RESEARCH ARTICLE

Motility patterns of filamentous sulfur bacteria, *Beggiatoa* spp.

Rita Dunker[1], Hans Røy[2], Anja Kamp[1] & Bo Barker Jørgensen[1,2]

[1]Max Planck Institute for Marine Microbiology, Bremen, Germany; and [2]Center for Geomicrobiology, Århus University, Århus, Denmark

Correspondence: Rita Dunker, Max Planck Institute for Marine Microbiology, Celsiusstrasse 1, 28359 Bremen, Germany. Tel.: +49 421 202 8630; fax: +49 421 2028 690; e-mail: rdunker@mpi-bremen.de

Received 10 October 2010; revised 2 March 2011; accepted 16 March 2011.
Final version published online 3 May 2011.

DOI:10.1111/j.1574-6941.2011.01099.x

Editor: Alfons Stams

Keywords
Beggiato; gliding motility; chemotaxis; random walk.

Abstract

The large sulfur bacteria, *Beggiatoa* spp., live on the oxidation of sulfide with oxygen or nitrate, but avoid high concentrations of both sulfide and oxygen. As gliding filaments, they rely on reversals in the gliding direction to find their preferred environment, the oxygen–sulfide interface. We observed the chemotactic patterns of single filaments in a transparent agar medium and scored their reversals and the glided distances between reversals. Filaments within the preferred microenvironment glided distances shorter than their own length between reversals that anchored them in their position as a microbial mat. Filaments in the oxic region above the mat or in the sulfidic, anoxic region below the mat glided distances longer than the filament length between reversals. This reversal behavior resulted in a diffusion-like spreading of the filaments. A numerical model of such gliding filaments was constructed based on our observations. The model was applied to virtual filaments in the oxygen- and sulfide-free zone of the sediment, which is a main habitat of *Beggiatoa* in the natural environment. The model predicts a long residence time of the virtual filament in the suboxic zone and explains why *Beggiatoa* accumulate high nitrate concentrations in internal vacuoles as an alternative electron acceptor to oxygen.

Introduction

The filamentous sulfur bacteria of the genus *Beggiatoa* are best known as white mats on sulfidic sediments or on decomposing organic debris (e.g. Jørgensen & Revsbech, 1983; Sweerts *et al.*, 1990; Sayama, 2001; de Beer *et al.*, 2006, Fig. 1a and b). Their most widespread habitat, however, is hidden within the mm to cm thick suboxic zone of coastal sediment, where neither oxygen nor sulfide is detectable (e.g. Jørgensen *et al.*, 2010, Fig. 1c). There are no consistent trends in the distribution of *Beggiatoa* biomass down through the suboxic zone. The highest densities can often be found just below the few mm thick oxic zone and at the diffusion front of sulfide (Mussmann *et al.*, 2003; Preisler *et al.*, 2007), in the middle (Hinck *et al.*, 2007), or the distribution can be more even throughout the suboxic zone (Jørgensen *et al.*, 2010). These distribution patterns have been ascribed to a negative tactic response to oxygen, by which *Beggiatoa* avoid high oxygen concentrations (Møller *et al.*, 1985). Similarly, the bacteria appear to have a negative tactic response to sulfide (Hinck *et al.*, 2007; Preisler *et al.*, 2007). It is not understood how *Beggiatoa* navigate in the suboxic zone in the absence of sulfide and oxygen gradients.

There are detailed studies of the chemotactic behavior of various swimming sulfur bacteria and of the formation of mats and veils of these organisms (Thar & Fenchel, 2001, 2005; Thar & Kühl, 2001; Fenchel & Thar, 2004). Some swimming sulfur bacteria reverse their swimming direction at a critical oxygen concentration of 1–10 μM oxygen (Thar & Fenchel, 2005). Others orient by the biased random walk well known from *Escherichia coli*. The filamentous *Beggiatoa*, however, do not swim, but glide by excretion of mucus. The chemotactic responses available to *Beggiatoa* in order to form complex community structures therefore appear to be limited to simple reversals of the gliding direction. *Beggiatoa* reverse in response to chemical clues (Nelson & Castenholz, 1982; Møller *et al.*, 1985; Huettel *et al.*, 1996; Høgslund *et al.*, 2009), for instance when gliding from an anoxic into an oxic zone. They also respond to blue light, but not to red light, by a step-up phobic response (Nelson & Castenholz, 1982). The reversals occur either over the entire filament or, when the filament is long, only over the leading end of the filament that first becomes exposed to oxygen (Møller *et al.*,

