

Can Bacteria Walk?

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Abstract:- The "walking mechanism" of bacterial cells and the role of their legs and falagellae in their transfer or walking is the subject of this article.

Additionally, the structure of bacterial cells and the role of their own thermal diffusion field, electric and magnetic fields constructed by the geometric Dirichlet boundary conditions in their transfer or walking mechanism have been thoroughly discussed.

Experimental study and theoretical analysis show that the bacterial cell colony is negatively charged due to the presence of free electrons which produce an electric field flowing radially outward from the colony and a circulating magnetic field surrounding the colony.

Bacterial cells can move radially outward and cross foodless spaces through the force mechanism of the bacterial colony's electric fields in addition to the swimming and crawling motion of the bacteria's legs and flagella.

I. INTRODUCTION

Colonies of bacterial cells are the main source of disease and food. Bacterial microbes are unicellular microorganisms lacking a nuclear membrane, metabolically active and when they grow, beyond a certain limit, under appropriate conditions of food, humidity and oxygen, they divide by binary fission. This means that the microscopic growth of individual bacterial cells is ultimately arrested by the bacteria self-dividing into two daughter cells. This process is called binary fission.

Provided no external effects occur, the resulting daughter cells are genetically identical to the parent cell. Obviously, this results in an exponential growth of bacterial colonies.

The temporal evolution of bacterial colonies goes through three main phases, namely the lag phase, the logarithmic or exponential growth phase and finally the exponential death or decay phase [1].

The physical interactions of developing bacterial cells with each other and with their growth environment (Agar food, temperature, humidity, etc.), significantly affect the structure and movement dynamics of bacterial colony biofilms.

The currently burgeoning field of theoretical and experimental studies of bacteria, particularly the movement or walking of bacterial cells, focuses on the role of their legs and falagellae Fig1.

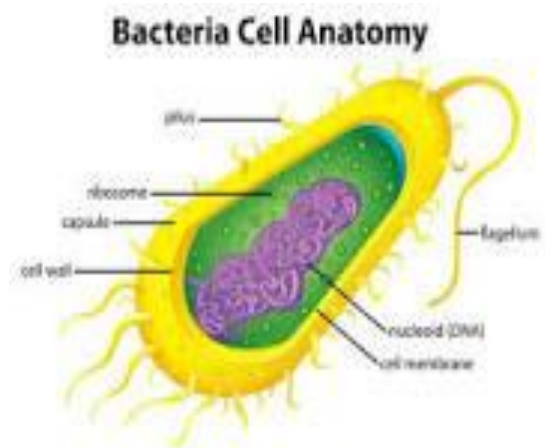


Fig. 1: Bacteria cell anatomy

The "walking mechanism" of bacterial cells and the role of their legs and falagellae in their transfer or walking is the subject of this article.

Katsumi Imada et al 2018 [2] states that many motile bacteria swim and swarm toward favorable environments using the flagellum, which is rotated by a motor embedded in the inner membrane. The bacterial flagellum is a mobile organelle composed of thousands of protein subunits. He adds that Gram-negative bacteria, such as Escherichia coli and Salmonella, swim by rotating helical filamentous organelles called the flagellum. The flagellum rotates at a relatively high speed and is driven by a reversible rotary motor embedded in the cell membrane at the base of the filament.

Sandra Postelet al 2016 [3] stated that Flagella are crucial for bacterial motility and pathogenesis. The flagellar capping protein (FliD) regulates filament assembly by chaperoning and sorting flagellin (FliC) proteins after they traverse the hollow filament and exit the growing flagellum tip. In the absence of FliD, flagella are not formed, resulting in impaired motility and infectivity.

He asserts that "many bacteria, including several that cause disease in humans, have long, whip-like appendages called flagella that extend well beyond their cell walls. Flagella can spin and propel bacteria through liquids, such as water or blood, and they are mostly made up of thousands of copies of a single protein called flagellin. During the construction of flagella, the flagellin proteins are placed in their appropriate positions by another protein called FliD, several copies of which form a cap at the end of the flagella. Without FliD, bacteria cannot properly assemble flagella and therefore cannot swim; it also hinders their ability to cause disease.

