Available online at www.sciencedirect.com
SCIENCE DIRECT
Sedimentary Geology 185 (2006) 131–145
Sedimentary Geology
www.elsevier.com/locate/sedgeo

Sulfate reducing bacteria in microbial mats: Changing paradigms, new discoveries

L.K. Baumgartner [a], R.P. Reid [b], C. Dupraz [c], A.W. Decho [d], D.H. Buckley [e], J.R. Spear [f], K.M. Przekop [a], P.T. Visscher [a,*]

[a] Department of Marine Sciences, University of Connecticut, 1080 Shennecossett Rd., Groton, CT, United States
[b] Marine Geology and Geophysics, Rosenstiel School of Marine and Atmospheric Science, University of Miami, 4600 Rickenback Causeway, Miami, FL 33149, United States
[c] Institute of Geology, University of Neuchâtel, Rue Emile-Argand 11, 2007 Neuchâtel, Switzerland
[d] Department of Environmental Health Sciences, Arnold School of Public Health, University of South Carolina, Columbia, SC 29208, United States
[e] Department of Crop and Soil Sciences, 705 Bradford Hall, Cornell University, Ithaca, NY 14853, United States
[f] Molecular, Cellular, and Developmental Biology, University of Colorado, 347 UCB, Boulder, CO, United States

Abstract

Sulfate reducing bacteria (SRB) have existed throughout much of Earth's history and remain major contributors to carbon cycling in modern systems. Despite their importance, misconceptions about SRB are prevalent. In particular, SRB are commonly thought to lack oxygen tolerance and to exist only in anoxic environments. Through the last two decades, researchers have discovered that SRB can, in fact, tolerate and even respire oxygen. Investigations of microbial mat systems have demonstrated that SRB are both abundant and active in the oxic zones of mats. Additionally, SRB have been found to be highly active in the lithified zones of microbial mats, suggesting a connection between sulfate reduction and mat lithification. In the present paper, we review recent research on SRB distribution and present new preliminary findings on both the diversity and distribution of δ-proteobacterial SRB in lithifying and non-lithifying microbial mat systems. These preliminary findings indicate the unexplored diversity of SRB in a microbial mat system and demonstrate the close microspatial association of SRB and cyanobacteria in the oxic zone of the mat. Possible mechanisms and further studies to elucidate mechanisms for carbonate precipitation via sulfate reduction are also discussed.

Keywords: Sulfate-reducing bacteria; Biofilms; Carbonate precipitation; Lithification; Stromatolites; Microbial mats

1. Introduction

Sulfate reducing bacteria (SRB) have been important organisms through much of Earth's 4.6 Ga history (Shen and Buick, 2004). Isotopic evidence indicates that sulfate reduction evolved at least 3.7 Ga ago, well before the evolution of oxygenic photosynthesis and cyanobacteria (Shen et al., 2001; Shen and Buick, 2004). SRB are major contributors to modern microbial mat systems: they can be responsible for up to 80% of carbon oxidation in marine sediments (Canfield et al., 1993).

Despite their important role in microbial mats through geologic time, SRB have been the subject of a wide range of misperceptions. Although many traditional dogmas regarding SRB have been overturned within the last two decades, SRB remain misunder-

* Corresponding author. Fax: +1 860 405 9153.
E-mail address: pieter.visscher@uconn.edu (P.T. Visscher).

0037-0738/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.sedgeo.2005.12.008

stood. Misconceptions regarding SRB result from the fact that these bacteria are predominantly anaerobic heterotrophs, i.e., they do not use oxygen as the main electron acceptor in their metabolism of carbon and H_2. Thus, SRB have been misperceived as lacking oxygen tolerance and being limited to anoxic zones such as the deeper subsurface layers of microbial mats. The incorrect assumption that SRB are restricted to anoxic zones has affected the perception of their effect on carbonate precipitation and lithification, important processes that preserve microbial mats in the geologic record. Understanding of the role of SRB in microbial mats is further complicated by confusion between the terms "aerobic" and "anaerobic", which refer to metabolic processes that use or do not use oxygen, versus "oxic" and "anoxic", which refer to the environment in which the organism lives.

The goal of this paper is to summarize current knowledge regarding the role of SRB in microbial mats and review research that links SRB activity to lithification of microbial mats. Misperceptions regarding oxygen tolerance and distribution of SRB will be discussed, as will the research that has challenged traditional dogma regarding SRB. First, to place new findings in context, we review traditional views of microbial mats and the role of SRB within these mats. Then we examine research that has changed those views. Finally, we present new data on the prevalence of SRB in actively lithifying mat systems.

2. Background and historical perspective

2.1. Introduction to microbial mats: the key players

Microbial mats are organosedimentary, layered systems (van Gemerden, 1993). The biotic portion of microbial mats is, as the name implies, microbial. The majority of the diversity in these mats is bacterial, although archaea and eukarya are also present (Spear et al., 2005; Papineau et al., 2005). The microorganisms in mats live in close proximity to each other, exchanging nutrients and organic carbon in tightly coupled biogeochemical cycles. Microbes can be classified by their metabolism—that is, their method of obtaining energy and carbon for biomass. By this classification, three of the most important groups of microbes in microbial mats are the oxygenic phototrophs, aerobic heterotrophs, and anaerobic heterotrophs.

Phototrophs are the primary producers of the mat system. They use light energy to fix inorganic carbon into organic carbon through photosynthesis. In typical microbial mat systems, the most productive phototrophs are the cyanobacteria (CYN), sometimes mistakenly referred to as blue-green algae. CYN are oxygenic photosynthetic bacteria that reduce carbon dioxide to organic carbon with water as electron donor, producing oxygen:

$$CO_2 + H_2O \rightarrow O_2 + [CH_2O] \quad (1)$$

($[CH_2O]$ is a simple notation for photosynthate).

Heterotrophs consume organic carbon for energy and biomass. Heterotrophs are often classified by their preferred terminal electron acceptor. Aerobic heterotrophs (HET) use oxygen as an electron acceptor during the respiration of organic carbon:

$$O_2 + [CH_2O] \rightarrow CO_2 + H_2O \quad (2)$$

Anaerobic heterotrophs use an alternative terminal electron acceptor (i.e., a compound other than oxygen). The key anaerobic heterotrophs in the mat system are sulfate reducing bacteria (SRB), which, as their name implies, reduce sulfate to sulfide while oxidizing organic carbon:

$$SO_4^{2-} + 2[CH_2O] + OH^- \rightarrow HS^- + 2HCO_3^- + 2H_2O \quad (3)$$

2.1.1. Calcium carbonate precipitation in microbial mats

Microbial metabolic processes, as described above, can promote the precipitation and dissolution of calcium carbonate. These processes, in turn, influence lithification and the preservation of the microbial mat in the geologic record. At the simplest level, precipitation is related to the saturation index (SI), which corresponds to log (IAP/K_{SP}), where IAP is the ion activity product and the K_{sp} is the solubility product. The IAP is proportional to the ion concentration through an activity coefficient. When the solution is well diluted, this coefficient is close to 1 and the IAP equals the ion concentration. However, under other conditions, IAP and ion concentration can differ considerably. If the SI is near zero or higher, the solution is saturated ($SI \geq -0.2$) or supersaturated ($SI \geq 0.2$) with respect to the calcium carbonate. In absence of specific inhibition (e.g., complexation by organic or inorganic compounds), experiments have shown that calcium carbonate will certainly precipitate if SI exceeds 0.8 (Kempe and Kazmierczak, 1994; Zeebe and Wolf-Gladrow, 2001). The SI is closely related to pH and the associated alkalinity that reflect the distribution of the

non-conservative ions in solution (i.e., distribution between carbonate species).

If sufficient calcium is present in solution, microbial metabolisms that induce an increase in alkalinity will move the equilibrium toward calcium carbonate precipitation, whereas metabolisms that consume alkalinity will result in calcium carbonate dissolution. Both CYN and SRB are predicted to facilitate precipitation of calcium carbonate by increasing the amount (or availability) of carbonate ions in solution. CYN increase alkalinity through carbon dioxide uptake during photosynthesis. SRB impact the pH (and thus the alkalinity) through consumption of sulfate and production of sulfide that increases the carbonate alkalinity during organic matter decomposition. In marine and many hypersaline systems that have relatively high DIC concentrations and are well-buffered, this results in calcium carbonate precipitation (Arp et al., 2003). Assuming no other process is removing these free ions (e.g., complexation by organic compounds), both CYN and SRB should precipitate carbonate.

