Motility of the giant sulfur bacteria *Beggiatoa* in the marine environment

Dissertation

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Motility of the giant sulfur bacteria *Beggiatoa* in the marine environment

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Summary

This thesis deals with aspects of motility in the marine filamentous sulfur bacteria *Beggiatoa* and thus aims for a better understanding of *Beggiatoa* in their environment. *Beggiatoa* inhabit the microoxic zone in sediments. They oxidize reduced sulfur compounds such as sulfide with oxygen or nitrate. *Beggiatoa* move by gliding and respond to stimuli like oxygen, light and presumably sulfide. Using these substances for orientation, they can form dense mats on the sediment surface.

The first manuscript is dedicated to the response of gliding motility to changing temperatures in *Beggiatoa* filaments from arctic, temperate and tropical marine environments. The optimum temperature and the overall temperature range of gliding motility were determined in these filaments. The temperature range of gliding correlated with the climatic origin of the filaments with a high temperature range for tropical, an intermediate range for temperate, and a low temperature range for arctic filaments. Likewise, the optimum temperature for gliding depended on the climatic origin of the filaments. At *in situ* temperatures filaments glided at 17-55 % of the gliding speed at the optimum temperatures, and were accordingly well adapted to the temperature regime of their origin. The cold adapted filaments were unaffected by transient freezing of the surrounding seawater. Cold acclimatization of temperate filaments for various weeks extended the temperature range at the cold end, indicating that the *Beggiatoa* community was adapted to seasonal temperature changes. The temperature dependent gliding is presumably subject to an enzymatic control.

An examination of *Beggiatoa* in arctic fjord sediments on the west coast of the archipelago Svalbard demonstrated that the filaments grow well under permanently cold conditions. Abundant populations of *Beggiatoa* were found at two of the eleven sites, while another six contained filaments at low numbers. In only rare cases the filaments formed mats on the sediment surface but lived within the sediment wherefore the filaments had not been noticed previously. The main source of sulfide in these sediments was bacterial sulfate reduction, as evident by the high sulfate reduction rates measured. The *Beggiatoa* examined stored nitrate intracellularly at concentrations above 100 mM and belonged phylogenetically to the large, marine, nitrate-storing *Beggiatoa* as

determined by 16S rRNA analysis. *Beggiatoa* could not be quantified by conventional microscopy-based cell counting techniques, because they were too scarce. However, due to their large cell diameters of 2-52 µm they constituted up to 15 % of the prokaryotic biomass in the sediments.

The work for the third manuscript focused on chemotactic patterns of single filaments. Observations of *Beggiatoa* filaments in transparent agar medium were complemented by a model based on the observations that explained the distribution of *Beggiatoa* in sediment where no direct observation is possible. Filaments within the preferred micro-environment where oxygen and sulfide concentrations were low anchored at their position by gliding shorter distances than filament length between reversals. This behavior led to the formation of a mat. Filaments in the oxic region above the mat and in the sulfidic, anoxic region below the mat glided distances longer than the filament length between reversals. This reversal behavior resulted in long trails and a diffusion-like spreading of the filaments, oftentimes leading them back into the mat. A model for *Beggiatoa* behavior was applied to virtual filaments in the suboxic zone, i.e. the oxygen and sulfide free zone of the sediment which is a main habitat of *Beggiatoa* in the natural environment. The model predicted a long residence time of a virtual filament in the suboxic zone and explained why *Beggiatoa* accumulate high nitrate concentrations in internal vacuoles as an alternative electron acceptor to oxygen.

Zusammenfassung

Diese Doktorarbeit ist der Motilität der marinen, filamentösen Schwefelbakterien *Beggiatoa* gewidmet und zielt auf ein besseres Verständnis von *Beggiatoa* in ihrem Lebensraum ab. *Beggiatoa* bewohnen die mikrooxische Zone von Sedimenten. Sie oxidieren reduzierte Schwefelverbindungen wie Sulfid mit Sauerstoff oder Nitrat. *Beggiatoa* bewegen sich durch Gleiten und sprechen auf Reize wie Sauerstoff, Licht und vermutlich Sulfid an. Indem sie diese Stoffe zur Orientierung nutzen, können sie dichte Matten auf der Sedimentoberfläche bilden.

Das erste Manuskript beschäftigt sich mit der Reaktion der Gleitbewegung auf Temperaturänderungen bei *Beggiatoa*-Filamenten aus dem arktischen, gemäßigten und tropischen marinen Lebensraum. Die Optimaltemperatur und der Gesamttemperaturbereich der Gleitbewegung wurden in den Filamenten bestimmt. Der Temperaturbereich der Gleitbewegung korrelierte mit der klimatischen Herkunft der Filamente mit einem hohen Temperaturbereich für tropische, einem mittleren Bereich für gemäßigte und einem niedrigen Temperaturbereich für arktische Filamente. Ebenso war die Optimaltemperatur der Gleitbewegung abhängig von der klimatischen Herkunft der Filamente. Bei *in situ*-Temperaturen glitten die Filamente mit 17-55 % der Gleitgeschwindigkeit bei Optimaltemperatur, und waren dementsprechend an die Temperaturbedingungen ihres Herkunftsortes gut angepasst. Die an kalte Bedingungen angepassten Filamente überdauerten vorübergehendes Einfrieren des umgebenden Meerwassers unbeschädigt. Akklimatisierung der gemäßigten Filamente an kalte Bedingungen für mehrere Wochen erweiterte ihren Temperaturbereich am unteren Ende. Dies zeigt, dass die *Beggiatoa*-Gemeinschaft an jahreszeitlich bedingte Temperaturänderungen angepasst ist. Die temperaturabhängige Gleitbewegung ist vermutlich einer enzymatischen Kontrolle unterworfen.

Eine Untersuchung von *Beggiatoa* in arktischen Fjordsedimenten an der Westküste der Inselgruppe Svalbard zeigte, dass die Filamente gut unter dauerhaft kalten Bedingungen wachsen. Große *Beggiatoa*-Populationen wurde an zwei von elf der untersuchten Stellen gefunden, wobei an sechs weiteren Stellen vereinzelte Filamente gefunden wurden. Nur selten bildeten die Filamente Matten auf der Sedimentoberfläche

sondern lebten im Sediment, weswegen sie bisher nicht wahrgenommen wurden. Die Hauptquelle für Sulfid in diesen Sedimenten war bakterielle Sulftreduktion, wie aus den hohen gemessenen Sulfatreduktionsraten ersichtlich. Die untersuchten *Beggiatoa* speicherten intrazellulär Nitrat zu Konzentrationen über 100 mM und gehörten aufgrund einer Analyse ihrer 16S rRNA phylogenetisch zu den großen, marinen, nitrat-speichernden *Beggiatoa*. Eine Quantifizierung von *Beggiatoa* mit herkömmlichen Mikroskopietechniken war nicht möglich aufgrund ihres spärlichen Vorkommens. Dennoch stellten sie aufgrund ihrer großen Zelldurchmesser von 2-52 µm bis zu 15 % der prokaryotischen Biomasse im Sediment dar.

Die Arbeit zum dritten Manuskript konzentrierte sich auf chemotaktische Muster einzelner Filamente. Beobachtungen von *Beggiatoa*-Filamenten in transparentem Agar-Medium wurden durch ein auf diesen Beobachtungen basierendes Modell ergänzt, das die Verteilung von *Beggiatoa* Filamenten im Sediment erklärte, wo keine direkte Beobachtung möglich ist. Filamente in ihrem bevorzugten Mikro-Lebensraum, wo die Sauerstoff- und Sulfidkonzentrationen gering waren, verankerten sich an ihrer Position, indem sie kürzere Strecken zwischen den Umkehrbewegungen glitten als ihre jeweilige Filamentlänge. Dieses Verhalten führte zur Ausbildung einer Matte. Filamente im oxischen Bereich über der Matte und im sulfidischen Bereich unter der Matte glitten längere Strecken als ihre jeweilige Filamentlänge zwischen den Umkehrbewegungen. Dieses Umkehrverhalten resultierte in langen Wegen und einer diffusionsartigen Verteilung der Filamente, die sie häufig zurück in die Matte führte. Ein Modell für das Verhalten von *Beggiatoa* wurde auf virtuelle Filamente in der suboxischen Zone angewendet, d.h. in der sauerstoff- und sulfidfreien Zone des Sediments, welches der hauptsächliche Lebensraum von *Beggiatoa* in ihrer natürlichen Umgebung ist. Das Modell berechnete eine lange Aufenthaltszeit eines virtuellen Filaments in der suboxischen Zone und erklärte, weshalb *Beggiatoa* hohe Nitratkonzentrationen als alternativen Elektronenakzeptor zu Sauerstoff in ihrer Vakuole speichern.

1. General Introduction

Filaments of the sulfur bacteria *Beggiatoa* have caught the eye of scientists early in the history of microbial research. By being large organisms compared to other members of the microbial world (e.g. Schulz & Jørgensen, 2001) and by their conspicuous appearance they received much attention ever since. *Beggiatoa* are highly interesting organisms in that they are not only visible to the naked eye but also have an exciting physiology and lifestyle. By observations of their sulfur metabolism Winogradsky, one of the pioneers in modern microbiology, developed the concept of chemolithotrophy in the late nineteenth century. *Beggiatoa* can form mats of remarkable density in environments of very low oxygen concentration (the so-called microoxic zone) on sulfidic sediments in both freshwater and marine habitats. These mats can prevent the efflux of toxic sulfide from deeper sediment layers to the water column by the ability of *Beggiatoa* to oxidize sulfide to elemental sulfur and sulfate. More recently, *Beggiatoa* were discovered to also populate the anoxic zone of the sediment above the diffusion front of sulfide. This finding motivated many recent studies on the physiology of *Beggiatoa*. Another exciting aspect of *Beggiatoa* is its metabolic diversity, thereby linking the sulfur, nitrate and carbon cycles. Gliding motility is an important ecophysiological characteristic of *Beggiatoa*. They orient in their environment by tactic responses towards chemical stimuli and light. By the help of mainly phobic responses they occupy the microoxic and/or anoxic layers of the sediment. Tactic motility patterns of *Beggiatoa* have been described as early as 1887 by Winogradsky (Winogradsky, 1887). Investigations on the gliding motility have aimed at the mechanisms of tactic behavior that underlie the gliding movement as well as the structural aspects of gliding. These questions are crucial to understand the ecology of *Beggiatoa*, and despite many have been answered many more remain to be targeted.

The work for this thesis was motivated by the observation of mat formation on top of sulfidic sediments and of single filaments in gradient cultures of oxygen and sulfide. In both cases filaments agglomerate at the oxic-anoxic boundary by gliding. This thesis approaches various aspects of the motility of *Beggiatoa*. The main focus is set on the mechanism of mat formation and the influence of temperature on gliding motility. The

first chapter gives an overview of the organism *Beggiatoa* and provides background information for the following manuscripts.

1.1. Characteristics of Beggiatoa

Classification

Members of the genus *Beggiatoa* belong to the familiy Thiotrichaceae (Order Thiotrichales) together with the genera *Thiothrix*, *Achromatium*, *Leucothrix*, *Thiobacterium*, *Thiomargarita*, *Thioploca* and *Thiospira*. They are all members of the γ-Proteobacteria. Due to their morphological similarities to filamentous cyanobacteria, *Beggiatoa* were described as *Oscillatoria alba* until the early nineteenth century (Pringsheim, 1949), and later were classified as apochlorotic cyanobacteria (Reichenbach & Dworkin, 1981). The classification of *Beggiatoa* was revised with the development and use of molecular techniques for phylogeny, especially the 16S rRNA gene sequencing. *Beggiatoa* and *Thioploca* and the non-motile, non-filamentous *Thiomargarita* form a monophyletic group within the proteobacterial γ-subdivision which does not include the third genus of filamentous sulfur bacteria, *Thiothrix* (Teske*, et al.*, 1999). The monophyletic group of *Beggiatoa*/*Thioploca* is split further into different clades. Freshwater *Beggiatoa* appear to be the most distantly related clade (Ahmad*, et al.*, 2006). Marine, non-vacuolated *Beggiatoa* form another clade. The vacuolated *Beggiatoa* and *Thioploca* fall into the clade of large vacuolated sulfur bacteria (Ahmad*, et al.*, 2006). Hence, the large *Beggiatoa* may be closer related to Thioploca than to the narrow, non-vacuolated *Beggiatoa* (Teske*, et al.*, 1999). Within the different clades distinct clusters can be identified which may be based on filament size and spatial distribution (Kojima & Fukui, 2003, Mussmann*, et al.*, 2003). Yet, size alone cannot be used as a classification criterion because there are indications of a genomic microdiversity among filaments of the same size class (Mussmann*, et al.*, 2007).

Morphology

Beggiatoa filaments are composed of individual, cylindrical cells separated by a peptidoglycan layer that is shared between each two adjacent cells (Strohl*, et al.*, 1982, Fig. 1A). The end cells of the filaments are commonly rounded, but tapered and curved filament ends have been observed (eg. Kojima & Fukui, 2003). A complex, multi-layered cell envelope covers the entire filament (Strohl*, et al.*, 1982, de Albuquerque*, et al.*, 2010, Fig. 1A). The most obvious characteristic of *Beggiatoa* is the bright, white appearance of the filaments in contrast to the usually dark sediment which often indicates the presence of *Beggiatoa* at first sight. The white appearance is caused by spherical intracellular periplasmic sulfur inclusions (Fig. 1B). These sulfur inclusions are present as zero-valent colloidal sulfur, coated by a proteinaceous membrane envelope (Strohl*, et al.*, 1981, Kamyshny*, et al.*, 2009). The storage of intracellular sulfur is a characteristic that all colorless sulfur bacteria have in common. Besides sulfur, *Beggiatoa* cells can also store polyphosphate (Strohl & Larkin, 1978a, Høgslund et al., in preparation, J. Brock, personal communication) and poly-β-hydroxybutyrate (Strohl & Larkin, 1978a, Strohl*, et al.*, 1982). These cytoplasmic inclusions presumably serve as energy storage which is deposited at conditions of high energy supply and can be exploited during starvation.

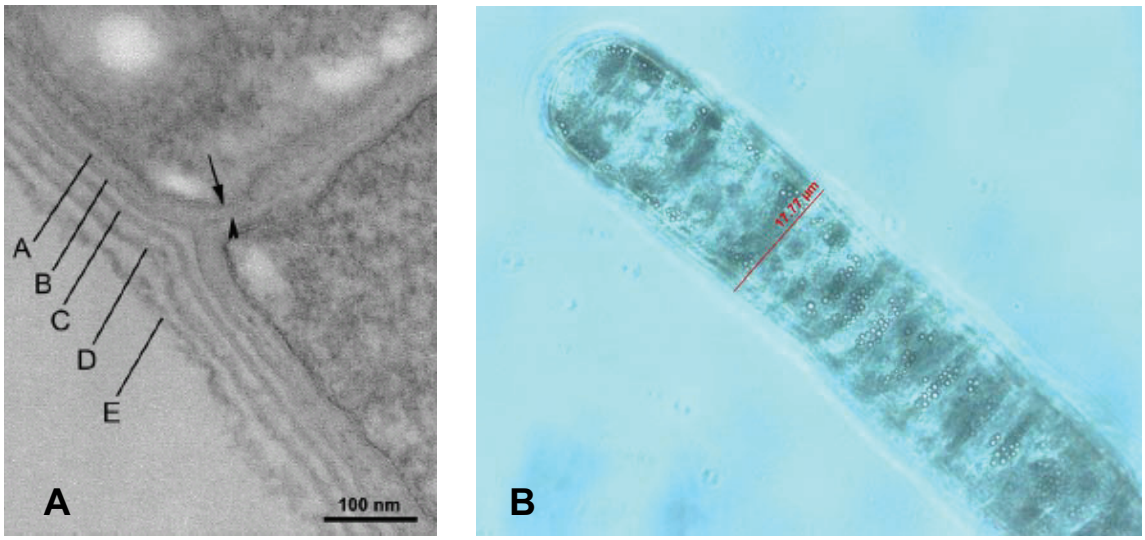

Figure 1: A: Transmission electron micrograph showing the layered envelope (A-E) and the peptidoglycan layer in between two adjacent cells (arrows, from de Albuquerque *et al.* (2010)). B: Filament of ~18 µm diameter with disc-shaped cells. Sulfur inclusions are visible as white spheres.

The filaments can grow up to several centimeters length and can consist of hundreds to thousands of cells. The width of *Beggiatoa* filaments ranges from 1.5 to nearly 200 µm (Nelson*, et al.*, 1982b, Larkin & Henk, 1996). The narrow filaments have ratios of cell length to cell width of 1-8 whereas the filaments ≥ 5 µm have length to width ratios of 0.1-0.9 (Teske & Nelson, 2006, Fig. 1B). Cells of filaments wider than about 5 µm have a central vacuole that can occupy more than 80% of the cross-sectional area of the cells (Jannasch*, et al.*, 1989, McHatton*, et al.*, 1996). The vacuole content is liquid and acidic (Beutler*, et al.*, 2009). The large, vacuolated *Beggiatoa* store nitrate intracellularly at concentrations of up to 370 mM, presumably in the vacuoles (Mussmann*, et al.*, 2003). In wide cells the cytoplasm is compressed towards the cell boundaries by the vacuole. The disc-shaped cells have a large surface to volume ratio which in theory is beneficial for substrate uptake. However, because the cells are arranged as stacks of discs the large surface is mostly covered by the adjacent cell. The habitat of the widest filaments is dominated by advective transport and thus mitigates the problems that arise concerning the surface limited solute uptake (see below).

Beggiatoa cells divide by binary fission. Only the cell membrane and the peptidoglycan layer are involved in the septation (Strohl & Larkin, 1978a). Filaments divide by the formation of sacrificial cells (necridia, Strohl & Larkin, 1978b). The lysis of a dead cell provides a breaking point for the filament. The formation of a loop or bend within the filament favors the rupture of the filament in the area of a sacrificial cell (Kamp*, et al.*, 2008). Sacrificial cell death can occur simultaneously at various points within the filament and leads to the formation of several daughter filaments within few hours (Kamp*, et al.*, 2008).

Physiology

Energy metabolism

The physiology and metabolism of *Beggiatoa* is remarkably versatile. *Beggiatoa* can gain energy by using sulfur compounds as electron donors or live on organic carbon compounds. So far sulfide, thiosulfate and sulfur have been identified to serve as electron donors for lithotrophy. It seems that marine strains preferably live as lithotrophs due to the better supply with sulfide in the marine environment (Hagen & Nelson, 1996, Hagen

& Nelson, 1997). Freshwater strains of *Beggiatoa* oftentimes gain energy by oxidizing dissolved organic carbon compounds such as acetate (Burton & Morita, 1964, Pringsheim, 1964, Strohl & Larkin, 1978a, Nelson & Castenholz, 1981a, Nelson & Castenholz, 1981b). *Beggiatoa* produce sulfur inclusions in the presence of sulfide and in some strains also thiosulfate. Sulfide is oxidized in two steps: the first step is the oxidation to elemental sulfur. Sulfur can be oxidized further to sulfate with a concurrent release of protons.

Oxygen and/or nitrate are the terminal electron acceptors for the oxidation of reduced sulfur compounds in lithotrophic *Beggiatoa*. Oxygen can only be used depth where oxygen still penetrates into the sediment while internally stored nitrate can be used as oxidant under anoxic conditions. *Beggiatoa* can reduce nitrate to either nitrogen gas by denitrification or to ammonia by the dissimilatory reduction of nitrate to ammonia (DNRA, Table 1). Under which conditions one or the other process dominates has not been fully understood. A highly reducing environment and the presence of reduced sulfur compounds seem to be inhibitory to NO- and N_2O-reductases (Brunet & Garcia-Gil, 1996). In large marine strains DNRA is therefore the predominant process (Graco*, et al.*, 2001, Sayama*, et al.*, 2005), due to a generally higher concentration of reduced sulfur compounds compared to most freshwater environments. In freshwater strains both pathways have been demonstrated (Sweerts*, et al.*, 1990, Kamp, 2007).

The transfer of electrons to oxygen yields more energy than the electron transfer to nitrate due to the lower reduction potential of nitrate. At 4 °C and a pH of 7.5 the

Table 1: Summarized equations for the oxidation of sulfide with oxygen and nitrate.

Electron acceptor for sulfide oxidation	Equation
Oxygen	$HS^- + 2O_2 \rightarrow SO_4^{2-} + H^+$
Nitrate (DNRA)	$HS^- + NO_3^- + H_2O + H^+ \rightarrow SO_4^{2-} + NH_4^+$
Nitrate (complete Denitrification)	$5HS^- + 8NO_3^- + 3H^+ \rightarrow 5SO_4^{2-} + 4N_2 + 4H_2O$

reduction of oxygen to water yields -747.6 kJ mol^{-1} per molecule HS^- whereas the reduction of nitrate to ammonia yields -427.7 kJ mol^{-1} per molecule HS^-, considering common ambient and intracellular concentrations of the reactants and products (10 µM O_2, 0.1 µM HS^-, 28 mM SO_4^{2-}, 150 mM NO_3^-, 1 mM NH_4^+, Jørgensen & Nelson, 2004).

The uptake mechanism of nitrate into the vacuole against a several thousand fold concentration gradient relative to the ambient water is not yet understood. The acidic vacuole content points towards an energy-consuming accumulation of protons (Beutler*, et al.*, 2009) by the concerted action of vacuolar ATPases and pyrophosphatases (Mussmann*, et al.*, 2007). The protons may be exchanged for nitrate by an NO_3^-/H^+-antiporter.

Growth

Winogradsky's observations of the sulfur inclusions in *Beggiatoa* led him to propose the concept of chemolithotrophy with the oxidation of sulfide to elemental sulfur (Winogradsky, 1949). Chemolithotrophy in *Beggiatoa* was demonstrated by an increasing growth yield with increasing sulfide concentration and carbon fixation from carbon dioxide in some marine strains (Nelson & Jannasch, 1983, Hagen & Nelson, 1996). In large vacuolated *Beggiatoa* high ribulose bisphosphat carboxylase-oxygenase activities and CO_2 fixation rates were measured, also suggesting autotrophic growth (Nelson*, et al.*, 1989, McHatton*, et al.*, 1996). Autotrophic growth can be sustained for several hours after depletion of the internal nitrate storage as based on calculations of the chemoautotrophic ribulose bisphosphat carboxylase-oxygenase activity in large *Beggiatoa* from a cold seep environment (McHatton*, et al.*, 1996). Yet, obligate autotrophy among *Beggiatoa* seems to be rather the exception than the rule, and a mixotrophic or heterotrophic nutrition has been identified for most examined strains. *Beggiatoa* that live heterotrophically seem to be extremely limited in the number and variety of substrates (Nelson & Castenholz, 1981a). In the presence of organic carbon sources they still acquire sulfur granules from sulfide or thiosulfate (Nelson & Castenholz, 1981b). In this case, sulfide might serve as a protection against harmful peroxides in catalase negative strains (Burton & Morita, 1964).

Under microaerobic conditions and in the absence of other N-sources some *Beggiatoa* have been shown to assimilate cell nitrogen from dinitrogen (Nelson*, et al.*, 1982b). N_2-fixation is suppressed in the presence of nitrate and ammonium. How widespread this highly energy consuming process is in the environment is not known.

1.2. *Beggiatoa* in their environment

Geochemistry of Beggiatoa inhabited sediments

Coastal sediments populated by *Beggiatoa* are characterized by active sulfur cycling and carbon mineralization processes. Oxidized and reduced sulfur compounds are constantly turned over by closely interrelated biotic and abiotic reactions. The oxidation of organic carbon compounds yields electrons for the microbial reduction of a variety of oxidized compounds in the sediment. In a typical redox cascade in coastal sediments oxygen as electron acceptor is followed by nitrate, manganese, iron and sulfate. These oxidation pathways have a vertical zonation which is determined by the free energy that the reaction produces. In *Beggiatoa* inhabited sediments oxygen is only present in the upper few mm below which the sediment is anoxic but often oxidized. Microsensor investigations found the nitrate penetration depth to be only few millimeters, only slightly deeper than oxygen penetration (Zopfi*, et al.*, 2001).

Sulfate reduction is one of the most important pathways of microbial respiration in coastal sediments (Jørgensen, 1982, Skyring, 1987). Sulfate reduction is active both in the oxidized and reduced zone of the sediment and can co-occur with metal oxidation (Jørgensen & Bak, 1991). Microbial reduction of iron and manganese oxides can locally exceed sulfate reduction in sediments rich in these metals (Canfield*, et al.*, 1993).

Another microbially mediated reaction involved in the sulfur cycle of sediments is the disproportion of intermediate oxidation products of sulfide by sulfate reducing bacteria. Disproportionation of elemental sulfur or thiosulfates provides an extra shunt of H_2S into the sulfur cycle (Jørgensen & Nelson, 2004). Eventually, disproportionation reactions may lead to the complete oxidation of H_2S to sulfate via abiotic production of

sulfur intermediates. A prerequisite is the scavenging of free sulfide which is inhibitory to sulfur disproportionation by iron or manganese oxides (Thamdrup, *et al.*, 1993).

Sulfide produced by sulfate reduction or disproportionation is gradually reoxidized either microbially or abiotically. Abiotically it can react with oxidized manganese or iron to form e.g. elemental sulfur, iron sulfide and pyrite (Fig. 2). With 5-20 % of pyrite being permanently buried in the sediment, 80-95 % of the sulfide is subject to reoxidation (Jørgensen, 1982). Sulfide oxidizing bacteria like *Beggiatoa* mediate the biotic reoxidation of sulfide in the presence of oxygen and/or nitrate and compete with or complement chemical reoxidation in the sediment (Preisler, *et al.*, 2007). The cycling of metal oxides and solid sulfur compounds is regulated by bulk transport mechanisms such as bioturbation.

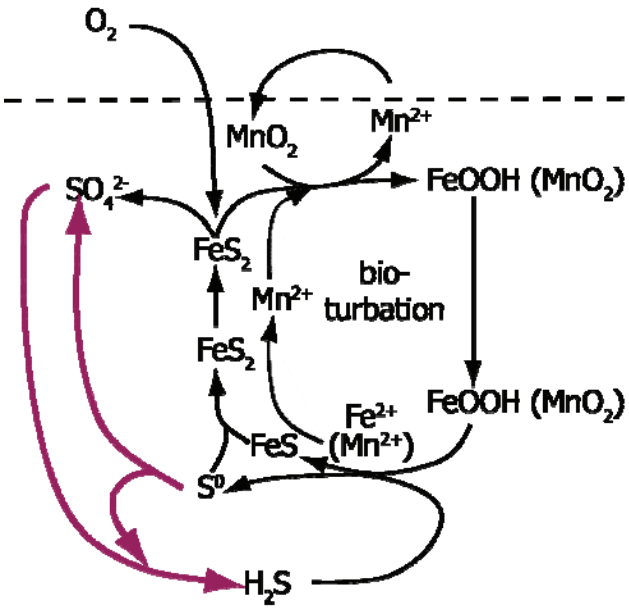

Figure 2: Sulfur cycling in the sediment. Biotic reactions, oxidation of reduced sulfur compounds and sulfate reduction are marked with purple arrows. Abiotic reactions (black arrows) involve iron and manganese. Bioturbation by meio- and macrofauna in the sediment transports oxidized compounds into deeper sediment layers where they are reduced, and reduced compounds up to the oxic sediment surface where they are oxidized. The dotted line marks the sediment-water interface. Modified after Jørgensen & Nelson (2004).

Habitats of Beggiatoa

Beggiatoa live in the microoxic zone of the sediment (Jørgensen & Revsbech, 1983, Møller*, et al.*, 1985). They can also live under anoxic conditions if an alternative electron acceptor is present and sulfide concentrations are low (Sayama, 2001). *Beggiatoa* occur in a wide range of habitats which comprise both freshwater and marine settings. The focus in this section is on marine environments. *Beggiatoa* inhabit sediments with high porosity and interstitial space for motility, whereas increasing density of the sediment selects for narrower filaments (Jørgensen, 1977). *Beggiatoa* prefer organic-rich soft mud over compact sandy sediment. *Beggiatoa* are commonly found at locations characterized by steep profiles of oxygen and sulfide. Habitats for non-vacuolated filaments are characterized by overlapping concentration gradients of oxygen and sulfide. Vacuolated forms of *Beggiatoa* can also occur in sediment where the concentration profiles of oxygen and sulfide are separated by a zone of varying depth where neither of the two compounds can be measured. Depending on the type of environment the mass transfer can be dominated by diffusion or by advection by local infauna, fluid seepage or degassing. Some typical habitats of *Beggiatoa* are given below, divided into coastal and deep sea environments.