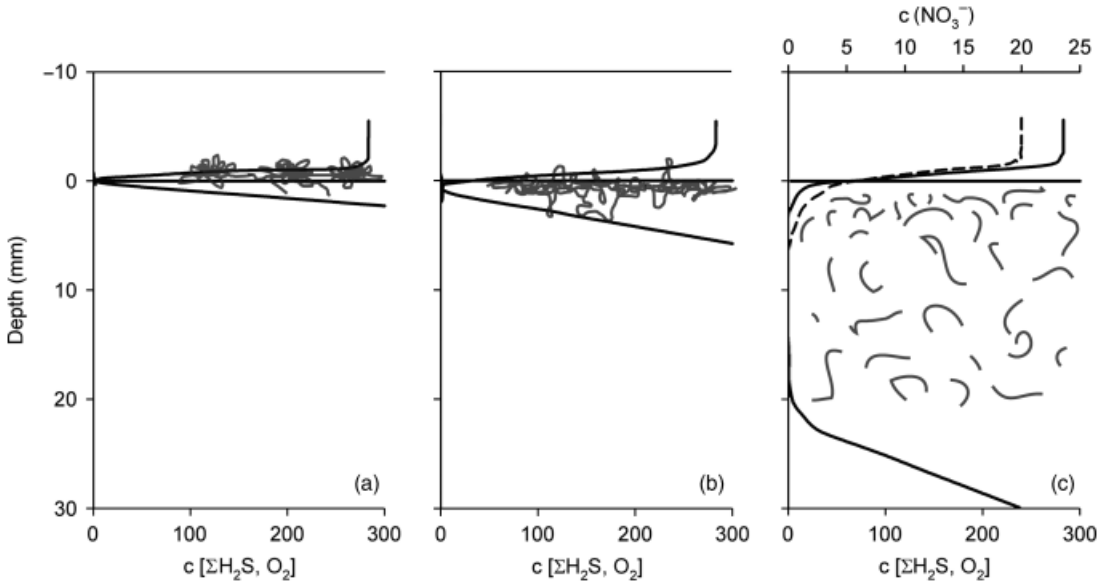

Fig. 1. Typical distribution patterns of *Beggiatoa* under different gradient conditions with O_2 diffusion from above and H_2S diffusion from below. (a) Tufts of filaments at oxygen declining to zero just above the sediment surface (compare with Møller *et al.*, 1985). (b) *Beggiatoa* layer at the sediment/water interface with oxygen and sulfide overlapping a few mm underneath the sediment surface (compare with Jørgensen & Revsbech, 1983). (c) *Beggiatoa* filaments spread out in the suboxic zone of the sediment with the oxygen and sulfide diffusion front separated by several cm (compare with Mussmann *et al.*, 2003; Preisler *et al.*, 2007). Dashed line: NO_3^-.

1985). On the surface of sulfidic sediment, the reversals effectively keep the individual *Beggiatoa* filaments within their preferred chemical environment. The mat can even respond to changes in oxygen supply by curling up in a tight mat or form a looser looping 'fur' (Fig. 1a and b).

The aim of the present study was to understand the mechanics of chemotaxis in *Beggiatoa* that live hidden in the three-dimensional matrix of the suboxic zone of coastal and estuarine sediments. We studied this by observing their motile behavior in transparent semi-solid medium and combined the results with a new computer model of *Beggiatoa* motility.

Materials and methods

Beggiatoa culture

The *Beggiatoa* used for this study were derived from scleractinian reef corals infected with the black-band disease in the Florida Keys (Richardson, 1996). The 6.3-μm-wide *Beggiatoa* originated from a culture from Douglas C. Nelson's laboratory and were isolated by allowing the filaments to glide and spread on sterile agar plates and by repeated passages of single filaments to fresh agar plates under reduced oxygen and elevated carbon dioxide concentrations (D.C. Nelson, pers. commun.).

The *Beggiatoa* culture was maintained at room temperature (18–20 °C) in agar tubes with opposing gradients of oxygen and sulfide and no nitrate present. The gradient tubes contained an anoxic, sulfide-rich agar plug at the bottom and oxic, sulfide-free agar on top. The concentration of Na_2S in the bottom agar was 4 or 8 mM. The top agar was soft (0.25%) and allowed *Beggiatoa* to glide through the medium. The preparation of the gradient agar tubes has been described in detail elsewhere (Nelson *et al.*, 1982; Kamp *et al.*, 2008).

After the preparation of the agar tubes, a 3–4-mm-thick whitish band of elemental sulfur developed in the tubes due to autocatalytic oxidation of sulfide with oxygen as sulfide diffused upwards. The sulfur precipitation marks the position of the oxic–anoxic interface in the top agar. Within a few hours after inoculation with *Beggiatoa*, a bacterial mat started to form in each tube at the oxic–anoxic interface at about 1 cm depth below the agar surface. The filaments were white due to intracellular sulfur globules and thus well visible in the agar tubes. Oxygen and sulfide concentrations were measured using Clark-type microsensors (Nelson *et al.*, 1986; Kamp *et al.*, 2006). Both declined to a few μM exactly at the horizontal mat. The motility patterns observed for this *Beggiatoa* culture were compared with the motility of temperate marine *Beggiatoa* from Århus Bay, Denmark (Dunker *et al.*, 2010). Tufts of the temperate *Beggiatoa* were picked from the sediment of a laboratory mesocosm and placed directly in the oxygen–sulfide transition in gradient tubes observed without awaiting growth.

To also study the behavior of the cultured *Beggiatoa* at the oxic–anoxic boundary in the absence of sulfide, the sulfidic bottom agar in the culture tubes was replaced by anoxic agar

that contained approximately 2.5 mM Ti(III)-citrate as a reductant and 10 mM thiosulfate as a reduced sulfur source. The *Beggiatoa* were inoculated into the freshly prepared tubes at the boundary of top and bottom agar about 50 mm below the agar surface.

