The genus *Beggiatoa* and its possible effects on the nutrient cycles of the Baltic Sea

Bachelor's thesis
Microbiology
(orig.) December 2012
(transl. & review) May 2014

Contents

1 Summary

The genus *Beggiatoa* consists of multicellular, filamentous and gliding bacteria that oxidize hydrogen sulfide with oxygen or nitrate and live in opposite gradients of sulfide and oxygen or nitrate. These bacteria form visible mats in the bottom of water bodies and are, thus, easy to spot especially in shallow waters. Maybe that is part of the reason why Winogradsky started researching cultures of *Beggiatoa*. Through his studies he discovered the concept of bacterial lithotrophy already in the 19th century. A period of inactivity in *Beggiatoa* studies followed after Winogradsky, but during the last 35 years a steadily growing body of research on *Beggiatoa* has been forming, which has elucidated the great metabolic, physiological and ecological diversity of these bacteria.

Beggiatoa takes part in several ecologically significant biogeochemical cycles in its habitats. The filaments accumulate e.g. elemental sulfur, polyhydroxybutyrate, polyphosphate and nitrate in high concentrations. In some cases a large part of the total content of the biomass, sulfur or nitrate of the sediment can be concentrated in *Beggiatoa* filaments. In addition, these filaments take active part in cycling of chemical substances. One of their most defining features is oxidation of sulfide. Sulfide is produced usually in the degradation of organic matter and it is toxic especially to the bottom fauna. There has been some controversy on the metabolic capabilities of the strains of *Beggiatoa* which use nitrate as their terminal electron acceptor (TEA), because some strains seem capable of Dissimilaritory Nitrate Reduction to Ammonium (DNRA) and others of denitrification. Beggiatoa seem to have also various other, possible ecologically relevant pathways as uncovered by a genomic study.

Beggiatoa inhabits sulfidic sediments with low oxygen concentrations and the Baltic Sea offers ideal conditions in many places for the growth of these bacteria. The extent of the sulfidic sediments in the Baltic Proper which suffer from hypoxia is on the order of 10^5 square kilometers and on many coastal areas the features of the sea bottoms and the large inputs of organic matter cause local anoxia. Accordingly, mats of *Beggiatoa* have been found both from the Baltic Proper in the Gotland Deep and also from the bottoms of the archipelagos. The biogeochemical cycles of nitrogen and phosphorus in the Baltic Sea needed for the growth of photoautotrophic organisms are still partly unclear despite a large research effort. The sediments are known to be very important in the processing of both of these nutrients.

Through the combination of its wide occurence and metabolic capabilities, *Beggiatoa* might have both local and Baltic Sea -wide effects that have been previously overlooked. More research is needed on both the physiology and ecology of *Beggiatoa* and also on its ecological effects in the Baltic Sea and other habitats.

2 Introduction

Genus *Beggiatoa* belongs to the γ-proteobacteria and the bacteria are filamentous, multicellular, gliding and oxidize hydrogen sulfide with either oxygen of nitrate (Teske & Nelson 2006). They are sometimes visible even to the naked eye when growing as large white mats on top of sediments. They are also easy to discern and identify microscopically because of their large size and the visible inclusions of elemental sulfur. The largest filaments of *Beggiatoa* discovered are found from the top of the prokaryotic size spectrum, with a diameter of 140 µm (Ahmad et al. 2006). The genus is also is also the source of a major discovery during the history of microbiology. Even though the chemolithotrophic capabilities of self *Beggiatoa* have been temporarily questioned (Teske & Nelson 2006), Winogradsky formulated the theory of chemolithotrophic growth of bacteria by examining cultures of *Beggiatoa* already in the year 1888 (Winogradsky 1888). Certain marine strains are known, in addition to sulfur, to accumulate soluble nitrate in internal vacuoles and polyphosphate and polyhydroxybutyrate as solids in inclusions (Teske & Nelson 2006). In this way they take part in the biogeochemical cycles of carbon, nitrogen, phosphorus, sulfur and oxygen.

Beggiatoa occurs in many environments which have a low oxygen concentration (Teske & Nelson 2006). Also the hypoxic sediments in the area of the Baltic Sea are opportune for the occurrence of *Beggiatoa* and mats of *Beggiatoa* have been found from a large part of these environments (Jonsson & Jonsson 1988, Rosenberg & Diaz 1993, Emeis et al. 2000, Preisler et al. 2007). In certain investigated areas a large part of the bacterial biomass of the whole sediment (Jørgensen 1977) and nitrate appears to be concentrated in *Beggiatoa* filaments (McHatton et al. 1996). Even though the importance of *Beggiatoa* in many ecosystems has been recognized, on the Baltic Sea their occurrence and their effect on the nutrient cycles has not been investigated thoroughly. These bacteria seem to have at least locally substantial effects in many parts of the Baltic Sea.

The purpose of this study is to review the general features of the genus *Beggiatoa*, to introduce the relevant research about the genus in connection to the Baltic Sea and on the basis of this information to draw conclusions about the effects of *Beggiatoa* on the nutrient cycles of the Baltic Sea. Section 3 deals with the morphology and motility, taxonomy, genetics, metabolism, and ecology of *Beggiatoa*. Subsection 3.5 deals with the metabolic capabilities of the genus, concentrating on the processing of carbon, nitrogen, phosphorus and sulfur. Subsection 3.6 looks over the ecology of *Beggiatoa* on a general level, after which the observations made of *Beggiatoa* in their habitats in the Baltic Sea are covered in the subsection 3.7. Because the vacuolated strains seem to be more common on the Baltic Sea, the ecology section concentrates mostly on the features of the vacuolated *Beggiatoa*. The basic features of the Baltic Sea are covered in the section 4, concentrating on hypoxic and anoxic

bottom areas, which are important both as a niche habitat of the *Beggiatoa* and for the biogeochemical nutrient cycles. Subsection 4.2 outlines the currently known features of the cycles of nitrogen, phosphorus and sulfur in the Baltic Sea, combining the possible effects of *Beggiatoa* with these. Section 6 synthesizes the results of this literary review and makes suggestions about the possible needs of further research in this field.

3 *Beggiatoa*

3.1 Overview of the genus *Beggiatoa*

Beggiatoa is prevalent in both fresh and saline waters in the sediments, where a sulfide gradient originating from either organic or inorganic sources overlaps with an oxygen gradient (Teske & Nelson 2006). For *Beggiatoa*, sulfide is the most usual electron donor, with oxygen as the electron acceptor, but some strains can use nitrate as the TEA instead of oxygen. *Beggiatoa* grows usually inside the sediment or on the top of the seafloor as mats or tufts, the color of which varies from white to yellow and orange. As they grow, the filaments follow the overlapping interface of oxygen and sulfide by gliding and compete efficiently against the chemical oxidation of the sulfide, which occurs naturally in the sediment. Thus, *Beggiatoa* plays a part in keeping the sulfide, toxic to many aquatic organisms, out of the water column. The mat forming in the interface of the sulfide-oxygen -gradients can be under one millimeter thick (Jørgensen & Revsbech 1983), but some mats that grow in the deeps of the Guyamas region in California and use nitrate instead of oxygen as their electron acceptor can have a thickness of even 30 cm (McHatton et al. 1996).

In freshwater *Beggiatoa* has been found from e.g. eutrophicated lakes (Sweerts et al. 1990), stream waters (Faust & Wolfe 1961), rice paddies (Joshi & Hollis 1977), hot springs (Nelson & Castenholz 1981a) and water treatment plants (Williams & Unze 1985). In marine or brackish waters *Beggiatoa* is common on anoxic and hypoxic seafloors (Graco et al. 2001), coastal areas with a high organic content in the sediment (Jørgensen 1977, Mußmann et al. 2003), in connection to black smokers and cold seeps (McHatton et al. 1996), on salt marshes (Strohl & Larkin 1978) and e.g. in the black band disease as a part of the moving biofilm that causes tissue death and gradually destroys the coral (Ducklow & Mitchell 1979, Richardson 1996). *Beggiatoa* has also been found from the oceanic abyssal seafloor with local occurrences in connection to whale carcasses (Bennet et al. 1994). In addition to sulfide, prerequisites for the occurrence of *Beggiatoa* are a sufficient porosity of the sediment and space for the movement of the filaments (Jørgensen 1977).

A wide range of bacteria with different types of metabolic and morphological features is included in the genus *Beggiatoa* (Ahmad et al. 2006). However, the approved taxonomical classification of the strains of the genus does not reflect the

actual phylogeny accurately. According to the current criteria, bacteria classified as *Beggiatoa* still posses the common features of being multicellular, filamentous, moving by gliding and oxidizing sulfide to elemental sulfur, which is stored inside the cells as granules (Teske & Nelson 2006). The thickness of *Beggiatoa* filaments varies between 1 to 140 µm and their length from a few micrometers to 10 cm. The filaments of *Beggiatoa* are stained Gram-negatively, which their known phylogeny supports.

3.2 Morphology and motility

Beggiatoa can be roughly divided into three main categories. The freshwater strains are heterotrophic, their filaments are narrow and they do not contain vacuoles (Strohl & Larkin 1978, Nelson & Castenholz 1981a). This group includes e.g. the strain B18LD, which is the type strain for *Beggiatoa alba* (Mezzino et al. 1984), and the filaments of which have a diameter of 3.15 µm. There are two categories of marine *Beggiatoa*. The first category, narrow autotrophic strains, like MS-81-6 do not contain vacuoles (Nelson et al. 1982, Nelson & Jannasch 1983). The filaments of this strain have a diameter of about 4.4 µm. The second category is larger autotrophic marine strains that can store nitrate in concentrations up to 370 mM in their vacuoles (McHatton et al. 1996, Mußmann et al. 2003). These bacteria have also been studied extensively, even though they have not yet been successfully isolated in pure culture. The diameters of filaments of these strains vary between 5 to 140 µm (Ahmad et al. 2006). There are also exceptions to the general categories introduced here, e.g. strain *Beggiatoa* D-402, which is chemolithotrophic and obtains its energy by oxidizing thiosulfate (Grabovich et al. 2001), and an enrichment culture isolated from fresh water that oxidizes sulfide with nitrate and consists of filaments with a diameter of about 3 µm (Kamp et al. 2006).

