Cell population migration cycle and response to environmental pH in *Bacillus subtilis*

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Running title: Cell population migration cycle in *B. subtilis*

Abstract

Microbial populations are ubiquitous and some form a robust, multicellular structure called biofilm that protects the cells from environmental damage. The dispersion cycle is critical for controlling the population of important target microbes (both pathogenic and beneficial). Here, we show that the hysteresis of *Bacillus subtilis* cell-type regulation with respect to auto-inducing signal strength triggers the migration cycle of the cell population. We investigate migration cycle and its dependence on environmental conditions by quantitative analysis of cyclically expanding, concentric circular colonies. Next, we construct an input/output model that controls cell types in response to environmental conditions and signal density. On the basis of this model, we propose a migration cycle model for cell populations. The proposed model will widely predict biofilm-related phenomena and provide the basis for the description of highly self-regulating multicellular systems.

Introduction

Microbial populations form colonies and they often exist stably in a robust system called biofilm[1-8]. The structural robustness of biofilms makes it difficult to kill disease-related microorganisms[9-11]. In addition, myriads of useful microorganisms are also being discovered, so controlling the population of target microorganisms is an urgent issue[12-14]. The process of biofilm formation is as follows: (i) bacteria attach themselves to suitable surfaces available in the environment; (ii) they proliferate while secreting extracellular polymeric substances (EPS) to form a biofilm; (iii) when the colony becomes large and the cell density increases, the outer wall is destroyed and mobile cells are released. These released cells wander around in search for a new environment suitable for growth. When they identify an environment that satisfies their growth requirements, they become non-migrating cells and colonize that environment. To summarize, these cells return to the first phase of biofilm formation as described in (i) above. This cycle of repeating a series of phases is called the life cycle of a bacterial biofilm. The concept of life cycle was originally used for individual organisms, whereas in a biofilm, it is used for

a group of cells. Therefore, it involves multilevel interactions, such as information transfer within and between groups. However, the details of the biofilm life cycle generation mechanism are not well understood.

A phenomenon similar to the life cycle of biofilms is the formation of concentric circular colonies that embody the migration cycle of bacterial cell populations. (Fig. 1). *Proteus mirabilis*[15-18] and *Bacillus subtilis*[18-23] are known to form concentric colonies. During their formation, the dominant cell population for colony growth switches periodically between migrating and non-migrating states. In *B. subtilis*, the migrating cell population consists of motile cells, which can swim by bundling and rotating multiple flagella[24]. Particularly, two-dimensional collective movement, called swarming, by motile cells plays a central role in rapid space expansion during the migration phase of the life cycle[25-27]. There are three possible factors that cause the cell population to become non-migrating—without swarming motility—: (i) switching to an immotile cell type; (ii) cessation of hyperflagellation; and (iii) insufficient concentration of surface-wetting molecules, such as surfactin. In the case of this *B. subtilis* concentric colony, the non-migrating cell population is in the form of matrix-producer-like chains of proliferating cell populations, and the cells do not move at all[21]. Therefore, the major mechanism underlying the stoppage of swarming involves the predominance of immotile cell types, most of which are matrix producers. Although the regulation of surface-wetting molecule secretion and hyperflagellation should also be involved in the stabilization of the cycle, we will consider this subject in a future research. Thus, here, we focus on cell type switching. A matrix producer does not move, and it proliferates while producing extracellular matrices[28]. The number of cells increases because of cell division, but in this phase, intercellular adhesion is strong and separation is rare[29, 30]. Therefore, the cell group looks like a thread that continues to grow, and its growth mode is called chaining or bundling. The colony not only expands two dimensionally but also increases in thickness (grows in the direction perpendicular to the medium plane). Cells called surfactin producers[31, 32] are known to coexist to assist the slow two-dimensional expansion in this case, improving sliding mobility

on the medium surface[33], although it is far behind the expansion speed during the migration phase. In this way, the repetition of the two phases, the migration phase in which cells rapidly spread on the surface of the medium and the growth phase in which migration stops and the thickness increases, exactly represents the migration cycle of *B. subtilis* concentric colonies.

Although the process of concentric colony formation is known in this way, the detailed mechanism remains unclear. This is because of the lack of information on environment-dependent control patterns between different cell types, mobile and non-mobile. By focusing on *B. subtilis*, various mutually exclusive cell types have been discovered [24]. The balance between motile cells and matrix producers is particularly important for the migration cycle of the cell population. The genes related to each of these two types are mutually exclusive because of self-activation and mutual repression. When this mutual exclusivity becomes stronger, hysteresis occurs in cell-type regulation and a migration cycle is induced in the cell population, resulting in the formation of concentric circular colonies. Specifically, whether or not concentric colonies are formed depends on the presence or absence of hysteresis during cell-type control of the cell population and on environmental conditions. Investigating the conditions for forming concentric colonies is essential for understanding the life cycle generation mechanism of cell populations.

However, the conditions for forming concentric colonies of *B. subtilis* have not yet been clearly determined, although the need for agar concentrations of about 0.7% in solid medium and high nutrient conditions is known[19, 21]. Particularly, the agar concentration is required to be in the range of 0.65–0.75%. In addition, the incubation temperature is preferably 25–35°C, which is lower than the usual 35–37°C[19], and long-term pre-drying of the medium surface is desirable[21]. The situation seems to be complicated by the effect of temperature and surface moisture other than agar and nutrient concentrations. Therefore, we introduced environmental pH as a new parameter and attempted to clarify the growth condition requirements of *B. subtilis*.