Photosynthesis by cyanobacteria leads to calcium carbonate precipitation. The basic equation for photosynthesis is:

$$CO_2 + H_2O \rightarrow [CH_2O] + O_2 \quad (1)$$

The excessive consumption of carbon dioxide during photosynthesis necessitates reestablishment of the carbonate equilibrium:

$$HCO_3^- \rightarrow CO_2 + OH^- \quad (4)$$

This produces alkalinity, which allows the precipitation of calcium carbonate to take place:

$$Ca^{2+} + HCO_3^- \rightarrow CaCO_3 + H^+ \quad (5)$$

The proton that is produced in Eq. (5) is removed:

$$H^+ + OH^- \rightarrow H_2O \quad (6)$$

and the overall reaction is:

$$2HCO_3^- + Ca^{2+} \rightarrow [CH_2O] + CaCO_3 + O_2 \quad (7)$$

As a result, two bicarbonate ions are removed, one to become photosynthate ($[CH_2O]$) and the other to precipitate as calcium carbonate (assuming SI is reached). Aerobic heterotrophy can be viewed simplistically as a reversal of photosynthesis, and the summarized equation for HET indicates dissolution:

$$[CH_2O] + CaCO_3 + O_2 \rightarrow 2HCO_3^- + Ca^{2+} \quad (8)$$

Similar to the equations for CYN, the equations for SRB indicate carbonate precipitation. The basic equation for sulfate reduction from above,

$$SO_4^{2-} + 2[CH_2O] + OH^- \rightarrow HS^- + 2HCO_3^- + H_2O \quad (3)$$

can be combined with the equations for carbonate equilibrium and carbonate precipitation:

$$HCO_3^- \rightarrow CO_2 + OH^- \quad (4)$$

$$Ca^{2+} + HCO_3^- \rightarrow CaCO_3 + H^+ \quad (5)$$

$$H^+ + OH^- \rightarrow H_2O \quad (6)$$

In sum, the balanced equation is:

$$SO_4^{2-} + 2[CH_2O] + OH^- + Ca^{2+} \rightarrow CaCO_3 + CO_2 + 2H_2O + HS^- \quad (9)$$

For every sulfate and every two organic carbons consumed, one calcium carbonate can potentially precipitate. Note that the consumption of sulfate and production of sulfide in this reaction changes the alkalinity somewhat in favor of precipitation (sulfate (sulfuric acid) is a much stronger acid than (hydrogen) sulfide).

It should be noted that the sulfide produced by SRB can serve as electron donor for sulfide-oxidizing bacteria (SOB). These chemolitho(auto)trophic organisms gain energy from the oxidation of sulfide, predominantly with oxygen, during which calcium carbonate dissolves (Visscher and Stolz, 2005). Coculture of SRB and SOB in chemostat cultures has been demonstrated at oxygen saturation (van den Ende et al., 1997). Under these conditions, assuming equal rates of sulfide production and consumption, no net calcium carbonate precipitation would occur. However, initial precipitation in microbial mats takes place on a micrometer scale (Fig. 1). On this small scale, oxygen can be low due to aerobic consumption, resulting in incomplete sulfide oxidation (Visscher and Stolz, 2005), which yields additional calcium carbonate precipitation rather then dissolution (Visscher and Stolz, 2005). Additionally, sulfide oxidation is coupled to photosynthesis through the requirement for oxygen, whereas sulfate reduction is not (Visscher et al., 1998). Therefore, on the micrometer scale, sulfate reduction and sulfide oxidation are uncoupled during a diel cycle, and sulfate reduction during nighttime causes net calcium carbonate precipitation.

Oxygenic photosynthesis by CYN has been implicated as a mechanism for carbonate precipitation in

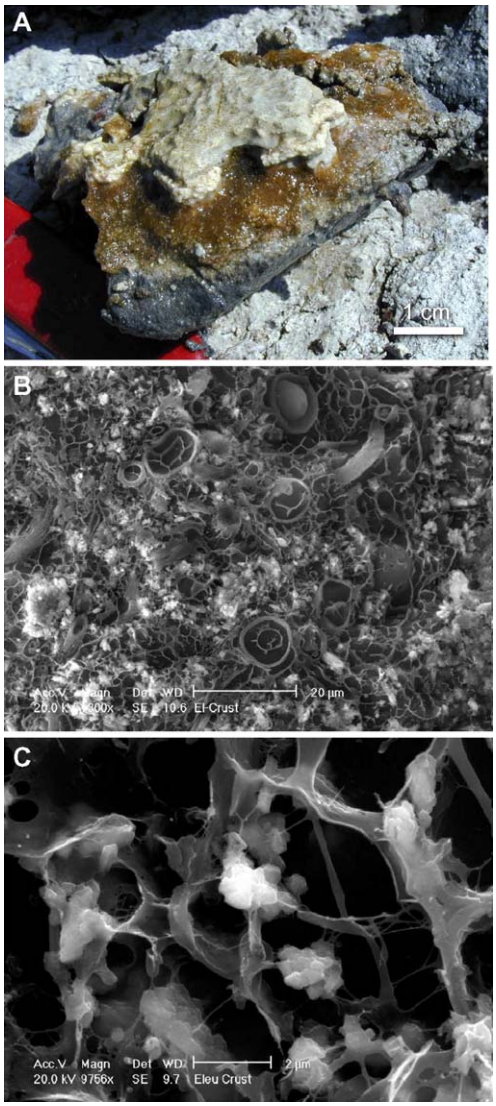

Fig. 1. High-Mg calcite precipitation in lithifying microbial mat, Salt Pan, Eleuthera, Bahamas. This precipitation takes place in the zone of maximal sulfate reduction, as documented with $^{35}SO_4^{2-}$ Ag-foil (Dupraz et al., 2004) A) Macroscopic sample showing the carbonate crust forming at the top of an orange-pigmented *Gloeocapsa* mat. B) Photomicrograph illustrating coccoid bacteria embedded in EPS (alveolar structure) and the microstructure of precipitates (white spots) as seen with FEG-ESEM using cryofixation (low temperature ESEM). The onset of high-Mg calcite is initiated in the alveolar structure of the EPS matrix, progressively replacing the organic structure. C) Details of the high-Mg calcite precipitation within the alveolar structure of the EPS. The precipitation is initiated as small spheres of calcium carbonate and progressively develops into larger, smoothly angular crystals. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

sediments (Krumbein et al., 1977; Freytet and Verrecchia, 1998). Indeed, freshwater microbial deposits often show calcite impregnation of organic matter such as cyanobacterial sheaths (e.g., Freytet and Plet, 1996; Freytet and Verrecchia, 1998). However, few cyanobacterial casts are found in recent and fossil marine environments (e.g., Grotzinger and Knoll, 1999), suggesting that another metabolic group may be responsible for mat lithification in these environments.

Research over the past two decades and recent new discoveries are evidence that SRB may be a missing link in our understanding of carbonate precipitation and lithification of microbial mats. As examples: Lyons et al. (1984) modeled the effects of biotic and abiotic processes on carbonate precipitation in continental shelf sediments, and found that sulfate reduction rates most closely corresponded with rates of carbonate precipitation. In a study of porewater geochemisty and flux experiments in Solar Lake, Sinai, Walter et al. (1993) demonstrated that sulfate reduction creates dissolved carbon dioxide, which is then removed through carbonate precipitation. Additionally, sulfate reduction, as indicated by pore water enrichment in heavy sulfate, was found to be a controlling factor in dolomite precipitation in Lagoa Vermelha, Brazil (van Lith et al., 2002). SRB cultures from Lagoa Vermelha have precipitated dolomite in controlled laboratory experiments (Warthmann et al., 2000). These experiments and others demonstrate the importance of SRB in carbonate precipitation.

2.2. Traditional dogma regarding sulfate reducing bacteria

SRB are traditionally viewed not only as anaerobic heterotrophs, but also as organisms that are incapable of tolerating oxygen and thus are restricted to anoxic zones. This dogma arose from both theory and observation. Two theories contributed to this view of SRB. First, SRB were thought to be poisoned by oxygen, because of the oxygen sensitivity of pyrophosphatase, the enzyme involved in the activation of sulfate to APS (Postgate, 1959). As is mentioned above, isotopic studies revealed that SRB evolved well before the advent of atmospheric oxygen (Shen and Buick, 2004). This suggested that SRB would not have originally needed to evolve mechanisms to deal with oxygen and the deleterious effects of reactive oxygen species such as superoxide radicals and peroxide. These compounds exist in the oxic environment and are created when the cell reduces oxygen. Reactive oxygen species can damage cell components. Survival under oxic conditions would require adaptations to remove these reactive oxygen compounds, and it has been assumed that SRB lacked these adaptations. However, laboratory and field observations have shown that some SRB can tolerate oxy-

gen. These experiments will be discussed in Sections 3.1 and 3.2.

The second theory contributing to the misunderstanding of SRB involves themodynamics. Thermodynamic considerations suggest that SRB should be outcompeted in the oxic zone by the more energetically favorable metabolism of the aerobic heterotrophs. The Gibbs free energy yield from HET metabolism is greater than that yielded by SRB ($\Delta G^{o\prime} = -480$ and -64 kJ/mol of [CH_2O], respectively). However, microbial mats are typically anoxic at night, which creates an exclusive environment uniquely suitable for the SRB during that period (Visscher et al., 2002). This allows SRB to compete with HET. Additionally, as is discussed in Section 3.1.2, researchers believe that SRB may create anoxic microniches within the oxic zone of a mat that allow the SRB to outcompete HET.

