The bacterium *Serratia marcescens* produces a plethora of multicellular shapes of different colorations on solid substrates, allowing immediate visual detection of varieties. Such a plasticity allows studies on multicellular community scale spanning two extremes, from well-elaborated individual colonies to undifferentiated cell mass.

For a single strain and medium, we obtained a range of different multicellular bodies, depending on the layout of initial plating. Four principal factors affecting the morphogenetic pathways of such bodies can be distinguished: (1) amount, density and distribution pattern of founder cells; (2) the configuration of surrounding free medium; (3) the presence and character of other bacterial bodies sharing the same niche; and (4) self-perception, resulting in delimitation towards other bodies. The last feature results in an ability of well-formed multicellular individuals to maintain their identity upon a close mutual contact, as well as in spontaneous separation of cell masses in experimental chimeras. We propose an “embryo-like” colony model where multicellular bacterial bodies develop along genuine ontogenetic pathways inherent to the given species (clone), while external shaping forces (like nutrient gradients, pH, etc.) exert not formative, but only regulative roles in the process.

**Introduction**

The morphogenesis of multicellular organisms is usually exemplified by the model case of animal ontogeny, i.e., as an irreversible process resulting in an *individual*. Many multicellular life forms, however, might be bound by much looser morphogenetic constraints, allowing alternative developmental pathways controlled by external or internal cues as, e.g., in plants. Not only a variety of shapes can arise from a zygote or a spore; some ontogenetic strategies allow also vegetative propagation by buds, cuttings, founder cells and similar body fragments. Some life forms can thus be “parsed” (even to the level of single cells) and give rise to new shapes. We focused on such plasticity in a model prokaryotic organism—the bacterium *Serratia marcescens*, whose natural coloration greatly facilitates in vivo observation of developing multicellular structures.

Bacteria cover an admirable range of multicellular life forms, even within a single species or strain. They may grow as undifferentiated cell masses or suspensions; given, however, opportunities (free space, nutrients, ground texture, neighbors, etc.), they give rise to sophisticated body forms—societies created by billions of cells—often following morphogenetic pathways akin to those observed in genuine individuals. *S. marcescens* is known for its extreme variability of multicellular bodies formed under different settings.

As nutritional conditions are a prominent factor, the variability of bodies tends to be described as an epiphenomenon of nutrient limitation and/or metabolite buildup in the medium. Often the morphogenesis of bacterial bodies is depicted or modeled as if molded by external forces like, e.g., the behavior of iron particles in a magnetic field, or as a crystallization-like process, with bacteria playing role of “molecules”. Decisive for a genuine embryonic development are, however, not the nutrients and bulk metabolites but specific signals. The participating cells are interpreting them in a context-dependent, semantic manner, which results in cell populations proceeding along inherent morphogenetic trajectories. Here we show that this indeed appears to be the case also in the development of multicellular bacterial bodies.

**Results**

Classification of bacterial bodies. Under standardized nutrient conditions and in the presence of glucose, *Serratia marcescens* CNCTS 5965 gives rise to distinctly shaped and colored colonies (see below), while only smooth red colonies were obtained on identical media without glucose. On glucose-containing media, the original bacterial population occasionally segregated clones lacking the red pigment. After several rounds of selection, we derived two stable variants of the strain, colored (F) and white (W), which no longer segregated and which have been used in all the subsequent experiments.

Surprisingly, colonies of both variants exhibited a finite growth, reaching, under optimal settings, a maximum diameter of 15 mm.
Ontogeny of bacterial colonies

Figure 1. Colonies of the F clone exhibit limited growth. Colonies were started by sowing (open symbols) or dropping (closed symbols; see Methods) at time 0; average diameter from 6 colonies ± standard deviation is shown for each time point.

Advantage, since colonies can be planted to desired coordinates on the dish. Even more importantly, mature large F and W colonies can be viewed as fully developed specimens of a lineage-specific “body plan”, while small colonies, or other forms described below, may be interpreted as instances of an incompletely accomplished development towards the prototype F or W colony pattern.

Next, we investigated initial and boundary conditions allowing development of colonies or colony-like bodies. In particular, cell density and the size and layout of the planting area (dot, drop or smear), can be understood as two dimensions of a bacterial “morphospace” that may act in mutual discordance (Fig. 4).

