Identification of a *Thiomicrospira denitrificans*-Like Epsilonproteobacterium as a Catalyst for Autotrophic Denitrification in the Central Baltic Sea†

Ingrid Brettar,1 Matthias Labrenz,1‡ Sébastien Flavier,2 Julia Bötöl,1 Harri Kuosa,3 Richard Christen,2 and Manfred G. Höfle1*  

GBF-German Research Center for Biotechnology, Department of Environmental Microbiology, Mascheroder Weg 1, D-38124 Braunschweig, Germany; UMR6543 CNRS-Université de Nice Sophia Antipolis, Centre de Biochimie, Parc Valrose, F-06108 Nice, France; and TVärminne Zoological Station, FI-10900 Hanko, Finland†  

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Identification and functional analysis of key members of bacterial communities in marine and estuarine environments are major challenges for obtaining a mechanistic understanding of biogeochemical processes. In the Baltic Sea basins, as in many other marine environments with anoxic bodies of water, the oxic-anoxic interface is considered a layer of high bacterial turnover of sulfur, nitrogen, and carbon compounds that has a great impact on matter balances in the whole ecosystem. We focused on autotrophic denitrification by oxidation of reduced sulfur compounds as a biogeochemically important process mediating concomitant turnover of sulfur, nitrogen, and carbon. We used a newly developed approach consisting of molecular analyses in stimulation experiments and in situ abundance. The molecular approach was based on single-strand conformational polymorphism (SSCP) analysis of the bacterial community RNA, which allowed identification of potential denitrifiers based on the sequences of enhanced SSCP bands and monitoring of the overall bacterial community during the experiments. Sequences of the SSCP bands of interest were used to design highly specific primers that enabled (i) generation of almost complete 16S rRNA gene sequences using experimental and environmental DNA as templates and (ii) quantification of the bacteria of interest by real-time PCR. By using this approach we identified the bacteria responsible for autotrophic denitrification as a single taxon, an epsilonproteobacterium related to the autotrophic denitrifier *Thiomicrospira denitrificans*. This finding was confirmed by material balances in the experiments that were consistent with those obtained with continuous cultures of *T. denitrificans*. The presence and activity of a bacterium that is phylogenetically and physiologically closely related to *T. denitrificans* could be relevant for the carbon budget of the central Baltic Sea because *T. denitrificans* exhibits only one-half the efficiency for carbon dioxide fixation per mol of sulfate oxidized and mol of nitrate reduced of *Thiobacillus denitrificans* hypothesized previously for this function.

The Baltic Sea is the world’s largest brackish water environment. It is strongly influenced by anthropogenic loads of nitrogen (6, 43). The central Baltic Sea is characterized by a pronounced salinity gradient (depth, 60 to 80 m) that inhibits vertical mixing. Due to the combined effect of hydrography and anthropogenic pollution, oxygen deficiency and sulfide accumulation occur in the deep water below the halocline, which mainly affects the large basins of the central Baltic Sea, such as the Gotland Basin. Denitrification plays a major role in the nitrogen budget of the Baltic Sea (31).

The Gotland Basin is the largest basin of the central Baltic Sea, and the station in the Gotland Basin that we studied is considered representative of the central Baltic Sea. This site has been studied for many decades, and thus there is an excellent database that includes microbiological parameters and data from denitrification investigations (3, 30, 42; for online databases on the Baltic Sea see http://www.helcom.fi). The oxic-anoxic interface has been identified as a major site of biogeochemical cycling in many ecosystems with anoxic basins, such as the Black Sea, the Cariaco Trench, the Baltic Sea, and fjords (5, 8, 16, 35, 36). High turnover of carbon, nitrogen, and sulfur compounds has been demonstrated for the oxic-anoxic interfaces of the central Baltic Sea (2, 5, 8, 30). Autotrophic denitrification driven by sulfide oxidation has been shown to be a major pathway for nitrogen loss from the central Baltic Sea (2, 3) because processes at oxic-anoxic interfaces have considerable importance. In addition to the oxic-anoxic interface on top of anoxic deep water, these biogeochemical processes may occur to a much larger extent during occasional interleaving of oxidized water masses in the anoxic deep water and thus have a pronounced impact on the material balance of the Baltic Sea as a whole (24).