Support for the theories that SRB were limited to anoxic zones of microbial mats was provided by visual observations of microbial mats. In the upper layers of a microbial mat, coloring is assumed to indicate the dominant organisms: the vibrant pigments of the phototrophs indicate the presence of cyanobacteria, and purple (sulfur and non-sulfur) bacteria and green (sulfur and non-sulfur) bacteria. This (inaccurate) logic was followed down into the black layer of mats (the black is indicative of iron sulfides produced when sulfide is formed). This black anoxic zone was assumed to be the location where all of the anaerobic sulfate reduction occurred. Given the aforementioned theories that SRB could not tolerate oxygen, this view was logical. However, along with those theories, this limited view of SRB extent has been overturned with more exacting measurements of SRB activity and abundance, as will be discussed in Section 3.2.

Theory and observation resulted in the dogma that SRB are anaerobic organisms incapable of surviving in the oxic zone. However, SRB activity has been shown to co-occur with lithification in the oxic zone of microbial mats. This co-occurrence (discussed in Section 3.2.2.) further demonstrates the activity of SRB and strengthens the connection between SRB and lithification.

3. Opposing the dogma: a chronicle of recent research

In the past two decades a variety of studies on both cultures and whole microbial mats have disproven the traditional notion that SRB are not oxygen tolerant and are incapable of surviving and competing in the oxic zone. This research is summarized below.

3.1. Laboratory culture studies

3.1.1. The "Obligate Anaerobe" theory falsified

The first challenges to the paradigm that SRB are oxygen intolerant came from laboratory culture experiments by Cypionka et al. (1985). These researchers exposed various strains of SRB to oxygen, demonstrating that SRB survive long oxic periods and maintain their capacity for sulfate reduction. Later work provided examples of the ability of SRB to reduce both oxygen and nitrate for energy in oxic conditions (Dilling and Cypionka, 1990; Dannenburg et al., 1992). This respiration was only microaerophilic, and was reduced or halted by increased concentrations of oxygen.

Additional studies examined the oxygen tolerance and preferred electron acceptors of a few SRB strains. Different strains of SRB were found to have different oxygen tolerances, which caused certain strains to move to different concentrations in an oxygen gradient (Marschall et al., 1993). This differential tolerance will be discussed further below (Section 3.1.2). Oxygen was again found to be toxic: with high concentrations or prolonged exposure, cell viability and motility were decreased and eventually halted in the seven strains investigated. In fact, to date, no culture strain has been reliably observed to grow beyond one division while exposed to high oxygen concentrations, with one study finding that growth was affected by oxygen concentrations of 1.5–2% of atmospheric oxygen (van Niel and Gottschal, 1998, also reviewed in Brune et al., 2000 and Cypionka, 2000). However, SRB that can utilize oxygen appear to do so preferentially. Exposure of SRB strains to different combinations of electron acceptors demonstrated that SRB utilize electron acceptors based on energy yield: first oxygen, then nitrate/nitrite, and then sulfur compounds (e.g., sulfate, sulfite, thiosulfate, and elemental sulfur) (Krekeler and Cypionka, 1995). In other words, if a small amount of oxygen is present, it will be utilized first until it is gone. Then nitrate will be consumed, and the SRB will finally turn to reduction of sulfur compounds.

Further studies have clarified the biochemical mechanisms of oxygen reduction in some strains of SRB. For example, Chen et al. (1993) discovered that *Desulfovibrio gigas* uses a pair of proteins to reduce oxygen while oxidizing NADH (in a 1 : 2 ratio). In this metabolic reaction, the production of hydrogen peroxide, which can create free radicals that are damaging to cells, is avoided. Similar adaptations were later discovered in other strains (reviewed in van Niel and Gottschal, 1998), but not all SRB possess the capability to deploy these mechanisms. A number of additional

methods for oxygen respiration have been found (van Niel and Gottschal, 1998, reviewed in Fournier et al., 2004). Finally, additional types of metabolism may be available to SRB for energy generation under oxic conditions. In thiosulfate disproportionation, a carbon-independent reaction, part of the sulfur compound is oxidized to sulfate while the other is reduced to sulfide in a process that is similar to organic fermentation reactions (Bak and Cypionka, 1987). This inorganic fermentation has been reported to be abundant in near-shore and shelf sediment where oxygen penetrates deep into the sediment (Jørgensen and Bak, 1991).

3.1.2. Adaptations to oxygen

Along with understanding the SRB metabolism of oxygen, researchers have examined potential protective and competitive mechanisms for SRB living in oxic environments. Because the SRB had already diverged into several groups before oxygen adaptations were required (Fournier et al., 2004), it is not surprising that different groups and strains of SRB display different adaptations to and tolerances for different oxygen concentrations. However, although some SRB are now known to utilize oxygen, oxygen can still be toxic, partially due to the presence of reactive oxygen species.

The simplest mechanisms for oxygen adaptation are physical. A few species of SRB are motile, and will move to their "preferred" oxygen concentration (Marschall et al., 1993; Eschemann et al., 1999) where they can continue metabolism and possibly grow. SRB will also form clusters or flocs, allowing them to create anoxic micro-environments (Sigalevich et al., 2000a). Similar mechanisms were proposed for the ability of SRB to grow aerobically when in coculture with facultative anaerobes—the facultative anaerobe could remove the oxygen, creating a microniche in which the SRB would grow (van den Ende et al., 1997; Sigalevich et al., 2000b). The creation of microniches would also allow SRB to compete with HET despite the thermodynamic favorability of aerobic heterotrophy.

Along with simplistic physical mechanisms for tolerating oxygen exposure, SRB have also been found to have more complex biochemical adaptive mechanisms. First, some SRB have an abundance of chemical-sensing proteins in their membranes (*E. coli* have four, the SRB *Desulfovibrio vulgaris* has ~16) (Voordouw, 1995). These proteins appear to allow the cell to sense oxygen concentration or redox potential and move or not move depending on the favorability of the environment.

As was discussed above, cells that remain in an oxic environment must have protection from the cell component damage caused by peroxide and superoxide radicals. To remove these compounds, SRB of the genus *Desulfovibrio* (*Dsv.*) have enzymes such as superoxide dismutases, which destroy superoxides. The expression of superoxide dismutase in *Dsv. gigas* appears to be linked to an oxygen-sensing mechanism: when oxygen or an oxygen radical is detected, the protective dismutase would be expressed and remove superoxides (Silva et al., 1999). Mutated strains of *Dsv. vulgaris* were used to measure the importance of periplasmic superoxide dismutase versus cytoplasmic superoxide dismutase (Fournier et al., 2003) The study found that the periplasmic dismutase protected the cell under microaerophilic conditions, when external superoxides are the greater threat. Cytoplasmic dismutase protected the cell under fully oxic conditions, when the cell conducts oxygen reduction and generates superoxides in the cytoplasm (superoxides cannot penetrate the cell membrane).

3.2. Studies on field samples

3.2.1. Oxic-tolerant sulfate reducing bacteria in microbial mats

Challenging the perception that SRB were confined to the anoxic zones of microbial mats (Section 2.2), field measurements reported the existence of SRB in the oxic zone of microbial mats well before oxygen tolerance by SRB was observed in the laboratory (i.e., Jørgensen and Cohen, 1977, reviewed in Visscher et al., 1992). The culture studies discussed above bolstered these field observations, and encouraged further examination of both activity and abundance of SRB throughout the microbial mat profile.

Canfield and Des Marais (1991) reported measurements of sulfate reduction in the oxic zone of hypersaline mats at Guerrero Negro, Baja California Sur, Mexico. They injected ^{35}S-labeled sulfate into cores and measured the production of ^{35}S-labeled sulfide with time. Interestingly, some the highest rates of sulfate reduction measured were in the oxic zone of the mat during the day (several μM sulfate reduced min^{-1}). Fründ and Cohen (1992) reported similar results, although much lower rates, for a mat growing in a controlled experimental setting.

Additional measurements of SRB activity and estimates of SRB abundance throughout the mat were presented by Visscher et al. (1992). Sulfate reduction rates were measured with isotope injection into cores and SRB abundances were measured with most probable number (MPN) counts. MPN is a statistical method in which the sample is serially diluted with medium that selects for the organism of interest. The lowest sample

concentration at which growth occurs indicates the initial density of the organism. Sulfate reduction rates were reported to be highest near the mat surface (650 nmol cm^{-3} d^{-1}), coinciding with the highest numbers of SRB (10^7–10^8 cells cm^{-3}). Other studies have presented additional evidence of sulfate reduction under oxic conditions in microbial mats (e.g., Canfield and Des Marais, 1993; Teske et al., 1998; Wierenga et al., 2000; Jonkers et al., 2003) as well as in other systems (e.g., marine sediments, Bühring et al., 2005).

