Chapter 19

Ecology of Phototrophic Sulfur Bacteria

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Summary

Anoxygenic phototrophic sulfur bacteria florish where light reaches sulfidic water layers or sediments. Their often dense communities have continuously attracted the attention of microbiologists. Although the major fraction of the existing diversity of phototrophic sulfur bacteria remains to be explored, ecophysiological studies have revealed a number of selective factors which govern the growth and the survival of phototrophic sulfur bacteria in the environment. Some novel aspects of the ecology of phototrophic sulfur bacteria have become apparent recently. Representing the most extremely low-light adapted photosynthetic organisms known to date, a brown-colored *Chlorobium* strain colonizes the chemocline of the Black Sea and is capable of maintaining a stable population at 0.0007% of surface light intensity. Besides the light intensity, the spectral composition of ambient light is a selective factor for the composition of anoxygenic phototrophic communities. A strong competition for infrared light occurs in laminated microbial benthic mats where phototrophic sulfur bacteria occupy their niches according to their long wavelength absorption properties. During evolution this apparently has led to the formation of a novel type of pigment-protein complex which was recently detected in a benthic *Chromatiaceae* species. Thirdly, the capability to establish a highly specialized symbiosis with motile Proteobacteria enabled some species of green sulfur bacteria to acquire motility. In these phototrophic consortia, a rapid

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signal transfer exists between the two partners and permits a scotophobic response toward light required by the immotile green sulfur bacterial epibiont. The isolation and characterization of dominant species of phototrophic sulfur bacteria and an improved understanding of their particular niche has also implications for the interpretation of molecular fossils of these bacteria which have been detected in sedimentary rocks of all geological eras and interpreted as evidence for the existence of extended oceanic anoxia in the past.

I. Introduction

Anoxygenic phototrophic sulfur bacteria occur where light reaches anoxic layers in the water column or aquatic sediments. Since the antiquity, colored waters or sediments occurring in various natural environments were described by natural scientists. First observations of blood-red lakes and swamps were reported from the Nile area. Red coloration of a crater lake near Rome was described by Pliny in 208 BC and reddish waters were observed at the seashore near Venice in the year 586 AD (Kondratieva 1965).

The first to describe unicellular motile phototrophic sulfur bacteria was Ch. G. Ehrenberg (1883), who discovered dense accumulations of purple sulfur bacteria, then named *Monas okenii* (now *Chromatium okenii*) at the sediment surface of a small polluted pond near Jena in Eastern Germany. Since then, dense communities of purple and green sulfur bacteria (Fig. 1) have continuously attracted the attention of microbiologists due to their conspicuous reddish, green or brown coloration and have stimulated numerous investigations of their environments, their morphology, physiology as well as repeated cultivation attempts (e.g., Winogradsky, 1887; Engelmann, 1988; Bavendamm, 1924; van Niel, 1931).

Earlier investigations of the community composition and physiology of these bacteria included measurements of relevant environmental parameters and physiological rates in situ (e.g., Sorokin, 1970). Elaborate cultivation techniques were developed based upon the insights into their ecological niches (Pfennig, 1993). More recently, a suite of culture-independent molecular methods have been established und permitted novel insights into the ecophysiology and population biology of phototrophic sulfur bacteria.

Abbreviations: *Bchl* bacteriochlorophyll

The current chapter will focus on the ecology of purple sulfur bacteria (members of the *Chromatiaceae* and *Ectothiorhodospiraceae*) and the green sulfur bacteria (*Chlorobiaceae*). Besides providing a condensed view on the ecology of these groups, novel aspects are addressed including extreme low-light adaptation, low maintenance energy requirements, the formation of symbioses in phototrophic consortia and, finally, the analysis of fossil phototrophic communities.

II. Habitats and Natural Populations of Phototrophic Sulfur Bacteria

A. Ecological Niches

1. Light Quantity and Quality

Typically, accumulations of phototrophic sulfur bacteria have been observed between 2 and 20 m, rarely down to 30 m depth in pelagic environments (Montesinos et al., 1983; Guerrero et al., 1987b; Gorlenko, 1988; van Gemerden and Mas, 1995; Herbert et al., 2005). In such environments, values for the light transmission to populations of phototrophic sulfur bacteria range from 0.015 to 10% (Parkin and Brock, 1980a; van Gemerden and Mas, 1995). *Chromatiaceae* so far have been found in chemocline environments down to depths of ≤20 m. The tight correlation between anoxygenic photosynthesis and the available irradiance suggests that light is the main environmental variable controlling the activity of phototrophic sulfur bacteria.

Since the accumulation of phototrophic sulfur bacterial cells results in an increased self-shading, they can only extend over a limited vertical distance, which is reciprocally related to the amount of biomass present. Accordingly, the densest pelagic communities of

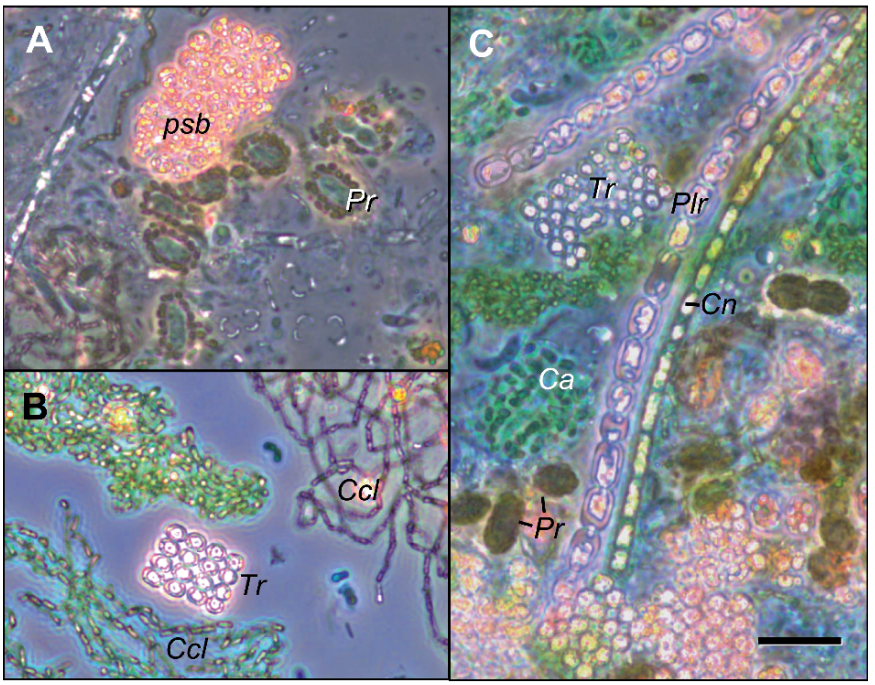

Fig. 1. Typical composition of a pelagic community of phototrophic and chemotrophic bacteria. Samples from the chemocline of dimictic Lake Dagow (North Brandenburg, Eastern Germany; obtained in September 2006) were left overnight after sampling for sedimentation of microbial cells. A. Purple sulfur bacteria (*psb*) containing numerous yellowish sulfur droplets. *Pr*, brown-colored phototrophic consortium "*Pelochromatium roseum*" in a partically disaggregated state. B. Brown- and green colored forms of *Chlorobium clathratiforme* (*Ccl*) and platelet-like microcolony of the purple sulfur bacterium *Thiopedia rosea* (*Tr*). C. Intact "*Pelochromatium roseum*" (*Pr*), one disintegrated phototrophic consortium "*Chlorochromatium aggregatum*" (*Ca*). Green-colored epibionts surround the central chemotrophic bacterium. *Tr*, *Thiopedia rosea*; *Cn*, filamentous green bacterium *Chloronema* sp. (*Chloroflexaceae*); *Plr*, filamentous cyanobacterium *Planktothrix rubescens*. Highly refractile and irregular intracellular regions in the latter three bacteria are gas vacuoles. Bar, 10 µm (*See Color Plates*).

phototrophic sulfur bacteria (up to 28 mg bacteriochlorophyll·l^{-1}; Overmann et al., 1994) extend over a depth range of 10 cm (Overmann et al., 1991a) whereas the least dense population in the Black Sea (0.068–0.94 µg BChl*e*·l^{-1}) is spread out over a depth interval of 30 m (Repeta et al., 1989; Manske et al., 2005). Communities of phototrophic sulfur bacteria in littoral sediments of sandy beaches, salt marshes or intertidal mudflats live in a significantly steeper light gradient and growth is limited to the uppermost 1.5–5 mm (van Gemerden and Mas, 1995). At the same time, biomass densities of 900 mg bacteriochlorophyll·dm^{-3} can be attained in these latter systems (van Gemerden et al., 1989).

In purple bacteria, the size of the photosynthetic antenna is in the range of 20–200 bacteriochlorophyll *a* per reaction center (Zuber and Cogdell, 1995). The photosynthetic antenna of green sulfur bacteria consist of specialized intracellular structures (so-called chlorosomes) and are significantly larger than those of other anoxygenic phototrophs with about 5000–8000 bacteriochlorophyll molecules connected to one reaction center (Frigaard et al., 2003). In addition, the theoretical quantum requirement for the CO_2-fixation of purple sulfur bacteria is 8–10.5 mol quanta·(mol CO_2)$^{-1}$, but only 3.5–4.5 mol quanta·(mol CO_2)$^{-1}$ for green sulfur bacteria (Brune, 1989). While the values for purple sulfur bacteria have been verified experimentally, much higher values than theoretically

expected were reported so far for green sulfur bacteria. This discrepancy warrants further investigations. In addition to their larger antenna, green sulfur bacteria exhibit lower maintenance energy requirements and higher sulfide tolerance than other phototrophic sulfur bacteria (Overmann and Garcia-Pichel, 2000) and hence are especially well adapted to low-light habitats.