Even though the genus *Beggiatoa* belongs to the Gram-negative γ-proteobacteria (Ahmad et al. 2006), the structure of the cell walls and membranes of the bacteria differ from the usual three-part structure of other Gram-negative bacteria (Strohl et al. 1982, De Albuquerque et al. 2010). The strain B15LD, which is almost identical to the type strain B18LD of *Beggiatoa alba* (Mezzino et al. 1984), appears have in total 5 outer structures when examined with Transmission Electron Microscopy (TEM) (Strohl et al. 1982). Only the peptidoglycan layer and the cellular membrane surround single cells in the filament. In addition, the peptidoglycan layer is covered by four membranes that surround the whole filament continuously. The periplasmic sulfur granules are also covered by an extra membrane structure. Two *Beggiatoa* strains isolated from salt-water lagoons near Rio de Janeiro had a differing number of membrane structures when examined by TEM (De Albuquerque et al. 2010). A narrow strain had five membrane structures, while a vacuolated thick strain had only four membranes. The complicated outer structure might have to do with the harsh

conditions faced by these organisms in their habitats. In addition to the sulfur granules contained within membranes, the cells can also contain similarly stored granules of polyhydroxybutyrate (Strohl & Larkin 1978) and polyphosphate (Brock et al. 2011).

Single cells in the filaments of *Beggiatoa* are round and have intercellular spaces that are sometimes hard to discern (Teske & Nelson 2006). Cells of the narrow marine or freshwater strains have a diameter of 2 to 5 µm and their length is about 1.5 to 8 times their thickness. However, the disc-shaped cells of the vacuolated marine strains can have a diameter of over 100 µm, with a length of only 0.1 to 0.9 times their thickness. In all of the cultured strains the cells at the end of the filaments are rounded.

The filaments move by gliding and they can usually also stretch, bend or twist while moving. The speed of the gliding varies between 1 to 8 µm s^{-1} (Teske & Nelson 2006). The gliding mechanism is apparently dependent on string-like structures in the outer membrane and trans-peptidoglycan channels (De Albuquerque et al. 2010). The filaments can also divide by forming necridias, single cells in the middle of a filament that are killed (Kamp et al. 2008). Apparently, this cuts the intercellular communication between the segments of the filament surrounding the necridia, which is important for the filament's coordinated moving. The segments start to move in different directions as a consequence of the break in the communication between them, possibly form various loop structures and ultimately separate from each other at the necridia.

There are many taxes involved in the movement of *Beggiatoa* (Teske & Nelson 2006). The filaments move away from e.g. high oxygen (Møller et al. 1985) and sulfide concentrations (Preisler et al. 2007), and also from light exposure (Møller et al. 1985, Nelson & Castenholz 1982). These taxes guide the movement of the *Beggiatoa* in its environment to a place where it can optimally utilize its electron donors and acceptors (Preisler et al. 2007).

3.3 Taxonomy

Beggiatoa differs from the morphologically and phylogenetically closely related *Vitriocella* genus by its ability to oxidize sulfide and store elemental sulfur (Teske & Nelson 2006). Another close relative, *Thioploca*, lives in clusters of filaments that share a common slime sheath, unlike *Beggiatoa* whose filaments grow separately.

The genus *Beggiatoa* was originally though to be related to cyanobacteria such as *Oscillatoria* because of their similar morphology and gliding motility (Reichenbach 1981). However, on the basis of 5S rRNA sequences *Beggiatoa* was situated in an early branching clade of the γ-proteobacteria, which are only very distantly related to cyanobacteria (Stahl et al. 1987). When comparing 16S rRNA sequences the *Beggiatoa* forms an uniform clade, with a nested clade that contains the strains

that store nitrate in their internal vacuoles (Ahmad et al. 2006). It is notable that this nested clade of vacuolated bacteria also contains bacteria from genera *Thioploca* and *Thiomargarita*. Outside its slime sheath *Thioploca* is morphologically and metabolically very similar to vacuolated *Beggiatoa* strains and its slime sheath might not be a phylogenetically significant feature after all. Filamentous growth appears similarly to not be a very conserved feature, because *Thiomargarita* grows in long chains of round cells instead of filaments, despite its inclusion in the *Beggiatoa* clade. The narrow freshwater strains of *Beggiatoa* form their own clade that branches off inside the genus from its root, while the narrow marine strains branch later on as their own clade from the vacuolated strains. The relationships of *Beggiatoa* strains studied by Ahmad et al. (2006) in relation to other free-living and endosymbiotic sulfur oxidizing bacteria is also presented in a phylogenetic tree (Figure 1).

A taxonomic revision of the genus *Beggiatoa* through the comparison of 16S rRNA sequences would be justified on the basis of this previous study (Ahmad et al. 2006). A good example of the need of revision is the discovery of a group of bacteria living in extremely saline environments, which delineate clearly from the main *Beggiatoa* clade on the basis of their 16S rRNA sequences, but which morphologically resemble vacuolated *Beggiatoa* strains (Hinck et al. 2011). The name *Candidatus* Allobeggiatoa was suggested as the new name of the bacteria in this clade. These bacteria have a diameter of 6 to 14 µm and as the marine *Beggiatoa* strains they also accumulate nitrate in internal vacuoles. However, these strains can store nitrate in their vacuoles at a concentration of 650 mM, which is almost twofold compared to the highest concentration of nitrate (370 mM) measured from *Beggiatoa* strains (Mußmann et al. 2003).

3.4 Genetics

The genetics of the genus *Beggiatoa* have been studied only little, because only a few strains have been successfully isolated in pure culture (Teske & Nelson, 2006). The G + C content of *Beggiatoa alba* is from 40 to 42.7 mol % (Mezzino et al. 1984). *Beggiatoa alba* has from two to three similar plasmids with a size of about 12.8 x 10^6 M_r (Minges et al. 1983), and using a chromatographic method, the genome size of *Beggiatoa alba* strain B18LD was calculated to be 3 Mb (Genthner et al. 1985). To the best of our knowledge, there has been only one study on the genomics of *Beggiatoa*, in which the sequences obtained from two single filaments of a vacuolated strain were analyzed (Mußmann et al. 2007). The filaments originated from a sediment sample covered by a *Beggiatoa* mat at the time of the sampling, taken from the Eckenförde Bay in Germany on the coast of the Baltic Sea. The genome size of the *Beggiatoa* forming this mat was estimated to be about 7.4 Mb by optical mapping.

Two filaments of *Beggiatoa* had genes related to sulfur oxidation, respiration with

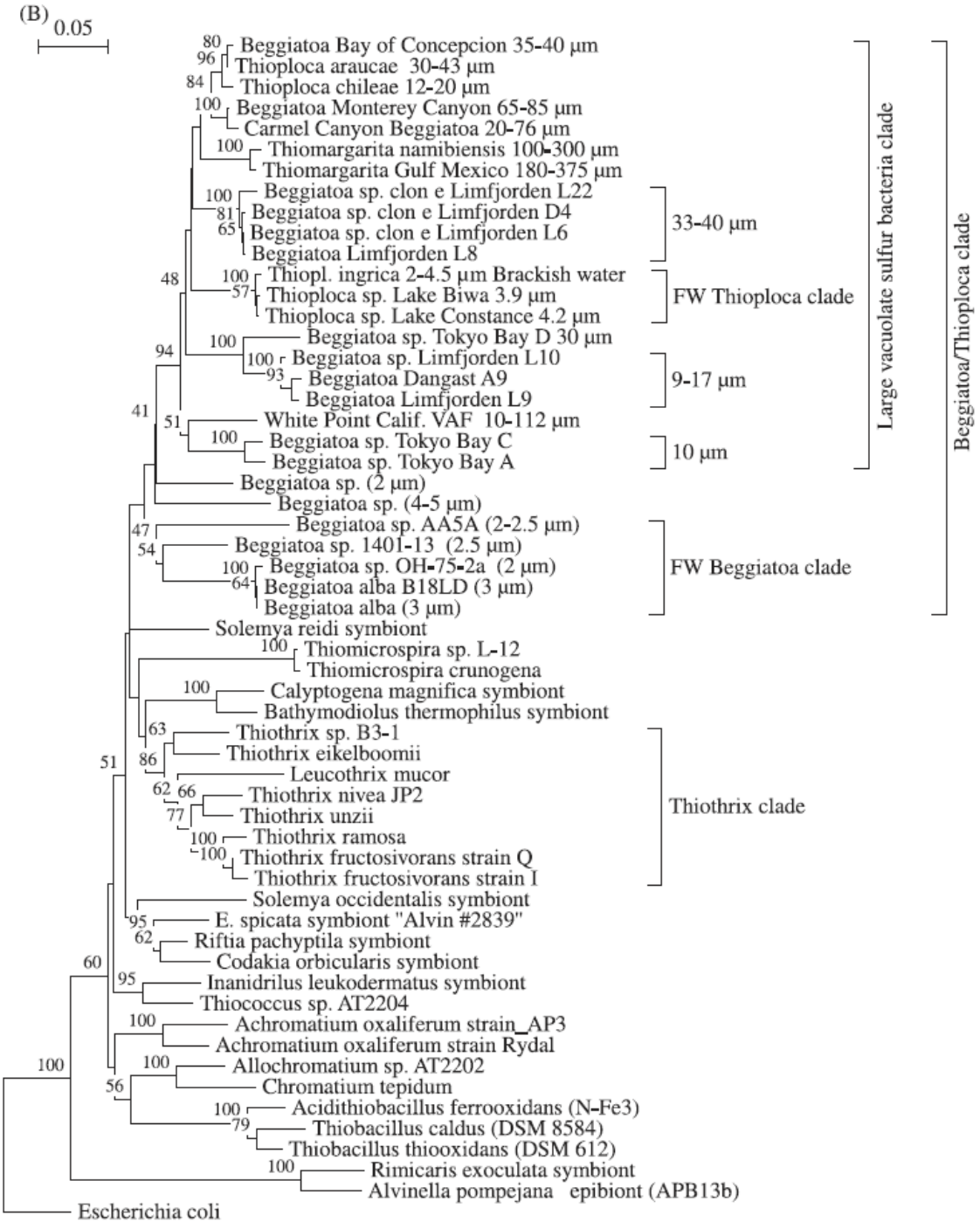

Figure 1: Phylogenetic relations of cultures of *Beggiatoa* and related uncultured strains using the neighbor-joining method (Ahmad et al. 2006). Other free-living and endosymbiotic sulfur oxidizing bacteria inside the γ-proteobacteria were used in the comparison. The size of the 16S rRNA fragment used to construct the tree was 498 nucleotides. Bootstrap values (out of 1000 repeats) over 40% are indicated at the nodes of the tree. *Escherichia coli* was used as the outgroup.

nitrate and oxygen, and carbon dioxide binding (Mußmann et al. 2007). There was also evidence from horizontal gene transfer between *Beggiatoa* and cyanobacteria. These genes of probably cyanobacterial origin contained e.g. genes for non-ribosomal peptide synthases and for exoproteins containing a hemagglutinin domain. The non-ribosomal peptide synthases are important e.g. for the production of secondary metabolites like antibiotics. The genes involved in the production of exoproteins are probably important for the gliding mechanism, the formation of slime sheaths and the self formation of the filaments.

