Growth-induced self-organization in bacterial colonies

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Chapter 1

Introductions

1.1 Mechanics of cellular systems

Ever since the genius speculations of Erwin Schrödinger on the physical nature of living systems and living processes, including heredity, consciousness, and how living matter evades the decay to thermodynamical equilibrium, in his famous book *What is life* [1], physics has been closely entangled with biological systems. On the one hand, successful applications of physics have greatly advanced our understanding of a plethora of biological phenomena spanning various scales: from the dynamics of protein folding [2, 3] to the mechanics of cell membranes [4, 5], to the collective behavior of animal groups [6–9]. In addition to these, the exciting development of electronic and optical imaging technologies makes it possible to observe and study biological systems at high spatial and temporal resolutions, based on which quantitative relations can be built. Typical examples include the first discovery of DNA's double-helix structure with X-ray radiation by Francis Crick and James Watson in 1953 [10], and the state-of-the-art scanning electron cryomicroscopy that can produce extremely high magnification images (up to 1000000×) with sub-nanometer resolution [11]. On the other hand, the inherent complexity of biological systems makes them excellent platforms for physicists to learn new physics, and expand our knowledge of nature from both the physical and biological perspectives in an integrated way.

In the last few decades, special emphasis has been placed on the mechanics of cellular systems, which exhibit a variety of fascinating phenomena that are interesting to physicists, biologists, and engineers. These studies usually lead to new understanding of the role of mechanics in biological processes [4, 5, 14–18], the discovery of amazing material properties, or extensions of physical laws to living systems [19–23]. Excellent examples are:

- Cell differentiation pathways, usually believed to be a pure biochem-

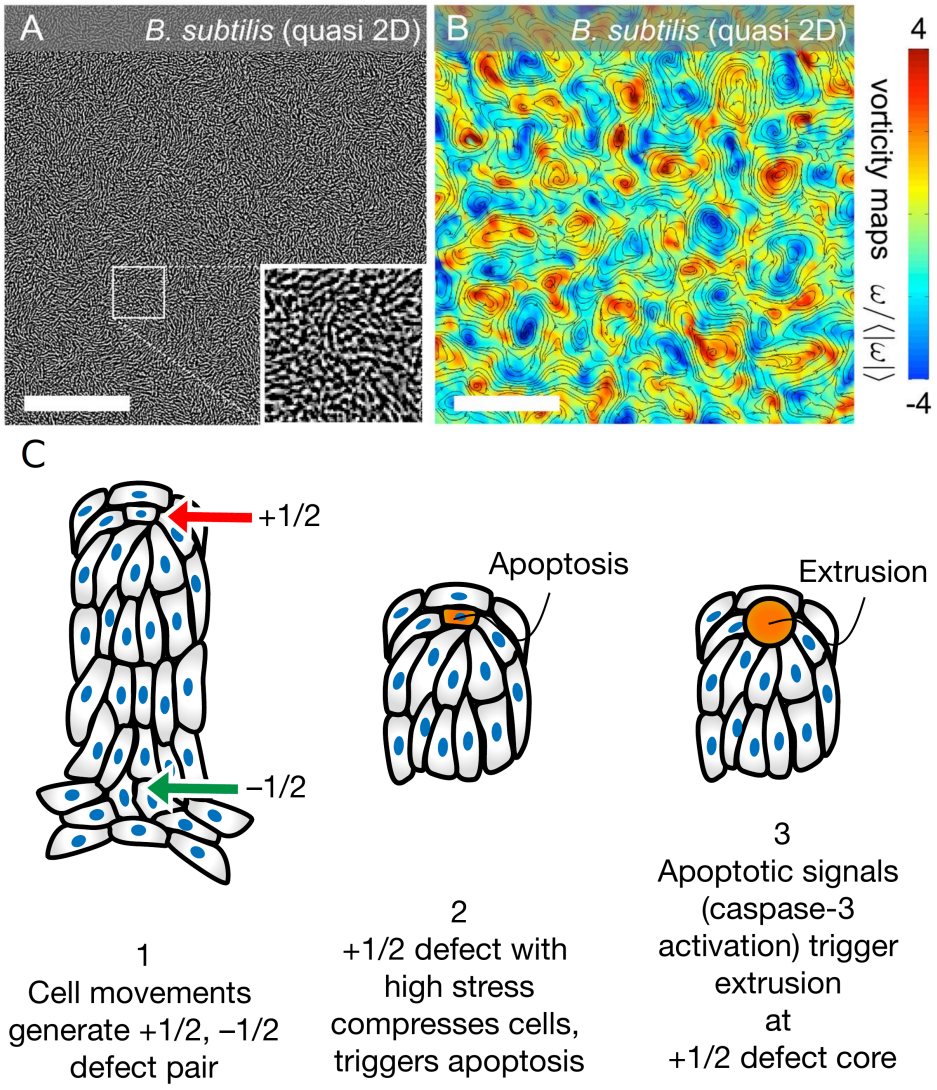

Figure 1.1. Recent discoveries on the mechanics of cellular systems. (A-B) Turbulent flow of bacterial suspension at low Reynolds numbers, adapted from [12]. (A) Experimental snapshot of a highly concentrated, homogeneous quasi-2D bacterial suspension. (B) Flow streamlines v and vorticity fields ω in the turbulent regime (Scale bars, 50 μm). (C) Topological defects in cell orientation can focus mechanics stresses and trigger cell apoptosis and extrusion, adapted from [13].

ical process, can be regulated by mechanical forces [24, 25];

- A suspension of swimming bacteria can effectively reduce the viscosity of the fluid, and can even turn it into a "superfluid" [26];
- Active turbulence: turbulent flow at low Reynolds numbers [12, 27–30] (Figs. 1.1A-B);
- Topological defects of cell orientation in epithelia govern cell death and extrusion [13] (Figs. 1.1C);
- Liquid-solid transition of tissue monolayer, controlled by cell motility and mechanical interactions among cells [31, 32], and fluidization of tissues by cell division and apoptosis [33];
- Motility induced phase separation [34–36].

Despite presenting enormous differences in terms of length and time scales, mechanical interactions, and biological nature, these intriguing phenomena in cellular systems can ultimately be ascribed to a few underlying properties. First of all, unlike normal material, the building blocks of cellular systems usually have complex structures or high internal degrees of freedom [5, 14]. In addition, the interactions among building blocks can be highly complex, involving specific mechanical interactions or cell-cell communications [5, 14, 37], which, collectively, can lead to the emergence of biological functionality. Second, cellular systems are usually heterogeneous (in space) and anisotropic [7, 19, 38]. Heterogeneity and anisotropy can be inherent to the building blocks (their shapes or mechanical interactions), but they can also be triggered by external stimuli. Both properties indicate the breaking of certain symmetry, hence can give rise to nontrivial spatial patterns or anisotropic material properties. Last but not least, cellular systems are living systems, which means that they can actively organize themselves to fulfill certain biological functions or respond to mechanical or biochemical cues. The activity enables the system to harvest energy locally and transfer it into motion or specific mechanical forces, which is fundamentally different from classical nonequilibrium systems where the energy injection is at the macroscopic scale and at the boundary of the system [6, 7]. These properties, individually or collectively, can promote different types of functions or material properties that ultimately distinguish cellular systems from the nonliving world.

These considerations raise a question of special physical interest: what are the general principles that govern the mechanics of cells at different

scales? Or in other words, how certain microscopic dynamics or mechanical interactions can give rise to specific macroscopic mechanical properties? In the past few decades, much has been done to address this question, theoretically and experimentally. Especially, the expanding community of *active matter* has made an enormous effort to connect the microscopic properties, e.g. particle shape or activity like self-propulsion, to macroscopic material properties such as rheology and mechanical stresses [6, 7, 19–23, 39–42]. These advances are very exciting and promising, but there's still a long way to go on the mechanics of biological systems. The research work presented in this thesis is motivated by the same general question and is devoted, specifically, to the problem of growth-induced self-organization in bacterial colonies.