Coastal environments

Coastal environments comprise shallow bays, fjords and intertidal flats (eg. Jørgensen, 1977, Sayama, 2001, Mussmann*, et al.*, 2003, Preisler*, et al.*, 2007). *Beggiatoa* are also widespread in upwelling regions, which are characterized by high productivity. In these environments sulfide production by sulfate reduction is usually driven by freshly deposited organic material such as settling algal blooms, sea grass, macroalgae and waste products from aquaculture (Fig. 3). High local sulfide concentrations and a constant upward diffusion of sulfide supply *Beggiatoa* and other sulfur oxidizing organisms with sulfide. *Beggiatoa* also occur in sediments of coastal regions where sulfide does not accumulate to concentrations much above detection limit. In these regions high sulfate reduction rates support growth of *Beggiatoa* (Jørgensen*, et al.*, 2010). In coastal zones the filaments either form mats on the sediment surface (Glud*, et al.*, 2004) or do not occur in

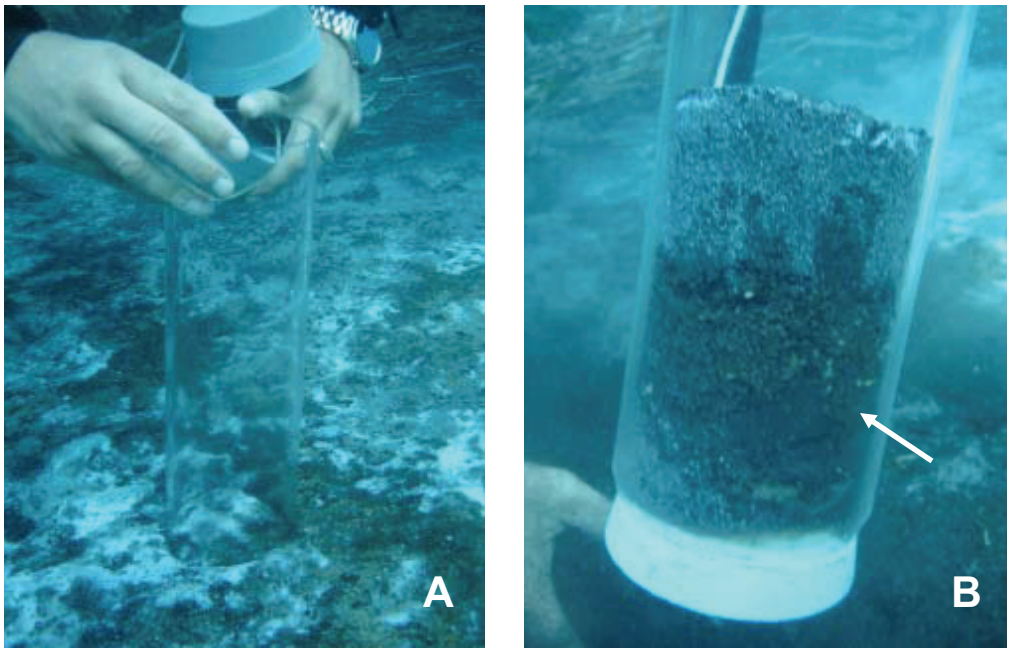

Figure 3: A: A mat of sulfur oxidizing bacteria including *Beggiatoa* in a shallow bay at the island of Elba, Italy. B: Below the sandy surface sediment a layer of mud containing sea grass (brown layer with pieces of *Posidonia* visible, marked by arrow) provides a source of sulfide. Image courtesy of Miriam Weber and Christian Lott © Hydra/M. Weber.

visible mats but are present in high numbers within the top centimeters of the sediment (Mussmann*, et al.*, 2003, Jørgensen*, et al.*, 2010).

Reef corals infected with the black band disease provide another niche within the coastal habitat. *Beggiatoa* are members of a microbial consortium that among others comprises cyanobacteria and sulfate reducing bacteria (eg. Richardson, 1996). This microbial consortium promotes the degradation of coral tissue.

Phototrophic microbial mats often harbor *Beggiatoa*, typically the smaller forms. These mats are characterized by a close and compact association of several functional microbial groups organized in often multicolored thin layers (Cohen & Gurevitz, 2006). Steep light intensity profiles exist within the mat (Jørgensen & Marais, 1988). Different microbial and chemical processes succeed in a very narrow layering. During daytime, when photosynthesis is the dominant process in mats, *Beggiatoa* are found below the zone of oxygen production. At night, respiration prevails and *Beggiatoa* migrate to the mat surface (eg. Garcia-Pichel*, et al.*, 1994).

Deep sea environments

In the deep sea *Beggiatoa* occur spatially limited to "hot spots" on the ocean floor. *Beggiatoa* form mats at cold environments like mud volcanoes (eg. de Beer*, et al.*, 2006), cold seeps (e.g. Ahmad*, et al.*, 1999) and areas with gas hydrates (e.g. Zhang*, et al.*, 2005) where water enriched in a wide spectrum of inorganic compounds and gasses seeps to the sediment surface either from deeper sediment layers or from crevices and cleavages in the oceanic crust. Geologically active areas such as spreading or subduction zones and the associated hydrothermal vents also provide good conditions for *Beggiatoa* (Jannasch*, et al.*, 1989, Nelson*, et al.*, 1989). *Beggiatoa* from these environments are not only among the filaments with the widest filament diameter found so far, they also form mats of surprising thickness of up to several centimeters (Jannasch*, et al.*, 1989, Gundersen*, et al.*, 1992).

Special habitats for *Beggiatoa* in the deep see are whale carcasses sunken to the sea floor (Fig. 4). The lipid-rich bone marrow of the whale bones provides substrate for the heterotrophic sulfate reducing microbial community (Deming*, et al.*, 1997, Treude*, et al.*, 2009). The produced sulfide is metabolized by sulfide oxidizers that can form dense mats on the surface of the bone (S. Grünke, personal communication).

Drift wood sunken to the sea floor also provides a source of organic carbon. Similar communities comprising several functional groups as those on whale bones develop on these sunken wood logs (Palacios*, et al.*, 2009).

Figure 4: Bone of a Minke whale, ~15 cm diameter. The white patchy covering is a community of sulfur oxidizing bacteria, among others, comprising *Beggiatoa* and *Arcobacter* species. Image courtesy of Hans Røy.

Life in mats and suboxic zones

Mats

Beggiatoa commonly form mats on sediments where oxygen and sulfide overlap just beneath the sediment surface (Jørgensen & Revsbech, 1983, Nelson*, et al.*, 1986a, Nelson*, et al.*, 1986b). *Beggiatoa* mats are not mats in the sense of consolidated microbial conglomerates like the phototrophic microbial mats that are held together and compacted by exopolymeric substances. The *Beggiatoa* mat is rather a tangle of randomly oriented filaments that can grow as an even surface coverage or arranged in knots of filaments often termed tufts. *Beggiatoa* mats range between a few hundred µm to several cm in thickness (e.g. Jørgensen & Revsbech, 1983, Jannasch*, et al.*, 1989, McHatton*, et al.*, 1996). *Beggiatoa* can also form a mat on sediment with a sulfide-free suboxic zone below the sediment surface (Dunker, 2005, Preisler*, et al.*, 2007). The appearance of the mat differs depending on the oxygen supply: At low oxygen flux to the mat the surface is a smooth layer with loose filament tufts and wide loops formed by the filaments. At high oxygen flux the filaments accumulate in denser tufts (Møller*, et al.*, 1985). The tufts are connected by strands of single filaments, giving the mat a web-like appearance. The tuft pattern is not stable. Tufts constantly form and disintegrate by the gliding motion of the filaments. Sometimes underneath the mat the anoxic sediment surface is visible if the mat is not too dense. Oxygen fluxes above tufts are higher than above smooth mats and above sediments without aggregates (Dunker, 2005) and the centre of the tufts is anoxic (Møller*, et al.*, 1985). Tuft formation hence presumably protects the filaments from too high oxygen concentrations. When the oxygen concentration in the upper sediment layer is above a tolerable limit for the filaments they retract into the sediment.

Mass transport of substrates to the mat can be either by diffusion or by advection. In the first case the mat is surrounded by an unstirred boundary layer of commonly 0.3-0.5 mm thickness (e.g. Rasmussen & Jørgensen, 1992). This layer is caused by friction between the water and the sediment surface. High concentrations of oxygen and sulfide rule in the surrounding environment whereas the mat lives under very low oxygen and sulfide concentrations. This results in steep concentration gradients of these substrates towards the *Beggiatoa* mat, which constantly consumes oxygen and sulfide and thus helps to maintain or even increase the concentration gradients of these compounds.

Mats at hydrothermal vents can grow as thick as a few centimeters because substrates are supplied by advective mass transport, thereby overcoming diffusion limitation. Advective flow of nutrients towards the cell can also satisfy the metabolic needs of the individual cells with a wide diameter of over 100 µm (Gundersen*, et al.*, 1992, Schulz & Jørgensen, 2001). A lining of cytoplasm between the central vacuole and the outer cell membrane provides a short diffusion distance for the nutrients within the cell. The physiological and ecological benefit of developing cells of a diameter that large is not yet understood.

Suboxic zone

Efficient reoxidation of sulfide either by abiotically or microbially mediated reactions can cause a depletion of sulfide in the oxidized zone, resulting in an intermediate zone where neither oxygen nor sulfide is detectable. The onset of the sulfidic zone is characterized by a steep gradient of sulfide and hence an upward diffusion of sulfide. Nevertheless, in the sulfide-free zone the production of sulfide is possible by bacterial sulfate reduction. Due to concurrent reoxidation it is not detectable. Sediment-dwelling *Beggiatoa* shuttle between the oxygen diffusion front where sulfide and/or internally stored sulfur are oxidized with oxygen, and the onset of the sulfide diffusion front where internally stored nitrate is used as oxidant. The storage of nitrate as electron acceptor and sulfur as electron donor is hence an important prerequisite to colonize the suboxic zone. *Beggiatoa* in the suboxic zone are randomly distributed (Preisler*, et al.*, 2007, Jørgensen*, et al.*, 2010) and perform a random walk-like locomotion (Dunker*, et al.*, submitted). The sulfide diffusion front is not fixed and depends on the metabolic activity of *Beggiatoa*. The diffusion front is pushed downwards if *Beggiatoa* consume more sulfide. Increased nitrate supply and hence intracellular uptake by *Beggiatoa* allows them to deplete sulfide in deeper layers. A characteristic pH profile with a pH minimum where oxygen disappears and a pH maximum at the sulfide diffusion front mirrors the spatially separated reactions that produce or consume protons (Dunker, 2005, Sayama*, et al.*, 2005).

Lately, an alternative mechanism was identified that could cause the sulfide diffusion front to move to deeper sediment layers. Free electrons can shuttle from sulfide

in the sediment to oxygen in the water without the action of redox reactions (Nielsen*, et al.*, 2010). The electrical currents are transmitted through the sediment by bacterial structures such as nanowires, chemical electron carriers or minerals like pyrite. The sulfide front rises or falls in dependence of the oxygen supply to the sediment. So far, this mechanism has only been shown in defaunated sediment. Whether sulfide oxidizing bacteria in natural sediments have to compete for sulfide with the electrical currents that run through the sediment remains to be demonstrated.

Beggiatoa are not only found in a vast variety of habitats, but they also thrive in sediments of all climatic zones. The next chapter provides an overview over the characteristics of temperature response in general and of specific adaptations that are required to colonize cold habitats.

1.3. Temperature response in *Beggiatoa*

General aspects of temperature response

Biological processes are highly temperature-dependent. All organisms have a specific temperature range at which they are physiologically active. Their activity increases exponentially from the minimum temperature for activity to the optimum temperature. At the optimum temperature (T_{opt}) they have reached their maximum activity. Beyond the optimum temperature the activity drops until it reaches the maximum temperature which it the highest temperature at which physiological activity can still be detected. Generally, the T_{opt} is above the *in situ* temperature at which the organisms live (Isaksen & Jørgensen, 1996, Knoblauch & Jørgensen, 1999, Dunker*, et al.*, 2010).

The above described temperature response has been observed for growth rates of a large number of microorganisms (e.g. Isaksen & Jørgensen, 1996, Knoblauch & Jørgensen, 1999, Reynolds, 2006) as well as for processes for energy generation like sulfate reduction (Arnosti*, et al.*, 1998), anaerobic oxidation of methane (Kallmeyer & Boetius, 2004, Treude*, et al.*, 2005), anaerobic ammonium oxidation (Dalsgaard &

Thamdrup, 2002, Dosta*, et al.*, 2008), denitrification (Rysgaard*, et al.*, 2004), and CO_2 fixation (Nelson*, et al.*, 1989). A similar temperature response curve can also be observed for activities directly related to energy generation such as motility (Crozier & Federighi, 1924, Crozier & Stier, 1926, Halfen & Castenholz, 1971). The general explanation for the similar temperature response in all these different biological processes is that enzyme activity increases with increasing temperature leading to higher rates up to a critical T_{opt}.

The temperature range in which organisms are active depends on the climatic conditions in which they live. Typically, organisms that live in warm environments are active at a high temperature range whereas organisms with a cold habitat have a low temperature range for activity (eg. Thamdrup & Fleischer, 1998, Robador*, et al.*, 2009). Information about the occurrence of *Beggiatoa* in different climatic regions is scarce, especially in the cold environment. They occur in the tropical zone (eg. Richardson, 1996) at temperatures that seldom drop below 20°C and are at maximum above 30°C. Other types of filaments have been found at the other extreme of the temperature range in the arctic zone (Glud*, et al.*, 2004, Jørgensen*, et al.*, 2010). These filaments even have to withstand transient freezing. *Beggiatoa* at great water depth live at permanently low temperatures of about 1-6°C (e.g. de Beer*, et al.*, 2006).

The temperature range and temperature optimum for gliding motility of filaments from different climatic origins has remained unexplored. The acclimatization potential of a *Beggiatoa* population and the mechanism behind has also not been evaluated. Cold adapted organisms that are incubated at higher temperature than *in situ* temperature can acclimatize to the new ambient temperature and increase their activity at that temperature (e.g. Robador*, et al.*, 2009). How *Beggiatoa* acclimatize to changing temperatures and if the acclimatization occurs within a single population or if the temperature change causes a shift in the community remains to be shown.

Enzymatic cold adaptation

The Arrhenius equation and its natural logarithm is very helpful to understand reaction kinetics and has been used by many biologists to describe temperature regulation of physiological processes (Isaksen & Jørgensen, 1996, Arnosti*, et al.*, 1998, Knoblauch

& Jørgensen, 1999). The reaction rate (Equ. 1) and its natural logarithm (Equ. 2) can be calculated as

$$\nu = Ae^{-\frac{E_a}{RT}} \qquad \text{(Equation 1)}$$

$$\ln \nu = \ln A - \frac{E_a}{RT} \qquad \text{(Equation 2)}$$

where ν is the rate of activity, A is a constant, E_a is the activation energy (kJ mol^{-1}), R is the gas constant (8.31 J K^{-1} mol^{-1}), and T is the temperature (K).

By plotting the natural logarithm of the rate (Equ. 2) against the inverse temperature a plot with a linear range is obtained. The linear range represents the interval at which the rate of a temperature dependent reaction increases exponentially. The exponential increase is typical for a temperature range where enzymes are intact and work properly (Arrhenius, 1908). From the slope of the linear range E_a can be calculated. High E_a values indicate a strong temperature dependence of the activity whereas low E_a values as they are often found for organisms living in permanently cold habitats point towards a weak temperature dependence (Low*, et al.*, 1973, Lonhienne*, et al.*, 2000).

In past work, cold adaptation in microorganisms has been described in various aspects (Deming, 2002). Besides changes in membrane fluidity, the amino acid composition of proteins and the stability of enzymes have been examined and discussed (review by Feller & Gerday, 2003). One of the main outcomes was that the reactivity of enzymes in the cold requires conformational flexibility. Cold adapted enzymes have more flexible catalytic sites than their mesophilic counterparts which facilitates substrate binding to the catalytic site of the enzyme (Hochachka & Somero, 1984). The most important implication of this is that psychrophilic enzymes can maintain high reaction rates at low temperature (Lonhienne*, et al.*, 2000). The higher catalytic activity at low temperatures in cold adapted enzymes compared to their mesophilic counterparts is caused by the lower number of molecular interactions that have to be disrupted during the activation process (Feller & Gerday, 1997). It is achieved at the expense of thermostability of the enzyme (Fig. 5). The thermostability of psychrophilic enzymes has been increased experimentally by amino acid substitution using directed evolution

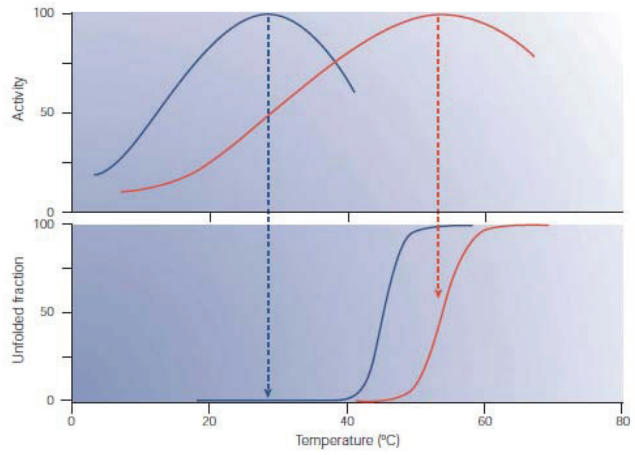

Figure 5: Stability of the catalytic site of psychrophilic enzymes (blue) compared to mesophilic enzymes (red). The catalytic site of the psychrophilic enzyme is inactive long before the protein unfolds. The mesophilic enzyme reaches its maximal activity when half the enzyme is already unfolded (from Feller & Gerday (2003)).

methods, in which the activity at low temperatures has not been compromised (Miyazaki, *et al.*, 2000), emphasizing the importance of amino acid substitution for temperature dependent characteristics of enzymes (e.g. Lonhienne, *et al.*, 2001).

Organisms from the cold have not only different enzymes, but also other proteins and vital molecules have specific adaptations that enable them to function in the cold. The production of carotenoids may contribute to membrane stability at low temperatures (Fong, *et al.*, 2001). Proteins involved in protein biosynthesis (Thomas, *et al.*, 2001, Williams, *et al.*, 2010), surface layer proteins (Williams, *et al.*, 2010) and most likely many more that remain to be discovered are differently expressed in psychrophilic and mesophilic organisms.

1.4. Gliding motility in *Beggiatoa*

Characteristics of gliding motility in Beggiatoa

Gliding motility in *Beggiatoa* is surface associated. Gliding is much slower compared to swimming, but the slow locomotion and hence slower translocation across oxygen and sulfide gradients may be compensated by lower energetic costs for motility (Mitchell & Kogure, 2006). The filaments glide by a left-handed helical rotation around

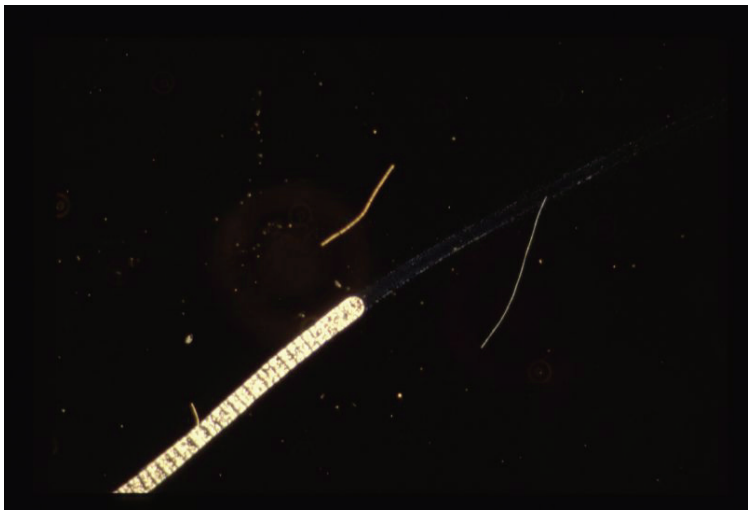

Figure 6: Darkfield micrograph of a gliding *Beggiatoa* filament. The filament is gliding to the left bottom. The slime trail is visible as a refracting trace. Image courtesy of Bo Barker Jørgensen.

their long axis (Møller, et al., 1985). They leave a slime sheath behind as they advance (Fig. 6). This slime sheath is only loosely associated with the filament.

If one end of the filament reverses and the other end does not, then both ends of the filaments glide into opposing directions. If the gliding movement is towards each other then the central part of the filament bends. As a consequence, the slime sheath can rupture in that region. The gliding speed of *Beggiatoa* depends on several factors such as filament width and climatic origin. Narrow filaments of around 1-10 µm are commonly slower than medium sized filaments of about 15-30 µm from the same climatic origin. Filaments of more than 100 µm width glide slower than narrower filaments from the same origin (Nelson, et al., 1989). Being presumably enzyme controlled, the gliding speed is also dependent on the ambient temperature. Until now, little is known about the temperature response of gliding. Gliding speed in *Beggiatoa* is identical in either direction. However, stimulation experiments with light suggest that there is a temporal polarity in the filaments. Stimulation of the leading part of the filament with light induced reversals whereas stimulation of the trailing part decreased the reversal frequency (Nelson & Castenholz, 1982a). The polarity is presumably inverted only after the reversal. If the polarity is also present with the application of other stimuli than light remains to be shown. The distance that *Beggiatoa* filaments can glide depends on gliding speed, their nitrate storage capacity and nitrate concentration in the vacuole, reduced sulfur supply

and the nitrate reduction rate. With an internal nitrate concentration of up to 370 mM, *Beggiatoa* filaments can cover distances of several meters if gliding in a linear path (Preisler, et al., 2007).

Possible mechanisms of gliding

Gliding locomotion is shared among a phylogenetically heterogeneous group of prokaryotic organisms (Reichenbach & Dworkin, 1981). This group comprises both unicellular and multicellular, filamentous organisms. Much research has been going on to reveal the underlying mechanisms of gliding, yet little is known about the mechanism of gliding motility in *Beggiatoa*. When *Beggiatoa* was described first by Trevisan in 1842 he noted that its "thallus" is wrapped in slime (Trevisan, 1842). Gliding filaments and also gliding single cells all move by leaving a slime trail behind them. The slime is a polysaccharide synthesized in the cells and extruded through pores on the cell surface (Halfen, 1979, Larkin & Strohl, 1983). In how far these slime threads are of major importance for the motility of gliding organisms has been debated (Burchard, 1981, Reichenbach & Dworkin, 1981, Larkin & Strohl, 1983). They also may function as adhesive to the surface on which the filaments glide (Ridgway & Lewin, 1988).

The gliding locomotion of filaments has mainly been studied on species of the Familiy Oscillatoriaceae (Halfen & Castenholz, 1971, Hoiczyk & Baumeister, 1998, Hoiczyk, 2000) as well as in the Flexibacteraceae (Burchard, 1982, Ridgway & Lewin, 1988). Although, against earlier assumptions (Reichenbach & Dworkin, 1981), *Oscillatoria* and *Beggiatoa* are not phylogenetically related, they certainly show striking structural similarity (eg. Pringsheim, 1949, Strohl*, et al.*, 1982, Mussmann*, et al.*, 2007). Filamentous gliding cyanobacteria possess certain structural elements that seem to be important for gliding motility (Hoiczyk, 2000, Read*, et al.*, 2007). These are an external layer outside of the cell membrane composed of a surface layer (S-layer), proteinaceous oscillin fibrils on top of the S-layer around the filament and the junctional pore complex organelles, organized in rows or girdles. All these features are also present in *Beggiatoa* although in details they differ from those described for filamentous cyanobacteria (Hoiczyk & Baumeister, 1995). The S-layer analysis of *Beggiatoa alba* revealed a complex pattern, which comprises five layers on the surface of the cytoplasmic

membrane (Strohl*, et al.*, 1982). Longitudinally arranged fibrils of the same diameter (6-13 nm) as in cyanobacteria were also present on the surface. Moreover, pores of about 15 nm diameter arranged in parallel rows, presumably in a spiral arrangement were described in *Beggiatoa* from the Gulf of Mexico (Larkin & Henk, 1996).

In many of these organisms gliding is accompanied by a rotation of the filament. The fibrils in rotating cyanobacterial filaments are helically arranged. The helical orientation of the fibrils and the pitch at which they are arranged coincided with the handedness and momentum of revolution. In non-rotating cyanobacteria these fibrils were absent (Hoiczyk, 2000). Hence, a motility mechanism of contracting fibrils was proposed that produced unidirectional waves which propagated along the filament surface (Halfen & Castenholz, 1970). However, these fibrils were not evident in all gliding organisms (Reichenbach & Dworkin, 1981).

In the more recent literature the hypothesis of propulsion by slime excretion through the pores on the filament surface was proposed (Hoiczyk & Baumeister, 1998, Wolgemuth*, et al.*, 2002, Fig. 7). Not much is known about the pores of *Beggiatoa* exept that they are arranged in parallel rows and traverse the cell surface. Much more research has been conducted on the pores involved in gliding in other bacterial families. For the cyanobacteria *Phormidium* and *Anabaena* it could be shown that mucilage strands were

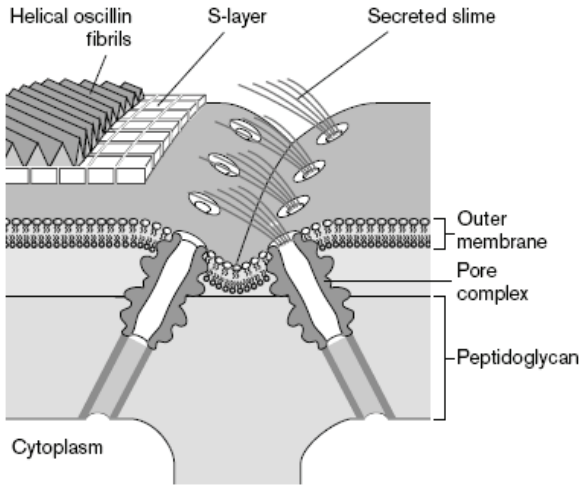

Figure 7: Diagram of the secretion process of slime based on ultrastructural data of the gliding, filamentous cyanobacterium *Phormidium tunicatum* (from Hoiczyk & Baumeister (1998)).

secreted and elongated at the same speed at which the filament glided (Hoiczyk & Baumeister, 1998). The slime is not excreted passively during locomotion but its excretion was opposite to the direction of movement and is therefore likely to be actively involved in filament locomotion. The junctional pores, through which the slime is excreted are localized close to the cross wall junctions in cyanobacteria (Halfen & Castenholz, 1971, Hoiczyk & Baumeister, 1998). The pores on the cell surface actually are just the opening of a complex structure termed pore complex organelles that span the cell membrane and the outer membrane. Presumably, they are present in sets or groups, one opposite the other. In fact, in *Beggiatoa* the pores on the cell surface are arranged like that (Larkin & Henk, 1996). This arrangement may be responsible for the reversals in gliding direction, in which at times one row of pores is active and after a reversal the opposite row.

A model developed for gliding *Myxococcus xanthus* cells suggested a mechanism of slime extrusion that yielded enough motive force to propel the cell forward (Wolgemuth*, et al.*, 2002). The authors suggested that in the pore complex organelles the hydration of slime fibers causes osmotic swelling and expansion which ultimately lead to the extrusion of the slime (Fig. 8). How the slime polymer is introduced into the pore complex is not explained yet. Calculations show that if the slime swelling occurs in many pores simultaneously this mechanism would produce enough force to propel single cells and even filaments forward, given a threshold number of pores (Wolgemuth*, et al.*, 2002, Robinson*, et al.*, 2007).