Two additional characteristic shapes were observed under such “suboptimal” conditions.

Confluent colony (“concol”) develops upon spreading a bacterial suspension (approx. 15–150 cells/cm²) over an area up to a diameter of 15 mm, i.e., similar to, or slightly exceeding, the size of a mature, fully developed colony (Fig. 5A). A concol can also arise if colonies in a sparse sowing meet prior to achieving their fully differentiated state (see Figs. 2A and 3B). Three types of concols can be observed for the F strain; bodies of the W form are analogous, albeit white (see Fig. 2B):

(i) At lower densities (approx. 20 cells/cm²), a small number of colonies is initiated; they soon merge, establishing a center consisting of several red spots (corresponding approximately to the number of founder cells), surrounded by an elevated common red rim reminiscent of an F colony (as in Figs. 2A and 3B; compare also Fig. 6C).

(ii) Densities up to 150 cells/cm² produce concols with a single granular red center surrounded by a white laced zone, which later
Ontogeny of bacterial colonies

(iii) If the diameter of the inoculated area exceeds the 15–20 mm limit, cells no longer establish a concol, the only exception being elongated concols developing when one dimension in the spread is limited to about 7 mm (Fig. 2, lower left of each dish). Again, however long such a body might be, it never grows far into the unoccupied area beyond its borders.

Unlike colonies, concols do not develop in the same manner after planting multicellular inocula in a dense spacing instead of sowing a cell suspension; results of such experiments will be described in more detail below.

If the density of spreads is even higher than in concols (>150 CFU/cm²) and, at the same time, the planting area exceeds the diameter of 10–15 mm, distinguishable colonies never appear. Instead, the F bacteria develop into a macula—a red carpet without apparent inner structuring but with a well-developed rim as the last remnant of the F-type developmental pathway (Fig. 5B, right). Best results were again obtained with a single body per dish: the development of a macula requires a certain amount of unoccupied medium in its surrounding. Smears as those in Figure 10 represent maculae elongated in one dimension. An analogous phenotype was obtained also for the W form.

We further focused on three factors affecting morphogenesis of bodies described: availability of unoccupied substrate, the presence of other bodies, and internal processes governing body shaping.

Behavior towards unoccupied medium. A dense suspension, spread evenly over the whole dish, results in a population of small, separated colonies (Fig. 3A and 6A). Spreading on dishes of varying density of spreads is even higher than in concols (>150 CFU/cm²) and, at the same time, the planting area exceeds the diameter of 10–15 mm, distinguishable colonies never appear. Instead, the F bacteria develop into a macula—a red carpet without apparent inner structuring but with a well-developed rim as the last remnant of the F-type developmental pathway (Fig. 5B, right). Best results were again obtained with a single body per dish: the development of a macula requires a certain amount of unoccupied medium in its surrounding. Smears as those in Figure 10 represent maculae elongated in one dimension. An analogous phenotype was obtained also for the W form.

We further focused on three factors affecting morphogenesis of bodies described: availability of unoccupied substrate, the presence of other bodies, and internal processes governing body shaping.

Behavior towards unoccupied medium. A dense suspension, spread evenly over the whole dish, results in a population of small, separated colonies (Fig. 3A and 6A). Spreading on dishes of varying densities (Fig. 5A). Best-looking concols developed from a single spot of bacterial suspension per dish.
Ontogeny of bacterial colonies

---

**Figure 6. Effects of plating geometry.** (A) Growth of dense colonies (about 300 CFU/dish) sown evenly over the whole surface of agar; (B) removal of surrounding agar leads to development of single colonies (wet tampons maintaining humidity can be seen in the dish); (C) a concol grown from a suspension of the same plating density, surrounded by free agar; (D) nutrient broth is not a sufficient substitution for the agar removed; (E) a combination of a concol and colonies developing at the interface of free space and agar. Cultures are at least 20 days old, but the same phenomena are well-developed already after 10–14 days.

---

**Figure 7. Transfer of colonies.** Circular plugs were removed from a field of 4-day colonies (left) and transferred into a hole in a fresh agar dish (middle), or to a damp chamber (right). Fresh agar induced colonies to merge into a macula within 8 days (all dishes photographed at the same time).

