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Uniform modeling of bacterial colony patterns with varying nutrient and substrate

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HIGHLIGHTS

- A unified model that reproduces the entire bacterial morphology diagram.
- Quantitative comparison between experimental and simulated temporal and spatial scales.
- A comprehensive comparison between experimental and numerical parameters.
- Investigation of the physical mechanisms underlying the formation of ring patterns.

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ABSTRACT

Bacteria develop complex patterns depending on growth condition. For example, *Bacillus subtilis* exhibit five different patterns depending on substrate hardness and nutrient concentration. We present a unified integro-differential model that reproduces the entire experimentally observed morphology diagram at varying nutrient concentrations and substrate hardness. The model allows a comprehensive and quantitative comparison between experimental and numerical variables and parameters, such as colony growth rate, nutrient concentration and diffusion constants. As a result, the role of the different physical mechanisms underlying and regulating the growth of the colony can be evaluated.

1. Introduction

In nature, bacteria cope with a wide range of environmental conditions. To take advantage of their surroundings, bacteria need to adapt to the external environment using various ways such as variation of movement, communication techniques and adaptation of their rate of reproduction [1–25]. If nutrients are deficient for a sufficiently long period of time, some strains of bacteria such as *B. subtilis (Bacillus subtilis)* enter a special stationary state of a spore, which enables them to survive until more favorable conditions return.

Laboratory experiments can test the growth of bacterial colonies under various environmental conditions, for example, by changing the amount of food (the peptone level), or the hardness of the substrate (the agar concentration) on which the bacteria grow in a Petri dish. With plenty of nutrients and a soft substrate, bacteria move individually inside the agar and reproduce at a maximal rate. However, when the agar is hard, bacteria have to cooperate and can only move collectively on top of the substrate.

Environmental conditions change the microscopic behavior of bacteria. As a result, the pattern of the entire colony may change, creating a morphology diagram [24,13,2]. Fig. 1 shows such a morphology diagram obtained by Rafols [2] for *B. subtilis.* The diagram is divided into five regions corresponding to different types of patterns, depending on the hardness of the substrate (horizontal axis) and the initial nutrient level (vertical axis) [2,13]. For high nutrient concentration and soft substrate, colonies form disk like patterns. However, under harsher conditions, the colony exhibits a fractal-like shape as described in the next section, see also [1,5,6,26,27,7,9,26,13,28,16,24,25]. The main purpose of this article is to present a unified model that reproduces all the different patterns observed in Fig. 1 by varying only the initial nutrients level and two parameters that correspond to the hardness of the substrate. This approach

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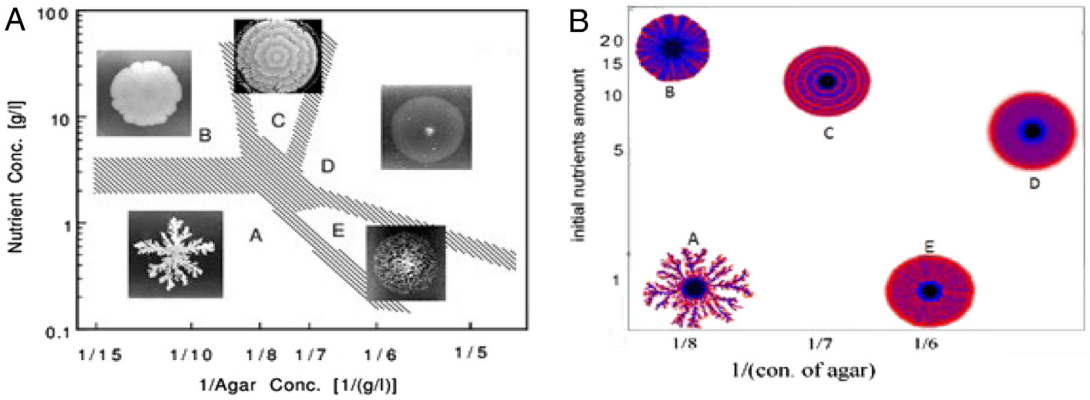

Fig. 1. (A) Experimental morphology diagram of *B. Subtilis*, reproduced from [2]. (B) Simulated patterns reproduce the experimental results. Simulations vary in initial nutrient concentration (y axis), agar concentration (x axis) and the bacterial diffusion coefficient.

allows a comprehensive comparison between experimental and numerical variables and parameters, such as colony growth rate, nutrient concentration and diffusion constants.