Except for concentric colonies, the dominant cell type is often defined as either a motile cell or matrix

96 producer. First, colonies formed by swarming motile cells are formed under an agar concentration of
97 0.4–0.7%. These colonies are classified into two types according to the nutrient concentration: disk-
98 like (high nutrition)[34, 35] and dense branching morphology (DBM)-like (low nutrition)[36-39]. On the other
99 hand, the slowly expanding colonies dominated by matrix producers are formed on a solid medium
100 with an agar concentration of 0.7% or more. These colonies are also classified into two types, namely,
101 Eden cluster-like (high nutrition)[40-42] and diffusion-limited-aggregation (DLA)-like (low nutrition)[43-
102 46], depending on the nutrient concentration[44, 45]. In general, the classification is based on these five
103 types. A detailed examination revealed that a small number of motile cells were mixed even on the
104 surface of a hard medium with an agar concentration of 1%, which affected macroscopic colony
105 morphology and expansion rate[47]. Particularly, a crater-like pattern in which cells gather at the tip of
106 the colony is formed by only slightly lowering the environmental pH from the intracellular pH of 7.4.
107 This is due to chemotaxis toward nutrients, which is a phenomenon that does not occur during
108 swarming[27]. Chemotaxis is also observed in concentric colonies. Specifically, in the inner region after
109 the end of the migration phase, slow swimming migration of the cell population occurs because of
110 chemotaxis from the inside to the outside, and as a result, the ring tip becomes longer. The mechanism
111 of this phenomenon is the same as that for crater-like pattern formation. On the other hand, when the
112 agar concentration is low (0.5%), swift colony expansion via swarming is observed because the energy
113 required for migration is low due to the availability of abundant water on the surface of the medium.
114 However, under certain conditions, i.e., optimum pH of 7.4, the dominant cell types become matrix
115 producers rather than motile cells, and lacework-patterned colonies may be formed[23]. Thus, other than
116 concentric colonies, different stress parameters, such as environmental pH, influence the choice
117 between motile cells and matrix producers.
118 Here, we investigate the effect of environmental pH on cell-type selection and concentric *B. subtilis*
119 colony formation. By quantitative analysis of culture experiments, we clarify the range of
120 environmental pHs in which concentric colonies are formed, and the environmental pH dependence of

the temporal and spatial cycles within that range. Further, by combining the mathematical model of cell-type regulation on the basis of the conventional gene regulatory network and analysis of its mathematical properties, we propose a model for type regulation of cell population that changes depending on the environment. Our proposed model provides a reasonable explanation for the formation of concentric circular colonies and subsequent biofilm life cycle generation.

Results

Concentric colonies embody the biofilm life cycle

We prepared a medium with a high nutrient concentration and an intermediate agar concentration of 0.7% among the range of solid agar media (containing 0.2% or more agar), and inoculated *B. subtilis* onto the center. When the plate was cultured at about 35°C, concentric colonies were formed on the medium surface (Fig. 1). This is a simple model for biofilm life cycle generation, and its temporal and spatial cycles embody that life cycle. We captured two-dimensional transmitted light images of this colony using a scanner (Fig. 1a). The image data were converted to cell density distribution (Fig. 1b), using which a 3D structure was reconstructed (Fig. 1c), and the cross-section was analyzed (Fig. 1d). By performing such quantitative analyses in a time series, not only the two-dimensional cell density (colony thickness) but also the dynamics of cell proliferation and migration were analyzed (Fig. 2). The boundaries of colonies were identified at each time and the depth of each terrace was measured (Fig. 2a, b). The concentric colonies of *B. subtilis* have distorted boundaries unlike those of *Proteus mirabilis*. Therefore, the colony radius r was defined from area A of the inner area of the colony: $r = \sqrt{A/\pi}$. Then, from the increase in the colony radius, the migration and growth phases of *B. subtilis* biofilm, and their combined life cycle were observed (Fig. 2c).

Microscopic observation of phase switching in concentric colonies

Using phase-contrast microscopy, we investigated how the concentric colony formation phase

transition occurred, and how the cell state and its spatial distribution changed. First, the termination process of the growth phase, in which matrix producers are dominant, begins with the appearance of motile cells just before the colony boundary (Fig. 3a). There, motile cells continue to grow and form ponds wherein cell swarming occurs. The surrounding cells are partially separated from each other by autolysins[29, 30, 48, 49], and the pressure of proliferating motile cells is also applied, destroying many points in the colony border area.

Among those destroyed points, the breakage starts at points on the outer side of the colony boundary, and motile cells flow out from there to form branches (Fig. 3b, c, Supplementary Video S1). The migration phase where motile cells are dominant is shown in Fig. 3d-f. In this phase, the formed branches quickly expand outward because of swarming (Fig. 3d, Supplementary Video S2). Then, the number of break points near the root increases, and the gap between branches is filled with outflow cells from the inside (Fig. 3e, f, Supplementary Videos S3, S4). The end of the migration phase begins when the branches stop growing (Fig. 3g). Immediately thereafter, the swarming area shrinks from the tip of the branch toward the inner root (Fig. 3g-i). When there is no swarming region, the growth phase begins. In the growth phase, the cells do not move and proliferate, and the cell density increases. The cell density is considered to be measured by the cells themselves by the concentration of the secreted quorum-sensing factor ComX[50-52]. When the concentration exceeds a certain threshold, motile cells reappear, and transition to the migration phase occurs (Fig. 3a). This is one round of the life cycle of *the B. subtilis* cell population.