Molecular phylogenetic analyses are also enhancing our knowledge of SRB distribution within microbial mats. With molecular methodology, there are two ways to examine an environmental community through nucleic acids (DNA or RNA). In the first method, nucleic acids are chemically/physically extracted from environmental samples and then (through various methods) the sequence of the nucleic acids is determined and compared to known sequences in a database. In the second method, the sequences in the database are used to develop probes with fluorescent tags that will bind to specific sequences from organisms of interest (i.e., with complementary sequences). This second technique was used to examine the distribution of different groups of SRB in mats from Guerrero Negro, and demonstrated that different genera of SRB resided at different depths within the mat (Risatti et al., 1994; Minz et al., 1999). These results concur with subsequent findings of differential oxygen preference and tolerance in different groups of SRB (Section 3.1.1).

3.2.2. Spatial correlation between sulfate-reducing bacteria and mat lithification

As a complement to studies showing the presence of SRB in the oxic zone of microbial mats, recent research has linked SRB activity to carbonate precipitation in surface, oxygenated zones. In particular, Visscher et al. (2000) and Dupraz et al. (2004) used the "silver foil technique" to demonstrate co-occurrence of SRB activity and mat lithification in Bahamian stromatolites and lithifying mats from a hypersaline pond. The "silver foil technique" allows for a micro-scale two dimensional profile of sulfate reduction. In addition, this method uses a sliced sample, and the other side of the slice interface can be preserved for thin sectioning or other analysis. To measure sulfate reduction, ^{35}S-labeled sulfate is coated onto a sheet of silver foil. This sheet is then incubated against a vertical section of sample for a known period of time. Sulfate reducing activity will transform the labeled sulfate to labeled sulfide, which will then bind to the silver foil. The foil can then be washed and areas of $Ag^{35}S$, indicating reduction, can be visualized by radiography. This two-dimensional measurement of sulfate reduction can then be directly compared to the thin-section image from the same interface. With this comparison Visscher et al. (2000) and Dupraz et al. (2004) demonstrated the spatial correlation between SRB activity and carbonate precipitation throughout the mat profile (including the oxic zone). Furthermore, these studies demonstrated that the lithified mat layers exhibited the highest rates of sulfate reduction, suggesting that sulfate reduction may be directly linked to lithification.

3.3. The state of the science

Although research has demonstrated that SRB are present and active in the oxic zone of microbial mats, many questions remain. Much of our understanding of oxygen tolerance in SRB comes from cultured strains (see Section 3.1). When the cultivation medium and incubation conditions are not chosen carefully, culture techniques only retrieve 1–10% of the organisms that occur in the environment (Amann et al., 1995; Pace, 1996). At best, culture methods retrieve 30–40% of environmental organisms (Visscher and van Gemerden, 1993). Given what we already know about the range of oxygen tolerances in *cultured* SRB, we can only imagine the many adaptations that exist in the uncultured SRB.

Additionally, although correlations have been established between SRB and lithification (Section 3.2), we still do not understand the mechanism behind carbonate precipitation by SRB. Further studies are being conducted to attempt to assess both the diversity and micro-scale distribution of SRB, as well as the correlations between diversity and lithification. While these studies are ongoing, preliminary results will be discussed in the next section.

4. Current research

New techniques are emerging, particularly in phylogenetics, which may result in improved understanding of the range of oxygen tolerance in SRB and the interrelationships between SRB and mat lithification. Studies discussed in Section 3.2.1 demonstrate how phylogenetics can aid our understanding of SRB in microbial mats. Two phylogenetic techniques that are used to examine environmental populations via their nucleic acids are of particular interest. These methods, phylogenetic sequencing and in situ hybridization, have both become increasingly useful and available for the examination of environmental systems such as microbial mats.

Phylogenetic sequencing is rapidly becoming easier and less expensive. Both difficulty and cost have kept researchers from being able to fully survey diversity in the complex microbial mat community, but molecular surveys are moving closer and closer to that goal, allowing researchers to examine the community differences within and between microbial mats. Genetic sequencing can be used to measure the diversity of SRB communities via analyses of 16S rDNA or functional genes. Additionally, genome sequencing has been used to indicate new enzymes as potential oxygen adaptations (Fournier et al., 2003). Comparisons of genetic diversity within a mat or between mat systems can point to certain organisms as being common in or even necessary to a layer with carbonate precipitation. Preliminary data from one such comparison is presented in Section 4.1.

Fluorescence in situ hybridization (FISH) is being honed by the ever-larger database of target sequences available from phylogenetic sequencing. A FISH probe can be designed to target almost any level of diversity (e.g., a single organism, a genus such as *Desulfovibrio*, or a domain such as the bacteria, or even one given organism). When this probe is hybridized to the target DNA, the target organism will fluoresce under certain wavelengths of light. This allows researchers to visualize population differences under an epifluorescent or confocal microscope (Pernthaler et al., 2001). In the case of oxygen tolerance, these probes could be designed to target SRB with a given enzyme and visualize their distribution along a mat profile. And in the case of carbonate precipitation, if sequencing indicates that a certain organism or group of organisms are key to precipitation, FISH would allow us to visualize the distribution of that organism throughout the mat on a micron scale. If the organism occurred only in lithified layers, or occurred with precipitated grains, that would be a further measure of the importance of that organism for precipitation and lithification. Preliminary data from a FISH study of SRB and stromatolites are discussed in Section 4.2.

4.1. Diversity of sulfate reducing bacteria in lithifying and non-lithifying microbial mats: 16S rDNA sequencing

To determine if specific SRB are associated with mat lithification, we are examining SRB diversity in lithifying and non-lithifying microbial mats from a closed hypersaline lagoon (Salt Pan, Eleuthera, Bahamas). This microbial mat system, which was previously described by Dupraz et al. (2004), contains mats with surface lithification (lithifying) and mats with no lithification (non-lithifying) under similar physical and chemical regimes. With few possible environmental changes between the outer portion of the pond where the lithifying mats occur and the inner portion where the non-lithifying mats occur, microbial community differences have been invoked as one potential mechanism for the difference in lithification.

Our goal is to examine differences in SRB diversity between the lithifying and non-lithifying mats. The majority of known (cultivated) SRB belong to a group of bacteria known as the δ-proteobacteria (Devereux et al., 1989). The close relatedness of these bacteria makes them an ideal candidate for initial examinations of correlations between lithification and the SRB community.

To examine these environments through sequencing, 16S rDNA was physically/chemically extracted from the samples of both lithifying and non-lithifying mat and amplified, cloned, and sequenced according to previously published methods (Papineau et al., 2005). Essentially, the rDNA is copied via polymerase chain reaction (PCR) with a δ-proteobacteria-targeted primer set (delta96F AGTARAGYGGCGCAC, universal 1492R GGTTACCTTGTTACGACTT). This environmental δ-proteobacteria DNA was then inserted into vector and into *E. coli* clones, which replicate each individual DNA separately. The DNA is again reamplified via PCR and sequenced using fluorescent tags inserted during reamplification. The raw sequence chromatogram is processed via several software packages (including ARB, http://www.arb-home.de/ and PAUP* 4.0, http://paup.csit.fsu.edu/) and the sequences are compared to sequences from other environmental and cultured organisms. Unique sequences were submitted to GenBank, accession numbers DQ109877-DQ109946.

In this initial study, we present 70 sequences representing the majority of the δ-proteobacteria. 23 and 47 contiguous sequences were retrieved, respectively, from the lithifying and non-lithifying mats. This low number of sequences does not represent adequate coverage of the full diversity of either mat type, but does give us a preliminary idea of the δ-proteobacterial diversity in these mats. Of these sequences, 64% and 91%, respectively, were actually δ-proteobacteria (Fig. 2). The other sequences represented organisms from other bacterial divisions (e.g., Bacteriodetes, Spirochetes, Chloroflexi). Our knowledge of bacterial diversity has increased since the delta96F probe was designed, and more specific primers could be designed with the current database.