3.5 Metabolism

All bacteria of the genus *Beggiatoa* can oxidize sulfide and store the end product sulfur intracellularly (Teske & Nelson 2006). For their other metabolic capabilities the strains of the genus can differ greatly from each other. The lifestyles of different *Beggiatoa* strains cover a wide arrangement of almost all non-photosynthetic trophic modes from obligate heterotrophy to facultative and obligate chemolithoautotrophy.

3.5.1 Carbon

Winogradsky developed his idea of chemolithoautotrophy by examining cultures of *Beggiatoa* and measuring the substances consumed and produced by them (Winogradsky 1888). However, the conclusive proof of autotrophic growth of *Beggiatoa* was obtained only when a pure culture was isolated (Nelson et al. 1982). Autotrophic strains use the Calvin cycle to bind carbon dioxide with the help of Ribulose-1,5-Bisphosphate Carboxylase/Oxygenase (RuBisCO) (Nelson & Jannasch 1983, Hagen & Nelson 1996). The activity of RuBisCO in an obligate chemoautotrophic strain MS-81-1c is reduced when acetate is added to the growth medium (Hagen & Nelson 1996). The addition of acetate also increases the growth of the strain by about 20%, which means that this bacteria can use the acetate as an extra carbon source also when growing autotrophically. In a facultatively chemolithoautotrophic strain MS-81-6 the addition of acetate reduces the activity of RuBisCO and also increases the activity of 2-oxoglutarate dehydrogenase, which facilitates the use of two-carbon compounds in oxidative respiration. In autotrophic conditions the 2-oxoglutarate dehydrogenase is not expressed, so that the autotrophically bound carbon can be used in the building of cellular components.

The large vacuolated *Beggiatoa* strains seem to be capable of autotrophic metabolism (McHatton et al. 1996). Strains from the Monterey Canyon in California have substantial RuBisCO and 2-oxoglutarate dehydrogenase activity. These vacuolated strains also seem to use nitrate as their TEA instead of oxygen. The narrow freshwater strain D-402 is capable of chemolithotrophic and mixotrophic growth by oxidizing thiosulfate (Grabovich et al. 2001). The strain also seems to have several enzymes of the Calvin cycle.

Most of the freshwater *Beggiatoa* strains are heterotrophic (Teske & Nelson 2006). Apparently many strains possess complete Krebs (citric acid) and glyoxylate cycles. Consequently, heterotrophic strains of *Beggiatoa* can use a variety of organic molecules which they also store as different storage compounds. The freshwater *Beggiatoa* strain OH-75-2a is capable of heterotrophic growth using acetate, pyruvate, lactate or ethanol as the single carbon source (Nelson & Castenholz 1981a). Together with acetate also the intermediate compounds of the Krebs cycle increase the growth of this strain. In addition to using organic compounds with two or more carbons, the *Beggiatoa alba* strains B18LD and OH-75-2a can also grow with methanol as their only carbon source (Jewell et al. 2008).

Mixotrophic *Beggiatoa* uses the oxidation of sulfide to produce energy, while saving the organic carbon skeletons for the purpose of increasing its biomass (Teske & Nelson 2006). Mixotrophy has been suspected as the trophic mode for many freshwater strains, but it has only been found in one marine strain of *Beggiatoa*, MS-81-6 (Hagen & Nelson 1996).

3.5.2 Nitrogen

In addition to their diverse trophic modes, the bacteria of the genus *Beggiatoa* differ greatly also in their use of nutrients (Teske & Nelson 2006). The vacuolated marine strains can accumulate nitrate in their vacuoles in 20 000 times the concentration of the surrounding sea water (McHatto et al. 1996). The vacuoles of filaments isolated from Dangast in Germany contained nitrate at a concentration of 370 mM (Mußmann et al. 2003). In a recent study, it was discovered that the vacuoles of a close relative of *Beggiatoa* isolated from hypersaline conditions contained nitrate at a concentration of 650 mM (Hinck et al. 2011).

Autotrophic vacuolated *Beggiatoa* can use its stored nitrate in anoxic conditions as the TEA (McHatton et al. 1996). In vacuolated marine strains of *Beggiatoa* the activity of the nitrate reductases seemed to be localized into the membranes. When *Beggiatoa* reduces nitrate dissimilatively, the end product seems to be ammonium instead of nitrogen (Vargas & Strohl 1985, Sayama et al. 2005, Preisler et al. 2007). This process, called Dissimilatory Nitrate Reduction to Ammonium (DNRA), reduces nitrate to ammonium (Conley et al. 2009). Ammonium, as a soluble nutrient, is then left to circulate in the water to support autotrophic production. However, a study of single vacuolated filaments isolated from marine conditions found genes linked with denitrification instead of those linked with DNRA (Mußmann et al. 2007). A complete denitrification pathway that would lead to the release of nitrogen gas could not be found, but only genes encoding the reducing enzymes forming the pathway from nitrate (to nitrite to nitric oxide) to nitrous oxide. In the same study the production of nitrous oxide, also known as laughing gas, was also proven chemically with a culture of *Beggiatoa* isolated from the Håkon Mosby mud vol-

cano in the Barents Sea. However, Bacterial and perhaps archaeal nitrifiers live in close connection to sulfide oxidizing *Beggiatoa* in microbial mats of a hydrothermal sediment in the Guyamas Basin (Winkel et al. 2013). These organisms convert the ammonia venting from the subsurface into nitrate, which can be used by the *Beggiatoa* in the sulfide oxidation.

An enrichment culture composed of freshwater *Beggiatoa* strains is capable of sulfide oxidizing with nitrate in anoxic conditions (Kamp et al. 2006). However, these filaments do not contain the vacuoles normally linked with storage of nitrate. Heterotrophic strains of *Beggiatoa* can metabolize a variety of nitrogen compounds (Teske & Nelson 2006). *Beggiatoa* alba can use ammonium, nitrate, nitrite and casamino acids as its nitrogen sources (Mezzino et al. 1984). In addition to these sources, the strain B18LD can use urea, aspartate, asparagine, alanine and thiourea (Vargas & Strohl 1985). The nitrate reductase of this strain is of the assimilatory type, which means that it functions only in binding of nitrogen to the cell mass of the bacterium instead of in energy production.

3.5.3 Phosphorus

Like all other living organisms, *Beggiatoa* needs phosphorus in its metabolism (Teske & Nelson 2006). Still, especially the metabolism involved with polyphosphate makes *Beggiatoa* special in this respect. Polyphosphate is an intercellular storage molecule that can be used by both eukaryotic and prokaryotic organisms (Kornberg 1995). Its tasks can vary depending on the needs of the cell and the location of the molecule inside it. These tasks are e.g. storage of energy and phosphate, chelation of metals, buffering of alkaline pH, transformation in competent cells (uptake of DNA into the cell) and involvement in the tolerance to stress and changing environmental conditions. The presence of polyphosphate in cells of *Beggiatoa* has been shown with e.g. methylene blue (Strohl & Larkin 1978), DAPI staining (Brock & Schulz-Vogt 2010) and TEM combined with X-ray microanalysis (De Albuquerque et al. 2010). Genes that are involved in the uptake of phosphorus and the formation of polyphosphate were found from the genomes of two vacuolated marine strains of *Beggiatoa* (Mußmann et al. 2007).

The accumulation of polyphosphate and the release of phosphate from filaments of *Beggiatoa* is dependent on environmental conditions (Brock & Schulz-Vogt 2010, Schulz & Schulz 2005). Oxygenated conditions cause a *Beggiatoa* strain to accumulate phosphate (Brock & Schulz-Vogt 2010). Anoxia together with an increasing concentration of sulfide however, causes a breakdown of polyphosphate and its subsequent release from the cells. The released phosphate can then be deposited as phosphorite minerals in the sediments or stay dissolved in the water. The cause for phosphate release was earlier though to have a connection with the use of acetate in anoxic conditions (Schulz & Schulz 2005), but this claim has not found support in a

later study (Brock & Schulz-Vogt 2010). The actual reason for the release is still not known for certain, but it might have to do with the stress induced by sulfide when a suitable TEA is missing in anoxic conditions. A more recent study conducted in the southwest Baltic Sea at the entrance to Eckernförde Bay also supports these results of phosphate release from *Beggiatoa* in anoxic conditions (Dale et al. 2013). A large increase in the dissolved phosphate in the sediments and subsequently a large flux of phosphorus into the water column was linked to *Beggiatoa* in a modeling approach.

3.5.4 Sulfur

The formation of visible sulfur inclusions through oxidation of sulfide is one of the defining features of *Beggiatoa* and evident in all of the strains (Teske & Nelson 2006). However, the patterns in the use of sulfur between them differ and depend on the habitat and trophic mode of the strain. Sulfide can be oxidized by *Beggiatoa* either dissimilatorily, assimilatorily, or utilizing both pathways depending on the needs of the cell. Certain *Beggiatoa* strains can also use thiosulfate as the substrate for their enzymes instead of sulfide. For the assimilatory metabolism, some strains that can utilize sulfate do not need sulfide at all. Certain heterotrophic strains of *Beggiatoa* might use thiosulfate and accumulated sulfur as electron acceptors for toxic peroxides similarly to the utilization of catalases in some other organisms. Another possible use of these compounds is as TEAs in the oxidation of organic compounds, which gives these bacteria the ability to tolerate temporary anoxia in their environment.