---

The fact that a bacterial body responds by developing a rim, however, disappeared at the border facing neighboring colonies, whereas the cut edge remains flanked by free colonies. Upon transfer of small (ca 1 cm$^2$) discs cut out from a continuous carpet of mature dormant colonies (hundreds per dish; Fig. 7 left) into an opening of identical diameter in an empty agar field (from the same batch of dishes, Fig. 7 middle), colonies resumed their development, expanded somewhat beyond the confines of the transferred agar plug and merged into a macula. Similar discs transferred into an empty dish without agar remained unchanged (Fig. 7 right). Such a behavior took place even with older (24 days) cultures. (Note that a comparable number of CFU—approx. 20—sown in the middle of the dish would result in a concol, as in Fig. 6C; however, here we did not transfer freshly sown cells but established colonies).

Thus, bodies seemingly in a final stage of their ontogeny can resume development if challenged by fresh unoccupied medium. However, even in such a case they remain spatially restricted, suggesting the involvement of specific regulatory processes as opposed to simple nutrient limitation.

**Close neighborhood effects.** To examine the mutual behavior of bodies sharing the same dish, we followed the development at several plating configurations.

First, dishes were sown with a suspension of the F strain at hundreds of CFU per dish, which should produce small colonies, and an inoculum of the same strain sufficient to start a colony or a macula was planted (dropped or dotted) upon this field immediately. Whereas the colonies of the background developed normally, only an undifferentiated blotch lacking structures of a typical macula or colony developed on the planting site, while controls developed normally (Fig. 8A–F). Although inhibition was observed even if the number of cells in the planting exceeded that in the field by several orders of magnitude, it was more profound on a dense field and/or upon planting a diluted suspension. As expected, dropping of inocula corresponding to a colony or macula to fields of 2–4 days old colonies led to even more dramatic impediment of development (not shown).

If such a dense bacterial field was applied only to one half of a dish, and a macula-producing inoculum was planted immediately at the interface of the occupied and free agar, a typical macula with a well-developed rim appeared in the direction of the free agar; its rim, however, disappeared at the border facing neighboring colonies.
Ontogeny of bacterial colonies

(Fig. 8G), reminiscent of the situation at the interface of free agar and empty space (Fig. 6E).

Multiple comparably strong inocula of $10^2$–$10^5$ cells planted close to each other developed to normal F colonies, neither inhibited nor merged into a concol (Fig. 8H). However, colonies developing from small inocula did produce a concol (Fig. 8I). This indicates the existence of a limit inoculum size deciding the result of a tradeoff between tendencies to merge into a concol, or to develop a full-fledged colony, at a given distance (see also the overlap of configurations in the morphospace phase diagram at Fig. 4).

Long-distance signaling between bodies. F colonies incidentally growing in close vicinity develop their coloration quicker than their peers growing sparsely; from such a colored center the reddening front spreads into the surroundings (Fig. 9). The effect suggests that colonies can induce final phenotypes in their neighbors.

To get a closer insight, a smear of approx. $10^8$ cells was applied to a line on agar, and, at the same time, colonies were planted 1–2 cm from that line. The smear developed into a macula that accelerated the coloring of colonies, as compared to the controls, to a degree proportional to the distance between both bodies (Fig. 10A). A trench cut into the agar did not prevent acceleration of color development, suggesting participation of a signal traveling through the gas phase (Fig. 10B). A macula of W cells also exerted an accelerating effect on F colony coloration (Fig. 10C).

Surprisingly, also an F macula induced weak pink coloration in W colonies, together with disruption of colony structure (Fig. 10D; for structure disruption see also Fig. 11A). Similar disruption of W colony structure was observed also in the vicinity of a W macula (not shown). Thus, an F body can induce the production of pigment in W colonies. The coloring, albeit fainter, appeared also in a configuration with the trench (not shown). Cells from the pink crescent gave rise to normal W colonies, even after five rounds of co-cultivation with an F macula as described above, indicating a transient, regulatory nature of the observed changes.

Chimeric bodies. Upon collision of growing F and W colonies, the two bodies do not merge into a concol with a common rim but maintain a clear-cut boundary instead. As in the case described above, the W colony develops a pink coloration adjacent to the F neighbor (Fig. 11A). While this phenomenon might be analogous to the above-described collision of large colonies that also do not merge (Fig. 8H), it prompted us to investigate the behavior of bodies originating from mixed suspensions.