The proposed mechanisms of gliding may not have general applicability to all types of gliding organisms. Presumably, several mechanisms of gliding coexist and are performed by different groups of gliding organisms (Jarrell & McBride, 2008). Yet, distinct features have been identified in a high number of unrelated gliding organisms. For *Beggiatoa* few studies were made and the mechanism of gliding in *Beggiatoa* has to be inferred on the base of what is known for other gliding organisms.

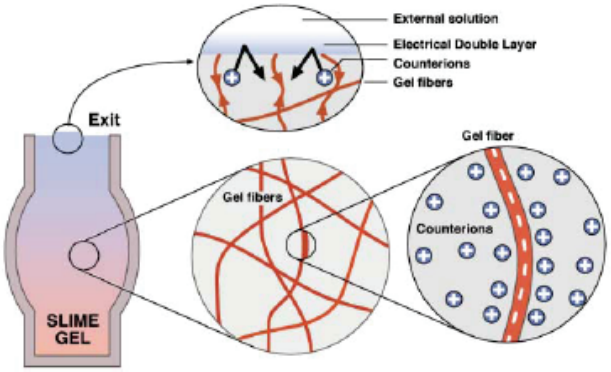

Figure 8: Negatively charged slime fibers are coated with positive charges. The electrical field that builds up between the different charges acts as a semipermeable membrane that allows water to enter but prevents ions to leave. The water that enters through the pore opening swells the slime which cannot expand the reinforced cell walls of the pore. Hence, the slime can leave the pore complex only through the pore opening (from Wolgemuth, *et al.* (2002)).

1.5. Chemotactic responses

Mechanisms of orientation in the environment

Orientation in the environment requires a gradient of either chemical or physical nature. Organisms able to orient towards stimuli have developed a variety of mechanisms to position themselves at the physiologically and energetically most favourable position within a gradient environment (Fenchel, 2002, Mitchell & Kogure, 2006). Oftentimes these gradients are transient, e.g. a gradient of nutrients that forms around a decaying particle of organic matter (Blackburn, *et al.*, 1998) or a sulfide gradient that extends into the diffusive boundary layer during periods of high sulfide production (Thar & Fenchel, 2005). If the oxic-sulfidic boundary lies within the diffusive boundary layer then only free swimming bacteria can position themselves at the oxic-anoxic boundary. Most studies investigated motility in water but few considered tactic movements of swimming bacteria in sediment (Barbara & Mitchell, 1996, Fenchel, 2008), despite sediment habitats with microbial mats are often characterized by steep solute gradients. In few cases chemotaxis has been studied in microorganisms from permanently cold environments (Allen & Deming, 2002), but as *Beggiatoa* have been found in arctic

sediments where they can form mats (Jørgensen*, et al.*, 2010) there is no doubt that *Beggiatoa* orient by chemotaxis also at low temperatures and that their mechanism of chemotaxis is adapted to the cold.

The biased random walk of *Escherichia coli* and other heterotrophic bacteria is probably the most thoroughly investigated chemotactic response (Brown & Berg, 1974, Berg, 1975, Fenchel, 2008). These bacteria swim following a relatively straight path which is interrupted by occasional turns. By decreasing the turning frequency when moving up a concentration gradient of an attractant they obtain a net movement towards the attractant. Contrary to the tactic behavior of *E. coli* some marine bacteria in microbial mats form microlaminations by increasing their turning frequency (Barbara & Mitchell, 1996). A similar response was observed for most motile bacteria that orient towards oxic-anoxic interfaces above sulfidic sediment. These organisms form bacterial mats, bands and veils by reversing when moving into suboptimal oxygen concentration. The strategies are versatile: Some bounce between a narrow range of oxygen concentrations and reverse whenever it is too high or too low (Fenchel & Thar, 2004, Fig. 9C). Other organisms need another repellent besides oxygen that delimits the mat to the anoxic side which oftentimes is sulfide (Møller*, et al.*, 1985, Thar & Kühl, 2001). The formation of mats and veils is advantageous in an environment where the energy sources, namely oxygen and sulfide are present in opposing gradients (Thar & Kühl, 2001, Thar & Fenchel, 2005). Among the investigated swimming bacteria were ovoid and spherical cells (Thar & Fenchel, 2001, Fenchel & Thar, 2004), spirilla and vibrios (Thar & Fenchel, 2005) and purple sulfur bacteria (Thar & Kühl, 2001, Fig. 9D). All swimming cells moved presumably with flagella although these were not always visible. The investigated organisms had a negative response towards oxygen concentrations above 1 to 10 µM (Thar & Kühl, 2001, Fenchel & Thar, 2004, Thar & Fenchel, 2005, Fischer & Cypionka, 2006).

Some swimming organisms seem to only react towards oxygen like "*Candidatus* Ovobacter propellens" which does not reverse its swimming direction in the completely anoxic and hence sulfidic zone (Fenchel & Thar, 2004). Of the above described swimming organisms only the phototrophic *Marichromatium* seems to respond towards low sulfide concentrations under anoxic light conditions (Thar & Kühl, 2001). *Beggiatoa*

react towards oxygen, light and probably sulfide and nitrate. The response towards sulfide may be a major difference of swimming organisms that stay at the surface of sulfidic sediments and the large filamentous sulfur oxidizers that besides forming mats on the sediment surface also populate the suboxic zone.

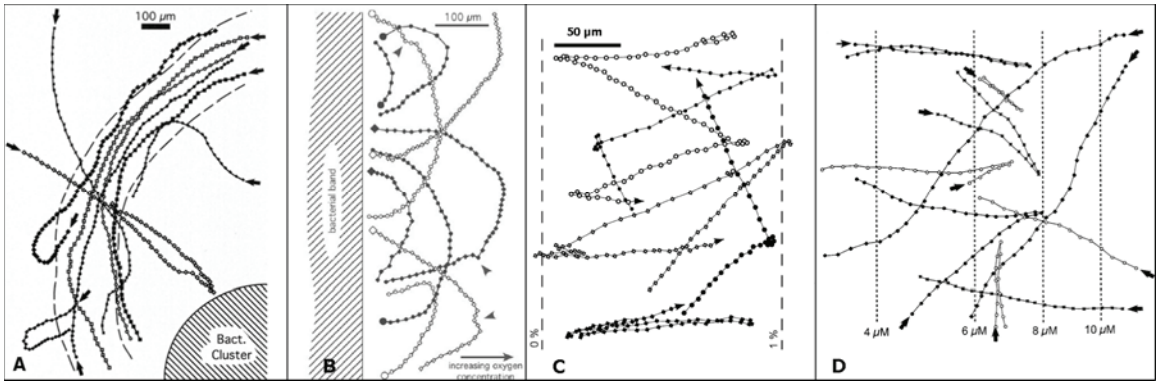

Figure 9: Motility patterns in swimming sulfur bacteria to keep track of the optimal oxygen concentration. A: *Thiovulum majus* (from: Thar & Fenchel (2001)), B: Gram negative vibroid bacterium from sulfidic sediment (from: Thar & Kühl (2003)), C: "*Cand.* Ovobacter propellens" (from: Fenchel & Thar (2004)), D: *Marichromatium gracile* (from: Thar & Kühl (2001)).

Swimming bacteria modulate their motility with different swimming speeds, turning angles, run lengths and rotation rates. They can rotate around the long or short axis, and have straight or helical swimming tracks. By alterations of one or more of these parameters the bacteria can adapt their specific motility pattern to stay within their preferred limits of the oxygen gradient. The turns in swimming direction usually occur within angles of 170-270° (Fenchel & Thar, 2004, Thar & Fenchel, 2005). *Thiovulum majus* and "*Candidatus* Ovobacter propellens" cells perform U-turns when they leave the isopleths of optimal oxygen concentration. They steer perpendicular to the oxygen gradient by swimming in a helical path by a mechanism called helical klinotaxis (Thar & Fenchel, 2001, Thar & Kühl, 2003, Fig. 9A and B). During stable conditions of optimal oxygen concentration some cells are able to attach by mucus stalks (Fenchel & Glud, 1998, Thar & Kühl, 2002, Thar & Kühl, 2003) that in some cases have even been observed to grow, following the oxygen gradient if it moved (Thar & Fenchel, 2005).

Sensing of stimuli can be achieved either by spatial sensing or by temporal sensing. Spatial sensing requires two sensing regions that are positioned at a certain distance from each other on the cell surface. Temporal sensing requires only one sensing

region. This type of sensing depends the comparison of a precedent signal to the actual signal and demands a memory unit for the precedent signal (Thar, 2002). Spatial sensing was believed to be exclusively practicable for larger cells, which now has been disproved both theoretically (Dusenbery, 1998) and experimentally (Thar & Kühl, 2003).

Reversals in Beggiatoa

Reversals in gliding direction in *Beggiatoa* are triggered by suitable chemical cues among which light, oxygen and/or sulfide at low concentrations seem to play a key role. They also occur at random intervals when the filaments are unstimulated (Nelson & Castenholz, 1982a, Møller*, et al.*, 1985, Dunker*, et al.*, submitted). *Beggiatoa* filaments reverse the direction of movement at an angle of about 180° (Fig. 10A and B). The change of direction happens abruptly. There is no evident deceleration before the filaments stop. After the filament stopped, it pauses for 1-4 s at its stopping position until it resumes motility (Fig. 10C and D). The resumption of motility is sometimes accompanied by a sudden jerk into the direction of gliding as if held back by a rubber band that snaps. Besides the jerk that happens within a few hundred milliseconds there is

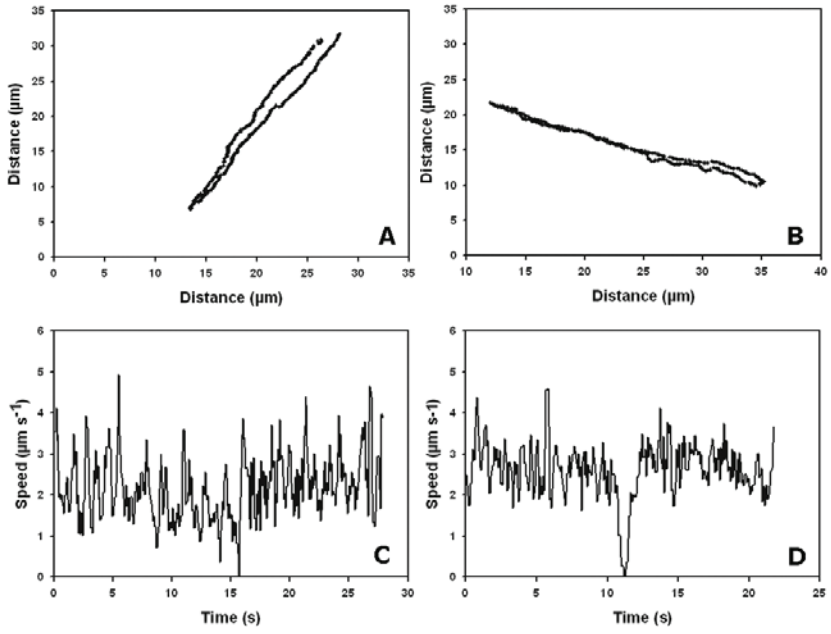

Figure 10: Direction (A and B) and speed (C and D) at reversals of two *Beggiatoa* filaments (own data).

no evident acceleration. After reversing the filaments continue to glide in the opposite direction at the former gliding speed.

The reversal of gliding direction in filaments is achieved by a concerted reversal of all cell of the filament rather than by the independent reversal of each single cell of the filament. The concerted action requires a suitable signal that is propagated along the length of the filament. Coordination between the cells involves presumably a molecular signal. The diffusive transport of a chemical signaling substance along the length of the filament does not seem to be involved in signal transduction, because the diffusion time is longer than the reaction time in the filament. Similar to the short reaction time in *Beggiatoa*, reversals in the filamentous bacterium *Flexibacter polymorphus* takes place within < 0.1 s (Ridgway & Lewin, 1988). This rapid signal transmission could be achieved by an electrical signal like membrane depolarization. Some gliding filaments reversed the direction when they encounter an obstacle (Ridgway & Lewin, 1988) and increased their reversal frequency when they glided in medium of increased viscosity (Halfen & Castenholz, 1971). This observation may point to the involvement of mechanoreceptors in signal transduction. At this time only speculations about the putative signal transmission pathway in filamentous bacteria are possible. However, close observation of the behavior allows excluding some signaling mechanisms like a diffusing chemical signal.

Tactic responses in Beggiatoa

Oxygen

Beggiatoa react to changes in oxygen concentration with a step-up phobic response (Møller*, et al.*, 1985, Nelson*, et al.*, 1986b). An increase in the experienced oxygen concentration leads to a reversal whereas the filaments do not react when oxygen concentration decreases. Reversals take place after a lag phase which is commonly less than 60 seconds. The filaments react to oxygen changes as low as 5 % air saturation per minute. The sensitivity towards weak oxygen gradients provides that *Beggiatoa* are able to orient even when gliding at an acute angle to the horizontal (Møller*, et al.*, 1985). A prerequisite seems to be that the oxygen change happens at low initial oxygen

concentrations. It is not known whether filaments exposed to oxygen concentrations higher than 10 % air saturation do reverse when they experience an oxygen change of the same magnitude. Short filaments (0.6-0.8 mm) usually reverse as a whole whereas in longer filaments partial reversals are common (Dunker*, et al.*, submitted). Partial reversals lead to coil and loop formation and may subsequently guide the filament into a new direction (Møller*, et al.*, 1985). The reversal behavior leads to the accumulation of *Beggiatoa* at a zone of microoxic conditions. *Beggiatoa* are rarely observed in zones above 10 % of air saturation. By actively avoiding high oxygen concentrations the filaments accumulate in a mat that is sharply defined towards the oxic but less sharp towards the anoxic side (Jørgensen & Revsbech, 1983). Different *Beggiatoa* strains are differently sensitive to oxygen (Nelson*, et al.*, 1986b). In the microoxic niche of a mat *Beggiatoa* is limited by oxygen diffusivity to the mat rather than by oxygen uptake kinetics (Nelson*, et al.*, 1986b). The molecular sensing mechanism of oxygen and light has been studied in various flagellated bacteria in which flagella beating is dependent on the proton motive force (Armitage, 1997), but the mechanism how *Beggiatoa* filaments sense changes in the oxygen concentration is still poorly understood.

Nitrate

Orientation towards their substrates nitrate and nitrite is a common behavior in denitrifying bacteria (Lee*, et al.*, 2002). The role of nitrate in tactic responses of large sulfur bacteria has been explored mainly in the large sulfur oxidizing filaments of the sheath-building *Thioploca* that are closely related to *Beggiatoa*. They inhabit a similar environment than the large vacuolated *Beggiatoa* (Gallardo, 1977). In the suboxic zone they shuttle in their sheath between sediment surface, where they accumulate nitrate internally and deeper sediment layers where they oxidize sulfide (Huettel*, et al.*, 1996). The orientation of the sheaths in the sediment thereby provides a vertical direction of filament movement (Schulz*, et al.*, 1996). Thioploca orient to nitrate by emerging from the sediment when nitrate is present in the water (Huettel*, et al.*, 1996). The same authors proposed that the ascent and the descent, respectively, are triggered by the interaction of tactic responses and by the balance of the internal electron acceptor (nitrate) and external

electron donor (sulfide). Despite large vacuolated *Beggiatoa* use nitrate as an electron acceptor, a positive nitrate taxis has not yet been undoubtedly shown.

Sulfide

In sediment with massive occurrence of *Beggiatoa* they can be almost exclusively responsible for the rapid oxidation of sulfide (Jørgensen & Revsbech, 1983). However, *Beggiatoa* have never been observed concurrent with high sulfide concentrations neither in their natural environment (eg. Hinck*, et al.*, 2007, Preisler*, et al.*, 2007) nor in cultures (Jørgensen & Revsbech, 1983, Nelson*, et al.*, 1986b). The concentration limit that they tolerate seems to be specific to the community and is around 10-350 µM (Jørgensen & Revsbech, 1983, Nelson*, et al.*, 1986a, personal observation). Many authors proposed an avoidance reaction to sulfide to explain the observation that *Beggiatoa* are absent in the sulfidic zone of the sediment and formed mats with a defined boundary towards the sulfidic side (e.g. Nelson*, et al.*, 1986b, Garcia-Pichel*, et al.*, 1994, Preisler*, et al.*, 2007). In experiments with gradient cultures of oxygen and sulfide downward migration was observed in older agar tubes with declining steepness of the sulfide gradient (A. Kamp, personal communication). In aged tubes up to 86 % of the mat is in the anoxic region (Nelson*, et al.*, 1986b). This behavior implies an orientation towards the sulfide boundary. The experimental proof for a phobic sulfide response is not easy, because the orientation towards microoxic conditions may hamper a clear response towards sulfide. It is clear, though, that *Beggiatoa* form mats in the absence of sulfide (Jørgensen & Revsbech, 1983, Dunker*, et al.*, submitted).

Light

Beggiatoa react towards visible light with a maximum action at blue light (430 nm, Nelson & Castenholz, 1982a). As the response to oxygen, the response to light is a step-up phobic response. The filaments reverse after a short lag phase when stimulated. The reversal frequency increases with increasing initial light intensity and increasing light intensity gradient. The light intensity gradient necessary to cause a reversal decreases at higher light intensities (Nelson & Castenholz, 1982a). The reaction towards light in *Beggiatoa* is presumably not linked to energy generation like in photosynthetic

organisms. Yet, it has been suggested that the light may interact with a component of the electron transport chain and that the change in output of the electron transport system may invoke the tactic response. The ecological benefit of the photophobic response is the protection from photo-oxidative cell damage. The photophobic response is essential when *Beggiatoa* live in microbial mats associated with photosynthetic organisms like cyanobacteria and diatoms. In these mats, oxygen concentrations correlate positively with incident irradiance and the presence of cyanobacteria (Garcia-Pichel*, et al.*, 1994). *Beggiatoa* may migrate to the sediment surface during darkness when the oxygen concentration is low just beneath the sediment surface. The onset of the light period leads to an increasing oxygen concentration in the uppermost few hundred μm below the nighttime position of the migrating *Beggiatoa* layer (Garcia-Pichel*, et al.*, 1994). A phobic response to light that overrides the phobic response to high oxygen concentrations may help *Beggiatoa* to avoid the zone of elevated oxygen concentration during illumination (Møller*, et al.*, 1985).

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2. Results

2.1. Manuscript 1:

Temperature regulation of gliding motility in filamentous sulfur bacteria, *Beggiatoa* spp.

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Abstract

The response of gliding motility to changing temperatures was studied in filaments of the large sulfur bacteria *Beggiatoa* from arctic, temperate and tropical marine environments. The general shape of the gliding speed versus temperature curves from all three locations was similar but differed in the maximal gliding speed of the filaments, optimum temperature, and the temperature range of motility. The optimum temperature and the overall temperature range of gliding motility accorded to the climatic origin of the filaments with a high temperature range for tropical, an intermediate range for temperate, and a low temperature range for arctic filaments. The temperature controlled decrease in gliding speed at low temperatures was reversible while the drop in speed at high temperatures was due to irreversible thermal damage in individual filaments. Filaments from the Arctic and cold acclimatized filaments from the temperate zone were unaffected by transient freezing of the surrounding seawater. At *in situ* temperatures filaments glided at 17-55 % of the gliding speed at the optimum temperatures, indicating that they were well adapted to the temperature regime of their origin. Our results point towards an enzymatic control of temperature dependent gliding motility.

Introduction

Gliding motility is a characteristic property of filamentous sulfur bacteria of the genus *Beggiatoa*. *Beggiatoa* occur widespread in sediments with steep concentration gradients of oxygen and sulfide. The gradients either overlap (Jørgensen & Revsbech, 1983) or are separated by a suboxic zone of varying depth (Sayama *et al.*, 2005, Preisler *et al.*, 2007). *Beggiatoa* are typically found at low concentration of oxygen and sulfide, both of which provide stimuli for a negative chemosensory response (Møller *et al.*, 1985, Nelson *et al.*, 1986, Kamp et al., 2006, Preisler *et al.*, 2007). The necessity to constantly reorient according to alterations in the chemical cues or to shuttle in the suboxic zone between the sulfide front and the oxic water column renders motility a crucial function in the ecology of *Beggiatoa*.

Beggiatoa filaments rotate around the long axis when gliding (Møller *et al.*, 1985, Larkin & Henk, 1996), similar to members of the cyanobacterial family Oscillatoriaceae (Hoiczyk, 2000). The mechanism of gliding motility in *Beggiatoa* involves pores on the cell surface through which exopolymeric slime is extruded which forms a mucilaginous trail as the filament proceeds (Larkin & Strohl, 1983, Larkin & Henk, 1996). A nozzle-like organelle through which the slime is extruded seems to be a general feature involved in the motility in gliding cyanobacteria (Hoiczyk & Baumeister, 1998) and in a variety of phylogenetically unrelated organisms (Pate & Chang, 1979, Wolgemuth *et al.*, 2002, Robinson *et al.*, 2007). It is not known whether the nozzle-like organelle is also present in *Beggiatoa*, but since cyanobacteria and *Beggiatoa* share many structural genes (Mussmann *et al.*, 2007) it is likely that they glide by a similar mechanism. The propulsive force that drives the cells forward results from a combination of physicochemical factors and exopolymer production (Wolgemuth *et al.*, 2002). Enzymatic reactions are necessary to produce the exopolymeric slime and to ultimately energize motility. Enzyme kinetics are highly temperature dependent (Russell, 1990, Somero, 1995, Feller & Gerday, 1997, Nedwell, 1999) and therefore the speed of gliding motility is a function of temperature (Crozier & Stier, 1926).

Biological temperature dependent processes, such as growth, are generally described by cardinal temperatures. The optimum temperature (T_{opt}) is the temperature of the maximum rate, while the minimum temperature and the maximum temperature (T_{max}) are the lowest and highest temperatures, respectively, where activity is still detected. T_{opt} for growth is generally below T_{opt} for respiration or other energy generating activities. T_{opt} for activities other than growth is commonly far from optimal for the organisms which then are on the verge of thermal damage, as evident from the steep decline in activity just above T_{opt}.

The effect of temperature on the metabolism and growth of microorganisms in the environment has been studied extensively (Thamdrup & Fleischer, 1998, Knoblauch & Jørgensen, 1999, Rysgaard *et al.*, 2004). These studies show that active organisms are adapted to the *in situ* temperature regime. Due to slow growth of environmental organisms the adaptation does, however, not necessarily track seasonal temperature variations (Robador *et al.*, 2009). Temperature is an important characteristic of the

microbial environment and some motile organisms have even been shown to orient in a thermal gradient by thermotacic response (Maeda *et al.*, 1976, Paster & Ryu, 2008), but the regulation of microbial motility by temperature has widely been ignored. Few studies exist on the temperature dependence of motility of microorganisms and especially of gliding motility (Crozier & Federighi, 1924, Crozier & Stier, 1926, Halfen & Castenholz, 1971, Ridgway & Lewin, 1988). In our study we compare temperature dependent gliding motility of *Beggiatoa* filaments from arctic, temperate and tropical marine environments. The aim of this study was to assess how gliding speed changes with temperature, and how the motility of filaments from the different climatic zones are adapted to the *in situ* temperature ($T_{in\ situ}$).

Materials and Methods

All filament types used for this study had the typical morphology described for *Beggiatoa* (Teske & Nelson, 2006). They were colorless filamentous organisms with cylindrical cells and highly refracting sulfur globules in the cells (supporting information, Fig. S1A-C). All filaments moved by gliding and were visible to the naked eye.

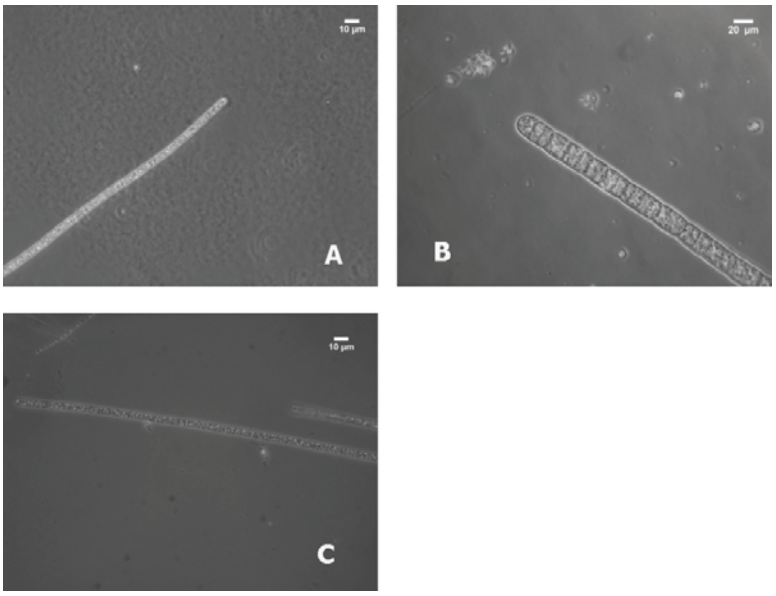

Figure S1: Light micrographs of (A) a tropical filament, (B) a filament from Aarhus Bay and (C) an arctic filament. 400fold magnification, phase contrast. Image S1A courtesy of Anja Kamp.

Tropical filaments

The tropical *Beggiatoa* used for this study belong to the microbial consortium associated with the black-band disease of corals in the Florida Keys, USA (Richardson, 1996). Water temperature at 15 m depth in the Florida Keys varies between 18 °C in winter and 32 °C in summer (Hudson & Anderson, 2007). The clonal culture originated from the laboratory of Douglas C. Nelson where it was isolated as follows (D. C. Nelson, personal communication): Single filaments of 6.3 µm diameter were isolated by allowing these to glide and spread on sterile agar plates and by repeated passages of single filaments to fresh agar plates (Nelson & Castenholz, 1981, Nelson *et al.*, 1982). After isolation the culture was maintained in culture tubes with opposing gradients of oxygen and sulfide at room temperature (Nelson & Jannasch, 1983). The culture was used for various ecophysiological studies (Kamp *et al.*, 2008).

Temperate filaments

Temperate sediment was sampled in Knebel Vig, (56° 12.9' N, 10°27.9' E), a cove at the northern part of Aarhus Bay, Denmark (Troelsen & Jørgensen, 1982). Box cores were taken in April 2006 from 11 m water depth at 5.4 °C water temperature and transported to the laboratory. The sediment was carefully placed in a laboratory flume of 3 m length and 0.3 m width without disturbing the layering and kept in darkness. Seawater of *in situ* salinity (25 ‰), oxygen concentration (50 % air saturation) and temperature (13ºC) circulated in the flume at a flow velocity of 1-2 cm s^{-1}. For the cold acclimatization of these temperate *Beggiatoa* an intact box core from Knebel Vig was stored at 4 °C in the dark, covered with seawater. It was inoculated with tufts of filaments from the flume. The filaments were allowed to acclimatize and grow for two months before the experiment. The bottom water temperature in Aarhus Bay during the year of sampling ranged from 3 °C in February to 16 °C in August (Dale *et al.*, 2008). Cultivation of filaments at 13 °C represented the summer conditions while cultivation at 4 °C adapted them to winter conditions. The *Beggiatoa* filament widths ranged from 5 to 35 µm. For gliding speed measurements 20-25 µm wide filaments were used.

Arctic filaments

Arctic sediment was collected in August 2008 in the small lagoon of Ymerbukta (78°16.8' N, 14°03' E) on the west coast of Spitsbergen, one of the main islands of the Svalbard archipelago. The temperature of both water and surface sediment was 6.5 °C. Sediment cores of 80 mm diameter were sampled by hand from spots with partial white *Beggiatoa* coverage from 30 cm water depth. The cores were stored at 2-5 °C for 4 days before the measurement of gliding speed. The filaments were of two size classes of 2 µm and 8-10 µm in diameter. The latter were used for gliding speed measurements.

Measurements of gliding speed

Gliding speed was observed in a custom-made, thermostat-controlled polycarbonate chamber (Fig. 1) placed under a light microscope (Zeiss Axioskop). The chamber had two compartments separated by a glass slide. The upper compartment was made by a metal spacer cut out in the center. It had a capacity of 2 ml and was filled with sea water and *Beggiatoa* filaments. Water of the desired temperature mixed with antifreeze fluid circulated through the lower compartment. Before each experiment the water temperature in the upper compartment was measured at each temperature increment used in the experiment by inserting a thermocouple temperature sensor (NiCr-Ni, diameter 1mm, Thermocoax, France). The small volume of the upper compartment and the continuous flushing of the lower compartment assured fast heat exchange between the compartments.

