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Complex pattern formation of marine gradient bacteria explained by a simple computer model

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Abstract

We report on the formation of conspicuous patterns by the sulfide-oxidizing bacterium *Thiovulum majus* and a recently described vibrioid bacterium. These microaerophilic bacteria form mucus veils on top of sulfidic marine sediment exhibiting regular spaced bacterial patterns (honeycombs, interwoven bands, or inverse honeycombs). A simple qualitative computer model, based on chemotaxis towards oxygen and the ability of the bacteria to induce water advection when attached, can explain the formation of the observed patterns. Our study shows that complex bacterial patterns in nature can be explained in terms of chemotaxis and resource optimisation without involvement of cell–cell signalling or social behavior amongst bacteria.

Keywords: Self-organisation; Chemotaxis; *Thiovulum*; Microbial pattern formation; Quorum sensing; Computer model

1. Introduction

Pattern formation and self-organisation in biological systems is an intriguing phenomenon, which challenges the analytic mind in order to reveal the underlying principles. In recent years, especially the self-organisation of bacteria and eukaryotic microorganisms has gained much research interest as simple model systems, which might give clues about the evolutionary development of the concerted action of cells in higher organisms. It was argued, that microorganisms developed mechanisms for self-organisation in order to cope with unfavourable environmental conditions [1]. An example is the formation of macroscopic aggregates of purple sulfur bacteria under oxic conditions. These phototrophic bacteria are only able to synthesize their light-harvesting bacteriochlorophyll under anoxic or microoxic conditions. The formation of aggregates solves this problem, as the bacterial respiration in the surface layer of the aggregates keeps the inner regions anoxic [2]. A similar oxygen-defence mechanism has been observed for sulfate-reducing bacteria, which grow best under anoxic conditions. If a motile population of these bacteria is exposed to oxygen, the cells aggregate by negative aerotaxis. The center of the aggregate is again kept anoxic by oxygen respiration in the surface layers [3].

Microbial self-organisation requires some kind of cell-to-cell interaction. Several interaction types in a diversity of systems have been proposed as central for microbial self-organisation. Researchers with a background in physical science have especially investigated pattern formation of bacterial agar plate cultures under nutrient limitation (for a review see [4]). Under such conditions, bacterial colonies of *Escherichia coli* and *Salmonella typhimurium* form highly symmetric

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spatio-temporal patterns of rings, stripes, or spots [5,6]. Other bacterial species develop colonies resembling fractal tree-patterns or dynamic vortices [7,8]. All observed patterns could so far be explained by a ''chemotactic feedback'', that is, individual bacteria react chemotactically to diffusion gradients of oxygen or metabolites (e.g., aspartate) excreted by other bacteria. Keller and Segel [9,10] provided the first detailed mathematical analysis of such systems. Explicit computer simulation of the chemotaxis and the diffusion processes could convincingly reproduce the observed patterns [7,8,11]. The heterogeneity of natural environments exhibiting strong spatio-temporal fluctuations normally prevents the formation of such highly symmetric patterns in nature. However, a remarkable exception is the complex process of fruiting body formation by Myxobacteria, which also relies on a ''chemotactic feedback'' mechanism [12].

Another microbial self-organizing mechanism is ''quorum sensing'', where cell-to-cell interaction is based on diffusible autoinducer molecules (e.g., peptides or acylated homoserine lactones) excreted by the bacteria. However, the bacteria do not react chemotactically to the autoinducer, but respond by changing their gene expression patterns [13]. Quorum sensing was first demonstrated for the autoinduction of bioluminescence in bacteria living in symbiosis with fish. Many other bacterial species exhibit quorum sensing [14], for example *Pseudomonas aeruginosa* when forming a biofilm [15]. It has thus been speculated that quorum sensing plays a predominant role in the organisation of natural microbial communities such as complex biofilms [16] or marine snow [17].