The sequences from the Salt Pan mat represented a broad range of δ-proteobacteria (Fig. 2). These sequences included representatives from all of the δ-

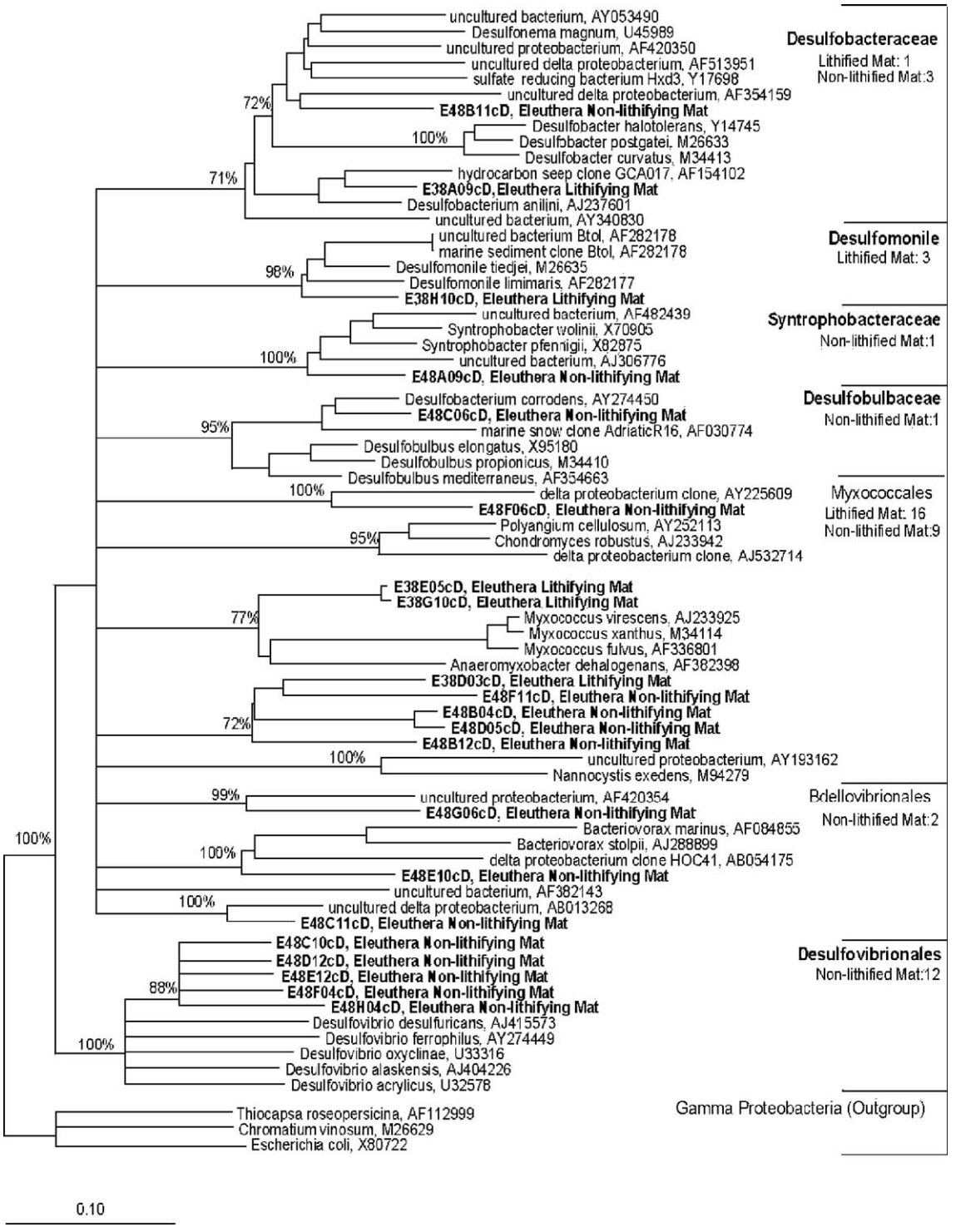

Fig. 2. Phylogenetic tree showing representative δ-proteobacterial sequences from the lithifying ("E3") and non-lithifying ("E4") mats (Salt Pan, Eleuthera, Bahamas) in relation to other δ-proteobacteria. Tree was constructed using PAUP* 4.0. Sequences from this study are in bold. Groups (on the right hand side) containing known sulfate-reducing bacteria are in bold. Numbers on the right hand side represent the number of sequences from each sample that were related to the indicated group (out of 23 and 47 sequences for the lithifying and non-lithifying mats, respectively). Percentages represent the percent of 1000 trees in which a given branch point appeared (calculated by neighbor joining bootstrap, PAUP* 4.0).

proteobacterial groups known to contain SRB except for the *Desulfomonadales*. Because the sample size is too small to have sampled the full diversity, the low number of groups represented by the sequences from the lithifying mat should not be interpreted as an indicator of the true diversity. In a larger sample set with less specific primers (universal primers 515F GTGCCAGCMGCCGCGGTAA and 1391R: GACGGGGCGGTGWGTRCA), the lithifying mats do contain a wider diversity of δ-proteobacteria, including more sequences representative of SRB-related organisms (Baumgartner et al., in preparation). However, the 515F/1391R sample set also indicates that the lithifying mat may contain a lower overall diversity than the non-lithifying mat. This observation seems logical if one considers the mat structure: the lithifying mat is very thin, (~0.1 cm depth) while the non-lithifying mat is much thicker (~1.5 cm deep). This depth difference would provide a wider range of niches in the non-lithifying mat (e.g., the oxygen gradient might be wider, allowing for a wider range of oxygen concentrations). The greater number of niches could equal greater diversity. Therefore, diversity differences may not in themselves indicate anything about the mechanism for lithification.

Although the goal of this initial study was to search for sequences representing SRB that could be further studied as agents of lithification, we also demonstrated how little we know about SRB. Even the current GenBank database of published culture and environmental sequences (http://www.ncbi.nlm.nih.gov/entrez/) does not include many sequences that are closely related to the Salt Pan sequences. One measure of relatedness is percent identity, which can be used as a loose guide to how related the organisms represented by those sequences would be. While exact percentages cannot be placed on any level of relatedness, any sequences with <97% identity are unlikely to be representatives of the same species (Stackebrandt and Goebel, 1994). Sequences from the lithifying mat averaged 92.5% identity by BLAST to sequences in the GenBank database, and 100% of those sequences were most closely related to environmental sequences in an ARB database containing >16,000 sequences. From the non-lithifying mat, the sequences averaged only 90.8% identity and 93% of the sequences were most closely related to environmental sequences in the database. The sequences from both mats were often most closely related to other sequences obtained from Salt Pan mats or hypersaline mats from Guerrero Negro, Baja California (Ley et al., submitted for publication). All of these data indicate that the environmental δ-proteobacterial sequences are from organisms that are very different from those in our databases, and from the well-studied cultures. Given the diversity of oxygen tolerances seen in the well-studied cultures, one can only imagine the diversity of SRB adaptations that exist in these microbial mats.

4.2. Distribution of sulfate reducing bacteria in stromatolites: fluorescence in situ hybridization (FISH)

To examine the distribution of SRB throughout a lithifying microbial mat, we used fluorescence in situ hybridization (FISH) to pinpoint SRB in stromatolites from Highborne Cay, Bahamas. Stromatolites are lithified layered sedimentary structures formed by microbes; these laminated structures formed massive reefs that dominate the fossil record for 80% of Earth history. Marine stromatolites in the Bahamas are the only known examples of modern stromatolites forming in an open marine environment. (e.g., Reid et al., 2000). Highborne Cay is an open, subtidal system with modern stromatolites (Reid et al., 2000 and references therein). Layering in the Bahamian stromatolites reflects an alternation of lithified and unlithified laminae. FISH was used to examine the correlation between SRB abundances and lithified layers in a living stromatolite.

FISH was conducted according general methods modified from Snaidr et al. (1997), Llobet-Brossa et al. (1998), and Amann et al. (1995). The 16S rRNA probe SRB385 (CGGCGTCGCTGCGTCAGG) targets sulfate reducing bacteria of the δ-proteobacteria and Gram-positive bacteria (Loy et al., 2003). This probe, with a fluorescent label, was allowed to enter the cells in a prepared sample of stromatolite. The probe attached to the abundant ribosomal RNA in cells of the target organism (SRB), and excess probe was removed by washing. The distribution of the SRB in the sample was then visualized with an epifluorescent microscope.

The FISH survey further demonstrates the ability of SRB to survive at the oxic zone of the stromatolite surface. The green-fluorescing SRB are highly abundant at the mat surface, intermingling with the red-fluorescing cyanobacteria at the top left of Fig. 3. This representative image provides further proof of the ability of SRB to not only survive oxic conditions, but to thrive with cyanobacteria.

These close microspatial associations of SRB and cyanobacteria illustrate the close metabolic coupling of these two groups of bacteria. A recent study of stromatolite mats showed that ^{14}C-labeled exudates released by cyanobacteria were rapidly (i.e., within 4 h) taken up and mineralized to $^{14}CO_2$ by the heterotrophic

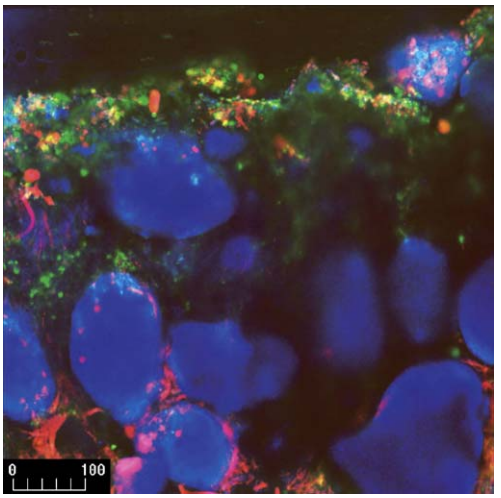

Fig. 3. Low-magnification confocal scanning laser microscopy (CSLM) cross-sectional image of the surface of a Highborne Cay stromatolite mat. Note abundant clusters of sulfate-reducting bacteria (green fluorescence) at the mat surface, as detected using a fluorescence in-situ hybridization (FISH) oligoprobe (SRB 385). Ooids (blue autofluorescence) and cyanobacteria (red autofluorescence) are also shown. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

community over a diel cycle. Further, specific substrates, such as the uronic acid monomers of cyanobacterial EPS were rapidly mineralized by SRB (Decho et al., 2005). This suggests that the microspatial proximity of SRB and cyanobacteria represent a close metabolic coupling; one that may fuel heterotrophic carbonate precipitation. Finally, the SRB do appear to form clusters, which may support the theory that they are associated with microniches of low oxygen concentration (see Section 3.1.2).