For the experiments the temperature was changed in 2-3 °C increments, starting at 20 °C for filaments from the tropics and from Aarhus Bay and 2-4 °C for arctic and cold acclimatized filaments from Aarhus Bay. The gliding speed of the filaments at each temperature (n= 1-64) was recorded either by a) simultaneous observation of the filaments through the microscope, timing of the speed with a stop watch, and measuring the gliding distance with a calibrated ocular micrometer or b) analyzing sequences of images taken by an attached digital camera (Canon Power Shot A 620) with an image analysis software (Image J, National Institutes of Health, USA). Measurements proceeded until the filaments stopped moving at each end of their temperature range or until the water in the chamber froze. After the filaments stopped, the temperature was lowered

again from the high end or raised again from the low end to test whether the immobilization was reversible.

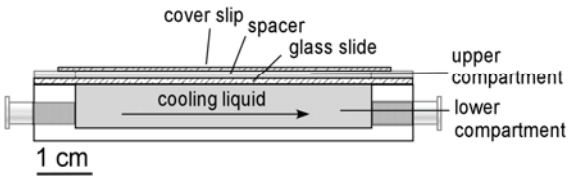

Figure 1: Longitudinal section of the microscope chamber which provided defined temperatures during gliding speed recordings with *Beggiatoa*.

Arrhenius plots and Q_{10}

Activation energies (E_a) and temperature coefficients (Q_{10}) serve as indicators for the temperature response of biological processes. The Q_{10} value is here the factor by which the gliding speed increases when the temperature is raised by 10 °C. The activation energy can be calculated from the slope of the logarithmic form of the Arrhenius function

$\ln v = \ln A - E_a/RT$

where v is the process rate or the speed, A is a constant, E_a is the activation energy, R the gas constant (8.31 kJ mol^{-1}), and T the absolute temperature (K). For each experiment Arrhenius plots of ln(v) as a function of T^{-1} (K) were calculated. The slope of each Arrhenius plot (E_a/R) was determined from which E_a was calculated. The linear range of the slope was found by first calculating a linear regression and then omitting data points from the cold and warm extremes until the fit of the line had a R^2 value of ≥ 0.93.

Q_{10} values were calculated by the following equation:

$$Q_{10} = e^{E_a(T_2-T_1)/RT_1T_2}$$

where T_1 is the lower temperature and T_2 is T_1+10. Hence, in the linear range Q_{10} values change slightly according to the temperature interval for which they were calculated, decreasing at high temperature intervals.

Results

Gliding speed

There was no correlation between gliding speed and filament diameter. The wide temperate *Beggiatoa* glided at a maximum speed of 5.6 ± 0.6 and 6.0 ± 0.6 µm s^{-1} for filaments grown at 4 °C and 13 °C, respectively (Fig. 2B, C). The narrower arctic filaments glided much slower at 1.6 ± 0.2 µm s^{-1} at T_{opt} (Fig. 2D) but the yet narrower tropical filaments at 6.1 ± 0.6 µm s^{-1} (Fig. 2A) were as fast as the wide temperate filaments.

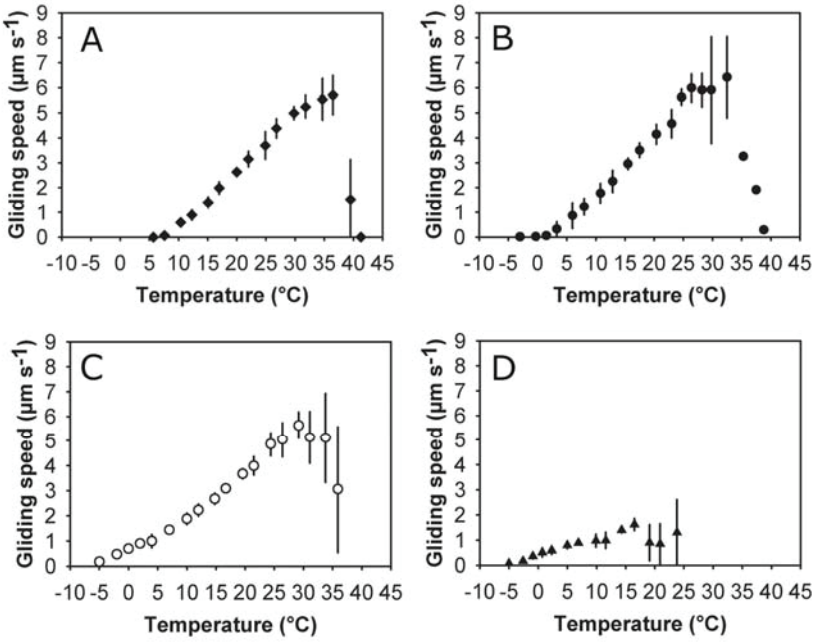

Figure 2: Gliding speeds of *Beggiatoa* at different temperatures. Tropical filaments from the black band coral disease grown at 20°C (A), from a temperate environment, Aarhus Bay, grown at 13°C (B), cold acclimatized filaments from Aarhus Bay grown at 4°C (C), and arctic filaments from Ymerbukta, Svalbard, growing at 6ºC (D). The vertical bars show standard deviations.

At 5 °C and below, the arctic *Beggiatoa* glided almost as fast as the cold acclimatized temperate filaments despite the lower maximal gliding speed at T_{opt} (Fig. S2). Hence in the temperature range from 5 °C to -5 °C gliding speed relative to the maximal speed was higher in arctic filaments than in cold acclimatized temperate

filaments. Below 6 °C, arctic filaments glided faster than the wider temperate filaments grown at 13 °C (Fig. S2).

Characteristics of the gliding speed– temperature relation

The shapes of the curves of the relation between temperature and gliding speed were similar for all filaments (Fig. 2A-D). They showed an increase in gliding speed with temperature until a maximum speed at T_{opt} and just above T_{opt} a sharp decline to T_{max}. This temperature dependent response is familiar from other biologically catalyzed processes where T_{opt} is usually close to the T_{max} at which all activity comes to a halt.

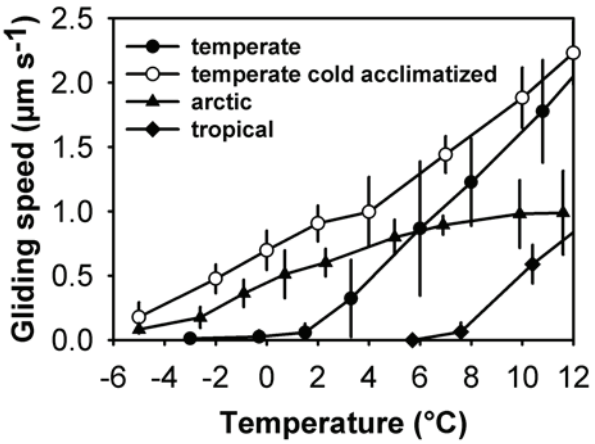

Figure S2: Gliding speeds of *Beggiatoa* filaments from all climatic regions plotted with expanded scales between -5 °C and 15 °C.

The activity range occurred at the relatively highest temperatures for tropical filaments, intermediate temperatures for temperate filaments, and coldest temperatures for arctic filaments. Arctic filaments had the narrowest temperature range for gliding while temperate Aarhus Bay filaments had the widest (shaded bars in Fig. 3). Cold acclimatization of Aarhus Bay filaments extended their temperature range of gliding by 7 °C towards lower temperatures relative to the non-acclimatized filaments, without affecting the T_{opt} or the T_{max}.

The T_{opt} correlated with the climatic origin of the filaments. Tropical filaments exhibited the highest T_{opt} of 37 °C, temperate filaments an intermediate T_{opt} of 30 °C, and arctic filaments a low T_{opt} of 17 °C (Fig. 2, open arrows in Fig. 3). According to the

classification into thermal groups, which is actually based on growth (Morita, 1975), tropical and temperate filaments were mesophilic, cold-acclimatized temperate filaments showed a psychrotolerant response and arctic filaments were psychrophilic. In filaments from all tested locations the T_{opt} for gliding was above the $T_{in\ situ}$. Yet, gliding motility of the filaments from the respective origins was well adjusted to the prevailing $T_{in\ situ}$ despite the slower gliding speed at $T_{in\ situ}$ than at T_{opt}. At their $T_{in\ situ}$ arctic filaments reached 55 % of the gliding speed observed at T_{opt}. At the minimum temperature for gliding at -5 °C they still maintained 11 % of the gliding speed at T_{opt}. For temperate filaments grown at 13 °C and 4 °C gliding speed at $T_{in\ situ}$ was 35 % and 17 % of the T_{opt}, respectively. Tropical filaments glided at $T_{in\ situ}$ with 46 % of the speed at the T_{opt}. Similar observations have been made for temperature dependent metabolic rates. Metabolic rates and growth rates at $T_{in\ situ}$ were commonly 10-40 % of those measured at the T_{opt} (Arnosti *et al.*, 1998, Knoblauch & Jørgensen, 1999, Rysgaard *et al.*, 2004).

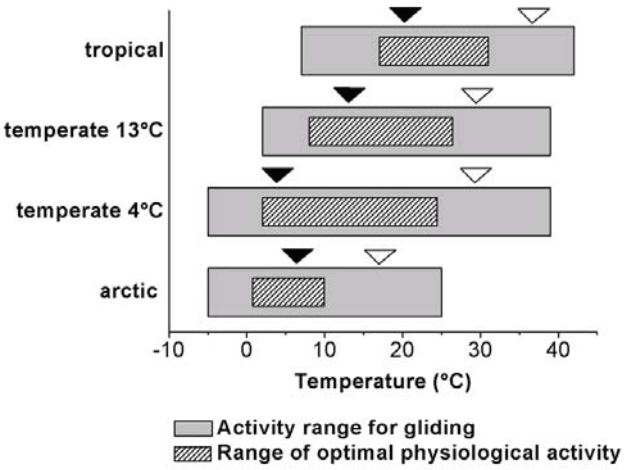

Figure 3: Activity ranges of gliding motility in *Beggiatoa* filaments from different climatic origins. Filled arrows indicate the temperature, "$T_{in\ situ}$", at which the Beggiatoa were growing, open arrows indicate T_{opt}. The shaded bars indicate the maximal range of gliding motility, the hatched bars indicate the range of optimal physiological activity. $T_{in\ situ}$ is always within the range of optimal physiological activity and T_{opt} is always outside of it.

Temperature range of physiological adaptation

The range of optimal physiological activity is narrower than the overall range of gliding activity. To illustrate this we calculated Arrhenius plots for each temperature-

speed curve (Figure 4A-D). In the Arrhenius plots an exponential dependence in the temperature-speed curves results in a linear relationship. E_a can be calculated from this exponential dependence. An exponential variation of a reaction rate with 1/T is commonly observed in enzymatically catalyzed reactions (Arrhenius, 1908). At the extreme low and high ends of the temperature range the speed decrease deviates from linearity. Provided that enzymatic reactions control the decline in gliding speed with decreasing temperatures, the temperature range with constant and low E_a should be the temperature range to which the filaments are physiologically optimized (hatched bars in Fig. 3).

Corresponding to the overall activity range of gliding, the temperature range in which the Arrhenius plot was linear was narrowest for the arctic filaments (Fig 3, 4D), indicating that they were physiologically optimized to a narrow range around $T_{in\ situ}$. Slightly broader was the linear range for tropical filaments (Fig 3, 4A). Aarhus Bay filaments had the broadest range of optimal physiological activity (Fig. 3, 4B, C). Their optimal range was further shifted downward by cold adaptation. The range of optimal physiological activity always included $T_{in\ situ}$, but never T_{opt} (compare open triangles and hatched bars in Fig. 3). The $T_{in\ situ}$ that arctic filaments experience during winter is not known, but it is definitely below -5 °C. During the freezing period in winter they might thus survive in an immobilized state.

Response to extreme temperatures and freezing

The reduction in speed at the low end of the temperature range occurred simultaneously in all individual filaments from each climatic origin as evident from the small error bars at the low end of the temperature range. The speed decrease with decreasing temperature at the low end of the temperature range was fully reversible in these experiments (Fig. S3). The arctic and the cold acclimatized *Beggiatoa* community from Aarhus Bay were even unaffected by transient freezing of the surrounding seawater and still glided at -5 °C in unfrozen water (Fig. 2C, D). Upon sudden freezing of the super-cooled water at -5 °C the filaments immediately stopped gliding. Microscopic observation did not reveal whether also the cytosol of the cells was frozen. After thawing, the filaments did not show visible damage under the light microscope and they resumed

gliding at their temperature dependent speed unaffected by the previous freezing (Fig. S3A, B).

At the high end of the temperature range, above T_{opt}, an increasing number of cells in the individual filaments appeared damaged when observed under the microscope. Increasingly large error bars illustrate a variation in lethal temperature between filaments. The speed reduction above T_{opt} was irreversible in all filaments tested, indicating permanent cell damage.

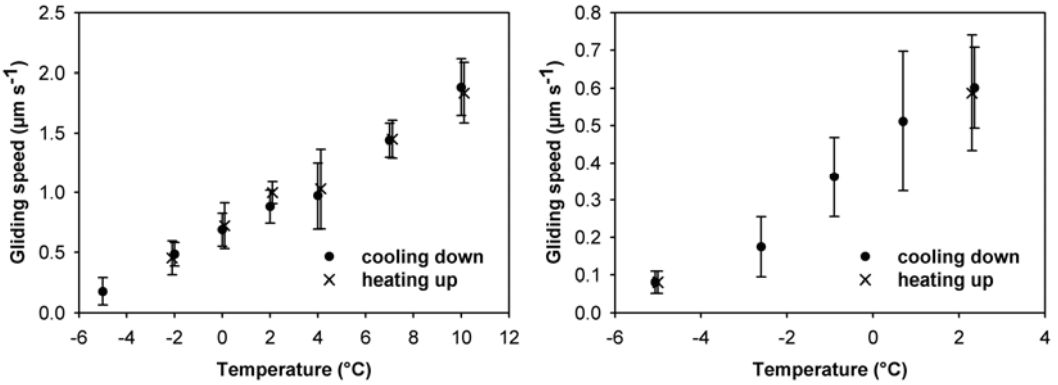

Figure S3: Reversible temperature control of gliding speed in (A) filaments from Aarhus Bay cultivated at 4 °C and (B) arctic filaments. The water in the chamber with the filaments was cooled to -5 °C in increments until freezing. Filled circles indicate the gliding speeds while decreasing the temperature; crosses indicate the gliding speeds while increasing the temperature again.

Discussion

Temperature response and dependence of gliding motility

To evaluate the temperature response of the filaments from the different climatic origins we analyzed the temperature range of optimal physiological adaptation. The steepness of the slope of the Arrhenius plots, as calculated from this range, is an indicator for the adaptedness to this temperature (Fig. 4). All filaments roughly doubled their gliding speed when increasing the temperature by 10 °C (Table 1). The E_a value of 58 kJ mol^{-1} for Aarhus Bay filaments adapted to summer conditions was slightly higher than for tropical, cold acclimatized Aarhus Bay filaments and arctic filaments (49, 50 and 46 kJ

mol $^{-1}$). The E_a value of tropical filaments was comparable to that of cold acclimatized Aarhus Bay filaments.

Our results were similar to the Q_{10} and E_a values of enzymatic processes in other bacteria from cold climatic regions (Thamdrup & Fleischer, 1998, Knoblauch & Jørgensen, 1999, Rysgaard *et al.*, 2004). Enzymatic processes from cold environments are often less temperature dependent than those from temperate or warm environments in

Table 1: Upper part: Temperature responses, *in situ* and optimum temperatures for filaments from the different climatic origins and the corresponding activation energies and Q_{10} values. Lower part: Activation energies and Q_{10} values of gliding motilities and respiration of gliding bacteria. Temperature ranges for which Q_{10} values were calculated are in parentheses.

Origin of filaments	T response of gliding speed	$T_{in\ situ}$ (°C)	T_{opt} (°C)	E_a (kJ mol^{-1})	Q_{10}	source
Tropical	Mesophilic	20	37	49	2.1 (19-29 °C)	this study
Temperate	Mesophilic	13	30	58	2.3 (12-22 °C)	this study
Temperate (cold acclimatized)	Mesophilic	4	30	50	2.1 (8-18 °C)	this study
Arctic	Psychrotolerant	6.5	17	46	2.0 (0-10 °C)	this study
Gliding motility of *Beggiatoa alba*	—	—	—	35.2	—	Crozier and Stier, 1926
Gliding motility of *Oscillatoria*	—	—	—	38.7	—	Crozier and Federighi, 1924
Gliding motility in *Oscillatoria princeps*	—	30-40	42	144[a)]	—	Halfen and Castenholz, 1971
Gliding motility of *Flexibacter polymorphus*	—	—	35	61.13	2.06 (15-35 °C)	Ridgway and Lewin, 1988
Respiration of *Flexibacter polymorphus*	—	—	40	58.62	2.64 (15-35 °C)	Ridgway and Lewin, 1988

[a)] E_a was calculated from the published plot.

that the reaction rates are not affected as strongly by changes in temperature (Somero, 1995, Feller & Gerday, 2003). This results from a lower reaction enthalpy $\Delta H^{\#}$ and thus low E_a and Q_{10} values in cold adapted enzymes (Low *et al.*, 1973, Lonhienne *et al.*, 2000). Gliding motility in *Beggiatoa alba* and in an *Oscillatoria* strain also had low E_a of 35 and 39 kJ mol^{-1}, respectively (Crozier & Federighi, 1924, Crozier & Stier, 1926, Table 1). Yet,

it cannot be generalized that gliding motility is an activity of low temperature dependence. The E_a estimated for gliding motility in *Oscillatoria pinceps* from a hot spring (Halfen & Castenholz, 1971) is at 144 kJ mol^{-1} much higher than in the other organisms, which is probably due to the constantly high temperature of the spring water.

A low E_a for gliding motility was also found in *Flexibacter polymorphus* (Ridgway & Lewin, 1988). The E_a for respiration is very similar to that of gliding motility (Table 1). The correlation between E_a values for gliding motility and for metabolic rates that energize motility point towards a direct relation between gliding motility and energy metabolism. In this case the temperature controlled gliding speed decrease at the cold end of the temperature range would be related to increasingly slow kinetics of enzymatically catalyzed metabolic processes. This argument is further supported by the temperature dependent gliding speed curves that had shapes similar to those often observed in temperature-metabolism curves. E_a values were constant over a wide temperature range as apparent from the Arrhenius plots (Fig. 4A-D). Similar observations have been made for *Beggiatoa alba* and *Oscillatoria* (Crozier & Federighi, 1924, Crozier & Stier, 1926).

Both these findings suggest that the speed of gliding depends on the rate of chemical (enzymatic) reactions rather than on physical factors such as the viscosity of the ambient medium or on cell membrane fluidity and permeability of *Beggiatoa*. Additionally, the temperature response at the extreme ends of the temperature range points to an enzymaticspeed control. Highly reversible cold denaturation, as observed for some globular proteins (Privalov, 1989), could have played a role in the reversibility of gliding speed of the filaments. Impaired protein and membrane integrity due to high temperatures was probably the main reason why filaments stopped gliding at the high end of the temperature range. Thermally induced cell lysis cannot be stopped (Morita, 1975) which explains that the filaments did not resume motility when cooling down again after having exceeded the T_{max}.

Our hypothesis of an enzymatic control of gliding speed might be tested further by observation of filaments in media of different viscosity but at the same temperature. Gliding speed in *Oscillatoria princes* and *Flexibacter polymorphus* was found to decrease with increasing viscosity of the medium but showed a linear decrease instead of a curve

with a skewed slope towards the maximal gliding speed as found for temperature dependent gliding speed (Halfen & Castenholz, 1971). We would expect a similar result for viscosity-dependent gliding speed in *Beggiatoa* because of the structural similarities of the motility apparatus of filamentous cyanobacteria and *Beggiatoa* (Larkin & Strohl, 1983, Larkin & Henk, 1996, Mussmann *et al.*, 2007).

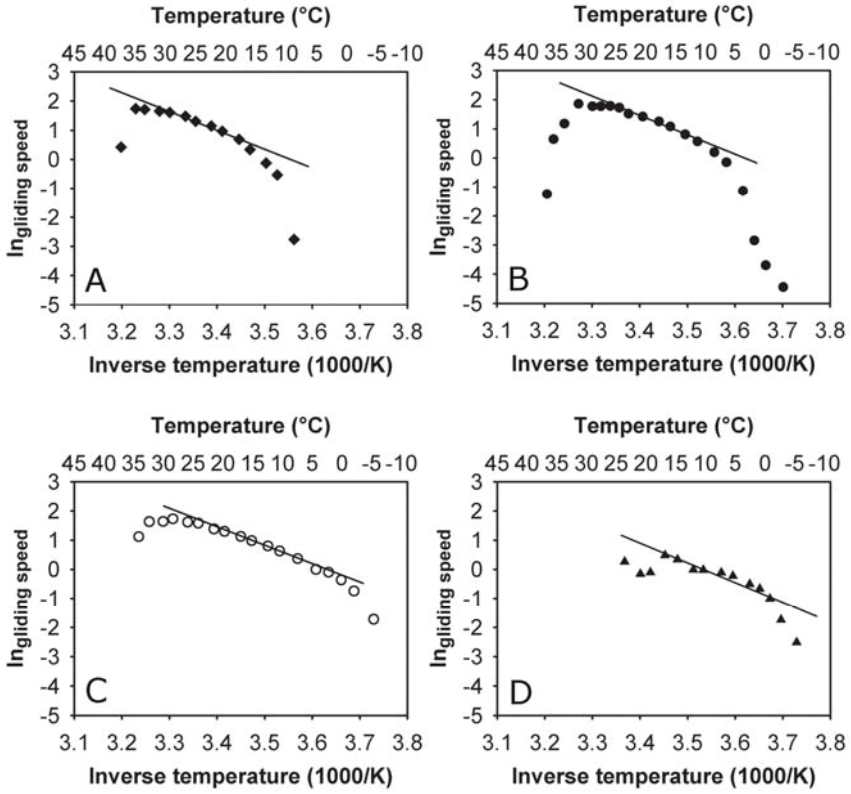

Figure 4: Arrhenius plots of graphs in Figure 2 that correspond to the *Beggiatoa* types A-D.

Adaptation potential in Beggiatoa from the temperate zone

After an acclimatization period of two months the community of *Beggiatoa* from Aarhus Bay had expanded the low temperature range of gliding from +1ºC to less than -5 ºC, i.e. to below the freezing point of seawater (Fig. 3). The range of optimal physiological activity was also extended towards lower temperatures so that the winter temperature of 3 °C was within the range of optimal physiological activity (longer linear range in the corresponding Arrhenius plot as compared to filaments from Aarhus Bay that

were not cold acclimatized, Fig. 4B, C). T_{opt} and T_{max} were unaffected by the cold acclimatization (Fig. 2C, 3)

The shift towards a more cold adapted population could be due to either a) a physiological acclimatization in individual filaments or b) an undetected shift in the community composition from mesophilic to psychrotolerant forms (Sieburth, 1967, Thamdrup & Fleischer, 1998). There is a continuous range of filament diameters in natural *Beggiatoa* communities (Jørgensen, 1977), and each size class can contain multiple taxonomic units based on 16S rRNA phylogeny (Mussmann *et al.*, 2003). Thus, it is possible that cold and warm adapted species that cannot be distinguished in the microscope could coexist in temperate sediments. If the cold acclimatization was due to a gradual shift in species composition rather than to a physiological adaptation we would expect to see the gliding pattern of both the mesophilic and the psychrotolerant individuals at low temperature in our experiments. However, all filaments tested showed the same shift in temperature dependence as evident by the low statistical variance of the data. Additionally, it appears unlikely that a different community would have the same T_{opt} and T_{max} for gliding. Phylogenetic analyses would, however, be required clear this question.

Temperature response of a mixed community versus a clonal culture

The arctic and the temperate filaments were from mixed samples with a variety of filament diameters and hence were presumably phylogenetically more diverse than the clonal tropical culture. Tropical filaments had the most sharply defined T_{opt} and showed less variance in gliding speed at the high end of the temperature range than temperate and arctic filaments (Fig. 2A). The T_{opt} of the tropical filaments was closest to the T_{max} for gliding of all tested filament types. We attribute this to the clonal nature of the tropical strain. However, the homogeneity of the speed versus temperature curves of the temperate and arctic community, expressed in small error bars, comes close to that of the tropical strain. Thus, the temperature response of filaments of one size class from a natural community is surprisingly close to that of a single strain.

Conclusions

The motility of *Beggiatoa* communities is adapted to the prevailing temperatures in their habitat with distinct temperature responses for arctic, temperate and tropical populations. Temperate populations that experience high seasonal temperature fluctuations are able to adapt to the range encountered throughout the year. Our results suggest that temperature dependent gliding speed is mainly enzymatically regulated. Therefore, *Beggiatoa* filaments can unequivocally be placed into thermal groups without the need to test the growth or metabolic response to temperature. Our results show that a single size class from a specific habitat shows an equally homogeneous behavior towards temperature change as a clonal culture.

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2.2. Manuscript 2:

Filamentous sulfur bacteria, *Beggiatoa* spp., in arctic marine sediments (Svalbard, 79º N)

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Abstract

Fjord sediments on the west coast of the arctic archipelago Svalbard were surveyed to understand whether large filamentous sulfur bacteria of the genus *Beggiatoa* thrive at seawater temperatures permanently near freezing. Two sediments had abundant populations of *Beggiatoa* while at half of the other sites sporadic occurrences were observed. We conclude that *Beggiatoa*, although previously unnoticed, occur widespread in arctic fjord sediments. *Beggiatoa* ranged in diameter from 2 to 52 µm and, by those tested, stored nitrate in vacuoles at up to 260 mM. The 16S rRNA gene sequence of a 20 µm wide filament is closely associated with other large, marine, nitrate-storing *Beggiatoa*. The *Beggiatoa* mostly occurred in the upper 2-5 cm of oxidized surface sediment between oxygen and the deeper sulfidic zone. In spite of very low or undetectable sulfide concentration, sulfate reduction provided abundant H_2S in this zone. The total living biomass of *Beggiatoa* filaments at one study site varied over three years between 1.13 and 3.36 g m^{-2}. Due to their large size, *Beggiatoa* accounted for up to 15% of the total prokaryotic biomass, even though filament counts at this site were rather low comprising <1/10.000 of the bacterial numbers on a cell basis.

Introduction

Filamentous, sulfide-oxidizing bacteria of the genus *Beggiatoa* occur widespread in marine surface sediments that have a sufficiently high production of sulfide from bacterial sulfate reduction. Such sediments occur in the eutrophic coastal zone and in highly productive upwelling systems along continental margins, often associated with low oxygen concentration in the bottom water. *Beggiatoa* spp. also occur as benthic mats in many other marine environments where sulfide is introduced by advective flow, e.g. at cold seeps or near hydrothermal vents (Jørgensen & Boetius, 2007).

Beggiatoa spp. are most often noticed when growing as white mats on the seafloor, yet the most widespread occurrence is probably as scattered filaments hidden within the uppermost few cm of the sediment. This more diffuse distribution requires a designated approach to find and quantify the organisms, although they are conspicuous as

they have the size of micro- or meiofauna. It is a property unique for these large, multicellular bacteria that they can be identified and counted by simple light microscopy. By that approach it has been demonstrated that *Beggiatoa* generally occur in the 'suboxic zone' between the few mm thick oxic zone at the sediment surface and the diffusion front of sulfide starting several cm below (Jørgensen, 1977; Mußmann *et al.,* 2003; Jørgensen & Nelson, 2004; Preisler *et al.*, 2007).

Beggiatoa display tactic responses that enable them to avoid both increasing oxygen and increasing sulfide concentration (Møller *et al.,* 1985; Preisler *et al.,* 2007). The microaerophilic organisms only accumulate at the sediment surface when the population density is sufficiently high to create a steep oxygen gradient in the diffusive boundary layer, thereby ensuring low oxygen concentration at the exposed mat surface (Jørgensen & Revsbech, 1983). It was proposed that a prerequisite for the sub-surface occurrence of *Beggiatoa* in sediments is the presence of a distinct sulfide zone which prevents filaments from getting lost at depth without a chemotactic clue (Preisler *et al.,* 2007).