Image acquisition

The motile behavior of individual filaments was recorded in time-lapse images of agar tube cultures ($n = 8$). The tubes were photographed from the side using a software-controlled digital monochrome camera (Sony XCD-X710) at time intervals of 20 or 30 s (Fig. 2). A small lens aperture and a long viewing distance ensured that the filaments were in focus throughout most of the tube. A red light LED (Luxeon Star/O LXHL-ND98, peak wavelength 635 nm, Lumiled^{TM}) with a collimating lens was used to illuminate the agar tube. *Beggiatoa* have been observed to have a phototactic response to blue, but not to red light. Images were taken consecutively for up to 5 days.

Single reversals in *Beggiatoa* at a high temporal resolution were studied with Århus Bay filaments on microscope slides. Eighteen filaments with diameters between 5.3 and 31 μm were observed under a light microscope (Zeiss Axioskop). Movies at 15 frames s^{-1} and a 400-fold magnification were filmed using a digital camera (Canon Power Shot A 620). This resulted in a time resolution of 67 ms. Spatial calibration was provided by an object micrometer.

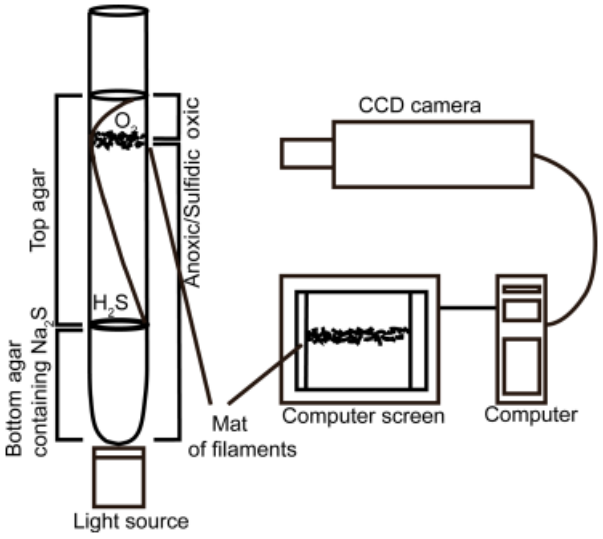

Fig. 2. Experimental setup for image acquisition of *Beggiatoa* filaments in gradient agar tubes. The different agar types are specified to the left and the chemical zonation to the right of the tube. Oxygen and sulfide concentration profiles are indicated with curves.

Image analysis

The dispersal of filaments was followed from time-lapse movies of filaments in agar tubes. Trails of individual filaments in agar tubes in, above and below the mat were followed by recording the x and y coordinates of a narrow region in the center of the filaments from frame to frame in sequences of consecutive images using IMAGEJ and ImageTool (IMAGEJ, National Institutes of Health, USA; ImageTool, University of Texas Health Science Center, USA). Image analysis of filaments in the bacterial mat was performed in culture tubes that were not yet densely populated to ensure that individual filaments could be tracked. Reversals of filaments within the mat and above and below the mat were determined in sequences of images taken over a defined time period. Three to 49 reversals were scored per filament, depending on the length of the observed filament. Reversals of filaments were scored when the entire filament reversed. This was typically the case in filaments $\leq$ 2 mm long. Filaments in which only a part of the filament reversed were not recorded. The distance traveled between reversals was calculated from the time between reversals and the gliding speed at the ambient temperature (2.4 ± 0.3 μm s^{-1}, Dunker *et al.*, 2010).

For high-resolution analysis of single reversals, the Århus Bay filaments on microscope slides were filmed at a high magnification until they performed a reversal and a few seconds thereafter. Only the tip of the filament was visible in the field of view due to the high magnification. The speed of each filament a few seconds before, during and after a reversal was determined using the image analysis software IMAGEJ by following the tip of the filament from frame to frame.

Results

Trails of filaments

The gliding speed of *Beggiatoa* filaments at 18 °C was the same within and outside of the mat. However, we observed remarkable differences in motility behavior depending on their position relative to the mat. Trails of individual filaments in the mat remained within a confined area with a radius equal to or shorter than the length of the filament (Fig. 3; Supporting Information, Video S1). Filaments above and below the mat, however, showed a substantial displacement over 2–3 h (Fig. 3, Video S2). The curves A and B in Fig. 4 show the average distances glided away from the origin within the mat and in the sulfidic zone, respectively, as a function of time.