The first “whole-genome shotgun sequencing” analysis of open ocean microbial communities was performed recently, and it generated a plethora of genetic information (39). Understanding the biogeochemical and ecological implications of this genetic information will be one of the great challenges in the years to come. Identification of bacterial catalysts for im-
portant biogeochemical processes is particularly relevant for (i) understanding factors that regulate biogeochemical processes by relating knowledge about cultured bacteria to in situ processes and rates and (ii) using the bacterial catalysts identified as indicators of in situ processes on a global scale (25). Identification of the bacterial catalysts to the species level is needed to determine such links. Studies of the biogeochemical functions of bacterial catalysts should yield results that are most relevant for an ecosystem if they are done under natural conditions; i.e., the experimental conditions should maintain the physicochemical conditions and the microbial "background" community of the environment as much as possible.

While denitrification in aquatic environments is generally considered a heterotrophic process, autotrophic denitrification is especially relevant in aquatic environments with anoxic waters, where it can represent a significant nitrogen sink (3). The bacterial autotrophic denitrification process is currently not well understood due to a lack of knowledge regarding the bacteria that are responsible and the factors regulating the process. Consortia consisting of different bacterial taxa, as well as single taxa, are conceivable mediators. Therefore, the aims of this study were to identify the bacterial catalysts responsible for autotrophic denitrification in the Gotland Deep and to obtain insight into metabolism in this environment. To do this, the activities of autotrophic denitrifiers in water samples obtained around the oxic-anoxic interface were specifically stimulated to obtain a mechanistic understanding of the biogeochemical processes involved. Newly developed molecular tools were used to study the responsible bacterial catalysts at the species level in situ and during stimulation experiments.

**MATERIALS AND METHODS**

**Study site, sampling, and physicochemical parameters.** All seawater samples were obtained at station BY15 (57.19°N, 20.03°E) in the Gotland Deep, a major anoxic basin in the central Baltic Sea, on 17 and 18 September 1998 using Niskin bottles mounted on a conductivity-temperature-depth rosette. H2S were determined aboard RV "Polyvalent". Anoxic basin in the central Baltic Sea, on 17 and 18 September 1998 using Niskin bottles obtained at station BY15 (57.19°N, 20.03°E) in the Gotland Deep, a major interface (175 m; 0.3 ml liter−1N2O, 0.3 ml liter−1NO3, no H2S). The physicochemical conditions and the microbial “background” community of the environment as much as possible.

**Experimental design.** Water samples obtained from the oxic-anoxic interface (138 m; 0.4 μmol liter−1N2O, 5.7 μmol liter−1H2S, no NO3) above the interface (120 m; 0.3 ml liter−1O2, 8.0 μmol liter−1NO3, no H2S), and below the interface (175 m; 24 μmol liter−1H2S, no NO3) were incubated at 5°C in the dark, in gas-tight 1.2-liter bottles after sequential addition of 100 μmol KNO3 (at the start of incubation) plus 50 μmol KNO3 (after 44 h of incubation), 100 μmol liter−1Na2SO3, 25 μCi liter−1H14CO3−. The controls did not contain substrates. All samples were handled so that changes in the in situ partial gas pressures (O2, H2S) were avoided (2); i.e., all bottles were filled until they were overflowing and capped so that they were air tight (Teflon-coated butyl rubber septa) and did not contain gas bubbles, and all additions added were kept under a nitrogen atmosphere. Three replicates were used for each assay. The time of incubation was within the linear range of in situ denitrification rates demonstrated by Brettar and Rheinheimer (2). After 44 h and 88 h samples were analyzed to determine the levels of N compounds (NO3−, NO2−, NH4+) and H14CO3− uptake into cells and into exuded material (9, 33); the bacterial biomass was harvested by filtration as described previously (40) and was stored frozen for later analysis.

As shown in a previous study, nitrate removal can be used to estimate autotrophic denitrification in the chemolow layer of the central Baltic Sea (1, 2). At least 75% of the nitrate consumed was recovered as gaseous nitrogen com-

**RESULTS**

In situ conditions during the study in the central Baltic Sea. The physicochemical and bacterial parameters determined (Fig. 1) can be considered typical for the summer stratification

