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True Chemotaxis in Oxygen Gradients of the Sulfur-Oxidizing Bacterium *Thiovulum majus*

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Observations of free-swimming *Thiovulum majus* cells show that these bacteria exhibit a phobic response as well as true chemotaxis in oxygen gradients. Both phenomena of their chemotactic behavior are integrated into a single model of helical klinotaxis, which is demonstrated by computer simulations.

Many motile prokaryotes are able to accumulate in regions which are favorable for their physiological adaptations. In order to achieve this they have to sense physical parameters (e.g., light, magnetic fields, gravity, or concentrations of chemical substances) of their environment. If bacteria change their motility patterns in response to chemical substances, this behavior is called chemotaxis. Following the terminology given by Dusenbery (9), chemotaxis can on principle be realized in two different ways. True taxis is given if responses to chemical gradients are directional, i.e., the moving direction of the organism is correlated to the direction of the chemical gradient. If the responses are undirectional, the behavior is called a kinesis, although traditionally microbiologists have used the term "chemotaxis" to describe all kinds of chemosensory behavior. Further, the sensor principle at the cellular level for sensing chemical gradients can be either temporal or spatial. In the first case the organism samples the chemical concentration along its swimming path. Information about the chemical gradient is obtained in combination with an internal memory for the sensed signal. Spatial sensing is given if the gradient is directly sensed along the cell body. This requires at least two independent sensor regions on the cell surface, while temporal sensing can be realized with a single sensor region.

The chemotactic behavior of *Escherichia coli* is the best understood among the prokaryotes (1). It exhibits a motility pattern called random walk. Straight swimming paths are repetitively interrupted by tumbling, i.e., random direction changes. When swimming through a chemical gradient, the bacterium senses a temporal change in chemical concentration. Chemotaxis is realized by modulating the tumbling frequency in response to this change, which results in a biased random walk (3). Phobic responses are closely related to the biased random walk. The chemical gradient is again sampled by temporal sensing. If a certain trigger value is exceeded, swimming direction is reversed. Thus, the bacteria are effectively trapped within a certain region, as can be seen, e.g., for microaerophilic bacteria forming a narrow band at oxic-anoxic interfaces (2).

All bacterial chemotactic responses reported until now can be described by models with temporal sensing and a run-tumble behavior as described for *E. coli*. It is not a true taxis in that the migration of the cells is a statistical phenomenon. It is generally assumed that prokaryotes are too small for spatial sensing due to physical constraints. A detailed theoretical analysis showed recently, however, that the actual size limit of the cell diameter for spatial sensing is less than 1 μm (10).

The sulfide-oxidizing bacterium *Thiovulum majus* shows many features which are unusual for a prokaryote (12). The spherical cells have a diameter of 5 to 10 μm. Flagella cover most of the cell surface. Swimming speeds are among the highest known for bacteria (up to 600 μm s^{-1}) (14), and the swimming path is always a left-handed helix. Their physiological adaptation requires the simultaneous presence of sulfide and oxygen, a condition found in opposing sulfide-oxygen gradients in marine sulfidic sediments. They often form conspicuous white veils on top of these sediments. *Thiovulum* prefers an oxygen concentration of about 4% air saturation (about 10 μM O_2 at 30% salinity and 20°C) independent of the present sulfide concentration (12). The cells show two different mechanisms to keep their position at their preferred isopleth of an oxygen gradient. Either they attach to a solid surface with a mucous stalk of up to 100-μm length or they remain free swimming and form narrow bands by a strong chemotactic response. An earlier study reported that cells keep within the band by a phobic response called "U-turn" (12) or "steered turning" (4, 13). It resembles the phobic response as described above. The bacteria, however, do not simply reverse swimming direction but perform a "U-turn" by gradually changing their direction. Thus, the cells return whenever they swim outside the preferred region. It was not believed, however, that the cells could directly orient themselves in an oxygen gradient.