Reversals

Reversing filaments stopped abruptly from one image to the next (67 ms), with no evident deceleration. They then paused for 1–4 s until they abruptly resumed motility in the

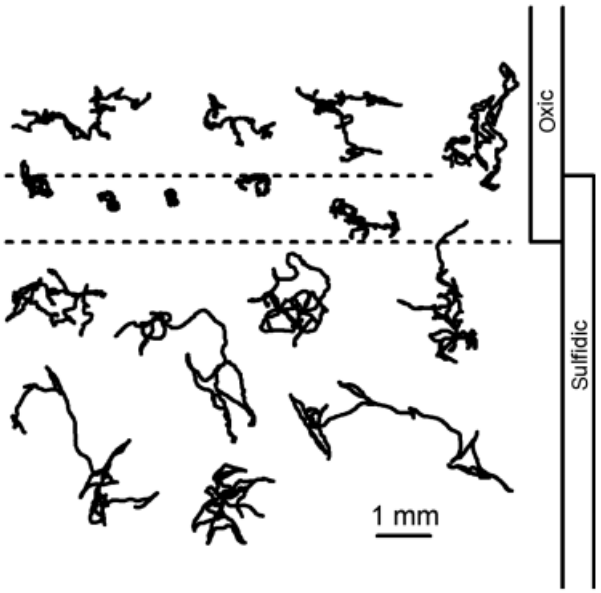

Fig. 3. Trails of filaments observed for 126–166 min. The dashed horizontal lines indicate the upper and the lower boundary of the *Beggiatoa* mat. Filaments above the mat move in the oxic agar, and those below the mat in sulfidic agar. The lower three filaments are modeled filaments that move in random trails in the sulfidic region.

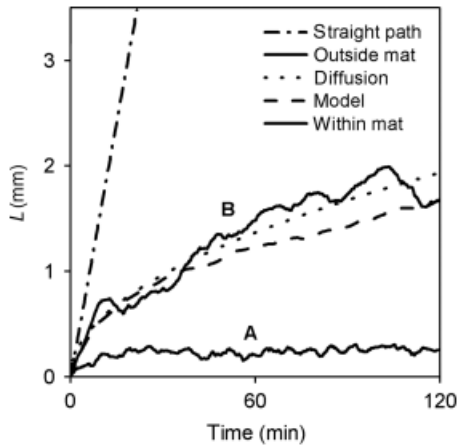

Fig. 4. The net distances over time that individual filaments glided away from their origin over 2 h. Solid curves show observed data. (A) A *Beggiatoa* filament gliding within the mat with both oxygen and sulfide present at low concentrations. (B) A *Beggiatoa* gliding in the sulfidic zone without oxygen. Dotted line: theoretical net diffusion distance of a particle with a similar effective diffusion coefficient as the filament in (B). Dashed line: modeled net movement of a filament outside the mat. Dotted and dashed line: movement of a filament that is theoretically gliding along a straight path.

opposite direction at the full former gliding speed. The resumption of motility was sometimes accompanied by a sudden jerk in the new direction of gliding as if the filament was held back by a force that snapped.

The mean reversal frequency was tied to filament length and to the vertical position in the culture tube. In Fig. 5a–c, the average distance glided between reversals is plotted against the filament length. A 1 : 1 line separates the plots into two areas. Note that a filament must glide longer between reversals than its own length in order to move away from its point of origin. Filaments outside of the mat glided longer than their own length between reversals (Fig. 5a and c). Filaments within the mat glided shorter than their own length, a reversal pattern that essentially anchored the filaments in the mat to one spot (Fig. 5b). Two particular filaments were observed as they moved from outside and into the mat, either from the oxic or from the sulfidic zone. Once within the mat, these filaments changed their reversal behavior immediately and glided shorter distances than outside of the mat (filled and open data points connected by dotted lines in Fig. 5a and c).

The frequency distribution of gliding distance relative to the position outside or within the mat was further analyzed for a number of randomly selected individuals. Five filaments of 0.18–1.34 mm length were observed for each zone, scoring 7–32 reversals of each. For the *Beggiatoa* gliding within the mat, we found that nearly all distances glided between reversals were shorter than the filament length (Fig. 6b). For filaments gliding above and below the mat, nearly all distances glided were longer than the filament length (Fig. 6a and c).

In the absence of sulfide in the bottom agar, *Beggiatoa* still formed a well-defined band at the sulfide-free oxic–anoxic boundary. More filaments persisted in the anoxic zone, however, in contrast to the very few filaments that were observed to glide into the sulfidic zone in agar tubes with sulfide.

Discussion

Chemotactic mechanism of reversals

The reversal of *Beggiatoa* filaments is apparently achieved by the synchronous reversal of all cells in the filament rather than by independent reversals by each single cell of the filament. Synchronous reversal also takes place when only the leading end of a filament is exposed to the chemical stimulus that triggers reversal, for example to a step-up in oxygen concentration or in light intensity. Synchronous reversal must require some form of communication between cells along an entire filament of up to several mm length. The diffusive transport of a chemical signal would be much too slow to initiate the coordinated reversal of an entire filament within 67 ms. The mean molecular diffusion time of small molecules along a 1–5-mm-long filament would be 10 min to 3 h, which highly exceeds the reaction time of the entire filament. The concerted action therefore appears to be