Marko Nedeljkovic, 2021 [4] stated that the bacterial flagellum is one of the most complex and dynamic biological nanomachines known and has attracted attention since its discovery in the late 19th century. The history of flagellar research has gradually moved from biological, purely morphological studies, to biochemical and biophysical studies at the atomic level, enabling current understanding to the point of being able to modify it for various purposes. The bacterial flagellum is a complex and dynamic nanomachine that propels bacteria through liquids. It consists of a basal body, a hook and a long filament.

Flagellar assembly is a complex and energetically costly process triggered by environmental stimuli and, therefore, highly regulated at the transcriptional, translational, and post-translational levels. Besides its role in locomotion, the filament is critically important in several other aspects of bacterial survival, reproduction, and pathogenicity, such as adhesion to surfaces, secretion of virulence factors, and formation of biofilms.

W. Wilson et al 2007 stated that [5], Here, an agent-based 3D model is formulated to describe the establishment of single expanding bacterial colonies by the physical force of their growth. With a single set of parameters, the model captures key dynamic characteristics of colony growth by

nonmotile, non-EPS-producing *E. coli* cells on hard agar. The model, supported by experiments on colony growth in different nutrient types and concentrations, suggests that the radial expansion of colonies is not nutrient-limited as commonly believed, but by **mechanical forces**.

"We announce again that the subject of this article is how the macroscopic growth of the bacterial colony follows the mechanical forces of the electric and magnetic fields in accordance with W. Wilson."

Xiaowei Zhao finds that [6]

The flagellum is one of the most sophisticated self-assembling molecular machines in bacteria. Propelled by the proton motive force, the flagellum rotates rapidly clockwise or counterclockwise, which ultimately controls the mobility and behavior of bacteria.

Wolfram Siede 2018 [7] performed a **groundbreaking experiment** to study the microscopic and macroscopic growth of bacterial colony in a multi-hole perforated plate Fig 2. He stated that, based on cutting holes in standard medium agar plates where mature mycelium is cultured "In terms of ease, time required and spore yield", we have found this method to be superior to others.

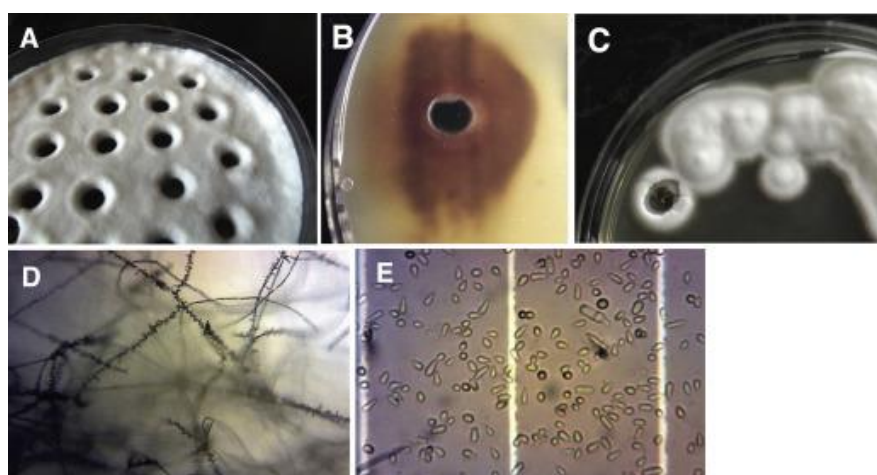


Fig. 2: Macroscopic growth of bacteria colony containing macroscopic holes in the agar

He explained, To start, an existing microconidia sample (or any source of spores/cells) is used for plating or streaking at a density that leads to a dense mycelium cover of a petri plate within a few days. After two days incubation at 30 °C, plates are typically sealed with Parafilm to prevent excessive drying and as a means to avoid spreading of spores of this BSL2 organism. After about 8 days of incubation in total (in the dark), holes of ~6 mm diameter are cut into the now mycelium-covered agar, preferably by using a flamed cork borer or a similar cutting tool (Fig. 1A–C). The circular agar pieces are removed and discarded. The resulting holes should be not placed less than 10 mm apart. After continued incubation, additional growth may become evident around the holes (Fig. 2A). In roughly the same area, developing red pigmentation should be visible when the plate is viewed from the bottom (Fig. 2B). This appears to accompany formation of spores which are mostly microconidia in our case (Fig. 2D). In another *Trichophyton*

species, carotenoid production has been associated with arthrospore formation. The question arises: do bacteria have legs?