Here we report new observations indicating that *Thiovulum* is capable of orienting itself in oxygen gradients. This enables the bacterium to stay more efficiently within the narrow band of optimum oxygen concentration. The "steered turning" and the present observations can be integrated into a single model called helical klinotaxis, which is demonstrated by computer simulations. The theory of helical klinotaxis was proposed by Crenshaw (6–8) and was later experimentally demonstrated for protists (13). But to our knowledge this is the first time that helical klinotaxis is demonstrated for a prokaryote. The underlying mechanism in helical klinotaxis is that cells that are sufficiently large to avoid a strong influence of rotational Brownian motion tend to swim in a helical trajectory. If the cells swim in a chemical gradient they will experience a periodic change in

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concentration for each period of the helical path. If the cells change the rotational velocity whenever they experience a changing concentration then the axis of the helical trajectory will bend so that cells migrate along the gradient. This is true taxis in the sense that the migration of the cells is not a statistical drift; rather, their moving direction is correlated with the direction of the gradient.

Experiments. Sulfidic sediment samples were collected during winter and springtime from Nivå Bay (Denmark) and were kept darkened at room temperature. The overlying water was constantly aerated. After about 3 days the sediment surface was covered by dense *Thiovulum* veils. Samples (~0.3 ml) were collected from the veil with a pipette and filled into a flat microslide capillary (40 by 10 by 1 mm^3). Most regions inside the filled capillary were oxic due to the preparation procedure. Microoxic regions (<10 μM) developed around small sulfidic debris particles (diameter, 0.5 to 2 mm) or around *Thiovulum* clusters (diameter, 50 to 500 μm). The clusters were remnants of the original veil and consisted of 10 to 100 *Thiovulum* cells connected to each other by their mucous stalks. The oxygen concentration was kept low due to the active respiration of the cells. Microsensor measurements showed typical oxygen gradients of 2 to 10 μM per 100 μm (data not shown). Swimming cells were recorded with a charge-coupled device camera attached to a microscope and a video recorder. Tracks of individual cells were obtained by analyzing the video tape frame by frame (time steps, 0.04 s) using the software program LabTrack (DiMedia, Kvistgaard, Denmark).

Free-swimming *Thiovulum* cells accumulated in narrow bands of about 100-μm diameter around the microoxic regions (Fig. 1A and B). Swimming speeds were between 250 and 500 μm s^{-1} (mean, 390 μm s^{-1}). The cells showed true taxis by keeping their swimming direction aligned with the band. Thus, the bacteria circled around the microoxic regions and followed their preferred oxygen isopleth. Figure 1A shows the cells steering around a corner of a debris particle. Some tracks with a pronounced helical swimming path showed that the steering was performed by changing the helix parameters. *Thiovulum* cells outside the band (i.e., in regions with an oxygen concentration of >10 μM) showed higher swimming speeds, between 425 and 625 μm s^{-1} (mean, 530 μm s^{-1}). These cells also exhibited chemotactic behavior within a certain distance (0.5 to 1 mm) from the microoxic region. Their swimming path was always bent towards the center of the microoxic region (Fig. 1C). The bending curvature became more pronounced when the cells were closer to the center. Thus, some cells showed a spiral swimming path towards the microoxic region and were finally "captured" by the band. Phobic responses (U-turns) were also observed. Cells leaving the band around a microoxic region reversed swimming direction after 100 to 500 μm by "steered turning" (Fig. 2A). This response was observed on both sides of the band.

Simulation. A simulation model (4) based on helical klinotaxis was modified for analyzing the chemotaxis of *Thiovulum*. The motility of the bacterium has both a translational and a rotational component, which are defined by tangential velocity $\vec{v}$ and rotational velocity $\vec{\omega}$. If the vectors are not aligned and do not change in time, it results in a helical swimming path (6). Cells can change their swimming direction by varying these vectors (7). We assumed in our model a constant tangential velocity, $\vec{v}$, of 400 μm s^{-1}. Thus, the rotational velocity can be split up into two components: a rotation around the axis aligned to $\vec{v}$ (rotational velocity ω_1) and a rotation around an axis perpendicular to $\vec{v}$ (rotational velocity ω_0). ω_0 was kept constant at 6 rad s^{-1}, while ω_1 was modulated by an intracellular signal, σ ($0 \leq \sigma \leq 1$):

$$\omega_1 = \omega_c (1 - \sigma) \tag{1}$$

where the constant ω_c = 30 rad s^{-1} (12). In the model of Brown and Berg (5) for chemotaxis in *E. coli*, it is assumed that the cells react to changes in the fraction P of receptor molecules that are reversibly bound to the attractant molecules:

$$P = \frac{C}{K_D + C} \tag{2}$$

where K_D is the dissociation constant and C is the ambient attractant concentration. The sensed signal is proportional to $\partial P/\partial t$. In the case of microaerophilic microbes, such as *Thiovulum*, there is an optimum oxygen concentration and cells respond negatively to positive as well as negative deviations from this concentration. For *Thiovulum* it was previously shown that responses to oxygen concentrations that are too low and too high seem equally intense (12). Due to this apparent symmetry and since we are ignorant with respect to how the sensory mechanism may work in *Thiovulum*, we replaced equation 2 by

$$P = \exp\left(\frac{-(C_0 - C)^2}{2\,\Delta C^2}\right) \tag{3}$$

where C is the ambient oxygen concentration, C_o is the optimum oxygen concentration of *Thiovulum*, and ΔC is a constant. This modified model ensures that P is maximal at the preferred oxygen concentration of *Thiovulum*. We assume that a signal, σ, is generated in response to the derivative in time of P:

$$\sigma = \begin{cases} 0 & \text{for } \dfrac{\partial P}{\partial t} \geq 0 \\ \mathrm{Min}\left(1, -\alpha \dfrac{\partial P}{\partial t}\right) & \text{for } \dfrac{\partial P}{\partial t} < 0 \end{cases} \tag{4}$$

where α is a time constant. Only negative changes in time of P result in a signal of $\sigma \neq 0$. As values for C_0, ΔC, and α we used 10 μM, 25 μM, and 6 s, respectively, in our simulation. We are aware that this model probably does not reflect any reality regarding signal reception and transduction in *Thiovulum* cells but only describes the empirical fact that the cells change their rotational motility in response to adverse oxygen concentrations with time. A degree of randomness was introduced by multiplying the signal, σ, by a random number between 0.9 and 1.1.

The swimming path of single cells in a given geometry of oxygen gradients was simulated on a computer in time steps of 0.05 s. A detailed description of the simulation technique can be found in Blackburn and Fenchel (4). At each step the intracellular signal, σ, was calculated (equation 4), which gave the new actual rotational velocity, $\vec{\omega}$, of the cell (equation 1). An underlying assumption of the finite time steps was that the cell used an internal memory of 0.05 s for the temporal sensing of the oxygen gradients. The position of the cell after the next time step was calculated from the tangential velocity, $\vec{v}$, and the