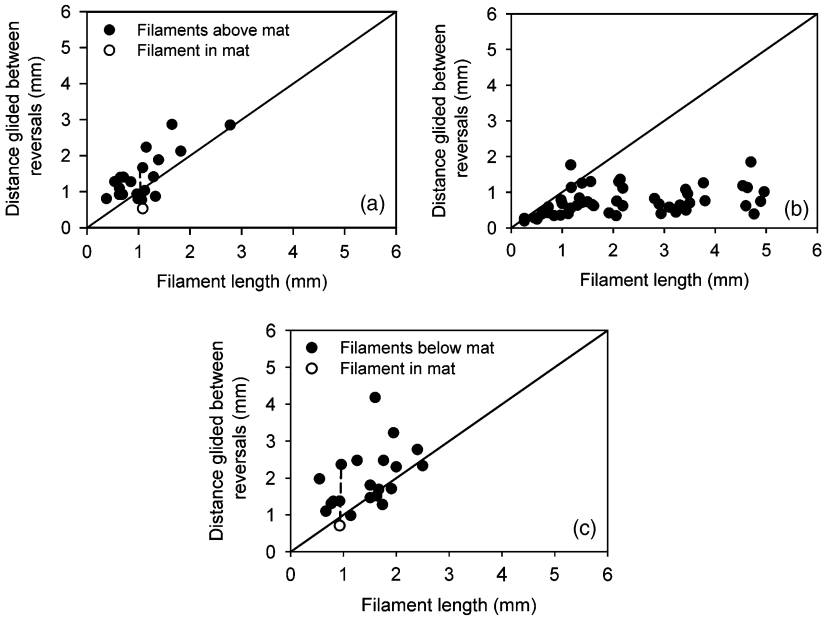

Fig. 5. Average gliding distances between reversals of filaments of different lengths at different positions relative to the mat. (a) Above the mat, $n = 20$; (b) within the mat, $n = 52$; (c) below the mat, $n = 20$.

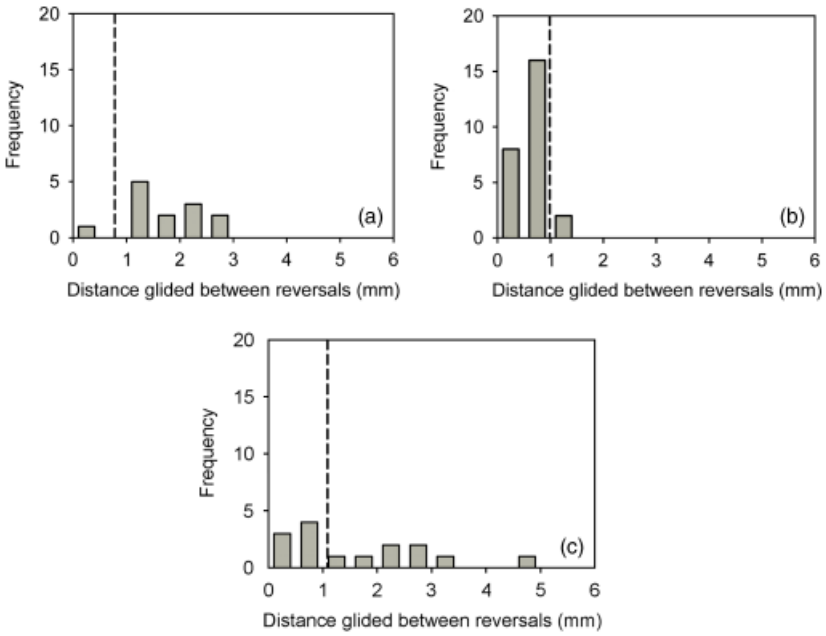

Fig. 6. Length of distances that individual filaments glided between reversals. Dashed line: Filament length. (a) Above the mat, (b) within the mat, (c) below the mat.

coordinated by an electrochemical signal that propagates rapidly along the length of the filament. A cell-to-cell communication could theoretically take place via mechanoreceptors in the cell walls or by another fast-responding process.

Halfen & Castenholz (1971) described reaction times of 1–2 s for the multicellular, filamentous cyanobacteria, *Oscillatoria princeps*, to complete a reversal while reaction times of < 0.1 s have been observed for the multicellular, filamentous, heterotrophic *Flexibacter polymorphus* (Ridgway & Lewin, 1988). The authors proposed an electrochemical signal involving membrane depolarization for this rapid signal transduction. Gliding filaments of *F. polymorphus* also reversed their gliding direction when they encountered an obstacle (Ridgway & Lewin, 1988), and both *F. polymorphus* and *O. princeps* increased their reversal frequency when they glided in a medium of higher viscosity (Halfen & Castenholz, 1971; Ridgway & Lewin, 1988). Both observations may suggest the involvement of mechanoreceptors.

Chemotaxis in surface mats

The tactic mechanisms described in detail by Møller *et al.* (1985) also involve sideways motion such as twisting loops. This is obviously not possible inside the sediment matrix or even in semi-liquid agar cultures. Reversal due to a negative step-up response to oxygen would in itself not prevent *Beggiatoa* from migrating from a surface mat and deep down into the anoxic sediment below. Therefore, an additional negative response to sulfide was proposed (Møller *et al.*, 1985). The mat formation that we observed in the absence of sulfide suggests that sulfide is not a requirement for mat formation. The 'anchoring' mechanism by frequent reversals at the oxic–anoxic interface provides a mechanism by which *Beggiatoa* can maintain their position at the zero oxygen level in nonsulfidic sediment. In their natural environment, *Beggiatoa* is sharply confined above the sulfide diffusion front, also when this front is located several cm below the oxic–anoxic interface (Fig. 1). Thus, *Beggiatoa* do indeed appear to possess a negative response to sulfide.