1.2 Mechanics driven self-organization in bacterial colonies

Bacteria successfully colonize a plethora of surfaces by producing hydrated extracellular polymeric matrix, generally composed of proteins, exopolysaccharides and extracellular DNA (Fig. 1.2) [43–47]. The extracel-

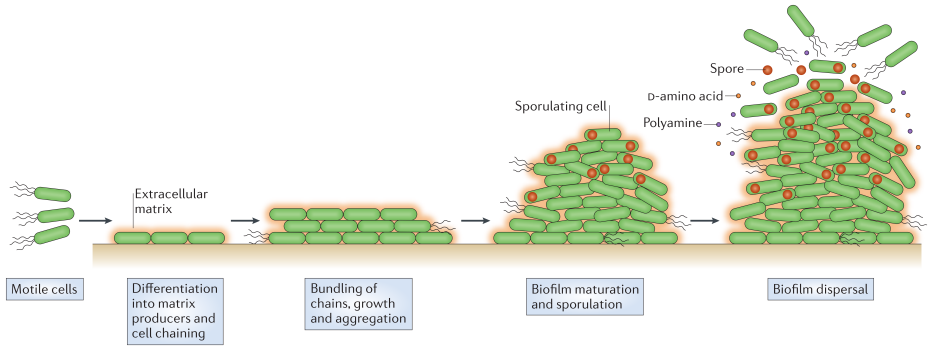

Figure 1.2. The formation of a biofilm occurs in several stages, comprising the development, maturation and disassembly of the bacterial community. At the initiation of biofilm formation, motile cells with flagella differentiate into non-motile, matrix-producing cells that stop separating and form chains that are surrounded by extracellular matrix. In mature biofilms, matrix-producing cells sporulate. In aged biofilms, some cells secrete small molecules such as D-amino acids and polyamines, which break down the extracellular matrix and allow the cells to disperse in the environment. Adapted from [43].

lular matrix (ECM), together with the bacterial cells, forms a slime layer of organism that is usually referred to as *biofilm*. A biofilm is more than a collection of ECM and cells, but a functional ecosystem that can grow, adapt, and actively respond to external stimuli [44–46]. Such surface-associated communities play a crucial role in the pathogenesis of many chronic infections–from benign dental caries in the oral cavity [48, 49] to life-threatening cystic fibrosis and catheter-related endocarditis [50]. For planktonic species (i.e., freely swimming), the life of a biofilm starts with cells undergoing a phenotypic shift whereby motile cells turn sessile (i.e. surface-associated), and thereafter continues growing in size via the formation of an exponentially growing monolayer of tightly packed and partially aligned cells (Fig. 1.2) [43, 51–55]. Colonies originating from a single bacterium initially develop as a flat monolayer and, upon reaching a critical population, invade the third dimension forming multiple layers [43, 51, 56]. After this, the multilayered structure becomes thicker, and the colony undergoes a transition from a planar sheet to a bulk material. As the biofilm becomes mature, some cells at the surface experience a reversed phenotypic shift, become motile again and start to initiate a new cycle.

In contrast to planktonic populations of motile cells (freely swimming, gliding, or swarming), cells in a sessile colony lack motility. Since most bacteria found in nature exist predominantly as surface-associated colonies [57], they are permanently exposed to a range of surface-specific forces [54]: time-varying internal stress due to growth, contact forces due to interactions with the neighboring cells and substrate they are growing on, or shear stresses due to ambient flows in the system. Our understanding of the mechanics of bacterial growth is still in its infancy, specifically in light of the wide range of mechanical cues that single cells overcome to successfully colonize surfaces. Although it has been long known that mechanical forces play a critical role in the development and fitness of eukaryotic cells and, in addition, can regulate key molecular pathways [58], the cornerstones of major discoveries in bacterial communities have relied on biochemical pathways triggered exclusively by chemical stimuli [52]. Only recently has the role of mechanics in the ecophysiology of prokaryotic cells come to the forefront [38, 51, 53–56, 59–62], highlighting the governing biophysical principles that drive colony formation.