Both the FISH study discussed here and the sequencing study discussed in Section 4.1 are ongoing, and continuing observations will expand our knowledge of both the diversity and distribution of SRB in these modern examples of lithifying mat systems. With this knowledge, we will further define the links between SRB diversity and mat lithification and preservation in the geologic record.

5. Future research

The discussion above summarizes evidence demonstrating that SRB are oxygen tolerant and can survive in the oxic zone of microbial mats, including zones of lithification. We have also shown how new phylogenetic techniques can be used to further our understanding of SRB diversity and distribution, thereby clarifying the mechanisms by which SRB affect carbonate precipitation and the lithification of microbial mats. These concepts open the door to an exciting array of future investigations.

5.1. Potential mechanisms for sulfate-reducing bacteria induced precipitation

The actual mechanism(s) of carbonate precipitation by SRB are still unknown. As was mentioned in Section 2.1.1, SRB promote precipitation through their metabolic processes. Additionally, SRB may induce precipitation by consuming exopolymeric substances (EPS). These cyanobacterial exudates may initially inhibit carbonate precipitation by accumulating free Ca^{2+} in an organic matrix that inhibits $CaCO_3$ precipitation (Trichet and Défarge, 1995; Arp et al., 1999; Dupraz et al., 2004). However, degradation of the EPS (by SRB and other heterotrophs) results in release of this Ca^{2+}. This release creates localized regions of elevated Ca^{2+} concentrations, which favors precipitation. Scanning electron micrography of the lithifying mats from Salt Pan (Section 4.1) demonstrates the interrelationship of bacteria, EPS, and carbonate precipitates (Fig. 1).

The structure of EPS is also modified through partial degradation by bacteria (Dupraz and Visscher, 2005), especially SRB (Visscher et al., 1999, 2002; Decho et al., 2005), and by environmental factors, such as UV-irradiation. These modifications of EPS produce exposed carboxyl groups whose chemical and structural characteristics may provide nucleation sites that induce calcium carbonate precipitation (Trichet and Défarge, 1995) and affect carbonate mineralogy (e.g., calcite, vaterite; e.g., Reitner, 1993, Braissant et al., 2003). The precipitation nucleus is the portion of a molecule that initially attracts (and binds) the free Ca^{2+} ion via a unidentate bridge (i.e., single bond). Subsequent complexation of a carbonate results in the formation of the initial calcium carbonate precipitate. Preliminary evidence suggests that in the presence of a compatible geochemical environment, partially-degraded EPS or other organic molecules may serve as precipitation nuclei and initiate the precipitation process (Défarge et al., 1996; Sprachta et al., 2001; Trichet et al., 2001; Kawaguchi and Decho, 2002; Gautret et al., 2004). The further complexation of organic molecules to the growing precipitate results in an amorphous (rather than crystalline) mineral matrix. This process, however, is still not well understood.

5.2. Transplants and flume studies

Transplant studies are currently under way in Salt Pan, Eleuthera, Bahamas to examine the effects of

physical environmental factors on community structure and lithification. In these experiments, lithifying mat is placed within the non-lithifying mat zone and vice versa. With time, changes in lithification and bacterial community can be examined within these mats. Similarly, samples of lithifying mat and stromatolite may also be studied in flumes. In the flumes, chemical characteristics (e.g., alkalinity) or physical parameters (e.g., light regime) can be controlled or altered to examine their effects upon lithification in the mats. The effects of these environmental changes can be examined microscopically, isotopically, and phylogenetically, all under a controlled setting.

In either of these studies, if the mats do begin to change and gain or lose lithifying characteristics, it will be helpful to understand how the diversity of SRB and other microbial groups changes in concert. Perhaps the loss or gain of one subgroup of SRB will be sufficient to tip the balance between precipitation and dissolution. These community changes can be measured: the database that is being prepared as part of the sequencing effort (Section 4.1) can be used as an important baseline for comparisons of community changes through techniques such as terminal restriction fragment length polymorphism (T-RFLP). T-RFLP provides a rapid assessment of community diversity and, in conjunction with large database of known sequences from the environment, can pinpoint changes in the abundance of key groups of organisms.

5.3. Cultures and co-cultures

The uncultured diversity measured by the delta96F sequencing (Section 4.1) demonstrates how much more we have to learn about this diverse group. Most particularly, we understand the physiology of only a limited number of SRB strains. Ongoing culture studies are expanding our library of cultivated strains of SRB. Interestingly, one preliminary result of this culturing is the cultivation of multiple strains of sulfide-tolerant fermenting bacteria (non sulfate-reducing) (Przekop et al., unpublished). These fermenters may be living in close association with SRB, perhaps working synergistically with the SRB to remove oxygen (as was discussed in Section 3.1.2).

These synergistic relationships will also be examined in co-culture studies. Co-cultures of two or more strains can be examined for their ability to tolerate oxygen, as has been done before (van den Ende et al., 1997; Sigalevich et al., 2000b). In addition, co-cultures can be used to create mat-like artificial biofilms. The associations that occur in these biofilms and the ability of these biofilms to precipitate carbonate will clarify both mat community dynamics and potential mechanism for lithification. For instance, the metabolic coupling between cyanobacteria and SRB observed in FISH (Fig. 3) implies that cyanobacterial exudates are rapidly and regularly funneled to the SRB and the broader heterotrophic community. This presents the interesting speculation that cyanobacterial exudates, in addition to representing a carbon source for SRB, may also contain chemical signaling molecules. These signaling molecules may facilitate metabolic regulation within/between bacterial communities (Bassler, 1999, 2002). Such associations may be examined in the future through controlled laboratory studies of the co-culture biofilms, and examinations of field samples for signaling molecules.

5.4. Phylogenetics

Once sequencing has been completed, sequences of SRB strains can be examined (via GenBank) for known genes (and their functions). The distribution of organisms with a given gene within the microbial mat can be analyzed, potentially revealing subgroups of SRB that have similar (or complementary) sets of genes. These subgroups may in turn suggest key metabolic processes for a given mat layer (e.g., processes that are important to oxygen tolerance or carbonate precipitation).

Once these target genes have been identified, they can in turn be targeted for further experimentation, including FISH examination of their distribution (or the distribution of organisms known to have those genes), or examination of the activities of those genes under given regimes (e.g., via green fluorescent protein, an assay that allows researchers to view protein activity under the microscope).

6. Summary and conclusions

Our understanding of the role of SRB in the environment continues to evolve. Over the last two decades, researchers have come to understand the ability of some SRB to survive in oxic conditions and even respire oxygen and nitrate (Cypionka et al., 1985; Krekeler and Cypionka, 1995). SRB have also been found to have a wide array of adaptive mechanisms for tolerating the free radicals produced under oxic conditions (Fournier et al., 2003). In the environment, researchers have demonstrated that SRB not only survive in the oxic zone of microbial mats, but even exhibit some of their highest rates of sulfate reduction during oxic conditions (e.g., Canfield and Des Marais, 1991). Finally, SRB have been

shown to be highly active in lithified zones of microbial mats, suggesting they may be directly involved in lithification (Visscher et al., 2000; Dupraz et al., 2004). This research has greatly clarified the physiology of cultured SRB and the role of SRB in microbial mats, but many questions remain. Given the wide diversity of uncultured SRB, what other physiological adaptations remain unknown? By what mechanism do SRB affect carbonate precipitation and lithification? Only further research, both in situ and under laboratory conditions, can answer these questions.

Acknowledgements

We thank two anonymous reviewers for their helpful comments. Our ongoing research on SRB activity in microbial mats is supported by National Science Foundation (EAR 0221796 and BGS 0311929). Research Initiative on Bahamian Stromatolites (RIBS) contribution number 33 and Center for Integrative Geosciences number 3.

References

Amann, R.I., Ludwig, W., Schleifer, K., 1995. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiol. Rev. 59, 143–169.

Arp, G., Theil, V., Reimer, A., Michaelis, W., Reitner, J., 1999. Biofilm exopolymers control microbialite formation at thermal springs discharging into the alkaline Pyramid Lake, Nevada, USA. Sediment. Geol. 126, 159–176.