The autotrophic marine strain of *Beggiatoa* MS-81-6 can compete efficiently against inorganic sulfide oxidation (Nelson et al. 1986a). The strain can oxidize sulfide with oxygen about three magnitudes faster than the corresponding inorganic process. The MS-81-6 strain oxidizes sulfide partly to elemental sulfur and partly directly to sulfate. This relation most likely depends on the steepness of the sulfide gradient - in a steeper gradient the filaments oxidize a larger part of the sulfide to elemental sulfur. However, when the concentration of sulfide decreases in the environment, they can oxidize stored elemental sulfur to cover the energy needs of the cells. Thus, by temporarily storing elemental sulfur the bacteria can increase their range of possible habitats and tolerance to changes in the concentrations of sulfide and oxygen. While growing in an oxygen-sulfide gradient, the strain MS-81-6 is a facultative chemolithotroph and the strain MS-81-1c is an obligate chemolithotroph (Hagen & Nelson 1996). The strain MS-81-6 can grow in a medium containing only sulfide as the energy source, but when given organic compounds it can change its metabolism towards heterotrophy by strongly downregulating the activity of RuBisCO and upregulating the activity of 2-oxoglutarate dehydrogenase. In turn, the strain MS-81-1c cannot use several organic compounds at all in its metabolism. Acetate is used by MS-81-1c only as a supplemental carbon source.

The heterotrophic freshwater *Beggiatoa* strain OH-75-2a most likely uses accumulated elemental sulfur as the TEA during temporary anoxia (Nelson & Castenholz 1981b). By respiring accumulated sulfur, the strain reduces it back to sulfide when cultured under anoxia in a medium containing organic compounds. Thiosulfate seems to reduce the lag phase in fresh cultures of this strain, probably through a mechanism similar to that of catalase in other organisms. The OH-75-2a strain also has a similar enzyme for the oxidation of sulfide as the facultatively autotrophic marine strain MS-81-6 (Hagen & Nelson 1997). However, the activity of this enzyme was much lower than in the strain MS-81-6 and thus it probably functions only in assimilation in the strain OH-75-2a.

The *Beggiatoa* freshwater strain D-402 is capable of chemolithotrophic growth with thiosulfate (Grabovich et al. 2001). In heterotrophic conditions the activity of RuBisCO decreases radically also in this strain. In the type strain B18LD of *Beggiatoa alba*, which is of the freshwater type, sulfide oxidation might compete against acetate oxidation for oxygen (Schmidt et al. 1987). The type strain oxidizes sulfide only to elemental sulfur, which is apparently never oxidized further to sulfate. Like the strain OH-75-2a, the type strain B18LD can reduce the stored sulfur back to sulfide to survive periodical anoxia.

The vacuolated marine strains oxidize sulfide with their stored nitrate (McHatton et al. 1996). Due to this, the sulfide and oxygen gradients utilized by these organisms do not have to overlap, unlike in the unvacuolated strains' habitats. In a mixed culture the process consists of two phases (Sayama et al. 2005). The bacteria first oxidize sulfide to sulfur in anoxic conditions with the stored nitrate, which is reduced to ammonium. The sulfur is stored inside the cells and the ammonium is released in the surrounding water. Then the filaments glide upwards in the sediment to oxic conditions and oxidize the stored elemental sulfur into sulfate. In these oxic sediments they also supplement their nitrate storages, before gliding back to the anoxic conditions. Marine vacuolated *Beggiatoa* have also genes linked to various other types of sulfur metabolism e.g. to thiosulfate oxidation, sulfide oxidation without nitrate, elemental sulfur reduction or thiosulfate reduction into sulfide, and dimethyl sulfoxide (DMSO) reduction to dimethyl sulfide (DMS) (Mußmann et al. 2007).

3.6 Ecology

This and the following sections concentrate only on the ecology of vacuolated *Beggiatoa* strains, because they are the most common and influential types of *Beggiatoa* in the conditions prevalent in the Baltic Sea.

In its habitat, *Beggiatoa* needs sulfide or thiosulfate as its energy source, together with an oxidizer (Teske & Nelson 2006). *Beggiatoa* can use sulfide from both biogenic sources, as from the reduction of organic matter in anaerobic conditions,

and geothermal sources. Based on the source of the sulfide, strains of *Beggiatoa* can be autotrophic producers or alternatively they can recycle the energy released in the degradation of organic matter back into the ecosystem. *Beggiatoa* uses either dissolved oxygen or nitrate as its oxidizer and certain strains can store the latter of these in their vacuoles in high concentrations. The internal nitrate concentration of the vacuoles can be even 100 to 400 mM, which is many thousand-fold higher than the concentration in their environment. Even though the energy efficiency of sulfide oxidation with nitrate is lower than with oxygen, oxygen can not be stored in as high concentrations as nitrate because of its low solubility in water. Already the first comprehensive studies of the vacuolated *Beggiatoa* strains presented a hypothesis of the shuttling of the bacteria between separately located gradients, enabled by their intracellular energy storages (McHatto et al. 1996). Theoretically, these strains can oxidize sulfide for 4.8 hours in anaerobic conditions by using the nitrate stored in their vacuoles. Considering the distance between then gradients and the gliding speed of the filaments, it is more than enough for the survival of the bacteria. Several subsequent studies have also given support to this behaviour (Sayama 2001, Mußmann et al. 2003, Preisler et al. 2007).

3.6.1 Habitats

Beggiatoa strains that use only oxygen as their TEA need overlapping gradients of sulfide and oxygen, to whose interface the filaments concentrate as a thin mat (Teske & Nelson 2006). The gradients do not have to overlap for the marine vacuolated strains. The vacuolated strains can store both their electron donor sulfur and electron acceptor nitrate intracellularly. Using these storages the filaments can shuttle between spatially separated sources of nitrate and sulfide or utilize temporally occurring conditions in a single location in mixing waters. The habitats of these bacteria are marine regions with generally low oxygen contents where their energy source, sulfide, is available from either an organic or inorganic source. The largest occurrences of vacuolated *Beggiatoa* and metabolically similar *Thioploca* are found from coastal upwelling regions, like the coasts of Chile and Peru. They are also very common around deep-sea black smokers and cold seeps. In these environments the mats of filamentous bacteria cover large areas and might reach a thickness of 30 cm (McHatton 1996). Vacuolated *Beggiatoa* commonly inhabit also certain much-researched coastal regions, with high autotrophic production and usually hypoxic sea floors (Teske & Nelson 2006). On these sea floors the filaments usually live inside the sediment and with their nitrate storage capabilities they can occur up to a depth of 2 to 4 cm while oxidizing sulfide. Hence, visible mats might not form at all at the sediment surface (Mußmann et al. 2003).

In some habitats of *Beggiatoa*, the filaments can comprise the majority of the microbial biomass of the sediment (Jørgensen 1977). Even though the number of the

filaments may not be very high, their volume is many magnitudes higher than that of the average bacterium in these habitats. The biomass of *Beggiatoa* filaments in the sediments of Limfjorden, Denmark, was measured to be from 5 to 20 g m^{-2} with a maximum of 48 g m^{-2} in 1977. A later measurement of Limfjorden sediments in 2003 gave a result of 14 to 16 g m^{-2} (Mußmann et al. 2003). In Dangast, Germany, the biomass is much lower than in Limfjorden, from 0.6 to 1 g m^{-2}. The biomass of a mat on the Eckenförde Bay in the Baltic Sea was estimated to be about 3 g m^{-2} (Preisler et al. 2007). The biomass of a *Beggiatoa* mat can thus be roughly equivalent to e.g. the macrofaunal biomass of a similar soft sediment, like Gdańsk Bay in the southern Baltic Sea (which varies from 1.54 ± 0.60 to 80.89 ± 107.28 g m^{-2}) (Drgas et al. 1998).

3.6.2 The formation and movement of *Beggiatoa* mats

The positioning of a *Beggiatoa* mat in the sediment and above it is controlled by the negative taxes of the filaments against high concentrations of oxygen, light (phototaxis) and sulfide (Nelson & Castenholz 1982, Møller et al. 1985, Preisler et al. 2007). When a gliding filament recognizes a rising concentration of oxygen, this part of the filament changes its gliding direction after a lag of a few tens of seconds (Møller et al. 1985). The filament collects on the interface of the gradients, caused by the changes in gliding directions in its different parts. Mats of *Beggiatoa* can be very stable (Nelson et al. 1986b). A cultivated mat stayed active in a sulfide-oxygen gradient for several weeks in the laboratory. The phobic reaction of *Beggiatoa* towards sulfide has been shown to exist only quite recently (Preisler et al. 2007), even though it has been assumed much earlier to be important for the movement of the filaments (Møller et al. 1985). The vacuolated strains might otherwise have a problem with getting lost deep in the sediment, but it is prevented by this taxis (Preisler et al. 2007). If they had no signal to turn back, the filaments could randomly glide so far from the nitrate or oxygen gradient that their energy reserves would run out before they could resupply.

Filaments of *Beggiatoa* also have a negative phototaxis - they react to light by changing their gliding direction away from it (Nelson & Castenholz 1982). Thus, Beggiatoa has a circadian rhythm regulated by sunlight on shallow, illuminated bottom areas. *Beggiatoa* mats living together with photosynthetic organisms depend heavily on the negative phototaxis and negative oxygen taxis for their survival (Møller et al. 1985). A *Beggiatoa* mat can sometimes be observed to follow the gradient of oxygen and sulfide above the photosynthetic organisms on certain shallow bottoms during the night. In the morning, the photosynthetic organisms start producing oxygen again and a new sulfide-oxygen gradient forms below them. The environment the *Beggiatoa* filaments find them in is now unfavorable because of the produced oxygen, and they need to move against a rising oxygen gradient to escape

it. The negative phototaxis is stronger than the negative oxygen taxis, which makes the filaments return back to the sediment and to the new gradient.

