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Self-engineering capabilities of bacteria

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Under natural growth conditions, bacteria can utilize intricate communication capabilities (e.g. quorum-sensing, chemotactic signalling and plasmid exchange) to cooperatively form (self-organize) complex colonies with elevated adaptability—the colonial pattern is collectively engineered according to the encountered environmental conditions. Bacteria do not genetically store all the information required for creating all possible patterns. Instead, additional information is cooperatively generated as required for the colonial self-organization to proceed.

We describe how complex colonial forms (patterns) emerge through the communication-based singular interplay between individual bacteria and the colony. Each bacterium is, by itself, a biotic autonomous system with its own internal cellular informatics capabilities (storage, processing and assessment of information). These afford the cell plasticity to select its response to biochemical messages it receives, including self-alteration and the broadcasting of messages to initiate alterations in other bacteria.

Hence, new features can collectively emerge during self-organization from the intracellular level to the whole colony. The cells thus assume newly co-generated traits and abilities that are not explicitly stored in the genetic information of the individuals.

Keywords: biocomplexity; bacterial colonies; self-organization; gene-networks; bacterial communication; natural self-engineering

1. INTRODUCTION

The motivation of this article is to present the observations and the conceptual challenges posed by self-engineering of bacteria during colonial development. We review some of the exciting discoveries about the cooperative behaviour of bacteria in colonies, guided by the assumption that they might shed new light on the foundations and evolution of biocomplexity in general. The review is aimed at researchers from different disciplines—microbiology, biology, chemistry, physics, mathematics and computer science. To make the presentation comprehensible to such a wide audience, we avoid the use of the specialized terminology of these different disciplines and limit the experimental and computational details.

Today, there are an increasing number of strains of disease-causing bacteria that can resist multiple drugs; bacteria are clearly capable of developing antibiotic resistance at a higher rate than scientists can develop new drugs (Levy 1998; Liebes *et al.* 1998; Miller 1998). We seem to be losing a crucial battle for our health. To reverse this course of events, we have to outsmart the bacteria by taking new avenues of study, which will in time lead to the development of novel strategies to fight them (Norris *et al.* 1999). But for that to happen, we must first reverse our current notion about bacteria as just simple solitary creatures with limited capabilities. These most fundamental of all organisms are cooperative beasts that lead complex social lives in colonies whose populations outnumber that of people on earth.

The idea that bacteria act as unsophisticated, uncommunicative and uncooperative cells stems from years of laboratory experiments where the bacteria are grown in Petri dishes under benign conditions. Not surprisingly, a bacterium that is not under any stress will strive to reproduce as fast as possible and need not worry about complex strategies that require large-scale coordination. However, when these versatile organisms are exposed to hostile environmental conditions, namely when the odds are against survival as individuals, they adopt a more complex strategy and employ a wide range of tactical behaviours to enable a proper adaptive response. One aspect of these behaviours has to do with self-engineered spatial organization of the colony (Kuner & Kaiser 1982; Matsushita & Fujikawa 1990; Ohgiwari *et al.* 1992; Ben-Jacob *et al.* 1994, 1998, 2000*a*; 2005; Salmond *et al.* 1995; Shapiro 1995; Dworkin 1996; Wirth *et al.* 1996; Shapiro & Dworkin 1997; Ben Jacob & Levine 1998, 2001, 2004; Shapiro 1998; Dunny & Winans 1999; Rosenberg 1999; Shimkets 1999; Strassmann 2000; Velicer *et al.* 2000; Crespi 2001; Bassler 2002; Di Franco *et al.* 2002; Miller 2002;

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Ben-Jacob 2003; Velicer 2003; Mok *et al.* 2003; Xavier & Bassler 2003; Komoto *et al.* 2003; Ben Jacob *et al.* 2004; Levine and Ben Jacob 2004; Ben Jacob & Shapira 2005), with the bacteria forming different patterns as needed to function. For example, there is evidence that colony structure can enhance antibiotic survival rate, allowing enough time for genetic experimentation in search of resistance; exactly how this works in detail is only now being elucidated. Some recent findings show that colony structures are modified in the presence of antibiotics (Ben Jacob *et al.* 2000*b*; Golding & Ben Jacob 2001; Ron *et al.* 2003) in ways which might optimize bacterial survival and that the bacteria might be using a sort of short-term epigenetic memory which enables them to keep track of the previous exposure to antibiotic.

Remarkably, the bacteria utilize pattern-formation mechanisms that we have begun to understand only in last few decades, mostly through the study of the physics and mathematics of self-organization in non-living systems (Levine & Ben Jacob 2004). In a real sense, the bacteria are billions of years ahead of us in their harnessing of pattern formation. In this article, we would like to guide the reader through some of the remarkable patterns that have been discovered in one class of bacteria, Paenibacillus. These patterns have been created by mimicking hostile conditions in a laboratory setting by growing the colony in a Petri dish containing a very low level of nutrients and/or a hard surface (high concentration of agar gel) preventing normal bacterial motion. As opposed to merely being objects of aesthetic beauty, they are striking evidence of an ongoing cooperation that enables the bacteria to achieve a proper balance of individuality and sociality as they battle for survival. We will try to convey a feeling for the multiple challenges faced by scientists when attempting to make sense of this incredibly diversity of structures. Our language will be that of nonlinear mathematics, all the while recognizing that models that focus on specific aspects of the observed patterns are necessarily incomplete when compared to the vast richness of the bacterial dynamics.

Compared to pattern formation in non-living systems (Kessler *et al.* 1988; Langer 1989; Ben-Jacob & Garik 1990; Ben-Jacob 1993), bacterial self-organization involves an additional inherent degree of plasticity: the building blocks of the colony are themselves living organisms, each with internal degrees of freedom, internally stored information and internal assessment of external chemical messages (Shapiro 1995; Shapiro & Dworkin 1997; Ben-Jacob *et al.* 1998, 2000*a*; Shapiro 1998; Ben-Jacob 2003). These afford each bacterium plasticity to respond flexibly and even alter itself, by means of modifying its genetic expression patterns. One well studied example is the increase of competence (the ability of a cell to import snippets of DNA) under colonial stress (Macfadyen *et al.* 1998; Bdejov 2003). It would, therefore, be interesting to learn whether competence and genetic transformation are related to colony structure (A. Minsky 2005, personal communication). At the same time, efficient adaptation of the colony to adverse growth conditions requires self-organization on all levels—which can only be achieved via cooperative behaviour of the individual cells. This function is enabled by bacteria communication that makes use of a broad repertoire of biochemical agents. At the same time, each bacterium has equally intricate intracellular communication mechanisms involving, for example, signal transduction networks (Ptashne & Gann 2002; Searls 2002; Hellingwerf 2005). These are used to generate intrinsic adaptive response to the chemical messages (Ben-Jacob 2003). Biochemical messages are also used for the exchange of meaningful information across colonies of different species, and even with other organisms (Kolenbrander *et al.* 2002). These abilities of bacterial communication are relevant in many fields from agriculture (communication with plants) to medicine. For example, bacteria in our gut can evaluate the state of our own body by sensing ('listening') to our hormones (Ben-Jacob 1997, 2000; Cohen 2001; Lyte 2004).

As we are discovering, bacterial communication-based cooperation encompasses colony morphogenesis, which includes coordinated gene expression, regulated cell differentiation and division of tasks. Collectively, bacteria can glean latent information from the environment and from other organisms, process the information, develop common knowledge, and thus learn from past experience (Levine & Ben Jacob 2004). The colony behaves much like a multi-cellular organism, or even a social community with elevated complexity and plasticity that afford better adaptability to whatever growth conditions might be encountered (Levine & Ben Jacob 2004).

2. BRANCHING PATTERNS OF LUBRICATING BACTERIA

To illustrate the ability of bacteria to cope with conflicting environmental constraints, we begin with the branching patterns exhibited by the *Paenibacillus dendritiformis* lubricating bacteria (Ben-Jacob *et al.* 1998). This class of bacteria has developed a sophisticated strategy to move on hard surfaces—they collectively excrete specialized chemicals to form a layer of lubricant (Ben Jacob *et al.* 2000*a*). In detail, they create on top of the agar crust a layer of lubricating fluid with a well defined envelope within which they can swim, even on the hard surface, and thus let the colony expand as we will explain below in greater details. The exact mechanisms and the chemical agents employed by the *P. dendritiformis* bacteria are yet to be discovered. However, based on accumulated knowledge from other bacterial species (Harshey 2003; Matsuyama *et al.* 1993), it is reasonable to expect that two classes of chemical agents are used to perform two distinct functions: (i) extraction of fluid from the substrate (probably by polysaccharides) and (ii) regulation of the surface tension and viscosity of the lubricating layer (probably by surfactants). Microscope observations reveal that as they swim, they push the layer forward, paving their own way.

A dilemma arises when, in addition to the motion difficulty, the available food is not sufficient to sustain a dense population (Matsuyama *et al.* 1992, 1993; Matsushita *et al.* 1998; Kozlovsky *et al.* 1999; Golding

et al. 1999; Harshey 2003; Ron *et al.* 2003; Julkowska *et al.* 2005) When such conditions are mimicked in Petri dishes with hard substrates of low nutrient concentration, new and interesting phenomena are observed, as shown in figure 1. The logic behind this pattern-forming behaviour starts by noting that the engineering task faced by the bacteria is not simple, as the production of the lubricant requires collective action of dense bacterial population, which the food-depleted substrate cannot sustain. The solution comes in the form of a branching structure of the colony—within each branch the bacterial density is sufficiently high, yet the average population density of the colony is sufficiently low to match the availability of food. Based on model simulations (in which the effects of different parameters are tested), we realize that the lubricant properties and its production rate have to be carefully adjusted to generate specific branch structures with specific widths according to fit the substrate hardness and food level (Matsuyama *et al.* 1992, 1993; Golding *et al.* 1999; Kozlovsky *et al.* 1999; Harshey 2003; Ron *et al.* 2003; Julkowska *et al.* 2005). In summary, then, this adaptable self-engineering can be viewed as the solution to a challenging self-consistency mathematical problem between two contradictory constraints—the need for high bacterial density for movement and the lack of sufficient level of food to support high bacterial densities.