Arp, G., Reimer, A., Reitner, J., 2003. Microbialite formation in seawater of increased alkalinity, Satonda Crater Lake, Indonesia. J. Sediment. Res. 73, 105–127.

Bak, F., Cypionka, H., 1987. A novel type of energy metabolism involving fermentation of inorganic sulphur compounds. Nature 326, 891–892.

Bassler, B.L., 1999. How bacteria talk to each other: regulation of gene expression by quorum sensing. Curr. Opin. Microbiol. 2, 582–587.

Bassler, B.L., 2002. Small talk: cell-to-cell communication in bacteria. Cell 109, 421–424.

Baumgartner, L.K., Dupraz, C., Buckley, D.H., Spear, J.R., Pace, N.R., Visscher, P.T., in preparation. Spatial and temporal changes in the microbial communities of lithifying and non-lithifying microbial mats.

Braissant, O., Cailleau, G., Dupraz, C., Verrecchia, E.P., 2003. Bacterially induced mineralization of calcium carbonate in terrestrial environments: the role of exopolysaccharides and amino acids. J. Sediment. Res. 73, 485–490.

Brune, A., Frenzel, P., Cypionka, H., 2000. Life at the oxic–anoxic interface: microbial activities and adaptations. FEMS Microbiol. Rev. 24, 692–700.

Bühring, S.I., Elvert, M., Witte, U., 2005. The microbial community structure of different permeable sandy sediments characterized by the investigation of bacterial fatty acids and fluorescence in situ hybridization. Environ. Microbiol. 7, 281–293.

Canfield, D.E., Des Marais, D.J., 1991. Aerobic sulfate reduction in microbial mats. Science 251, 1471–1473.

Canfield, D.E., Des Marais, D.J., 1993. Biogeochemical cycles of carbon, sulfur, and free oxygen in a microbial mat. Geochim. Cosmochim. Acta 57, 3971–3984.

Canfield, D.E., Jørgensen, B.B., Fossing, H., Glud, R., Gundersen, J., Ramsing, N.B., Thamdrup, B., Hansen, J.W., Nielsen, L.P., Hall, P.O.J., 1993. Pathways of organic carbon oxidation in three continental margin sediments. Mar. Geol. 113, 27–40.

Chen, L., Liu, M., Le Gall, J., Fareleira, P., Santos, H., Xavier, A.V., 1993. Rubredoxin oxidase, a new flavor-hemo-protein, is the site of oxygen reduction to water by the "strict anaerobe" *Desulfobivrio gigas*. Biochem. Biophys. Res. Comm. 193, 100–105.

Cypionka, H., 2000. Oxygen respiration by *Desulfovibrio* species. Ann. Rev. Microbiol. 54, 827–848.

Cypionka, H., Widdel, F., Pfennig, N., 1985. Survival of sulfate-reducing bacteria after oxygen stress, and growth in sulfate-free oxygen-sulfide gradients. FEMS Microbiol. Ecol. 31, 39–45.

Dannenburg, S., Kroder, M., Dilling, W., Cypionka, H., 1992. Oxidation of H_2, organic compounds and inorganic sulfur-compounds coupled to reduction of O_2 or nitrate by sulfate-reducing bacteria. Arch. Microbiol. 158, 93–99.

Decho, A.W., Visscher, P.T., Reid, R.P., 2005. Cycling and turnover of natural exopolymers from a marine stromatolite. Palaeogeogr. Palaeoclimatol. Palaeoecol. 219, 71–86.

Défarge, C., Tribble, J., Sansone, F.J., Trichet, J., Jaunet, A.-M., Robert, M., 1996. Texture of microbial sediments revealed by cryo-scanning electron microscopy. J. Sediment. Res. 66, 935–947.

Dilling, W., Cypionka, H., 1990. Aerobic respiration in sulfate-reducing bacteria. FEMS Microbiol. Lett. 71, 123–128.

Devereux, R., Delaney, M., Widdel, F., Stahl, D., 1989. Natural relationships among sulfate-reducing bacteria. J. Bacteriol. 171, 6689–6695.

Dupraz, C.D., Visscher, P.T., Baumgartner, L.K., Reid, R.P., 2004. Microbe–mineral interactions: early carbonate precipitation in a hypersaline lake (Eleuthera Island, Bahamas). Sedimentology 51, 745–765.

Dupraz, D., Visscher, P.T., 2005. Microbial lithification in marine stromatolites and hypersaline mats. Trends Microbiol. 13, 429–438.

Eschemann, A., Kühl, M., Cypionka, H., 1999. Aerotaxis in *Desulfovibrio*. Environ. Microbiol. 1, 489–494.

Fournier, M., Zhang, Y., Wildschut, J.D., Dolla, A., Voordouw, J.K., Schriemer, D.C., Voordouw, G., 2003. Function of oxygen resistance proteins in the anaerobic, sulfate-reducing bacterium *Desulfovibrio vulgaris* Hildenborough. J. Bacteriol. 185, 71–79.

Fournier, M., Dermounn, Z., Durand, M., Dolla, A., 2004. A new function of the *Desulfovibrio vulgaris* Hildenborough [Fe] hydrogenase in the protection against oxygen stress. J. Biol. Chem. 273, 1787–1793.

Freytet, P., Plet, A., 1996. Modern freshwater microbial carbonates: The *Phormidium* stromatolites (Tufa-Travertine) of southeastern Burgundy (Paris basin, France). Facies 34, 219–237.

Freytet, P., Verrecchia, E.P., 1998. Freshwater organisms that build stromatolites: a synopsis of biocrystallization by prokaryotic and eukaryotic algae. Sedimentology 45, 535–237.

Fründ, C., Cohen, Y., 1992. Diurnal cycles of sulfate reduction under oxic conditions in cyanobacterial mats. Appl. Environ. Microbiol. 58, 70–77.

Gautret, P., Camoin, G., Golubic, S., Sprachta, S., 2004. Biochemical control of calcium carbonate precipitation in modern lagoonal microbialites, Tikehau Atoll, French Polynesia. J. Sediment. Res. 74, 462–478.

Grotzinger, J.P., Knoll, A.H., 1999. Stromatolites in Precambrian carbonates: evolutionary mileposts or environmental dipsticks? Ann. Rev. Earth Planet. Sci. 27, 313–358.

Jonkers, H.M., Ludwig, R., De Wit, R., Pringault, O., Muyzer, G., Niemann, H., Finke, N., De Beer, D., 2003. Structural and functional analysis of a microbial mat ecosystem from a unique permanent hypersaline inland lake: 'La Salad de Chiprana" (NE Spain). FEMS Microbiol. Ecol. 44, 175–189.

Jørgensen, B.B., Cohen, Y., 1977. Solar Lake (Sinai): 5. The sulfur cycle of the benthic cyanobacterial mat. Limnol. Oceanogr. 22, 657–666.

Jørgensen, B.B., Bak, F., 1991. Pathways and microbiology of thiosulfate transformations, and sulfate reduction in a marine sediment (Kattegat, Denmark). Appl. Environm. Microbiol. 57, 847–856.

Kawaguchi, T., Decho, A.W., 2002. A laboratory investigation of cyanobacterial extracellular polymeric secretions (EPS) in influencing $CaCO_3$ polymorphism. J. Cryst. Growth 240, 230–235.

Kempe, S., Kazmierczak, J., 1994. The role of alkalinity in the evolution of ocean chemistry, organization of the living systems and biocalcification processes. Bull. Inst. Oceanogr. (Monaco) 13, 61–117.

Krekeler, D., Cypionka, H., 1995. The preferred electron acceptor of *Desulfovibrio desulfuricans* CSN. FEMS Microbiol. Ecol. 17, 271–278.

Krumbein, W.E., Cohen, Y., Shilo, M., 1977. Solar Lake (Sinai): 4. Stromatolitic cyanobacterial mats. Limnol. Oceanogr. 22, 635–656.

Ley, R.E., Harris, J.K., Wilcox, J., Spear, J.R., Miller, S.R., Bebout, B.M., Maresca, J.A., Bryant, D.A., Pace, N.R., submitted for publication. Unexpected diversity and complexity from the Guerrero Negro hypersaline microbial mat. Appl. Environ. Microbiol.

Llobet-Brossa, E., Rossello-Mora, R., Amann, R., 1998. Microbial community composition of Wadden Sea sediments as revealed by fluorescence in situ hybridization. Appl. Environ. Microbiol. 7, 2691–2696.

Loy, A., Horn, M., Wagner, M., 2003. ProbeBase: an online resource for rRNA-targeted oligonucleotide probes. Nucleic Acids Res. 31, 514–516.

Lyons, W.B., Long, D.T., Hines, M.E., Gaudette, H.E., Armstrong, P.B., 1984. Calcification of cyanobacterial mats in Solar Lake, Sinai. Geology 12, 623–626.

