard for the defectors to imitate. This ongoing intelligence clash with defectors is beneficial to the group as it helps the bacteria to improve their social skills for better cooperation.

By contrast, in multi-colonial communities (e.g. sub-gingival plaque) social intelligence is usually used for cooperation between colonies of different species [11,17,41]. For example, each colony develops its own expertise in performing specific tasks for the benefit of the entire community and they all coordinate the work.

Some bacteria undertake the task of keeping valuable information that is costly to maintain and can be hazardous for the bacteria to store. Frequently, such information is directly transferred by conjugation following chemical courtship that is played by the potential partners; bacteria resistant to antibiotics emit chemical signals to announce this fact. Bacteria in need of that information, upon receiving the signal, emit pheromone-like peptides to declare their willingness to mate. Sometimes, the decision to mate is followed by exchange of competence factors (peptides). This pre-conjugation communication modifies the membrane of the partner cell into the penetrable state needed for conjugation.

A third example is the non-winning rock–paper–scissors game played between strains of *Escherichia coli:* some strains (C) produce colicins that kill other colicin-sensitive (S) strains; these then out-compete colicin-resistant (R) strains that close the circle by out-competing C strains [53]. Expectedly, in this game of no prevailing strategy all three strains survived. However, in a recent *in vivo* version played by feeding the strains to different mice, strains (C) tend to loose with time [54].