The predominant *Beggiatoa* populations in marine sediments are filaments with diameters of several µm to several tens of µm. Filaments of these size groups were found to accumulate nitrate in internal vacuoles, often up to concentrations of several hundred mM (McHatton *et al.,* 1996; Ahmad *et al.,* 1999; Muβmann *et al.,* 2003; Preisler *et al.,* 2007). *Beggiatoa* use nitrate as an alternative electron acceptor when living in the suboxic zone. Where studied, nitrate is not denitrified but is rather reduced to ammonium (McHatton, 1998). Internal nitrate accumulation and reduction to ammonium is a property shared with other large, marine sulfur bacteria of the genera *Thioploca* with whom *Beggiatoa* form a monophyletic group (Otte *et al*., 1999; Jørgensen & Nelson, 2004; Jørgensen *et al.,* 2005b).

In coastal environments *Beggiatoa* have so far mostly been observed under temperate conditions where the organic sedimentation is high and where experimental measurements of sulfate reduction demonstrate high sulfide production rates. In these moderately warm sediments *Beggiatoa* have gliding motility and express highly developed phobic responses to oxygen and sulfide. At temperatures above 5-10ºC the filaments glide at a velocity of 2-4 µm s^{-1} and effectively migrate within their preferred

environmental zone (R. Dunker, in press). Coastal sediments of the Arctic and Antarctic have temperatures permanently near 0ºC which may require a psychrophilic adaptation of the indigenous *Beggiatoa* communities, not only of their metabolic rate but also of their gliding motility, in order to metabolize and orient effectively. Such a psychrophilic adaptation has been demonstrated for arctic communities of sulfate reducing bacteria (Knoblauch *et al.,* 1999; Knoblauch & Jørgensen, 1999) but was not yet described for the large, sulfide oxidizing bacteria such as *Beggiatoa*. The present study therefore aimed to explore the occurrence of *Beggiatoa* spp. in arctic sediments with respect to their distribution, size spectrum and biomass and to understand whether these large bacteria are equally well adapted to permanently cold environments as to temperate environments. Concurrent studies of the temperature regulation of their metabolic rate, expressed through their gliding velocity, will be published elsewhere (R. Dunker *et al*., in press).

Materials and Methods

Field sampling and sediment properties

Sediment was collected during summer in 2003, 2005 and 2008 from different fjords on the west coast of Spitsbergen, the main island of the archipelago Svalbard bounding the North Atlantic and the Arctic Ocean. The location and relevant characteristics of these sites are given in Fig. 1 and Table 1. All sites were located between 78° and 80° northern latitude and, apart from a small lagoon, had temperatures permanently near 0ºC. In several of the 70-200 m deep Spitsbergen fjords, cores of 15 cm diameter and 20-30 cm depth were collected with a HAPS corer (Kanneworff & Nicolaisen, 1973) during cruises with MS FARM (Longyearbyen). Sub-cores were taken immediately on board using 26 mm or 36 mm inner diameter acrylic tubes. The cores were kept submerged in seawater at ca 0°C during the following 1-3 days on the ship. Back in the laboratory, cores were transferred to an incubator at 0.5 ±0.5°C until further sub-sampling within 1-2 days.

Porosity, density, and ignition loss of the sediments were determined at 2 cm depth intervals. Core segments of defined volume were transferred to pre-weighed crucibles and the sediment weight was determined before and after drying at 105°C

overnight. Porosity was determined from the water loss per volume of sediment upon drying. Porosity data were used for the calculation of sulfate reduction rates.

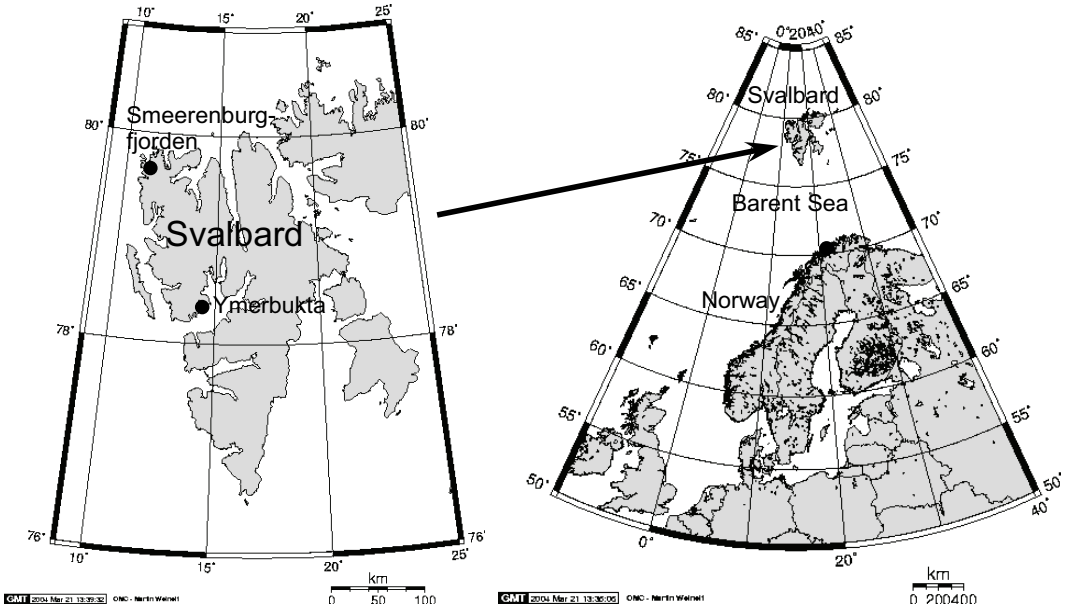

Figure 1: Map of Svalbard with sampling sites.

Biomass determination

Sediment sub-cores were sectioned into 0.5 cm depth increments. Subsamples of 20-30 mg were taken with a clean mini-spatula, transferred to a tarred microscope slide and weighed to ±0.2 mg accuracy. The sediment was immediately wetted with drops of seawater, mechanically suspended and smeared over the surface of the slide, and finally covered with a large cover slip. *Beggiatoa* filaments were quantified by direct light microscopy according to Jørgensen (1977) by scanning the entire slide systematically using the 10 × objective. The length (10 ×) and diameter (40 ×) of all detected filaments were measured by using a calibrated ocular micrometer. Only motile, colorless,

Table 1. Sampling sites visited during field campaigns in 2003, 2005 and 2008. A general description of the sediment and an indication for the occurrence of *Beggiatoa* are provided. Stations are all located in fjords on the west and north coast of the island Spitsbergen. Water depths and sediment temperature at the time of sampling are indicated. Areal rates of sulfate reduction (SRR) were integrated over the top 0-15 cm of the sediment and represent the mean of three cores. Two numbers are given when measurements were conducted during different years.
*Data from Sawicka *et al*., in press, measured in 2007

Station	Location	Coordinates		Depth (m)	Temp. (°C)	SRR (mmol m^{-2} d^{-1})	Description
J	Smeerenburgfjorden	79°42'815N	011°05'189E	214	0.4°C	2.71	Light gray until 2-4 cm, dark gray below, silt and very fine sand, rich in macrofauna Many *Beggiatoa* of different diameters
DA	Ymerbukta	78°16'61N	014°02'69E	0.3	6.5°C	2.04*	Light brown until 2-4 cm, silt with fine sand of 30-80 µm, gray to black fine sand below, patches of *Beggiatoa* or cyanobacteria overlying black sediment Many *Beggiatoa* of mostly 5 and 12 µm
A	Adventfjorden	78°15'44N	015°30'90E	69	0.4°	4.48	Gray-brown until 5-6 cm, mottled gray below; silt and fine sand of 30-100 µm diameter, not very sulfidic No *Beggiatoa*
O	Borebukta	78°19'557N	014°27'760E	94	1.4°	2.50/1.59	Gray-brown until 3 cm, gray below, silt and very fine sand of <30 µm One *Beggiatoa* filament of 6 µm on top
BN	Ymerbukta S	78°15'352N	013°58'417E	104	0.3°	1.99	Gray-brown until 4 cm, gradually into gray below, silt-clay, no sand, pelletized Three *Beggiatoa* of 15-20 µm in top 2 cm
E	St. Jonsfjorden W	78°32'599N	012°17'909E	168	1.6°	1.87	0-3 cm gray-brown, gray below, silt and fine sand One *Beggiatoa* filament of 4 µm at 2 cm
F	Kongsfjorden	78°55'234N	012°13'912E	114	1.8°C	1.33/1.11	Silty sediment colored red from hematite Few scattered *Beggiatoa* of 7 and 20 µm
S	Blomstrandhalvoya N	78°59'929N	011°59'213E	77	0.0°	2.40	Red-gray, with depth dark gray mottled in red, silt and fine sand of 50-150 µm Few *Beggiatoa* of 4 and 7 µm in top 2 cm
R	Blomstrandhalvoya S	78°57'424N	012°09'873E	90	0.0°	1.32	Red, with depth gray mottled in red, silt and very fine sand of <30 µm No *Beggiatoa*
K	Raudfjorden	79°46'144N	012°04'433E	154	-0.5°	2.09	Gray-brown to 6 cm, mottled gray below, silt No *Beggiatoa*
I	Magdalenefjorden	79°34'052N	011°03'597E	124	-1.0°	1.87	Gray-brown to 5 cm, mottled gray below, silt Three *Beggiatoa* of 18-20 µm

multicellular filaments with distinct sulfur inclusions were counted as *Beggiatoa*. Some *Beggiatoa*, in particular those of smaller diameters, may thereby have been missed and the counts thus represent minimum values.

Beggiatoa filaments were also quantified by a modified approach whereby a weighed sediment sample of ca 0.5 g was added to 10 ml seawater and suspended. A 0.35 g subsample of suspension was then smeared on a glass slide and the filaments counted. This procedure improved the statistical representation of the bacterial density in case these had a patchy distribution. Determination of length and diameter was also done by digital photography using imaging software (ImageTool, The University of Texas Health Science Center, San Antonio, USA),

For quantification, diameters were grouped in 2-3 μm increments. Based on the mean dimensions of each diameter group, *Beggiatoa* biomass was calculated using the cylinder volumes of the filaments and assuming a boyant density of 1 g cm^{-3}. The boyant density of bacteria is generally 1.1-1.2 g cm^{-3} (e.g. Tamir & Gilvarg, 1966; Bakken, 1985) but *Beggiatoa* filaments include also liquid vacuoles with a density of 1.0. The volume fraction of vacuoles in the cells increases with filament diameter and has not been determined. It should therefore be noted that the term "biomass" is equivalent to biovolume and includes vacuole volume. All population data were recalculated from gram wet weight to volume (cm^3) of sediment based on determined sediment densities. Adding up the data over the entire depth interval yielded the population size as fresh biomass per sediment area (g m^{-2}).

Phylogenetic analyses

Single filaments intended for phylogenetic analyses were stored separately at -20°C, preserved in 1 × TE buffer (Promega Corporation, Madison, WI). Prior to PCR amplification, each filament was separated from the TE buffer by centrifugation for 3 min at 5000 × *g* and dissolving in 5 μL of PCR-grade water (Sigma-Aldrich Biochemie GmbH, Hamburg, Germany). The complete volume of 5 μL was then used as template in the following amplification reaction. Universal bacterial primers GM3F (5'-AGAGTTTGATCMTGGC-3'; Muyzer *et al.*, 1995) and GM4R (5'-TACCTTGTTACGACTT-3'; Muyzer *et al.*, 1995) were used for amplification of nearly

full length 16S rRNA gene sequences. Amplification reactions were set up as follows: 1 × MasterTaq Buffer with 1.5 mM Mg^{2+}, 0.3 mg mL^{-1} BSA (Sigma-Aldrich Biochemie GmbH), 250 µM of each dNTP (Roche, Mannheim, Germany), 0.5 µM of each primer (Biomers, Ulm, Germany) and 0.025 U μL^{-1} MasterTaq (5Prime, Hamburg, Germany) in a total volume of 50 µL. After an initial denaturation for 15 min at 95°C, 30 cycles of 95°C/1 min, 42°C/1 min and 72°C/3 min were performed before a final elongation step at 72°C for 10 min.

PCR products were purified using the QIAquick PCR Purification Kit (Qiagen, Hilden, Germany), ligated into pGEM-T Easy vector (Promega Corporation) and transformed into One Shot TOP10 Chemically Competent *E. coli* (Invitrogen Corporation, Karlsruhe, Germany) according to the manufacturer's instructions. Three representative clones were selected for plasmid preparation (Montage Plasmid $Miniprep_{HTS}$ Kit; Millipore GmbH, Schwalbach, Germany). Purified plasmids were subjected to *Taq* cycle sequencing with an ABI Prism 3130x Genetic Analyzer (Applied Biosystems, Foster City, CA).

Partial sequences were assembled with Sequencher 4.6 software (Gene Codes Corporation, Ann Arbor, MI) and manually checked. Examination for chimeric signals was done by using the Pintail program (Ashelford *et al.,* 2005) with nearest neighbors obtained with the SILVA-based Webaligner (http://www.arb-silva.de/aligner/; Pruesse *et al.*, 2007). No genuine chimeric signals were detected. Three sequences obtained from the same filament width were nearly identical (similarity values of 99.5-99.7%). Finally, clone S3678 was chosen for further phylogenetic analyses.

A phylogenetic tree was constructed with the neighbor joining and maximum likelihood (RAxML) algorithm, as included in the ARB software package (Ludwig *et al.*, 2004). Initial calculations were conducted with nearly full length sequences (≥ 1200 bp) and by applying different filters. Partial sequences L41043, L40999 and AF129012 were subsequently inserted by applying parsimony criteria and without allowing changes in the overall tree topology. Deltaproteobacterial sequences were used as outgroup. A consensus tree based on the different reconstruction approaches was built, wherein unstable branching orders were visualized by multifurcation.

The sequence of the uncultured *Beggiatoa* sp. clone S3678 from Smeerenburgfjorden (Station J) has been submitted to the EMBL database under accession no. FN561862.

Chemical measurements

Sediment sub-cores were sectioned in 1 cm depth increments and pore water was obtained by squeezing through 0.45 µm pore size membrane filters under N_2 according to Reeburgh (1967). Sulfate was measured by non-suppressed ion chromatography (Waters IC with conductivity detector). A subsample was diluted 30-50-fold in double distilled water and membrane-filtered just before analysis.

Profiles of O_2, H_2S and pH were measured with microsensors in cores mounted in a mini-flume which provided a constant temperature of 0ºC and a water flow of 1-2 cm s^{-1} at 1 cm above the sediment surface (Jørgensen *et al.*, 2005a). O_2 and H_2S were measured with Clark-type microelectrodes. The O_2 electrode had an internal reference and a guard cathode (Revsbech, 1989). The tip size of the O_2 sensors was 10-20 µm, the stirring sensitivity <1% and the 90% response time ~1 s. A two-point calibration was made by positioning the sensor in air-saturated seawater and in anoxic sediment. The H_2S electrode (Jeroschewski *et al.*, 1996) had a tip diameter of 20-50 µm, stirring sensitivity <2 %, and a 90 % response time of ~3 s. The H_2S electrode was calibrated in anoxic, stirred 0.2 M phosphate buffer at pH 7.5 to which appropriate volumes of 100 mM sodium sulfide solution were consecutively added. The electrode current was read after each addition and a subsample of the calibration solution was fixed in 2% Zn-acetate for later photometric analysis of the sulfide concentration (Cline, 1969). Total sulfide was calculated from the H_2S concentrations using the sediment pH and a pK_1 of 6.87 as calculated from the seawater salinity and temperature of the incubated cores (Millero et al., 1988). The pH was measured with freshly filled liquid ion exchange microsensors (de Beer *et al.*, 1997), calibrated with standard pH buffers.

With the help of a dissection microscope, all sensors were positioned vertically above the sediment surface. Data acquisition started above the sediment and the electrodes were moved downwards in 100 µm steps by using a micromanipulator with computer-controlled stepper motor (Faulhaber, Germany). The O_2 and H_2S electrode

currents were read by a pA-meter and the pH potential was determined with a mV-meter. Data were transferred to an A-D converter (National Instruments, USA) and stored on a laptop computer.

For the determination of nitrate concentrations in *Beggiatoa* vacuoles, 50-100 filaments were picked for each analysis with a clean glass needle into 1 mL of a NaCl-solution isotonic to seawater. After gentle centrifugation, 0.9 mL of the supernatant water was collected separately. Water and *Beggiatoa* samples were acidified at pH 1 with 6 M HCl and stored frozen. Three freeze-thaw cycles between liquid N_2 and 90°C warm water ensured breakage of the vacuoles and release of the nitrate. Nitrate was analyzed with a chemoluminescence NO/NOx analyser (Eco Physics, Germany) with an isotonic NaCl solution as control. The supernatant samples were also analyzed to check for nitrate leakage before freezing. The mean biovolume of *Beggiatoa* filaments was determined by photographing at random individual filaments (n=73) from a bulk sample of the mat. The length and width of each photographed filament was determined with calibrated image analysis software (ImageTool). The mean biovolume was used to calculate the internal nitrate concentration.

Sulfate reduction rates

Sulfate reduction rates (SRR) were measured by whole core injection using ^{35}S-labelled sulfate (Jørgensen, 1978). At each 1 cm depth interval, 2 µl carrier-free $^{35}SO_4^{2-}$ tracer (~100 kBq) was injected and the core was incubated for 8-12 h at *in situ* temperature. Sulfate reduction was stopped by mixing 1 or 2 cm depth sections with 10 ml cold Zn-acetate (20% w/v) and freezing. The samples were later treated with cold chromium distillation according to Kallmeyer *et al*. (2004). Sulfate reduction rates were calculated according to Jørgensen (1978):

$$SRR = [\text{sulfate}] \times (^{35}\text{S-CRS}/^{35}\text{S-sulfate}) \times (1.06/t)\ \text{nmol}\ SO_4^{2-}\ \text{cm}^{-3}\ \text{d}^{-1} \qquad \text{(Eq. 1)}$$

where [sulfate] is the sulfate concentration in nmol cm^{-3} of wet sediment, ^{35}S-CRS is the radioactivity of total reduced sulfur at the end of the incubation, ^{35}S-sulfate is the initial radioactivity of sulfate added to the experiment, 1.06 is a correction factor for the expected isotope discrimination against ^{35}S-sulfate versus the bulk ^{32}S-sulfate by the sulfate-reducing bacteria, and t is the incubation time measured in days.

Results

Filamentous bacteria observed and quantified microscopically in sediments from Svalbard fjords were recorded as belonging to the genus *Beggiatoa* based on the following criteria (Strohl, 2005): A) The filaments were freely motile and were not surrounded by a visible sheath common to several filaments. B) The filaments ranged in diameter from 2 to 50 μm, were multicellular, and had rounded, never tapered, terminal cells. C) The cells were always rich in light-refracting, spherical sulfur globules. In the wider filaments, with diameters >5 μm, sulfur globules were distributed in the periphery of the individual cells, typical of the morphology of *Beggiatoa* spp. containing nitrate vacuoles. Filaments of similar appearance, but devoid of sulfur globules, were not counted.

Occurrence of Beggiatoa around the arctic archipelago Svalbard

We made a survey of *Beggiatoa* in fjords on the west and north coast of the main Svalbard island, Spitsbergen. Sediment cores were sampled, generally in the deeper and central part of the fjords, and the cores were screened for *Beggiatoa* using the described approach. For each site, a total of 5-10 samples of ca 30 mg wet sediment each were screened from the oxidized surface zone. Thus, a total of 150-300 mg sediment was completely screened per station. The results are summarized in Table 1.

The main conclusion from this survey was that only two out of eleven investigated sites, i.e. Smeerenburgfjorden and Ymerbukta, had abundant *Beggiatoa* during all three sampling years. At six sites, only one or a few *Beggiatoa* were found with diameters ranging mostly from 4 to 20 μm. At the remaining three sites no *Beggiatoa* were found. Given the volume of sediment screened, those sites had statistically <3-6 filaments cm^{-3}, if any.

Filament frequency and dimensions

During the entire study, close to one thousand *Beggiatoa* filaments were counted and measured. In Fig. 2 the frequency distribution and mean dimensions are shown after an arbitrary classification of all filaments into different diameter groups. When compiling

data from all locations and years, there was an almost continuous variation in diameters but a strong variation in their frequency (Fig 2A). The two narrowest size classes, 2-3 µm and 4-5 µm, were the most abundant and comprised 77% of all *Beggiatoa* filaments counted. Filaments narrower that 2 µm were not found, although they were particularly searched for in several of the sediment samples. There were notably many *Beggiatoa* with diameters of 11-22 µm. A small number of very wide filaments of >23 µm diameter were also found. The widest filament encountered was 52 µm in diameter. The mean filament length of each diameter class increased with the filament width (Fig. 2B). The narrowest filaments of 2-5 µm had a mean length of 0.25 mm while the widest were about 1.5 mm long. The longest individual filaments were >3 mm in length.

The biomass (living wet weight) of individual filaments was calculated based on their measured volume, assuming a density of 1 g cm^{-3}. It should be noted that a large part of this biomass is not active cytoplasm, but is comprised of vacuoles inside the cells. As shown in Fig. 2C, the mean biomass of whole filaments increased >1000-fold from 1.3 ng of the 2-3 µm class to 1500 ng of the 30-50 µm class. Due to the large difference in biomass per filament between narrow and wide diameters (Fig. 2C), the widest filaments often dominated the biomass in spite of their low numbers. Typically, the 5% widest filaments comprised 50% of the total *Beggiatoa* biomass. It is striking that, in spite of the small mean individual filament lengths ranging from 0.25 to 1.5 mm, the accumulated length of all filaments reached 50 cm per cm^3 of sediment and was typically

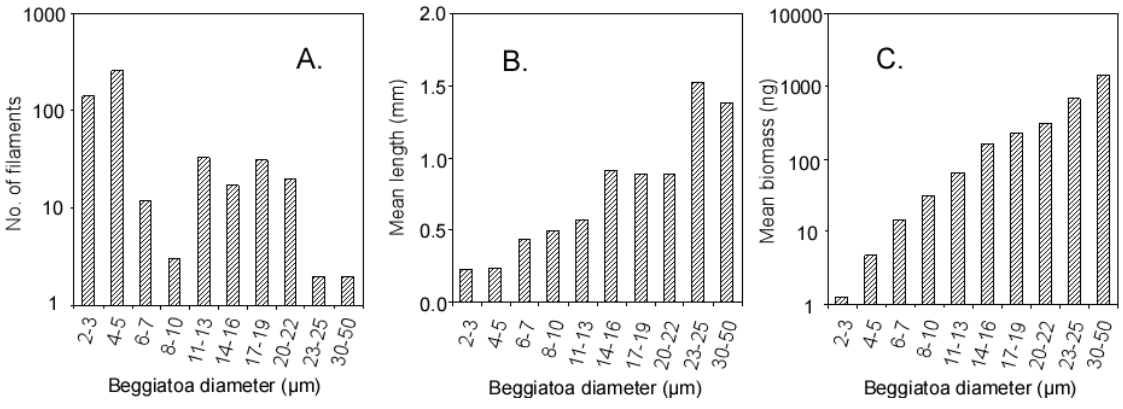

Figure 2. Size frequency and dimensions of all *Beggiatoa* measured. A) Frequency distribution of filament diameters. B) Mean filament lengths of different size groups. C) Mean fresh biomass of different size groups. Notice log scales in frames A) and C).

a few tens of cm per cm^3.

There was a rather constant ratio between filament (i.e. cell) diameter and cell length (i.e., the height of the cylinder-shaped individual cells) for the different size classes. The narrow filaments had relatively longer cylindrical cells than the wide filaments which had flatter disk-shaped cells. Thus, among the 2-5 µm wide filaments the mean cell diameter was 3.7±1.1 µm and the mean cell length was 5.4±0.4 µm. Among the 17-22 µm wide filaments the mean cell diameter was 19.2±0.5 µm and the mean cell length was 9.0±1.2 µm. The mean volume of the two size classes of cells was 18 μm^3 and 830 μm^3, respectively, i.e. 100 and 5000-fold larger than the mean size of other sediment bacteria with a mean volume of 0.2 μm^3. The mean number of cells in each filament was 50 cells for the 2-5 µm size class and 160 cells for the 17-22 µm size class. The latter, multicellular filaments were thereby close to a million-fold larger than the mean size of other sediment bacteria.

Smeerenburgfjorden Beggiatoa community

Station J was situated in central Smeerenburgfjorden at 214 m water depth (Table 1) and was particularly rich in *Beggiatoa*. The station was sampled during three different summers in 2003, 2005 and 2008. The Smeerenburgfjorden is a channel on the north-west coast of Spitsbergen and connects to the ocean both towards the west and the north. The sediment was a silty mud mixed with fine sand and rich in burrowing macrofauna, particularly in tube-building polychaetes. The upper 2-4 cm of sediment was oxidized and light gray to brown in color while the sediment below was light to dark gray and black due to iron sulfides with a mottled appearance due to heterogeneity caused by ventilation and mixing by the fauna.

During sampling in 2003 and 2005 *Beggiatoa* occurred until 2.5 cm depth in the oxidized surface sediments (Fig. 3). *Beggiatoa* filaments with a narrow size range of 2-2.5 µm width dominated in numbers in 2005 and made up 70% of all *Beggiatoa* counted there. In the more sulfidic (based on coloration) of two sediment cores analyzed in 2005, nearly all of these narrow filaments were found in the top 0-5 mm while the wider filaments of >5 µm mostly occurred sub-surface at 0.5-2.5 cm depth (Fig. 3A). Filament numbers reached 1600 cm^{-3} for the 2-2.5 µm group and 500 cm^{-3} for the >5 µm wide

group. Due to the large size difference, however, the >5 µm Beggiatoa completely dominated the biomass (Fig. 3B). Comparison with 2003 data shows a high reproducibility in biomass distribution between these two years (Fig. 3C).

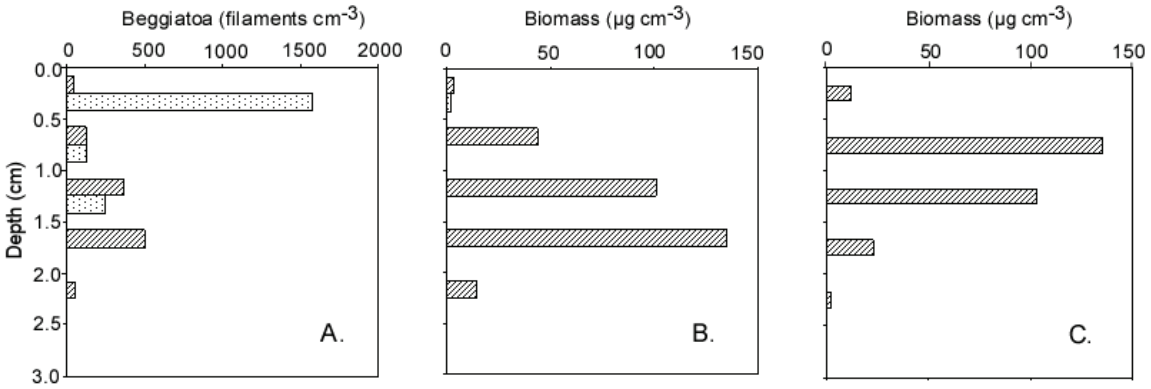

Figure 3. Depth distribution of *Beggiatoa* in sediment of Station J, Smeerenburgfjorden, during two sampling seasons. A) Numbers of filaments in 2005. B) Biomass of filaments in 2005. In A) and B) dotted bars show 2-2.5 µm wide filaments while hatched bars show >5 µm wide filaments. C) Biomass of filaments in 2003.

In order to understand the parameters controlling *Beggiatoa* distribution, high-resolution microprofiles of oxygen, sulfide and pH were recorded in 2008 in sediment cores retrieved from Station J. The oxidized zone was deeper that year, 5 cm judging from sediment color. The primary oxygen front penetrated only 1-2 mm into the sediment (Fig. 4A). Free sulfide (total H_2S) was detected from 2 mm depth and downwards at very low concentration, reaching 3 µM at 2-3 cm depth. The pH was 7.9 in the overlying seawater and showed a sharp minimum of 7.4 at the oxic-anoxic interface at 1.5 mm. A broad pH maximum of 8.0 occurred in the middle of the oxidized zone. Although free H_2S was hardly detectable, sulfate reduction took place throughout the sediment reaching maximum values of up to 17 nmol cm^{-3} d^{-1} at 2-3 cm depth (Fig. 4B). *Beggiatoa* were observed throughout the oxidized zone with high abundances and biomass down to 4.5 cm (Fig. 4C).