When dense suspensions containing a mixture of cells of F and W lineages were dropped in a manner that would produce colonies under monoclonal conditions, colony-sized chimerical bodies (i.e., about 10–12 mm in diameter) lacking the typical F morphology developed, with a red center and white periphery. The proportion of colors roughly corresponded to the ratio of F and W cells in the original suspension (Figs. 11B and 12A middle). Material dotted directly from the red or white part of a chimera, or sown from suspensions prepared from local samples, maintained the characteristics of the place it was taken from, suggesting segregation of both constituting clones into delimited territories, while a suspension

Figure 8. Development of bodies on dense bacterial fields. (A and B) Maculae from about 1500 cells, (C and D) those from about 150 cells, sown on (A and C) free agar and (B and D) a background of about 200 CFU per dish. (E and F) A similar layout with inocula normally giving rise to F colonies (3000 cells above, 300 below, respectively); (A–F) 36 days, but identical results after 14 days. (G) Development of a macula at the interface of small-colony field and free agar (6 days). (H) Three high-density drops (100–150 cells) in close vicinity develop to delimited F phenotypes, without merging into a concol; the same result was obtained with larger inocula up to $10^5$ cells, while (I) drops containing only 10–15 cells in the same configuration give rise to a typical concol (H and I, 15 days).

Figure 9. Spread of colony coloring from “reddening centers”. The same dish is shown at three time points.

82 Communicative & Integrative Biology 2008; Vol. 1 Issue 1
starting to appear in the oldest bodies). 2–4 days after such homogenization the body re-established its red center and white margin (Fig. 12A). The younger the chimera at the time of disruption, the more complete was the pattern regeneration; in 4 days-old chimeras, the regeneration capacity is nearly lost (see bottom row of Fig. 12A).

When bacteria from white or red parts of such regenerated bodies were replanted, they produced colonies whose character corresponded to their kin in the region the dot was taken from, indicating that reconstitution involves cell migration rather than phenotype switching in individual cells. Material from red parts of incompletely reconstituted older (4 days) chimeras produced mixed bodies, consistent with imperfect redistribution of both cell types, possibly due to increasing rigidity of older chimeras, which impedes their complete homogenization.

Unlike chimeras, F colonies did not regenerate their original pattern after homogenization at 2 to 3 days, producing only variegated bodies (Fig. 12B). Obviously, cells of the same clonal origin do not perceive their sisters as “strangers”, regardless of their (color) differentiation status, and, thus, they do not migrate to segregate the two colored variants.

**Discussion**

Prior to the advent of the DNA era, bacterial colonies were mostly viewed as epiphenomena or diagnostic tools revealing “true” performances taking place at cellular level, such as pathogenicity, biochemical processes, mutations or even life cycles (reviewed in ref. 16). For example, Mary I. Bunting in a series of papers on colony plasticity (so called “dissociation”) in *Serratia* seeks after trends going on not in colonies proper, but in suspensions that gave birth to such colonies. (For a review written as an epilogue of the whole period (see ref. 17).
**Ontogeny of bacterial colonies**

*Bacillus, Caulobacter*, often encompassing multicellular stages with multiple cell types (e.g., *Myxobacteria, Streptomycetes, Cyanobacteria*). Finally, multi-species consortia such as mats, plaques, stromatolites, etc., joined the company. A colony is no longer perceived as a heap of cells, which stick together only because they cannot disperse quickly enough. Finally, in the 1980s Shapiro18-20 introduced multicellularity of colonies to common awareness.

The structure of multicellular bacterial bodies might simply reflect environmental differences—often transient—caused by metabolism of growing bacterial masses, such as nutrient limitation or accumulation of various metabolites in the medium, which has been the standard explanation in earlier works.21,22 Shapiro,14,19,20 on the other hand, takes the radial symmetry as one of the arguments suggesting developmental coordination of processes within the bacterial body (such as synchronous switching of transposons), i.e., active participation of cell assemblies in true morphogenetic processes. A plethora of colony shapes can also be acquired by treatment as simple as changing the density of agar support.21 The recent studies of E. Ben Jacob and others go even further, proposing genuine semantical communication between bacteria comprising the colony.23