**Nucleic acid extraction, community fingerprinting by SSCP analysis of 16S rRNA RT-PCR, and sequencing.** Nucleic acid extraction from frozen filters and quantification were performed by parallel extraction of RNA and DNA as described by Weinbauer et al. (40). Prior to reverse transcription (RT)-PCR, RNA extracts were purified from DNA by incubation with DNase I (Roche Diagnostics, Mannheim, Germany) for 60 min at 37°C. The primer used for 16S rRNA amplification from environmental RNA was described by Schierweig and Tebbe (32) (primer set Com1/Com2 amplying positions 519 to 926 [Escherichia coli numbering] of the 16S rRNA gene). RT-PCR amplification was performed using the C. therm DNA as described by Schierweig and Tebbe (32). Realignment of individual bands excised from the SSCP gels was performed as described by Pohler et al. (25), and this was followed by cycle sequencing (ABI Prism BigDye terminator cycle sequencing Ready Reaction kit; Applied Biosystems, Foster City, CA) using the primers described above. SSCP fingerprints were analyzed as described by Pohler et al. (25), using the Gelcompare II software (Applied Maths, Inc., Austin, TX). For cluster analysis the Dice coefficient, fuzzy logic, and complete linkage were used.

**Design of specific primers and quantitative RT-PCR.** Specific, 16S rRNA-targeted primers (Ost1F and Ost1R) for the Thiomicrospra denitrificans-like bacterium were designed based on the sequences obtained from the excised bands of the SSCP gel using the sequence between positions 610 and 850 (E.coli numbering) of the 16S rRNA molecule as described in detail by Höfle et al. (11). The Thiomicrospra denitrificans-like bacterium was represented by a double band (i.e., two bands that had the same sequence [see the supplemental material] but had slightly different migration distances on the SSCP gel). The occurrence of double bands is a well-known phenomenon on SSCP gels that is caused by different conformational states of the single-stranded DNA under nondenaturing conditions (32).

**Phylogenetic analysis of 16S rRNA gene sequences.** Phylogenetic trees were constructed by using three different methods (BIONJ using a Kimura two-parameter correction, maximum likelihood using the Global option, and maximum parsimony). The BIONJ program from Gascuel (7) and the DNAIDIST, ML, and MP programs from PHYLIP (Phylogeny Inference Package, version 3.573c; distributed by J. Felsenstein, Department of Genetics, University of Washington, Seattle) were used. A tree (see Fig. 3C) was drawn from the neighbor-joining topology, and it included information for bootstrap values (1,000 replications) and confirmation of each branch by the other two methods.

**Nucleotide sequence accession number.** The 16S rRNA gene sequence obtained for the Thiomicrospra denitrificans-like bacterium has been deposited in databases under accession number AJ810529.
in the Gotland Deep, a station representative of the central Baltic Sea, in periods when there is stagnant deep water (2). The water was well oxygenated above the halocline (enhanced salinity gradient from 60 to 80 m). Below 60 m, the oxygen level decreased, and oxygen was absent at depths below 130 m; the sulfide concentration increased from the oxic-anoxic interface down to the sediment. The nitrate level increased in the low-oxygen layer; low concentrations of nitrate were present in the layer of the chemocline containing low concentrations of sulfide (138 m; 5.7 μmol liter⁻¹ H₂S, 0.4 μmol liter⁻¹ NO₃⁻, no O₂). Bacterial abundance was highest in the surface water and declined with depth until just above the oxic-anoxic interface, where the number of bacteria began to increase (Fig. 1B).

**Biogeochemical stimulation experiments and bacterial community response.** The activity and abundance of the bacterial catalysts were monitored for up to 88 h under conditions that were close to the in situ physicochemical conditions during the experiments, i.e., after addition of thiosulfate and nitrate to samples obtained from the chemocline itself (138 m; with concomitant occurrence of nitrate and reduced sulfur compounds in situ), from above the chemocline (120 m), and from below the chemocline (175 m). Nitrate removal and H¹⁴CO₃⁻ uptake (Fig. 2) were observed as indicators of autotrophic denitrification. Nitrate removal data can be considered estimates of autotrophic denitrification in the deep water of the central Baltic Sea, as shown in a previous study by Brettar and Rheinheimer (2). In samples obtained from the chemocline (138 m), carbon dioxide fixation was almost linear for the 88-h observation period, and the uptake for the total incubation period was 177 μg C liter⁻¹. Samples obtained from 175 m showed a slightly higher level of CO₂ fixation (about 206 μg C liter⁻¹ for the total period [88 h], with lower CO₂ fixation during the first 44 h). Samples obtained from 120 m showed a much lower level of CO₂ fixation, 22 μg C liter⁻¹ for the total period; in the first 44 h no CO₂ fixation was detected. The high CO₂ fixation rates of the samples obtained from 138 and 175 m corresponded to a high level of nitrate consumption, about 100 μmol N liter⁻¹; the low level of CO₂ fixation in samples obtained from 120 m was accompanied by a low level of nitrate consumption. Thus, samples obtained from the chemocline and below the chemocline showed much higher activity of autotrophic denitrification than the sample obtained from above the interface. Assuming that the rate for the 88-h period was linear, the CO₂ fixation rates of the anoxic samples corresponded to rates of 49 μg C liter⁻¹ day⁻¹ (138 m) to 57 μg C liter⁻¹ day⁻¹ (175 m).