In the chemocline of the Black Sea, brown-colored green sulfur bacteria form an extremely dilute, but detectable population. The uppermost sulfidic water layers were detected at 80–120 m depth, while light attenuation in the overlying water layers is comparable to other stratified aquatic systems. As a consequence, the Black Sea chemocline is characterized by a very extreme low-light situation. According to recent measurements conducted with an integrating quantum meter, maximum in situ light intensities reach only 0.0022–0.00075 µmol Quanta × m^{-2} s^{-1} during winter, corresponding to 0.0007% of surface light intensity (Manske et al., 2005), while other environmental factors correspond to those prevailing in other oxic/anoxic habitats of phototrophic sulfur bacteria (Overmann and Manske, 2006). Combining the data on available light intensities and on concentrations of photosynthetic pigments, it can be calculated that each bacteriochlorophyll *e* molecule of the green sulfur bacteria on average absorbs one photon every 8 hours (Overmann and Manske, 2006).

Culture-independent 16S rRNA gene sequence analyses of the bacterial community present in the Black Sea chemocline revealed that one single and novel phylotype (BS-1) of green sulfur bacteria persisted over more than 13 years (Manske et al., 2005). These bacteria thus form a single population under the extreme conditions in the Black Sea chemocline, while other types of anoxygenic phototrophic sulfur bacteria could not be detected. Its continuous presence suggests a specific adaptation of phylotype BS-1 to the specific environmental conditions in the Black Sea chemocline and a high competitive advantage in situ. Subsequent $H^{14}CO_3^-$ - incorporation studies indicated that phylotype BS-1 is in fact capable of exploiting the minute light quantum flux available in situ.

From water samples obtained during the US-Turkish expedition of the RV *Knorr*, the first successful enrichment of the chemocline bacterium could be established (Overmann et al., 1992). This bacterium was isolated again from chemocline water samples recovered in 2001 from a depth of 95 m in the central western basin (Manske et al., 2005) and permitted first insights into its specific mechanisms of adaptation to extreme low-light conditions. In comparison to all other green sulfur bacteria tested, the Black Sea isolate incorporated $H^{14}CO_3^-$, oxidized sulfide and grew significantly faster at light intensities ≤1 µmol Quanta × m^{-2} s^{-1}.

Acclimation to very low light intensities in most phototrophic organisms involves an increase in the size of the photosynthetic unit (Göbel, 1978; Drews and Golecki, 1995; Sanchez et al., 1998). In the Black Sea isolate, the intracellular concentration of light-harvesting pigments is twice as high than in other green sulfur bacteria (Overmann et al., 1992) and chlorosomes are twofold larger than in another strain investigated (Fuhrmann et al., 1993). A conspicuous feature of the low-light-adaptation of the green sulfur bacterium from the Black Sea is the presence of geranyl homologs of BChl*e*, which had never been described for any other isolate of green sulfur bacteria. The structure of the esterifying alcohols in the bacteriochlorophylls of green sulfur bacteria may influence the function of the light-harvesting chlorosomes (Steensgard et al., 2000). Interestingly, geranyl ester isobutyl/ethyl [I, E]-Bchle_G an unusual Bchl *e*- homologue, and minor amounts of ethyl/methyl, ethyl/ethyl and propyl/ethyl-Bchle_G were detected in the chemocline as well as in cultures of strain BS-1. Upon low-light-adaptation of the culture of BS-1, the composition of these homologues changes towards a strong dominance of the higher alkylated [I, E]-Bchle_G (Manske et al., 2005). The alkyl side chains of the BChl tetrapyrrol system are directly involved in the aggregation of BChl molecules (van Rossum et al., 2001). Accordingly, a higher degree of alkylation leads to a red shift of the Q_y absorption maximum by 7–11 nm, which has been hypothesized to increase the energy transfer efficiency of the chlorosomes (Borrego and Garcia-Gil, 1995). The extremely efficient low light utilization of the Black Sea isolate comes at a price, however, since its specific physiological rates at saturating light intensities are much lower than in other phototrophic sulfur bacteria. The low specific metabolic rates reached under

light-saturation may be caused by a reduction in the intracellular enzyme levels which may represent a way to decrease maintenance energy demand of the BS-1 cells (see section II.B).

Besides the available light intensity, the composition of the underwater light spectrum is a selective factor for the composition of anoxygenic phototrophic communities and differs considerably between pelagic and benthic habitats. In many lacustrine habitats, light absorption by phytoplankton exceeds that of humic substances or water itself and light of the bluegreen to green wavelength range reaches layers of phototrophic bacteria. In contrast, infrared light is an important source of energy in benthic microbial mats.

In general light absorption by anoxygenic phototrophs in the free water column is mediated by carotenoids and the short wavelength (Soret) bands of bacteriochlorophylls. In coastal and most lacustrine waters, the in vivo-absorption spectrum of *Chromatiaceae* which contain the carotenoid okenone matches the available light of the green wavelength range. Accordingly, okenone-bearing *Chromatiaceae* dominate in 63% of all natural communities investigated (van Gemerden and Mas, 1995) which has been explained by their higher efficieny of light absorption compared to species containing other types of carotenoids (Guerrero et al., 1987b; Overmann et al., 1991a). Dominant *Chromatiaceae* are obligately photolithotrophic, lack assimilatory sulfate-reduction, cannot reduce nitrate, and assimilate only few organic carbon sources. Obviously, metabolically versatile species of the *Chromatiaceae* have no selective advantage in most pelagic habitats.

Humic substances in lakes are of terrestrial origin and absorb light of the ultraviolet and blue portion of the spectrum. As a consequence, light of the red wavelength range prevails in lakes containing humic substance as the major light-absorbing constituents. Under these conditions, green-colored species of green sulfur bacteria have a selective advantage over their brown-colored counterparts, or over purple sulfur bacteria (Parkin and Brock, 1980b).

In benthic microbial mats, radiation of the visible wavelength range is strongly attenuated by mineral and biogenic particles. In quartz sand, light attenuation occurs preferentially in the wavelength range of blue light due to the reflection by sand grains (Kühl et al., 1994), while the absorption of infrared light by the sediment particles is low and absorption by water is negligible due to the short optical pathlength. As a consequence, the red and infrared portion of the spectrum penetrate the deepest in benthic environments; the irradiance reaching phototrophic sulfur bacteria may be reduced to <1% of the surface value for light in the visible region, while >10% of the near infrared light is still available (Kühl and Jørgensen, 1992). Under these conditions, the long wavelength (Q_y) absorption bands are significant for light-harvesting in anoxic sediment layers, and variations in the type of bacteriochlorophyll (Bchl*a* or Bchl *b*) and in the fine structure of the pigment-protein complexes thus are the means of ecological niche separation.

Populations of phototrophic microorganisms impose strong absorption signatures on the spectrum of the scalar irradiances, creating different niches in a vertical sequence by the successive absorption of different wavelength bands in the red and infrared portion of electromagnetic radiation (Pierson et al., 1987). This may lead to the formation of up to five distinctly colored layers which (from top to bottom) comprise diatoms and cyanobacteria, cyanobacteria alone, *Chromatiaceae* containing Bchl*a*, *Chromatiaceae* containing Bchl*b*, and *Chlorobiaceae* (Nicholson et al., 1987). Only these benthic habitats are known to harbor distinct blooms of Bchl*b*-containing *Chromatiaceae*. The absorption spectra of whole cells of phototrophic bacteria seem to have evolved in such a way that almost the entire electromagnetic spectrum suitable for electrochemical reactions can be exploited.

Yet, no phototrophic sulfur bacterium was known which could absorb light of the wavelengths between 900 and 1020 nm until recently. Because of the strong competition for infrared light in sediment ecosystems, an effective absorption in this wavelength range by other types of photosynthetic antenna complexes would be expected to be of selective advantage in microbial mats. The isolation of purple nonsulfur α-Proteobacteria with long wavelength absorption maxima at 911 nm (Glaeser and Overmann, 1999) and 986 nm (Pfennig et al., 1997) indicates that the diversity of the pigment-protein complexes in photosynthetic Proteobacteria is greater than previously assumed. More recently, a purple photosynthetic sulfur bacterium with an absorption

maximum at 970 nm was isolated from a littoral microbial mat (Permentier et al., 2001). Since this bacterium contain bacteriochlorophyll *a* as the photosynthetic pigment like most other members of the *Chromatiaceae*, the different in vivo absorption spectrum must be the result of differences in the non-covalent binding of Bchl*a* to the light-harvesting proteins.

Deep sea hydrothermal vents represent a novel potential habitat of phototrophic sulfur bacteria identified recently (Beatty et al., 2005). Black smokers are thought to emit geothermal radiation at wavelengths commensurate with the absorption spectrum of phototrophic organisms (Van Dover et al., 1996). Indeed, a novel phylotype of obligately photolithoautotrophic green sulfur bacterium could be isolated from water samples originating from the TY black smoker located at 2391 m depths on the East Pacific Rise (Beatty et al., 2005). It remains to be elucidated whether the isolated bacterium is a typical and long-term resident of hydrothermal vents or whether it also thrives in habitats known for other phototrophic sulfur bacteria.

2. Reduced Sulfur Compounds and Redox Potential

A combination of two photosystems as in oxygenic phototrophs is required for the thermodynamically unfavorable utilization of water as an electron donor for photosynthesis. Due to the simpler architecture of their photosystems, all anoxygenic phototrophic bacteria depend on electron donors which exhibit standard redox potentials more negative than water (e.g., H_2S, H_2, acetate). This molecular feature thus is one major reason for the narrow ecological niche of anoxygenic phototrophic bacteria in extant ecosystems. Most phototrophic sulfur bacteria grow preferentially by photolithoautotrophic oxidation of reduced sulfur compounds. Other inorganic electron donors utilized include H_2, polysulfides, elemental sulfur, thiosulfate, sulfite and iron. In the green sulfur bacteria, polysulfide utilization is inhibited by sulfide. In addition to reduced sulfur compounds, molecular hydrogen serves as electron donor in the majority of green sulfur bacteria, and in the metabolically more versatile species of purple sulfur bacteria like *Allochromatium vinosum* and *Thiocapsa roseopersicina*. In green sulfur bacteria which lack assimilatory sulfate reduction, a reduced sulfur source is required during the growth with molecular hydrogen as electron donor. In microbial mats, polysulfides and organic sulfur compounds may be significant as photosynthetic electron donor. Polysulfide oxidation has been reported for *Chlorobium limicola*, *Ach. vinosum* and *Tca. roseopersicina* while dimethylsulfide is utilized and oxidized to dimethylsulfoxide by the purple sulfur bacteria *Thiocystis* sp. and *Tca. roseopersicina* (van Gemerden and Mas, 1995).