3.6.3 The effects of *Beggiatoa* on its habitat

The occurrence of *Beggiatoa* has many effects on the surrounding sediment and the water phase above it (Teske & Nelson 2006). A mat of *Beggiatoa* can effectively use in its metabolism all of the forming sulfide and diffusing oxygen in the habitat and keep the interface of the gradients relatively stable (Nelson et al. 1986b). Vacuolated filaments of *Beggiatoa* are also able to keep the gradients of nitrate and sulfide separated, and thus they can monopolize the use of nitrate in the sediment (Sayama et al. 2005). However, in certain cases vacuolated *Beggiatoa* might be less able to oxidize sulfide (Mußmann et al. 2003). A population of *Beggiatoa* in Limfjorden, Denmark, could oxidize only 50% of the sulfide produced in this habitat. Also a Baltic Sea community consisting of vacuolated *Beggiatoa* could oxidize only a fraction of the sulfide present in its environment (Preisler et al. 2007). Instead, sulfide was oxidized mostly chemically with Fe(III) to elemental sulfur. Sulfide oxidation is an important process in the sediment, especially because sulfide is highly toxic to bottom fauna and other organisms living in the sediment (Wang & Chapman 1999).

Beggiatoa mats present on the Bay of Concepción changed the sediment from an ammonium sink to a source of ammonium during the hypoxia caused by large algal blooms in the summer (Graco et al. 2001). At the same time hypoxia and the large inputs of organic matter from the bloom increased the production of sulfide in the sediments, which inhibited nitrification and denitrification. These effects together increased eutrophication on the bay, partly because nitrogen was recycled more efficiently back to the algae due to the involvement of *Beggiatoa*. Eutrophication can further be increased in these environments because *Beggiatoa* competes for nitrate against denitrifiers and increases the proportion of recycled nitrogen to nitrogen released by the denitrifiers (Sayama 2001).

Many grazing organisms living in the sediment, e.g. nematodes, can use *Beggiatoa* as their food source (Freckman 1988), and *Beggiatoa* and other bacteria living in the sediment can use the elemental sulfur gathered by the filaments as their energy source (Granth & Bathmann 1987). *Beggiatoa* mats can be resuspended by waves or other currents to the water phase. If this happens, the organisms living in the sediment lose both the nutrients and the elemental sulfur included in the filaments.

Vacuolated marine *Beggiatoa* have genes for the processing of nitrogen, sulfur and phosphorus, and also for gliding mechanisms discovered in previous studies (Mußmann et al. 2007). In addition, they seem to share many metabolic pathways with other organisms. The figure summarizing the energy-producing pathways of the vacuolated marine strain is drafted on the basis of these pathways (Figure 2).

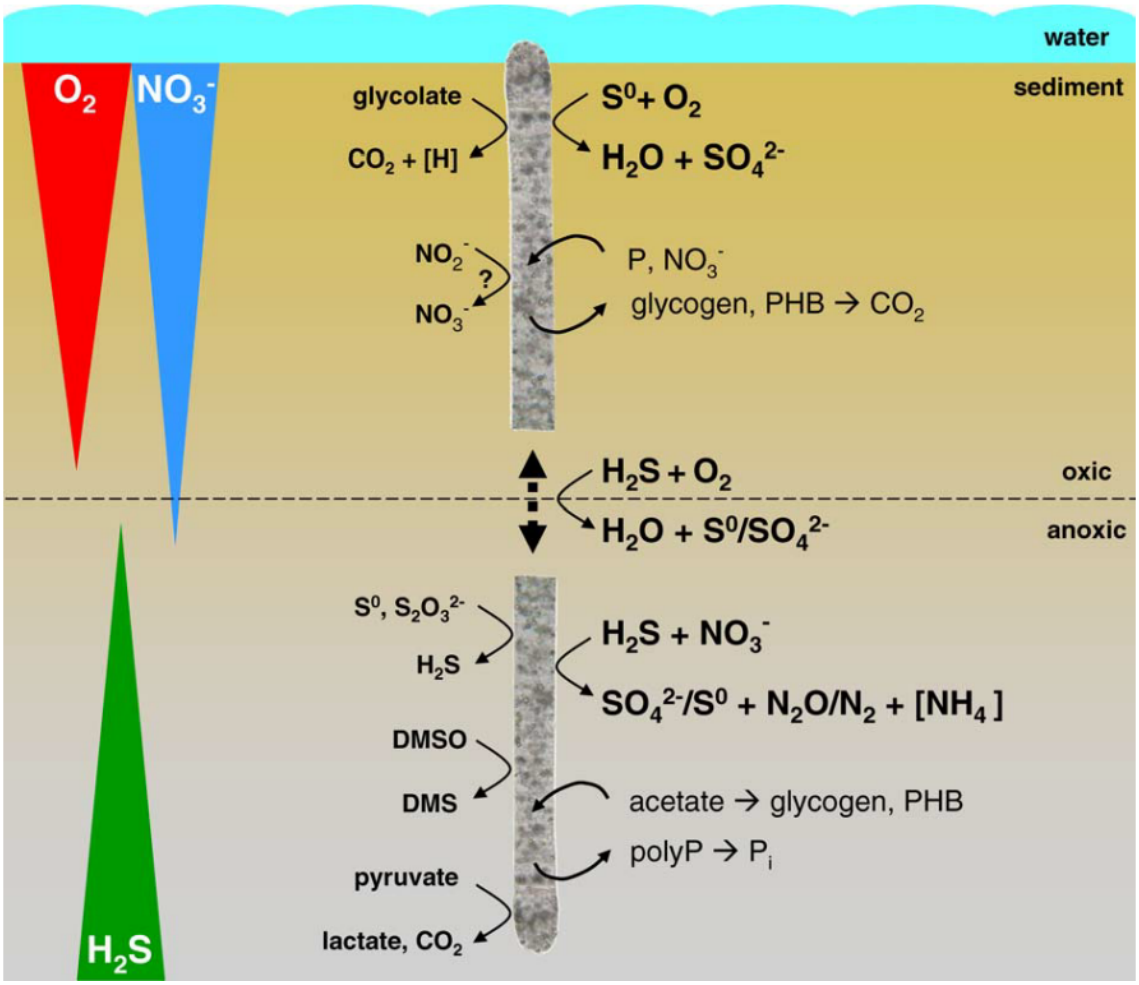

Figure 2: The potential energy-yielding pathways of a vacuolated *Beggiatoa* strain in gradients of oxygen, nitrate and sulfide in the top layer of a marine sediment (Mußmann et al. 2007). © 2007 Mußmann et al. Creative Commons Attribution License. Reproduced with permission. doi:10.1371/journal.pbio.0050230.g005

The pathways are also discussed briefly in previous chapters about the metabolism of *Beggiatoa*.

3.7 Occurrence of *Beggiatoa* on the Baltic Sea

Mats and filaments of *Beggiatoa* inhabit many bottom areas with low oxygen concentrations on the Baltic Sea (Jonsson & Jonsson 1988, Rosenberg & Diaz 1993, Vallius 2006, Preisler et al. 2007). Usually the mats themselves have not been the focus of these studies, but their presence has been casually noted, often as additional evidence on the anoxia of the sediments. *Beggiatoa* occurs on the Eckenförde Bay in Kiel in the southern Baltic Sea, where thin white bacterial mats cover the sediment (Preisler et al. 2007). However, most of the filaments apparently lie inside the sediment, which means the visible mat is just a fraction of the biomass of the whole *Beggiatoa* community. *Beggiatoa* filaments cover almost all of the sediment surface north of Gotland at the depth of 83 to 190 m (Jonsson & Jonsson 1988).

Apparently *Beggiatoa* also covers parts of the sediment at a depth of 225 to 243 m on the east side of Gotland (Emeis et al. 2000). The photographic examination of the sediment profiles in the Stockholm archipelago showed that a *Beggiatoa* mat covered the sediment in most of the sampling locations (Rosenberg & Diaz 1993). It is likely that the bottom ares in this region with a depth of over 20 meters (25 km^2) were in large part covered by *Beggiatoa* at the time of the sampling during 1991. A video imaging study in the region of Tammisaari on the Gulf of Finland showed that mats of *Beggiatoa* covered large areas of the anoxic sediments (Vallius 2006). Furthermore, *Beggiatoa* can live inside the sediment when the surface of the sediment is oxic, in which case their occurrence can also easily be overlooked (Mußmann et al. 2003).

4 The Baltic Sea

4.1 Features of the Baltic Sea

The body of water currently know as the Baltic Sea is young on a geological time scale. It is one of the largest pools of brackish water in the world and it is situated completely on top of the continental shelf (Matthäus 1995). The position on top of the continental shelf is the main reason for the shallowness of the sea. The average depth of the Baltic Sea is only 55 meters and the maximum depth is 459 meters. The water exchange with the North Sea is limited efficiently by the shallow and narrow Danish straits Darrs and Drodgen. The sea is situated on a humid climatic region, and excluding evaporation, it receives a water surplus of approximately 475 km^3 from its catchment area. This water flows out to the North Sea through the Danish straits in the surface layer, and the outflow causes a continuous inflow of saline water near the sea floor back to the Baltic Sea. The inflow maintains both the low salinity level in the Baltic Sea and the salinity stratification, which prevents the deep waters with low oxygen mixing to the oxygenated surface waters.

4.1.1 Hypoxia in the Baltic Proper

After the freshwater Ancylus Lake stage ended, the Baltic Sea has been a brackish water body for approximately the last 8000 years (Bianchi et al. 2000). During the Littorina Sea 7000 - 4000 years ago the salinity was probably about two-fold compared to the current salinity, and the salinity stratification developed already at that time. Laminated sediments from the Littorina Sea are proof from the lack of bioturbation by bottom fauna during this time period, and thus also prove that the sea floor naturally has a low oxygen concentration. The deepest parts of the Landsort deep (>250 m) have probably been anoxic or almost anoxic during the whole Holocene (Conley et al. 2009). The conditions in deepest parts of the Baltic

Sea have changed from oxidizing to reducing periodically during the last centuries (Matthäus 1995). Cold climate conditions seem to correlate with good oxygen conditions and warm climate conditions with reducing conditions. However, the oxygen concentration and salinity of the deepest parts of the Baltic Proper have decreased since the year 1952 and a some of the deeps have been almost continuously anoxic.

The increasing hypoxia and anoxia of the sea floor are caused by the imbalance between biological processes consuming oxygen and the physical processes supplying oxygen (Conley et al. 2009). From the beginning of the 20^{th} century anthropogenic inputs of nutrients to the Baltic Sea have continually increased, which has led to a higher algal production and thus to larger inputs of organic matter to the sea bottom (Vahtera 2007). When the high amount of descending dead organic matter is degraded on the bottom, oxygen is consumed and the extent of the hypoxic and anoxic sea floor area increases. The sediments on these areas return to their original biological and chemical state slowly, because when the conditions improve it takes a long time for the bioturbating zoobenthos to spread back to previously inhospitable areas which have had low oxygen levels (Conley et al. 2009). Also the bottom fauna can not develop very far in its succession on sea floors that have fluctuating oxygen levels. On these areas only species that inhabit the top layer of the sediment, and thus do not extensively mix the sediment, can colonize the sediment before the next anoxic period. This worsens the oxygen situation on these bottom areas even further.