Effect of environmental pH on periodically expanding colonies

The conditions for forming concentric colonies of *B. subtilis* have been investigated. Particularly, under optimal conditions (incubation temperature around 35–37°C), concentric circular colonies are formed only in an extremely narrow range of agar concentration of 0.65–0.68%, even if nutritional conditions are suitable[21]. It can be inferred that other stress parameters, such as low temperature[19], are

necessary for the formation of concentric colonies. We investigated the effect of environmental pH as a stress parameter. Conditions other than environmental pH were optimal, and the agar concentration was set at an intermediate level of 0.7% (Fig. 4). First, in the neutral pH region, no periodically growing colonies appeared. Matrix producers were the predominant cell type, and colonies grew by increasing their volume via proliferation (Fig. 4a, b). As the environmental pH decreased, many, very-short migration phases appeared around pH 6.8. Under the pH condition close to this transition point, the variation in space was very strong, the periodicity was not clear, and the pattern was far from that of concentric circles. When the pH decreased below 6.5, concentric circular colonies that expand periodically were formed. When the pH was lower than 5.3, colony formation itself became unstable, and the colony formation stopped halfway or no colonies were formed. Finally, below pH 5.1, colonies were not always formed. In summary, the stable pH range for cyclic growth of colonies was 5.3–6.5.

Next, we investigated the properties of concentric colonies in this pH range. First, the circularity of the ring was constant, although there was some variation in the pattern transition region near the neutral pH (Fig. 4c). The spatial period (width of the swarm ring) seemed to increase monotonically as pH decreased (Fig. 4d). Specifically, in the pattern transition region (pH 6.0–6.5), the spatial period sharply increased with decreasing pH, and then it had a substantially constant period in the range of pH 5.3–6.0.

To clarify how these differences in colony morphology were produced, we examined the periods of each growth phase and migration phase (Fig. 4e). We found that while the total time period was almost constant, the growth phase duration decreased as the pH decreased; conversely, the duration of the migration phase increased. Specifically, the finer spatial patterns reflected shorter migration periods, while the total length of the life cycle of the cell population seemed homeostatic.

Environment-dependent regulation of cells and cell populations

We investigated the mechanism of control of the two different states of the cell population (migration

192 and growth phases). Cell population control should be understood on the basis of cell-level regulation
193 in response to environmental conditions The regulation between the two cell types, motile cells and
194 matrix producers, is well studied, particularly the switch between planktonic and biofilm states[24, 53-56].
195 The two cell types are designed to have mutually exclusive gene expression states. We created a simple
196 mathematical model to investigate cellular state dynamics (Fig. 5a). In this model, cell states can be
197 described according to four group variables by grouping coordinated genes and their products: (1) S:
198 A group represented by Spo0A~P, which also includes phosphorelay of Spo0F, Spo0B, and Spo0A;
199 (2) H: SigH; (3) A: AbrB; (4) C: A group represented by ComK, driven by the ComX-ComP-
200 ComA pathway.
201 The output of this control system is the cell type. In this case, it is a matrix producer when S is
202 high and a motile cell when S is low. Sporulation is initiated when S is continuously very high.
203 However, predominant sporulation occurs at a nutrient-depletion stage much later than colonization.
204 Therefore, it is not necessary to consider sporulation here. On the other hand, the inputs of the cell
205 state control system include external environmental conditions and auto-inducing signals that represent
206 cell density. There are various environmental conditions. For example, poor nutritional conditions may
207 be input to activate S, but we considered the formation of periodic growth colonies, and the initial
208 nutrient concentration was sufficiently high. Therefore, the nutritional conditions were constant and
209 sufficient at the time of colony formation. As an auto-inducing signal related to cell-type control, a
210 small peptide ComX is secreted by *B. subtilis* cells[50-52]. In the following equation, its concentration is
211 denoted as X, which is an input from C to the cell-type regulation system[50, 51, 57]. The input mode of
212 environmental pH is of significant interest at this stage. According to previously performed studies,
213 H or A is the candidate input point for external pH in the current model[58, 59].
214 Here, we considered the most minimal and smooth dynamic model of these four variables
215 ("Methods"). The curve of the set of equilibrium points can be divided into two types: with respect to
216 the increase and decrease in X, the variable that represents the cell state (for example, S) is

monotonic and non-monotonic (Fig. 5b, c)[60]. In the latter case, the curve is an S-shaped curve with two turning points. Specifically, there are two cases where cell-type control is either not hysteretic (Fig. 5b) or hysteretic (Fig. 5c), considering the increase/decrease in the cell density signal X. The latter hysteretic control is necessary for biofilm life cycle generation and concentric colony formation. When there is hysteresis in the choice of cell state for increasing and decreasing X, a life cycle is created for the cell population as follows. Migration phase: the cell density information X at the growth front decreases as the cell population disperses in the motility state, and when it falls below a certain threshold X_1, the cell population switches to the matrix-producing state. Growth phase: as the cell population grows and matures in the matrix-producing state, X increases, and when it exceeds a certain threshold $X_2\left(>X_1\right)$, the cell population switches to the motility state again.

