Chapter 8
Interplay Between Sibling Bacterial Colonies

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8.1 Introduction

8.1.1 Using Intelligence to Cope with Stress

Bacteria in the wild are frequently exposed to harsh conditions, the sources of which include, but are not limited to, a lack of available nutrients, overcrowding and space limitations, the presence of enemies, and extreme environmental conditions, such as high temperatures and dryness. Their responses to stress can consist of radical behaviors, such as the deadly competition often observed between individuals of the same species [1–5].

The first and most fundamental of all organisms, bacteria have evolved mechanisms to ensure their survivability when faced with harsh conditions [6, 13]. They lead rich social lives in complex hierarchical communities, collectively sense the environment to glean information, learn from past experience, and make decisions. To engage in such complex, cooperative behavior, bacteria utilize highly sophisticated chemical communication mechanisms whose chemical language includes semantic and even pragmatic aspects [7].

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S.J. Hagen (ed.), *The Physical Basis of Bacterial Quorum Communication*, Biological and Medical Physics, Biomedical Engineering, DOI 10.1007/978-1-4939-1402-9_8

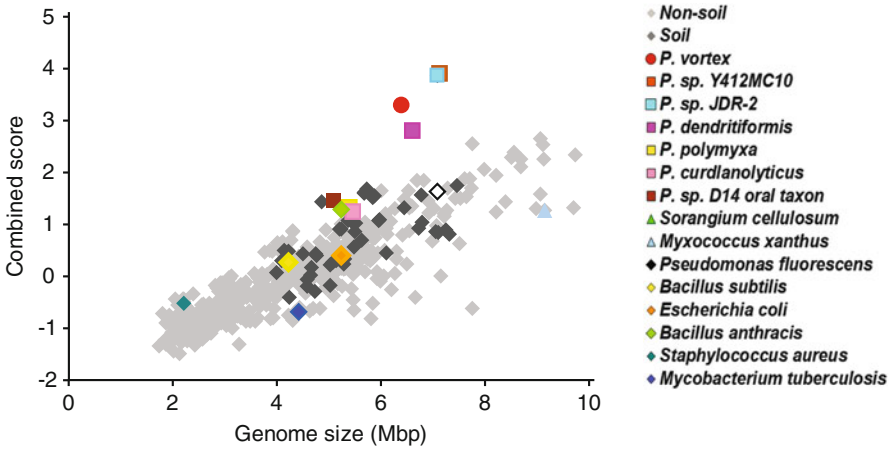

Fig. 8.1 The combined social IQ score as a function of genome size for 502 bacterial species. The *y*-axis represents the relative combined score (relative to the averaged score divided by the standard deviations). The social IQ scores of the "smart" *P. dendritiformis* are nearly three standard deviations above normal. Image was taken from [8] with minor modifications

The bacterial capacity to cope with stress is based on each species' distinctive abilities. While some bacteria are "goofs," others are "smart" and are able to survive a large variety of complex situations. Bacterial survivability is correlated with the microbial signal transduction system, which can be viewed as an information processing network comprising multiple sensory and transduction/output elements [8]. Comparative genomic studies revealed that, in general, microbial signal transduction system size increases as genome size grows [8]. Moreover, bacteria with elevated adaptability for survival in highly versatile and complex environments were found to have significantly larger, more sophisticated, and more diverse signal sensing and processing systems (Fig. 8.1). The relative numbers of the genes behind these systems can give a sense of "bacterial IQ," a measure that was found to be remarkably high for the *Paenibacilli* spp. (Fig. 8.1).

8.1.2 Paenibacillus dendritiformis

First identified in 1993, *Paenibacilli* spp. have been detected in a wide range of environments such as soil, water, the rhizosphere, vegetable matter, insect larvae, and in clinical samples. Recent years have witnessed increased interest in *Paenibacillus* spp., many of which were found to be important for industrial, agricultural, and medical applications. These bacteria produce extracellular substances, such as polysaccharide-degrading enzymes and proteases, which catalyze a wide variety of synthetic reactions in fields ranging from cosmetics to biofuel production. In addition, some *Paenibacillus* spp. also produce antimicrobial substances that affect

a wide spectrum of micro-organisms such as fungi, soil bacteria, plant pathogenic bacteria, and anaerobic pathogens such as *Clostridium botulinum*.

In line with the generally "high IQ" score obtained for members of *Paenibacillus* spp., extensive work on *P. dendritiformis* has revealed that they possess a large array of quorum communication mechanisms alternatingly capable of promoting fast colonization under growth-favorable conditions or of supporting survival strategies when confronted with a harsh environment. Five such mechanisms are listed below.

1. Differentiation: *P. dendritiformis* form and reshape large, intricately organized colonies comprising billions of cells [9–14]. Colony formation is enabled by differentiation, which confers on populations of otherwise identical, individual bacteria the capacity for cooperative self-organization. Once part of a colony, the bacteria can better compete for food resources and they enjoy greater protection against antibacterial assaults.
2. Lubrication: On hard surfaces, *P. dendritiformis* have the ability to secrete a lubricant that both reduces surface friction and that also facilitates rapid migration [13]. Such materials are secreted by the bacteria only when their cell count in a niche has become high enough, indicating that lubricant secretion is dependent on a quorum mechanism.
3. Swarming: Robust swarmers [15, 16], *P. dendritiformis* can swarm on a large variety of agar concentrations, including hard agar (2 %) and agar with limited nutrient levels. In fact, unlike *Escherichia coli* (require glucose for swarming), *Bacillus subtilis* (require high levels of moisture), and *Serratia marcescens* (unable to swarm at 37 ºC, i.e., mammalian body temperatures), *P. dendritiformis* swarm under most laboratory conditions. As in other species, however, the bacteria must reach sufficient cell density to swarm.
4. Sporulation: As with many other Gram-positive species, *P. dendritiformis* may enter sporulation mode if nutrient levels are insufficient for growth [2]. Dependent on population density, sporulation is enhanced by the self-secretion of subtilisin, which inhibits the growth of sibling *P. dendritiformis* colonies. Both the sporulation and swarming circuits of *P. dendritiformis* resemble those of *B. subtilis* and are dependent on the local bacteria concentrations.
5. Resistance: Another challenge bacteria often encounter is antibiotic stress. While some species are naturally resistant to specific types of antibiotics, others use persister cells [17] or small colony variants [18] to ensure their survival. These cells, which typically constitute very small fractions of bacterial populations, either do not reproduce or they reproduce very slowly. Phenotypically, however, they are considerably more resistant to the antibiotic than are regular cells from the same population, and as such, their survival ensures strain continuity. An additional *P. dendritiformis* survival mechanism that assists in fighting antibiotic attacks is the existence in this species of two well-characterized motile strains, or morphotypes, which are known as the *T* [19] and *C* [20, 21] morphotypes and which have the same 16S rRNA ribosome. While the two strains differ in terms of the corresponding survival strategy associated with each [13, 22], in most cases the two morphotypes will grow independently under the specific conditions