A particularly interesting demonstration of the mechanical aspects of bacterial organization was illustrated in Refs. [38, 55, 59, 60], upon con-

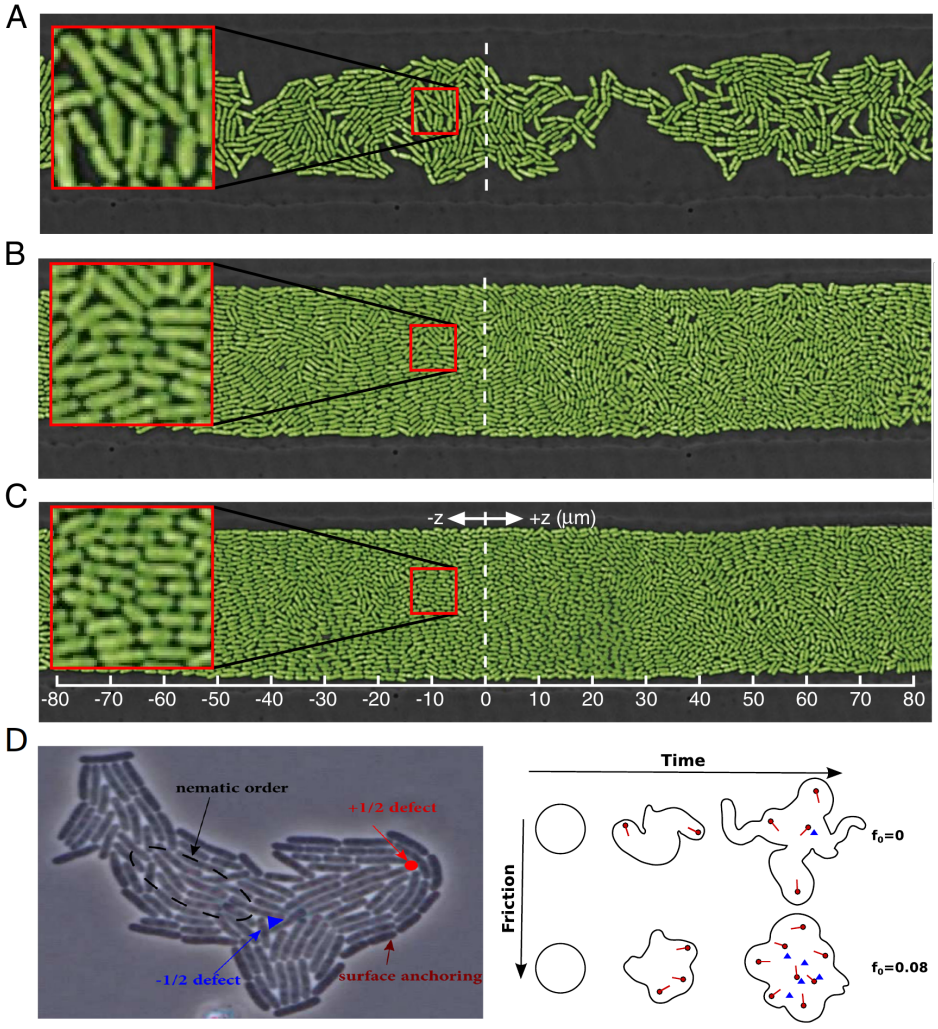

Figure 1.3. (A-C) Snapshots of a growing bacterial colony confined in a microfluidic channel at (A) 60, (B) 90, and (C) 138 minutes from the beginning of the experiment. Adapted from [38]. (D) A growing *E. coli* colony shows nematic ordering, $\pm 1/2$ topological defects, and tangential alignment to the interface (left). (Right) The spatiotemporal evolution of a growing colony is affected by the dynamics of topological defects and by friction with the substrate. With increasing friction, the defect density increases and the average defect velocity drops, resulting in a more isotropic morphology. Adapted from [63].