AbrB is upregulated and SigH is suppressed as the environmental pH decreases in the weakly acidic region of interest[58, 59]. These two are potential candidates for inputting environmental pH information. It is not easy to determine which of these two elements, which suppress each other in the regulatory network, is upstream for inputting external pH information. According to the detailed mathematical analysis of the equilibrium state of this model, a situation in which hysteresis occurs with a decrease in environmental pH and concentric colonies are formed can be realized only when AbrB is the input point for pH (Fig. 5a, d; "Methods")[60] . Therefore, we proposed a model of cell-type regulation using AbrB as the input point for environmental pH information (Fig. 5a). On the basis of this cell-type regulation model, the generation mechanism of the life cycle of the cell population in the parameter range for hysteretic control could be explained (Fig. 5c), and the dependence on environmental conditions could be understood.

Discussion

We have presented a possible mechanism of formation of *B. subtilis* concentric colonies that expand periodically and embody the life cycle of the cell population. By performing culturing experiments

and their quantitative analysis, the dynamics of colony growth and its environmental pH dependence were clarified from a multilevel perspective—from the cell level to the macroscopic morphological level. We sought to understand patten formation under the assumption that the predominant cell type switches between motile cells and immotile cells, such as matrix producers. Based on the accumulation of molecular biology research to date, the outline of the gene regulatory network responsible for the regulation between these two cell types of *B. subtilis* has been established. We proposed a model of cell-type regulation by environmental conditions and cell density by combining concentric colony culture experiments under different environmental pH conditions. The mathematical properties of the minimal smooth model describing the dynamics also suggested the input location of environmental pH in the cell-type regulation system. The obtained model should be a general control mechanism that selects an appropriate cell population structure depending on the situation. Particularly, the S-shaped curve of the set of equilibrium points obtained by this mathematical model analysis makes it possible to understand the hysteretic control of the cell population that forms concentric colonies, and subsequently, the migration cycle generation mechanism.

In the cell population migration cycle, phase switches are dependent on cell density. We investigated the switching mechanism and found that the migration cycle was stably modulated via hysteretic control of cell type. Further, the effect of environmental conditions can be predicted. At present, however, environmental changes and cell type control were investigated via concentric circle colony formation experiments under environmental pH (and only neutral to acidic) conditions. In the future, we plan to elucidate how *B. subtilis* cells collectively cope with fluctuations in a large range of pH, particularly via the study of the responses at high pH. Moreover, to predict the response to changes in other environmental conditions, such as nutrient concentration, temperature, and oxygen concentration, it is necessary to understand a wider gene regulatory network. Nevertheless, the conditions for biofilm life cycle generation can be better organized as per the presence or absence of hysteresis. Of note, this classification will probably be applicable to biofilm life cycle studies focusing on other microbial

266 species.

267 A morphological diagram was created for understanding the colony morphology of *B. subtilis* in the
268 1990s[34, 61], in which one of the five colonies was formed depending on the two parameters, nutrient
269 concentration and agar concentration, in the medium. These five colonies are called Eden cluster-like[40-
270 42], DLA-like[43-46, 62, 63], concentric ring-like, disk-like[34, 35], and DBM-like[36-39], respectively. The concept
271 of cell type was not established at the time, but it is now well-understood, and the colony pattern can
272 be explained as follows. The dominant cell type is immotile cells, such as matrix producers, in Eden
273 cluster-like and DLA-like colonies; disk-like and DBM-like colonies have motile cells as the
274 predominant cell type. Finally, in the concentric ring-like colony, the dominant cell type switches
275 between motile cells and immotile cells. Thus, among the five patterns, concentric colonies had
276 multiple dominant cell types, which created unsolved problems. The mechanism of pattern formation
277 was unknown, and it was not understood or reproduced by a mathematical model[64]. Our proposed
278 model for cell-type regulation has presented one possible mechanism for this problem.

279 Furthermore, the concentric colonies presented uncertainties, even with respect to the formation
280 conditions, such as an extremely narrow agar concentration range[21] and the dependence on culture
281 temperature[19]. Apparently, parameters other than agar and nutrient concentrations were likely involved
282 in the formation of concentric colonies. In other words, introducing some kind of a stress parameter is
283 effective for concentric pattern formation. We obtained the conditions to stably form concentric
284 colonies by introducing environmental pH. By drawing the morphology diagram in a three-parameter
285 space, it is possible to understand the entire cell population morphology correctly and in detail. By
286 incorporating our cell-type regulation model into a multilevel morphogenesis model, we expect to
287 obtain a simulation model that accurately predicts the growth of multicellular systems with non-
288 uniform cell state distribution.

289 Robust biofilms and other complex cell populations of microorganisms are social structures involved
290 in the organization of diverse cell types. Therefore, understanding the mode of state regulation of each

cell should be the first key to understanding and controlling the behavior of the cell population as a whole. Therefore, higher-order structures of cell populations are self-organized in a trans-hierarchical manner: from gene-level and cell-level control to microscopic structures, macroscopic morphology and function of cell populations, and interaction with the surrounding environment. Thus, the construction of multilevel models for controlling the state of cell populations will strongly advance the research on various multicellular societies.

Methods

Experimental methods

B. subtilis wild-type strain OG-01 (JCM32485) was used[44, 65]. A very small quantity of bacteria (endospores) was incubated overnight (10–16 h) in 5 mL of Luria–Bertani liquid growth medium (LB Broth Base (Lennox L Broth Base), Gibco BRL Life Technologies, cat. no. 12780-052) at 35°C with shaking at 140 rpm. For inoculation on solid agar media, this final pellet was diluted with the buffer to an OD_{600} of 0.5.