Chemotaxis in gradient cultures and subsurface mats

Figure 3 shows three zones with different motility patterns in the agar cultures. Within the *Beggiatoa* band where oxygen and sulfide overlap, the filaments moved in a confined sphere defined by their own filament length due to frequent reversals. Below the band, in the sulfidic agar, the filaments followed random trails with only a few reversals. The horizontal and vertical component of the random walk contributed about equally to the motion of the filament. The random walk sometimes brought the filaments back into the mat, but filaments were also observed to ultimately stop and die in their track. This apparently occurred when they had depleted their nitrate reserve. Thus, the sulfide gradient in the tubes seems to provide an insufficient cue for the organisms for a spatially oriented net movement. In the oxic zone above the mat, the general pattern was similar to that below the mat. When the vertical component of the motion became predominant, however, the filaments generally moved into the mat at the oxic–anoxic interface and remained there (e.g. rightmost filament in the oxic zone, Fig. 3).

The *Beggiatoa* were able to 'anchor' in the mat by systematically gliding shorter distances between reversals than their own length. A similar pattern was observed in trichomes of the filamentous, sheath-building marine *Thioploca* when their sheaths were chopped into pieces of different lengths (Høgslund *et al.*, 2009). Trichomes from the short pieces always reversed after a shorter distance than trichomes from the long pieces, and this gliding behavior kept the *Thioploca* trichomes within the truncated sheath. In this case, the 'anchored' reversal pattern was even effective with both ends of the filaments cut off. Similar, yet unknown mechanisms may be used by the closely related *Beggiatoa* and *Thioploca* to couple reversal probability to their filament length, while achieving slightly different objectives. Also, *F. polymorphus* filaments of different lengths reverse with a frequency that is inversely proportional to their filament length (Ridgway & Lewin, 1988). Even unicellular microorganisms can aggregate in preferred microniches by increasing their reversal (tumbling) frequency and thereby decreasing their diffusion coefficient (e.g. Schnitzer *et al.*, 1990).

The paths of individual *Beggiatoa* filaments below the oxic–anoxic interface resemble a random walk (Fig. 4). The average displacements from the point of origin (L) over time (t) by random walk follow the mathematical description of diffusion in space:

$$L = \sqrt{\frac{D4t}{\pi}} \quad (1)$$

where L is the mean net distance moved from the starting point, D is the effective diffusion coefficient and t is the time. Equation 1 can be used in a nonlinear fit to observed L vs. t data (Fig. 4) to estimate the value of D. The best fit to the data in Fig. 4, curve B, is found with a D value of $0.1\,\mathrm{mm^2\,min^{-1}}$ ($1.8 \times 10^{-5}\,\mathrm{cm^2\,s^{-1}}$). Thus, *Beggiatoa* filaments appear to spread with a similar diffusion coefficient as small molecules or swimming bacteria (Fenchel, 2008), even though the bacteria swim an order of magnitude faster than the *Beggiatoa* glide. The reason is that the path of *Beggiatoa* is much less convoluted than that of micrometer-size bacteria that get bumped around by Brownian motion and tumble about every second.

Model of *Beggiatoa* motility in the suboxic zone

The anchoring by high reversal frequency can explain how *Beggiatoa* are able to remain in the oxygen–sulfide interface in the gradient tubes. We will now explore how the filaments navigate in the suboxic zone where sulfide and oxygen is separated by many millimeters (Fig. 1c). We first describe the random walk of *Beggiatoa* in the absence of chemical clues by building a simple kinematic computer model of a *Beggiatoa* filament. The physical properties of the model filament were taken from a typical *Beggiatoa* filament from Fig. 3. It had 285 cells, each 2.7 μm in length and 6.3 μm in diameter. Thus, the entire filament was 0.77 mm long. It glided at a speed of $2.7\,\mu\mathrm{m\,s^{-1}}$ at 17 °C. The probability density of reversal as a function of time since the last reversal was calculated from the frequency chart in Fig. 6c.

The computer model keeps track of the position in space of every single cell and of the direction that the lead cell is pointing. It also keeps track of the time since the last reversal. The model is incremented in 1-s steps. Thus, the

lead cell will move forward by 2.7 μm for each time step. All other cells are simply following the cell in front of them. A stochastic error of a few degrees in the direction of the apical cell was allowed for each 1-s iteration (Fig. 7). The decision to reverse was evaluated stochastically for each time-step according to the probability density function. Thus, the model has only one free parameter, namely the maximum stochastic error on the direction (α) of the lead cell.

The initial calibration of α was carried out by visually comparing the modeled filament tracks with real *Beggiatoa* tracks in transparent media while manipulating α. This simple method proved surprisingly sensitive. Too large an α value made the modeled filament coast in stiff paths while too small a value would curl up the filament in a small ball. Numerical confirmation was obtained by comparing the mean displacement from the origin as a function of time between modeled and real *Beggiatoa* (Fig. 4). Both verification methods considered the vertical and one horizontal dimension because this is the information available from the real *Beggiatoa*. The tuned model provided a virtual *Beggiatoa* with the same displacement rate as the real *Beggiatoa* from which it was modeled. The pattern of motility was indistinguishable from that of live *Beggiatoa* (Fig. 3, Video S3). Thus, the model provides an excellent mechanistic description of how *Beggiatoa* filaments move by random walk through a uniform environment with no chemotactic cue.