Sulfide frequently becomes the growth-limiting factor at the top of the phototrophic sulfur bacterial layers where light intensities are highest, but sulfide has to diffuse through the remainder of the community. The affinity for sulfide during photolithoautotrophic growth varies between the different groups of anoxygenic phototrophs and has been shown to be of selective value during competition experiments. *Chlorobiaceae* and *Ectothiorhodospiraceae* exhibit five to seven times higher affinities for sulfide than *Chromatiaceae* (van Gemerden and Mas, 1995). On the contrary, affinities for polysulfides are similar for *Chlorobiaceae* and *Chromatiaceae*.

Because light and sulfide occur in opposing gradients, growth of phototrophic sulfur bacteria is confined to a narrow zone of overlap and only possible if the chemical gradient of sulfide is sufficiently stabilized against vertical mixing. In open water, like lakes or lagoons, stratification of oxic and anoxic water layers is maintained by density differences. Stratification can be transient if caused by temperature differences, or permanent (as in so-called meromictic lakes) if caused by higher salt concentrations of the bottom water layers. Benthic environments of phototrophic sulfur bacteria are characterized by a lower frequency of turbulent mixing and by diffusion as the dominant means of mass transport. As a result, and because of the higher rates of sulfate reduction, gradients of sulfide are much steeper in these environments.

In some habitats of phototrophic sulfur bacteria, redox conditions change rapidly within hours. This is particularly true for intertidal sediments. Certain small-celled species of the *Chromatiaceae* (*Allochromatium vinosum*, *Marichromatium gracile*, *Thiocapsa roseopersicina*, *Tca. rosea*, *Thiocystis minor*, *Tcs. violascens*, *Tcs. violacea*, *Thiorhodovibrio winogradskyi*) which

are typical inhabitants of these fluctuating environments, as well as most of the *Ectothiorhodospiraceae* have adapted to these conditions and can switch to an aerobic chemolithotrophic growth mode and oxidize sulfide or thiosulfate with molecular oxygen. Under oxic conditions, the synthesis of pigments and of pigment-binding proteins of the photosynthetic apparatus ceases and the cells become colorless. Concomitantly, the activities of the respiratory enzymes NADH dehydrogenase and cytochrome oxidase and respiratory activity are increased. However, growth affinities of chemolithoautotrophically growing cells of *Tca. roseopersicina* are lower than for the directly competing colorless sulfur bacteria which may explain why no natural populations of purple sulfur bacteria are known which grow permanently by chemotrophy. All *Chlorobiaceae* are obligate anaerobes.

3. Temperature and Salinity

Although green and purple sulfur bacteria typically form conspicuous blooms in non-thermal aquatic ecosystems, moderately thermophilic members have been described from hot spring mats (Castenholz et al., 1990). *Chlorobaculum tepidum* (formerly *Chlorobium tepidum*) occurs in only a few New Zealand hot springs at pH values of 4.3 and 6.2 and at temperatures up to 56°C. *Thermochromatium tepidum* (formerly *Chromatium tepidum*) was found in several hot springs of western North America at temperatures up to 58°C and might represent the most thermophilic proteobacterium (Castenholz and Pierson, 1995).

Of the purple sulfur bacteria, most members of the *Chromatiaceae* are typically found in freshwater and marine environments, whereas the *Ectothiorhodospiraceae* inhabit hypersaline waters. About 10 species of *Chromatiaceae* are halophilic (Imhoff, 2005a). Members of the marine subgroup I of the green sulfur bacteria forming extraordinarily dense blooms could be isolated from a hypersaline (30–70&ppercnt; salinity) athalassohaline lake in the semi-arid Ebro region (Spain) (Vila et al., 2002).

4. Mixotrophy and Organotrophy

Organic carbon as it is present in microbial biomass is considerably more reduced than CO_2. Given the high energy demand of CO_2-fixation, the capability for assimilation of organic carbon compounds would be expected to be of selective advantage in natural populations of phototrophic sulfur bacteria if limited by light or low sulfide concentrations. Acetate represents one of the most important intermediates during the degradation of organic matter and almost all phototrophic sulfur bacteria are capable of assimilating this compound. At limiting concentrations of sulfide, the cell yield of green sulfur bacteria is increased three times in the presence of acetate, i.e. under mixotrophic growth conditions.

Green sulfur bacteria are the least versatile of all phototrophic sulfur bacteria with all species growing obligately photolithoautotrophic. Only acetate, propionate and pyruvate are assimilated as carbon compounds during mixotrophic growth and a few strains are capable of using fructose or glutamate in addition. A number of *Chromatiaceae*, like *Allochromatium vinosum* and other small-celled members of the family, as well as the *Ectothiorhodospiraceae* are capable of using organic carbon compounds not only as carbon source but also as the only electron donating substrate (Imhoff, 2005a, b). These latter versatile *Chromatiaceae* utilize a wide range of organic carbon compounds and usually are capable of assimilatory sulfate reduction. The affinity for acetate is 30 times higher than that of green sulfur bacteria. Still, the metabolic flexible *Chromatiaceae* rarely form dense blooms under natural conditions, from that organotrophy confers only a limited selective advantage to the cells.

5. Motility and Taxis

Sedimentation represents a significant loss process for natural populations of phototrophic sulfur bacteria in pelagic habitats. The minimum buoyant density which has been determined for phototrophic cells devoid of gas vesicles was $1010\,kg \cdot m^{-3}$ (Overmann et al., 1991b). Actively growing cells which contain storage carbohydrate and elemental sulfur can easily attain much higher buoyant densities of up to $1046\,kg \cdot m^{-3}$ (Overmann and Pfennig, 1992) whereas freshwater has a considerably lower density (in the order of $996\,kg \cdot m^{-3}$). In the stably stratified pelagic

habitats of phototrophic sulfur bacteria, the difference in buoyant density of the cells to that of the surrounding water would result a sedimentation of bacteria out of the photic zone towards the lake bottom. Many species of phototrophic sulfur bacteria use vertical migration, mediated by tactic responses and/or the formation of gas vesicles to change their vertical position in the light and sulfide gradients of their environment.

In its pelagic habitat, *Chromatium okenii* may display diurnal migrations with a vertical amplitude of about 2 m (Sorokin, 1970). Vertical migrations of *Thiocystis minor* extended over a vertical distance of 30–35 cm (Pedrós-Alió and Sala, 1990). Planktonic anoxygenic phototrophs, unlike some planktonic cyanobacteria, do not seem to perform vertical migrations mediated by changes in gas vesicle content, but rather employ these cell organelles to maintain their vertical position within the chemocline (Overmann et al., 1991b; Overmann et al., 1994).

About two thirds of the *Chromatiaceae* species and all known species of the *Ectothiorhodopiraceae* swim by means of flagella, whereas only one benthic species of the *Chlorobiaceae*, *Chloroherpeton thalassium*, moves by gliding. True phototaxis is the ability to move towards or away from the direction of light, but is not found in phototrophic sulfur bacteria. Instead, these bacteria employ the scotophobic response to accumulate in regions of higher light intensity by changing the direction of movement in reaction to abrupt changes in light intensity (Armitage, 1997). As a result, cells accumulate in the light and at wavelengths corresponding to the absorption maxima of photosynthetic pigments. The formation of flagella in *Chromatium* and *Allochromatium* species is induced by low sulfide concentrations and low light intensities.

In laboratory cultures of *Chromatium* sp. and *Marichromatium gracile*, a combined effect of chemotaxis and photoresponses can be observed under the microscope: the cells accumulate around air bubbles in the absence of light but move away if illuminated (Armitage, 1997; Thar and Kühl, 2001). Motile *Chromatiaceae* are found in many microbial mats and exhibit diurnal vertical migrations in response to the recurrent changes in environmental conditions. Vertical migrations of *Chromatium* spp. and of *Thermochromatium tepidum* have been documented for populations in ponds, and intertidal or hot spring microbial mats (Castenholz and Pierson, 1995). In these environments, cells migrate upwards to the surface of the mat and enter the overlaying water as a result of a positive aerotaxis during the night. It is assumed that this migration into the microoxic layers enables the cells to grow chemoautotrophically by oxidation of sulfide or intracellular sulfur with molecular oxygen. In contrast, microbial mats of intertidal sediments are typically colonized by the immotile purple sulfur bacterium *Thiocapsa roseopersicina*. Cells form aggregates together with sand grains, apparently as an adaptation to the hydrodynamic instability of the habitat (van den Ende et al., 1996).

Although all known pelagic species of green sulfur bacteria are nonflagellated, some of them have acquired motility by forming highly specific symbioses with a chemoheterotrophic motile Betaproteobacterium (Overmann and Schubert, 2002). These associations, termed phototrophic consortia (see section II.A.6), exhibit a scotophobic response and accumulate in a spot of white light. The action spectrum of this response corresponds to the absorption spectrum of the green sulfur bacterial epibionts, indicating that the scotophobic behavior is based on a rapid signal transfer between green sulfur bacterial epibionts and the colorless motile bacterium (Fröstl and Overmann, 1998).