In this study, we report on complex spatial patterns formed by marine microaerophilic bacteria kept in enrichment cultures under quasi-natural conditions. The patterns were formed by the sulfide-oxidizing bacterium *Thiovulum majus* and a recently discovered vibrioid bacterium [18]. Both species thrive in oxygen-sulfide counter-gradients at the oxic–anoxic interface on top of sulfidic marine sediment. The bacteria aggregate chemotactically at their preferred oxygen concentration of 2–10 μM, where they can attach themselves with a mucus stalk [18–21]. Detachment occurs under unfavourable oxygen concentrations [20]. Large accumulations of these bacteria form macroscopically visible whitish veils, which are built up by their excreted stalks. The bacteria are attached on the upper surface of the veils where their flagella-driven rotation induces local convection cells. By this advective water mixing the bacteria can enhance their oxygen uptake up to 40 times [18,22]. The bacteria are typically not homogeneously distributed on the veils; rather they form regular patterns (Fig. 1). Here, we show that the formation of these patterns can be explained solely by the chemotactic behavior of the bacteria in combination with their ability to induce advective water mixing when attached.

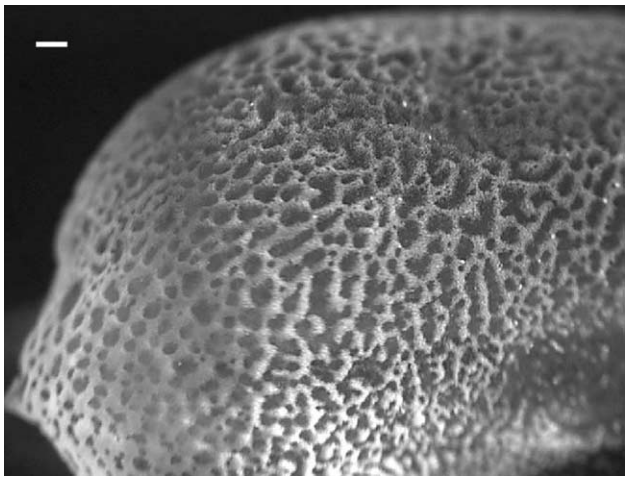

Fig. 1. *Thiovulum majus* veil on top of sulfidic sediment in an enrichment culture. Bright areas are colonized by the bacteria (stereo microscopic photograph, scale bar ca. 1 mm).

2. Material and methods

Marine sulfidic sediment was sampled from a shallow bay (Nivå Bay, Denmark) and kept in the laboratory under in situ conditions (20 °C, covered with ~100 mm air saturated seawater (250 μM O_2) and advective water currents of ~10 mm s^{-1}). Sulfide produced by sulfate-reducing bacteria in the anoxic sediment was released into the overlying water, and removed by chemical and biological oxidation with O_2 in a ~5 mm thick water layer above the sediment surface. Opposing sulfide-oxygen gradients within this layer provide ideal growth conditions for motile microaerophilic bacteria, which formed within 1–3 days macroscopic visible veils spanning depressions on the sediment surface. The bacterial distribution on the veils was documented by a standard monochrome CCD-camera (EHD, Germany) mounted on a dissection microscope.

3. Results and discussion

Within 3 days after their formation, larger veils (>1 cm^2) typically showed regular patterns in the distribution of bacteria. A honeycomb pattern could be observed for the vibrioid species, where the attached bacteria populated the rims of regularly spaced holes measuring ~0.3 mm in diameter (Fig. 2(a)). A similar pattern could be observed for *T. majus* with regularly spaced holes measuring ~0.5 mm in diameter (Fig. 2(b)). At lower bacterial density, veils of *T. majus* exhibited patterns of interwoven bands (Fig. 2(c)) or regularly spaced ~0.5–1 mm wide clusters forming an ''inversed honeycomb'' pattern (Fig. 2(d)).