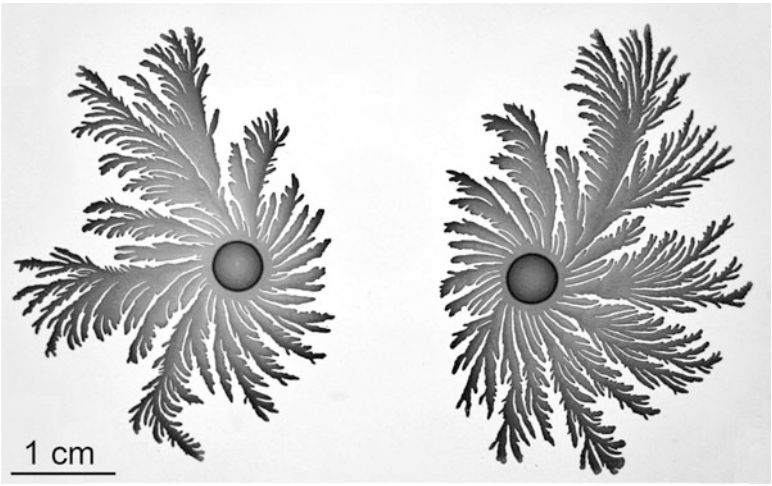

Fig. 8.2 Two neighboring *P. dendritiformis* colonies growing on 1.6 % agar with 1.5 g/L peptone

(temperature, humidity, agar rigidity, and food level) that are suitable for it. When subjected to antibiotic stress, however, a strain can switch to the other morphotype if it is better suited to survival in the antibiotic-tainted environment. Once favorable conditions return, cells can revert to the original morphotype. The majority of the work in this chapter focuses on the *T* morphotype.

Mechanisms that support the collective behavior of bacteria change the population of a single colony into a multi-cellular organism. Under such collective conditions, therefore, one expects that there may also be competition between sibling colonies (taken from the same culture), as suggested by the growth inhibition shown in Fig. 8.2.

8.2 Competition Between Sibling Colonies

8.2.1 Early Observations

In the early 1990s, Fujikawa and Matsushita [23] showed that sibling *B. subtilis* colonies grown on nutrient-poor agar in the same Petri dish did not merge. Each colony expanded in the direction of its neighboring colony but stopped short of making physical contact, leaving a gap of a few millimeters between the two colonies. Under rich growth conditions, however, the authors did not observe the phenomenon [24], and the colonies spread in homogenous patterns. The reason for the gap, they concluded, was nutrient depletion. Their observations illustrate a phenomenon similar to diffusion limited aggregation (DLA) processes [25], in

which the consumed nutrients diffuse through the medium, creating intricate branch patterns with gaps where nutrients were depleted.

About two decades later, a study by Gibbs et al. [26] on the interaction between *Proteus mirabilis* colonies showed that different *P. mirabilis* strains formed boundaries between colonies while those of sibling colonies of the same strain did not contain any boundaries. A fundamental requirement for boundary formation in the Gibbs et al. study is that bacteria exhibit the ability to discriminate between self and nonself. Swarms of mutants with deletions in the *ids* gene cluster did not merge with their parent. Thus, although Gibbs et al. suggested that the *ids* genes are involved in the ability of *P. mirabilis* to distinguish self from nonself, the specific mechanism of inhibition and its evolutionary advantage are still unclear.

The first study to show some sort of competition between **sibling** colonies that was not a result of food depletion focused on the *T* morphotype of *P. dendritiformis* [27–29]. Although simulations suggested that colonies were repelled due to some signaling factor secreted to the medium, the physical basis of that competition, its mode of action, and the chemicals involved in the process have still not been resolved.

8.2.2 Competition Between P. dendritiformis Colonies

Subsequent quantitative studies investigated the competition between *P. dendritiformis* colonies of the *T* morphotype [1–3]. In the first study [1], two sibling colonies were inoculated simultaneously on an agar plate, and their time development was monitored using an integrated incubation-imaging system to track bacterial colony growth over a period of a week (Fig. 8.3a). The initial development of each colony was virtually the same as that of a single, isolated colony: after a lag of 18 h, the two colonies began to expand outward, in the process developing intricate, branched patterns within well-defined, circular envelopes. The speed of envelope growth was isotropic and constant (Fig. 8.3b, c).

However, after a well-defined time that depended on the initial separation distance between the two colonies, the rates of growth of the colony growth fronts facing each other began to decelerate until growth there stopped altogether, leaving a gap between the pair of colonies (Fig. 8.3a). Other areas of the colonies, however, continued to grow unhindered. Surprisingly, the deceleration observed in the colony growth along the fronts that were opposite each other was independent of the initial separation distance, indicating a threshold mechanism. This suggested the presence of an inhibitory chemical. Indeed, colonies grown at different initial separation distances are affected the same way when they sense an inhibiting level above a threshold.