fining a monolayer of nonmotile duplicating bacteria in a microchannel. Depending on the channel length, the bacterial population was observed to evolve either into a highly ordered colony [38, 55, 60], with all the cells parallel to each other and to the channel wall (Figs. 1.3A-C), or, for longer channels, into disordered structures consisting of multiple domains of aligned cells with no global order [59]. A strikingly similar behavior was identified by Wioland *et al.* in suspensions of swimming bacteria [64]. Theoretical study also revealed that in a monolayered colony without boundary confinement, topological defects in the orientation of cells were created, which were found to regulate the morphological development of the colony (Fig. 1.3D) [63]. The friction between the dividing cells and underlying substrate drives anisotropic colony shapes toward more isotropic morphologies, by mediating the number density and the velocity of topological defects. Indeed, a recent experiment found that increasing the adhesion, i.e. the "effective friction", between cells and the substrate resulted in a more circular colony shape, although it was not clear whether or not this was triggered by the creation of topological defects [65]. In addition, the mechanical interactions among neighboring cells were also found to be responsible for the mono-to-multilayer transition in bacterial colonies, by competing with the vertical restoring forces from the ECM or from the agarose gel on top [56, 65, 66]. More recently, the development of new imaging techniques makes it possible to inspect the internal structure of a three-dimensional growing colony down to the scale of individual bacterium [67, 68]. Compared to the 2D counterparts, the 3D colonies show much richer organizations, in terms of local cellular order and the global biofilm architecture, as a consequence of the intricate mechanical interactions among cells and between cells and the substrate/ECM [67, 68].

When coupled with other factors, mechanical interactions can promote many other interesting phenomena. For example, cell-cell repulsive forces can account for the nonequilibrium transition from circular to branching colonies often observed in the lab (Figs. 1.4A-B), upon tuning the intake of nutrient from the substrate [62]. More interestingly, mechanical interactions can also affect the biological evolution by regulating the relative motion of cells in the colony [69]. Specifically, a mutation arising at the colony's frontier can either die out and extinct, or survive and persist, and the probability distributions of the two fates are determined by the mechanical properties of the system, such as the cell aspect ratio, the cell