To test the generality of the model, we applied it to a known example with temporally dynamic *Beggiatoa* distributions in a hypersaline microbial mat (Hinck *et al.*, 2007). The *Beggiatoa* community here consisted of 6–8-μm-thick filaments that stored nitrate in internal vacuoles to concentrations of 4–42 mM. The halophilic filaments were most closely related to narrow, nonvacuolated marine *Beggiatoa*. During the day, this mat had overlapping oxygen and sulfide zones 8–10 mm below the sediment surface, similar to the gradient tubes, and the *Beggiatoa* were confined to a narrow mat. At night, however, oxygenic photosynthesis stopped and oxygen retreated to the upper 2 mm (compare to Fig. 1c). Contrary to expectation, however, the *Beggiatoa* did not move up to spread evenly in the suboxic zone between oxygen and sulfide (Hinck *et al.*, 2007). We modeled this scenario by seeding our model with 1000 virtual *Beggiatoa* filaments according to the observed depth distribution during the day (Fig. 8, white bars). We then allowed the filaments to glide randomly for 14 h corresponding to the time from dusk to dawn *in situ*. Filaments were forced to reverse if they passed into the oxic layer above 2 mm or into the sulfide front at 10 mm. The model setup (number of cells, etc.) was identical to the description above, except that the gliding speed of the filaments was set to 1.4 μm s^{-1}, which was the gliding speed of the halophilic strain. The distribution after 14 h of free-roaming virtual *Beggiatoa* (Fig. 8, black bars) was close to the *in situ* distribution (Hinck *et al.*, 2007, Video S3). Thus, the model shows that the halophilic *Beggiatoa* did not disperse throughout the oxygen- and sulfide-free zone during the night because the filaments glided too slowly to spread effectively within the 14 h of darkness.

The good reproduction by the model of *Beggiatoa* migrations in the hypersaline mat encouraged us to apply it to more general *Beggiatoa* populations. A main habitat for marine *Beggiatoa* is the anoxic, but oxidized (suboxic) zone of coastal and estuarine sediments (Jørgensen, 1977; Mussmann *et al.*, 2003; Jørgensen *et al.*, 2010, Fig. 1c). We picked *Beggiatoa* from such a few cm thick surface layer in sediments of Århus Bay (Denmark) and confirmed that the filaments had similar motility patterns in transparent media as the *Beggiatoa* culture used in this study (data not shown). We then went on to apply the model directly to these *Beggiatoa* living within anoxic marine sediment without further tuning of the modeled filaments.

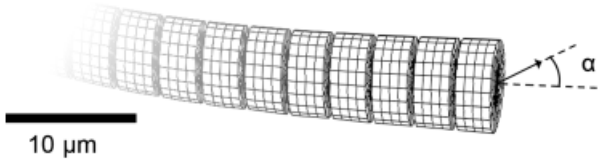

Fig. 7. Schematic representation of the computer model of *Beggiatoa* motility. At each iteration, the apical cell moves forward by one cell length into its gliding direction and all other cells follow in the footsteps of the cell in front of it. A small stochastic error (α) is induced on the direction each step. Note that the magnitude of α is exaggerated in the figure.

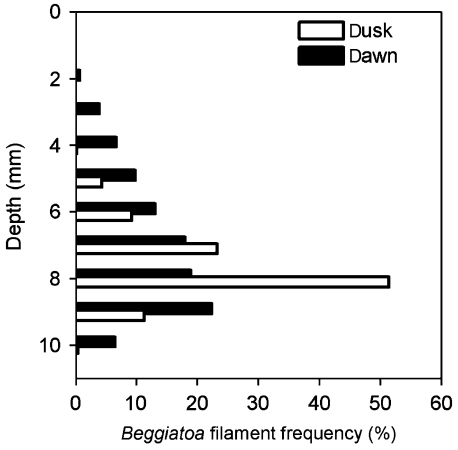

Fig. 8. Daytime distribution of *Beggiatoa* in a hypersaline mat plotted as white bars (data redrawn from Hinck *et al.*, 2007). Black bars show the distribution of *Beggiatoa* predicted by the model after 14 h of darkness (compare with Hinck *et al.*, 2007, Fig. 1).