These results motivated us to investigate why the colonies stopped growing on their adjacent growth fronts while other areas of the colonies seemed unaffected. In contrast to what has been observed in studies of *B. subtilis* growth [23], food depletion can be ruled out as the primary cause since, given the length and time scales for colony growth, the diffusion constant of peptone in the agar was found to

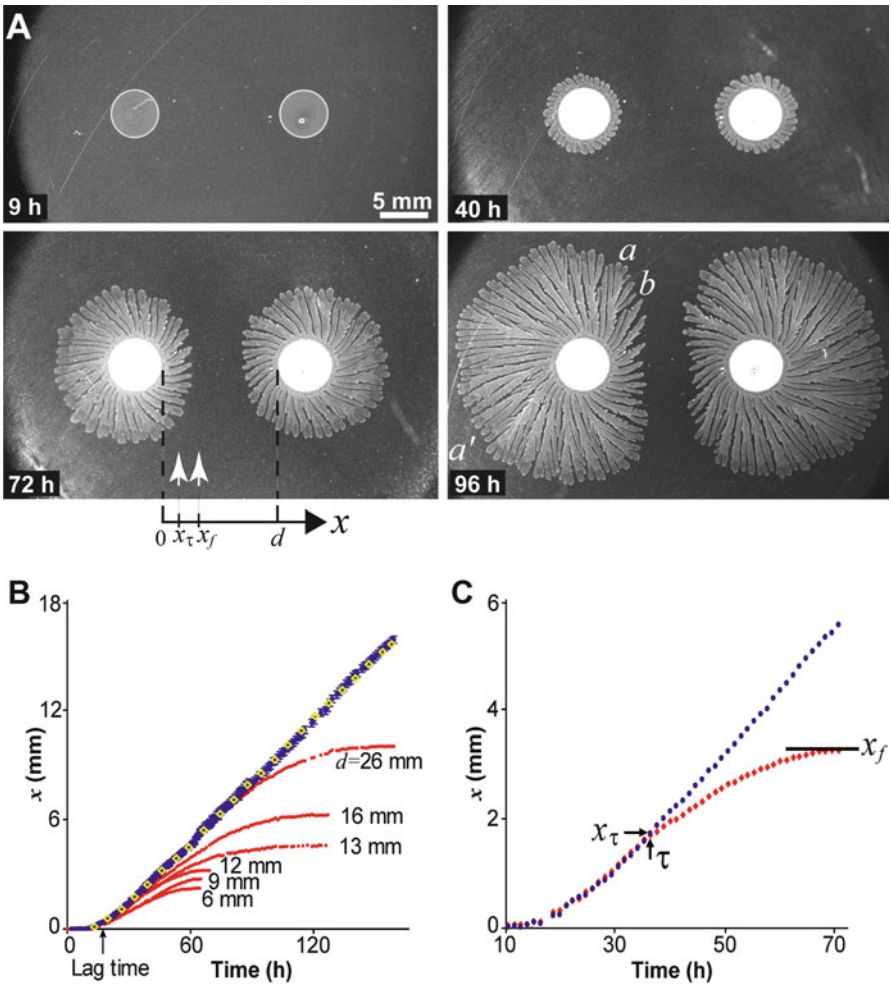

Fig. 8.3 Interaction between neighboring *P. dendritiformis* colonies growing on 1.5 % agar with 2 g/L peptone. (**a**) Images of adjacent growing colonies: 9 h after inoculation, no growth; 40 h, the onset of inhibition; 72 h, inhibited growth; 96 h, growth has stopped in region *b* but continues in regions *a* and a'. The labels on the *x*-axis (under panel **a**) indicate the initial distance *d* separating the two colonies (here 12 mm); the position $x_\tau(d)$ is where growth begins to decelerate, and the position $x_f(d)$ is where growth stops. (**b**) Position *x* of the growth front as a function of time for initial colony separation distances *d* of 6–26 mm; *blue* symbols aligned on a *straight line* correspond to the uninhibited growth in region a', whereas *red* symbols, aligned on the *curved lines* represent growth toward region *b* [see data for 72 h and for 96 h, (**a**)]. The *yellow diamonds* (on the *straight line*) represent the growth of a single colony for the same conditions. For both the single colony and for neighboring colonies with any separation *d*, there is the same lag time (18 h), followed by growth with a speed of 0.11 mm/h. (**c**) A well-defined transition (at $\tau = 36$ h, $x_\tau = 1.8$ mm) from uninhibited to inhibited growth for the colony in (**a**). Growth speed decelerates, and the growth front stops at x_f. Image was taken from [1] with minor modifications

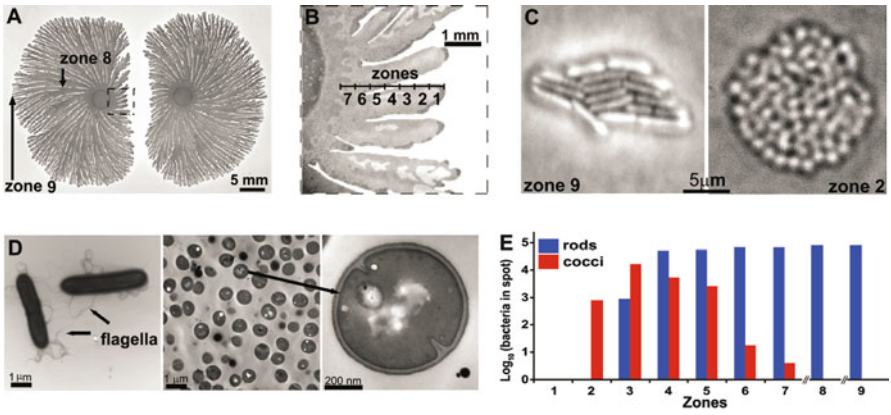

Fig. 8.4 Switch from rods to cocci. (**a** and **b**) Low (**a**) and high (**b**) magnifications of a competing colony. (**c**) Colonies formed from single bacteria taken from zone 9 (rods) and from zone 2 (cocci). (**d**) Transmission electron microscopy of rod-shaped, motile *P. dendritiformis* cells (*left*). Cocci (cross-section image) are shown at the same magnification as are the rods (*middle panel*) and at a higher magnification, revealing incipient cell division (*right*). (**e**) Number of bacteria recovered from each zone (250 × 250-μm area) are indicated in panels **a** and **b**. No bacteria were recovered from zone 1, and only cocci grew from zone 2. The number of rod-shaped bacteria increased with the distance from the inhibited interface, and only rods were recovered from zones 8 and 9. Image was taken from [3] with minor modifications

be too large (1.6×10^{-5} cm^2/s). Therefore, it was not clear why the bacteria simply stopped moving. Two possible explanations are that they entered the sporulation stage or that they died.