One third of the species of *Chromatiaceae* (including *Lamprobacter*, *Lamprocystis*, *Thiocapsa*, *Thiodictyon*, *Thiopedia* and *Thiolamprovum* spp.) (Fig. 1), some green sulfur bacteria (Fig. 1B) but only one species of *Ectothiorhodopiraceae* (*Ets. vacuolata*) habor gas vesicles. This pattern reflects the distribution of these bacteria in nature, with gas vesicle-bearing *Chromatiaceae* typically colonizing low-light stratified environments, and *Ectothiorhodospiraceae* usually inhabiting more shallow saline ponds and sediments. Gas vesicles are cylindrical structures with conical ends and species-specific lengths and widths and are filled with a gas mixture which corresponds to that in the surrounding medium. Gas vesicle formation in *Chlorobium clathratiforme* is detected exclusively at light intensities $<5\,\mu mol\ Quanta \cdot m^{-2} \cdot s^{-1}$ (Overmann et al., 1991b) which appears to be the reason for the rare observation of gas vesicles in pure cultures that are routinely incubated

at higher light intensities. Similarly, a transfer of the purple sulfur bacterium *Lamprocystis purpurea* to the dark was found to result in an increase in the specific gas vesicle content by a factor of 9 (Overmann and Pfennig, 1992). *Ectothiorhodospira vacuolata* forms gas vesicles during the stationary phase.

By comparison, the vertical migration based on flagellar movement and gas vesicle formation have different advantages under natural conditions. Whereas the movement by flagella requires a continuous supply of metabolic energy (the proton motive force), gas vesicle formation requires an initially higher, but one-time investment for the phototrophic cell. Gas vesicles, once formed, help to keep the bacterial cell at the appropriate vertical position without any further demand for energy. In accordance with this view, species like *Lamprobacter modestohalophilus* or *Ectothiorhodospira vacuolata* which are capable of both, gas vesicle synthesis as well as flagellar movement, use flagella during exponential growth but become immotile and form gas vesicles upon entry in the stationary phase. Gas vesicle formation therefore may represent an adaptation to conditions of starvation in these species. It has been estimated that flagellar movement is sustained at underwater irradiances of 0.2 µmol Quanta$\cdot m^{-2}\cdot s^{-1}$ (Overmann and Garcia-Pichel, 2000). Indeed, a dominance of gas-vacuolated forms over motile *Chromatiaceae* is usually observed in lakes where irradiances are below 1 µmol Quanta$\cdot\ m^{-2}\times s^{-1}$ (Fig. 1).

However, a lower limit appears to exist, below which gas vesicle formation does not represent a selective advantage for phototrophic sulfur bacteria due to its metabolic burden. The extremely low-light adapated *Chlorobium* BS-1 from the Black Sea chemocline exhibits an extremely low maintenance energy requirement but is not capable of gas vesicle synthesis. Apparently, synthesis of the proteinaceous gas vesicle sheaths becomes too energy-demanding under the severe light limitation in the Black Sea chemocline.

6. Syntrophy and Symbioses

In the laboratory, stable associations between green sulfur bacteria and sulfur- or sulfate-reducing bacteria can be established readily (Warthmann et al., 1992). These associations are based upon a cycling of sulfur compounds but not carbon. Simultaneous growth of the two partner bacteria is fueled by the oxidation of organic carbon substrates and light. In a similar manner, cocultures of *Chromatiaceae* with sulfate-reducing bacteria have been established in the laboratory. Interestingly, cellular aggregates consisting of the sulfate-reducing Proteobacterium *Desulfocapsa thiozymogenes* and small-celled *Chromatiaceae* were observed in the chemocline of a meromictic alpine lake (Tonolla et al., 2000).

A commensalistic relationship may exist between coccoid epibiotic bacteria and the purple sulfur bacterium *Chromatium weissei* (Clark et al., 1993). The unidentified epibionts attach to healthy *Chromatium* cells but do lyse the host cells like the morphologically similar parasite *Vampirococcus* (Guerrero et al., 1987a). Possibly, the epibiont grows chemotrophically on carbon compounds excreted by the purple sulfur bacterium.

The most spectacular type of association involving phototrophic bacteria is represented by the so-called phototrophic consortia. Phototrophic consortia (Fig. 2) consist of epibionts arranged in a regular fashion around a central chemotrophic bacterium and are regarded as the most highly developed interactions between different species of prokaryotes (Overmann and Schubert, 2002). Eight different types of motile phototrophic consortia can be distinguished based on the overall morphology of the association and the color of the epibionts (Glaeser and Overmann, 2004). In addition, two immotile forms ("*Chloroplana vacuolata*" and "*Cylindrogloea bacterifera*") are recognized (Fig. 2). Fluorescence in situ hybridization identified the epibionts as green sulfur bacteria (Tuschak et al., 1999) and the central rod-shaped colorless and motile bacterium as a member of the Betaproteobacteria (Fröstl and Overmann, 2000). Based on their distinct morphology, intact phototrophic consortia can be specifically collected from natural communities by micromanipulation and the 16S rRNA gene sequences of the green sulfur bacterial epibionts can be determined. Employing this technique, an unexpected diversity of 19 different types of epibionts have recently been identified in a culture-independent manner. All epibionts represent distinct and novel phylotypes that are often only distantly related to known species of green sulfur

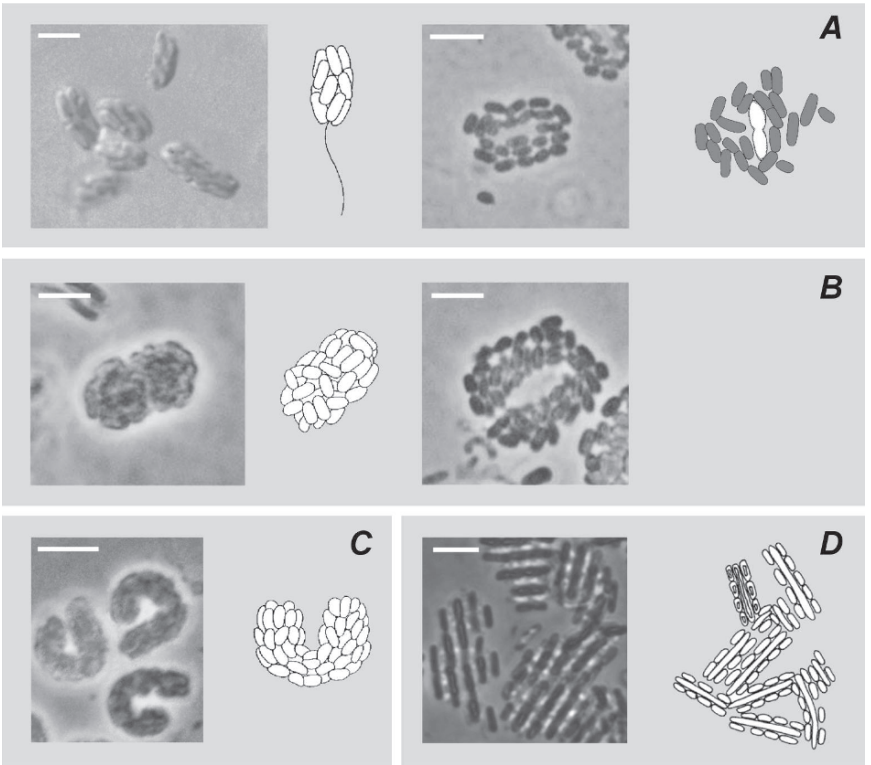

Fig. 2. Light microscopic and schematic views of five different types of phototrophic consortia. A. Differential interference contrast image and schematic view of "*Chlorochromatium aggregatum*", phase contrast photomicrograph of the disaggregated state and schematic view of "*Pelochromatium roseum*" (from left to right). B. Phase contrast photomicrograph in the intact state, schematic view, and the disaggregated state of "*Pelochromatium latum*". C. Phase contrast photomicrograph and schematic view of "*Chlorochromatium glebulum*". D. Phase contrast photomicrograph and schematic view of "*Chloroplana vacuolata*". Bars, 5 μm. Taken from Overmann (2006).

bacteria (Glaeser and Overmann, 2004). None of the epibiont 16S rRNA sequences have so far been detected in free-living green sulfur bacteria, suggesting that the interaction in phototrophic consortia is an obligate one. Based on the comparative phylogenetic analysis, the epibiont sequences are not monophyletic. Thus, the ability to form symbiotic associations either arose independently from different ancestors or was present in a common ancestor prior to the radiation of green sulfur bacteria and the transition to the free-living state in independent lineages. With regard to the phylogenetic affiliation of the central bacterium, a recent molecular analysis of the phototrophic consortium "*Chlorochromatium aggregatum*" revealed that this bacterium represents an isolated phylogenetic lineage distantly related to *Rhodoferax* spp., *Polaromonas vacuolata* and *Variovorax paradoxus* (Kanzler et al., 2005).

Maximum rates of light-dependent $H^{14}CO_3^-$ fixation were observed in a natural population of phototrophic consortia, suggesting that the green sulfur bacterial epibionts grow autotrophically like their free-living relatives. This conclusion was substantiated by the stable carbon isotope ratios ($\delta^{13}C$) of farnesol, tetradecanol, hexadecanol and hexadecenol which are esterifying alcohols of BChl*e* and biomarkers of the epibionts (Glaeser and Overmann, 2003a). Intact phototrophic consortia exhibit a scotophobic response in which the bacteriochlorophylls of the epibionts function as light sensors, whereas the central bacterium confers motility. Hence, a rapid signal transfer exists between the two partners and permits phototrophic consortia to accumulate at

preferred light intensities and wavelengths (Fröstl and Overmann, 1998). Phototrophic consortia are attracted by sulfide and 2-oxoglutarate, which indicates a potential role of these compounds in the metabolism of the consortia. Microautoradiography of consortia in natural water samples revealed that 2-oxoglutarate is incorporated only in the presence of both light and sulfide (Glaeser and Overmann, 2003b). Because the green sulfur bacterial epibionts grow autotrophically, 2-oxoglutarate most likely is taken up and utilized by the central bacterium while sulfide is the electron-donating substrate of the epibionts. These results indicate that incorporation of 2-oxoglutarate by the central bacterium is regulated by the metabolic state of the epibiont cells.