The maximum biomass of *Beggiatoa* varied between the three years from 70 to 150 µg cm^{-3} (Fig. 3 and 4). The total biomass of *Beggiatoa* (in g of wet biomass per m^2) was 1.38 g m^{-2} in 2003, 1.13 g m^{-2} in 2005 (mean of two cores with 0.76 and 1.49 g m^{-2}, respectively) and 3.36 g m^{-2} in 2008.

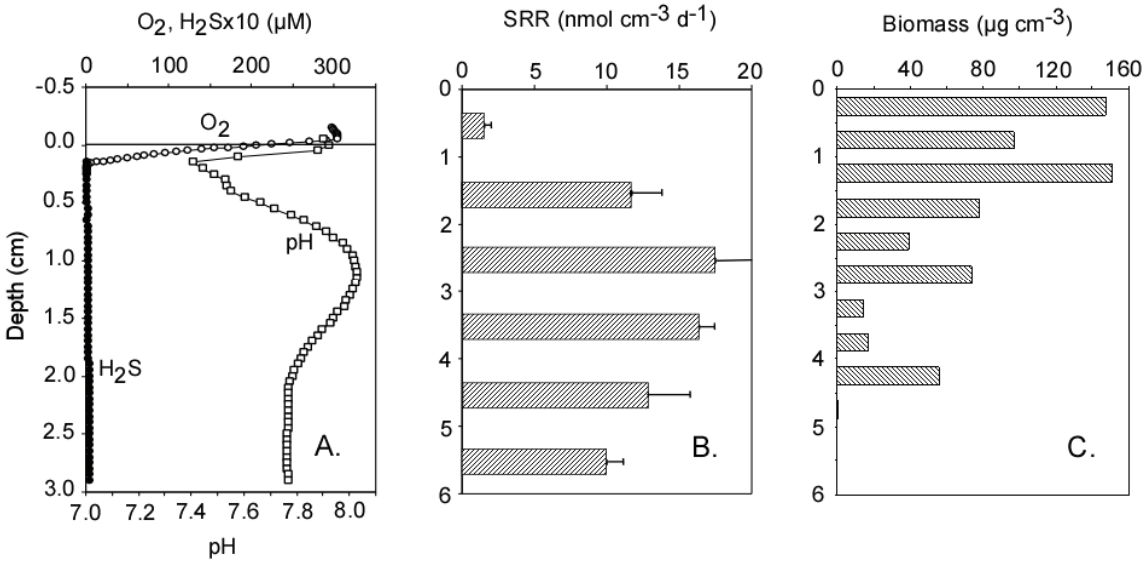

Figure 4. Station J, Smeerenburgfjorden, in 2008. A) Microsensor measurements of O_2, total H_2S and pH. B) Sulfate reduction rates (SRR) measured by ^{35}S-tracer technique (depth resolution 1 cm). C) Depth distribution of *Beggiatoa* wet biomass. Note difference in depth scales.

Ymerbukta Beggiatoa community

The other site visited during all three years was a protected lagoon in Ymerbukta on the north coast of Isfjorden (Station DA, Table 1). Due to the shallow water depth, 20-30 cm, and the 24 h of daylight during summer, the sediment surface was relatively warm, 6-7ºC at the time of sampling. The sediment was mixed silt and fine sand of mostly 20-80 µm grain size and was light brown and oxidized to 4 cm depth. The sediment below was grey to black fine sand.

Beggiatoa occurred throughout the 4 cm deep oxidized zone (Fig. 5) and two distinct size classes prevailed. As in Smeerenburgfjorden, there was a distinct difference in the depth distributions of narrow and wide *Beggiatoa*. There was a predominance of *Beggiatoa* filaments with a narrow size range of 5±0.5 µm width which comprised 90% of all filaments counted at this site. Most of the wider *Beggiatoa* were 12 µm in diameter. The narrow 5 µm *Beggiatoa* had highest density at 1-2 cm depth while the wider 12 µm *Beggiatoa* had maximum at 2-3 cm depth (Fig. 5A). Due to their much larger individual filament size, the biomass of the 12 µm filaments was overall highest (Fig. 5B). The mean areal biomass was 0.167 g m^{-2} of which the 5 µm wide filaments accounted for 40%.

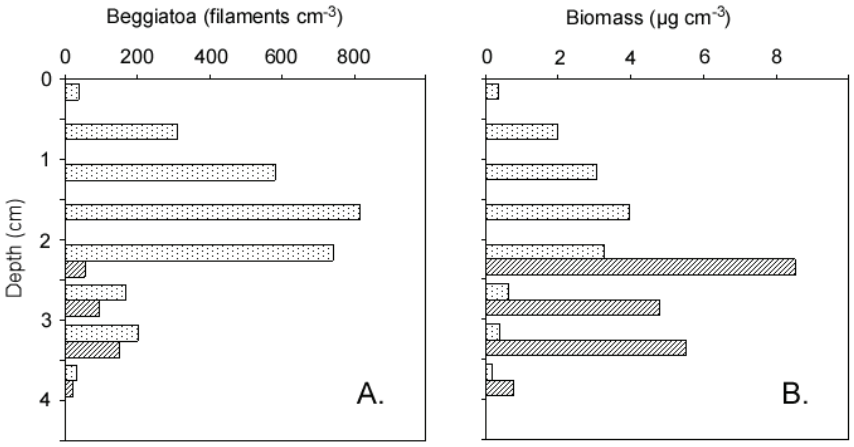

Figure 5. Depth distributions of *Beggiatoa* in sediment at Station DA, Ymerbukta lagoon, 2005. A) Numbers of filaments per cm^3 sediment. B) Biomass of filaments per cm^3 sediment. Dotted bars: 5 µm filaments. Hatched bars: >5 µm filaments. Mean of two cores.

On the sediment surface were also scattered patches of white mats of *Beggiatoa*, typically a few hundred cm^2 in size and dominated by 2 µm and 8-10 µm wide filaments. Some patches were green to blue-green and were dominated by *Oscillatoria*-like cyanobacteria with filaments of mostly 18 or 25 µm in diameter. Interestingly, these filamentous cyanobacteria often occurred down to >4 cm depth in the sediment. The cyanobacterial patches also contained many *Spirulina*-like cyanobacteria, some *Beggiatoa*, and different pennate diatoms. The sediment beneath the white and green patches was gray to black and highly sulfidic.

In a studied patch with *Beggiatoa* mat on the surface oxygen penetrated only to 0.4 mm depth (Fig. 6A), while the penetration depth just outside the visible *Beggiatoa* mat was 0.8 mm (Fig. 6B). In both cases, sulfide overlapped a few hundred µm with oxygen but did not reach the sediment surface. The *Beggiatoa* mat was only about 1 mm thick and covered the O_2-H_2S interface and the uppermost front of the sulfide zone. Maximum sulfide concentration was found at 1 cm depth and was twice as high below the *Beggiatoa* mat (1100 µM, Fig. 6A) as just outside the mat (600 µM, Fig. B). These near-surface peaks of sulfide were extremely high and indicated intensive sulfate reduction driven by a high pool of organic matter in the sediment. Accordingly, we found abundant remains of decomposing macroalgae buried just under the sediment surface, probably brought into the lagoon during storms and covered by fine-grained sediment

during calm weather. The steep decrease in sulfide below the peak indicates that a large pool of reactive Fe(III) was also mixed into the sediment and was precipitating different iron-sulfide minerals and, thus, causing the black color of the sediment.

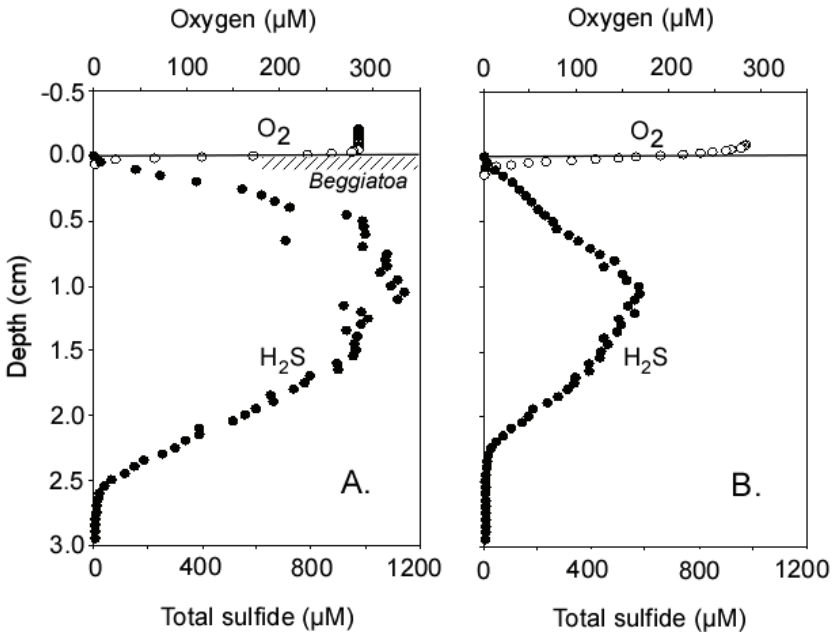

Figure 6. Oxygen and sulfide (total H_2S) profiles in sediment from a shallow lagoon in Ymerbukta (Station DA). A) Patch of white *Beggiatoa* mat. B) Sediment just outside of the visible *Beggiatoa* mat.

Nitrate-accumulating arctic Beggiatoa

Beggiatoa filaments were used to either measure the internal nitrate concentration or to determine their phylogenetic affiliation. Two parallel batches of 16-20 µm wide filaments from Station J in Smeerenburgfjorden contained 86 and 134 mM nitrate, respectively, while a batch of 13-15 µm wide filaments contained 260 mM nitrate. The meadian nitrate concentration for these large *Beggiatoa* was thus ca 130 µM. In contrast, *Beggiatoa* of 8-10 µm diameter from a mat in Ymerbukta contained only 2.7 ±0.2 mM nitrate.

Phylogenetic analysis of 20 µm wide *Beggiatoa* from Smeerenburgfjorden (clone S3678; Fig. 7) revealed that the closest relatives include nitrate-storing *Beggiatoa* spp. from the brackish Limfjorden, Denmark (AF532775) and from an intertidal mud flat at Dangast, German Wadden Sea (AF532769). All these *Beggiatoa* are relatively large, with diameters between 9-17 µm, and accumulate nitrate in intracellular vacuoles (Mußmann

et al., 2003). Information on possible nitrate-storage in the closest relative, a marine, uncultured *Beggiatoa* from Tokyo Bay (AB108786), was not given by the original investigators, but seems likely due to the presence of an internal vacuole (Kojima & Fukui, 2003).

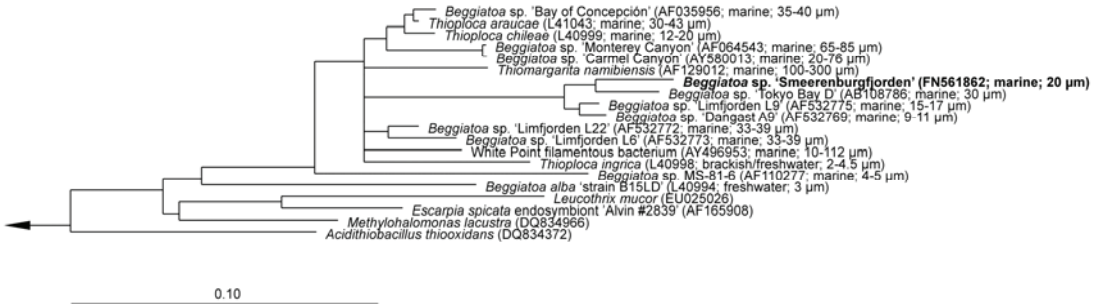

Figure 7. Phylogenetic 16S rRNA gene based tree showing the affiliation of the uncultured *Beggiatoa* sp. clone S3678 from Smeerenburgfjorden in Svalbard (FN561862, indicated by bold type) to selected reference sequences within the Gammaproteobacteria. Following each accession number, information on the general habitat (marine/freshwater) and cell diameter is given in parentheses. The bar indicates 10% estimated phylogenetic divergence.

Discussion

The fjords of Svalbard provide some of the most extreme arctic, coastal sediments, given the latitudes ranging between 78° and 80° N, continuous daylight in summer for 3-4 months and corresponding dark night in winter, and seawater temperatures always near the freezing point. Our study demonstrates for the first time the widespread, yet scattered occurrence of large filamentous sulfur bacteria, *Beggiatoa* spp., in such permanently cold sediments. An earlier study of a conspicuous white bacterial mat of unidentified Gammaproteobacteria in Young Sound on the NE coast of Greenland also reported the presence of *Beggiatoa*, however in very low numbers (Glud *et al.,* 2003).

The occurrence of *Beggiatoa* is best known from sites where sulfide reaches the sediment surface and where thin, visible mats are formed at the narrow oxygen-sulfide interface (Jørgensen & Revsbech, 1983). Previously, white mats of sulfur bacteria have been observed in the cold deep-sea and also in the Arctic. These mats are, however, often associated with seep systems where sulfidic pore fluid emerges at the sediment surface. A

remarkable example is the Håkon Mosby mud volcano in the deep North Atlantic west of Spitsbergen, where dense *Beggiatoa* mats have been observed at 1250 m water depth thriving at a temperature of -0.5°C (de Beer *et al.,* 2006; Niemann *et al*, 2006). It is therefore not a new finding that marine *Beggiatoa* can live at near-zero temperatures provided that sufficient sulfide is available. In this study, *Beggiatoa* mats on the sediment surface were only observed in the shallow lagoon of Ymerbukta where microbial sulfate reduction coupled to decomposing macroalgae just beneath the sediment surface provided a high sulfide flux from below. At most other stations *Beggiatoa* were found to thrive within the sediment at depths down to 2-5 cm.

Controls on Beggiatoa distribution

There is no indication that low temperature is directly inhibitory to the growth and distribution of *Beggiatoa*. The arctic communities of *Beggiatoa* are apparently cold adapted and at some sites large communities are able to thrive at temperatures permanently near the freezing point. Recent measurements of the gliding speed of 8-10 µm wide Svalbard *Beggiatoa* showed that these were moderately psychrophilic with optimum at 17ºC. They continued their gliding motility even down to -5ºC and immediately recovered, without motility loss, from a transient freezing at that temperature (Dunker *et al.,* in press). Since the upper part of the lagoonal sediment in Ymerbukta freezes solid in winter, it is an important observation that both *Beggiatoa* and sulfate reducing bacteria from this environment survive freezing without or with only little detrimental effect on their metabolic rates (R. Dunker *et al*., in press; Sawicka *et al.,* in press; Mountford *et al*. 2003). By comparison, *Beggiatoa* from a sediment of the temperate North Sea were immobilized, and probably killed, by transient freezing, but survived freezing provided that the population had been cold adapted over a few months (Dunker *et al*., in press).

The availability of H_2S is critical for *Beggiatoa* to establish a community in marine sediments. The sulfate reduction rates measured in the permanently cold Svalbard sediments, 1.3-4.5 mmol m^{-2} d^{-1} (Table 1), tend to be of similar magnitude as rates measured in temperate sediments (Canfield *et al.,* 2005; Jørgensen & Kasten, 2006). The key control on these rates is provided by the phytoplankton productivity in the water

column and thus the deposition and burial of degradable organic matter in the sediment. The fjords of Svalbard are relatively deep, typically 70-200 m, with steep rocky coasts and hardly any terrestrial organic material coming from the barren and extensively ice and snow-covered land. Yet, the primary productivity during the ice-free season is relatively high and the rates of microbial metabolism (oxygen uptake, metal reduction and sulfate reduction) in the sediments is correspondingly high (Glud *et al.,* 1998; Vandieken *et al.,* 2006; Arnosti & Jørgensen, 2006).

The most common, yet mostly unnoticed occurrence of marine *Beggiatoa* is within the top several cm of slightly oxidized sediment (Jørgensen, 1977). This zone is characterized by the abundance of oxidized iron minerals which provide the sediment with a light gray to brown color. The zone generally has no detectable sulfide, yet often a high rate of sulfate reduction. The produced sulfide is thus turned over immediately, mostly by reaction with oxidized iron minerals, but also by *Beggiatoa*. It is a prerequisite for the occurrence of *Beggiatoa* in this zone that the filaments are able to glide through the sediment. It has been noticed in temperate sediments that a mixture of silt and very fine sand may physically exclude *Beggiatoa* because gliding motility is not possible (Jørgensen, 1977).

The occurrence of visible *Beggiatoa* mats on the sediment surface was associated with buried macroalgae which were the source of intense sulfide production. The oxygen flux into the *Beggiatoa* mat was more than twice the oxygen flux just outside the *Beggiatoa* patch (61 and 24 mmol m^{-2} d^{-1}, respectively, Fig. 6). The corresponding sulfide fluxes were 34 and 13 mmol m^{-2} d^{-1}, respectively, yielding flux ratios of 2.1:1 (O_2:H_2S) in the mat and 1.8:1 outside the mat. This corresponds in both cases to a complete oxidation of sulfide to sulfate with oxygen which would imply a 2:1 stoichiometry:

$$2\ O_2 + H_2S \rightarrow SO_4^{2-} + 2\ H^+ \qquad \text{(Eq. 2)}$$

A flux ratio of 2:1 or slightly higher was also found by microsensor studies of a temperate *Beggiatoa* mat (Jørgensen & Revsbech, 1983). This stoichiometry is characteristic of a community in steady state where the *Beggiatoa* biomass or their elemental sulfur content is not increasing (Nelson *et al.,* 1986). The complete sulfide oxidation to sulfate outside of the *Beggiatoa* mat could be due to other, non-conspicuous sulfide oxidizing bacteria, for example of the *Thiobacillus* or *Thiomicrospira* groups. A

purely chemical sulfide oxidation would expectedly not lead to a quantitative conversion to sulfate but rather to sulfur compounds of intermediate oxidation state, such as elemental sulfur or thiosulfate.

In the sediments where oxygen and sulfide were separated by an intermediate oxidized zone inhabited by *Beggiatoa* (Fig. 4) the pH profile showed a sharp minimum near the oxygen front. Since there was no significant gradient of free sulfide reaching the oxygen front, the pH minimum may be due to the oxidation of elemental sulfur to sulfate in *Beggiatoa* as proposed by Sayama *et al.* (2005). It may also be due to the re-oxidation of free Fe^{2+} produced by iron reduction in the intermediate zone. Preisler *et al*. (2007) concluded from a similar pH minimum in a *Beggiatoa* inhabited Baltic Sea sediment that the oxidant for Fe^{2+} was MnO_2 rather than O_2, both of which would produce excess H^+ and thus a pH minimum. On the contrary, other oxidation processes with MnO_2 involving pyrite, iron sulfide or organic matter consume H^+ and may contribute to the broad pH maximum in the oxidized zone (Preisler *et al.,* 2007). The pH maximum may also be caused by sulfide oxidation to elemental sulfur coupled to either nitrate respiration to ammonium in *Beggiatoa* or coupled to the reduction of oxidized iron in the sediment to form free Fe^{2+}.

In Table 1 we compare the occurrence of *Beggiatoa* to the sulfate reduction rates, i.e. to the rate of sulfide formation and, thus, presumably to the availability of sulfide for these sulfide oxidizing bacteria. Station A in Adventfjorden had the highest areal rates of sulfate reduction, yet *Beggiatoa* were not found. Perhaps this sediment was physically not accessible to *Beggiatoa* due to the lack of pore space for gliding motility. Smeerenburgfjorden sediment with abundant *Beggiatoa* was also in the higher end of the SRR range, but overall there was no clear correlation between rates of sulfide production and the occurrence of *Beggiatoa* (Table 1). It may be that suitable pore space is critical and that certain combinations of silt and fine sand prevent the gliding movement that is a prerequisite for *Beggiatoa* to thrive (Jørgensen, 1977).

Ecology of arctic Beggiatoa

The ability to store nitrate enable the large marine *Beggiatoa* of Svalbard to thrive in the oxidized but anoxic zone within the upper 2-4 cm of the sediment. Near the

sediment surface they may take up nitrate which in the bottom seawater in fjords on the west coast of Svalbard is mostly present at 1-10 µM concentration (Eilertsen *et al.,* 1989; Wang *et al.,* 2009). In the sediment below, they may effectively utilize the sulfide produced from bacterial sulfate reduction which proceeds throughout this zone although sulfide is near or below detection. Thus, in Smeerenburgfjorden sulfate reduction rates reached 17 nmol cm^{-3} d^{-1} at 2-3 cm depth where *Beggiatoa* were abundant, but where free sulfide concentrations did not exceed 3 µM (Fig. 4). With a measured porosity of 0.72 at that depth, this rate corresponds to 24 µM SO_4^{2-} reduced per day or 2 µM SO_4^{2-} reduced per hour. The turnover time of total sulfide in the *Beggiatoa* zone is therefore in the order of one hour. The experimentally determined SRR data show that *Beggiatoa* do indeed have sulfide available which they can oxidize with intracellularly stored nitrate. The low concentration and relatively fast turnover of free sulfide shows that the sulfide was oxidized at the depth where it was produced and did not diffuse away.

Even at the highest density of *Beggiatoa* found in Smeerenburgfjorden, they probably did not play an important role for the overall sulfide oxidation, as the following calculations show. The cell-specific rate of nitrate reduction in marine *Beggiatoa* of 24-30 µm diameter from a temperate sediment at 15°C was found to be 13 mM NO_3^- d^{-1} (Preisler *et al.,* 2007). Arctic *Beggiatoa* of this size class living at 0°C may have ca 5-fold lower metabolic rate, estimated from the difference in gliding speed (R. Dunker *et al.,* in press), i.e. about 3 mM NO_3^- d^{-1}. Since the dominant biomass of *Beggiatoa* in Smeerenburgfjorden belonged to the wider size range (Fig. 3B) which stored on the order of 130 mM NO_3^-, this rate of nitrate reduction could keep the filaments supplied with electron acceptor for (130/3 =) ca 40 days without a refill. If the filaments carry out dissimilatory nitrate reduction to ammonium and oxidize sulfide completely to sulfate, then the stoichiometry of nitrate reduction to sulfide oxidation is 1:1 and the cell specific rate of sulfide oxidation would also be about 3 mM sulfide d^{-1}:

$$H_2S + NO_3^- + H_2O \rightarrow SO_4^{2-} + NH_4^+ \qquad \text{(Eq. 3)}$$

This rate can be compared to the rate of sulfide production from the measured sulfate reduction. At 0-1 cm depth in the Smeerenburgfjorden sediment the biomass of *Beggiatoa* was 150 µg cm^{-3}. This is equal to 0.15 mm^3 of *Beggiatoa* biovolume per cm^3 of sediment and these *Beggiatoa* could oxidize ($0.15 \times 3 \times 10^{-6}$ =) 0.5 nmol sulfide cm^{-3} d^{-1}.

The measured SRR at 0-1 cm was equal to 2 nmol H_2S cm^{-3} d^{-1} produced, i.e. 4-fold higher. The same calculation for the 2-3 cm depth interval provide: 60 µg cm^{-3} of *Beggiatoa* biomass which could oxidize 0.2 nmol sulfide cm^{-3} d^{-1}. The measured SRR at 2-3 cm was equal to 17 nmol H_2S cm^{-3} d^{-1} or nearly 100-fold higher. The conclusion is that, even at their highest biomass density in Smeerenburgfjorden, the *Beggiatoa* did not contribute significantly to the overall rate of sulfide oxidation. This is the same conclusion as reached for a rather similar *Beggiatoa* population in Baltic Sea sediment (Preisler *et al.,* 2007).

This calculation neglects, however, that *Beggiatoa* are facultative aerobes and that they may have a much higher metabolic rate when, during their gliding movement, they are near the sediment surface and respire with oxygen. A more differentiated scenario may thus be that *Beggiatoa*, when moving around below the oxic zone, cover only their motility and maintenance energy through nitrate respiration. If at the same time sulfide is oxidized to elemental sulfur only they could oxidize four times more elemental sulfur, 12 mM sulfide d^{-1}, per mol of nitrate respired. When they pass the oxic surface zone they may then increase their metabolic rate through oxygen respiration while oxidizing elemental sulfur to sulfate. At the same time they could use some of the reducing power for CO_2 reduction and autotrophic growth. With a differentiated anaerobic and aerobic metabolism as suggested here, *Beggiatoa* might contribute much more than calculated to sulfide oxidation in the sediment.

For comparison, vacuolate marine *Beggiatoa* from Monterey Canyon were shown to consume oxygen at rates considerably greater than their rate of nitrate consumption (McHatton, 1998).A study of the related, *Thioploca chileae*, of 12-20 µm diameter showed that they may have a respiration rate of ca. 40 mM O_2 d^{-1} at 25ºC and a sulfide oxidation rate of 150 mM sulfide d^{-1} (Høgslund *et al.,* 2009), i.e. about ten-fold higher than the arctic *Beggiatoa* at 0ºC.

Mat-forming *Beggiatoa* of 8-10 µm diameter in Ymerbukta stored only 2.7 mM nitrate. It is possible that their chemoautotrophic life at the narrow interface of overlapping O_2 and H_2S (Fig. 6) is not as selective for a large internal nitrate storage as the subsurface life in Smeerenburgfjorden sediment with many hours or days of gliding without contact to the oxic surface zone.

Beggiatoa – members of the "rare biosphere"?

During the microscopic search for *Beggiatoa* in Svalbard sediments we also looked for other cells containing light refractive globules that could indicate sulfur bacteria with elemental sulfur inclusions. This search for morphologically conspicuous sulfur bacteria was generally without positive results. In one core from Smeerenburgfjorden, however, we found a distinct sheath which harbored five filaments of 23 µm diameter. The sheath was 3.5 mm long and 70 µm wide and the individual filaments were 0.5-1.1 mm in length and rich in light refractive inclusions. The terminal cells were rounded and it was thus not possible to distinguish the filaments morphologically from free-living *Beggiatoa*. The presence of a common sheath for a bundle of filaments, however, is diagnostic of the genus *Thioploca* and the diameter of 23 µm classified the filaments taxonomically to the species *T. chileae* (Jørgensen *et al.,* 2005b). In contrast to the observed filaments, *Thioploca* spp. most often have tapered terminal cells.

The scattered occurrence of *Beggiatoa* of highly variable diameters in many sediments indicates that these bacteria are widely present in the Arctic, but in low numbers. A different approach than used here would be required to scan a larger sediment volume (>>0.1 g) in the search for *Beggiatoa*, e.g. by extracting DNA and searching for *Beggiatoa*-related 16S rRNA genes, provided that appropriate primers for amplification were established. Such an environmental genomic approach, however, misses the unique advantage provided by the distinct morphology of *Beggiatoa*. *Beggiatoa* is of the size of micro- and meiofauna, rather than of the size of normal bacteria, and the organisms can therefore be quantified with similar sample volumes and techniques. Due to their extraordinary size, *Beggiatoa* are rarely recorded in marine sediments although they are widely distributed. The main reason is that they are not detected by normal direct bacterial counts using fluorescent stains such as acridine orange or DAPI. This becomes clear from the following example.

The densities of *Beggiatoa* filaments in Svalbard varied from <10 to 1000 filaments cm^{-3} sediment. By direct fluorescence counts of bacteria, about 1 µg of sediment may be scanned under the light microscope at 1000 × magnification. As the cell density of bacteria in normal fjord sediments is $>10^9$ cells cm^{-3} this would suffice to

count >1000 bacteria. However, in 1 µg of sediment the chance of finding just one *Beggiatoa* at the above densities is $<10^{-5}$ to 10^{-3}, i.e. highly unlikely. By the counting of *Beggiatoa* we routinely counted all filaments in 30 mg of sediment, i.e. in a 10^5-fold larger sediment volume than is needed to count all other bacteria.