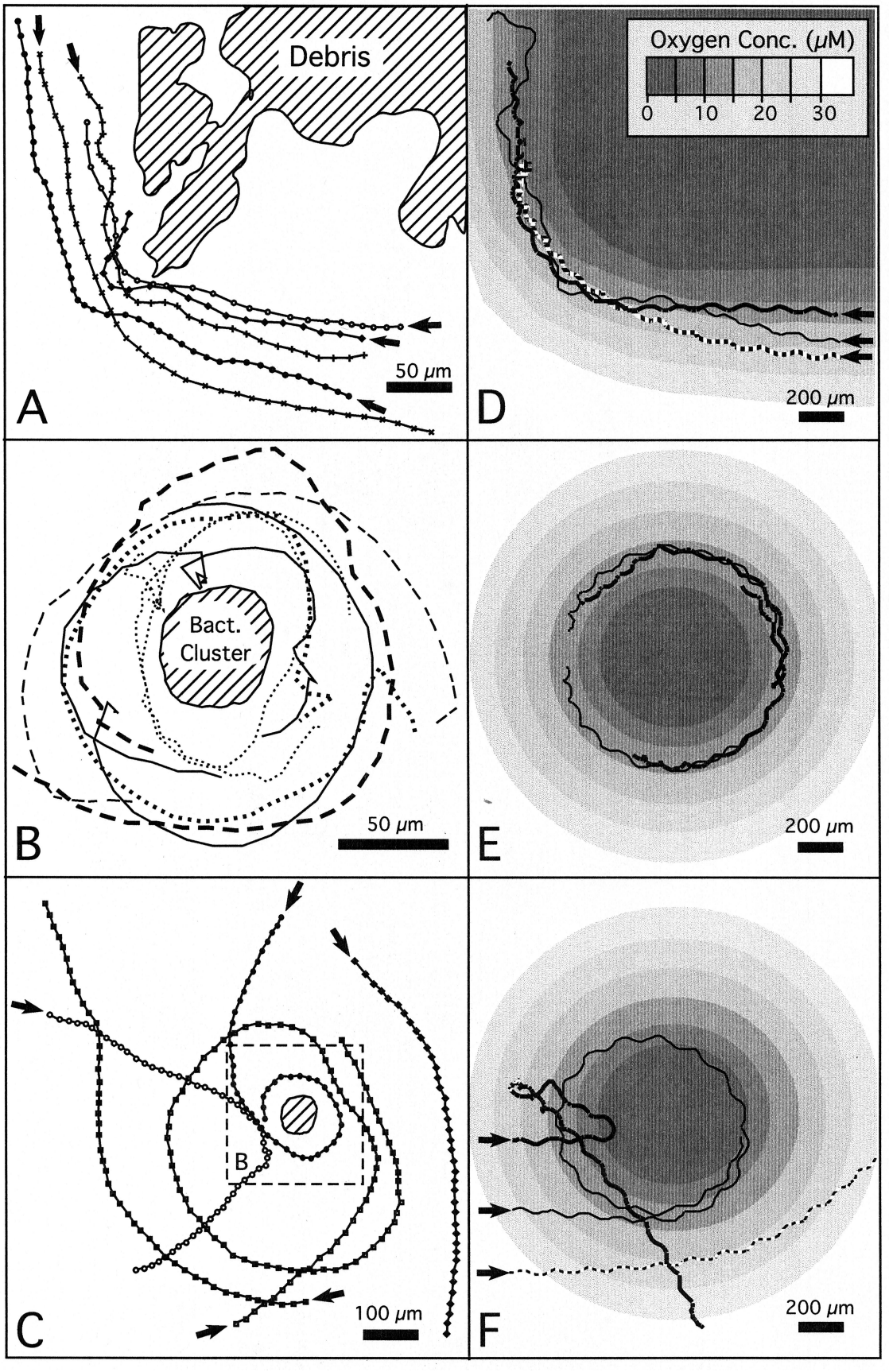

FIG. 1. Swimming paths of *Thiovulum* cells observed in experiments (A to C) and the simulated counterparts (D to F). Time steps between dots on the tracks (if indicated) are 0.05 s. (A and D) Steering around a corner along the preferred oxygen isopleth. (B and E) Circular tracks in a cylindrical gradient around a *Thiovulum* cluster. (C and F) Tracks outside the preferred oxygen isopleth. The dashed square in panel C indicates the region shown in panel B.