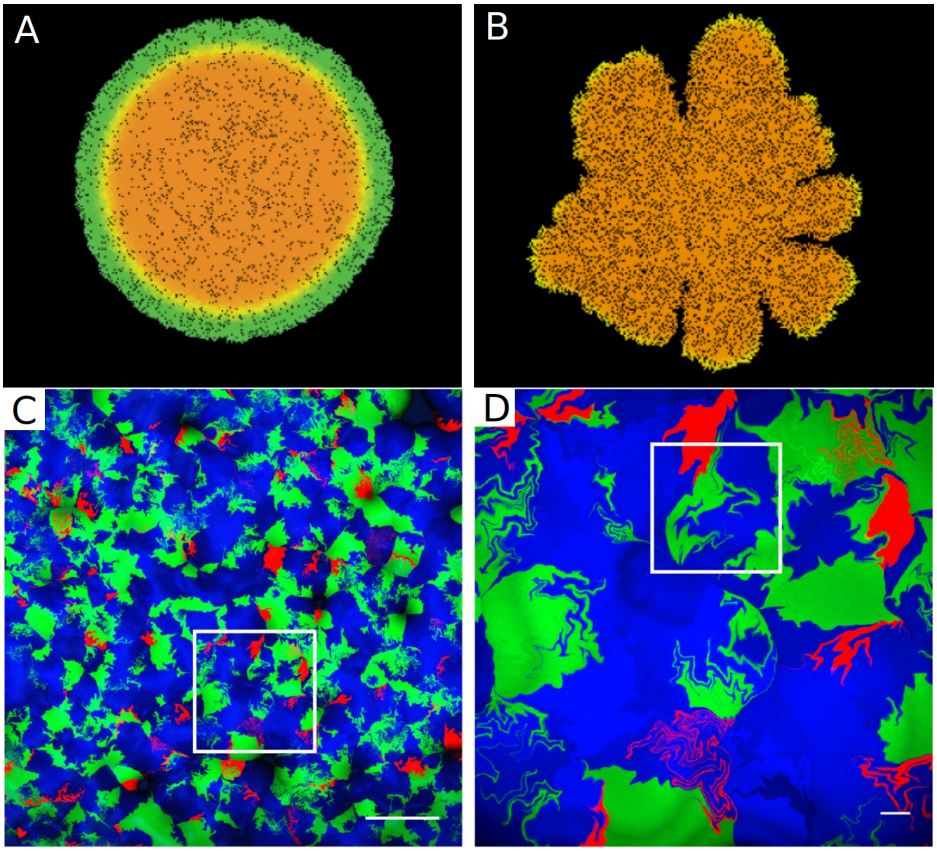

Figure 1.4. (A-B) Snapshots of growing bacterial colonies from the simulation of $N \sim 10^5$ cells, showing the mechanics-driven circular-to-branching transition. Panels (A) and (B) have, respectively, low and high values of branching parameter β, which depends on cell growth rate, the initial concentration of nutrient, and the consumption rate of nutrient. The green (orange) color corresponds to a high (low) local nutrient concentration. Adapted from [62]. (C-D) Fractal patterns in growing multi-species bacterial colonies. Different species are labeled with three different fluorescent proteins: mTurquoise2 (in blue), mRFP1 (in red) and sfGFP (in green). Scale bars, 1 mm in (C), 100 μm in (D). Adapted from [53].

orientation, and the friction from the substrate [69]. Moreover, in the case of multi-species biofilm, the buckling instability at the interface of different species can trigger the formation of striking fractal patterns with jagged, self-similar shapes (Figs. 1.4C-D) [53].

These works have greatly advanced our understanding of how mechanical interactions can drive diverse types of self-organizations in growing bacterial colonies. However, a general understanding of the underlying physics that govern different stages of the colonization process is still lacking. Specifically: what mechanical effects play a dominant role in the system dynamics? How to characterize them using the language of physics? And how do they originate from the microscopic dynamics of bacterial cells? Among these, a key problem is to understand the mechanical effects of cell growth. This is particularly important because of three reasons. First of all, a good knowledge of the role of cell growth is crucial to our comprehension of the morphological developments of growing cellular systems such as tissues or microbial colonies. Second, previous studies have shown that the activity of cells, e.g. self-propulsion or active alignment, can give rise to very specific mechanical properties at the macroscopic scale [6, 7, 19, 41]. Conversely, the effects of cell growth on the macroscopic organization of a bacterial colony have not been investigated with an equally systematic approach. Third, in sessile bacterial colonies, cell growth is the ultimate driving force of cell motion and colony expansion. Without cell growth, the frictional and adhesive forces from the substrate will quickly damp any cell motion and leave the system in a stationary state. It is thus important to understand how various mechanical interactions can arise as a consequence of cell growth.

In this thesis, we address these problems theoretically, with computer

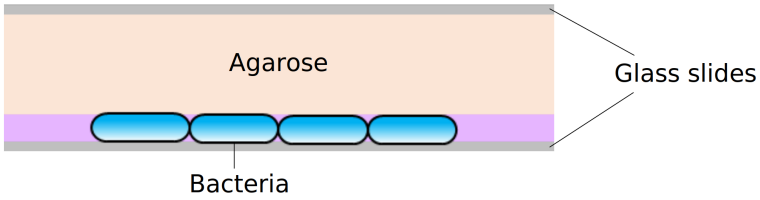

Figure 1.5. Sketch of the model system we used in the thesis. A growing colony of non-motile, rod-shaped bacteria is sandwiched between a hard glass slide and a relatively soft agarose gel. Cells take up nutrient from the agarose, and then grow, divide, and push each other away as they elongate.