In order to explain the underlying mechanism for the observed pattern formation, we developed a simple qualitative computer model taking our present

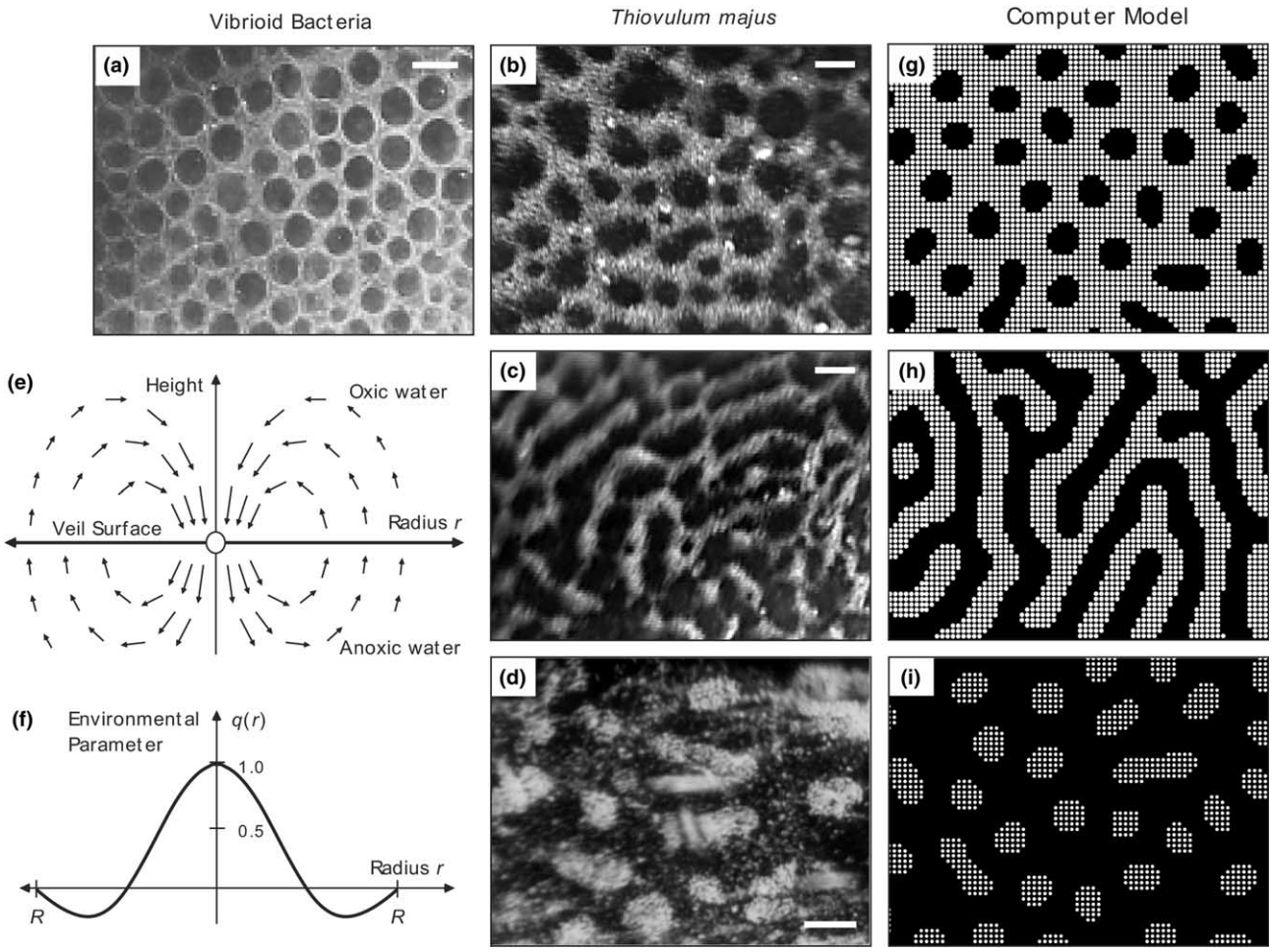

Fig. 2. Natural and simulated bacterial patterns on sulfidic sediments. (a), (b) Top-view of honeycomb patterns formed by veil-forming vibrioid bacteria (a) and the bacterium *Thiovulum majus* (b). Bright areas are colonized by bacteria, scale bars are 0.5 mm. (c), (d) Interwoven bands (c), and inverse honeycomb pattern (d) formed by *Thiovulum majus* at lower bacterial density. Scale bars are 0.5 mm. (e) Schematic drawing of the flow field around an attached bacterium (white circle), arrows denote the velocity vectors $\vec{v}$ of the water. (f) Radial dependence of the environmental parameter $q(r)$ around a single attached bacterium used for the computer simulation. High values of q represent favourable oxygen conditions. (g)–(i) Simulated bacterial patterns (white spots represent single bacteria): honeycomb pattern (g, simulation parameters $f = 0.75$, $R = 7$), interwoven bands (h, $f = 0.5$, $R = 7$), and inverse honeycomb pattern (i, $f = 0.25$, $R = 7$).