Bacterial community structure and composition were assessed by 16S rRNA-based SSCP community fingerprint analysis (Fig. 3A and B). The microbial community remained rather stable during the experiments compared to the community in the original sample, as revealed by cluster analysis of the SSCP community fingerprints (Fig. 3B). After substrate addition, only two bands showed a pronounced increase in intensity in samples from all depths (Fig. 3A). The increase was most pronounced in samples obtained from 120 m; for these samples the intensities of the bands increased from close to the level of detection to very high.

The 16S rRNA sequences of the two enhanced bands were
identical (see the supplemental material). These sequences were used to design highly specific primers in order to generate a complete 16S rRNA gene sequence using DNA extracted from in situ samples and experimental samples (11). Phylogenetic analysis of the almost complete sequence (1,422 nucleotides; accession no. AJ810529) revealed that the autotrophic denitrifier was an epsilonproteobacterium related to *Thiomicrospira denitrificans* DSM 1251^T^, which was the closest cultured neighbor (95.8% 16S rRNA gene sequence similarity) (22) (Fig. 3C). Identical complete sequences were obtained from all samples (i.e., from in situ samples obtained from different depths and from the experimental samples) (11). Another close relative is *Sulfurimonas autotrophica*, a sulfide oxidizer that is not able to denitrify (15). Due to the physiological similarity and phylogenetic relatedness to *Thiomicrospira denitrificans*, we designated the Baltic Sea bacterium the *Thiomicrospira denitrificans*-like bacterium.

The percentage of the *Thiomicrospira denitrificans*-like bacterium based on a comparison of its 16S rRNA with the total 16S rRNA of the community increased markedly after addition of thiosulfate plus nitrate in all samples, as assessed by quantification of the *Thiomicrospira denitrificans*-like bands from the community fingerprints (Fig. 4). The band intensities increased from initial values of 2% (120 m) to 15% (175 m) to final values of 48% (120 m) to 63% compared to all bands for samples from the three depths (average band intensities for three SCCP analyses).

The increase in the amount of *Thiomicrospira denitrificans*-like RNA on the SCCP gels was also observed via quantification of the *Thiomicrospira denitrificans*-like RNA fraction of the total 16S rRNA by real-time RT-PCR using the specific primers mentioned above and the appropriate environmental RNA (Fig. 4). The lower estimate for the *Thiomicrospira denitrificans*-like RNA fraction by real-time PCR at the beginning of the experiments (identical to in situ values) is consistent with the results of our calibration experiments for the SCCP analysis that showed enhanced sensitivity for low-abundance members of the bacterial community due to PCR bias (most pronounced below 1% RNA abundance [data not shown]).

According to real-time RT-PCR quantification, the maximum level of the *Thiomicrospira denitrificans*-like bacterium occurred in the RNA fraction around the chemocline layer (120 to 175 m) (18) (Fig. 1C). The size of the *Thiomicrospira denitrificans*-like 16S rRNA fraction of the total 16S rRNA in seawater samples obtained in the vicinity of the interface layer (120 to 175 m) ranged from 0.1% (120 m) to 0.9% (138 m) (18) (Fig. 1C). By using real-time PCR, a pronounced maximum and a large contribution of the *Thiomicrospira denitrificans*-like bacterium to the total bacterial DNA in the vicinity of the chemocline were estimated (Fig. 1C). Based on the 16S rRNA gene fraction, maxima for the numbers of *Thiomicrospira denitrificans*-like cells were determined for 138 and 175 m (7.1 × 10^4^ cells/ml at 138 m and 1.4 × 10^5^ cells/ml at 175 m, accounting for 8.3 and 15.1% of all bacterial 16S rRNA genes, respectively) (18). In summary, the major contribution of the *Thiomicrospira denitrificans*-like bacterium to the total bacterial DNA in the vicinity of the chemocline were estimated (Fig. 1C). Based on the 16S rRNA gene fraction, maxima for the numbers of *Thiomicrospira denitrificans*-like cells were determined for 138 and 175 m (7.1 × 10^4^ cells/ml at 138 m and 1.4 × 10^5^ cells/ml at 175 m, accounting for 8.3 and 15.1% of all bacterial 16S rRNA genes, respectively) (18). In summary, the major contribution of the *Thiomicrospira denitrificans*-like bacterium to the total bacterial DNA in the vicinity of the chemocline were estimated (Fig. 1C). Based on the 16S rRNA gene fraction, maxima for the numbers of *Thiomicrospira denitrificans*-like cells were determined for 138 and 175 m (7.1 × 10^4^ cells/ml at 138 m and 1.4 × 10^5^ cells/ml at 175 m, accounting for 8.3 and 15.1% of all bacterial 16S rRNA genes, respectively) (18).