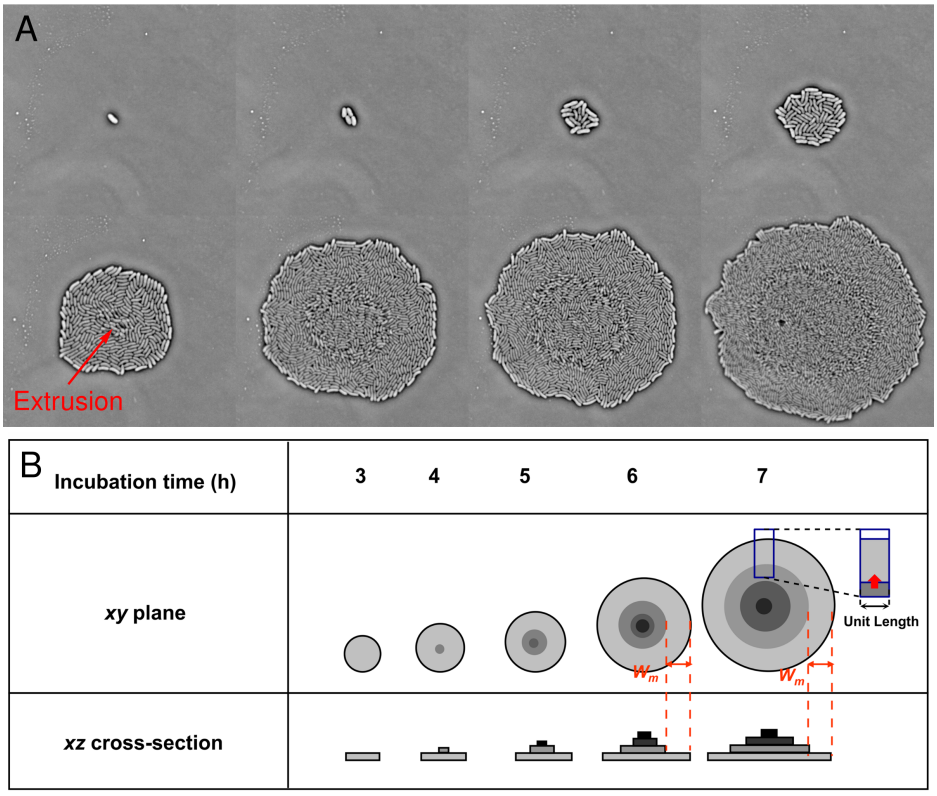

Figure 1.6. (A) Snapshots at different time points of a growing bacterial colony sandwiched between an agarose gel and a glass slide. Monolayer expansion was found at the initial stage (top images). At certain colony size, a second layer was formed through cell extrusion at the center of the colony (bottom, left), and then both layers expanded simultaneously. Images provided by Anupam Sengupta. (B) Illustration of a micro-colony development by viewing at different planes. Even though the diameter and layers of the bacterial micro-colony were increasingly expanding, the average width of the outermost monolayer reached a constant value after approximately 6 h of growth. The red arrow indicates the constant outward force per unit length. Adapted from [61].

simulations of discrete and continuum models. We use a rather simple model system that has been frequently used to study growing bacterial colonies [56, 61, 65], where a growing colony of nonmotile rod-shaped bacteria is sandwiched between a glass slide and an agarose gel (Fig. 1.5). Cells take up nutrient from the agarose and then grow and divide. In such a setup, cell motion can be instantaneously damped by frictional and adhesive forces, due to the interaction with the glass and the agarose gel [56, 65]. Nevertheless, the elongation of cells enables them to persistently push away their neighbors along their axes. These mutually pushing forces between neighboring cells can then help them overcome the damping forces, and drive continuous motion of cells in the whole colony. In addition, since the agarose on top is relatively soft compared to the glass side, cells are allowed to deform the agarose gel, at a cost of certain elastic energy. For this reason, the colony expands as a monolayer first (Fig. 1.6). Then, upon reaching a certain colony size, the in-plane stress can induce normal lifting forces to cells that outweigh the agarose compression, squeezing them out of the monolayer, and initiate the second layer of the colony (Fig. 1.6) [56, 61]. After this, more and more cells are transported from the first to the second layer, but at the same time, cells on the second layers are also growing and duplicating. This expanding second layer can then initiate the third layer, and subsequently more and more layers (Fig. 1.6) [61]. There are many interesting phenomena that we can dig into during this process, including the dynamics of the monolayer expansion, the mono-to-multilayer transition, and the dynamics of the mulitlayer expansion, where adjacent layers actively interact with each other through mechanical forces and mass transfer. Here in this thesis, we are interested in the initial stages, more specifically the monolayer expansion and the mono-to-multilayer transition. We focus on the mechanical effects of cell growth, and how their competition/collaboration with other mechanical interactions can give rise to self-organizations at different stages of the biofilm formation.