Yes, but to some extent it's true: the movies show the bacterium *Pseudomonas aeruginosa* swinging in an upright position and moving leg-like projections known as type IV pili to walk around a surface.

However, it is clear from Figure 2-A that bacteria cannot travel or walk from the outside of the circular Agar hole to the inside as if the barrier cannot be crossed.

Nevertheless, to our knowledge, previous work has never described the possibility that bacteria can travel or break out of the circular Agar hole and cross the seemingly impassable barrier.

In other words, can microscopic bacteria during colonial growth travel or wander into centimeter-sized areas without food? This question is attractive but never considered in all previous works ([2]-[7]).

In other words, they all confirm that the bacterial cell's flagella and legs are the basic mechanism beyond its motility but omit the role of its own electric and magnetic fields in this walk which may be the main one in crossing areas without food.

The present work is perhaps the first comprehensive theoretical and experimental study to fuse experimental microbiology with the foundations of theoretical mathematical physics to further reveal the growth properties of bacteria in two-dimensional geometric space.

Another reason for the current approach is that in the previous works cited above ([2]-[7]), the authors experimentally showed that bacterial cells have only a few nanometer legs and one or more micrometer flagella and that they play an important role in directing bacterial populations to food/Agar.

This leads to the conclusion in previous literature that a foodless hole or barrier of centimeter dimensions cannot be traversed by bacterial cells, a conclusion which is not entirely true.

In fact, the same bacteria can travel or walk from the inside of the circular food ring outwards as if there were no barrier Fig 4,5,6[8,9].