**DISCUSSION**

We demonstrated that an epsilonproteobacterium closely related to *Thiomicrospira denitrificans* was the major bacterial catalyst for denitrification in the Gotland Basin by a combined experimental-molecular approach and study of in situ abundance. This finding was supported by concomitant observations of autotrophic denitrification driven by oxidation of thiosulfate and an increase in the abundance of the *Thiomicrospira denitrificans*-like 16S rRNA fraction during stimulation experiments. The increase in abundance was determined by SCCP analyses as well as by real-time PCR. An additional indicator of increased activity of the *Thiomicrospira denitrificans*-like
**A**

[Image of a gel with bands labeled C44h, Th44h, Th88h, C88h]  
138 m oxic-anoxic interface  
120 m above interface

**B**

[Tree diagram showing bacterial relationships]

**C**

Tree diagram showing bacterial relationships:

- **Thiomicrospira denitrificans** DSM 12517 [L40808]
- **Baltic sea autotrophic denitrifier** [AJ810529]
- **Sto.thermonas autotrophica** OK10T [AB088431]
- *Helicobacter pylori* ATCC 43504T [U01330]
- *Wolinella succinogenes* ATCC 29453T [M26636]
- *Arcobacter cryaerophilus* CCUG 17801T [L14624]
- *Sulfurospirillum barnesi* SES3T [U41564]
- *Sulfurospirillum sp 181* [AF357199]
- *Campylobacter jejuni subsp. dolyei* CCUG 24567T [L14630]
- *Nitratireductor turgidus* M155-1T [AB175499]
cells was the 70- to 100-fold increase in the ribosome content observed in the stimulation experiments compared to the ribosome content in the original water samples obtained from 138 and 175 m (18). This indicated that there was an immediate growth and activity response after substrate addition (17).

Autotrophic denitrification at the oxic-anoxic interface in the central Baltic Sea was studied in more detail by Brettar and Rheinheimer (2). It was found that autotrophic denitrification was the major nitrate removal process in the water column, and the major fraction of nitrate consumed was reduced to dinitrogen (N₂). Nitrate reduction to ammonium was never observed in the samples.

The substrate turnover, as well as the community analyses, indicated that we were able to mimic in situ conditions during our experiments. The overall bacterial community composition remained intact, and increases in abundance were observed only for the bacteria responsible for autotrophic oxidation of reduced sulfur compounds. The carbon dioxide fixation rates for the bacteria responsible for autotrophic oxidation of nitrate and thiosulfate limitation conditions had the same ratios (37). The CO₂ fixation per mol of nitrate consumed by the epsilonproteobacterium *Thiomicrospira denitrificans* is only one-half as efficient as the CO₂ fixation by the betaproteobacterium *Thiobacillus denitrificans*. It has previously been suggested that the latter organism is a catalyst for denitrification in the Baltic Sea (2, 10). The reason for the different growth efficiency was attributed in part to the fact that *Thiomicrospira denitrificans* lacks adenosine phosphosulfate reductase (37). Additionally, it was shown that the energy needed for maintenance was twice as high for *Thiomicrospira denitrificans* (37). On the other hand, it was recently shown that *Thiomicrospira denitrificans* uses the more energy-efficient reverse tricarboxylic acid cycle for carbon dioxide fixation (13). Thus, the difference between the metabolic efficiencies of the two organisms is not fully understood yet.