knowledge about the bacterial behavior into account. It has been shown, that the attached bacteria can induce a radial symmetric flow field $\vec{v}(\vec{x})$ (i.e., the velocity vector $\vec{v}$ of the water at position $\vec{x}$; Fig. 2(e)) [22]. At the center oxygenated water is moved downwards towards the bacterium enhancing its oxygen uptake rate. Microscopic observations showed that free-swimming bacteria preferentially attached in close vicinity to already attached bacteria, benefiting from the locally enhanced oxygen supply. In contrast, free-swimming bacteria avoided a ring-shaped region around attached bacteria, which was anoxic due to upwelling sulfidic water.

At a small scale the bacterial veils are relatively flat structures. Thus, in the following we regard the veil as a two-dimensional plane. The above mentioned observations can now be qualitatively described by mapping an environmental parameter q on this two-dimensional plane, showing a radial symmetric sombrero-shape (Fig. 2(f)). High values of q correspond to favorable oxygen conditions for the bacteria. A possible mathematical description of the sombrero-shape is given by

$$q(r) = \begin{cases} \frac{\sin(2\pi R^{-1} r)}{2\pi R^{-1} r}, & \text{for } r \leqslant R, \\ 0, & \text{for } r > R, \end{cases} \quad (1)$$

with r denoting the radial distance from the attached bacterium and R the maximum distance influenced by the attached bacterium; the exact shape of $q(r)$ is not essential for the outcome of the computer simulation. The differential equations describing the water flow field at microbial size scales are linear [23]. Adding up the flow fields generated by single bacteria can thus approximate the total flow field generated by bacterial clusters. Following this argumentation, the total environmental parameter Q at a specific position on the veil generated by N attached bacteria

is calculated by $Q = q(r_1) + q(r_2) + \cdots + q(r_N)$, where r_i is the distance to the ith attached bacterium. In our model attaching bacteria will always prefer positions with a maximal value for Q.

The model was implemented (with LabView, National Instruments, USA) in a computer simulation on a dimensionless two-dimensional grid representing the veil surface (movie files showing several simulation runs are available as supplementary online information; the simulation software itself can be downloaded for Windows PCs from the authors' homepage: http://www.mbl.ku.dk/mkuhl/pattern). At the beginning of the simulation N bacteria are randomly distributed on the grid with a bacterial density f (i.e., the fraction of occupied grid cells). Each grid cell in the simulation can be occupied by a single bacterium only. The position of the ith bacterium is given by the integer numbers k_i and l_i corresponding to the row- and column-numbers of its grid cell. The model is dimensionless, i.e., distances are measured in number of grid cells, and each iteration step in the simulation consists of two procedures: First, the total environmental parameter is calculated for all grid cells as

$$Q(k,l) = \sum_{i=1}^{N} q\left(\sqrt{(k-k_i)^2 + (l-l_i^2)}\right), \qquad (2)$$

where the integer numbers k and l designate the row- and column-numbers of the grid cells. Secondly, the bacterium with the minimum value of Q at its position is moved to an unoccupied grid cell having a maximum value of Q. The iteration is resumed until the bacteria on the grid attain a steady state distribution. This iteration procedure mimics the observed bacterial behavior in their natural environment: Attached bacteria detach when experiencing unfavourable oxygen concentrations and locate, by help of true chemotaxis, a more favourable position where they reattach.