3. UTILIZING CHEMOTAXIS FOR NATURAL SELF-ENGINEERING

Once the notion of self-engineering (Nadav Raichman *et al.* 2004) is appreciated, one can study other possible mechanisms whereby the bacteria control the overall colonial pattern. Over the years, it has become clear that, in fact, there are many such possibilities, often relying on some sort of chemical signal that the cells can use to influence each other. One such possibility utilizes the notions of chemotaxis and chemotactic signalling.

Chemotaxis is cell movement in response to gradients in the concentration of a chemical agent (Budrene & Berg 1991, 1995; Berg 1993; Ben-Jacob *et al.* 1995; Blat & Eisenbach 1995; Maki *et al.* 2000; Charon & Goldstein 2002). The movement can be biased either towards higher concentrations (attractive) or away from high concentrations (repulsive). Bacteria are too short to detect chemical gradients, yet swimming bacteria found a smart solution to detect gradients and bias their movement accordingly. For attractive (repulsive) bias, they simply detect the concentration as they swim, and if the concentration increases (decreases) they delay their tumbling. The net result is biased random walk towards the higher (lower) concentration, which can be directly incorporated into a mathematical model as a drift term. The most familiar example of chemotaxis is attraction to an external chemical such as a nutrient. There is evidence that such chemotaxis occurs in these colonies and is responsible for an increased expansion rate and colony 'bushiness' at intermediate values of the nutrient concentration.

Very different patterns form at low nutrient levels (figure 2). To explain the mechanism, we recall that part of the branch-making dynamics relies on the cells going into a non-motile state further back from the colony front, where the nutrient levels is extremely low. In a variety of systems, there has emerged the idea that cells emit a repellent chemical as they are entering this state. For the repellent to accomplish its task, namely to persuade other cells to move away from such locations, the range of this signal must be fairly large. This can be tested by generic modelling, as illustrated in figure 2.

So, the picture that emerges is that the basic branching pattern is sculpted into a variety of forms by the combined action of a variety of chemotactic strategies. These strategies serve to coordinate the actions of otherwise independent cells so as to make maximal use of the resources at their disposal. As these different influences sort themselves out, changing conditions and changing bacterial strains always lead to new structures. Exactly how information from the outside is utilized to help decide which if any of these processes need to be turned on is still an open question. Yet, there are increasing hints that these decisions are made cooperatively, much like what is known to be true regarding the collective decision-making of bacteria to sporulate or the decision to share genetic information (Shapiro 1995, 1998; Levine & Ben Jacob 2004).

4. BREAKING THE REFLECTION SYMMETRY

Bacteria can cooperatively make drastic alterations of their internal genomic state, effectively transforming themselves into practically different cells. For example, the *P. dendritiformis* lubricating bacteria, when grown on poor substrates, can select their identity from the two available distinct cell types—the branching (B) and the chiral (C) morphotypes shown in figure 3 (Ben-Jacob *et al.* 1998, 2000*a*; Ben-Jacob 2003). On harder substrates, when higher bacteria densities 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 reminiscent of the patterns generated by starved *Bacillus subtilis* bacteria (Matsushita *et al.* 1998; Booth 2002). The engineering skill of the *P. dendritiformis* bacteria manifest itself once again during growth on softer substrates, in the formation of curly branches generated by the C morphotype shown in figure 3. This particular geometrical organization allows faster expansion even while continuing to use patches of food left behind as the branches are twisted inward. To bring this about, tan individual bacterium suppresses cell division and thereby elongates. Optical microscope observations during colony development reveal 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 with limited tumbling (Maki *et al.* 2000; Charon & Goldstein 2002), which yields an organized twist of the branches with a specified handedness. It is now understood how the preferred handedness of the twist resulted from the cell-cell interaction together with the inherent flagella handedness. Other bacterial strains such as *Bacillus mycoides*

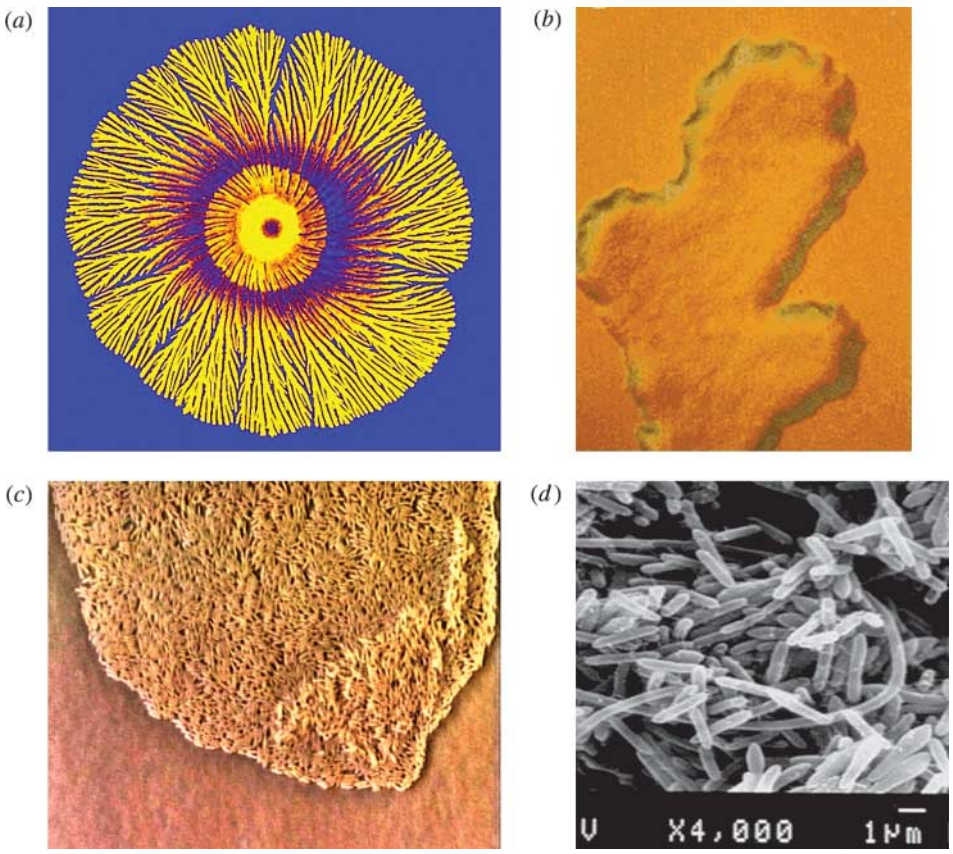

Figure 1. Example of branching colonial patterning: (*a*) typical branched colonial pattern formed by the *P. dendritiformis* bacteria when grown on hard and food depleted substrate. The substrate is prepared from an LB solution with about 2% agar and less than 2 g l^{-1} peptone. Twenty-two cubic centimetre of the substrate solution (in a liquid state) is poured into a standard Petri dish 8.8 cm in diameter. The solution is let cool and dry at 25 °C and 50% humidity for about 60 h until its weight is reduced by 1 g. The colony starts from a droplet (5 μl) inoculation at the centre of the Petri dish. The droplet is taken after 24 h bacterial growth in a shaker to guarantee that the stationary phase has been reached. The dark dot at the centre of the colony is the area of the inoculated droplet that usually contains about 10^4 bacteria. After inoculation, the bacteria go through an 'embryonic' colonial stage for several hours and only then does the colony start to expand outward on the surface (Ben-Jacob *et al.* 1998, 2000; Ben-Jacob 2003). It takes the colony about 2 days to reach the observed size of about 5 cm in diameter shown here. The observed pattern can be associated with quantified measures, as explained in appendix A. (*b*) A closer look at the branches through a polarized optical microscope to show the layer of lubricant collectively produced by the bacteria. (*c*) A snapshot from a video clip taken through an optical microscope with ×500 magnification. (Bacterial images and video clips are available from PhysicaPlus—the online magazine of the Israel Physical Society http://physicaplus.org.il and at Ben Jacob's home page http://star.tau.ac.il/~eshel/.) The video clip observations reveal bacteria swimming—segments of straight motion for about 1–3 s at a speed of about 1 μm s^{-1}, interrupted by bacterial tumbling terminating in a random new direction. That is why the bacterial movement is usually modelled as random walk. (*d*) A scanning electron microscope picture. Note the variability in the individual bacteria. It is now understood that phenotypic diversity is not arbitrary but collectively regulated to afford the colony elevated group flexibility (Booth 2002).

can exhibit similar chiral patterning (Di Franco *et al.* 2002).