To compare the stoichiometry for autotrophic denitrification by *Thiomicrospira denitrificans* with the stoichiometry observed in our experiments, the ratio of CO₂ fixation to NO₃⁻ consumption was calculated for the total incubation period (88 h) for the anoxic samples obtained from 138 and 175 m. For the 120-m sample the ratio could not be calculated due to the low turnover rates and concomitant use of oxygen. To calculate the ratios, the cellular uptake shown in Fig. 2 and the exudates containing labeled carbon (on average 11% of the incorporated labeled carbon) were compared to the amount of nitrate consumed. In the experiments with samples obtained from 138 m, the percentage of nitrate consumed versus CO₂ fixed was 97% of that of *Thiomicrospira denitrificans*; for samples obtained from 175 m the value was 110%. Thus, the ratios of...
CO₂ fixation to nitrate consumption were similar to the ratio for *Thiomicrospira denitrificans* (within 10% of the stoichiometry for *Thiomicrospira denitrificans*, as measured by Timmer-ten-Hoor [37]) but very different from the ratio for *Thiobacillus denitrificans*. Thus, the phylogenetic position corresponded to the physiological features.

From an ecological point of view, it is surprising that a bacterium with a physiology very similar to that of *Thiomicrospira denitrificans* controls autotrophic denitrification at the redoxcline in the central Baltic Sea, because the metabolism of this organism is only one-half as efficient in terms of growth yield per mol $\text{S}_2\text{O}_3^{2-}$ or $\text{H}_2\text{S}$ oxidized as the metabolism of the betaproteobacterium *Thiobacillus denitrificans* (37).

Sequences of *Epsilonproteobacteria* have been determined several times by cloning environmental DNA from ecosystems with an active sulfur cycle, such as hydrothermal vents or anoxic basins, in which their involvement in sulfur cycling was hypothesized (21, 29). Enrichment cultures of *Epsilonproteobacteria* have been analyzed, and involvement of these organisms in autotrophic sulfate reduction has been shown (e.g., *Sulfurospirillum* sp. strain 18-1) (4). Additionally, a set of *Epsilonproteobacteria* has recently been isolated, and some of these organisms are even able to oxidize sulfide using oxygen or nitrate (34); also, many new genera have been described recently (23), and the aerobic sulfide oxidizer *S. autotrophica* (15) is the closest relative of the Baltic Sea denitrifier among the new genera.

However, the specific role of members of the *Epsilonproteobacteria* in situ could not be demonstrated until now. To our knowledge, this is the first study that estimated the contribution of a specific member of the *Epsilonproteobacteria* under in situ conditions to a specific predominant biogeochemical process, such as autotrophic denitrification.

**Advantages of the combined experimental-molecular approach.** We used biogeochemical experiments to stimulate a biogeochemical process of interest, and we used a set of newly developed molecular tools in the experiments to identify and quantify the bacterial catalyst of interest. The core of the molecular analyses was the 16S rRNA-based community fingerprints that allowed us to monitor the community, identify the active members due to “highlighted” fingerprint bands, and use the sequences of the bands for rough phylogenetic identification and for generation of highly specific primers. These primers were used to generate almost complete 16S rRNA gene sequences for comprehensive phylogenetic analysis (20, 28) and to quantify the species of interest by real-time PCR (11, 18). The material turnover in the stimulation experiments was used to calculate the stoichiometry of the process.

A major advantage of fingerprint techniques is that they allow workers to obtain an immediate overview of the structure and composition of bacterial communities. This overview provides insight into changes in the bacterial community during experiments, an important aspect considering that interspecies competition is a major factor for activities and survival of bacteria in nature (14). We used RNA-based community fingerprints because RNA reflects actively growing bacteria better than DNA does (17). Combined in situ analyses and biogeochemical experiments reflected rapidly increasing activities and numbers of the bacterial catalyst in the Baltic Sea water samples.

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**FIG. 4.** Relative amounts of the *Thiomicrospira denitrificans*-like bacteria during the stimulation experiments. Quantification was based on the areas of the specific bands from replicate SSCP gels (circles) and quantitative RT-PCR (diamonds) as described by Labrenz et al. (18). Solid symbols indicate the results of experiments with thiosulfate and nitrate addition, and open symbols indicate the results obtained for controls with no addition. The experiments were identical to those whose results are shown in Fig. 2. (A) Results for samples obtained above the oxic-anoxic interface (120 m). (B) Results for samples obtained at the interface (138 m). (C) Results for samples obtained below the interface (175 m).
Under certain circumstances (e.g., dominance of a single specific process) the stoichiometry of a process can be calculated based on stimulation experiments. In our study, we were able to relate the stoichiometry of autotrophic denitrification (i.e., nitrate reduction and carbon dioxide fixation) to a specific taxon. For this relationship it was necessary to monitor the bacterial community to be sure that the abundance of only the specific taxon studied was increasing parallel to the biogeochemical process. Support for this relationship was obtained by quantification and assessment of the activity by real-time RT-PCR.