The deep water in the basins of the Baltic Sea can be substantially renewed only through major inflows of saline water from the North Sea (Matthäus 1995). The saline, oxygenated water is heavy and it moves along the sea floor, flushing the stagnated water from the basins to the edges where it can mix with less saline water. These inflows occur usually during wintertime and several years can pass between separate events. Their occurrence is influenced by the climatic conditions on the northern Atlantic, the North Sea and the Baltic Sea. For example, low winds and low river runoff to the Baltic Sea, which cause a drop in the water level, correlate positively with the occurrence of the inflow events. The latest clusters of major inflows occurred between the years 1948 and 1952 and between the years 1968 and 1972. Later on, there was a long pause, the stagnation period, between the years 1983 and 1992 when no inflow events occurred. This period ended in 1993 when a major inflow event occurred again. The latest significant major inflow event occurred in 2003 (Carstensen et al. 2014). The major inflows improve the oxygen situation of the sea floor, but also strengthen the salinity stratification because of their high density compared to the overlying waters with low salinity (Conley et al. 2009). Due to the strengthening of the salinity stratification, the largest area of hypoxic and anoxic sea floor (< 0.2 mL L^{-1} dissolved oxygen) was measured on the Baltic Sea in the year 1971 (70 000 km^2) after a streak of inflow events. Correspondingly,

the smallest area was measured in the year 1993 (11 050 km^2) after a long pause in major inflow events. The average extent of the sea bottom covered by hypoxic water between the years 1961 and 2000 has been 49 000 km^2. Currently the extent of hypoxic sea floor is over 60 000 km^2 (Carstensen et al. 2014).

4.1.2 Coastal hypoxia in the Baltic Sea

Low oxygen concentrations are a problem also on many coastal regions in the Baltic Sea (Conley et al. 2011). On these areas, like on the Baltic Proper, the biggest problem in terms of hypoxia is the anthropogenic nutrient loading. On bottom areas close to the shore the temperature stratification acts as a barrier for the mixing of waters instead of salinity stratification. However on the region of the Danish straits the large differences in the salinities of the water masses, together with a large autotrophic production, create an environment prone to hypoxia.

In the Stockholm archipelago and the Archipelago Sea of the Baltic Sea the complex topographic features of the bottoms create numerous small basins (Conley et al. 2011). If the water in these basins becomes stratified, the lower water layers are isolated from each other. Thus a large, localized input of organic matter e.g. a drifting algal mat can cause local hypoxia. In the Tammisaari region on the Gulf of Finland, the complex topography of the bottoms also seems to cause their hypoxia (Vallius 2006). Certain areas in the Tammisaari region have been continuously anoxic during the last 40 years. Local hypoxia is much rarer in the estuaries on the Gulf of Bothnia because of the smaller nutrient inputs to them (Conley et al. 2011). The efficient cycling of the water prevents the forming of hypoxic conditions on the coasts of Poland and the Baltic countries .

The worsening of the oxygen conditions on the coastal bottoms has been a continuous trend during the last hundred years (Conley et al. 2011). Some improvement in the oxygen conditions locally on certain areas has happened, but in most areas the situation has gotten worse. The oxygen conditions from 326 coastal bottoms in the Baltic Sea were examined from a period of 50 years. The results show that 30% of the sites have suffered from episodic hypoxia, 4% of them have been seasonally hypoxic and 1.5% were constantly hypoxic.

4.2 Nutrient cycles in the Baltic Sea and the possible effects of *Beggiatoa* on them

The most important inorganic nutrients that limit the photoautotrophic production on the Baltic Sea are nitrogen and phosphorus (Vahtera 2007). In the most eutrophicated parts of the Baltic sea, the production has been generally nitrogen-limited after the winter. The N:P-ratio in the water has been since the 1960's continuously under the so called Redfield ratio of 16:1, in which the algae use the nutrients. In the

spring bloom the growing algae use the nitrogen, leaving a surplus of phosphorus for which use they would need more nitrogen. These conditions give the competitive advantage to the nitrogen-fixing cyanobacteria which can fix atmospheric nitrogen gas and are thus limited in their growth usually only by phosphorus or trace elements.

Out of the total nutrient load and organic matter ending up in the Baltic Sea, about two thirds originates from the runoff of rivers and one third from point sources (Matthäus 1995, Vahtera 2007). It is estimated that in the Baltic Sea the nitrogen loading has quadrupled and the phosphorus loading has increased eight-fold since the beginning of the 20$^{\text{th}}$ century. About 40% of the nitrogen load ends up in the sea straight from atmospheric deposition and, depending on the estimate, about 10% to 25% is fixed by the cyanobacteria. There has been reliable information available about the phosphorus concentrations on the Baltic Sea since the year 1958 and about the nitrogen concentrations since the year 1969 (Matthäus 1995). The levels of both of the nutrients have considerably increased on average from the beginning of the measurements until the 1980's. Since the 1980's the concentrations have been quite stable on this high level, which is a signal for the continuing eutrophication of the Baltic Sea.

The stability of nitrogen in the Baltic Proper is only 5 years, unlike phosphorus, which residence time is 11 or 87 years depending on the estimation method (nutrient budget or modeling, respectively) (Vahtera 2007). Because the annual anthropogenic nutrient inputs have decreased, the anthropogenic nutrient loading currently does not have an increasing effect on the total nutrient levels of the Baltic Sea. However, previously higher levels of nutrient loading and its long duration have influenced the nutrient cycles together with the features of the Baltic Sea. This has led to a "vicious circle" of internal cycling, which makes it very difficult for the sea to recover by removing nutrients from the circulation through natural means.

4.2.1 Nitrogen cycling

The oxygen conditions in the water and on the sea floor have a well-known effect on the concentrations of nitrogen (Conley et al. 2009). Nitrogen can be removed from the water by bacteria through denitrification or anammox in gaseous forms (N2, N2O and NO). Denitrification requires nitrite and/or nitrate and anammox requires nitrite and ammonium as its substrates. The substrates can also be made available by nitrifying bacteria, who oxidize ammonium to nitrite and nitrate in oxic conditions. Denitrification removes about 23% of the annual nitrogen load on the Bay of Bothnia and 30% on the Bothnian Sea and the Gulf of Finland. The information is lacking for other parts of the Baltic Sea. According to the traditional view, anoxia in the sediment prevents nitrification and thus also reduces denitrification due to a lack of substrates. However, an increase in the area of anoxic sea floor seems to correlate negatively with dissolved nitrogen in the water. Nitrifica-

tion may occur in the oxic water column instead of the sediment, and its oxidation products nitrite and nitrate are quickly denitrified in the hypoxic and anoxic layer below it. The effect of this process of combined nitrification and chemolithotrophic denitrification might be locally large, but it seems to occur efficiently only during short time periods (Hietanen et al. 2012). The process requires circumstances in which anoxic water is mixed with oxic water and the abundant ammonium of the anoxic water is nitrified. When the products of nitrification, nitrate and nitrite, end up back in the anoxic water, they are denitrified in the water column with the help of sulfide. Compared to this process, the traditional denitrification that takes place in sediments with oxic top layers is a more certain way to remove nitrogen from the water, because it occurs on a much larger area and with a constant speed.

The nitrogen metabolism of the vacuolated *Beggiatoa* should be studied further. The mechanism of sulfide oxidation is probably associated with DNRA (Vargas & Strohl 1985, Sayama et al. 2005, Preisler et al. 2007). This means that *Beggiatoa* can increasingly recycle nitrate back to ammonium and thus increase eutrophication at least on a local scale. Ammonium is also toxic to many organisms living in the sediment (Ankley et al. 1990). Based on a genomic study, certain vacuolated *Beggiatoa* seem to have an incomplete denitrification pathway that produces nitrous oxide (Mußmann et al. 2007). Nitrous oxide is the third most important green house gas after carbon dioxide and methane, and perhaps also the most important compound currently depleting stratospheric ozone (Wuebbles 2009). Alternatively, a missing gene for the nitrous oxide reductase, which converts the nitrous oxide to nitrogen gas, might be located in the unstudied region of the genome (Mußmann et al. 2007). In any case even a partial denitrification pathway producing a gaseous product removes nitrogen from the water and reduces eutrophication. Vacuolated *Beggiatoa* can also monopolize the use of nitrate in shallow coastal sediments while accumulating it intracellularly (Sayama 2001). A large part of the total nitrate in these sediments can be located in the *Beggiatoa* filaments, which reduces the amount of nitrate available for the denitrifiers and thus increases eutrophication.

4.2.2 Phosphorus cycling

The amount of dissolved phosphate in the water correlates positively with the area of hypoxic sea floor in the Baltic Sea (Conley et al. 2009). The reason seems to be the release of phosphorus bound to iron oxyhydroxides in anoxic conditions when the P-binding Fe(III) is reduced to Fe (II), which in turn cannot bind phosphorus. This process is reversible and a change back to oxic conditions restores the P-binding capabilities of the sediment.

Phosphorus is removed from the Baltic Sea through two main routes, with approximately equal volumes. The routes are the flow of phosphorus-laden water though the Danish straits and the permanent burial of phosphorus into sediments.

The redox (oxygen) conditions of the sea floor have an effect only on the latter one of these routes. Phosphorus is buried into the sediment mostly as organic phosphorus and calcium phosphate (Ca-P), of which the latter can be further divided into authigenic (chemically formed from soluble phosphate) and biogenic (e.g. fish bones) calcium phosphate. Hypoxia and anoxia reduce the formation of Ca-P and the permanent burial of organic-P, but increase the preservation of biogenic Ca-P. Knowledge is still lacking on the effects of the redox conditions to the burial of organic phosphorus and biogenic Ca-P.