8.2.3 The Inhibited Region

To discover what caused the pattern of inhibited growth, bacteria were isolated from different regions of the two colonies and examined. These regions included the uninhibited area (zones 8 and 9), the colonies' centers (zone 7), and the area from the center to the inhibited tips (zone 1) (Fig. 8.4a, b). In zones 8 and 9, only rod-shaped motile bacteria were found, and in zone 8 some spores were also found, in line with what is expected from a single colony. Cells collected from the edges of the inhibited regions (zone 1) were dead, and no spores were found there, indicating an unexpected killing zone. However, microscopic examinations of cells from the inner regions within the zone of inhibition (zones 2–7) revealed small (0.7 μm in diameter), immotile but vegetative cocci in those zones that were not detected in zones 8 and 9 (Fig. 8.4c, d) [3]. The quantity of rods increased with increasing distance from the zones of inhibition of the competing colonies (Fig. 8.4e), and only cocci were recovered from the areas closest to the competing colonies (zone 2). No spores were found in zones 1–3.

A comparison of the DNA sequences of the 16S rRNA genes of the cocci and rods showed that they were identical, ruling out contamination. The cocci were thus considered to be a new phenotype of the *P. dendritiformis T* morphotype, but it was not clear what triggered their formation. To identify differences between rods and cocci to possibly shed light on what determines the formation of each form of *P. dendritiformis*, their growth and metabolism under different conditions were compared. Re-streaked multiple times for isolation and maintained as separate stocks, cocci and rods produced pure cultures in rich media and grew at the same rates during exponential phase.

The discovery of the new phenotype in the inhibited zones could not explain the deaths of cells at the tips (zone 1), but it suggested that cocci do not exist in a normal, vegetative colony and that they are the result of the competition between the colonies. Regardless of what the findings indicate about cocci, the zone of killing identified at the tips of the colonies dictated that we test for the existence of something more than a simple inhibitory element, i.e., for a killing factor. Material from the agar between two competing colonies was therefore extracted and re-introduced near a single, isolated, growing colony. The inhibitory influence of the extract on the colony and the changes in the colony's interior structure were found to be highly similar to those observed during colony–colony interaction. Moreover, bacteria collected from uninhibited regions grew normally in LB whereas that collected from the inhibited tips showed no growth, observations that are identical to those recorded with the two competing colonies.

8.2.4 The Role of Subtilisin

The extracted material was examined for its protein content [2]. Analyses by SDS-polyacrylamide gel and by Edman degradation sequencing revealed protein bands at 32, 30, and 12 kDa. In contrast, extracts from the agar surrounding single growing colonies showed only two protein bands, at 32 and 30 kDa.

BLASTP analysis revealed that the genes encoding the 32 and 30 kDa proteins were annotated in the *P. dendritiformis* genome as flagellin and subtilisin, respectively [2]. Flagellin had no effect on colony growth or morphology, but large amounts of subtilisin inhibited growth, and only dead cells were found in the inhibited area. Material extracted from agar near subtilisin-inhibited regions was found to contain the same 12 kDa protein detected in the region between two colonies. This suggested that high subtilisin levels trigger secretion of this third protein, which is involved in the inhibition process and cell death. The 12 kDa protein corresponded to a gene predicted to encode a larger, 173-amino-acid (20 kDa) protein that belongs to the DUF1706 family of conserved hypothetical proteins. The 20 kDa gene was named dfsB (dendritiformis sibling bacteriocin) and the 12 kDa protein was named Slf (sibling lethal factor).

To further characterize the effects of the proteins on *P. dendritiformis* colony growth, single growing colonies were exposed to different concentrations of

subtilisin. While low subtilisin levels promoted bacterial reproduction and colony expansion, at higher levels it initially promoted expansion. As exposure times grew, however, it eventually inhibited expansion. Moreover, very high subtilisin levels, as mentioned earlier, resulted in the death of cells at the inhibited interface. For the case of two competing colonies grown in a large Petri dish, the very early stages of growth were characterized by faster expansion (than in later stages) along the adjacent growth fronts, a finding that is likely because of the low levels of subtilisin secreted by both colonies.

In addition to the effect of subtilisin on cell growth, colony expansion is physically limited by surface tension—the colony cannot expand fast enough to create enough new space to accommodate the reproducing bacteria. When space and nutrients are in limited supply, therefore, increases in subtilisin help elevate bacterial density, thereby leading to nutrient stress. Further experiments [2] showed, however, that when space is not limited, subtilisin promotes reproduction independent of how much is added. But under space constraints, increases in bacterial density cause them to become overpopulated, which likely triggers their production of Slf to reduce the overall number of bacteria.

8.2.5 The Slf Toxin

The introduction of extracted Slf near a single growing colony inhibited that colony's growth. In fact, it was found to be an exclusively inhibitory protein, even at extremely low levels. In addition, when Slf was added to liquid cultures prior to their inoculation, no growth was detected. For grown liquid cultures, high levels of Slf lysed bacterial cells. More importantly, the evidence of cell lysis was visible at the edge of inhibited colonies (Fig. 8.5). Colony branches exposed to the lethal protein were destroyed within a few hours, indicating that Slf is the killing factor.