In phototrophic consortia, the immotile green sulfur bacteria not only functionally attain motility like their purple sulfur bacterial competitors, but are obviously capable of controlling the chemotactic and physiological response of the chemotrophic partner bacterium. The high numbers of phototrophic consortia found in many lakes, the fact that in some environments all cells of green sulfur bacteria occur in the associated state (Glaeser and Overmann, 2003a), and the repeated advent of epibionts during the green sulfur bacterial radiation indicate that this strategy must be of high competitive value under certain environmental conditions. Since phototrophic consortia have recently become available in enrichment cultures, they can now serve as suitable model systems for the investigation of the molecular mechanisms of cell–cell recognition and signal exchange, and for studies of the coevolution of nonrelated prokaryotes.

B. Physiology in Situ as Opposed to Growth in the Test Tube: Growth Rates, Low Maintenance Energy Requirements and Survival

Doubling times of phototrophic sulfur bacteria under natural conditions are significantly lower than in laboratory cultures. As an example, values for *Chromatiaceae* in lakes have been estimated to range from 1.5 to 238 days (Garcia-Cantizano et al., 2005). These data suggest that adaptation to low growth rates and survival may have played a significant role in the evolution of phototrophic sulfur bacteria.

In a careful study, the maintenance energy requirement of the purple nonsulfur Alphaproteobacteria *Rhodobacter capsulatus* and *Rba. acidophilus* was determined to amount to 0.012 mol quanta (g dry weight·h)$^{-1}$ (Göbel, 1978). Based on indirect estimates, the maintenance energy requirements of green sulfur bacteria are significantly lower than that of purple sulfur bacteria (van Gemerden and Mas, 1995). This may be explained by the fact that biosynthesis of proteins requires a major fraction of the energy expenditure of the bacterial cell and that the protein content of green sulfur bacterial antenna is much lower than that in purple sulfur bacteria: the paracrystalline rod-like structure of bacteriochlorophyll aggregates in the chlorosomes of green sulfur bacteria features a significantly lower protein:pigment mass ratio (0.5–2.2; Overmann and Garcia-Pichel, 2000) than the light-harvesting complexes of other anoxygenic phototrophs (3.9–6.7) or cyanobacteria (22.4).

Based on the value for the maintenance energy requirement of *Rba. capsulatus* and *Rba. acidophilus* given above it has been calculated that the minimum irradiance which would be required for survival of photosynthetic bacteria is 2 μmol Quanta·m^{-2}·s^{-1} (Overmann and Garcia-Pichel, 2000). By comparison, irradiances of this order or lower prevail in the natural habitats of phototrophic sulfur bacteria (Overmann and Manske, 2006). Yet, growth of natural populations can be observed under these conditions, indicating that phototrophic sulfur bacteria under natural conditions must exhibit significantly lower maintenance energy requirements than those of the laboratory cultures studied to date.

In particular, this holds true for the green sulfur bacterium from the Black Sea chemocline which forms the slowest growing population of anoxygenic phototrophic bacteria known to date (Overmann and Manske, 2006). As extrapolated from the laboratory growth rates or carbon fixation rates, green sulfur bacteria in the Black Sea attain doubling times of 3 years in summer and 26 years in winter (Overmann et al., 1992; Manske et al., 2005; Overmann and Manske, 2006). The green sulfur bacterium from the Black Sea chemocline thus represents an excellent model system for the study of low-light adaptation and, from a more fundamental perspective, a model system

for studies of mechanisms of adaptation towards extreme energy-limited environments.

Commensurate with the extremely long doubling times, the maintenance energy requirement of the Black Sea *Chlorobium* is significantly lowered compared to other green sulfur bacteria (Overmann et al., 1992). At the same time, the Black Sea strain exhibits significantly decreased specific rates of photosynthetic CO_2-fixation, sulfide oxidation and growth at saturating light intensities (section II.A.1). These observations suggest that the extraordinarily low maintenance energy requirement of this bacterium at least in part is accomplished by lowering the intracellular concentrations of metabolic enzymes.

The threshold light intensity supporting the photosynthetic growth of green sulfur bacteria is decreased in the presence of suitable organic carbon substrates like acetate (Bergstein et al., 1981). Although the environmental concentrations and the turnover rates of potential carbon substrates of phototrophic sulfur bacteria have rarely been determined (Bergstein et al., 1979), these compounds may support the survival of the cells.

C. Diversity of Phototrophic Sulfur Bacteria

In almost all freshwater and marine photic anoxic environments, green and/or purple sulfur bacteria (*Chlorobiaceae* and *Chromatiaceae*) represent the dominant anoxygenic phototrophs. *Ectothiorhodospiraceae* dominate in saline habitats. Only very few, and atypical, ecosystems have been described in which phototrophic Alphaproteobacteria (purple nonsulfur bacteria) outnumber the phototrophic sulfur bacteria. These latter habitats are aquatic systems heavily polluted with organic wastewaters in which low-molecular organic compounds occur at millimolar concentrations (Okubo et al., 2006).

Based on the current status of taxonomy (Overmann, 2001; Imhoff, 2003; Imhoff, 2005a, b), 41 different species of *Chromatiaceae*, 12 species of *Ectothiorhodospiraceae* and 17 species of *Chlorobiaceae* are currently recognized. In contrast, the ribosomal database project (rdp) database lists 429, 460 and 339 16S rRNA gene sequences for the above three families of phototrophic sulfur bacteria (Cole et al., 2006).

Culture independent analyses of natural communities based on 16S rRNA gene sequences routinely recover novel sequence types of green or purple sulfur bacteria from natural bacterial communities (Coolen and Overmann 1998; Overmann et al., 1999a; Elshahed et al., 2003; Glaeser and Overmann, 2003a; Koizumi et al., 2004; Martínez-Alonso et al., 2005; Tonolla et al., 2005). In addition, the dominant phylotypes determined by the culture-independent approach often do not match the phylotypes cultured from the same environment. It has to be concluded that the current number of species described do not reflect the full phylogenetic breadth of phototrophic sulfur bacteria and that the dominant species may differ considerably from known types with respect to physiology and ecology. The latter assumption has been substantiated by the discovery of several novel isolates of green (Vogl et al., 2006) and purple sulfur bacteria (Permentier et al., 2001) which exhibit conspicuously different physiological properties in comparison to the previously described species.

At low biomass densities, 16S rRNA gene sequences of phototrophic sulfur bacteria often cannot be detected PCR amplification methods using universal primer pairs (Coolen and Overmann, 1998; Vetriani et al., 2003), despite the presence of their specific pigment biomarkers. Therefore, specific molecular detection methods have been developed, which employ group-specific primers targeting 16S rRNA gene sequences of green or purple sulfur bacteria (Coolen and Overmann, 1998; Overmann et al., 1999a). In the case of green sulfur bacteria, the highly specific PCR method permits the detection of as little as 100 cells (Glaeser and Overmann, 2004).

III. Biogeochemical Significance of Phototrophic Sulfur Bacteria

In lakes haboring phototrophic sulfur bacteria, an average of 28.7% of the primary production is anoxygenic and a maximum fraction of 83% has been determined (Overmann, 1997). Anoxygenic photosynthesis depends on reduced inorganic sulfur compounds which originate from the anaerobic degradation of organic carbon und the concomitant sulfide production by sulfate- and sulfur-reducing bacteria. During anaerobic

degradation, a large fraction of reducing equivalents become trapped in sulfide due to the low growth yield of fermenting and sulfate-reducing bacteria. Since these reducing equivalents originate from carbon already fixed by oxygenic photosynthesis, the CO_2-fixation of anoxygenic phototrophic bacteria does not lead to a net increase in organic carbon of the entire oxic/anoxic stratified ecosystem. In a sense, then, capture of light energy by anoxygenic photosynthesis merely compensates for the degradation of organic carbon in the anaerobic food chain. The CO_2-assimilation by anoxgenic phototrophs has therefore been termed "secondary primary production" (Pfennig, 1978). Geothermal sulfur springs are the only exception since their sulfide is of abiotic origin (Elshahed et al., 2003). Yet, sulfur springs are rather scarce, and anoxygenic photosynthetic carbon fixation of these ecosystems thus appears to be of minor significance on a global scale.

When reoxidizing the sulfide, anoxygenic phototrophs do not need to divert part of the electron donor towards ATP generation and almost completely transfer them to CO_2. In contrast to the chemolithoautotrophic sulfide-oxidizing bacteria, green and purple sulfur bacteria therefore efficiently recycle the reducing equivalents present in sulfide where light reaches the sulfide-containing water or sediment layers at sufficient intensities (Overmann, 1997). As a result, phototrophic sulfur bacteria often form dense microbial biomass accumulations even at moderate supply of sulfide and can attain biomass concentrations up to 28 mg bacteriochlorophyll $a \cdot l^{-1}$ in the case of *Chromatiaceae* in a saline meromictic lake (Overmann et al., 1994) and 16.7 mg bacteriochlorophyll $d \cdot l^{-1}$ in the case of green sulfur bacteria thriving in a athalassohaline hypersaline lake (Vila et al., 2002). The dense accumulations of phototrophic sulfur bacteria in turn may feed organic carbon (which would otherwise be lost) into the carbon cycle of the overlaying oxic water or sediment layers (Overmann et al., 1996, 1999b). However, several instances have been reported in which predation of phototrophic sulfur bacteria is of minor importance due to the toxicity of hydrogen sulfide for grazing organisms (van Gemerden and Mas, 1995). More recent data indicate a significant transfer of phototrophic bacterial biomass into the aerobic grazing food chain via rotifers and calanoid copepods (Overmann et al., 1999b, c).