Beggiatoa accumulates phosphorus as polyphosphate and subsequently releases phosphate in anoxic conditions (Brock & Schulz-Vogt 2010). This might increase the availability of phosphorus to primary producers through recycling on eutrophic areas if the phosphate is released from the sediment to the water phase. The release of phosphorus linked to *Beggiatoa* has also been preliminarily observed on the Baltic Sea through a modeling approach, where bubbling of methane from the sediment led to mixing of the phosphorus containing sediment pore water into the water column (Dale et al. 2013). Another alternative to phosphorus release to the water column is authigenic formation of phosphorite (Ca-P minerals) in the sediment as a result of high pore water concentrations of phosphate, which occurs in connection to a close relative of *Beggiatoa*, *Thiomargarita* (Schulz & Schulz 2005). A model on the effects of *Beggiatoa* on the phosphorus cycling in the Baltic Sea showed that the reduction of sulfide by these bacteria may decrease the rate of iron sulfide formation in the sediments, and thus increase the phosphorus retention capability of the sediment (Yekta & Rahm 2011). These complicated mechanisms would need more research for the evaluation of their total effects on the phosphorus cycles in the Baltic Sea.

4.2.3 Sulfur cycling

Hydrogen sulfide is produced in water environments in the anaerobic degradation of organic matter with sulfate (Wang & Chapman 1999). Sulfide itself is toxic to e.g. bottom fauna, but it also reduces the toxicity of metals present in the sediment by forming insoluble metal sulfides and metal sulfide complexes. In the sediments of marine environments the concentration of sulfide can reach 28 mM. On the Baltic Proper the maximum concentration of sulfide was 61.80 µM L^{-1} in samplings conducted in 2009 (Hietanen et al. 2012). In the Baltic Sea sulfide accumulates below the redoxcline. In the Gotland deep sulfide was encountered at 116 meters in the winter and at 95 meters in the summer. In the Landsort deep sulfide was encountered at a depth varying between 70 and 85 meters. Sulfide present in the water phase is used in the Baltic Sea probably by e.g. chemolithotrophic denitrifiying bacteria. However, the circumstances for the occurrence of water column denitrification are transient. Organisms living in environments where sulfide is usually present are generally resistant to its effects, especially bivalves and polychaetes (Wang & Chap-

man 1999). Organisms living in marine sediments can tolerate many magnitudes higher concentrations of sulfide than those living in fresh water sediments, where sulfide does not commonly occur.

Beggiatoa detoxifies sulfide as a byproduct of its metabolism and thus makes the survival of sulfide sensitive organisms possible in its habitat (Teske & Nelson 2006). Sulfur accumulating bacterial mats can be resuspended from the sea floor (Grant & Bathmann 1987). Resuspension of the mats removes a potential sulfur source from other sulfur oxidizing bacteria in the sediment, to the bacteria habiting the water column. Vacuolated *Beggiatoa* seem to have pathways involved also in other types of sulfur metabolism e.g. the reduction of dimethyl sulfoxide (DMSO) to dimethyl sulfide (DMS) (Mußmann et al. 2007).

5 Conclusions

There has been a growing, albeit still scarce, amount of information about the occurrence of *Beggiatoa* in the Baltic Sea and their effects on its biogeochemistry. Also some of the studies contain conflicting evidence about these effects. *Beggiatoa* has been actively researched for the last 40 years and new discoveries about the capabilities of these bacteria have been made continuously during this time. Many of their metabolic routes and their ecological effects would still need clarifying and confirming. The genus *Beggiatoa* itself is in need of a taxonomic revision on the basis of the 16S rRNA sequences and it will probably be divided in several genera. The revision would be helpful for the study of the metabolic traits of relevant strains and also their identification in the environment. New genetic studies with bacterial strains representing several different types of *Beggiatoa* isolated from different environments would hopefully elucidate the broad metabolic capabilities of these bacteria and help to estimate their effects in their habitats.

The role that these bacteria play in the continuing eutrophication of the Baltic Sea might be greater than previously assumed. Beggiatoa likely habit many coastal areas on the Baltic Sea and they might cover large areas also on the Baltic Proper. Their nitrogen metabolism probably increases eutrophication, since DNRA seems to be the more likely main metabolic pathway. DNRA increases the recycling of nitrogen back to the use of primary producers as ammonia, while preventing the removal of nitrogen through denitrification in the sediment. For phosphorus, the total effect is more uncertain. In hypoxic and anoxic conditions *Beggiatoa* cleaves the stored polyphosphate and releases phosphorus into the pore water. The pore water phosphorus can then be either released into the water column (increasing recycling), or it can precipitate in the sediment as Ca-P minerals (decreasing recycling) if its concentration is very high. The oxidation of sulfide by *Beggiatoa* apparently has an effect on the phosphorus cycling and it is also important for the bottom fauna. Sul-

fide oxidation seems to increase the amount of P-binding sites in the sediment, thus reducing the levels of phosphate in the water, and it also makes the sediment more habitable for sulfide-sensitive organisms. As autotrophs, *Beggiatoa* bind carbon back to the sediment by utilizing sulfide, which energy originates from the degradation of organic matter. By being an important nutrient source for the macro-organisms living in the environment they channel the energy of the sedimenting organic matter to higher trophic levels.

The effect of *Beggiatoa* on the nutrient cycles of the Baltic Sea is at least local, perhaps even wide-ranging, but still mostly unknown. By investigating the function of these bacteria we could learn more about nutrient cycling in complex ecosystems, build better models on the basis of them and apply the gathered information to control and reduce eutrophication in this vulnerable ecosystem.

References

[1] Ahmad A, Kalanetra KM, Nelson DC. 2006. Cultivated *Beggiatoa* spp. define the phylogenetic root of morphologically diverse, noncultured, vacuolate sulfur bacteria. Canadian Journal of Microbiology 52, 591–598.

[2] Ankley GT, Katko A, Arthur JW. 1990. Identification of ammonia as an important sediment-associated toxicant in the lower Fox River and Green Bay, Wisconsin. Environmental Toxicology and Chemistry 9, 313–322.

[3] Bennett BA, Smith CR, Glaser B, Maybaum HL. 1994. Faunal community structure of a chemoautotrophic assemblage on whale bones in the deep northeast Pacific Ocean. Marine Ecology Progress Series 108, 205.

[4] Bianchi TS, Engelhaupt E, Westman P, Andren T, Rolff C, Elmgren R. 2000. Cyanobacterial blooms in the Baltic Sea: Natural or human-induced? Limnology and Oceanography 45, 716–726.

[5] Brock J & Schulz-Vogt HN. 2010. Sulfide induces phosphate release from polyphosphate in cultures of a marine *Beggiatoa* strain. The ISME Journal 5, 497–506.

[6] Brock J, Rhiel E, Beutler M, Salman V, Schulz-Vogt HN. 2011. Unusual polyphosphate inclusions observed in a marine *Beggiatoa* strain. Antonie van Leeuwenhoek 101, 347–357.

[7] Carstensen J, Andersen JH, Gustafsson BG, Conley DJ. 2014. Deoxygenation of the Baltic Sea during the last century. Proceedings of the National Academy of Sciences 111, 5628–5633.

[8] Conley DJ, Björck S, Bonsdorff E, Carstensen ,J Destouni G, Gustafsson BG, Hietanen S, Kortekaas M, Kuosa H, Markus Meier HE, Müller-Karulis B, Nordberg K, Norkko A, Nürnberg G, Pitkänen H, Rabalais NN, Rosenberg R, Savchuk O,P Slomp CP, Voss M, Wulff F, Zillén L. 2009. Hypoxia-related processes in the Baltic Sea. Environmental Science & Technology 43, 3412–3420.

[9] Conley DJ, Carstensen J, Aigars J, Axe P, Bonsdorff E, Eremina T, Haahti B-M, Humborg C, Jonsson P, Kotta J, Lännegren C, Larsson U, Maximov A, Medina MR, Lysiak-Pastuszak E, Remeikaitė-Nikienė N, Walve J, Wilhelms S, Zillén L. 2011. Hypoxia is increasing in the coastal zone of the Baltic Sea. Environmental Science & Technology 45, 6777–6783.

[10] Dale AW, Sommer S, Bohlen L, Treude T, Bertics VJ, Bange HW, Pfannkuche O, Schorp T, Mattsdotter M, Wallmann K. 2011. Rates and regulation of nitrogen cycling in seasonally hypoxic sediments during winter (Boknis Eck, SW Baltic Sea): Sensitivity to environmental variables. Estuarine, Coastal and Shelf Science 95, 14–28.

[11] De Albuquerque JP, Keim CN, Lins U. 2010. Comparative analysis of *Beggiatoa* from hypersaline and marine environments. Micron 41, 507–517.

[12] Drgas A, Radziejewska T, Warzocha J. 1998. Biomass size spectra of near-shore shallow-water benthic communities in the Gulf of Gdańsk, southern Baltic Sea. Marine Ecology 19, 209–228.

[13] Ducklow HW & Mitchell R. 1979. Observations on naturally and artificially diseased tropical corals: A scanning electron microscope study. Microbial Ecology 5, 215–223.

[14] Emeis K-C, Struck U, Leipe ,T Pollehne F, Kunzendorf H, Christiansen C. 2000. Changes in the C, N, P burial rates in some Baltic Sea sediments over the last 150 years—relevance to P regeneration rates and the phosphorus cycle. Marine Geology 167, 43–59.

[15] Faust L & Wolfe RS. 1961. Enrichment and cultivation of *Beggiatoa alba*. Journal of Bacteriology 81, 99–106.

[16] Freckman DW. 1988. Bacterivorous nematodes and organic-matter decomposition. Agriculture, Ecosystems & Environment 24, 195–217.

[17] Genthner FJ, Hook LA, Strohl WR. 1985. Determination of the molecular mass of bacterial genomic DNA and plasmid copy number by high-pressure liquid chromatography. Appliend and Environmental Microbiology 50, 1007–1013.

[18] Grabovich MY, Patritskaya VY, Muntyan MS, Dubinina GA. 2001. Lithoautotrophic growth of the freshwater strain *Beggiatoa* D-402 and energy conservation in a homogeneous culture under microoxic conditions. FEMS Microbiology Letters 204, 341–345.

[19] Graco M, Farias L, Molina V, Gutierrez D, Nielsen LP. 2001. Massive developments of microbial mats following phytoplankton blooms in a naturally eutrophic bay: Implications for nitrogen cycling. Limnology and Oceanography 46, 821–832.