Even at their highest density, *Beggiatoa* filaments comprise only a millionth of all bacteria in the sediment. Yet, due to the very large biomass of each filament, 1-1000 ng, *Beggiatoa* may comprise a significant fraction of the total prokaryotic biomass. In Smeerenburgfjorden sediment the mean total bacterial numbers were 3.4×10^9 cells cm^{-3} sediment in the top 0-2 cm (Ravenschlag *et al.,* 2000). With a typical biovolume of 0.2 μm^3 of sediment bacteria (Kuwae & Hosokawa, 1999) the living biomass per bacterial cell is 0.2 pg. The total prokaryotic biomass (excluding *Beggiatoa*) is thus 700 µg cm^{-3}. The total biomass of *Beggiatoa* at 0.5 cm depth was 50-100 µg cm^{-3}, i.e. 7-15% of the total prokaryotic biomass of other bacteria. This shows that, although a normal direct count of total bacteria in the sediment would not have detected any *Beggiatoa*, they may still account for a significant fraction of the total bacterial biomass. With respect to biomass and metabolic activity they may thus be an important component of the microbial community. With respect to numbers they are less than a millionth (on a per cell basis less than 1/10.000) and belong to the "rare biosphere" (Sogin *et al*., 2006), even at their highest densities. Such rare but widely distributed microorganisms constitute a seed bank of bacteria that may rapidly multiply in case conditions become favorable. Thus, *Beggiatoa* may suddenly appear as white biofilms on the coastal seabed when a phytoplankton bloom settles or the lower water column becomes oxygen depleted, even though *Beggiatoa* are highly conspicuous and none could be detected in the area before the event.

Conclusions

Our results show that large, nitrate-accumulating *Beggiatoa* occur widespread in permanently cold sediments of the high Arctic, similar to temperate sediments (Mußmann *et al.,* 2003; Preisler *et al.,* 2007). The bacteria are not inhibited in their metabolic rate or in their motility and chemotactic behavior by temperatures near freezing, but are well

adapted to the cold (Dunker *et al.,* in press). Only in two out of eleven investigated fjord sediments were *Beggiatoa* abundant and arctic *Beggiatoa* were found to contribute moderately to sulfide oxidation in the studied sediments. Nevertheless, our findings provide a new and interesting perspective on the biogeography of *Beggiatoa* by showing that these giant, conspicuous sulfur bacteria occur frequently as members of the rare biosphere in marine sediments.

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2.3. Manuscript 3:

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

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Abstract

The large sulfur bacteria, *Beggiatoa* spp., live from 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 gliding direction to find their preferred environment, the oxygen-sulfide interface. We observed chemotactic patterns of single filaments in transparent agar medium and scored their reversals and the glided distances between reversals. Filaments within the preferred micro-environment glided distances shorter than their own length between reversals which 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 that grow on sulfidic sediments or decomposing organic debris (e.g. Jørgensen & Revsbech, 1983, Sweerts*, et al.*, 1990, Sayama, 2001, de Beer*, et al.*, 2006). Their most widespread habitat, however, is within the few-mm to cm thick suboxic sediment where neither oxygen nor sulfide is detectable (Mussmann*, et al.*, 2003, Preisler*, et al.*, 2007, Jørgensen*, et al.*, 2010). The *Beggiatoa* biomass is often highest just below the few-mm thick oxic zone and at the diffusion front of sulfide (Mussmann*, et al.*, 2003, Preisler*, et al.*, 2007), yet it can be highest in the middle (Hinck*, et al.*, 2007). This distribution has been ascribed to a negative tactic response towards oxygen by which *Beggiatoa* avoid high oxygen concentrations (Møller*, et al.*, 1985). Similarly, the bacteria appear to have a negative tactic response towards sulfide (Hinck*, et al.*, 2007, Preisler*, et al.*, 2007). How

these tactic responses lead to the observed distribution patterns of *Beggiatoa* is not understood, especially not within the sediment where the organisms cannot be observed.

There are detailed studies of the chemotactic behavior of large swimming sulfur bacteria and of the formation of mats and veils of these organisms over sulfidic sediments (Thar & Fenchel, 2001, Thar & Kühl, 2001, Fenchel & Thar, 2004, Thar & Fenchel, 2005). 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 known from *E. coli*.

The filamentous *Beggiatoa* glide by excreting mucus in the opposite direction in which they move. The chemotactic responses available to *Beggiatoa* in order to form complex community structures therefore appear to be limited to simple reversals of gliding direction. These multicellular organisms reverse in response to chemical cues (Nelson & Castenholz, 1982a, 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, 1982a). The reversals occur either over the entire filament or, when the filament is long, only over the leading end of the filament which first becomes exposed to oxygen (Møller*, et al.*, 1985). The reversals effectively keep the individual *Beggiatoa* filaments, and thus the entire population, within their preferred chemical environment.

The aim of the present study was to understand the statistical mechanics of chemotaxis in *Beggiatoa,* living in the 3-dimensional matrix of the sub-oxic zone of coastal and estuarine sediments, by observing their motility behavior in transparent semisolid medium.

Material and Methods

Beggiatoa culture

The *Beggiatoa* used for this study were derived from scleractinian reef corals infected with the black-band coral disease in the Florida Keys, USA (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, personal communication). The clonal *Beggiatoa* culture was populated by an accompanying organism which is closely related to *Pseudovibrio denitrificans* (A. Bachmann, Diploma thesis 2007).

The *Beggiatoa* culture was maintained at room temperature (18-20° C) in agar tubes with opposing gradients of oxygen and sulfide. 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 mM or 8 mM. The top agar was soft (0.25 %) and allowed *Beggiatoa* to glide trough the media. The preparation of the gradient agar tubes has been described in detail elsewhere (Nelson*, et al.*, 1982b, Kamp*, et al.*, 2008).

After 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 indicated the position of the oxic-anoxic interface in the top agar. Within few hours after inoculation with *Beggiatoa*, a bacterial mat formed 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 with Clark type microsensors (Nelson*, et al.*, 1986a, Kamp*, et al.*, 2006) and both dropped to a few µM exactly at the horizontal mat. The motility patterns observed for this *Beggiatoa* culture were compared to 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, placed directly in the oxygen-sulfide transition in gradient tubes, and directly observed without further growth.

To study also 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 2.5 mM Ti (III)-citrate as reductant and 10 mM thiosulfate as reduced sulfur source at a pH of 7.7. 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

For the reversal frequency of *Beggiatoa* series of images were taken of agar tubes (n=8) with filaments. The tubes were photographed from the side by a software controlled digital monochrome camera (Sony XCD-X710) at time intervals of 20 or 30 s (Fig. 1). A small lens aperture and long viewing distance assured that the filaments were in focus through most of the tube. A red light LED (Luxeon Star/O LXHL-ND98, peak wavelength 635 nm, Lumiled^{TM}) with 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 high temporal resolution were studied with Århus Bay filaments on microscope slides. 18 filaments with diameters between 5.3 and 31 µm were observed under a light microscope (Zeiss Axioskop). Movies at 15 frames per second and a 400 fold magnification were filmed with a digital camera (Canon Power Shot A 620). This resulted in a time resolution of 67 ms. Calibration was done with an object micrometer and an image analysis software (ImageTool, The University of Texas Health Science Center at San Antonio, USA).

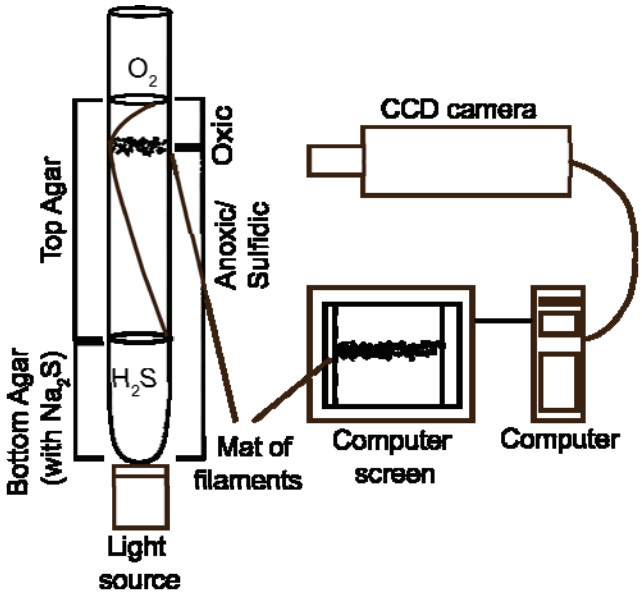

Figure 1: Experimental setup for image acquisition of *Beggiatoa* filaments in gradient agar tubes. The different agar types are specified to the left, the chemical zonation to the right of the tube. Oxygen and sulfide concentration profiles are indicated with curves

Image analysis

The dispersal of filaments was followed from time-lapse movies of filaments in agar tubes. Only filaments that glided in the focal plane were analyzed. 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). Image analysis of filaments in the bacterial mat was done 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. The traveled distance 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 the analysis of single reversals the Århus Bay filaments on microscope slides were filmed at 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 the high magnification. The movies were analyzed by resolving them into single images and stacking them. The speed of each filament a few seconds before, during and after a reversal was determined with the image analysis software Image J by following the tip of the filament from frame to frame.

Results

Trails of filaments

The gliding speed of *Beggiatoa* filaments at 18 ºC 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. 2). Filaments above and below the mat showed significant net movement over the observed time period (2-3 hours) whereas filaments within the mat departed only little from their original position (Fig. 3). The graphs A and B in Figure 3 show the

average distances glided away from the origin of all filaments scored within the mat and outside of the mat in the sulfidic zone, respectively. The standard deviations for filaments within the mat and outside of it were omitted for better readability of the plot. The standard deviations were largest for filaments gliding in the sulfidic zone and smallest for filaments gliding within the mat.

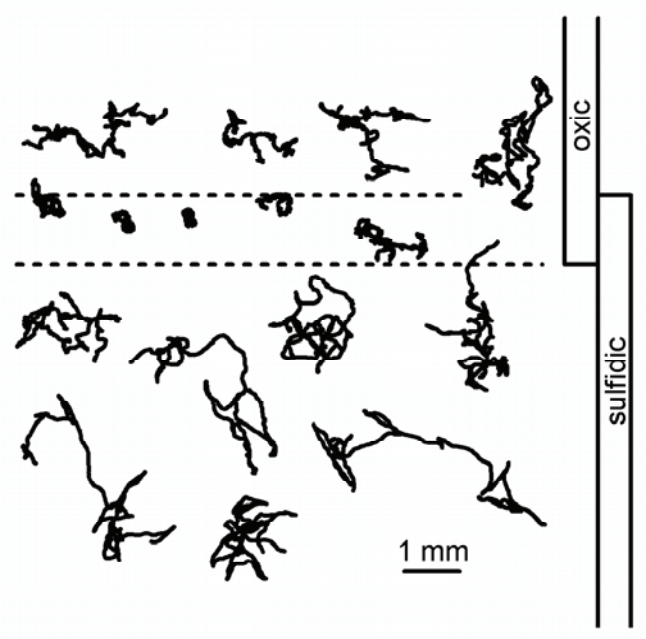

Figure 2: Trails of filaments observed for 126-166 minutes. The dashed horizontal lines indicate the upper and lower boundary of the Beggiatoa mat. Filaments above the mat move in the oxic agar, below the mat in sulfidic agar. The lower three filaments are modeled filaments that move in random trails in the sulfidic region.

The filaments moved a net distance by alternating long or short gliding distances after each reversal. After gliding back and forth in the old trail for distances of variable length the leading part of the filament usually found a new path. In none of the regions the filaments had any specific orientation relative to the mat or the oxygen or sulfide gradient.

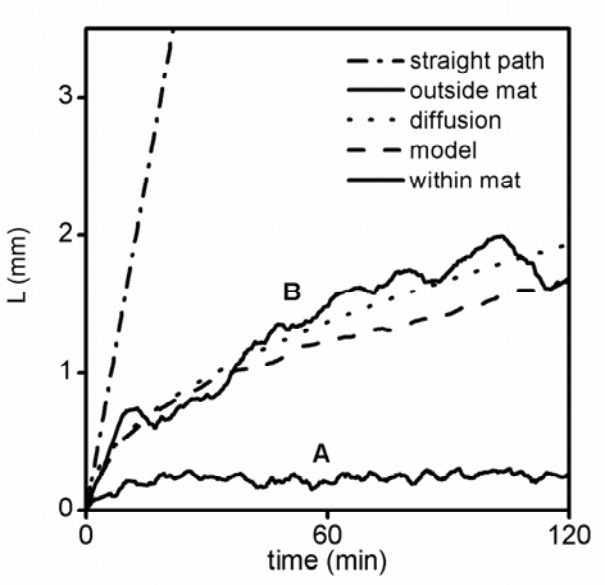

Figure 3: The net distances over time that individual filaments glided away from their origin over two hours. Solid curves show observed data. (A) A *Beggiatoa* gliding within the mat with both oxygen and sulfide present at low concentration. (B) A *Beggiatoa* gliding in the sulfidic zone without oxygen. 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.

Reversals

The change of gliding direction happened abruptly, meaning that at the time resolution used (67 ms) there was no evident deceleration before the filaments stopped. After a filament stopped, it paused for 1-4 s at its stopping position until it resumed motility in the opposite direction at the full former gliding speed. The resumption of motility was sometimes accompanied by a sudden jerk in the direction of gliding as the filament was held back by an elastic force that snapped. Besides the jerk, which happened within several hundred milliseconds according to our time resolution, there was no evident acceleration.

The reversal frequency was related to filament length. In Figure 4A-C the average distance glided between reversals is plotted against 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 (Fig. 4A and C). Filaments within the mat glided shorter than their own length, a reversal pattern that essentially anchored them to the same spot (Fig. 4B). Two particular filaments were observed as they moved from outside

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. 4A and C).

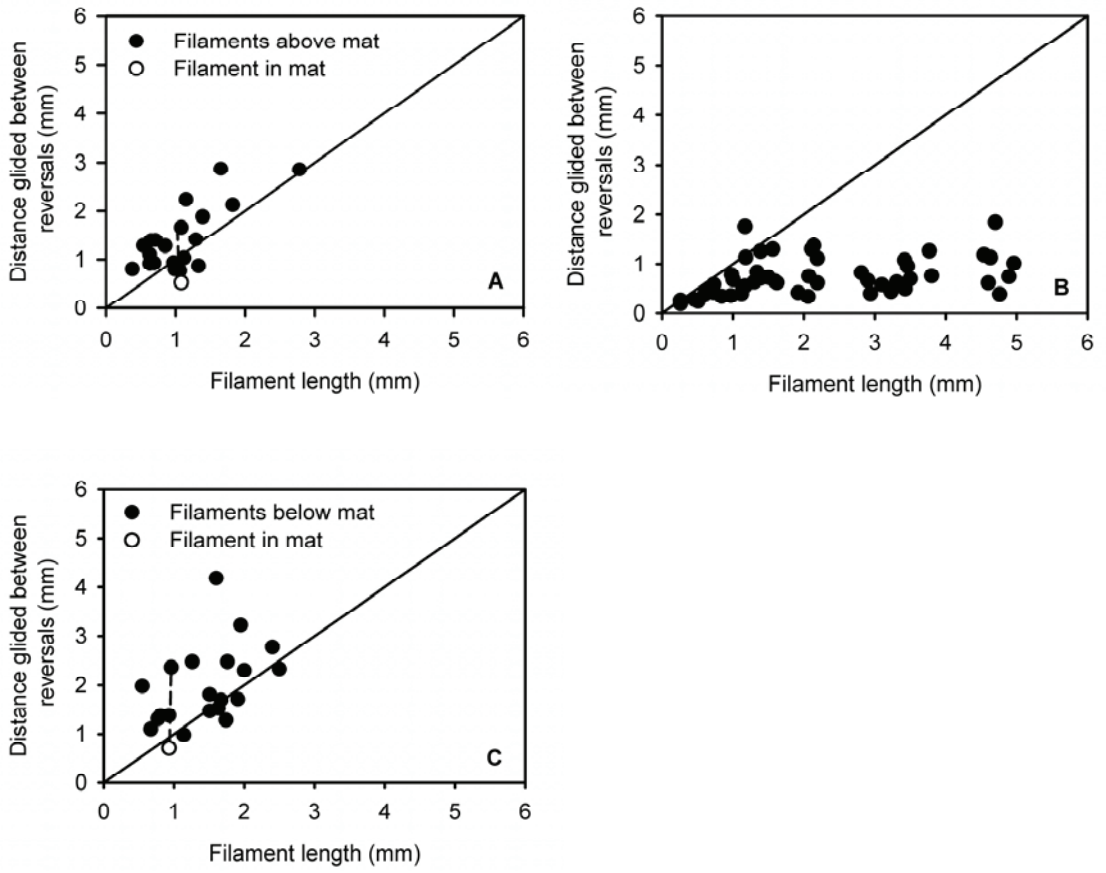

Figure 4: Average gliding distances between reversals of filaments of different length at different positions relative to the mat. A: Above the mat, n=20 B: within the mat, n=52 C: below the mat, n=20.

The patterns of reversal frequency relative to position outside or within the mat were further analyzed over time on a few specimens above, within, or below the mat. Five filaments of 0.18-1.34 mm length were observed for each position, scoring 7-32 reversals of each. For the *Beggiatoa* gliding within the mat, we found that nearly all glided distances between reversals were shorter than filament length (Fig. 5B). For the filaments gliding above and below the mat, nearly all glided distances were longer than filament length (Fig. 5A 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.

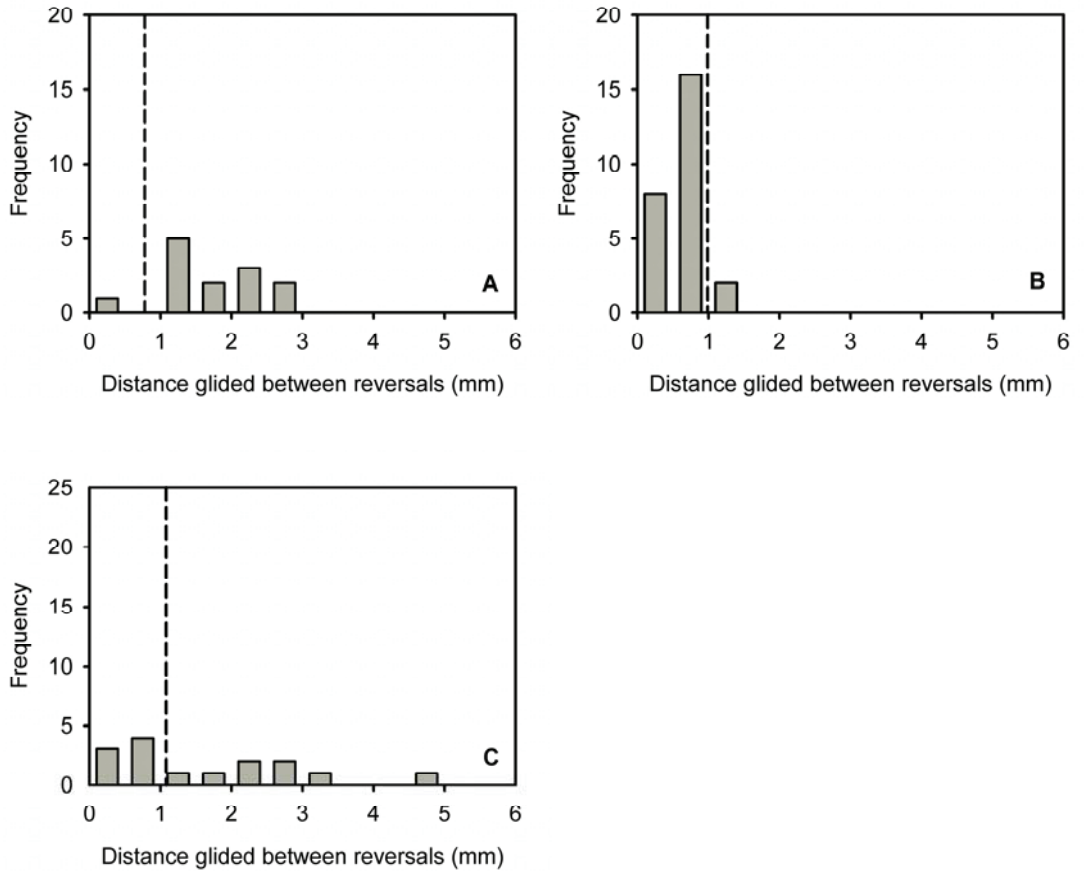

Figure 5: Length of distances that individual filaments glided between reversals. Dashed line: Filament length. A: Above the mat, B: within the mat, C: below the mat.

Discussion

The reversal of gliding direction in entire 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. The diffusive transport of a purely chemical signal would be much too slow to initiate the coordinated reversal of an entire filament within the used

time frame (67 ms). The mean molecular diffusion time of small molecules along a 1-5 mm long filament would be 10 minutes to 3 hours which highly exceeds the reaction time of the entire filament. The concerted action could be coordinated by an electrochemical signal that propagates along the length of the filament or by mechanical cell to cell communication via mechanoreceptors in the cell walls. If the jerky movement after the reversal may be linked to the exopolymeric slime threads extruded by the filament during locomotion remains to be investigated.

Halfen & Castenholz (1971) described reaction times of 1-2 s for the multicellular, filamentous cyanobacteria, *Oscillatoria princeps,* to complete a reversal. Even shorter reaction times of < 0.1 s have been observed for the multicellular, filamentous, heterotrophic bacteria, *Flexibacter polymorphus* (Ridgway & Lewin, 1988). The authors proposed an electrochemical signal involving membrane depolarization for this rapid signal transduction. Gliding filaments of *F. ploymorphus* 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 medium of higher viscosity (Halfen & Castenholz, 1971, Ridgway & Lewin, 1988). Both observations may suggest the involvement of mechanoreceptors.

Figure 2 shows three zones with different motility patterns. 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 few reversals. The horizontal and vertical component 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 happened when they had depleted their nitrate reserve. Thus, the sulfide gradient in the tubes seems to provide insufficient cue for the organisms for a spatially oriented net movement. In the oxic zone above the mat the general pattern was similar to 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 stayed there (e.g. rightmost filament in the oxic zone, Fig. 2).

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 sense and to remain confined within an optimal microenvironment. But also *Flexibacter polymorphus* filaments of different lengths reverse with a frequency inverse proportional to their filament length (Ridgway & Lewin, 1988). These observations point towards ubiquitous gliding response mechanisms in bacteria.

Reversals of gliding direction happened simultaneously over the entire filament length in the present experiments. Short filaments reversed completely when they entered the oxic zone (Møller*, et al.*, 1985). Long *Beggiatoa* filaments, however, that glided across a steep anoxic-to-oxic interface reversed with a delay of 20-60 s and then only in the leading end of the filament. The trailing end, still in the anoxic zone, would continue forward and thus cause the filament to bend to the side at the anoxic-oxic interface. This shows that the signal transduction along the filament, which induces trailing cells to also reverse, may be interrupted at some point within the filament (Kamp*, et al.*, 2008). Signal transduction in multicellular prokaryotes requires further study to explain such behavior.

The described tactic behavior of single filaments towards oxygen concentrates the long *Beggiatoa* filaments close to the oxic-anoxic interface and also results in mat formation. A reversal due to a negative step-up response to oxygen would in itself not prevent the filaments from migrating deep down into the anoxic sediment. Therefore, an additional negative response to sulfide was proposed (Møller*, et al.*, 1985). Our observations complement the findings of Møller*, et al.* (1985) by providing a mechanism that keeps *Beggiatoa* confined to the oxic-anoxic interface independent of a negative sulfide taxis.

Our experiments with *Beggiatoa* in gradient cultures without sulfide in the bottom agar suggest that sulfide is not necessary for *Beggiatoa* to form a mat. The filaments rather orient towards the oxic-anoxic interface. However, without sulfide many filaments remain below the mat in the anoxic zone whereas in tubes with sulfide filaments are quite rare in the sulfidic zone. Hence, sulfide helps to confine the *Beggiatoa* population to the microoxic zone above the depth where dissolved sulfide first appears. These experiments suggest that *Beggiatoa* performed a negative tactic response to sulfide, but the mechanism could not be elucidated with our experiments. The sulfide gradient in the tubes is comparable to sulfide gradients found in nature (data not shown, e.g. Preisler*, et al.*, 2007). In their natural environment *Beggiato*a do not occur below the sulfide diffusion front, which further supports the hypothesis of a negative response towards sulfide.

The paths of individual *Beggiatoa* filaments below the oxic-anoxic interface resemble a random walk (Fig 2). Average displacements from the point of origin (L) over time (t) by random walk followed the mathematical description of diffusion:

$$D = \pi L^2 / 4t \qquad \text{(Equation 1)}$$

$$L = \sqrt{\frac{D4t}{\pi}} \qquad \text{(Equation 2)}$$

where L is the net distance moved from the starting point, D is the effective diffusion coefficient, and t is time. If Equation 1 is rearranged to isolate L as a function of t and D (Equation 2), this equation can be used in a parametric fit to observed L versus t data (Fig. 3) to estimate the value of D. The best fit to the data in Fig. 3, curve B, is achieved with D set to 0.1 $mm^2\ min^{-1}$ ($1.8\ 10^{-5}\ cm^2\ s^{-1}$). Thus, *Beggiatoa* filaments appear to spread with a similar diffusion coefficient as 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

The anchoring by high reversal frequency can explain how *Beggiatoa* are able to stay in the oxygen-sulfide interface in the gradient tubes. We will now explore the tactic mechanism by which the filaments find this interface in a simple kinematic model. We first describe the random walk of *Beggiatoa* in the absence of chemical clues. A typical *Beggiatoa* filament from Figure 2 had 285 cells, each 2.7 μm long and 6.3 μm in diameter, and the entire filament was 0.77 mm long. It glided at a speed of 2.7 μm s^{-1} at 17º C. Model iterations in 1 s increments imply that each cell along the filament moved to the position of the cell in front of it. Note that the model keeps track of the position of each individual cell in space and does not rely on a grid. A stochastic error of a few degrees in the direction of the apical cell was allowed for each one-second iteration. The probability of reversal, applied to the modeled filament, was calculated as a function of time from last reversal based on the reversal frequencies of an actual filament from Fig. 2. Thus, the model has only one free parameter, namely the maximum magnitude (α) of the stochastic error on the direction of the lead cell.

The initial calibration of the model was done by visually comparing the modeled filament tracks to real *Beggiatoa* tracks in transparent media while manipulating α. This simple method proved surprisingly sensitive. Numerical confirmation was obtained by comparing the mean displacement from the origin as a function of time between modeled and real *Beggiatoa* with the same gliding speed (Fig. 3). Both verification methods considered the vertical and the horizontal dimension since this is the information available from the real *Beggiatoa*. The results of the model were very similar to the real observed behavior of filaments (Fig. 2, video S1 in supplementary material). We thus conclude that the model provides a good mechanistic description of how *Beggiatoa* filaments move by "random gliding" through a uniform environment with no chemotactic clue.

We tested if the model could mimic the diurnal distribution pattern observed in a hypersaline microbial mat containing *Beggiatoa* (Hinck*, et al.*, 2007). During 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 in a narrow mat. At night, however, oxygenic photosynthesis stopped and oxygen retreated to the upper 2 mm. The

Screenshot of Video S1: Modeled *Beggiatoa* filaments in a virtual environment show the same distribution pattern like observed in agar tubes with opposing gradients of oxygen and sulfide.

Beggiatoa did not spread out evenly in the space between oxygen and sulfide (Hinck*, et al.*, 2007).We modeled this scenario by seeding the model with 1000 virtual *Beggiatoa* filaments according to the depth distribution during day (Fig. 6, white bars). We then allowed the filaments to glide randomly for 14 hours 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 halophile strain. The distribution after 14 hours of free roaming (Fig. 6, black bars) was close to the *in situ* distribution (Hinck*, et al.*, 2007). The halophile *Beggiatoa* did not disperse throughout the oxygen and sulfide free zone during night, even though the oxygen diffusion front was 5 mm above the sediment layer with the highest *Beggiatoa* biomass, because the filaments glided too slow in order to spread further within 14 hours of darkness.