The two possible morphotypes are inheritable; colonies of the B and C morphotype can develop equally well for some intermediate range of growth conditions. Yet, when starting with inoculation of B morphotypes bacteria on soft substrate, an intriguing phenomenon of spontaneous transitions is observed—first the bacteria develop a branching (B morphotype colony) and consequently after about a day or two many of the grown colonies exhibit B→C transitions (see figure 4*a*). The reverse C→B morphotype transitions are observed during growth on harder substrate. In both cases, the newly selected pattern is the one that maximizes the rate of colony expansion, hinting that the colonial morphotype manipulation is for better adaptability (Ben-Jacob *et al.* 1998). Based on model simulations, it was argued that the morphotype transitions are not likely to be simple single cell spontaneous phenotypic transitions (Ben-Jacob 2003). In particular, it has been suggested that the transitions may be autocatalytic and/or synchronized. By autocatalytic, we mean that the C morphotype cells emit signalling molecules that can trigger a B→C transitions in other cells. By synchronized, we mean that before transition, the B morphotype cells emit signalling molecules that can trigger the transition. The idea that transitions can be collectively determined is based on similar notions for transitions to sporulation or competence.

There seems to be an ongoing chemical messaging system that helps the cells collectively decide between the C and B patterns. For instance, we show in figure 4*b* a morphotype transition initiated in response to a

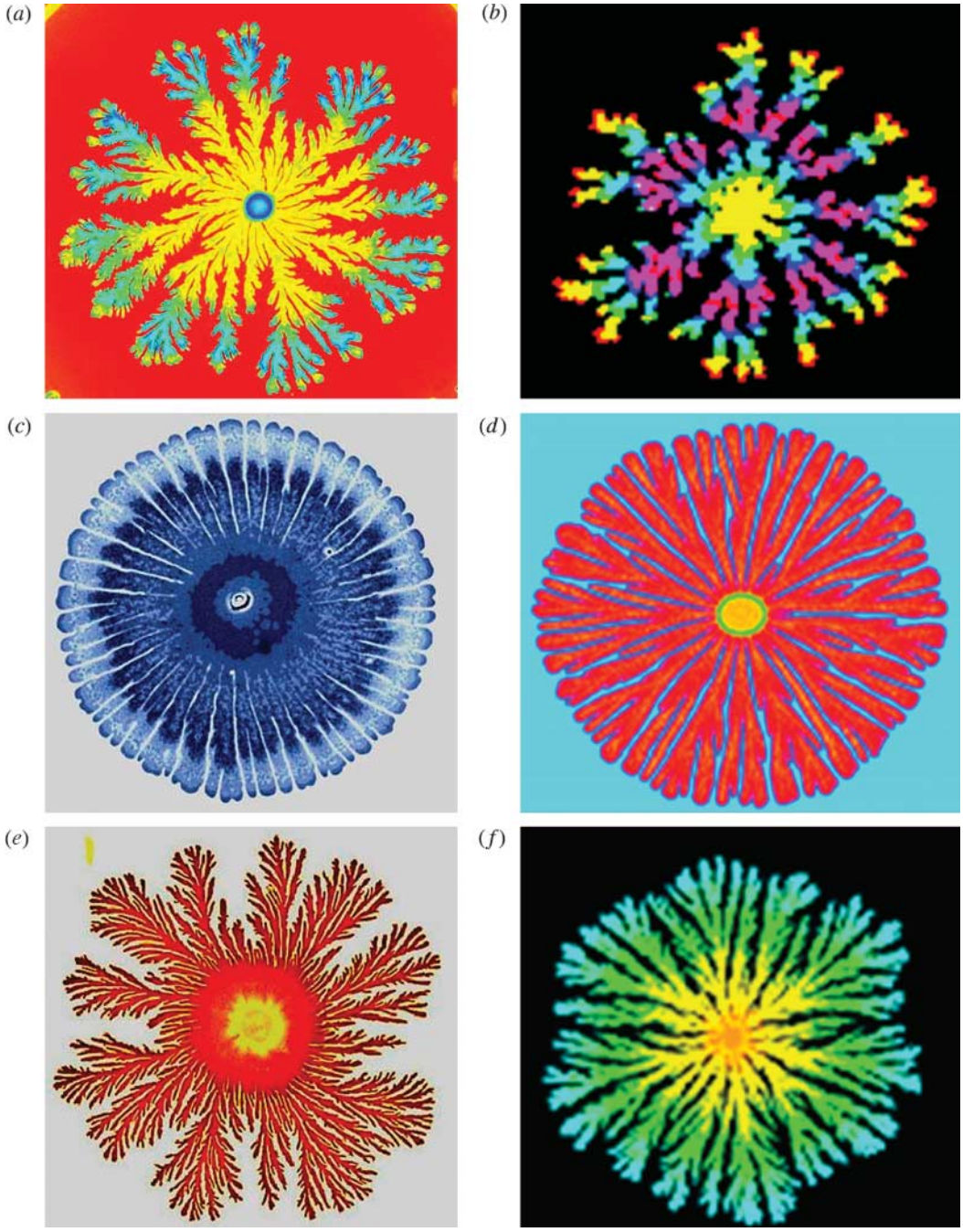

Figure 2. Examples of different branching patters formed during colonial developments of the *P. dedritiformis* bacteria. To self-engineer their colonial structure these bacteria regulate the balance between attractive and repulsive chemotactic signalling and food chemotaxis as well as their food chemotaxis. Generic modelling using interacting agents (see appendix A), of the growth can be used to test this idea by comparing the observed patterns shown on the left (*a*,*c* and *e*), with the results of model simulations shown on the right (*b*,*d* and *f*). The colours in the observed colonies are added according to the bacterial density. In the simulations (*b*,*f*) the different colours indicate different stages (times) of the growth. Top shows the typical pattern when food chemotaxis dominates the growth at intermediate levels of food depletion. Middle is the typical pattern at higher food levels when attractive chemotactic signalling is activated, and bottom for very low level when repulsive chemotactic signalling is intensified. Comparing the patterns one should keep in mind that the real colonies have almost million times more bacteria than (and presumably additional mechanisms as compared to) agents in the model.

geometrical stress encountered due to the presence of fungi. In this case, the colony adopts the chiral morphotype to give itself better structural (morphological) flexibility—the C morphotype can perform fine-scale structures that allow the colony to wrap around the fungus.

During morphotype transitions, the newly formed C bacteria (e.g. in the case of B→C transition), have to find their fellows within the large crowd of B cells and burst out as a group with a new identity. This process involves a 'dialogue' between C's within the majority of B's, as can be deduced from the picture shown in figure 4*c*. This growth starts from a mixed culture with a majority of C's, namely, an artificial context which 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's.

5. COOPERATIVE HIERARCHICAL ORGANIZATION

Some bacterial strains organize their colonies by generating modules, each consisting of many bacteria, which are used as building blocks for the colony as a whole. This behaviour is observed, for example, in the lubricating bacteria *Paenibacillus vortex* (Ben-Jacob *et al.* 1998, 2000*a*; Ben-Jacob 2003) that produce the bacterial vortices shown in figure 5, and in other strains like *Bacillus circulans* (Komoto *et al.* 2003) and *Paenibacillus alvi* (Cohen *et al.* 2000). Model simulations suggest (Cohen *et al.* 1996; Czirok *et al.* 1996) that a combination of short-range attractive and long-rage repulsive chemotactic signalling mechanisms can lead to the formation of the observed patterns, as is illustrated in figure 6.

This idea of hierarchical organization brings us to a key conceptual question regarding the role of mathematical models in describing these patterns. For some aspects of what we have been studying, models that treat the bacteria as relatively simple interacting particles suffice. As we ask more complex questions, however, more and more detailed levels of possibly relevant degrees of freedom can reveal themselves. For example, genetic degrees of freedom (i.e. the dynamics which determine which genes are expressed) are irrelevant if one merely wishes to explain branching but appear to be crucial if we want to understand observed transitions between different colony patterns. The vortex itself can be studied using simple notions (Levine *et al.* 2001), but the way in which the complex exchange of information takes place between the colony as a whole and the vortex substructures requires much more. If we take into account the incredible complexity present at all scales (from molecular up to ecological community) in the bacterial system, it is fair to say that there will always be phenomena at the macroscopic scale that are not captured by any sort of tractable model. An example of such phenomenon is presented in §6.

Of course, it is also true in pure physics that there are always additional levels of substructure; there however the macroscopic scale phenomena are shielded from these details and there is not much that we can learn about, say, superstrings, which could conceivably affect our view of, say, diffusively driven crystal growth (Ball 1999). However, as we have learned from the bacteria, this simple separation of scales does not seem to hold for living systems. Complete understanding of the colonial level patterning and in particular morphotype transitions is strongly coupled to the intracellular and gene-network dynamics and, new discoveries are being made all the time in this rapidly expanding research area. To pick one example, new discoveries about the role of micro-RNA in embryonic development and cell differentiation in multi-cellular eukaryotes (Bartel 2004) hint that small RNA might play crucial role in the morphotype transitions and in collective behaviour of bacteria in general (Lenz 2004).

6. GENERATION OF VORTICES SELF-IDENTITY

Maintaining the integrity of the individual vortices (the dotes in the colony level pictures in figure 5), while they serve as a higher-order building blocks of the colony requires advanced communication: each cell in a specific 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 that it can adjust its activities accordingly. This ongoing communication is particularly apparent when it comes to the birth of new vortices. New vortices emerge in the trail behind a vortex, following initiation signals that cause the bacteria there to produce more lubricating fluid. These cells then begin to move quite rapidly as a turbulent 'biofluid', until an eddy forms and becomes a new vortex. The entire process appears to be under continuous communication-based cooperative control: a vortex grows and moves, producing a trail of bacteria and being 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, that leaves home (the trail) as a new entity toward the colonization of new territory.

To illustrate the above notions, we show in figure 7, the patterns exhibited by bacteria inoculated from the centre of the colony vs. those created by bacteria taken from the leading vortices. Interestingly, even after several generations of growth the colonies started from inoculums from the centre and the leading vortices are different. These observations suggest that there must exist processes connected with the formation of bacterial collective self-identities (Ben Jacob & Shapira 2005). This phenomenology goes well-beyond what can be discussed using current modelling ideas.