Based on its migration in polyacrylamide gels, Slf secreted into the medium has an approximate molecular weight of 12 kDa, but the predicted protein sequence of the gene dfsB is 173 amino acids, or 20 kDa (Fig. 8.6). The segment in amino acid positions 5–169 (larger letters in Fig. 8.6) is associated with a conserved Pfam family domain in many bacteria. The bold segment (Fig. 8.6), associated with the isolated protein, starts with the sequenced part of the detected peptide (underlined) and continues downstream to the end of the protein. The smaller size of the purified protein (12 kDa) indicates that the protein is processed or cleaved during secretion. The DNA encoding the 20 kDa DfsB protein was cloned into an expression vector, and the protein was synthesized and purified. After purification, the 20 kDa protein was treated with subtilisin, and the products were examined by SDS–PAGE gel electrophoresis. The subtilisin treatment resulted in conversion of the 20 kDa protein to a 12 kDa protein that comigrated with the 12 kDa protein isolated from the agar medium. Colonies were exposed to both the uncleaved 20 kDa species and the 12 kDa processed protein. The 20 kDa protein had no effect on *P. dendritiformis*

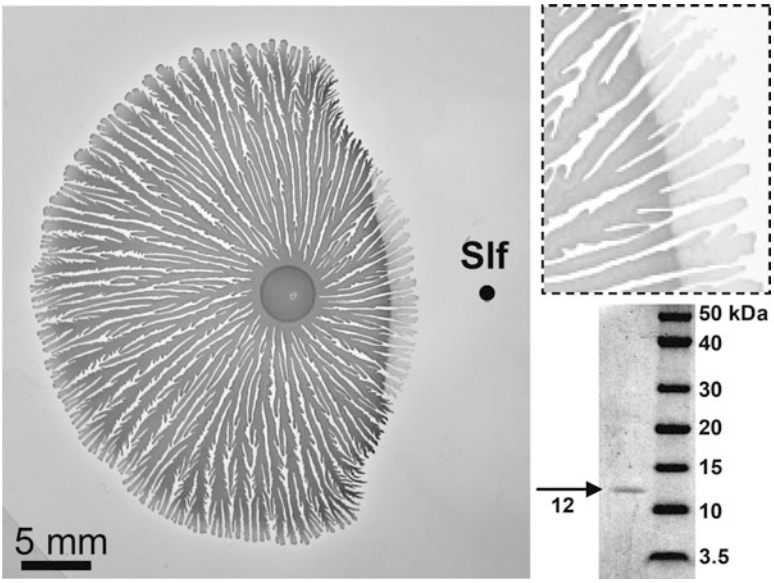

Fig. 8.5 Slf was introduced at the *black dot* near a single growing colony 4 days after inoculation. The faint region (shown at higher magnification in the inset) corresponds to lysed cells; growing branches were absent. Only a single band appeared in the gel electrophoresis results for the isolated Slf. Image was taken from [2] with minor modifications

MASYEYTSKEELKKTIHAAYLLLDGEFEGI
DDSQKDNRVPEVDRTPAEIIAYQLGWLHL
VMGWDRDELAGK**PVIMPAPGYKWNQLGGL**
YQSFYAAYADLSLTELRRLFRDTERQWLD
WIDTLSEEDLFTQSVRKWTGDKPNWPMAR
WIHINSAAPFKTFRAKIRKWKKHQRQA

Fig. 8.6 List of the 173 amino acids of the DfsB protein corresponding to the dfsB gene. *Large letters* indicate the segment at amino acid positions 5-169 that is associated with a conserved Pfam family domain. *Bold letters* indicate the segment of the isolated protein Slf. The detected peptide (Edman sequencing) is *underlined*. Image was taken from [2]

bacteria, but as was observed for the 12 kDa protein extracted from the area of inhibition between colonies, the processed 12 kDa fragment lysed growing colonies.

Tests of the toxic protein Slf on the *C* morphotype of *P. dendritiformis* showed that it is lethal, i.e., it lysed the cells. However, Slf had no effect on the closely related species *B. subtilis*. This suggests that similar to other bacteriocins, Slf has a narrow spectrum of activity.

8.2.6 *Reversible Phenotypic Switching Between Cocci and Rods*

The absence of cocci in areas of colonies not exposed to Slf suggested that the cocci were not preexisting in the population but were instead induced by exposure of the colony to Slf. Indeed, the addition of low levels of purified Slf to rod-like cultures resulted in phenotypic switching from rods to cocci, with the latter resistant to killing by Slf (Fig. 8.7). A more detailed metabolic profiling using Biolog Phenotype MicroArrays showed differences in carbon, nitrogen, phosphorus, and sulfur source utilization and in resistance to environmental stresses and antibiotics. In particular, cocci were much more resistant than rods were to osmotic stress and penicillin, indicating that there may be differences in the cell walls and membrane structures of the two morphologies. Thus, the cocci and rods exhibited striking differences in their abilities to survive and replicate under certain environmental and nutrient conditions.

Because the switch from rods to cocci was found to be an adaptive response to overcrowding [3], it seems likely that cocci could revert to the rod morphology under conditions that favor motile rods. Individual cocci inoculated on LB swarm plates (1 % agar) and monitored for the appearance of motile rods expanded slowly for the first 48 h, during which time only cocci were detected. After 50 h, rod-shaped, motile bacteria were observed at the colony edge. After an additional 6 h, rods multiplied and began to swarm in multiple layers in behavior similar to that observed in colonies initiated from single rods. At this stage cocci were not observed.

When multiple cocci colonies were present within a spot on the plate, the length of time required for cocci in each colony to switch to rods was proportional to the number of colonies initially present in the spot and to the proximity of those colonies to each other. This suggests that the transition from cocci to rods is not random but that it requires a secreted signal that is present in larger quantities when there are more colonies and when those colonies are situated closer to each other.

Such a signaling molecule may also be present in culture supernatants. To test for the presence of a secreted inducing signal, cocci were grown in LB broth for 18 h at 30 °C, and sterile supernatant from this culture was added to an equal volume of fresh medium prior to inoculation with cocci. In this culture, the shift to rods began at 18 h, whereas in culture without added supernatant, the transition did not occur until 22 h. This supports the hypothesis that a secreted factor, designated Ris (rod inducing signal), induces the switch from cocci to rods.