The good performance of the model with *Beggiatoa* in the hypersaline mat suggests that the model can describe the dispersal in the sediment. 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). We picked *Beggiatoa* from this few-cm thick surface layer in sediments of Århus Bay (Denmark) and observed that the filaments had similar motility patterns in transparent media as the

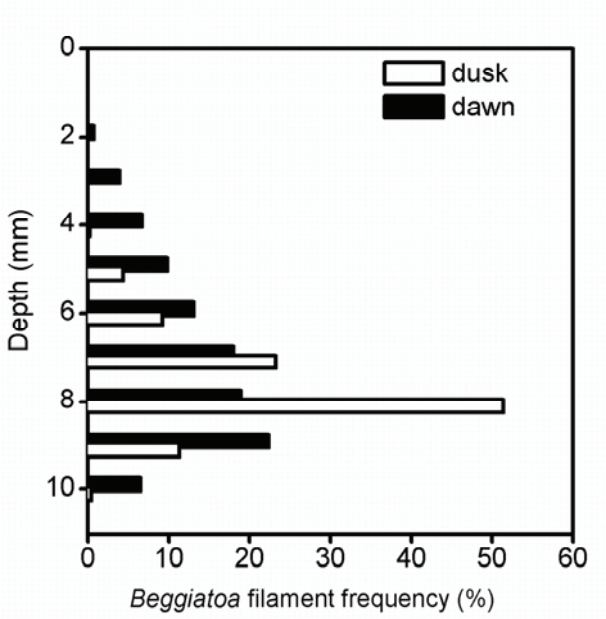

Figure 6: Daytime distribution of *Beggiatoa* in a hypersaline mat plotted as white bars (data redrawn from (Hinck*, et al.*, 2007)). Black bars show the distribution of *Beggiatoa* predicted by the model after 14 hours of darkness. Compare to (Hinck*, et al.*, 2007) Figure 1.

Beggiatoa culture used in this study (data not shown). Thus, we conclude that the model can be applied directly to *Beggiatoa* living within anoxic marine sediment. In this environment *Beggiatoa* has no direct access to oxygen but use instead nitrate accumulated in intracellular vacuoles as electron acceptor for their respiration and chemosynthetic metabolism.

We applied the mechanistic model of *Beggiatoa* movement to the suboxic zone using data typical of a coastal sediment such as that from Århus Bay. The typical oxygen and nitrate penetrations 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). In the model, as for the hypersaline *Beggiatoa*, a virtual filament in the suboxic zone is forced to reverse its direction of gliding if the lead cell reaches the oxic zone above or the sulfide front below. Otherwise it is allowed to roam freely in the sediment. We are interested in the frequency distribution of filament excursions into the suboxic zone where oxygen, nitrate and sulfide are not present in detectable amounts. Thus, we record the length of each random excursion from the moment the filament crosses from the nitrate containing zone above into the nitrate free zone, and until random gliding brings it back into the

nitrate zone. As expected, many excursions are short since the convoluted path gives a high probability of moving right back into the nitrate zone. Figure 7 (solid line) shows how the vast majority of the excursions are shorter than one day. To judge the importance of longer excursions we must, however, consider the time spent on excursions of various lengths rather than their mere frequency (Fig. 7, broken line). This reveals 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 these 10 days 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.

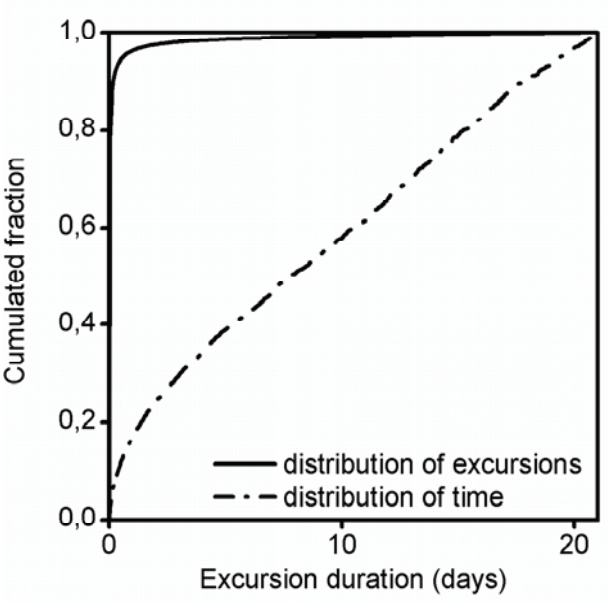

Figure 7: Solid line: The cumulated frequency of excursions of various durations. Dotted and dashed line: The cumulated time a model *Beggiatoa* spends on excursions of various durations.

This explains two enigmas about *Beggiatoa*: First, that there is no depth dependence in the nitrate concentration of *Beggiatoa* 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.

Although sulfide is not detectable in the suboxic sediment, radiotracer measurements of sulfate reduction reveal that sulfide is indeed produced in this zone. The

free sulfide is just turning over too fast to accumulate to detectable concentration, for example due to sulfide uptake by *Beggiatoa* (Jørgensen & Nelson, 2004). The endurance of *Beggiato*a in the suboxic zone is therefore not limited by sulfide availability but is ultimately limited by electron acceptor constraints. Preisler*, et al.*, 2007 observed that similar *Beggiatoa* depleted their nitrate storage by 13 mM per day when kept in 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 presumably died. The virtual *Beggiatoa* in Fig. 7 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 acceptor during long random trips.

In summary, it appears that *Beggiatoa* 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 maintain their optimal position within the suboxic environment. Simple random walk coupled to a long endurance will assure that they sooner or later find their way back to the surface where they may replenish their nitrate tanks. If both electron acceptor and sulfide are available they can stay 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

Lars Peter Nielsen and Dirk de Beer are thanked for helpful discussions on motility patterns in *Beggiatoa* which gave the impulse to the present study. We thank Heide Schulz-Vogt for supporting the work of Anja Kamp and for the use of the camera equipment of the Ecophysiology group at the Max Planck Institute in Bremen. We thank her and Douglas C. Nelson for providing *Beggiatoa* cultures originating from corals infected with the black-band coral disease. This work was financially supported by the Max Planck Society.

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2.4.

A new approach to study *Beggiatoa* spp. behavior in oxygen gradients

Background

The 1-200 µm thick filamentous sulfur bacteria *Beggiatoa* spp. occur in environments with opposing oxygen and sulfide gradients that oftentimes overlap (Jørgensen & Revsbech, 1983, Nelson*, et al.*, 1986b). Both elevated oxygen and sulfide concentrations are toxic to *Beggiatoa*. The phobic response of the population to oxygen is well known (e.g. Møller*, et al.*, 1985, Nelson*, et al.*, 1986b), however, the mechanism behind this behavior remains unclear. A key to the underlying mechanism is the close observation of the movement of single filaments under the microscope. The determination of the local oxygen concentration, however, represents a challenge. Microsensor measurements with an oxygen sensor inserted from the side proved impractical because the maneuverability of the microscope stage was impaired. Since the simultaneous measurement with an oxygen microsensor was unsatisfactory, we developed and applied an optical method to measure oxygen. Here we present this new approach to monitor the oxygen concentration in a microscope chamber filled with *Beggiatoa* filaments. The method allows to determine the oxygen concentration in two dimensions and to simultaneously follow the movements of the filaments. Thus the behavior of the bacteria can be correlated to the oxygen concentration they experience with high spatial and temporal resolution.

Since the development of optical methods for the measurement of oxygen they became increasingly popular and have been used for a variety of environmental applications (e.g. Glud*, et al.*, 2001, Polerecky*, et al.*, 2006) and combined with microscopy (Kühl*, et al.*, 2007). The technique relies on the fluorescence quenching of a luminescent dye by oxygen. The oxygen-sensitive dye is excited at an adequate wavelength and the emitted light is detected. Both the luminescence intensity, and the luminescence lifetime can be used to measure the oxygen concentration. The oxygen-sensitive dye can be immobilized in a matrix and applied as a thin layer to a transparent

surface, permitting the measurement of oxygen concentration in two dimensions in a variety of environments.

Materials and Methods

Oxygen was measured in a simple chamber constructed on a microscope slide with oxygen sensitive coating. All sides but one were sealed with black electrical tape and Vaseline. On the open side oxygen was allowed to diffuse into the chamber. The top consisted of a long cover slip. The final chamber was 26 mm x 5mm.

The oxygen sensitive coating

An object slide was coated with 4% Pt(II)meso-Tetra(pentafluorophenyl)porphyrin (Frontier Scientific Inc.) immobilized in polystyrene and dissolved in chloroforme. The coating was applied in a 10-20 µm thick transparent layer by knife-coating. The Pt-Porphyrin complex is a fluorophore and its fluorescence is quenched by oxygen. Thus the fluorescence intensity can be converted in 2-dimensional images of the oxygen distribution on the slide.

Measurements and calibration

The build-in mercury lamp of the epifluorescence microscope (Axioskop, Zeiss) was used as excitation light source for the oxygen measurements with an excitation filter in place (Schott BG12). The fluorescence intensity on the slide was imaged with a digital camera attached to the microscope (Canon Powershot A 620) with an emission filter in front of the objective (Kodak deep red wratten filter). A two-point area averaging calibration was performed with sea water at 0% and 100% air saturation, respectively. After calibration, the chamber was filled with water overlying a *Beggiatoa* laboratory culture and populated with *Beggiatoa* filaments. Oxygen and brightfield images were taken at intervals ranging from 10-60 min. Oxygen concentrations were calculated based on the fluorescence intensity of the fluorophore using a modification of the Stern-Volmer equation (Amao*, et al.*, 2000).

Results and conclusions

In the chamber filled with water from the *Beggiatoa* laboratory culture but without filaments oxygen is present at a low concentration and decreases gradually towards the center of the slide (Fig. 1A). The remaining oxygen is presumably consumed by other bacteria populating the laboratory culture. After 15 min a significant change in the distribution can be observed (Fig. 1B). After 90 min a stable gradient has developed. The

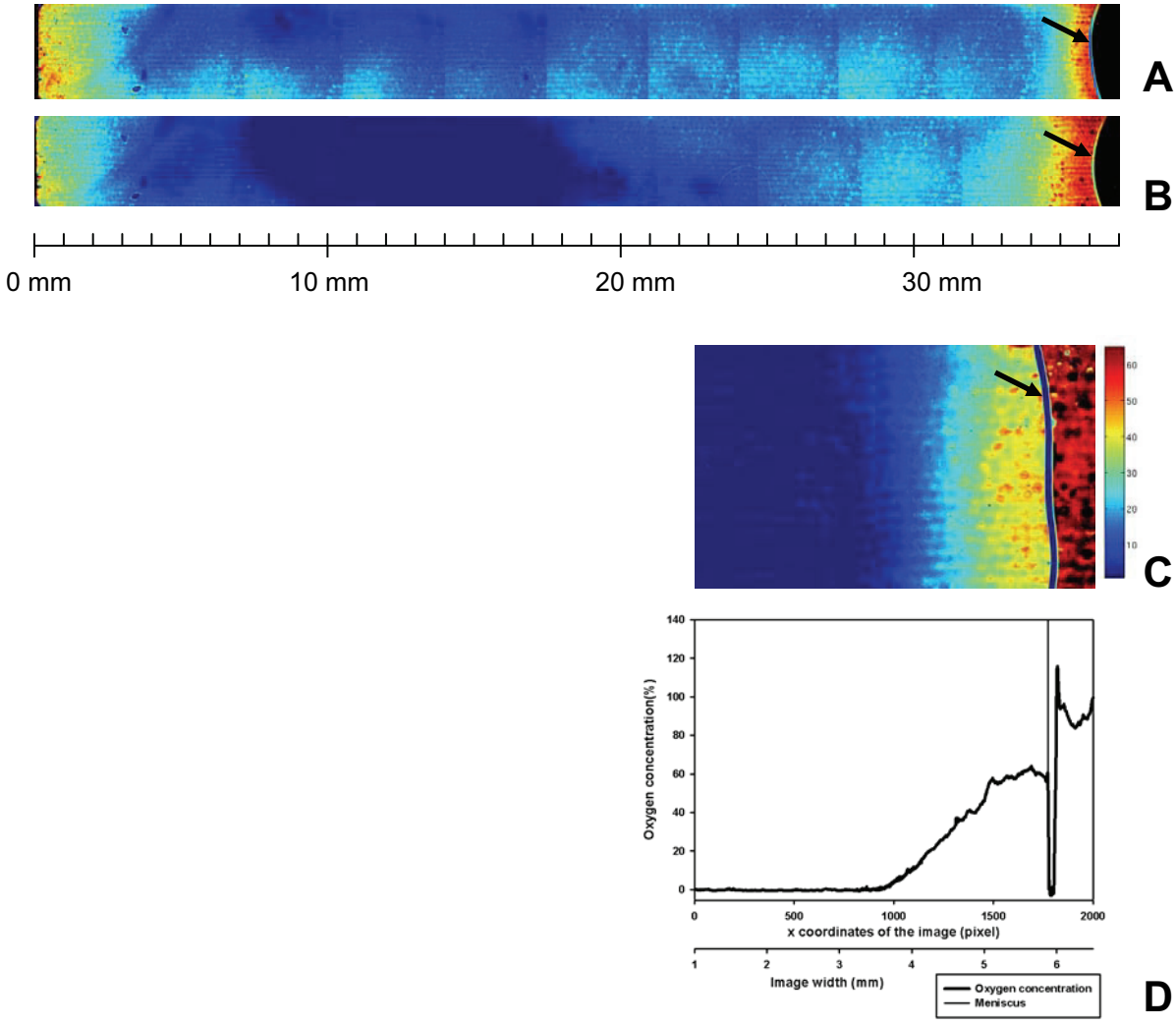

Figure 1: Distribution of the oxygen saturation in the slide chamber without *Beggiatoa* filaments A: immediately after filling in the medium, B: 15 minutes later C: 200 min later (note the different scale). D: Oxygen concentration profile through C. The apparently high oxygen concentration at the meniscus is an artifact. The dashed line indicates the presumable progress of the oxygen concentration. The menisci in the images are indicated by arrows. The chamber is 36 mm long.

oxygen saturation is 100 % at the meniscus and 0 % after 1.5 mm (Fig. 1C, D). The oxygen saturation in the chamber is high where oxygen diffuses in at the air/water interface at the right and at some leakage in the chamber on the left side (Fig. 1A, B).

When adding *Beggiatoa* filaments to the chamber, they accumulated where the oxygen saturation is < 10 % (Fig. 2A and B). This is in accordance with former observations (eg. Jørgensen & Revsbech, 1983). Some filament loops stick out but are never exposed to long terms of elevated oxygen concentration because the filaments glide constantly.

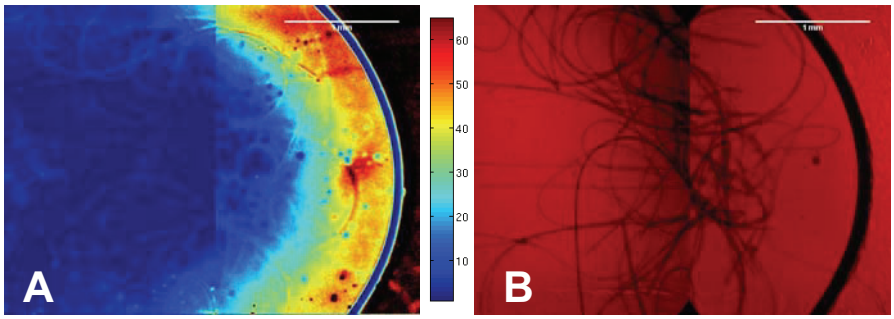

Figure 2: Meniscus and first 3 mm of slide after stabilisation of the gradient. A: Colour map of the oxygen saturation, B: Distribution of the *Beggiatoa* filaments. Bars correspond to 1 mm.

Oxygen and brightfield images from the *Beggiatoa* filaments could be obtained simultaneouly. This allowed monitoring of their movement and orientation in the oxygen gradient with high precision and in two dimensions. A stable oxygen gradient built up within the chamber that was maintained for at least 5 hours. Oxygen imaging not only contains more information on the oxygen distribution than one-dimensional microsensor profiles, it is also less invasive since no mechanical penetration of the chamber is necessary. Furthermore, the temporal resolution of measurements can be higher. Oxygen penetrating the chamber from the sides could be detected that would have been overlooked in conventional microsensor measurements. No other technique than microscopical observation needs to be used and the same optical path can be used for both oxygen and bright field imaging. This renders the method less invasive and benefits the mechanical stability of the setup compared to common microsensor measurements. Evaporation can be largely eliminated, because the open side of the chamber can be minimized.

Limitations of the method are given by artifacts at the medium interfaces and the sulfur inclusions of the *Beggiatoa* filaments that scatter light and therefore disturb oxygen imaging. Thus, the oxygen gradient around single filaments could not be measured.

This work was done in collaboration with J. Fischer, H. Røy and B. B. Jørgensen. It was presented at the 10th Symposium on Aquatic Microbial Ecology 2007 in Faro, Portugal as a poster presentation.

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3. Conclusion and Outlook

This thesis is a continuation of my Master's thesis on microsensor measurements on *Beggiatoa* mats from Århus Bay, Denmark (Dunker, 2005). Intriguing motility patterns were observed in the mats while changing the oxygen concentration in a laboratory mesocosm. Hence, the main objective of this dissertation was to study the motility patterns of single filaments and the underlying mechanisms. The observation of the single filaments should provide deeper insight in the behavior on community level.

First experiments aimed at the cultivation of the specimen from the laboratory mesocosm in agar tubes with opposing gradients of oxygen and sulfide. The attempts remained unsuccessful despite great effort. So far only narrow specimen have been cultivated (Nelson*, et al.*, 1982b, Nelson & Jannasch, 1983, Kamp*, et al.*, 2006, Hinck*, et al.*, 2007, Kamp*, et al.*, 2008), and the wider ones (> 10 µm diameter) resisted cultivation in tubes. The majority of the Århus Bay filaments were about 10-35 µm wide with few individuals of 5 µm width. In the following a different experimental platform was chosen: A custom-made microscope chamber with two compartments that allowed access from the side with a microsensor and the circulation of fluids in a compartment separate from the filaments. This experimental setup provided the base for the measurement of temperature dependent motility in *Beggiatoa* from different climatic origins.

The temperature response observed in single filaments of tropical, temperate and permanently cold *Beggiatoa* from the Arctic provided new insights in the temperature control of gliding motility. The temperature dependent gliding motility in *Beggiatoa* followed the same pattern as observed for the gliding motility of other filamentous gliding bacteria (Crozier & Federighi, 1924, Crozier & Stier, 1926, Halfen & Castenholz, 1971, Ridgway & Lewin, 1988) and for the physiology of enzymatically controlled processes in many other microorganisms (e.g. Thamdrup & Fleischer, 1998, Knoblauch & Jørgensen, 1999, Rysgaard*, et al.*, 2004). Hence, the main control over the gliding speed at changing temperatures is by enzyme kinetics of the enzymes involved in gliding motility and not by physical factors such as increasing viscosity of fluids at low ambient temperature (Dunker*, et al.*, 2010, chapter 2.1. of this thesis). In addition, the temperature range for gliding motility and the optimum temperature for gliding of filaments from

each climatic zone were identified, proving that the filaments from each origin were well adapted to the prevailing temperature regime. The acclimatization potential of a temperate *Beggiatoa* community to seasonal changes in ambient temperature was examined, showing that within months the filaments could extend their temperature range at the cold end and even became resistant to transient freezing while keeping their optimum temperature (Dunker*, et al.*, 2010). Other acclimatization experiments performed by Robador*, et al.* (2009), who acclimatized a sulfate reducing community to different temperatures resulted in a shift of the optimum temperature for sulfate reduction. Hence, acclimatization to variations in temperature either on seasonal scale or on a longer term seems to be a survival strategy of microorganisms in changing environments. The acclimatization occurs possibly within a single filament type but a community shift from psychrotolerant to mesophilic groups cannot be ruled out. It is questionable if this can be revealed with the currently available phylogenetic tools, because psychrophily is a polyphyletic characteristic (Sahm*, et al.*, 1999) which impedes the discrimination of temperature adaptations based on phylogeny. For instance, a *Beggiatoa* strain from the Arctic clustered with temperate strains from coastal regions instead with other permanently cold *Beggiatoa* from the deep sea (Jørgensen*, et al.*, 2010). More specific phylogenetic probes would be necessary to detect single *Beggiatoa* strains in the community to determine the mechanism behind the acclimatization process.

Our method is a helpful tool to quickly assess the temperature response of gliding motility in microorganisms and its application has revealed in recent experiments that Guaymas Basin *Beggiatoa* have a psychrophilic response to temperature (H. Røy, personal communication).

The first study was followed by a report on arctic marine *Beggiatoa* (Jørgensen*, et al.*, 2010, chapter 2.2. of this thesis). Although *Beggiatoa* from permanently cold environments have been described before (de Beer*, et al.*, 2006, Niemann*, et al.*, 2006), in this study *Beggiatoa* receive attention with regard to their chemical environment, spatial distribution, phylogeny and nitrate storage capacity. As our results on temperature dependence for the gliding motility suggested, the arctic *Beggiatoa* are equally well adapted to the permanent cold as temperate filaments are to temperate environments (Dunker*, et al.*, 2010). The filaments were abundant in some of the explored sediments

and were closely related to other large, marine, nitrate-storing *Beggiatoa*. They occurred predominantly in two different kinds of habitats: Sediment in shallow water which was characterized by overlapping gradients of oxygen and sulfide at the sediment surface, on top of which *Beggiatoa* formed mats, and sediment at several hundred meters depth where sulfate reduction rates were high but no sulfide was detectable down to 3 cm. In this sediment filaments were distributed throughout the upper 5 cm without forming mats on the sediment surface.

The large size of *Beggiatoa* results in a high total biomass in the sediment (7-15 %). This is in extreme contrast to the low numbers of filaments in the sediment (10-1000 filaments cm^{-3}, each comprising several hundreds to thousands of cells, compared to 10^9 bacterial cells cm^{-3} in fjord sediments). As to numbers, they belong to the "rare biosphere" as defined by Sogin*, et al.*, (2006, Jørgensen*, et al.*, 2010).

The study on *Beggiatoa* in oxygen/sulfide gradients elucidated motility patterns that lead to the typical distribution of *Beggiatoa* on top of the sediment and in the suboxic zone of sediments with a high sulfide flux (e.g. Jørgensen & Revsbech, 1983, Mussmann*, et al.*, 2003, Preisler*, et al.*, 2007, Jørgensen*, et al.*, 2010). The ability of filaments to anchor and form mats in their preferred habitat, namely low concentrations of oxygen and sulfide, was shown for the first time in such detail. The anchoring is accomplished by gliding shorter distances than filament length between the reversals. It is quite effective and filaments leave the mat very seldom if oxygen and sulfide overlap. If filaments leave the mat they lack the chemical cue provided by the mat environment, and the filaments switch to gliding long random trails until they reach the mat again or die in case they run out of internally stored nitrate (Dunker*, et al.*, submitted, chapter 2.3. of this thesis). Therefore, the main implication of gliding long random trails in the anoxic zone is the necessity to accumulate high concentrations of nitrate as alternative electron acceptor to oxygen as it has been described in many studies (e.g. Mussmann*, et al.*, 2003, Preisler*, et al.*, 2007). Given the high numbers of filaments in the suboxic zone where neither oxygen nor sulfide are present, the filaments may sense by an internal trigger when it is time to refill their sulfur storage. Consequently, they leave the mat and roam in the suboxic zone where sulfide is not detectable despite a constant sulfide production by sulfate reduction (e.g. Dunker, 2005, Jørgensen*, et al.*, 2010). The time period that filaments can glide in

the suboxic zone depends on their nitrate storage capacity, their gliding speed and their motility ("diffusivity"). To which depth they can advance in the sulfidic zone additionally depends on their tolerance of a high sulfide concentration.

In future studies this concept should be verified by correlating the gliding speed of the filaments, their distribution and nitrate storage capacity and the depth of the suboxic zone. For this purpose the model for *Beggiatoa* distribution provides a great tool to test how far *Beggiatoa* can spread within a given time frame. This is exemplified in hypersaline *Beggiatoa* from microbial mats that apparently do not obey our findings of gliding long random trails if separated from the oxygen front. Garcia-Pichel*, et al.* (1994) found that two *Beggiatoa* subpopulations were present in a mat: One followed the oxygen boundary to the surface of the mat during nighttime whereas the other stayed at the depth where both populations were located during daytime when oxygen penetration into the mat inhibited *Beggiatoa*. Hinck*, et al.*, (2007) discovered that the filaments from a hypersaline microbial mat did not at all follow the withdrawing oxygen front and only spread little during the dark period. Feeding the model with actual gliding speeds of the filaments (Hinck*, et al.*, 2007), it turned out that within the time available during dark period the modeled filaments distributed as observed in the real filaments (Dunker*, et al.*, submitted).

The role of sulfide remains to be clarified, since filaments in agar tubes also formed mats in the absence of sulfide (Dunker*, et al.*, submitted), and the mechanism of anchoring and gliding long random trails did also not rely on the presence of sulfide. Conditions of high sulfide concentrations were avoided, most likely due to a negative tactic response towards sulfide as proposed by some authors (Preisler*, et al.*, 2007). Filaments that glided for a too long period in the sulfidic zone died due to a depletion of their internal nitrate storage (Dunker*, et al.*, submitted), but also the toxicity of sulfide killed the filaments once they glided into regions of intolerable concentrations as it had been observed in some agar tubes. Sulfide may also be involved in inducing cell death of single cells in the filaments. Lysis of single cells within a filament is the main reason for filament breakage and multiplication (Kamp*, et al.*, 2008). Hence, sulfide does not only serve as electron donor but also may also play a role in the population ecology of *Beggiatoa*.

Our observations of the reversal behavior in *Beggiatoa* filaments give the direction for future investigations on the underlying mechanism of cell-to-cell communication. As reversals are completed faster than the diffusion time of a substance along the length of an entire filament, diffusion of a chemical signaling molecule can probably be ruled out. An electrical signal could be propagated along the filament in much shorter time than the diffusion of a molecule and could therefore play a role in signal transduction in *Beggiatoa* cells. Electrical signaling has been observed in filamentous cyanobacteria. A phobic reaction to light induced externally measurable potential changes in the cell membrane (Häder, 1978, Häder, 1987). The hypothesis of an electrochemical signal gains further support by the observation of Kamp*, et al.* (2008) that the movement direction within a filament becomes asynchronous at the position of dead cells within the filament, which points towards an interruption of cell to cell communication. No interruption of communication would occur during diffusion of a signaling substance.

Beggiatoa remain exciting organisms to study, and each finding opens more questions. Being key players in their habitats for the cycling of sulfur, nitrate and carbon the study of *Beggiatoa* and their close relatives *Thioploca* and *Thiomargarita* deserves incessant attention.

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Contribution to manuscripts

1. **Temperature regulation of gliding motility in filamentous sulfur bacteria, *Beggiatoa* spp.**

   R. Dunker, H. Røy, B. B. Jørgensen

   Concept by R. Dunker, H. Røy and B. B. Jørgensen, measurements by R. Dunker, writing of manuscript by R. Dunker with editorial help from H. Røy and B. B. Jørgensen.

2. **Filamentous sulfur bacteria, *Beggiatoa* spp., in arctic marine sediments (Svalbard, 79° N)**

   B. B. Jørgensen, R. Dunker, S. Grünke, H. Røy

   Concept by B. B. Jørgensen, biomass determinations and chemical analyses by B. B. Jørgensen and R. Dunker, sulfate reduction rates by B.B. Jørgensen, microsensors measurements by R. Dunker, phylogenetic analyses by S. Grünke, writing of manuscript by B. B. Jørgensen.

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

   R. Dunker, H. Røy, A. Kamp, B. B. Jørgensen

   Concept by R. Dunker, H. Røy and B. B. Jørgensen, imaging by R. Dunker and A. Kamp, image analyses by R. Dunker, writing of manuscript by R. Dunker with editorial help of H. Røy, A. Kamp and B. B. Jørgensen.

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Erklärung

Hierdurch erkläre ich, dass die Dissertation zum Thema „Motility of the giant sulfur bacteria *Beggiatoa* in the marine environment“ selbstständig verfasst und alle benutzten Hilfmittel sowie evtl. zur Hilfeleistung herangezogenen Institutionen vollständig angegeben wurden. Die Dissertation wurde nicht schon als Diplom- oder ähnliche Prüfungsarbeit verwendet.

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