A solution of 86 mM NaCl and 29 mM K_2HPO_4 was prepared. As a nutrient, 20 g L^{-1} peptone (Bacto Peptone, BD Biosciences, cat. no. 211677) was added to the solution, and the pH was then adjusted to a designated value by adding 6 M HCl, and 0.7% agar (Bacto Agar, BD Biosciences, cat. no. 214010) was added to the above liquid nutrient medium. After autoclaving at 121°C for 15 min, 20 mL of the solution was poured into each plastic Petri dish. The dishes were maintained at room temperature overnight and dried at 50°C for 90 min.

The pre-cultured bacterial suspension was inoculated using a sterilized needle at the center of the prepared solid medium surface. The dishes were incubated in a humidified box at 35°C for the designated time. Macroscopic colony-transmitted light images were obtained using a flat head scanner (GT-X980, Epson). Cell or cell cluster level morphology was observed by phase-contrast microscopy using an inverted microscope (IX83, Olympus) with a camera (DP80, Olympus).

315 **Mathematical model of dynamic cell-type regulation.**

316 To describe the dynamics of cell-type control (Fig. 5a), we consider the following minimal smooth

317 model:

318
$$\begin{aligned}\frac{dS}{dt} &= \frac{c_S H}{a_S + b_S C} - d_S S \\ \frac{dH}{dt} &= \frac{c_H}{a_A + b_A A} - d_H H \\ \frac{dA}{dt} &= \frac{c_A}{a_A + b_A S} - d_A A \\ \frac{dC}{dt} &= c_C X A - d_C C\end{aligned} \tag{1}$$

319 where $a_*, b_*, c_*, d_* \ \left(* = S, H, A, C\right)$ are positive constants. There are two cases where cell-type

320 control is non-hysteretic (Fig. 5b) and hysteretic (Fig. 5c), regarding the increase/decrease in the cell

321 density signal *X*. We set a parameter indicating the rate at which AbrB is activated from outside the

322 model system of cell-type regulation (equation (1); Fig. 5a) as follows:

323
$$I_A = \frac{b_H}{a_H} \times \frac{c_A / a_A}{d_A} \tag{2}$$

324 Similarly, we define the rate at which Spo0A~P and SigH are activated from outside the model

325 system as

326
$$I_{SH} = \frac{b_A}{a_A} \times \frac{c_S / a_S}{d_S} \times \frac{c_H / a_H}{d_H} \tag{3}$$

327 Particularly, the external activation rate of AbrB $I_A = I_{\mathrm{AbrB}}$ is the horizontal axis in Fig. 5d. Thus, the

328 following can be proved[60]: There exists $I_{SH}^* > 0$ such that

329 1. If $I_{SH} \le I_{SH}^*$, then the steady states are anhysteretic (Fig. 5b).

330 2. If $I_{SH} > I_{SH}^*$, then there exists $1 < I_{A,L} < I_{A,U}$ such that

(i) If $0 < I_A \leq I_{A,L}$, then the steady states are anhysteretic (Fig. 5b).

(ii) If $I_{A,L} < I_A < I_{A,U}$, then the steady states are hysteretic (Fig. 5c).

(iii) If $I_{A,U} \leq I_A$, then the steady states are anhysteretic (Fig. 5b).

Case 2 above (the external activation of Spo0A~P and SigH is larger than the threshold $I_{SH} > I_{SH}^*$) shows the situation in Fig. 5d, and it is also in agreement with the experimental results, suggesting that $I_A = I_{\mathrm{AbrB}}$ plays the role of a parameter corresponding to the environmental pH reduction. Note the mathematical feature that at least AbrB must be actively involved to reproduce the pH dependence of cell type control[60]. In the past literature, SigH or AbrB, especially SigH, was a candidate[58, 59], but at least SigH alone does not show the observed pH dependence of cell type control in the mathematical model.

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Author contributions

S.T., M.N. and W.S. conceived the study. S.T. and M.N. designed and performed the experiments, and analyzed the data. S.T. and M.N. constructed the mathematical model. S.T., M.N. and I.T. analyzed the model. S.T. and M.N. wrote the manuscript. All authors reviewed the manuscript.

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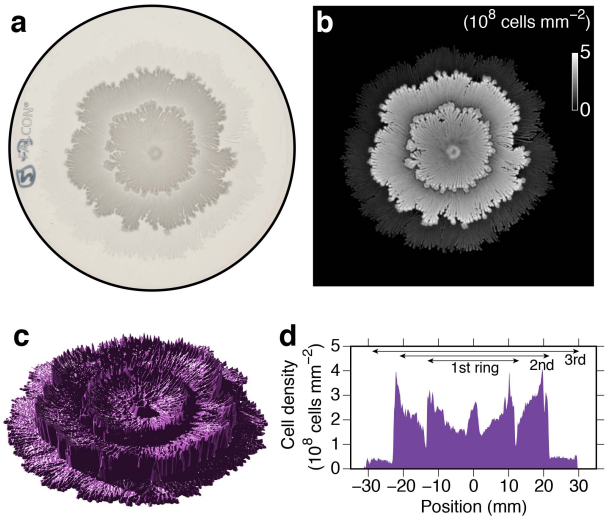