Rods grown in rich medium (LB) were also assessed for Ris production. Similar to the procedure followed with the coccus supernatant, the addition of rod supernatant to a culture of cocci also induced the shift from cocci to rods by 18 h. In contrast, however, exposure of the cocci culture to rod supernatant triggered the transition to the rod phenotype in more than 50 % of the cocci population by 18 h, compared to 3 % in the culture treated with coccus supernatant. The simplest explanation for this finding is that Ris is secreted in greater amounts by the rods, a scenario that may be part of a positive-feedback loop: cells that switch to the rod phenotype subsequently secrete the inducer in larger amounts, accelerating the

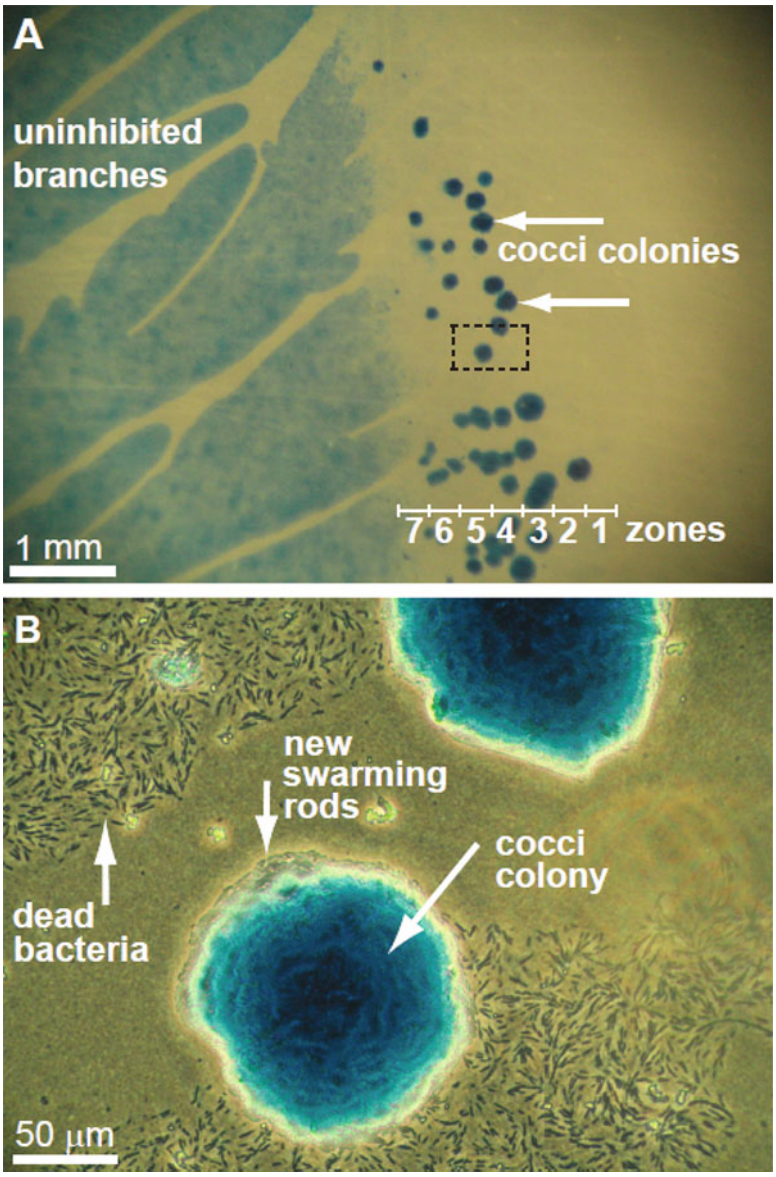

Fig. 8.7 Slf induces the switch from rods to cocci in a single growing colony. Purified Slf was placed 1 cm from the edge of the colony 4 days after inoculation. (**a**) Coccus colonies (*arrows*) in the inhibited regions 4 weeks after Slf introduction. (**b**) Higher magnification of the *marked rectangle* in panel **a**. Dead bacteria are visible in the inhibited region near coccus colonies. Rods are swarming from the edges of the coccus colonies. Image was taken from [3]

process of switching among the remaining cocci and ensuring that the transition is complete.

Ris was isolated from the culture supernatants of both rods and cocci by HPLC, individual fractions of which were tested for their ability to induce the phenotypic switch. Activity was associated with the fraction that eluted at 42 min from both rod and coccus supernatants. The fraction contained a single peak with maximum absorption at 214 nm. Placement of the isolated compound near a single coccus caused it to shift to the rod phenotype in less than 2 h, thus verifying that a specific secreted signal molecule induces the coccus to rod transition. However, Ris did not produce ninhydrin-positive spots on thin-layer chromatography (TLC) plates and did not absorb UV light at 280 nm, suggesting that it is not a peptide.

8.3 Mathematical Modeling: Self Regulation

The above results leave a key question unanswered: why is Slf produced by closely situated, neighboring colonies (or when subtilisin is added near single colonies) but not by single, isolated colonies?

To answer this question a mathematical model was developed. The model uses an approach in which bacteria, nutrients, prespores, subtilisin, and Slf are modeled as continuous fields, and the outer effective envelope of the colony is given by a smooth, time-dependent curve. The advantage of this approach is that the system is described as a free boundary problem, and the time evolutions of both the continuous fields and of the envelope can be modeled and simulated consistently and efficiently. This approach suits the problem at hand because it better describes the coarse-grained behavior of the colony rather than the exact shape of the edge of the lubrication layer. The envelope of growing neighboring colonies is depicted in Fig. 8.8a.