1.3 Outline of the thesis

Before discussing the main results, in chapter 2, we introduce the discrete and continuum models we used to characterize growing bacterial colonies. To perform "experiments" of growing bacteria *in silico*, we employ a molecular dynamics model of elongating hard rods. From the dis-

crete model, we are able to identify the self-organized patterns of growing bacterial colonies, and measure the mechanical properties of the system, which can not be easily done in experiments. In addition, in order to understand intuitively the results from our molecular dynamics simulations and gain further insights, we also describe our growing colony in the realm of continuum mechanics, more specifically the continuum theory of active nematics, with suitable extensions that are specific to the growing bacterial colonies.

With the models at hand, in chapter 3, we discuss the geometrical and mechanical properties of a freely expanding monolayer. We demonstrate that such an expanding colony self-organizes into a "mosaic" of microdomains consisting of highly aligned cells. The emergence of microdomains is mediated by two competing forces: the steric forces between neighboring cells, which favor cell alignment, and the extensile stresses due to cell growth that tend to reduce the local orientational order and thereby distort the system. This interplay results in an exponential distribution of the domain areas and sets a characteristic length scale proportional to the square root of the ratio between the system orientational stiffness and the magnitude of the extensile active stress. Based on these results, we develop a continuum theory for growing bacterial colonies by suitably extending the hydrodynamic equations of active nematics, and the simulations show the same qualitative results as we found in the discrete model. Our theoretical predictions are finally compared with experiments with freely growing *E. coli* micro-colonies, finding quantitative agreement.

In chapter 4, we study the self-organization of growing monolayer under lateral confinement. Whereas a freely expanding colony shows chaotic dynamics, where nematic domains are randomly forming and breaking, upon confinement, the colony exhibits a dramatically different behavior: it develops a global nematic order. To be specific, after a transient process of "chaotic" expansion, the growing bacterial colony develops a globally ordered state where the nematic director is normal to the direction of confinement. With computer simulations of the hard-rod model, we demonstrate a complex interplay among cell orientation, cell growth, and mechanical stresses. Especially, the combined effects of confinement and cell growth result in a globally anisotropic stress, where the stress components parallel to the direction of confinement are larger than their orthogonal counterparts. This anisotropic stress can drive cell to align along the direction of minimal stress (i.e. perpendicular to the direction

of confinement), and promote a global nematic order in the whole colony.

In chapter 5, we discuss how growth-induced stress can trigger the mono-to-multilayer transition in a bacterial colony. Using a combination of numerical simulations and analytical modeling, we demonstrate that the transition originates from the competition between growth-induced in-plane stresses and vertical restoring forces, due to the cell-substrate interactions. The mechanistic picture of this transition can be captured by a simple model of a chain-like colony of laterally confined cells. Mechanically, the transition is triggered by the mechanical instability of individual cell, thus it is localized and mechanically deterministic. Asynchronous cell division renders the process stochastic, so that all the critical parameters that control the onset of the transition are continuously distributed random variables. Upon modeling the transition as a Poisson process, we can approximately calculate the probability distribution functions of the position and time associated with the first extrusion. The rate of such a Poisson process can be identified as the order parameter of the transition, thus highlighting its mixed deterministic/stochastic nature.

Finally, we conclude our study with an outlook in chapter 6.