Marschall, C., Frenzel, P., Cypionka, H., 1993. Influence of oxygen on sulfate reduction and growth of sulfate-reducing bacteria. Arch. Microbiol. 159, 168–173.

Minz, D., Fishbain, S., Green, S.J., Muyzer, G., Cohen, Y., Rittmann, B.E., Stahl, D., 1999. Unexpected population distribution in a microbial mat community: Sulfate-reducing bacteria localized to the highly oxic chemocline in contrast to a eukaryotic preference for anoxia. Appl. Environ. Microbiol. 65, 4659–4665.

Pace, N.R., 1996. New perspective on the natural microbial world: molecular microbial ecology. ASM News 62, 463–470.

Papineau, D., Walker, J.J., Mojzsis, S.J., Pace, N.R., 2005. Composition and structure of microbial communities from stromatolites of Hamelin Pool in Shark Bay, Western Australia. Appl. Environm. Microbiol. 71, 4822–4832.

Pernthaler, J., Glőckner, F., Schőnhuber, W., Amann, R., 2001. Fluorescence in situ hybridization (FISH) with rRNA-targeted oligonucleotide probes. Meth. Microbiol. 30, 207–226.

Postgate, J.R., 1959. Sulphate reduction by bacteria. Ann. Rev. Microbiol. 13, 505–520.

Reid, R.P., Visscher, P.T., Decho, A.W., Stolz, J.F., Bebout, B.M., Dupraz, C., Macintyre, I.G., Paerl, H.W., Pinckney, J.L., Prufert-Bebout, L., Steppe, T.F., Des Marais, D.J., 2000. The role of microbes in accretion, lamination and early lithification of modern marine stromatolites. Nature 406, 989–992.

Reitner, J., 1993. Modern cryptic microbialite/metazoan facies from Lizard Island (Great Barrier Reef, Australia): formation and concepts. Facies 29, 3–40.

Risatti, J.B., Capman, W.C., Stahl, D.A., 1994. Community structure of a microbial mat: the phylogenetic dimension. Proc. Natl. Acad. Sci. U. S. A. 91, 10173–10177.

Shen, Y., Buick, R., 2004. The antiquity of microbial sulfate reduction. Earth Sci. Rev. 64, 243–272.

Shen, Y., Buick, R., Canfield, D.E., 2001. Isotopic evidence for microbial sulphate reduction in the early Archaean era. Nature 410, 77–81.

Sigalevich, P., Meshorer, E., Helman, Y., Cohen, Y., 2000a. Transition for anaerobic to aerobic growth conditions for the sulfate-reducing bacterium *Desulfovibrio oxyclinae* results in flocculation. Appl. Environ. Microbiol. 66, 5005–5012.

Sigalevich, P., Baev, M.V., Teske, A., Cohen, Y., 2000b. Sulfate reduction and possible aerobic metabolism of the sulfate-reducing bacterium *Desulfovibrio oxyclinae* in a chemostat coculture with *Marinobacter* sp. strain MB under exposure to increasing oxygen conditions. Appl. Environ. Microbiol. 66, 5013–5018.

Silva, G., Oliveira, S., Gomes, C.M., Pacheco, I., Liu, M.Y., Xavier, A.V., Teixeira, M., LeGall, J., Rodrigues-Pousada, C., 1999. *Desulfovibrio gigas* neelaredoxin: a novel superoxide dismutase integrated in a putative oxygen sensory operon of an anerobe. Eur. J. Biochem. 259, 234–243.

Snaidr, J., Amann, R., Huber, I., Ludwig, W., Schleifer, K.H., 1997. Phylogenetic analysis and in situ identification of bacteria in activated sludge. Appl. Environ. Microbiol. 63, 2884–2896.

Spear, J.R., Walker, J.J., McCollom, T.M., Pace, N.R., 2005. Hydrogen and bioenergetics in the Yellowstone geothermal ecosystem. Proc. Natl. Acad. Sci. U. S. A. 102, 2555–2560.

Sprachta, S., Camoin, G., Golubic, S., Le Campion, Th., 2001. Microbialites in a modern lagoonal environment: nature and distribution, Tikehau atoll (French Polynesia). Palaeogeogr. Palaeoclimatol. Palaeoecol. 175, 103–124.

Stackebrandt, E., Goebel, B.M., 1994. Taxonomic note: a place for DNA–DNA hybridization and 16S rRNA sequence analysis in the present species definition in bacteriology. Intern. J. Syst. Bacteriol. 44, 846–849.

Teske, A., Ramsing, N.B., Habicht, K.S., Fukui, M., Küver, J., Jørgensen, B.B., Cohen, Y., 1998. Sulfate-reducing bacteria and their activities in cyanobacterial mats of Solar Lake (Sinai, Egypt). Appl. Environ. Microbiol. 64, 2943–2951.

Trichet, J., Défarge, C., 1995. Non-biologically supported organomineralization. Bull. Inst. Oceanogr. (Monaco) 14, 203–236.

Trichet, J., Défarge, C., Tribble, J., Tribble, G., Sansone, F., 2001. Christmas Islands lagoonal lakes, models for the deposition of carbonate–evaporite–organic laminated sediments. Sediment. Geol. 140, 177–189.

van den Ende, F.P., Meier, J., van Gemerdern, H., 1997. Syntrophic growth of sulfate-reducing bacteria and colorless bacteria during oxygen limitation. FEMS Microbiol. Ecol. 23, 65–80.

van Gemerden, H., 1993. Microbial mats: a joint venture. Mar. Geol. 113, 3–25.

van Lith, Y., Vasconcelos, C., Warthmann, R., Martins, J.C.F., 2002. Bacterial sulfate reduction and salinity: two controls on dolomite precipitation in Lagoa Vermelha and Brejo de Espinho (Brazil). Hydrobiologia 485, 35–49.

van Niel, E.W.J., Gottschal, J.C., 1998. Oxygen consumption by *Desulfovibrio* strain with and without polyglucose. Appl. Environ. Microbiol. 64, 1034–1039.

Visscher, P.T., Stolz, J.F., 2005. Microbial mats as bioreactors: populations, processes and products. Palaeogeogr. Palaeoclimatol. Palaeoecol. 219, 87–100.

Visscher, P.T., van Gemerden, H., 1993. Sulfur cycling in laminated marine microbial ecosystems. In: Oremland, R.S. (Ed.), Biogeochemistry of Global Change: Radiatively Active Trace Gases. Chapman and Hall, New York, pp. 245–271.

Visscher, P.T., Prins, R.A., van Gemerden, H., 1992. Rates of sulfate reduction and thiosulfate consumption in a marine microbial mat. FEMS Microbiol. Ecol. 86, 283–294.

Visscher, P.T., Reid, R.P., Bebout, B.M., Hoeft, S.E., Macintyre, I.G., Thompson Jr., J.A., 1998. Formation of lithified micritic laminae in modern marine stromatolites (Bahamas): the role of sulfur cycling. Am. Mineral. 83, 1482–1493.

Visscher, P.T., Gritzer, R.F., Leadbetter, E.R., 1999. Low-molecular-weight sulfonates, a major substrate for sulfate reducers in marine microbial mats. Appl. Environ. Microbiol. 65, 3272–3278.

Visscher, P.T., Reid, R.P., Bebout, B.M., 2000. Microscale observations of sulfate reduction: correlation of microbial activity with lithified micritic laminae in modern marine stromatolites. Geology 28, 919–922.

Visscher, P.T., Hoeft, S.M., Surgeon, T.L., Rogers, R., Bebout, B.M., Thompson, J.S., Reid, R.P., 2002. Microelectrode measurements in stromatolites: unraveling the Earth's past? In: Taillefert, M., Rozan, T. (Eds.), Environmental Electrochemistry: Analyses of Trace Element Biogeochemistry. ACS Symposium Series, Oxford University Press, Washington, D.C., pp. 265–282.

Voordouw, G., 1995. The genus *Desulfovibrio*: the centennial. Appl. Environ. Microbiol. 61, 2813–2819.

Walter, L.M., Bischof, S.A., Patterson, W.P., Lyons, T.W., 1993. Dissolution and recrystallization in modern shelf carbonates: evidence from pore water and solid phase chemistry. Philos. Trans. R. Soc. Lond., A 344, 27–36.

Warthmann, R., van Lith, Y., Vasconcelos, C., McKenzie, J.A., Karpoff, A.M., 2000. Bacterially induced dolomite precipitation in anoxic culture experiments. Geology 28, 1091–1094.

Wierenga, E.B.A, Overmann, J., Cypionka, H., 2000. Detection of abundant sulphate-reducing bacteria in marine oxic sediment layers by a combined cultivation and molecular approach. Environ. Microbiol. 2, 417–427.

Zeebe, R.E., Wolf-Gladrow, D.A, 2001. CO_2 in Seawater: Equilibrium, Kinetics and Isotopes. Elsevier Oceanography Series, Elsevier, Amsterdam. 346 pp.