The main contribution of the model is the identification of a negative feedback loop that regulates the subtilisin concentration at the front of a growing colony. Figure 8.8b shows the profiles of the bacteria, nutrient, subtilisin, and Slf concentrations immediately after Slf was produced. One of the key features of the profile is that inside the colony, subtilisin concentration exceeds a critical threshold. However, this does not trigger Slf production because the motile bacterial concentration in this region is low. In line with the assumption that the exposure to subtilisin increases bacterial reproduction, it was found that high subtilisin levels at the front of a growing colony increase bacterial density. This, in turn, increases the colony expansion rate, and the bacteria at the front move farther away from the point of maximum subtilisin concentration. Subtilisin levels at the front subsequently decrease. In contrast, at low subtilisin concentrations, the opposite occurs, namely, decreases in bacterial reproduction and density and in colony expansion.

Combining the effects observed for high and low subtilisin concentrations, it was found that the subtilisin levels for a single colony reach a steady state in which the bacteria concentration is close to maximal, and therefore, the subtilisin

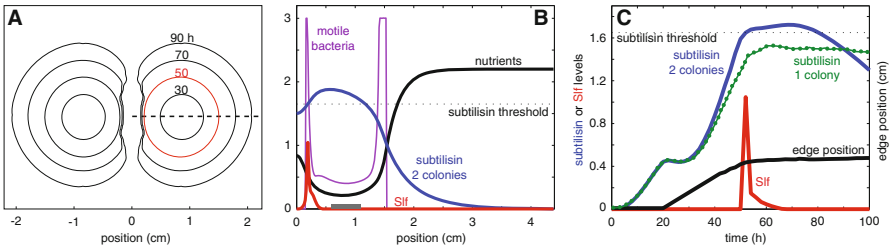

Fig. 8.8 Numerical simulation of colony competition. (**a**) The positions of the edges of competing colonies at 30, 50, 70, and 90 h. (**b**) Levels of motile bacteria (*purple*), subtilisin (*blue*), nutrients (*black*), and Slf (*red*) measured along the *horizontal dashed line* in A, 50 h after inoculation. The *gray rectangle* represents the initial inoculation droplet. For each panel, the model parameters and the initial conditions were the same; the initial distance between the colonies was 1.2 cm and the diameter of the dish was 8.8 cm, as in the experiments. (**c**) Subtilisin levels (in arbitrary units) at the moving edge of a colony as a function of time, both for a single colony (*green dots*) and for the inhibited interface in the case of two colonies (*blue line*). After 40 h, a competing colony senses its neighbor, and after 50 h the subtilisin level crosses a prefixed threshold (*horizontal dashed black line*). The *red curve* shows the level of Slf for the inhibited colony; no Slf is secreted in the single colony case. The *black line* shows the position of the edge of a colony growing toward a neighbor. The colony's edge starts to move after a lag time of 20 h, and then begins slowing down just before Slf is secreted. Image was taken from [2] with minor modifications

concentration at the front of the colony is regulated. This finding also accounts for the constant expansion rate of the colony. Likewise, an external source of subtilisin, such as a neighboring colony, can disrupt this regulatory mechanism (Fig. 8.8c). Simulations show that the disruption can happen via two routes: (1) added subtilisin from the neighboring colony and (2) nutrient depletion in the inhibited region between colonies, which increases the sporulation rate. The additional prespores, in turn, promote further increases in the subtilisin level. Another effect of nutrient depletion is the deceleration of colony expansion at its growth front. As explained previously, the bacteria at the front approach the area of maximum subtilisin concentration, and as a result, they reproduce faster than the colony can expand.

Bacterial stress under such conditions cannot be resolved through sporulation, as (1) sporulation requires additional nutrients, which may not be present, and (2) sporulating bacteria are assumed to secrete high levels of subtilisin, which would reduce the probability of colony survival. To ensure colony survival, therefore, it is suggested that the bacterial colony quickly reduce its population level. Indeed, the model predicts that the secretion of Slf will rapid lower the bacterial population, a finding that is consistent with laboratory observations.

8.4 Colonies of Closely Related Strains

The observations described so far summarize the results for the branching (*T*) morphotype of *P. dendritiformis*. Although its other morphotype, the chiral (*C*) morphotype [20, 21] has the same 16S rRNA ribosome, it differs in terms of several physical properties such as cell length, colony structure, and mode of migration [30]. We therefore decided to examine and compare the interactions between two *P. dendritiformis* sibling colonies of the *C* morphotype and between one each of the *T* and *C* morphotypes. In all cases the two colonies were inoculated simultaneously on the same plates. Compared with our results for the analyses of the interaction between two *T* morphotypes, our observations of interactions involving *C* morphotype (*C*–*C* or *T*–*C*) colonies indicated greater complexity, a finding rooted in the nature of the chiral morphotype and also due to the fact that we investigated the behavior of two distinct sub types of *C* (*C*+ and *C*−). Compared to the *C*− genome, that of *C*+ includes several additional segments. Bioinformatics analyses indicate that the extra segments are associated with a viral origin. Likewise, the *T* morphotype has *T*+ and *T*− sub types that correspond roughly to the +/− sub types of the *C* morphotype, and that undergo morphotype transitions to *C*+ and *C*−, respectively. Indeed, the sub types of the two morphotypes share many characteristics, but it is easier to induce morphotype transitions between the *T*− and *C*− sub types. Observations of colony–colony interactions described thus far (here, and in [1–3]) were of the *T*− sub type, but we are also working on interaction between *T*+ sub type sibling colonies (some preliminary work has already been done [27]).

Competition between two *C*− sub type colonies (Fig. 8.9a) showed results similar to those observed for the competition between two *T*− sub type colonies as well as between *T*− and *C*− sub type colonies. Also, Slf extracted from competing *T*− sub type [1] colonies lysed cells of a single growing *C*− sub type colony (see Fig. 8.5 in [2]). These competition results were, to an extent, expected because of the biological similarity between the two morphotypes (the genomes of the *T*− and *C*− morphotypes are very close).