561 **Fig. 1 Concentric ring-like colony embodies the life cycle. a** Raw image of transmitted light obtained
562 by a flat scanner. The center is the inoculation point. The diameter of the dish is 88 mm. **b** Two-
563 dimensional cell density. **c** Three-dimensional reconstruction of the colony. **d** Cross-sectional view.
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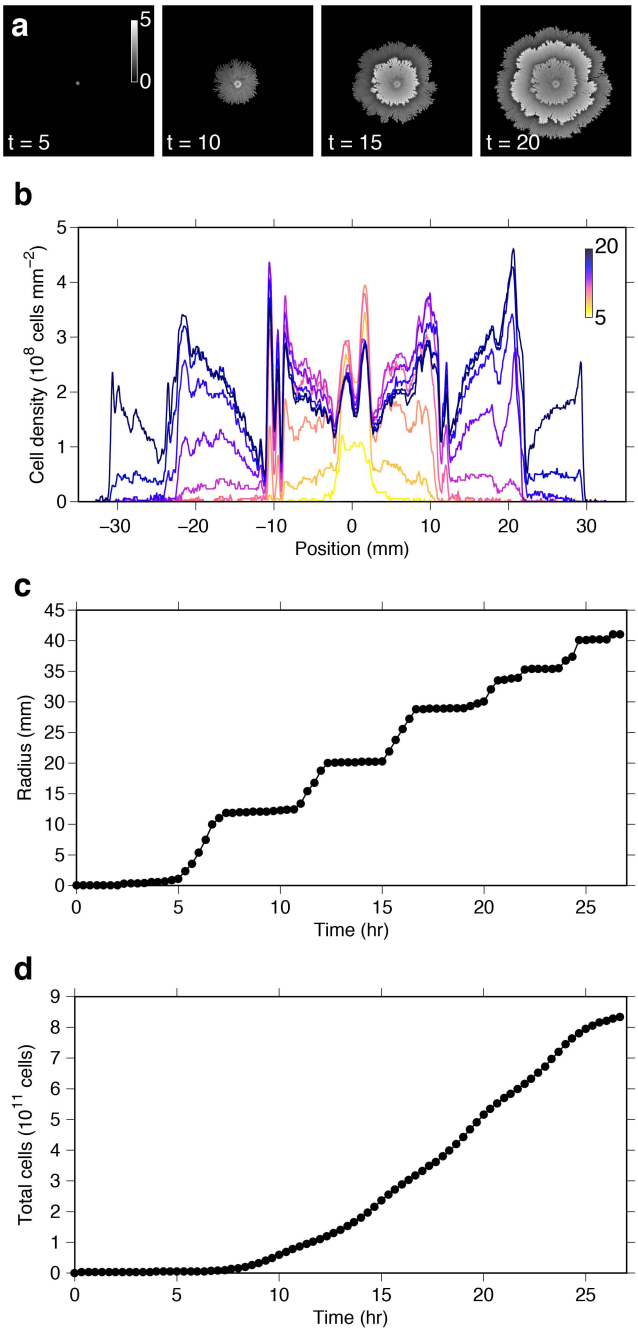

565 **Fig. 2 Growth dynamics of concentric ring-like colony. a** Two-dimensional cell density. Color bar,
566 cell density (10^8 cells mm^{-2}). Incubation time, 5, 10, 15, and 20 h after inoculation. **b** Cross-section
567 cell density. The center is the inoculation point. **c** Increasing colony radius. **d** Increasing total number
568 of cells.