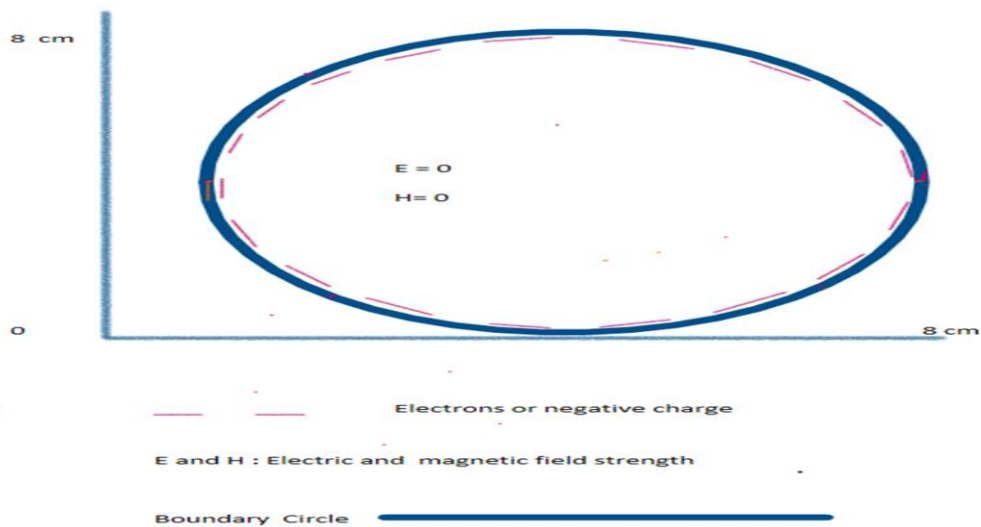


Fig. 3: HOLLOW Agar bacteria colony has no intrinsic E and H fields

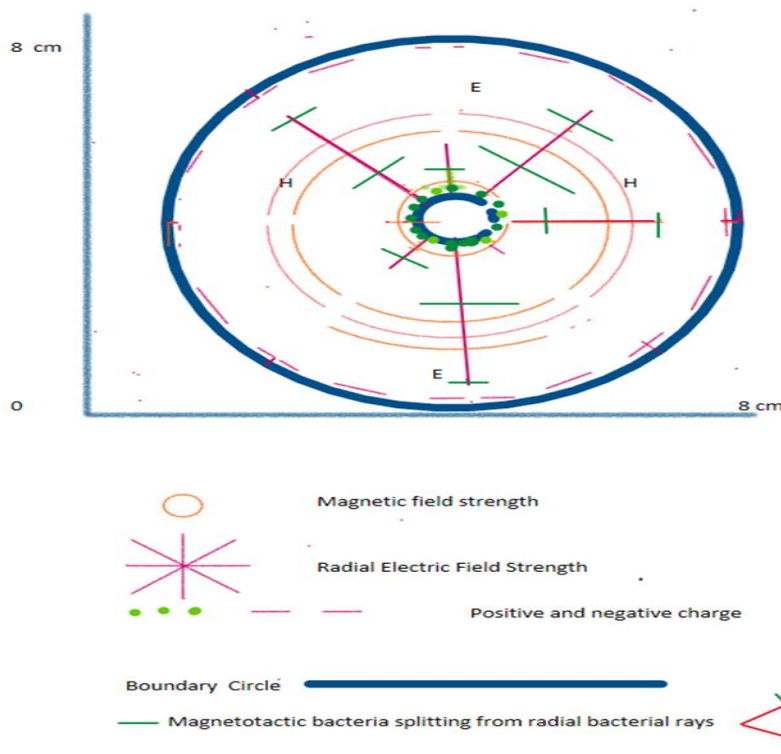


Fig. 4: Agar bacteria colony on two concentric rings with central food has its own radial E-field and circular H-fields

The "walking dynamics" of bacterial cells and the role of their own thermal diffusion field or electric and magnetic field constructed by the geometric boundary conditions of Dirichlet in their transfer or walking is the core subject of this article.

In addition, how the food barrier of centimeter dimensions can be crossed by bacterial cells where they are born and are transported by E continuously for days, as explained in the following section II.

II. THEORY

Experimental results showed that bacterial cells move outside their colony in a foodless area of centimeter dimensions in both radial and circular directions (Fig.4, Fig.5. Ref 1,8,9).

Moreover, it is difficult to imagine that flagella alone can direct bacterial cells in two orthogonal directions, radial and circular at the same time.

- Here it is best to assume that the growth of bacterial colonies in a foodless area is a process dominated by its own intrinsic E and H fields.

Note that in the case of concentric rings on an agar plate:

H is perpendicular to E, i.e. H has no radial component.

It is observed that the mechanism of development of the colonies over time begins with the accumulation and proliferation of the number of bacterial cells n at the internal and external circular borders [1,8,9].

This accumulation results in an increasing radial field E and an increasing radial current.

The numerical value of n bacterial cells can vary greatly from 10^5 to 10^9 /ml but at some critical points when the density n becomes high and hence its current becomes large enough, it produces an intense circular field H.

Therefore, the sufficiently developed bacterial colony in the symmetry between two concentric rings should provide simultaneous radial and circular exponential growth, which is the case of Fig.5,6.



Fig. 5: 7 days Growth of bacteria on a flat food surface (high quality Egyptian bread) maintained at 4 degrees Celsius with a pH of 7, normal air and humidity [1,8,9]

Inner ring radius $R1 = 1.5$ cm, outer ring $R2 = 4.5$ cm, and iron-rich food agar content 0.3 mg/1 g.

Fig.5. shows that bacterial macroscopic expansion or walking between two concentric rings of colonies follows:

- Its own electric field in the assumed radial direction along its pseudo-legs and also,

- Along its own magnetic field in concentric circles, assumed to be perpendicular to its pseudo-legs.

In short, the current theory that bacteria move or search for food via their pseudo-legs as the only driving force is not comprehensible.



Fig. 6: Early phase of bacterial colony patterns

Note that the starting radial rays of the field E are not yet complete and that the tiny elements of the circular field H are in their first phase of formation perpendicular to E.

In the case of the early phase where the n and E densities are low, no complete radial or circular pattern is observed. But both radial (E-Field driven) and circular (H-Field driven) beams progress at the same time although radial beams are much faster.

This instantly leads us to an alternative hypothesis that nature itself equips every bacterial cell with one or more free electrons as a "walking" mechanism to be able to survive and search for food.

The bacterial cell carries a negative charge $Q = N e$ on its surface, (where N is the number of free electrons and e is the electronic charge equal to -1.6×10^{-19} Coulomb).

However, we will not discuss in detail the origin or the mechanism of generation of negative charges in different types of bacterial cells in order to focus on the mechanism of its transport movement.