C+ sub types, however, behaved differently. On the one hand, two *C*+ sub type colonies grown in the same Petri dish merged (Fig. 8.9b), suggesting that *C*+/*C*+ competition does not occur either because the *C*+ morphotype does not produce enough subtilisin to trigger the secretion of Slf or because it somehow blocks the regulation circuit. On the other hand, experiments in which *C*+ sub type colonies were grown near colonies of the *T*− sub type (Fig. 8.9c) or near *C*− sub type colonies did show the deadly competition observed in the previous tests of the *T* morphotype.

These results demonstrate that the regulatory process behind the competition behavior of *P. dendritiformis* is highly intricate, mainly due to the extra segments of viral origin present in the genome of the *C*+ morphotype. Therefore, *P. dendritiformis* should be researched further for its potential to provide important clues about the role of virus-mediated horizontal gene transfer between bacteria.

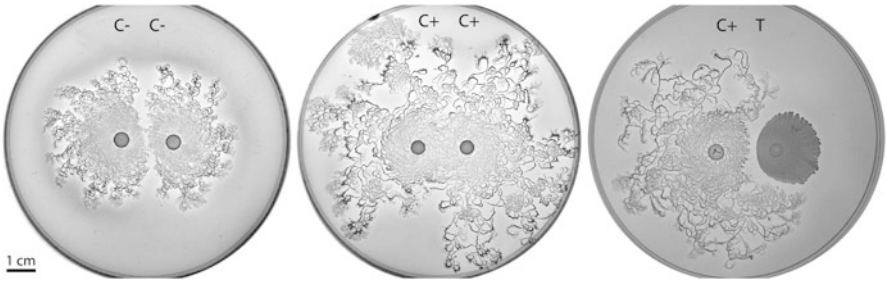

Fig. 8.9 Interaction between neighboring *P. dendritiformis* colonies growing on a 1.0 % agar with 2 g/L peptone. (**a**) Two *C*− colonies. A gap between the colonies is formed. (**b**) Two *C*+ colonies. No gap is formed. (**c**) Two colonies, one *C*+ and the other *T*, showing the gap that formed between them

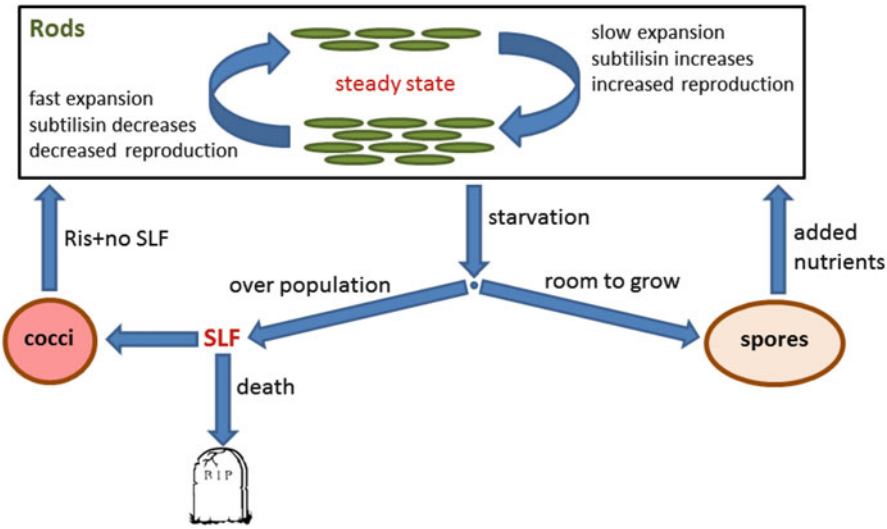

Fig. 8.10 A schematic diagram showing the different subtilisin-regulation stages of *P. dendritiformis*. At normal rich growth conditions, the cells are rods. The density at the tip of a growing colony is regulated by a negative feedback loop between the local concentration of subtilisin and the colony's expansion rate. Starvation or a close sibling colony disrupts this steady state, and as a result, bacteria either engage in sporulation or in the production of Slf. The presence of Slf in the growth medium quickly reduces the cell population and promotes the phenotypic transition of the bacteria into a more resilient cocci phenotype. When conditions are again favorable, cocci can switch back to the typical rod-shaped bacteria

8.5 Summary

The results indicate that *P. dendritiformis* has at least two mechanisms to deal with changing environmental conditions and to enable its long-term survival (Fig. 8.10). First, it has the ability to form spores that are highly resistant to harsh conditions.

The second mechanism is the formation of cocci—less resistant than spores but able to replicate even in the presence of Slf—which confers on cells near the leading edge of the colony the ability to continuously monitor the level of competition and the environment and to respond appropriately when sufficient nutrients are available for colony expansion. The shift between rods and cocci performed by *P. dendritiformis* requires the specific secreted bacterial signals Slf and Ris, which induce the relevant transition (rods to cocci or cocci to rods) in response to environmental cues. Thus, the population can be maintained as either rods or cocci under the appropriate conditions. For example, a culture consisting of all rods should contain high levels of Ris as the factor maintaining the population in the rod state. Under normal growth conditions, no Slf would be secreted and no transition to cocci would occur. In the event of sudden overcrowding, as in the case of encroaching colonies, Slf is produced, killing most of the rods at the leading edge. This killing of rods is essential to eliminate Ris production and enable the transition to cocci in response to low levels of Slf, a scenario that is apparent at the edges of the colonies on solid media, where cocci are found in areas that also contain dead rods (Fig. 8.7).

The ability to replicate and maintain the coccoid form in an inducible manner distinguishes this phenotype switching from other phenotypic changes—such as persistence cells, sporulation (which leads to a dormant state), or formation of cocci in stationary phase—as it allows *P. dendritiformis* to adapt to changing environmental conditions. Although this form of phenotypic switching has not been described previously, genes of unknown functions with homology to the *P. dendritiformis* Slf encoding gene are widespread in bacteria and yeast. Therefore, the lethal response to competition and associated phenotypic switching that have been observed in *P. dendritiformis* may be a common but previously unrecognized mechanism for regulation of population growth in nature.

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