Here we suggest viewing the development of multicellular bacterial bodies as a genuine counterpart of animal, plant or fungal ontogeny, i.e., production of multicellular bodies through intricate morphogenetic processes resulting from cell cooperation and communication that channel the development along species- or lineage-specific pathway (a “body plan”). We observed that bacteria can (i) produce multicellular bodies of limited size that adjust their development according to such parameters as layout of the inoculum, availability of unoccupied solid medium, or the presence of other bodies in the vicinity and (ii) distinguish between cells of identical and non-identical clonal origin upon co-cultivation. This suggests involvement of specific signals that are perceived in a context-dependent manner, similar to morphogenetic signals of multicellular eukaryotes.

**Embryology** of colonies. Bacteria in general (and our model *Serratia marcescens* in particular) are known to be inherently phenotypically plastic—minute variation in the environment changes the overall appearance of a multicellular body. Indeed, different colony phenotypes can be obtained in *S. marcescens* upon minor variation of media composition, including, e.g., variation in agar density—reviewed in refs. 24 and 25; and our unpublished data. However, here we focused on factors affecting the development of multicellular bodies under constant nutrient conditions on rich media, such as plating geometry and mutual interaction of bacterial bodies over short or long distance.

To our surprise, we found that our strain exhibits restricted growth, producing colonies whose size usually does not exceed the limit of 10–12 mm in diameter, while most strains described in the literature display unlimited growth of colonies, resulting in very big bodies.26,27 Also some other isolates of *S. marcescens* exhibit unlimited growth under the same conditions in our laboratory (not shown), indicating that the observed limitation is not due to exhaustion of the medium. It can be speculated whether such a limited growth does not result from domestication of a sort; domestication effects of laboratory life were described in bacteria15 as well as in yeast colonies.28 From our point of view, finite growth further justifies the embryological analogy, with fully developed mature colonies as final, “accomplished” stages of a genuine morphogenetic pathway, consummated and modified according to the parameters of the environment (a “morphospace” where geometry of the plating, chemical composition and its time course represent just some of multiple variables). Alternative developmental outcomes may then be viewed as different stages and/or endpoints of an endeavor towards such a final stage. In particular, three such alternatives deserve closer attention.

The first variant outcome is represented by small colonies that develop at densely populated plates, reaching their final appearance rapidly, which suggests an early quorum-based decision. Such colonies might be compared to “dormant” embryos, as frequently encountered in plants or some animals. Dormancy, however, can be overcome by discontinuing the supply of the quorum signal, e.g., by transfer to a fresh medium. At even higher plating densities, distinct colonies can no longer be established, and an unstructured lawn develops instead (not shown).

The quorum signal is unlikely to be constituted by a simple parameter such as concentration of nutrients in the medium or a signal molecule in the gas phase above it, since colonies apparently perceive directional information about the availability of unoccupied substrate, even if they do not attempt to expand onto it to a larger extent (see below). Studies on quorum signaling in *Serratia* revealed a plethora of specific chemical signals regulating the bacterial phenotype, such as, e.g., various acyl homoserine lactones. However, these phenomena were studied on bacteria grown in suspensions, i.e., the traits followed were biochemical.29,30 (Similarly, when Engelbert-Kulka et al.31,32 speak about the role of programmed cell death in multicellular bacterial bodies, they mean bacterial suspensions).

The decision between the pathway towards fully developed colonies and the “dormant” ones does not depend on the overall number of cells on a dish but on the amount of colony seeds (colony-forming units, CFU) that may be either unicellular or multicellular. Surprisingly, even large multicellular CFU containing millions of cells “respect” the quorum established by multiple unicellular initiating centers that make up only a negligible fraction of total cells present on the dish, thus further supporting the notion of specific signaling.

It is well documented (reviewed in refs. 14 and 33) that fully developed colonies can arise not only clonally from single cells, but also from a dense suspension, planted to a small area by dotting or dropping. Such a behavior reminds of “regulatory embryos” (like, e.g., those of mammals), which can start not only from a zygote but also from greater embryonic-cell masses or even chimaeras of different genetic backgrounds, or of regeneration of an anatomically complete plant from adventitious roots or shoots developing on a callus. However, establishing the colony body plan from such cell masses may be error-prone, as documented by occasional aberrant colonies. It also apparently takes some time before a colony from a multicellular inoculum acquires the ability to process environmental information, e.g., to monitor the presence of close neighbors. Thus, fully developed colonies form even in situations that soon lead to collision of growing bodies originated from closely planted large inocula.