7. ANTIBIOTIC DRIVEN SELF-ENGINEERED ORGANIZATION

In medicine, resistance to antibiotics is usually considered a binary (Yes/No) property: either a specific strain of bacteria is resistant to a specific antibiotic or it is not. The bacteria are, generally, regarded as being 'resistant' if they can tolerate the maximal concentration of the antibiotic which is non-toxic to the treated humans or animals. However, it is known that bacterial colonies are often more resistant than the individual cells, thereby blurring the borderline between resistance and sensitivity (Novick & Wiener 1957; Balaban *et al.* 2004; Stewart *et al.* 2005; and references there in). Moreover, antibiotics affect cells in different manners depending on the growth and the physiological state of the cells. New results show that susceptibility to antibiotic should be regarded as a quantitative property: the bacteria react to non-lethal levels of antibiotic even in concentrations well

below the critical (lethal) level (Ben Jacob *et al.* 2000*b*; Golding & Ben Jacob 2001). And in some cases, low levels can even enhance the colony development.

Although, the available arsenal of antibiotics contains a variety of functionally and structurally different compounds, resistance mechanisms have been developed for almost all of them. Resistance may involve decreased entry of the drug, changes in the receptor (target), metabolic inactivation or modification of the drug, or production of 'dummy' targets to adhere to the compound and lower its bioavailability. Resistance can be a cooperative process. The most compelling example is metabolic inactivation of an antibiotic occurring outside the cells: a significant reduction in the drug concentration can occur only with high concentration of the inactivating agent, which requires a dense population of bacteria.

The idea of 'higher complexity for better adaptability' is reflected by the bacteria's ability to engineer the colony shape to better adapt to the antibiotic stress, i.e. by adjusting a specific pattern to each kind of antibiotic (Ben-Jacob 2003). An example of bacterial self-engineered organization under antibiotic stress is shown in figure 8, where we demonstrate how the *P. vortex* bacteria utilize their cooperative, complexity-based plasticity to alter the colony structure in order to cope with the stress.

We study colonial patterning in responses to non-lethal stress of two different kinds of antibiotics: Septrin, a suppressor of cell growth and reproduction (it inhibits the metabolic pathway of tetrahydrofolic acid, a cofactor needed for the synthesis of DNA and RNA) and Ampicillin, a distorter of cell wall structure that might impair normal cell communication. When exposed to Septrin, the bacteria generate larger vortices (probably due to stronger attraction) that move faster away from the antibiotic stress (probably due to stronger repulsion by those left behind). These interpretations are based on comparison with model simulations. However, while they are interesting plausible explanations it should be kept in mind that there are other possible explanations. For example, starving bacteria risk running short of energy while Septrin-treated bacteria do not (one might even wonder if they have more ATP at their disposal) and taken together with a possible shift in the size distribution (fewer dividing cells), they may actually be more motile. This would lead to a colony with less mass and greater motility that would not have to change its signalling behaviour to produce a different morphology (A. Minsky 2005, personal communication).

The response to Ampicillin is puzzling: the patterns look as if the short-range communication required to form vortices is not affected, but the long-range communication required for coordinated movements of the vortices as units is tampered; yet, for some low levels of Ampicillin, the colonies can expand even faster than without the antibiotic. In short, currently we do not understand the colony level response to Ampicillin.

8. BACTERIAL COLLECTIVE MEMORY AND LEARNING FROM EXPERIENCE

We proposed that bacteria seem to be able to store information about 'past experience' (Ben Jacob *et al.* 2004). In figure 9, we show that bacteria exposed to the same antibiotic stress a second time can cope better with the stress. This effect can be erased (depending on the growth conditions and duration of exposure) by exposure to neutral conditions (i.e. growth on plates in the absence of antibiotic or in LB media) between the two encounters. Therefore, it seems that the bacteria can generate erasable, collective memory, as if to learn from their past experience. One possibility is that this effect is caused by a genotypic switch in the population, namely that the antibiotics select for some pre-existing (otherwise neutral) genetic variation. Another possibility involves heritable epigenetic states in the gene-network of the cells. Irrespective of the exact mechanism, it is clear that the bacteria can retain memory of the conditions and that this acquired memory is expressed in the colonial pattern in consequent colonial developments.

We should mention in this context the revolution that is taking place in our overall understanding of the power of genomic degrees of freedom. 'Natural genetic engineering' (Shapiro 1992), or 'genome cybernetics' (Ben-Jacob 1998), refers to the ability of the genome to perform information processing and alter itself accordingly (Wesson 1993). Genome cybernetics upon replication has been illustrated in ciliates (Kari & Landweber 2003), and more recent work shows that transposable elements can effectively re-program the genome between replications (Makalowski 2003). Small RNA molecules (Bartel 2004; Lenz 2004) have been shown to play a role in bacterial quorum sensing and might provide new intracellular and gene-network mechanisms needed to support some of these exotic processes. As our study of colonial self-organization continues, we expect to find cases where these extraordinary capabilities of the genome become dynamically coupled to the pattern self-organization; perhaps we have already seen some hints of this in the dialogues between cells that appear to underlie the more complex patterns found to date.

A new picture is thus emerging, one in which adaptable self-engineering can be viewed as the bacteria solution to a challenging self-consistency mathematical problem at the forefront of optimization and control in nonlinear dynamics. So it is reasonable to conclude that collectively, bacteria can glean information from the environment and from other organisms and interpret the information in an existential 'meaningful' way, i.e. by building an appropriate colony structure. It is perhaps even not so far-fetched to imagine that the bacteria can develop common knowledge and learn from past experience. The findings presented here about bacteria 'shaped to survive' as response to antibiotic stress appear to be pushing us in this direction.

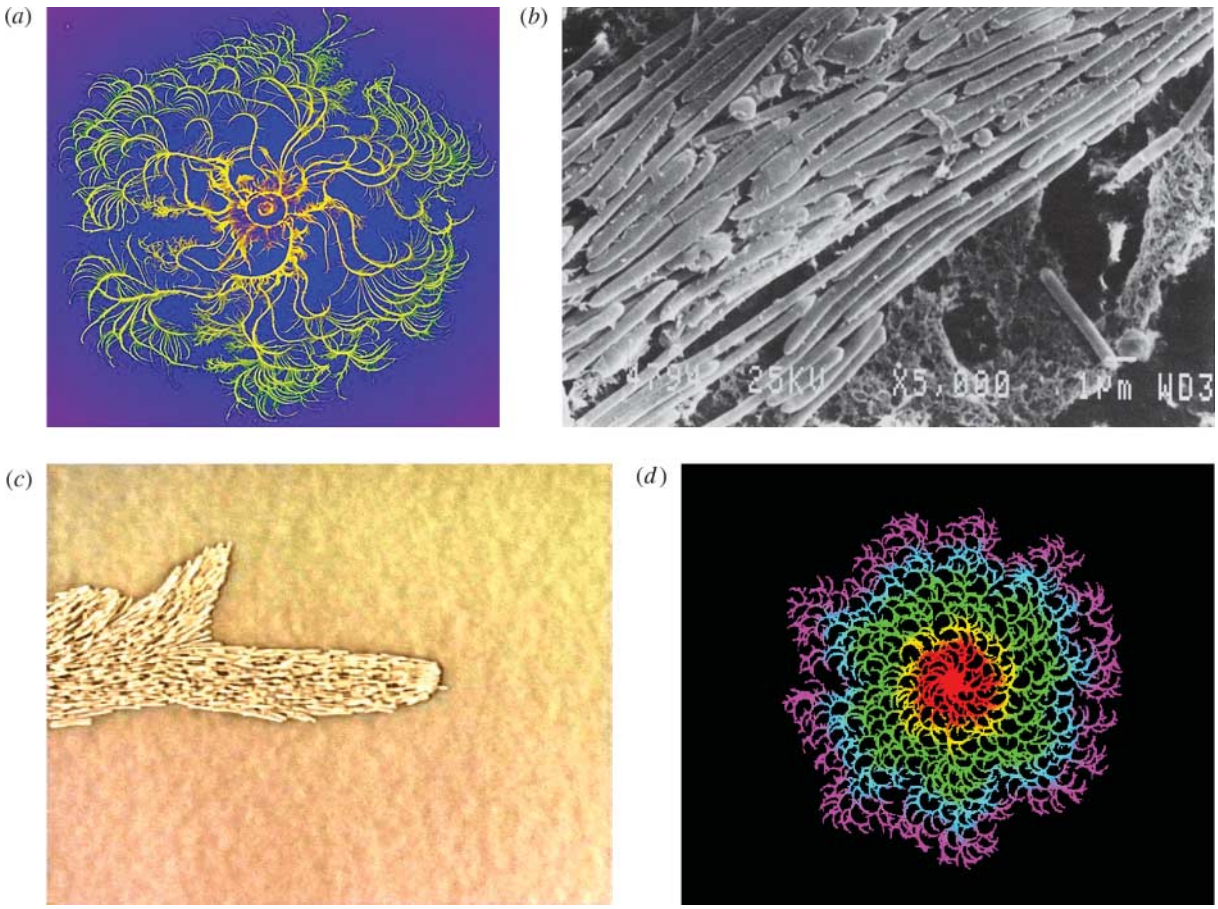

Figure 3. The chiral morphotype. Colony structure exhibiting the formation of curly branches is shown in (*a*). To bring this about, the bacteria suppress cell division and become elongated as is observed via scanning electron microscope (*b*). Upon elongation, the cells alter their collective movement 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 (*c*). (*d*) An example of model simulations using the walking spinors model.