Thus, anoxygenic primary production does only represent a net input of organic carbon to an ecosystem if (1) the anaerobic food chain within the system is fueled by additional allochthonous carbon from outside or by geothermal sulfide, and (2) aerobic grazers have access to the biomass of phototrophic sulfur bacteria. Based on experimental evidence, these conditions are met at least in some stratified aquatic environments (Overmann, 1997) where phototrophic sulfur bacteria can substantially alter the carbon and sulfur cycles.

As the closest analogue to past sulfidic oceans, the Black Sea has repeatedly been chosen as a model system for the study of the carbon and sulfur cycles in a large stratified marine water body and of the microorganisms relevant in these environments. The biomass of green sulfur bacteria in the chemocline of the Black Sea amounts to ≤0.8 mg BChl*e* m^{-2} (Manske et al., 2005) and hence is orders of magnitude lower than in any other environment studied so far (25–2000 mg BChl*e* m^{-2}; van Gemerden and Mas, 1995). The green sulfur bacteria in the chemocline of the Black Sea therefore represent the most dilute population of anoxygenic phototrophs known to date which can be attributed to the highly (i.e. four orders of magnitude more) sensitive method employed for the detection of their bacteriochlorophylls. Attempts to quantify photosynthetic activity in natural samples from the Black Sea chemocline have failed (Jørgensen et al., 1991) and stimulation of sulfide oxidation by light could not be detected in natural water samples (Repeta et al., 1989). Based on an extrapolation of light-limited rates of $H^{14}CO_3^-$ fixation, integrated anoxygenic photosynthesis contributes well below 1% to total photosynthetic carbon fixation in the Black Sea (Manske et al., 2005). Similarly, anoxygenic phototrophic sulfide oxidation accounts for ≤0.1% of total sulfide oxidation in the Black Sea chemocline. These estimates support the results of the modeling of sulfide fluxes which revealed that direct and indirect oxidation by molecular oxygen accounts for most, if not all of the sulfide removal in and beneath the chemocline of the Black Sea (Konovalov et al., 2001, 2003). Due to their low population density in the chemocline of the Black Sea, the green sulfur bacteria most likely are not

significant in the carbon and sulfur cycles of this environment.

IV. Phototrophic Sulfur Bacteria in the Past: Interpretation of Molecular Fossils

Today, habitats of phototrophic sulfur bacteria are restricted to a limited number of lacustrine environments, coastal lagoons and intertidal sandflats. Of these, the Black Sea currently represents the largest anoxic water body on Earth and covers 0.083% of the area of the planet. Its stratified water column comprises a ~60-m thick oxic top layer, a ~40-m-thick suboxic intermediate zone devoid of sulfide and oxygen, and a ~2000-m-deep sulfidic bottom zone (Murray et al., 1989). As a consequence, between 87 and 92% of the Black Sea water body remain permanently anoxic (Codispoti et al., 1991; Konovalov et al., 2001; Sorokin, 2002).

Due to the usually small size, the limited number, and the pronounced light limitation (see section II.A.1) of their contemporary habitats, the contribution of phototrophic sulfur bacteria to global photosynthetic CO_2-fixation is very small and has been estimated to amount to less than 1% (Overmann and Garcia-Pichel, 2000). In contrast, the entire Proterozoic ocean may have consisted of sulfidic deep water covered by a possibly 100 m-thick oxygenated surface layer (Anbar and Knoll, 2002), and its sulfidic pelagial may have persisted over 1000 million years. Furthermore, extended water column anoxia may also have occurred during the Phanerozoic, starting with the Ordovician, and including the Upper Devonian, Permian, Mid-Triassic, early Jurassic and Miocene (Messinian). The most distinct, and probably global, Mesozoic anoxic oceanic events occurred during the Toarcian (~187 Myr ago), Early Aptian (~132 Myr ago) and latest Cenomanian (~94 Myr ago). These events have been explained by an increase in atmospheric CO_2 due to an increased volcanism, with the associated greenhouse effect leading to increased continental weathering, and the resulting increased nutrient supply stimulating the productivity in Mesozoic Oceans together with an increased supply of biolimiting metals by submarine hydrothermal activity (Erba, 2004). Since an upper mixed layer of less than 20 m is relatively common in the present warm coastal and even open ocean (Kara et al., 2003), the existence of such large scale photic zone anoxia would appear to be a reasonable assumption.

All known green sulfur bacteria and about half of the species of purple sulfur bacteria are obligate anaerobic photolithoautotrophs (Overmann and Garcia-Pichel, 2000; Overmann, 2001). These bacteria only grow in an environment providing both, light and reduced sulfur compounds, and therefore represent suitable indicator organisms also for past photic zone anoxia of aquatic ecosystems. Three specific carotenoids (isorenieratene, β-isorenieratene, chlorobactene) occur in the different green sulfur bacteria (Overmann, 2001) and represent highly specific biomarkers which occur almost exclusively in this group (however, β-isorenieratane can also be formed by aromatization from β-carotene, which is widely distributed among different photosynthetic organisms; Koopmans et al., 1996a). Okenone so far has only been found in nine species of *Chromatiaceae* (Imhoff, 2005a). These biomarkers of phototrophic sulfur bacteria may survive over extended geological time periods and even some of their degradation products can be identified with sufficient reliability. Due to their specificity and chemical stability, pigment biomarkers thus offer the opportunity to reconstruct past ecosystems and numerous studies have taken the presence of isorenieratene and its geochemical derivatives like sulfurized isorenieratane (Repeta, 1993; Sinninghe-Damsté et al., 1993; Wakeham et al., 1995; Passier et al., 1999; Menzel et al., 2002), or degradation products of bacteriochlorophylls (Grice et al., 1996) as evidence for extended water column anoxia of ancient oceans, so called Oceanic Anoxic Events (Fig. 3).

Numerous marine deep-sea sediments and sedimentary rocks (some of them presently even located on land; Kohnen et al., 1991) were shown to contain such green sulfur bacterial biomarkers (Fig. 3). Since it may represent a modern analogue of past water column anoxia, the development of anoxic conditions in the Black Sea has been studied extensively. Bottom water anoxia was initiated 7000 to 8000 years ago by the intrusion of saltwater from the Mediterranean via the Bosporus strait (Ross and Degens, 1974). Within the subsequent 3000 years, the O_2–H_2S interface rose from

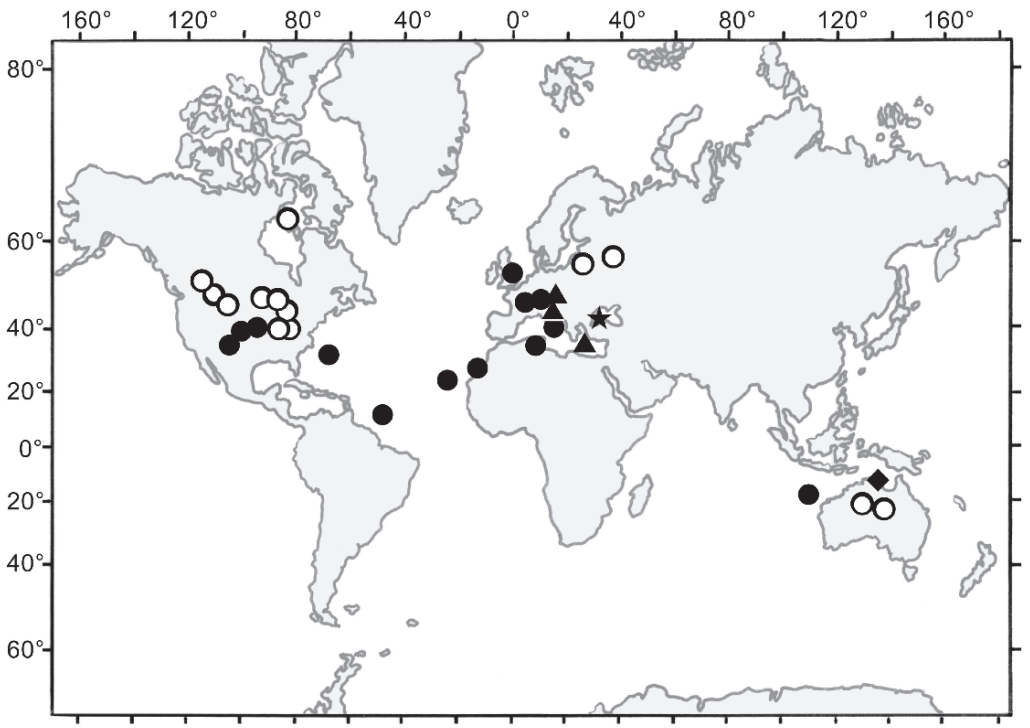

Fig. 3. Contemporary geographical location of fossil sediments and sedimentary rocks containing biomarkers of phototrophic sulfur bacterial as evidence for Oceanic Anoxic Events. Locations are distinguished according to their geological age.

★ Extant population of green sulfur bacteria in the chemocline (Manske et al., 2005) and isorenieratene in the Black Sea sediments (Sinninghe-Damsté et al., 1995).

▲ Cenozoic environments:, Pliocene/Pleistocene (isorenieratene; Rohling et al., 2006) and Pleistocene/Holocene (isorenieratene and 16S rRNA gene sequences; Coolen and Overmann, 2007) deposits in Eastern Mediterranean sapropels; C_{11}–C_{19} trimethyl substituted aryl isoprenoids in late Eocene/Early Oligocene shales of the Austrian Molasse Basin (Schulz et al., 2002), Northern Italian Marl sediments of the Messinian (Miocene) (Kohnen et al., 1991; Sinninghe-Damsté et al., 1995).