[20] Grant J & Bathmann UV. 1987. Swept away: resuspension of bacterial mats regulates benthic-pelagic exchange of sulfur. Science 236, 1472–1474.

[21] Hagen KD & Nelson DC. 1996. Organic carbon utilization by obligately and facultatively autotrophic *Beggiatoa* strains in homogeneous and gradient cultures. Applied and Environmental Microbiology 62, 947–953.

[22] Hagen KD & Nelson DC. 1997. Use of reduced sulfur compounds by *Beggiatoa* spp.: Enzymology and physiology of marine and freshwater strains in homogeneous and gradient cultures. Applied and Environmental Microbiology 63, 3957–3964.

[23] Hietanen S, Jäntti H, Buizert C, Jürgens K, Labrenz M, Voss M, Kuparinen J. 2012. Hypoxia and nitrogen processing in the Baltic Sea water column. Limnology and Oceanography 57, 325–337.

[24] Hinck S, Mußmann M, Salman V, Neu TR, Lenk S, de Beer D, Jonkers HM. 2011. Vacuolated *Beggiatoa*-like filaments from different hypersaline environments form a novel genus. Environmental Microbiology 13, 3194–3205.

[25] Jewell T, Huston SL, Nelson DC. 2008. Methylotrophy in freshwater *Beggiatoa alba* strains. Applied and Environmental Microbiology 74, 5575–5578.

[26] Jonsson P & Jonsson B. 1988. Dramatic changes in Baltic sediments during the last three decades. Ambio 17, 158–160.

[27] Jørgensen BB. 1977. Distribution of colorless sulfur bacteria (*Beggiatoa* spp.) in a coastal marine sediment. Marine Biology 19–28.

[28] Jørgensen BB & Revsbech NP. 1983. Colorless sulfur bacteria, *Beggiatoa* spp. and *Thiovulum* spp., in O_2 and H_2S microgradients. Applied and Environmental Microbiology 45, 1261–1270.

[29] Joshi M & Hollis J. 1977. Interaction of *Beggiatoa* and rice plant: Detoxification of hydrogen sulfide in the rice rhizosphere. Science 195, 179–180.

[30] Kamp A, Stief P, Schulz-Vogt HN. 2006. Anaerobic sulfide oxidation with nitrate by a freshwater *Beggiatoa* enrichment culture. Applied and Environmental Microbiology 72, 4755–4760.

[31] Kamp A, Røy H, Schulz-Vogt HN. 2008. Video-supported analysis of *Beggiatoa* filament growth, breakage, and movement. Microbial Ecology 56, 484–491.

[32] Kornberg A. 1995. Inorganic polyphosphate: Toward making a forgotten polymer unforgettable. Journal of Bacteriology 177, 491–496.

[33] Matthäus W. 1995. Natural variability and human impacts reflected in longterm changes in the Baltic deep water conditions—A brief review. Deutsche Hydrographische Zeitschrift 47, 47–65.

[34] McHatton SC, Barry JP, Jannasch HW, Nelson DC. 1996. High nitrate concentrations in vacuolate, autotrophic marine *Beggiatoa* spp. Applied and Environmental Microbiology 62, 954–958.

[35] Mezzino MJ, Strohl WR, Larkin JM. 1984. Characterization of *Beggiatoa alba*. Archives of Microbiology 137, 139–144.

[36] Minges CG, Titus JA, Strohl WR. 1983. Plasmid DNA in colorless filamentous gliding bacteria. Archives of Microbiology 134, 38–44.

[37] Møller MM, Nielsen LP, Jørgensen BB. 1985. Oxygen responses and mat formation by *Beggiatoa* spp. Applied and Environmental Microbiology 50, 373–382.

[38] Mußmann M, Schulz HN, Strotmann B, Kjær T, Nielsen LP, Rosselló-Mora RA, Amann RI, Jørgensen BB. 2003. Phylogeny and distribution of nitrate-storing *Beggiatoa* spp. in coastal marine sediments. Environmental Microbiology 5, 523–533.

[39] Mußmann M, Hu FZ, Richter M, de Beer D, Preisler A, Jørgensen BB, Huntemann M, Glöckner FO, Amann R, Koopman WJH, Lasken RS, Janto B, Hogg J, Stoodley P, Boissy R, Ehrlich GD. 2007. Insights into the genome of large sulfur bacteria revealed by analysis of single filaments. PLoS Biology 5, e230.

[40] Nelson DC & Castenholz RW. 1981a. Organic nutrition of *Beggiatoa* sp. Journal of Bacteriology 147, 236–247.

[41] Nelson DC & Castenholz RW. 1981b. Use of reduced sulfur compounds by *Beggiatoa* sp. Journal of Bacteriology 147, 140–154.

[42] Nelson DC & Castenholz RW. 1982. Light responses of *Beggiatoa*. Archives of Microbiology 131, 146–155.

[43] Nelson DC, Waterbury JB, Jannasch HW. 1982. Nitrogen fixation and nitrate utilization by marine and freshwater *Beggiatoa*. Archives of Microbiology 133, 172–177.

[44] Nelson DC & Jannasch HW. 1983. Chemoautotrophic growth of a marine *Beggiatoa* in sulfide-gradient cultures. Archives of Microbiology 136, 262–269.

[45] Nelson DC, Jørgensen BB, Revsbech NP. 1986a. Growth pattern and yield of a chemoautotrophic *Beggiatoa* sp. in oxygen-sulfide microgradients. Applied and Environmental Microbiology 52, 225–233.

[46] Nelson DC, Revsbech NP, Jørgensen BB. 1986b. Microoxic-anoxic niche of *Beggiatoa* spp.: Microelectrode survey of marine and freshwater strains. Applied and Environmental Microbiology 52, 161–168.

[47] Preisler A, de Beer D, Lichtschlag A, Lavik G, Boetius A, Jørgensen BB. 2007. Biological and chemical sulfide oxidation in a *Beggiatoa* inhabited marine sediment. The ISME Journal 1, 341–353.

[48] Reichenbach H. 1981. Taxonomy of the gliding bacteria. Annual Review of Microbiology 35, 339–364.

[49] Richardson LL. 1996. Horizontal and vertical migration patterns of *Phorrnidium corallyticum* and *Beggiatoa* spp. associated with black-band disease of corals. Microbial Ecology 32, 323–335.

[50] Rosenberg R & Diaz RJ. 1993. Sulfur bacteria (*Beggiatoa* spp.) mats indicate hypoxic conditions in the inner Stockholm Archipelago. Ambio 22, 32–36.

[51] Sayama M. 2001. Presence of nitrate-accumulating sulfur bacteria and their influence on nitrogen cycling in a shallow coastal marine sediment. Applied and Environmental Microbiology 67, 3481–3487.

[52] Sayama M, Risgaard-Petersen N, Nielsen LP, Fossing H, Christensen PB. 2005. Impact of bacterial NO_3^- transport on sediment biogeochemistry. Applied and Environmental Microbiology 71, 7575–7577.

[53] Schmidt TM, Arieli B, Cohen Y, Padan E, Strohl WR. 1987. Sulfur metabolism in *Beggiatoa alba*. Journal of Bacteriology 169, 5466–5472.

[54] Schulz HN & Schulz HD. 2005. Large sulfur bacteria and the formation of phosphorite. Science 307, 416–418.

[55] Stahl DA, Lane DJ, Olsen GJ, Heller DJ, Schmidt TM, Pace NR. 1987. Phylogenetic analysis of certain sulfide-oxidizing and related morphologically conspicuous bacteria by 5S ribosomal ribonucleic acid sequences. International Journal of Systematical Bacteriology 37, 116–122.

[56] Strohl WR & Larkin JM. 1978. Enumeration, isolation, and characterization of *Beggiatoa* from freshwater sediments. Applied and Environmental Microbiology 36, 755–770.

[57] Strohl WR, Howard KS, Larkin JM. 1982. Ultrastructure of *Beggiatoa alba* strain B15LD Journal of General Microbiology 128, 73–84.

[58] Sweerts J-PRA, de Beer D, Nielsen LP, Verdouw H, den Heuvel JCV, Cohen Y, Cappenberg TE. 1990. Denitrification by sulphur oxidizing *Beggiatoa* spp. mats on freshwater sediments. Nature 344, 762–763.

[59] Teske A & Nelson DC. 2006. The genera *Beggiatoa* and *Thioploca*, in: Dworkin M Falkow S Rosenberg E Schleifer K-H Stackebrandt E (Eds.), The Prokaryotes. Springer New York, pp. 784–810.

[60] Vahtera E. 2007. The role of phosphorus as a regulator of bloom-forming diazotrophic cyanobacteria in the Baltic Sea. Finnish Institute of Marine Research, Helsinki.

[61] Vallius H. 2006. Permanent seafloor anoxia in coastal basins of the northwestern Gulf of Finland, Baltic Sea. AMBIO: A Journal of the Human Environment 35, 105–108.

[62] Vargas A & Strohl WR. 1985. Utilization of nitrate by *Beggiatoa alba*. Archives of Microbiology. 142, 279–284.

[63] Wang F & Chapman PM. 1999. Biological implications of sulfide in sediment—a review focusing on sediment toxicity. Environmental Toxicology and Chemistry 18, 2526–2532.

[64] Williams TM & Unz RF. 1985. Isolation and characterization of filamentous bacteria present in bulking activated sludge. Applied Microbiology and Biotechnology 22, 273–282.

[65] Winkel M, de Beer D, Lavik G, Peplies J, Mußmann M. 2013. Close association of active nitrifiers with *Beggiatoa* mats covering deep-sea hydrothermal sediments: Nitrifiers in *Beggiatoa* mats. Environmental Microbiology (Epub Ahead of Print).

[66] Winogradsky S. 1888. Zur Morphologie und Physiologie der Schwefelbakterien, In: Beiträge zur Morphologie und Physiologie der Bakterien. Arthur Felix, Leipzig, p. 120.

[67] Wuebbles DJ. 2009. Nitrous oxide: No laughing matter. Science 326, 56–57.

[68] Yekta SS & Rahm L. 2011. A model study of the effects of sulfide-oxidizing bacteria (*Beggiatoa* spp.) on phosphorus retention processes in hypoxic sediments: Implications for phosphorus management in the Baltic Sea. Boreal Environment Research 167–184.