Two parameters need to be chosen for the model, the fraction f of grid cells occupied by bacteria, and the maximum influence distance R (in units of grid cells). For low bacterial densities ($f < 0.25$) the steady state distribution showed isolated circular colonies of bacteria randomly distributed on the grid (Fig. 2(i)). The diameter of the colonies as well as the minimum distance between the colonies was approximately R grid cells. With increasing f the isolated clusters merged with each other, resulting in a pattern of interwoven bands for $f = 0.5$ (Fig. 2(h)). The thickness of the bands is approximately R grid cells. Finally, for $f = 0.75$, the simulation reproduces the honeycomb-pattern of regular spaced holes (Fig. 2(g)). Thus, by variation of the bacterial density parameter f in the computer model, we could reproduce all essential features of the naturally observed bacterial patterns (Fig. 2(a)–(d)).

The striking similarity of natural and simulated patterns suggests, that the observed self-organisation can be explained by bacterially induced small-scale water currents in combination with bacterial chemotaxis towards oxygen. The honeycomb structure of the vibrioid bacteria (Fig. 2(a)) shows on average smaller hole-diameters than the one observed for *Thiovulum* (Fig. 2(b)). This can be explained by the fact, that attached vibrioid bacteria induce less strong advective water currents than the bigger cells of *Thiovulum* do [18]. Thus, the absolute influence radius R in case of the vibrioid bacteria should be smaller than the one of *Thiovulum.* However, as our qualitative model is based on dimensionless distances (i.e., number of grid cells), these differences in scaling are not considered by the simulation. Our goal was to demonstrate that our simple model can reproduce qualitatively the observed patterns. A quantitative model would require an explicit simulation of fluid dynamics, and a promising approach was recently presented by Cogan and Wolgemuth [24]. They simulated a simplified model of a one-dimensional bacterial veil and were able to predict the formation of convection cells and their scale dependence on the distance of the veil to the sediment surface.

The observed honeycomb-patterns (Fig. 2(a), (b), and (g)) result in a complex three-dimensional advective flow pattern with regions of upwelling water over the holes (dark areas) and downwelling water at the bacterial rim (bright areas). Interestingly, this three-dimensional flow pattern is very similar to the purely physical phenomenon of hexagonal flow patterns observed for Bénard-convection in fluids [25].

The mechanism we propose for pattern formation in the bacterial veils differs from other known interaction mechanisms. The involved bacteria do not actively excrete any signalling compounds, rather they use a chemotactic feedback mechanism in response to the ambient oxygen concentration. Further, the driving signal is not mediated by diffusion, but by advective transport generated actively by the bacteria. A related phenomenon has been described by Kessler and coworkers, who investigated bioadvective honeycomb-patterns close to the water surface in liquid pure cultures of aerotactic microorganisms (for a review see [26,27]). Such patterns were induced by the increased specific weight of local cell aggregations rather than a combined effect of flagellar motion. It was argued that this mechanism could increase the oxygen uptake across the air–liquid interface [27]. However, the ecological significance of such bioadvective patterns in natural environments remains questionable [28,29].

We report on bacterial patterns often seen on sulfidic natural sediments [18,22], where the bacterial veil (Fig. 2(b)–(d)) generates an advective flow pattern with regions of upwelling water over the holes (dark areas) and downwelling water at the bacterial rim (bright areas). This example of complex bacterial self-organisation in nature can be explained by a combination of bacterial chemotaxis and resource optimisation that does not imply any

direct communication or complex genetic programs, as it is found in quorum sensing. Quorum sensing has gained much research impetus in recent years, and some researchers propose that quorum sensing is the base for complex "social" interaction in microbial communities [30,31] or even the "language of bacterial communication" [32]. Quorum sensing can indeed regulate bacterial self-organisation, e.g., among bioluminescent bacteria or in the formation of bacterial colonies during biofilm development [12]. However, it's universal significance for microbial organisation is questionable [33], and a simpler functional explanation for many phenomena anticipated to be regulated by quorum sensing could be based on e.g., "diffusion sensing" [34]. Here, we have shown an example of complex bacterial self-organisation in nature, which can be explained by a simple mechanism, i.e., a combination of bacterial chemotaxis and resource optimisation that does not imply any direct communication or social behavior amongst the bacteria.

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Appendix A. Supplementary data

Supplementary online information is available on the Journal's homepage. Supplementary data associated with this article can be found, in the online version at doi:10.1016/j.femsle.2005.03.036.

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