● Mesozoic environments: black shales from the eastern equatorial Atlantic of the Coniacian-Santonian (Deep Ivorian basin, Lake Cretaceous; Wagner et al., 2005), from the southern part of the proto-North Atlantic Ocean of the late Cenomanian (Cretaceous) (Kuypers et al., 2002; Kuypers et al., 2004); late Cenomanian marls of the Western Interior Basin (USA) from Kansas to New Mexico (Simons and Kenig, 2001), late Cenomanian Italian, NE Tunesian and Indian Ocean black shales, early Aptian black shales from the Italian Marche-Umbria basin, Toarcian (Jurassic) black shales of the same region, of the Belluno through and the Paris basin, as well as Middle Lias German Basin Posidonia Shale (Pancost et al., 2004); early Jurassic organic rich bituminous facies from Yorkshire (UK; Bowden et al., 2006).

○ Paleozoic environments: isorenieratene derivatives in Upper Devonian Williston and Western Canada Sedimentary Basins of western Canada (Requejo et al., 1992; Hartgers et al., 1994; Koopmans et al., 1996b), in Middle and Upper Devonian black shales of the Illinois and Michigan basins (Brown and Kenig, 2004), of the Belarussian Pripyat River Basin (Clifford et al., 1998), of the Holy Cross Mountains in Poland (Joachimski et al., 2001) and of the Western Australia Canning Basin (Barber et al., 2003) and Late Ordovician Boas Oil Shale of Southampton Island (Koopmans et al., 1996b). Aryl and diaryl isoprenoids in middle Ordovician grey-green shales of the central US (Pancost et al., 1998). Isorenieratane was also detected in mid-Cambrian limestone of the Georgina Basin (Brocks et al., 2005).

♦ Proterozoic environments: 1.64-Gyr-old mid-Proterozoic records of *Chromatiaceae* (okenane) and *Chlorobiaceae* (chlorobactane and isorenieratane) (Brocks et al., 2005).

the bottom at 2200 m depth toward the surface (Degens and Stoffers, 1976). The presence of isorenieratene and its degradation products in subfossil Black Sea sediments suggests that photic zone anoxia occurred already more than 6000 years ago (Repeta, 1993; Sinnighe-Damsté et al., 1993). Recently, fossil 16S rRNA gene sequences of the low-light adapted green sulfur

bacterial strain BS-1 have been detected in old deep sea sediments of the Black Sea, indicating that extremely low-light conditions in the chemocline must have existed also during past photic zone anoxia (E. Marschall and J. Overmann, in prep.).

In the Eastern Mediterranean, isorenieratene (Menzel et al., 2002) and its diagenetic derivatives (Passier et al., 1999) were detected in 1.8–3.0-million year-old Pliocene as well as 120,000-year-old interglacial (Rohling et al., 2006) sapropels, suggesting that anoxic water layers reached the photic zone during sapropel formation and that the enhanced preservation of organic carbon was caused by anoxia of deep Mediterranean bottom water (Rossignol-Strick, 1985; Rohling and Hilgen, 1991; Passier et al., 1999). Older Cenozoic samples are the 34-Myr-old late Eocene/Early Oligocene shales of the Austrian Molasse Basin that contain C_{11}–C_{19} trimethyl substituted aryl isoprenoids (Schulz et al., 2002).

Isorenieratane, the derivative formed very rapidly via nonbiological reduction with sulfide (Hebting et al., 2006), as well as the typical diagenetic and catagenetic C_{32}/C_{33} diaryl isoprenoids and 2,3,6-/3,4,5-trimethyl-substituted aryl isoprenoids (Koopmans et al., 1996b; Clifford et al., 1998), or derivatives of bacteriochlorophylls *c*, *d* or *e* like methyl isobutyl maleimide (Grice et al., 1996; Pancost et al., 2004), have meanwhile been extracted from rocks deposited throughout Earth's history (Fig. 3). In black shales from the 94-Myr-old late Cenomanian, molecular fossils of chlorobactene have been detected. Since this particular carotenoid is present only in the green strains of *Chlorobiaceae* which are adapted to higher light intensities than their brown-colored counterparts which contain isorenieratene, it has been concluded that anoxic water layers extended as high as 15 m below sea surface at these times (Kuypers et al., 2002). Recently, okenane, chlorobactane and isorenieratane, the reduced but intact derivatives of okenone, chlorobactene and isorenieratene have been detected even in 1.64-Gyr-old northern Australian shales (Brocks et al., 2005).

However, isorenieratene has also been detected *Streptomyces griseus* and *Brevibacterium linens* (Krügel et al., 1999; Krubasik and Sandmann, 2000), which are actinobacteria and hence belong to a different bacterial phylum. Vice versa, certain strains of brown-colored green sulfur bacteria do not contain any detectable amounts of isorenieratene and β- isorenieratene which were originally thought to be typical for this group (Glaeser et al., 2002). Based on these uncertainties in the interpretation of fossil green sulfur bacterial pigments, additional highly specific biomarkers are required for a more detailed reconstruction of the paleoenvironment.

Green sulfur bacteria (*Chlorobi*) form a distinct and coherent phylogenetic lineage (Overmann and Tuschak, 1997; Imhoff, 2003; Chapter 14). Accordingly, 16S rRNA gene sequences are employed to trace the occurrence and species composition of green sulfur bacteria in the environment (Overmann et al., 1999a; Tuschak et al., 1999). Compared to the limited diversity of carotenoids, 16S rRNA gene sequences provide the opportunity to differentiate between the 80 different phylotypes of green sulfur bacteria which are recognized to date (A. Manske and J. Overmann, submitted). Analyses of 16S rRNA gene sequences permit a more differentiated view of past environmental conditions and may be used to identify typical marine members of the green sulfur bacteria, since the latter form a single, phylogenetically well-separated clade within the *Chlorobi* (Imhoff, 2003; Manske et al., 2005). Subfossil DNA may survive over 10,000–100,000 years under favorable conditions, particularly at low temperatures, high ionic strength, anoxic conditions and protection from enzymatic degradation by adsorptive binding to hydroxyapatite, sand or humic acids (Romanowski et al., 1991; Lindahl, 1993; Poinar et al., 1996; Crecchio and Stotzky, 1998; Willerslev et al., 2004). Indeed, intact DNA of anoxygenic phototrophic bacteria was extracted from up to 9,100-year-old holocene lake sediments and could be amplified by polymerase chain reaction and sequencing (Coolen and Overmann, 1998).

Recently, the 16S rRNA gene sequence of the chemocline strain *Chlorobium* BS-1 has also been detected in deep sea sediment layers of the Black Sea (A. Manske and J. Overmann, submitted). The occurrence of subfossil 16S rRNA gene sequences of the extremely low-light adapted green sulfur bacterium may now be used as a more specific biomarker to infer photic zone anoxia and to determine the vertical extent of the oxic

zone more precisely. In a parallel approach, subfossil 16S rRNA gene sequences were recovered from Eastern Mediterranean sapropels deposited between 8,000 and 217,000 years ago (Coolen and Overmann, 2007). Unexpectedly, however, all recovered sequences grouped with freshwater or brackish, rather than truly marine, types of green sulfur bacteria. In addition, green sulfur bacterial sequences could also be recovered from carbon-lean intermediate sediment layers deposited during times of an entirely oxic water column. It is therefore feasible that the molecular remains of green sulfur bacteria originated from populations which thrived in adjacent freshwater or estuarine coastal environments rather than from an indigenous pelagic population. Based on these novel findings, molecular biomarkers of anoxygenic phototrophic sulfur bacteria may not always represent autochthonous chemofossils.

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References

Anbar AD and Knoll AH (2002) Proterozoic ocean chemistry and evolution: a bioinorganic bridge? Science 297: 1137–1142

Armitage JP (1997) Behavioral responses of bacteria to light and oxygen. Arch Microbiol 168: 249–261

Barber CJ, Grice K and Alexander R (2003) Biomarkers for Paleoenvironmental conditions of Devonian source rock deposition in the Canning Basin (Western Australia) and Western Canadian Basin. 21st International Meeting on Organic Geochemistry, Book of Abstracts, Part II, PI/160

Bavendamm W (1924) Die farblosen und roten Schwefelbakterien des Süss- und Salzwassers. Verlag Gustav Fischer, Jena

Beatty JT, Overmann J, Lince MT, Manske AK, Lang AS, Blankenship RE, Van Dover CL, Martinson TA and Plumley GF (2005) An obligately photosynthetic bacterial anaerobe from a deep-sea hydrothermal vent. Proc Natl Acad Sci USA 102: 9306–9310

Bergstein T, Henis Y and Cavari BZ (1979) Investigations on the photosynthetic sulfur bacterium *Chlorobium phaeobacteroides* causing seasonal blooms in Lake Kinneret. Can J Microbiol 25: 999–1007

Bergstein T, Henis Y and Cavari BZ (1981) Uptake and metabolism of organic compounds by *Chlorobium phaeobacteroides* isolated from Lake Kinneret. Can J Microbiol 27: 1087–1091

Borrego CM and Garcia-Gil LJ (1995) Rearrangement of light harvesting bacteriochlorophyll homologs as a response of green sulfur bacteria to low light intensities. Photosynth Res 41: 53–65

Bowden SA, Farrimond P, Snape CE and Love GD (2006) Compositional differences in biomarker constituents of the hydrocarbon, resin, asphaltene and kerogene fractions: An example from the Jet Rock (Yorkshire, UK). Org Geochem 37: 369–383

Brocks JJ, Love GD, Summons RE, Knoll AH, Logan GA and Bowden SA (2005) Biomarker evidence for green and purple sulphur bacteria in a stratified Palaeoproterozoic sea. Nature 437: 866–870

Brown TC and Kenig F (2004) Water column structure during deposition of Middle Devonian-Lower Mississippian black and Green/gray shales of the Illinois and Michigan Basins: a biomarker approach. Palaeogeogr Palaeoclimatol Palaeoecol 215: 59–85