9. CONCLUDING REMARKS AND LOOKING AHEAD

In this article, we presented some of the remarkable patterns formed during colonial self-organization when the bacteria are exposed to various growth conditions, mimicking the ones they are likely to encounter in Nature. Endless simple yet ingenious methods were invented by the bacteria for developing the incredible diversity of observed complex patterns. They illustrate the bacterial schemata of 'Communication-Based Cooperation' for 'Complexity-Based Adaptability' which enable them to thrive and self-improve in a changing environment (Ben-Jacob 2003; Levine & Ben Jacob 2004).

We have shown how complex colonial forms (patterns), emerge through the communication-based singular interplay between individual bacteria (the micro-level) and the colony (the macro-level). Each bacterium is, by itself, a biotic autonomous system with its own internal cellular gel that possesses informatics capabilities (storage, processing and interpretation of information) (Hellingwerf 2005). These afford the cell certain freedom to select its response to biochemical messages it receives, including self-alteration and broadcasting messages to initiate alterations in other bacteria. Such self-plasticity and decision-making capabilities elevate the level of bacterial cooperation during colonial self-organization.

As the individuals in a growing colony begin to respond to the colony itself (i.e. information flow from the colony to the individual), these individuals response by regulating their movements, growth rates, various tasks they perform, the chemical signals they send to other bacteria and even their gene-network state (phenotypic state) according to the received signals. By doing so, the individual cells collectively alter the colony so as to increase its durability and adaptability.

The essential new lesson learned from bacteria is that colonial high complexity provides the degree of plasticity and flexibility required for better durability and adaptability of the whole colony to a dynamic environment. According to this picture, new features collectively emerge during biotic self-organization on every level, from the membranes and cytoplasm to the whole colony. The cells thus assume newly co-generated traits and abilities that are not explicitly stored in the genetic information of the individuals. For example, bacteria cannot genetically store all the information required for creating the colonial patterns. In the new picture, they do not need to, since the required information is cooperatively generated as self-organization proceeds by bacterial communication, informatics and self-plasticity capabilities. Thus, the

Figure 4. Morphotype transitions. (*a*) A spontaneous B→C morphotype transition. (*b*) An example of a stress induced morphotype transition when a colony of branching morphotype bacteria encounters fungi (the bright spot). (*c*) Sorting out the pattern by means of a B–C dialogue, as discussed in the text.

bacteria need only have genetically stored the guidelines for producing these capabilities and using them to generate new information as required.

We have illustrated these remarkable capabilities by exposing the bacteria to non-lethal levels of antibiotics. Recent findings even indicate that the bacteria purposefully modify their colonial organization in the presence of antibiotics in ways which optimize bacterial survival, and that the bacteria have a special collective memory which enables them to keep track of how they handled their previous encounters with antibiotic—learning from experience (Ben Jacob *et al.* 2004; Ben Jacob & Shapira 2005). This statement requires additional clarification. In the examples presented here, we showed that in second encountered with the same antibiotic stress the emerged colonial patterns expand faster. We also proposed (albeit without using here a quantified measure of complexity), that the colonial patterns are more complex (relative to the patterns generated when the bacteria are first encountered the antibiotic. It seems like a cyclic argument—higher complexity has an advantage and there is learning since in second encounter the patterns have higher complexity.

We wish to close by making note of one additional piece of evidence, arising from the phenomenon of bursts of sectors during colonial developments (Golding *et al.* 1999; Ben-Jacob 2003; Julkowska *et al.* 2005). This is a well documented but rarely studied phenomenon of bursts of sectors of mutations that overgrow (expand faster) the original colony (Shapiro 1995). Model simulations (Golding *et al.* 1999; Julkowska *et al.* 2005), suggest that segregation of mutants into distinct sectors as is shown in figure 10 is observed when the mutants have different growth characteristics, e.g. reproduction rates, motility, response to chemotaxis signalling, etc. Note that the faster expanding sectors have lower complexity in comparison with the wild type (original) colony. To compare the adaptability of the mutants and the wild type, we inoculate bacteria from the sectors and the original colony on a variety of growth conditions. We found that for most of the conditions, the wild type colonies expand faster, which indicates that they have higher adaptability. In other

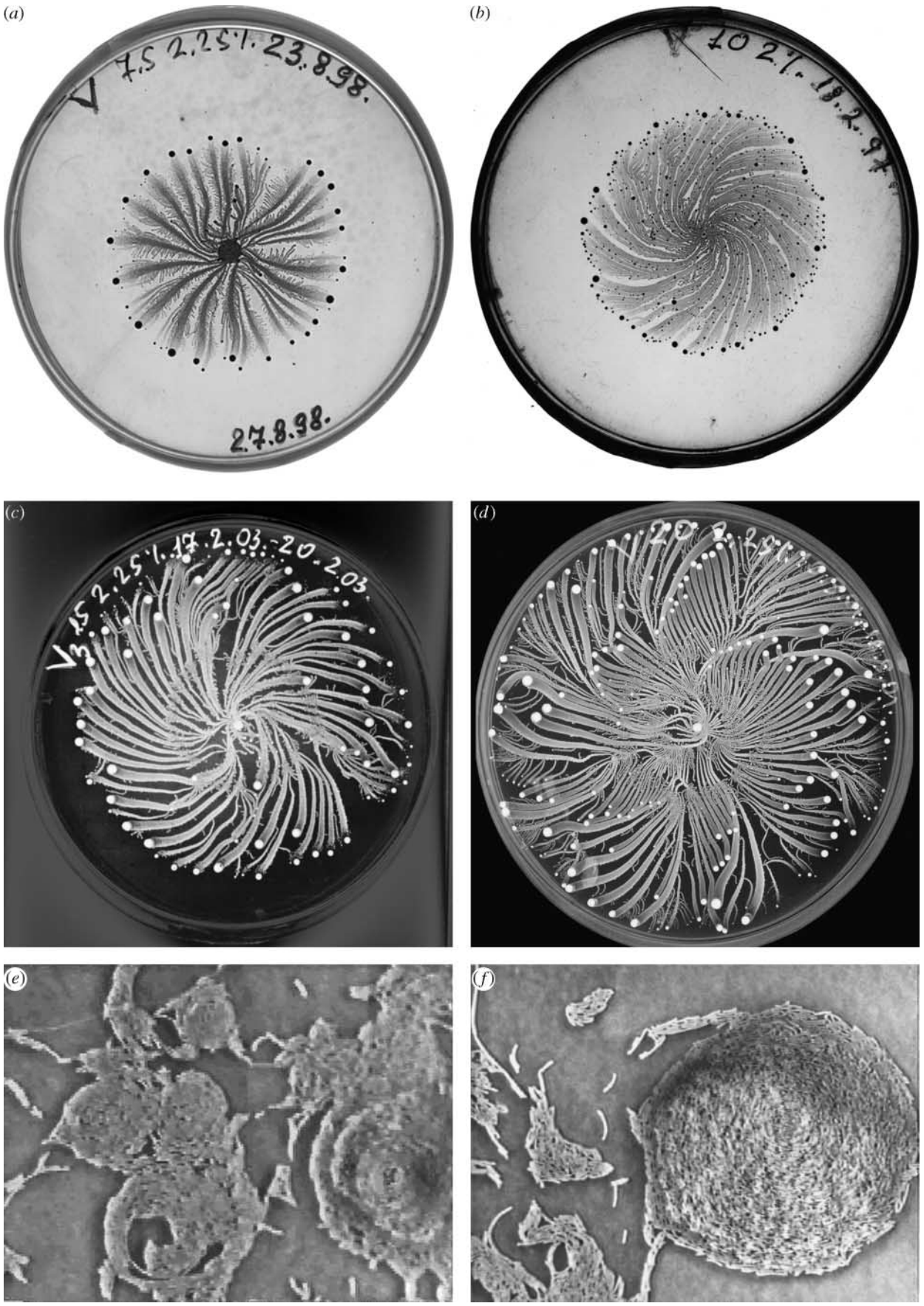

Figure 5. (*Caption opposite.*)

Figure 5. (*Opposite.*) Hierarchical colonial organization. Colonial patterns generated by the *Paenibacillus vortex* bacteria when exposed to different growth conditions. The pictures (*a–d*) show a whole colony view. The Petri dishes are 8.8 cm in diameter. The bacterial population of these colonies is larger than that of people on earth, yet they coordinate their behaviour. The peptone (food) level is 7.5 g l^{-1} (*a*), 10 g l^{-1} (*b*), 15 g l^{-1} (*c*) and 20 g l^{-1} (*d*). Each vortex (the condensed group of bacteria) is composed of many cells that swarm collectively around their common centre at about 10 μm s^{-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 the vortex rotation. The dynamics of the vortices is quite complicated and includes attraction, repulsion, merging and splitting of vortices. Yet, from this complex, seemingly chaotic movement, a colony with complex but non-arbitrary organization develops, as seen in pictures (*a–d*). Pictures (*e*) and (*f*) are snapshots from a video recording (Bacterial images and video clips are available from PhysicaPlus—the online magazine of the Israel Physical Society http://physicaplus.org.il and at Ben Jacob's home page http://star.tau.ac.il/~eshel/), taken during formation of new vortices (magnification ×500, so the pictures are about 100 μm wide; the bars are the individual bacteria).

words, the mutants are better fitted to the specific growth conditions in which they have emerged but lower adaptability. It has been proposed that their lower adaptability might go hand in hand with the lower geometrical complexity (Ben-Jacob 2003).