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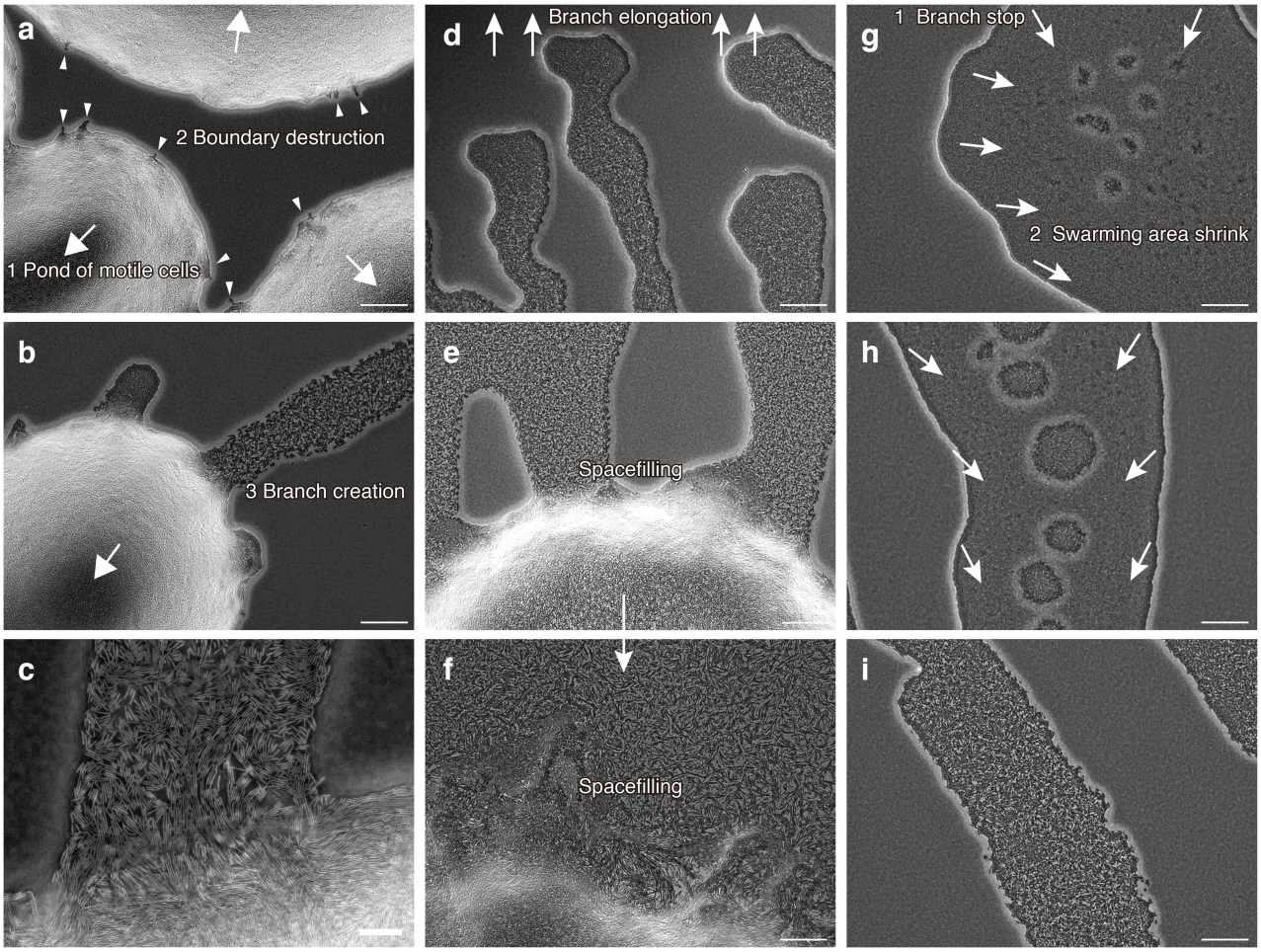
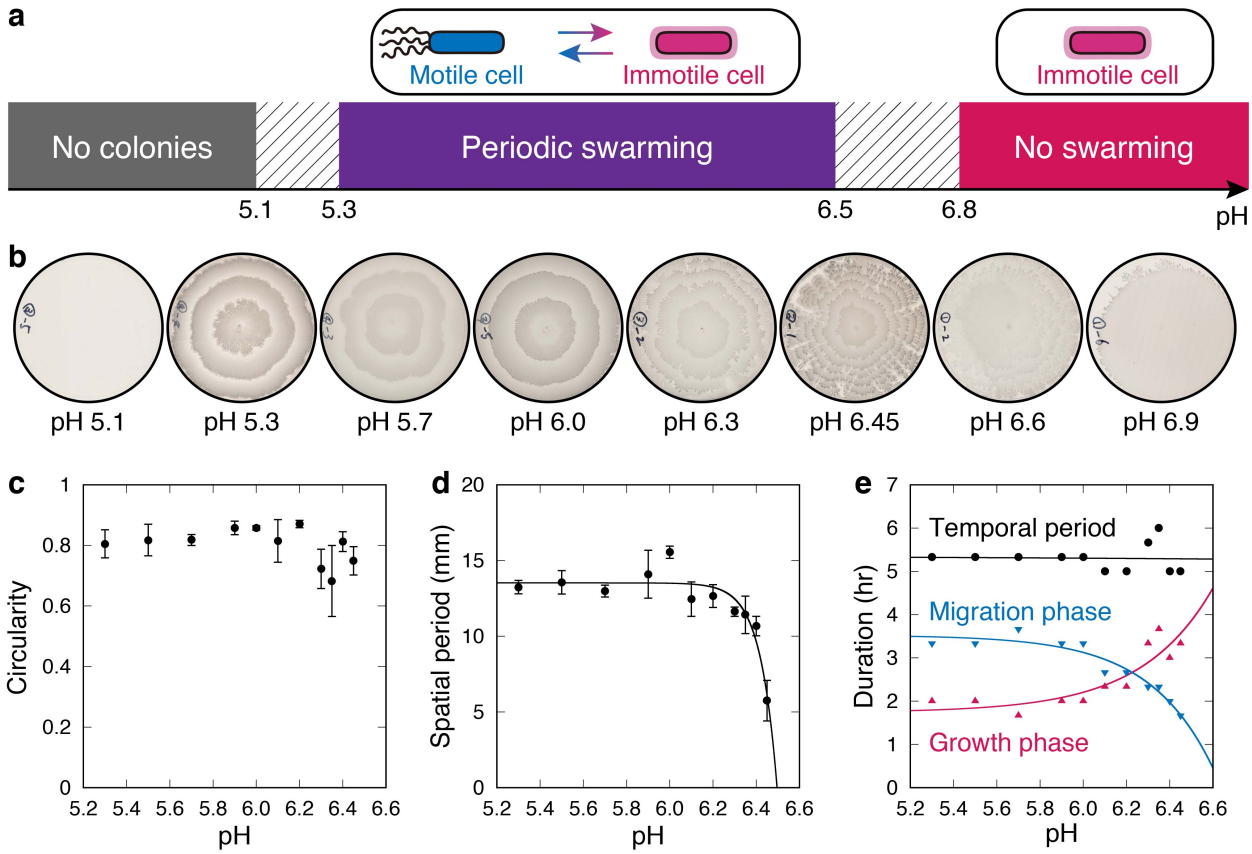
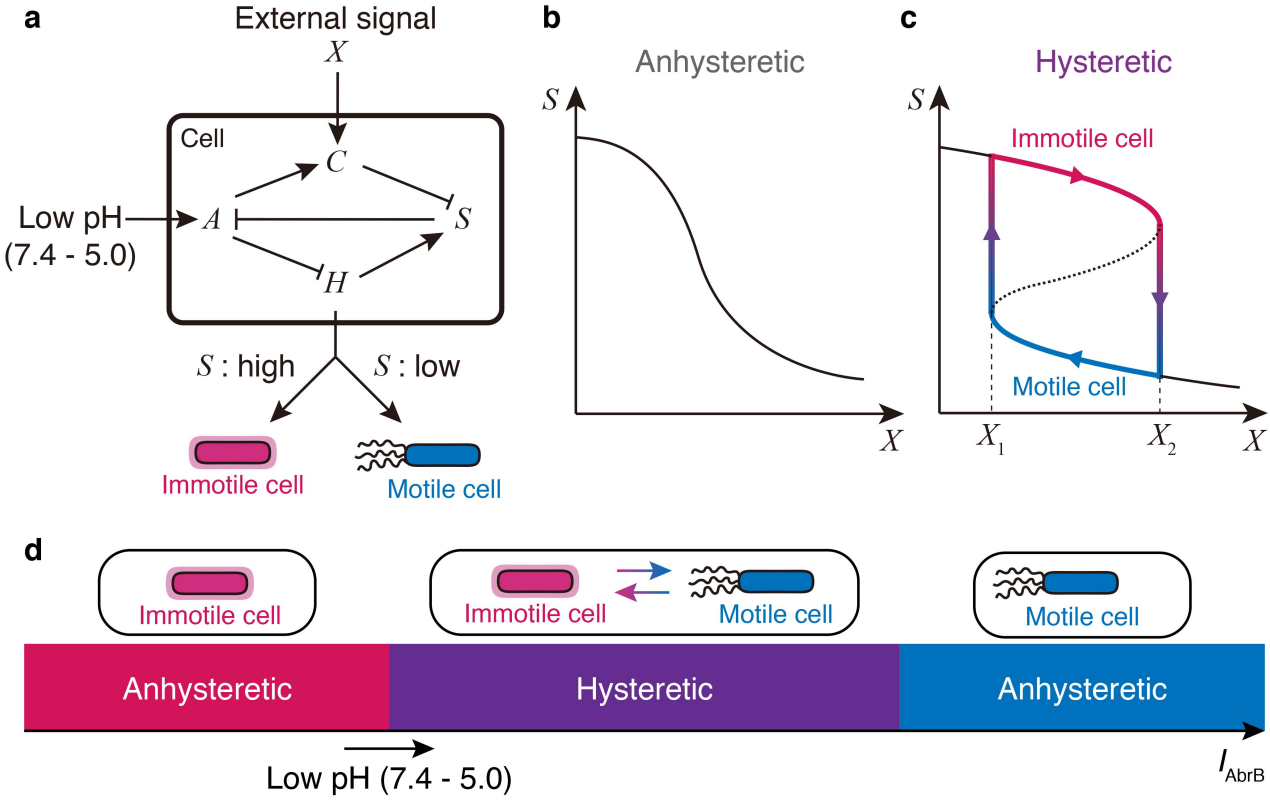