Plating of a dense suspension to an area greater than the maximum colony size leads to another variant developmental outcome—confluent colonies—that may be understood as a tradeoff between several suboptimal options. The development cannot get canalized to dormant small colonies, because of lack of the necessary quorum
ontogeny of bacterial colonies

Effects of inoculum layout. Our observations suggest that colonies adjust their development according to the parameters of surrounding space. A decisive physical or chemical cue may be provided by free, unoccupied agar medium. Moreover, this cue is likely to be specific, since if such information were carried by absolute concentration of nutrients (or by dilution of an inhibitory signal molecule), it would be provided also by liquid broth replacing the agar, or even by agar removal in case of an inhibitor. However, it has to be noted that even simple small molecules including bulk metabolites may play the part of a specific signal if they are “understood” as such (e.g., ethylene as a plant signaling molecule, or ammonia governing the morphogenesis of yeast colonies).

Multicellular communities obviously cannot arise in liquid media, as these do not provide structured environment with gradients of nutrients, information, and—later—support for the resulting bodies. Only a semi-solid support (e.g., agar) allows establishing (often transient) spatial structures that may mold more permanent multicellular bacterial bodies. A single colony on the dish may impose to its surroundings a steep gradient (of any compound), whose slope conveys information on extent of free space available to establish a big and well-structured colony. On the contrary, dense sowings may hinder formation of such steep gradients, resulting in the choice of the pathway towards small, unstructured “dormant” bodies. Such explanations, which may deserve a more formal treatment in terms of Turing-style reaction-diffusion models, may be adopted also for cases of more complex plating layouts such as e.g., sowings to an already occupied background.

Besides self-structuring, the growing bodies also influence the development of their neighbors, at least partly via signals propagated by the gas phase, similar to the case of yeast colonies. Searches for the “material basis” of structured gradients in the environment identified multiple candidate factors in yeasts; a similar outcome can be predicted also for bacteria, but remains to be proved experimentally.

A natural white variety derived from our pigmented Serratia strain allowed a deeper insight into the cell and body communication phenomena. Reddening of white colonies in the vicinity of a red streak suggests that the white clone retained its capacity to synthesize the pigment, but lost the ability to generate a releasing signal, thus developing the color only in the presence of signal(s) coming from outside. This feature is stable, as no signs of genetic assimilation were observed. Like the famous triton axolotl (Ambystoma), white colonies remain as if locked at a “larval” stage, never proceeding to the fully developed coloring. An alternative explanation—transfer of the dye from neighboring red cells—is extremely unlikely since the color-inducing signal is airborne.

Genetic mutations, in particular those caused by transposable elements, or stable regulatory states, can also cause morphological changes in microbial colonies. A population of E. coli was found to generate three metabolically distinguishable forms, which persist, in constant proportions, through hundreds of generations, and which specialize for distinct metabolic tasks while “cross-feeding” the rest of the population. Older authors also described such “differentiation-like” phenomena, showing that a suspension in its “optimal” phase produces colonies with greatest heterogeneity. Even for bacteria, true ontogeny where the developing body decides for alternative developmental trajectories towards different endpoints has been proposed in older works, opening the space for recognition and analysis of phenomena such as developmental plasticity or reaction norm of the strain. Indeed, we are inclined towards such an explanation of our results.

According to M. Barbieri, ontogeny represents a build-up of new information by re-iterating inputs from both genome and environment in an “organic memory”, which is virtually “empty” at the beginning of development. Elaborated embryonic and organogenic processes in multicellular eukaryotes are highly dependent on genetic regulatory networks (reviewed in ref. 42). Since the complexity of such possible networks in bacteria is far lower than that found in eukaryotes, it can be speculated that in bacteria the memory is more emancipated (less “hard-wired” into the genome).