We note that the example of bursts of sectors also provides additional support to the assumption that the colonial 'learning' does not simply reflects a genetic shift or selection of random mutations. Had it been a mutation that can generate a colony better adapted to the antibiotic we would expect to observe the emergence of a sector when the bacteria are first grown in the presence of the antibiotic stress.

To conclude, we now begin to realize the power of bacterial cooperation and self-engineering, which allow them to store past information when solving newly encountered problems. The new findings bear the promise to provide satisfactory explanations to bacterial threat for our health: that an increasing number of bacterial strains of disease-causing bacteria can today resist multiple antibiotic drugs. Bacteria are clearly capable of developing antibiotics resistance at a higher rate than scientists develop new drugs, and we seem to be losing a crucial battle for our health. To reverse this course of events, we must outsmart the bacteria by taking new avenues of study which will lead to the development of novel fighting strategies. One such promising direction is to perform gene-expression studies during colonial development, when bacteria are exposed to antibiotic stress and most challenging during bacterial learning from experience (Ben Jacob & Shapira 2005).

We expect that such and other future experiments will soon lead us to reverse the current notion of bacteria as mere solitary and simple creatures with limited capabilities, and recognize that bacteria are cooperative beasts that lead complex communal lives with rapidly evolving self-engineering skills (Ben-Jacob 2003; Ben Jacob *et al.* 2004; Ben Jacob & Shapira 2005). We might even discover that the last five decade's evolution in bacterial resistance to antibiotic is largely a result of their encounter with our socially irrational massive use of antibiotic materials in agriculture and human intakes.

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APPENDIX A. THE ART OF MODELLING

In physics and chemistry, models have long served as an indispensable research tool. But in the life sciences, the predictive power of models is still largely questioned.

One can easily fall into the 'reminiscence trap'—the tendency to devise a set of rules that will just mimic aspects of observed phenomena. The challenge being how to elicit the generic features and basic principles needed to explain behaviour from experimental observations and biological knowledge.

With present computational power, it is only natural to use computer models to study bacterial cooperation in attempt to reveal their secret strategies. In physics and chemistry, computer models have long served as an indispensable research tool. But in the life sciences many still question the value and predictive power of model simulations. The research strategy presented here assumes that models can provide a crucial research tool in the study of bacterial pattern formation. The usefulness and crucial role of modelling in the study of bacterial complex organization is illustrated in the communicating walker model presented below. We try to demonstrate that generic modelling allows in a natural way for both the physics (represented by chemical fields such as the nutrient concentration, any chemical signals, the lubricating fluid flow) and the biology (the response of individual cells to their sensed environment) to be represented in a computationally tractable format. Yet, models should never be viewed as precise analogues of the actual system, but instead as tools for unravelling cause and effect and for helping in the quest for as yet unknown biological phenomena at the single bacterium scale.

Models can also help with placing the phenomena of bacterial self-organization within the context of the

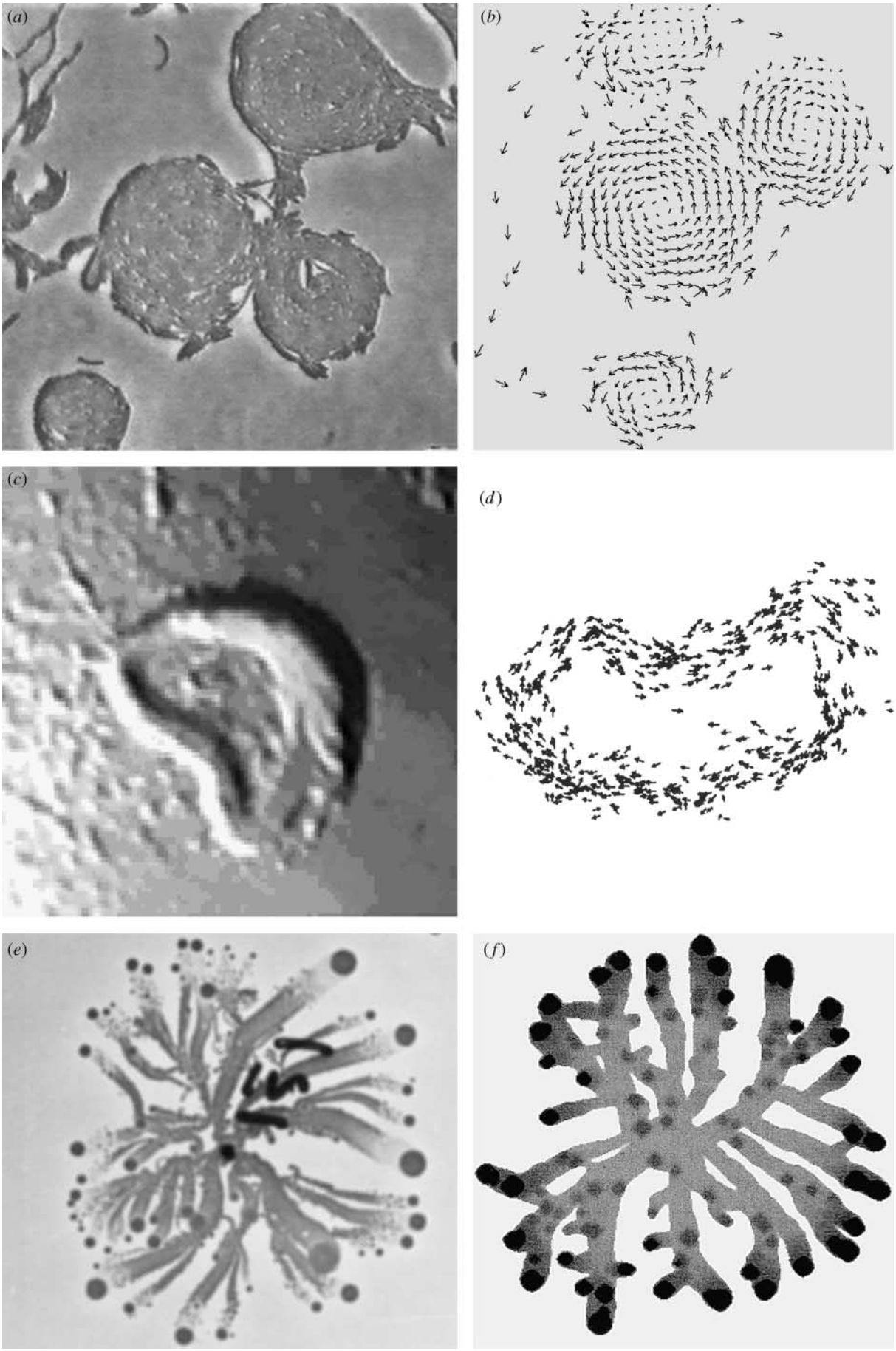

Figure 6. (*Caption opposite.*)

Figure 6. (*Opposite.*) Generic modelling of generation and organization of vortices. In this figure, we show comparison of the observed patterns on the left (*a*,*c* and *e*), with numerical simulations (Ben-Jacob 2003; Cohen *et al.* 1996; Czirok *et al.* 1996), on the right (*b*,*d* and *f*), to test the idea that vortices can be generated by gliding and swarming bacteria when attractive forces (e.g. by attractive chemotaxis) is in operation. Such forces keep the bacteria together (like ice-skating people who hold hands together) thus forcing them to perform a 'Merry go around' like motion. To be pushed out the bacteria inside the colony can emit repulsive chemotactic signalling. Results of numerical simulations of the self-generation of vortices via attractive chemotactic signalling are shown on top (*b*) and the effect of repulsive chemotactic signalling in which the vortices are 'pushed out' by repulsive chemotactic signalling is shown in (*f*). (*c*) and (*d*) a 'bagel' dynamics in which the bacteria form a close movement with an open centre. The arrows in (*d*) indicate the velocity of the bacterial moments in the model simulations.