570 **Fig. 3 Microscopic images of phase transition during colony expansion. a-c** Transition from the
571 growth phase to the migration phase. Motile cells appear slightly inside colony boundaries (large
572 arrows in **a** and **b**). The number of motile cells increased to form bacterial ponds where cells organize
573 to undergo swarming collective motion. The motile cells then destroy the boundary walls (small
574 triangles in **a**). Cells flow out from some of the outermost destruction points on the colony boundaries
575 to create rapidly expanding branches (**b** and **c**). **d-f** The migration phase. Numerous dense branches
576 grow outward (**d**). Gradual collapse of the colony boundaries at the roots of branches, and the space
577 around the roots is filled with the outflowing cells (**e** and **f**). **g-i** Transition from the migration phase to
578 the growth phase. First, the branches stop growing (**g**). Then, the swarming regions composed of motile
579 cells (small bubble-like regions in **g** and **h**; whole branch region in **i**) shrink from the outside to the
580 inside of the colony (**g**, **h** to **i**; **g** is the outermost boundary, **i** is the innermost). Scale bars, 100 μm
581 (except for **c**), 10 μm (**c**).



582 **Fig. 4 Effect of environmental pH on periodically expanding colonies. a** Environmental pH
583 conditions for forming periodically expanding colonies. **b** Colonies under different environmental pH
584 conditions. **c** Circularity of colony ring (defined by circularity of second ring). **d** Spatial period (width
585 of terrace). **e** Time period: growth phase, migration phase, and total period. Data represent mean ± s.d.,
586 $n = 6$ (**c** and **d**). All fitting curves are exponential plus constant (**d** and **e**).

587



588 **Fig. 5 A model for the response of cells and cell populations to environmental pH. a** A model for
589 cell-type selection for environmental pH and cell density. **b, c** Two types of cell-type controls.
590 Anhysteretic (**b**) and hysteretic (**c**). **d** A model for cell population-type selection. The horizontal axis
591 (I_{AbrB}) is a parameter indicating the external activation rate of AbrB (see "Methods").

592

593