For this, we propose the following hypotheses:

- The so-called legs are actually negatively charged appendages that extend radially and act like strings or ropes pulling the bacterial cells in a direction anti-parallel to its own intrinsic E and at the same time they influence the bacteria rich in iron via magnetic force, i.e. in circular motion normal to E.
- The higher the number of free electrons in a particular bacterial species, the greater its negative charge on its surface. This may be a function of the number of evolutionary stages it has passed through and therefore inhibits its ability to survive and adapt to food scarcity conditions over time in 2D and 3D geometric space.
- iii-The bacterial cell has one or more free electrons and the bacterial colony contains millions of them which produce a radial electric field and a circular magnetic field according to the EMW theory.

It is therefore the electromagnetic field of the macroscopic bacterial colony that is responsible for the macroscopic movement of bacteria passing through the holes without food.

This particular hole-crossing move is mistakenly called Leg March.

The experiment of Fig.2, Fig.5 and Fig.6 shows that the plane or surface macroscopic growth of a bacterial colony in the case of two concentric food rings follows two directions, i-its own electric field in the radial direction i.e. along its pseudo-legs and also ii - along its own magnetic field in concentric circles, i.e. perpendicular to its pseudo-legs.

But why do bacterial cells move or walk so slowly, taking days or even weeks to bridge a gap of a few centimeters?

The answer is that the bacterial cell contains one or a few N of free electrons and the electrostatic charge (Nxe where $e = -1.6 \times 10^{-19}$ C) force F_e divided by the heavy mass of the bacterial cell is extremely low.

$$F_e = N e E \tag{1}$$

A similar analysis shows that the magnetic force F_m is still much less,

$$F_m = N e V_d \times B, \dots, \tag{2}$$

$$F_m/F_e = \mu_e V_d$$

Since V_d the drift rate is practically zero, the F_m only appears for bacteria containing ferrous compounds (μ_e iron/ μ_e water > 1000)

Which may be the reason why the bacterium accelerates faster in the radial direction and its radial structure is completed long before its circular structure as shown in Figs 5,6.

III. EXPERIMENTAL RESULTS

A. Two sets of experiments were performed on the macroscopic growth of bacterial colonies, namely, 7 days Growth of bacteria on flat food surface (high quality Egyptian bread) maintained at 4 degrees Celsius with pH 7, normal air and humidity [1,8,9]

Food Agar has the shape of two concentric rings.

Inner ring radius $R_1 = 1.5$ cm, outer ring $R_2 = 4.5$ cm, and iron-rich food agar content 0.3 mg/1 g.

Figure 5 shows the late developed dense phase of bacterial colony experiments.

It also shows the well-developed complete radial and circular patterns of the hollow bacterial colony.

B. The same experimental conditions as case (a) except that the macroscopic growth of bacteria was observed in its early phase after four days.

Results of this experiment is presented on Fig 6 whereradial rays of the field E are not yet complete and that the tiny elements of the circular field H are in their first phase of formation perpendicular to E.

Details of the experiments (a,b) and experimental setup are explained in ref. 1,8,9.

Note that,

- The agar plate should not be compact but hollow with appropriate R_1 and R_2 ratios.
- Agar food itself is rich in iron compounds and/or iron oxide magnetite (Fe_3O_4), [more than 0.1 mg/g].

IV. CONCLUSION

The "walking mechanism" of bacterial cells and the role of their legs and falagellae in their transfer or walking are studied in depth.

Additionally, the structure of bacterial cells and the role of their own thermal diffusion field, electric and magnetic fields constructed by the geometric Dirichlet boundary conditions in their transfer or walking mechanism have been thoroughly discussed.

Experimental study and theoretical analysis show that the bacterial cell colony is negatively charged due to the presence of free electrons which produce an electric field flowing radially outward from the colony and a circulating magnetic field surrounding the colony.

Bacterial cells can move radially outward and cross foodless spaces through the mechanism of forcing the bacterial colony's own electric fields in addition to the swimming or crawling motion of the bacteria's legs and flagella.

In short, all bacterial cell types have one or more free electrons forcing them to move along radial and circular lines of their own electron and magnetic fields and this is why macroscopic bacterial growth can cross areas without food.

The higher the number of electrons in a given species of bacteria, the greater its negative free charge on its surface and the greater its ability to survive or adapt to conditions of food scarcity.

NB. All experiments in this article were produced using the author's laboratory.

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