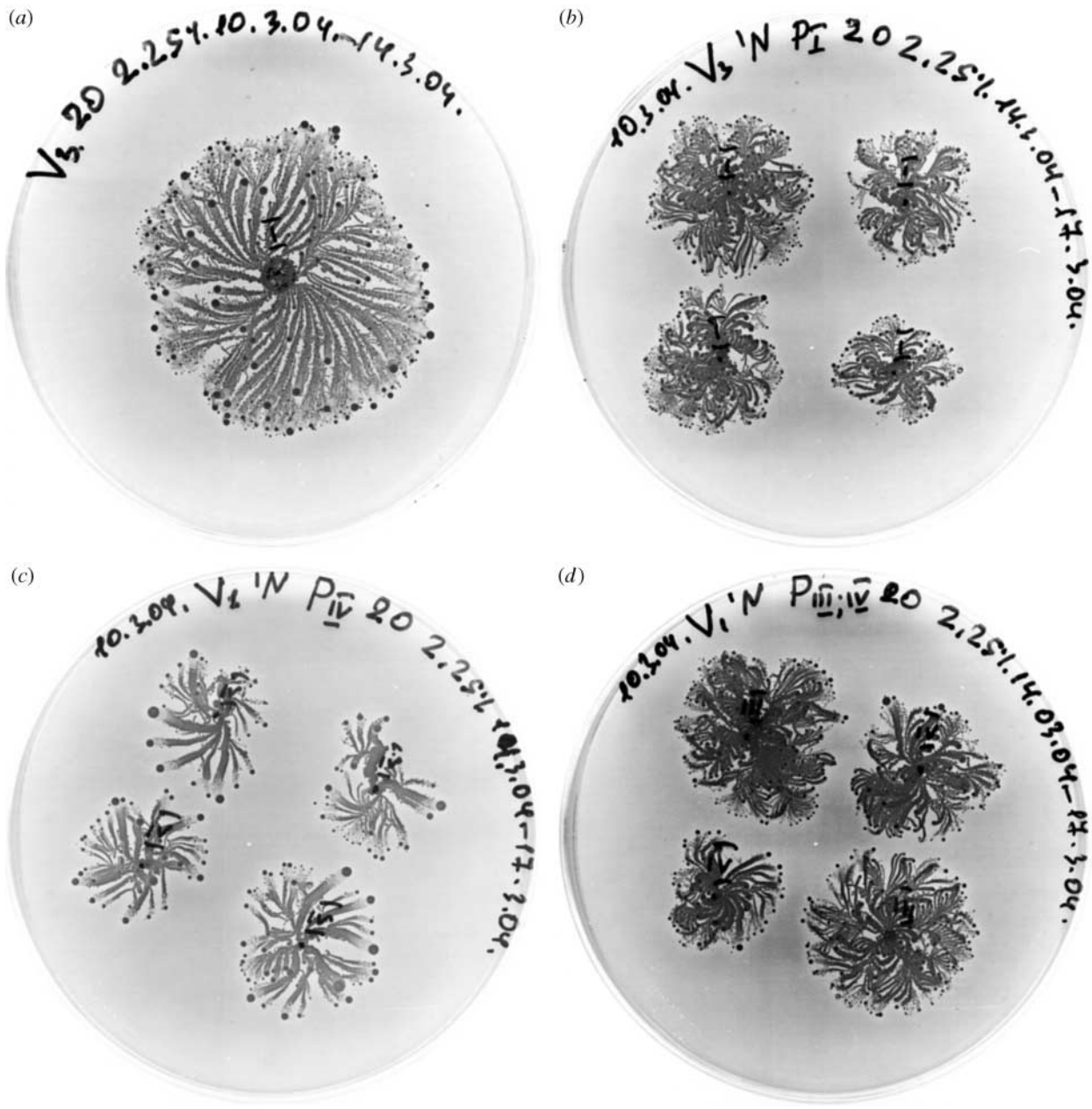

Figure 7. Vortices inheritable self-identity. (*a*) A picture of the 'mother' colony used as a source of bacteria for the consequent growth experiments. The pictures on the right (*b*) and (*d*), show two plates with four colonies each that started from bacteria taken (by picking) from the centre of the mother colony (*a*). These colonies have distinctively different patterns in comparison with those shown in (*c*). In this case, the colonies started from bacteria taken (by picking) from large vortices at the front of the mother colony.

proper mathematics of pattern-formation schema in non-living systems. However, doing so deserves special care. One must keep in mind the profound differences due to the functionality requirements standing behind the patterns generated by living systems for their survival and their ability to evolve and learn from experience. We thus propose to harness the new mathematical understandings of pattern formation in

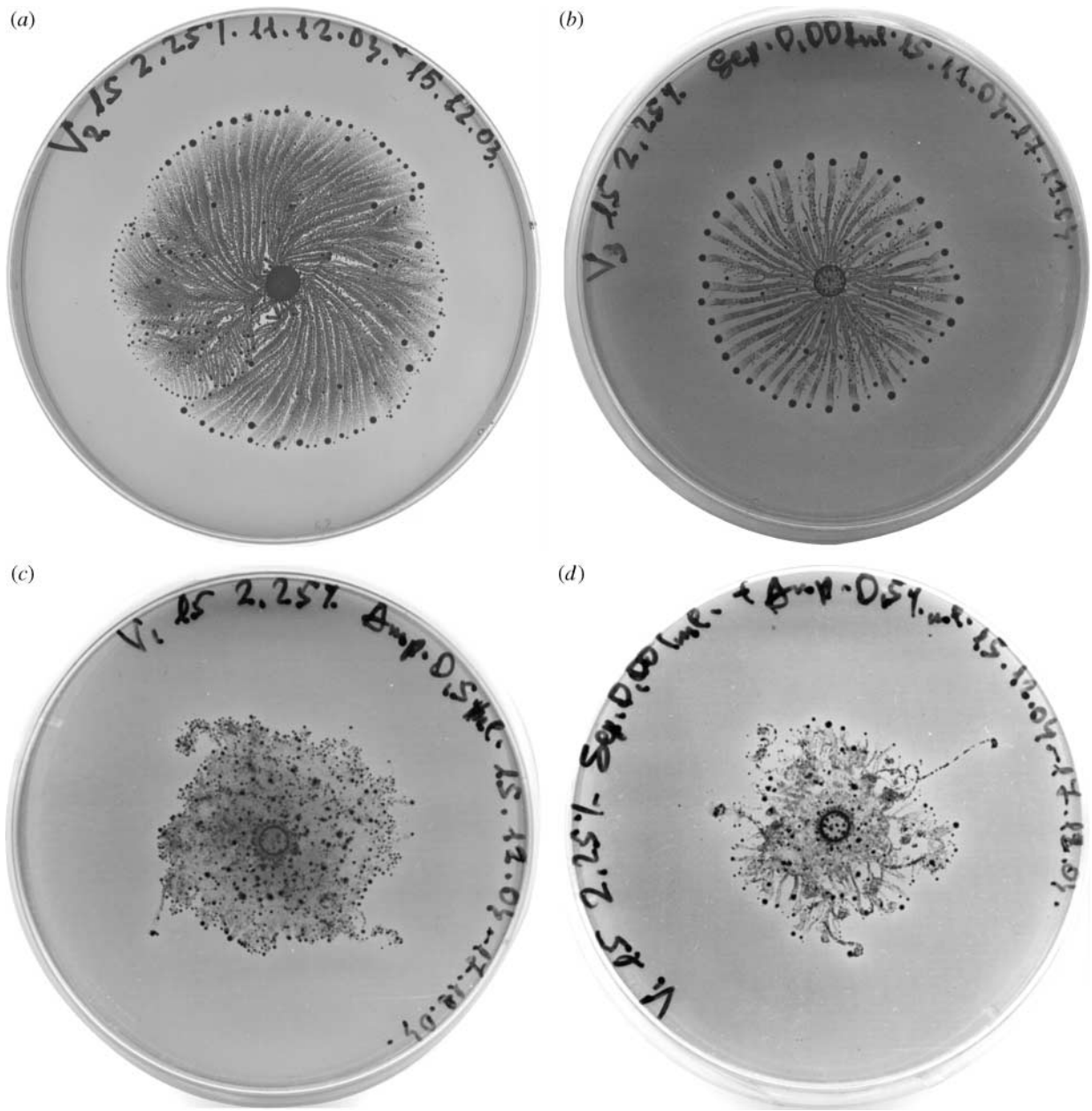

Figure 8. Higher complexity for better adaptability II. To further test this principle, we grow colonies of the *Paenibacillus vortex* bacteria in the presence of additional kinds of antibiotic. Growth conditions are 15 g l^{-1} peptone and 2.25% agar. (*a*) A typical colonial development for this specific strain for the specific growth conditions. The growth started at the same day from bacteria taken from the same test tube at the end of 24 h growth in LB media. (*b*) The response to Septrin (co-trimoxazole), which inhibits synthesis of folic acid and thus suppresses cell reproduction. Namely, the metabolic stress caused by Septrin is somewhat similar to that of food depletion. And indeed, there is clear similarity between this pattern and the one generated for growth in the absence of antibiotic stress but half the level of food (figure 5*a*). Yet, based on model simulations and microscope observations, it seems that in response to Septrin the bacteria enhance their cooperation by intensifying chemotactic attraction to form larger vortices. This clever strategy protects the bacteria, since the antibiotic is diluted in larger vortices by the lubricating fluid extracted by the bacteria. This is true provided that the larger vortices also move faster away from the antibiotic stress; and indeed, the bacteria also elevate repulsive chemotactic response to signals emitted by the bacteria behind the vortices, which helps drive the large vortices away faster. The pattern in the presence of Septrin (middle left) evolved in half the time of the pattern under food depletion (top right). This difference further supports the idea of enhanced cooperation in the presence of Septrin. (*c*) A disorganized colonial development in response to Ampicillin, which distorts cell wall structure and thus probably impairs the communication-based coordination. Surprisingly, when the bacteria are exposed to multiple stresses—Septrin and Ampicillin (*d*)—they can still survive in a way which is not understood. However, we found that we can outsmart them by first exposing them to one kind of antibiotic for the duration it takes them to adapt, and only then expose them to the other kind of antibiotic.

non-living systems for the study of patterning in the living world while avoiding the mathematization' of life.