In conclusion, the combined experimental-molecular approach allowed us to relate the precise phylogenetic position of a single bacterial taxon to its specific biogeochemical function in situ.

**General relevance of the presence of Thiomicrospira denitrificans-like bacteria in marine systems.** The presence and physiological responses of the autotrophic denitrifier at and around the oxic-anoxic interface are consistent with prior observations of in situ denitrification and carbon dioxide fixation in basins with sulfidic deep water in the central Baltic Sea (2, 5, 8). Reduced sulfur compounds were identified as a major electron donor for denitrification. This was explained by the low organic carbon supply from the euphotic layer for most of the year and by the availability of reduced sulfur compounds from the anoxic water (3). Turbulent vertical diffusion at the oxic-anoxic interface above the anoxic water, as well as lateral interleaving of nitrate-containing water masses into layers of anoxic deep water, can be perceived as mechanisms that promote intense autotrophic denitrification (2, 8, 24, 43). The presence of the autotrophic denitrifiers at the interface as well as in the anoxic deeper water layers explains the observed potential of the water masses for denitrification.

In addition to mixing of sulfide- and nitrate-containing water masses, storage of electron donors or acceptors by the autotrophic denitrifiers would increase their potential for denitrification when they come into contact with the complementary substrate. The observed high in situ CO$_2$ fixation rates support such a storage and/or transport mechanism, as shown for the sediment-dwelling gammaproteobacteria Thioploca spp. (12). Such mechanisms and many others (e.g., grazing resistance, motility, and competitive uptake mechanisms for nitrate and reduced sulfur compounds) could be selection factors that overcome the disadvantage of the lower growth yield of a Thiomicrospira denitrificans-like bacterium. Genome sequencing of Thiomicrospira denitrificans is currently under way (http://www.jgi.doe.gov), and the results will be a step toward predictive biogeochemistry. “Whole-genome sequencing” with stimulation experiments could provide more detailed information on the in situ genomics of the catalyst, as recently demonstrated by Tyson et al. (38) for an acid mining community with reduced biodiversity.

The lower carbon fixation rate of the Thiomicrospira denitrificans-like bacterium could be very relevant for the carbon budget of the deep water and the benthos of the central Baltic Sea, if we assume that this bacterium catalyzes a major fraction of the oxidation of reduced sulfur compounds at the chemocline. Rahm (27) calculated a yearly export of 50 g C m$^{-2}$ year$^{-1}$ from the euphotic zone to deeper water layers. Assuming a rate of 50 mg C m$^{-2}$ day$^{-1}$ as reported by Gocke (8) and Dettmer et al. (5) for a 5-m layer at the oxic-anoxic interface, dark carbon dioxide fixation would increase the yearly carbon input to the deep water by 91 g C m$^{-2}$. Due to the dependence of sulfur oxidation on mixing at the interface, very high variability must be taken into account, and it is difficult to predict rates. For the C budget of the Baltic Sea, it is an interesting question whether specific environmental conditions could promote replacement of the Thiomicrospira denitrificans-like bacterium by a more efficient sulfide oxidizer, such as Thioaucalis denitrificans, and thus greatly increase the amount of dark carbon dioxide fixation.

In conclusion, the combined biogeochemical-molecular approach used in this study allowed us to relate the precise phylogenetic position of a single bacterial population to its specific biogeochemical function in situ. This relationship can be used to predict the potential of a specific biogeochemical process when substantial amounts of 16S rRNA of a microorganism are detected in an environment. This study demonstrated the potential impact of a single bacterial taxon on the matter balance in a marine water column. Thus, it can serve as a good example to demonstrate the utility of identifying major bacterial catalysts in pelagic aquatic environments. In more general terms, our results indicate that ecological principles other than growth efficiency can play a dominant role as selection factors and thus influence the stoichiometry of a biogeochemical process relevant to marine carbon and nitrogen budgets.

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