Self/non-self recognition. We used the availability of two stable colored lines of our strain to investigate the behavior of bacterial bodies towards populations of the same species but different clonal origin. To our surprise, mixed suspensions attempted, more or less successfully, to segregate the two constituent clones into separate populations. Regeneration experiments confirmed that segregation is due to cell migration and not phenotypic switching or selective cell death. The ability of bacteria to travel long distance just to be able to reestablish zones of their own kin is an unexpected finding, and resembles a similar incompatibility observed in embryonic cells in cultures (a mixture of, e.g., ectoderm and mesoderm cells segregates in a similar manner). Moreover, it suggests that the ability to recognize “self” (i.e., cells of the same clone) from non-self (cells of related but distinct clonal origins), that has been even suggested as one of the criteria for assessing intelligence of a biological system, may be characteristic not only for multicellular animals or plants, but also for bacteria—and, indeed, possibly for all forms of life. The capacity for intraspecific self/non-self recognition may present one of the ancestral and fundamental features of living beings.
We believe that our observations document the existence of surprisingly sophisticated communicative potential within and between bacterial colonies that presents a challenge both for experimental and theoretical biologists. One possible pathway of future investigation obviously leads towards identification of the signaling compounds and molecular mechanisms involved. Theoretical (mathematical) models of colony development also should be able to incorporate or predict the observed colony/spacer interaction phenomena.

Materials and Methods

Strain and media. The strain S. marcescens CNCTS 5965 was obtained from the Czech National Institute of Health, and maintained in nutrient broth No. 2 (Imuna Pharm, ZB No. 2, containing 5 g beef extract, 5 g peptone, 6.25 g nutrient base No. 1, 6.25 nutrient base No. 2, 2.5 g NaCl per litre) with 15% glycerol at -80°C. Upon reviving from the frozen stock, the bacteria were colony-purified twice and their colony phenotype was verified prior to subsequent experiments.

Bacteria were grown under standard conditions comprising 9 cm dishes with a 4% nutrient broth with nutrient agar No. 2 (Imuna Pharm ZA No. 2, i.e., ZB No. 2 with 1.5% agar), complemented with 0.5% glucose unless stated otherwise. For liquid cultures, nutrient broth No. 2 was also used.

Inoculation procedures. All manipulations were performed in a laminar flow box, at room temperature. Cell suspensions for plating were prepared in PBS (22 mM KH₂PO₄, 50 mM Na₂HPO₄, 8.6 mM NaCl, 18.7 mM NH₄Cl; pH 7.4) from agar-grown mature but less than 3 weeks old bacterial bodies. Cell density of suspensions was adjusted according to optical density at 450 nm, calibrated by plating serial dilutions.

Methods of application to the agar surface were as follows:
- (1) Sowing (standard procedure, used unless specified otherwise): 100 μl of an adequately diluted bacterial suspension was spread over the whole surface of the 9 cm dish. Proportionally smaller volumes were applied if sowing to a smaller area (e.g., 40 μl to a 6 cm dish).
- (2) Dropping: a dense suspension was applied as a small drop (ca 1 μl) within a diameter of about 2 mm.
- (3) Dotting: Small amount of material was picked from a bacterial body using a sterile needle and transferred to a fresh surface.
- (4) Smear: 30 μl of bacterial suspension (approx. 10^8 cells) was applied to a line of approx. 5 cm.

In all cases, the suspensions soaked into agar within 2–3 min. Dishes were kept at 27°C for 2 days, and maintained at room temperature (ca 22°C) in closed but not sealed boxes containing a damp sponge to maintain humidity, wrapped in cloth to prevent insect infestation.

Documentation. Cultures were photographed using an Olympus digital camera under ambient light (except Fig. 12B that has been taken under artificial illumination with the aid of an Olympus dissecting microscope); black background was used for visualizing white colonies in some cases. Colony size measurements have been performed on the digital photographs, either manually or with the aid of the ImageJ 1.32i software (http://rsb.info.nih.gov/ij/).

All experiments were done repeatedly; figures shown were selected from an extensive collection of primary photos. Corel Photo Paint was used to assemble the plates but no image enhancement was performed.

Acknowledgements

This work was supported by the projects GACR 408/08/0796 and MSM 0021620845 to Anton Markoš, Anna Blahašková and Zdeněk Neubauer, and MSM 0021620858 to Fatima Cvrčková. We thank Alexander Nemec for providing the bacterial strain and Vladimír Vondrejs for critical reading of the manuscript. The authors declare that they have no financial interests in the outcome of this work, nor they are aware of any conflict of interests.

References