One can easily fall into the 'reminiscence trap'—the tendency to devise a set of rules that will just mimic aspects of observed phenomena. But the desire to avoid this naturally pushes one in the direction of including more biological details. Model building, indeed, is 'an art in its own right' and the skills can only be acquired

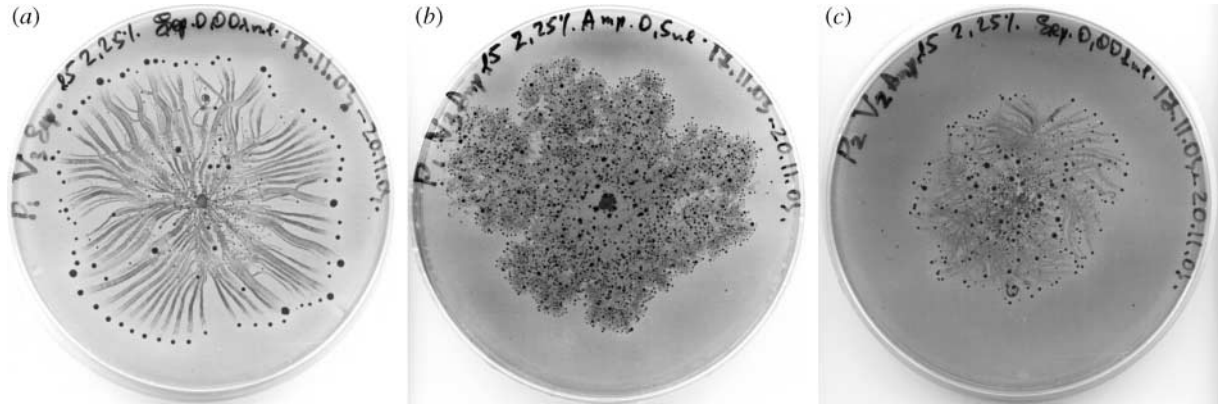

Figure 9. Collective memory and learning from experience. The pictures show colonial development in the presence of antibiotic started from bacteria taken (by picking), from colonies that were grown in the presence of the same antibiotic. In (*a*), we show colonial development in the presence of Septrin of bacteria taken from the colony shown in figure 8*b*. In (*b*), we show colonial development in the presence of Ampicillin of bacteria taken from the colony shown in figure 8*c* and in (*c*) we show colonial development in the presence of Ampicillin of bacteria taken from the colony shown in figure 8*b*. Note that the colonial development seems to be more enhanced. A very different pattern from both (*a*) and (*b*) is developed when bacteria grown in the presence of Ampicillin are exposed to Septrin as is shown in (*c*).

Figure 10. Bursts of sectors in colonies of *P. dendritiformis* bacteria. The darker shaded part of the colony is a sector formed by 'mutant' (we emphasize that the bacteria in the sector were not compared genetically with the wild type but their different phenotypic characteristic have been shown to be inheritable). Growth conditions are $1\,\mathrm{g\,l^{-1}}$ peptone and 1.75% agar (Golding *et al.* 1999; Julkowska *et al.* 2005).

by practice. The challenge is how to elicit the generic features and basic schemata needed to explain bacterial cooperative behaviour from experimental observations, general biological knowledge, the physics of pattern formation in non-living open systems and the mathematics of nonlinear dynamics (Levine & Ben Jacob 2004).

To illustrate the above we proceed to describe the communicating walker's model devised to study branching colonial patterning. This is a special hybrid model: on one hand the diffusion of nutrients are described by the continuum modelling approach or reaction–diffusion equations. And on the other hand, the bacterial cells are described by discrete walking and communicating cellular automatons in the spirit of the molecular dynamics modelling approach.

Even with today most advanced computers it is beyond computer power to handle 10th of billions of automatons which is the number of bacterial population. Therefore, in the model each walker is taken to represent about 100 000 cells.

We start the model by drawing an imaginary mesh of hexagonal boxes as is shown in the figure. At the beginning of the simulations, all the boxes have the same level of food C_o which set according to the initial concentration of food in the experiment.

As the simulations proceed the amount of food in each box is depleted as it is consumed by walkers in the box. In addition, food diffuses between the boxes according to the ordinary diffusion kinetics.

Unlike in ordinary cellular automata models each walker (i) is assigned an internal degree of freedom described by an 'internal energy' E_i. The internal energy is continuously used for metabolism. Yet, when sufficient food is available the walker can consume food fast enough to compensate for the metabolic needs and E_i increases until it reaches a threshold level and the walker replicates into two walkers. When the food level is not sufficient the internal energy decreases. After a walker is starved for a long interval of time E_i drops to zero and the walker 'freezes'. This 'freezing' represents the bacterial transition into a pre-spore state.

Proper modelling of bacterial collective lubrication poses a greater challenge. In the figure, we illustrate a relatively simple and computationally efficient way it can be done. First, the envelope of the lubricant is marked on the imaginary hexagonal mesh. The walkers perform a free random walk within the envelope. We then assign a counter to each segment of the envelope to count the number of times walkers hit the segment. After N_H, we move the segment one step forward. This requirement of N_H hits represents the colony

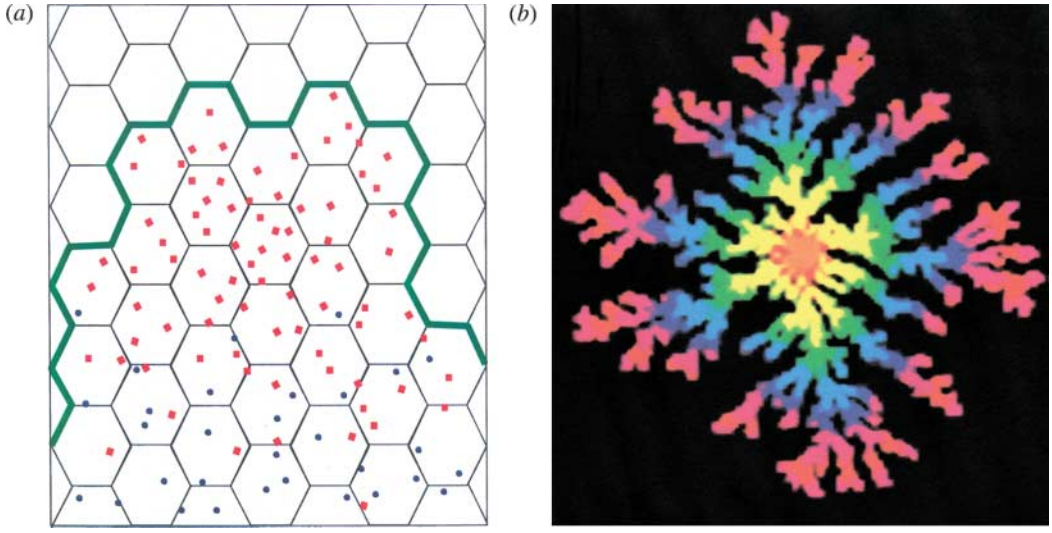

Figure 11. Typical results of the communicating random walker's model. On the left, we show the latice used in the simulations and the walkers. Red are the active ones and the blue the ones further back from the front that already froze. The picture on the right shows a typical result of numerical simulations. The different colours indicate different time stages of the simulations.

propagation through wetting of unoccupied areas by the cells. Note that N_H is related to the substrate hardness, as more lubrication fluid has to be produced (more 'collisions' are needed) to push the boundary on a harder substrate.

The next crucial step is relating the model's parameters with real bacterial growth. The food diffusion is known and the bacterial random walk is of about micron per second. The weight of a bacterial cell is about 10^{-12} g (1 pg). We assume that each cell consumes about 3 pg of food for reproduction: 1 pg for the materials of the new cell, 1 pg for metabolism and 1 pg to 'pay' for decreasing the entropy while converting the food into new cell organic substances. The maximal consumption rate per one cell is 3 pg during the minimal reproduction time which is about 25 min.

To include chemotactic signalling in the model, each walker emits the chemical agent according to internal state of the walkers—chemo-attractant is emitted when the internal energy is high and the chemo repellent is emitted by the frozen walkers. In addition the walker's random walk is biased according to the concentration gradient of the chemotactic chemicals (figure 11).

It is known that the flagella perform a propeller-like movement with a specific handedness resulting from the gear like structure of their molecular motors. Still, the observation of a pattern of strongly twisted, chiral branches, all with the same handedness, in swimming bacteria was unexpected since it was not clear how the flagella handedness can determine the global structure of the colony. In this regard, we point out that there are observations of chiral colonial patterns in elongated bacteria that result from a different mechanism—the bacteria form helical strings (Di Franco *et al.* 2002; Wand *et al.* in press). Microscope observations show that in the case of the C morphotype the bacteria do have flagella and that they show swimming in low density in fluid media. The microscope and electron microscope pictures shown in figure 3 illustrate that indeed the bacteria do not form entangled strings.

Flagella handedness has no effect on the tumbling of short bacteria as they make many turns before ending at a new angle. Optical microscope observations indicate that, in the case of chiral branching, the cells are much longer with liquid crystal like orientational interaction. This co-alignment of the longer bacteria limits their tumbling thus turning the two-dimensional random walk (of short bacteria) into a quasi-one-dimensional movement back and forth as the bacteria elongate. Only bacteria close to the branch tip can perform larger rotation which imposes a very weak bending on the advancement of the branch-tips. Put together the flagella handedness is made to act as a singular perturbation in a manner similar to that crystalline symmetry, thus leading to the colonial chiral patterning. These ideas can be tested by simulations of a version of the communicating walker's model in which the walkers tumble with a specific handedness and have an orientation interaction. Thus, the random walk becomes quasi-one-dimensional and only walkers close to the tip of the branches can twist. This model generates the observed broken reflection symmetry of the chiral morphotype as is illustrated in figure 3*d*.

We have seen how vortices contribute to the overall colony structure by leading the expansion over extremely hard substrates. The basic idea that needs to be incorporated in any model of the vortex is that the bacteria are self-propelled (Levine & Ben Jacob 2004). Of course, the communicating walker model already deals with particles propelled by their metabolic energy, but here the emphasis is on motion that remains coordinated for long-time, i.e. for situations where the tumbling into a new direction is suppressed by interactions with the neighboring cells. In this sense, there is a progression from bacterium motion in the branching pattern (random walker modulated by chemotaxis) to the chiral state (orientational effects lead to discrete reflection-symmetry breaking but are not strong enough to retain long-time coherence) to the vortex structure (with a breaking of the continuous rotational symmetry). An interesting mathematical

point is that it was used to be thought that picking a particular motion direction out of a continuous set of choices was impossible in two-dimensions. But the bacteria reminded us that facts about equilibrium structures, where the above was rigorously established, are routinely violated in non-equilibrium open systems (Levine & Ben Jacob 2004).

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