Biological background. As explained above, the experimental morphology diagram of *B. subtilis* reproduced from [2,13] in Fig. 1 is divided into five regions, each corresponding to a different pattern. With high agar and poor nutrients (region A), the colony has a branched structure. Adding nutrients (region B), branches become thicker and the colony has a round shape. On the other hand, decreasing the agar percentage, and keeping nutrient low (region E), conditions are harsh and a dense branching morphology is observed. However, with sufficiently high nutrients and soft substrate (region D), conditions are favorable and the colony forms an almost full disk. An interesting intermediate region occurs with high nutrient levels but moderate agar concentrations (region C). Then, bacterial colonies of some species, for example *B. subtilis*, can develop a ring-like pattern, suggesting that the colony alternates between two states—a fast expansion state similar to region D and a slow growth state, similar to region B [28]. Using our simulation results, in the following we give conditions under which ring patterns can occur and explain the phenomenon by alternating modes of movement, represented in the model by different types of diffusion.

Other bacterial species, for example Proteus mirabilis, have been found to form rings patterns [28,23,29]. It has been suggested that rings in Proteus mirabilis colonies are caused by a mechanism of alternating motility phases, more specifically, cyclic process of differentiation and de-differentiation of swimmers into swarmers [23,29].

Microscopic observations show that bacterial movement depends on the hardness of the substrate [2–5]. At very low agar concentrations (below 0.3%) bacteria move individually inside the agar. However, as agar powder creates a gel, the rigidity of the gel increases with the agar percentage [2–4]. Between 0.3 and 1% agar concentration some bacteria move on the surface while some inside [2]. However, bacteria can significantly change the physical properties of the medium, making it more suitable for bacterial movement, by secreting various materials such as enzymes, surfactants and other polymers [1,2]. At higher agar concentrations, the surface is semi-solid and bacteria cannot move at all inside. Instead, bacteria produce a layer of liquid to move in, essentially creating a lubrication layer to increase motility [2,4]. This is a collective endeavor that requires sufficient bacterial density. As a result, at medium and high agar concentrations the ability of bacteria to move depends on its density.

2. Modeling bacterial colonies

Different theoretical models describing the physical properties underlying pattern formation in bacterial colonies have been suggested and tested by simulations. The general approach underlying the models varies significantly. In [3,6,30,31], emphasis is given on the collective effect of bacteria on their environment. The main goal of [32] is to explore the ability of bacteria to differentiate into different types. Other models aim to expose the possible cell to cell interaction [33]. Many of these and other models take into consideration the influence of chemotaxis [1,5,18,31].

From the point of view of modeling, models can be divided into two main categories.

- Bacteria as discrete agents: The approach models bacteria, or an effective group of bacteria, as discrete self-propelled particles, moving and interacting with each other and the environment. In typical models, agents consume nutrients, multiply, sporulate or die [5,6,14]. This approach is particularly advantageous for modeling the internal state of bacteria, for example, energy levels or stress.
- Bacteria as a continuous density: The approach describes the evolution of the bacterial concentration function, typically in two-dimensions (2D) as a partial integro-differential equation, for example a reaction–diffusion equation. Additional constituents of the system, for example, nutrients, signaling factors and other chemicals or proteins are also modeled as a 2D density. See, for example [1,26,27,11,25].

In this paper, we follow the second, continuous, approach which allows us to model the large number of bacteria and directly compare simulation and experimental results.

Based on biological observations, we have constructed a unified reaction–diffusion model that reflects the experimental conditions. To our knowledge, this is the first model that explicitly takes into consideration the different component of the substrate hardness; agar concentration and lubricant creation by bacteria. This new description of substrate properties and its influence on the bacterial motion enables us to effectively capture the colony's expansion under different bacterial modes of motion (swarming, swimming). However, the model is essentially a phenomenological reaction–diffusion model. Such a modeling approach neglects many aspects of the bacterial dynamics such as surfactant production, run-and-tumble dynamics, signaling agents and more, [32] which are only captured in a phenomenological, coarse grained nature. None the less, this approach allows a quantitative comparison between experimental and simulation parameters such as diffusion coefficients and the bacterial and nutrient