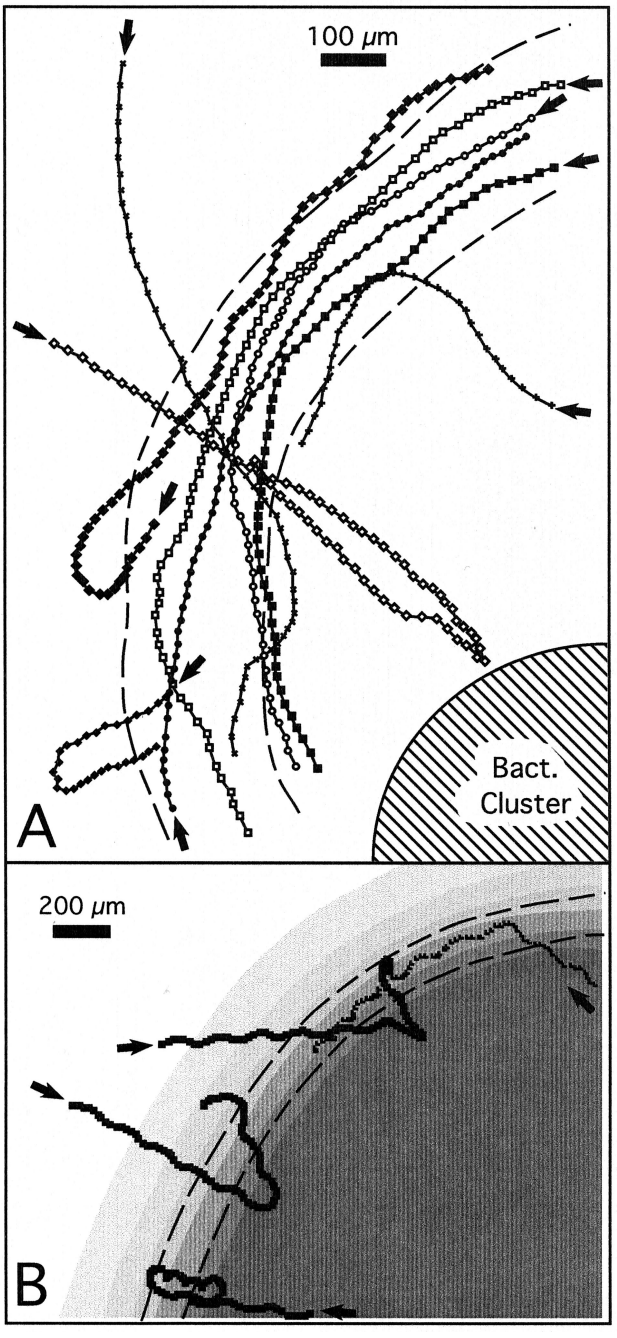

FIG. 2. Swimming paths of *Thiovulum* cells (A) and the simulated counterpart (B). Time steps between dots on the tracks are 0.05 s. Dashed lines enclose the band around the preferred oxygen isopleth. Several cells show the "steered turning" response when leaving the band.

rotational velocity, $\vec{\omega}$. The geometries of the oxygen gradients were chosen to be similar to the ones found in the experiments. No gradients perpendicular to the observation plane were assumed, which reflected the geometry of the glass capillary used in the experiments.

If simulated cells started swimming within and in parallel to their preferred band around the oxygen isopleth of 10 μM (Fig. 1D and E), they exhibited true taxis and kept swimming within the band. This resembles the behavior found for *Thiovulum* (Fig. 1A and B). Simulated cells starting outside their preferred band showed tracks which were bent towards the microoxic center (Fig. 1F) as also seen in the experiment (Fig. 1C). Cells approaching the preferred band at an oblique angle were "caught" on a circular track, but cells approaching at an obtuse angle showed a phobic U-turn response. The latter is demonstrated in more detail in Fig. 2B. Simulated cells leaving the preferred band at an obtuse angle performed a U-turn after about 200 μm and swam back to the band, which reflects the "steered turning" response of *Thiovulum* (Fig. 2A).

Gradient sensing at the cellular level. The demonstrated experiments and simulations show that the chemotactic behavior of *Thiovulum* can be described by helical klinotaxis. The model is appealing as it integrates two apparently different behaviors: "steered turning" and true taxis. Which behavior is actually expressed depends on the orientation of the cell towards the oxygen gradient. The "steered turning" response is most pronounced if the cell is swimming in parallel to the gradient, and the true taxis response is most pronounced if the cell is swimming perpendicular to the gradient (i.e., swimming in parallel to isopleths). At the cellular level the difference can be seen at the intracellular signal, σ. If swimming along isopleths, the cells experience a periodically modulated signal due to the helical geometry of their swimming path; i.e., the time period of the modulation is equal to the time period of their helical swimming path. Thus, the helix parameters are modulated periodically, which leads to the true taxis behavior (8). The situation is different for cells swimming along gradients. In this case the signal is not periodically modulated. A "steered turning" response is produced if the signal exceeds a certain trigger value.

The model of helical klinotaxis assumes that all receptor signals are integrated into a single intracellular signal, σ. In this case chemotaxis is based on temporal sensing. An unresolved problem of the model is how *Thiovulum* controls the action of the flagella in order to modulate its rotational velocity, $\vec{\omega}$. An alternative model of chemotaxis based on spatial sensing could give an explanation of how this is done. Groups of receptors on the cell membrane produce intracellular signals, which influence only the local flagella. The rotational velocity is produced by the concerted action of all flagella. Different groups of flagella would show a different behavior depending on the cell's orientation in an oxygen gradient, which would explain the modulation of the rotational velocity. All molecular studies of bacterial chemotaxis involved an intracellular signal transduction, which was based on intracellular diffusion of some chemical compound (1). Typical diffusion times over distances corresponding to the diameter of a *Thiovulum* cell are in the order of 10 ms, which is much shorter than the typical time period of 0.2 to 1 s of their helical swimming path (12). No local intracellular signal could be built up over time periods relevant for the motility which would be neccessary for spatial sensing. However, electron micrographs of sectioned *Thiovulum* cells show complex invaginations of the cell membrane (11) dividing the intracellular space into compartments. Thus, intracellular signal transductions in separate compartments can be independent of each other, and a local receptor-flagella coupling is again possible.

The present study has demonstrated that the versatile chemotactic behavior of *Thiovulum* can be described by helical klinotaxis. Future studies have to show whether this is realized on the cellular level by temporal or by spatial sensing.

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