The physical and chemical parameters for the model of *Beggiatoa* in the suboxic zone were taken from typical values for coastal sediment such as measured in Limfjorden or Kiel Bight (Mussmann *et al.*, 2003; Preisler *et al.*, 2007). In this environment, *Beggiatoa* has no direct access to oxygen, but instead uses nitrate accumulated in intracellular vacuoles as an electron acceptor in respiration and in their chemosynthetic metabolism. The typical oxygen and nitrate penetration into the sediment are 2 and 5 mm, respectively, and the sulfide front starts at 20 mm below the sediment surface (e.g. Preisler *et al.*, 2007, Fig. 1c). The *Beggiatoa* distribution was modeled in the suboxic zone as above, i.e. the filaments reversed their direction of gliding when the lead cell reached the oxic zone above or the sulfide front below. Otherwise, the filaments roamed around in the sediment without responding to chemical clues. We now analyze the frequency distribution of filament excursions in the suboxic zone where oxygen, nitrate and sulfide are not present in detectable amounts. In particular, we analyze whether a simple random walk enables the *Beggiatoa*s to acquire the substrates needed for their chemosynthetic metabolism. We compute the length and duration of each random excursion from the moment when a filament crosses from the nitrate zone above into the nitrate-free zone below and until random gliding brings it back into the nitrate zone.

As expected, many excursions are short because the convoluted path provides a high probability of moving right back into the nitrate zone. Figure 9 (solid line) shows how the vast majority of the excursions are shorter than 1 day. To assess the importance of longer excursions, we must consider both the time spent on excursions of various lengths and their frequency (Fig. 9, broken line). The calculations reveal that the model *Beggiatoa* spends most of its time within the suboxic zone during excursions several days long, the mean duration being around 10 days. This is 100 times longer than it would take a *Beggiatoa* to glide along a straight path from the bottom of the nitrate zone to the sulfide front and back. During a 10-day trip, the filament has followed a convoluted path, spent about an equal amount of time at all depths in the suboxic zone and progressively depleted its nitrate storage. This explains two enigmas about *Beggiatoa*: first, that there is no depth dependence in the nitrate concentration of *Beggiatoa* filaments in the suboxic zone. Second, that *Beggiatoa* store well over 100-fold as much nitrate as needed for a straight trip from the sediment surface to the sulfide front and back.

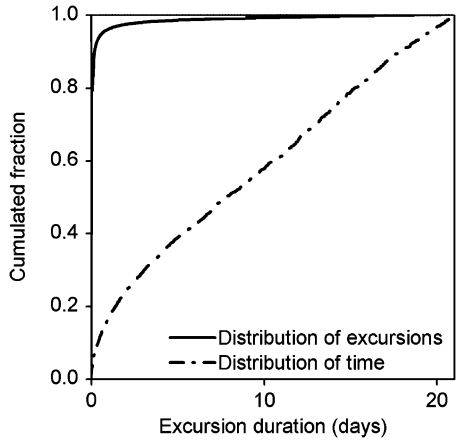

Fig. 9. Solid line: the cumulated frequency of excursions of various durations. Dotted and dashed line: the cumulated time a model *Beggiatoa* spends on excursions of various durations.

Although sulfide is not detectable in the suboxic sediment, radiotracer measurements reveal that the peak of sulfide production from bacterial sulfate reduction is found in this zone (e.g. Jørgensen *et al.*, 2010). The free sulfide produced turns over too fast to accumulate to a detectable concentration, for example due to sulfide uptake by *Beggiatoa* (Jørgensen & Nelson, 2004), by precipitation with iron minerals or by remote sulfide oxidation through biogeoelectric networks (Nielsen *et al.*, 2010). The *Beggiatoa* occurring in the suboxic zone are therefore not limited by sulfide availability, but by electron acceptor constraints. Preisler *et al.* (2007) observed that *Beggiatoa* similar to those modeled here depleted their nitrate storage by 13 mM day^{-1} when held in a nitrate-free anoxic sediment. The filaments remained motile for 21 days, corresponding to the time it would take to fully deplete their 270 mM internal nitrate reservoir. Then they stopped and apparently died. The virtual *Beggiatoa* modeled in Fig. 9 statistically initiated a trip longer than 21 days (a 'no return trip') once every 57 days. If the nitrate storage had been less, such trips with no return would be more frequent. Thus, *Beggiatoa* need nitrate far in excess of their consumption during the average short trip in the suboxic zone in order not to run out of electron acceptors during random long trips.

Conclusions

In summary, it appears that *Beggiatoa* in the suboxic zone use negative chemotactic responses to confine their distribution between the boundaries of oxygen/nitrate and sulfide. They do not require chemical clues or complex tactic mechanisms to remain within the suboxic environment. Simple random walk coupled to a long endurance will ensure that they find their way back to the surface sooner or later, where they may replenish their nitrate vacuoles. In the transition zone where both electron acceptors and sulfide are available, they stop net migration and remain anchored on the spot simply by increasing their reversal frequency. These reversals occur coordinated along the filament and probably involve electrochemical or mechanical cell-to-cell communication.

Acknowledgements

We thank Heide Schulz-Vogt for supporting the work of A.K. We thank her and Douglas C. Nelson for providing *Beggiatoa* cultures originating from corals infected with the black-band disease. This work was financially supported by the Max Planck Society, the Danish National Research Foundation and the Fonds der chemischen Industrie.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Video S1. Modeled *Beggiatoa* filament gliding trails shorter than or equal to its filament length.
Video S2. Model of a *Beggiatoa* filament gliding trails longer than its filament length and thereby changing its position.

Video S3. Twenty-four hours of a modeled *Beggiatoa* mat as that from Hinck *et al.* (2007): 14-h light conditions followed by 10 h darkness when the filaments spread out from the mat.

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