Brune DC (1989) Sulfur oxidation by phototrophic bacteria. Biochim Biophys Acta 975: 189–221

Castenholz RW and Pierson BK (1995) Ecology of thermophilic anoxygenic phototrophs. In: Blankenship RE, Madigan MT and Bauer CE (eds) Anoxygenic Photosynthetic Bacteria, Advances in Photosynthesis, Vol II, pp 87–103. Kluwer Academic, Dordrecht, The Netherlands

Castenholz RW, Bauld J and Jørgensen BB (1990) Anoxygenic microbial mats of hot springs: thermophilic *Chlorobium* sp. FEMS Microbiol Ecol 74: 325–336

Clark KJ, Finlay BJ, Vicente E, Lloréns H and Miracle MR (1993) The complex life-cycle of a polymorphic prokaryote epibiont of the photosynthetic bacterium *Chromatium weissei*. Arch Microbiol 159: 498–505

Clifford DJ, Clayton JL and Sinninghe-Damsté JS (1998) 2, 3, 6-/ 3, 4, 5-trimethyl substituted diaryl carotenoid derivatives (Chlorobiaceae) in petroleums of the Belarussian Pripyat River Basin. Org Geochem 29: 1253–1267

Codispoti LA, Friederich GE, Murray JW and Sakamoto CM (1991) Chemical variability in the Black Sea: implications of continuous vertical profiles that penetrated the oxic/anoxic interface. Deep Sea Res 38 (suppl. 2): S691–S710

Cole JR, Chai B, Farris RJ, Wang Q, Kulam-Syed-Mohideen AS, McGarrell DM, Bandela AM, Cardenas E, Garrity GM and Tiedje JM (2006) The ribosomal database project (RDP-II): introducing myRDP space and quality controlled

public data. Nucleic Acids Research; doi: 10.1093/nar/gkl889

Coolen MJL and Overmann J (1998) Analysis of subfossil molecular remains of purple sulfur bacteria in a lake sediment. Appl Environ Microbiol 64: 4513–4521

Coolen MJL and Overmann J (2007) 217,000-year-old DNA sequences of green sulfur bacteria in Mediterranean sapropels and their implications for the reconstruction of the paleoenvironment. Environ Microbiol 9: 238–249

Crecchio C and Stotzky G (1998) Binding of DNA on humic acids: effect on transformation of *Bacillus subtilis* and resistance to Dnase. Soil Biol Biochem 30: 1061–1067

Degens ET and Stoffers P (1976) Stratified waters as a key to the past. Nature 263: 22–27

Drews G and Golecki JR (1995) Structure, molecular organization, and biosynthesis of membranes of purple sulfur bacteria. In: Blankenship RE, Madigan MT and Bauer CE (eds) Anoxygenic photosynthetic bacteria, Advances in Photosynthesis, Vol II, pp 231–257. Kluwer Academic, Dordrecht, The Netherlands

Ehrenberg CG (1838) Die Infusionstierchen als vollkommene Organismen. Leipzig, 2 vol.

Elshahed MS, Senko JM, Najar FZ, Kenton SM, Roe BA, Dewers TA, Spear JR and Krumholz LE (2003) Bacterial diversity and sulfur cycling in a mesophilic sulfide-rich spring. Appl Environ Microbiol 69: 5609–5621

Engelmann TW (1888) Die Purpurbakterien und ihre Beziehung zum Licht. Bot Z 46: 661–669

Erba E (2004) Calcareous nannofossils and Mesozoic oceanic anoxic events. Mar Micropaleontol 52: 85–106

Frigaard N-U, Chew AGM, Li H, Maresca JA and Bryant DA (2003) *Chlorobium tepidum*: insights into the structure, physiology, and metabolism of a green sulfur bacterium derived from the complete genome sequence. Photosynth Res 78: 93–117

Fröstl JM and Overmann J (1998) Physiology and tactic response of "*Chlorochromatium aggregatum*". Arch Microbiol 169: 129–135

Fröstl JM and Overmann J (2000) Phylogenetic affiliation of the bacteria that constitute phototrophic consortia. Arch Microbiol 174: 50–58

Fuhrmann S, Overmann J, Pfennig N and Fischer U (1993) Influence of vitamin B_{12} and light on the formation of chlorosomes in green- and brown-colored *Chlorobium* species. Arch Microbiol 16: 193–198

Garcia-Cantizano J, Casamayor EO, Gasol JM, Guerrero R and Pedros-Alio (2005) Partitioning of CO_2 incorporation among planktonic microbial guilds and estimation of in situ specific growth rates. Microb Ecol 50: 230–241

Glaeser J and Overmann J (1999) Selective enrichment and characterization of *Roseospirillum parvum*, gen. nov and sp nov., a new purple nonsulfur bacterium with unusual light absorption properties. Arch Microbiol 171: 405–416

Glaeser J and Overmann J (2003a) Characterization and in situ carbon metabolism of phototrophic consortia. Appl Environ Microbiol 69: 3739–3750

Glaeser J and Overmann J (2003b) The significance of organic carbon compounds for in situ metabolism and chemotaxis of phototrophic consortia. Environ Microbiol 5: 1053–1063

Glaeser J and Overmann J (2004) Biogeography, evolution and diversity of the epibionts in phototrophic consortia. Appl Environ Microbiol 70: 4821–4830

Glaeser J, Baneras L, Rütters H and Overmann J (2002) Novel bacteriochlorophyll *e* structures and species-specific variability of pigment composition in green sulfur bacteria. Arch Microbiol 177: 475–485

Göbel F (1978) Quantum efficiencies of growth. In: Clayton RK and Sistrom WR (eds) The Photosynthetic Bacteria, pp 907–925. Plenum, New York

Gorlenko VM (1988) Ecological niches of green sulfur bacteria. In: Olson JM, Ormerod JG, Amesz J, Stackebrandt E and Trüper HG (eds) Green Photosynthetic Bacteria, pp 257–267. Plenum, New York, London

Grice K, Gibbison R, Atkinson JE, Schwark L, Eckhardt CB and Maxwell JR (1996) Maleimides (1*H*-pyrrole-2,5-diones) as molecular indicators of anoxygenic photosynthesis in ancient water columns. Geochim Cosmochim Acta 60: 3913–3924

Guerrero R, Esteve I, Pedros-Alio C and Gaju N (1987a) Predatory bacteria in prokaryotic communities. The earliest trophic relationship. Ann NY Acad Sci 503: 238–250

Guerrero R, Pedrós-Alió C, Esteve I and Mas J (1987b) Communities of phototrophic bacteria in lakes of the Spanish Mediterranean region. Acta Acad Aboensis 47: 125–151

Hartgers WA, Sinninghe Damsté JS, Requejo AG, Allan J, Hayes JM and de Leeuw JW (1994) Evidence for only minor contributions from bacteria to sedimentary organic carbon. Nature 369: 224–227

Hebting Y, Schaeffer P, Behrens A, Adam P, Schmitt G, Schneckenburger P, Bernasconi SM and Albrecht P (2006) Biomarker evidence for a major preservation pathway of sedimentary organic carbon. Science 312: 1627–1631

Herbert RA, Ranchou-Peyruse A, Duran R, Guyoneaud R and Schwabe S (2005) Characterization of purple sulfur bacteria from the South Andros Black Hole cave system: highlights taxonomic problems for ecological studies among the genera *Allochromatium* and *Thiocapsa*. Environ Microbiol 7: 1260–1268

Imhoff JF (2003) Phylogenetic taxonomy of the family *Chlorobiaceae* on the basis of 16S rRNA and *fmo* (Fenna–Matthews–Olson protein) gene sequences. Int J Syst Evol Microbiol 53: 941–951

Imhoff JF (2005a) Family I. Chromatiaceae. In: Garrity JM (ed) Bergey's Manual of Systematic Bacteriology, Vol 2B, pp 3–40. Williams and Wilkins, Baltimore

Imhoff JF (2005b) Family II. Ectothiorhodospiraceae. In: Garrity JM (ed) Bergey's Manual of Systematic Bacteriology, Vol 2B, pp 41–48. Williams and Wilkins, Baltimore

Joachimski MM, Ostertag-Henning C, Pancost RD, Strauss H, Freeman KH, Littke R, Sinninghe Damsté JS and Racki G (2001) Water column anoxia, enhanced productivity and concomitant changes in $\delta^{13}C$ and $\delta^{34}S$ across the Frasnian–Famennian boundary (Kowala Holy Cross Mountains/Poland). Chem Geol 175: 109–131

Jørgensen BB, Fossing H, Wirsen CO and Jannasch HW (1991) Sulfide oxidation in the Black Sea chemocline. Deep-Sea Res 38 (Suppl): S1083–S1103

Kanzler BEM, Pfannes KR, Vogl K and Overmann J (2005) Molecular characterization of the nonphotosynthetic partner bacterium in the consortium "*Chlorochromatium aggregatum*". Appl Environ Microbiol 71: 7434–7441

Kara AN, Rochford PA and Hurlburt HE (2003) Mixed layer depth variability over the global ocean. J Geophys Res 108: 3079 (doi:10.1029/2000JC000736)

Kohnen MEL, Sinninghe Damsté JS and DeLeeuw JW (1991) Biases from natural sulphurization in palaeoenvironmental reconstruction based on hydrocarbon biomarker distribution. Nature 349: 775–778

Koizumi Y, Kojima H, Oguri K, Kitazato H and Fukui M (2004) Vertical and temporal shifts in microbial communities in the water column and sediment of saline meromictic Lake Kaiike (Japan), as determined by a 16S rDNA-based anmalysis, and related to physicochemical gradients. Environ Microbiol 6: 622–637

Kondratieva EN (1965) Photosynthetic Bacteria. Akademiya Nauk SSSR Institut Mikrobiologii. S. Monson, Jerusalem

Konovalov SK, Ivanov LI and Samodurov AS (2001) Fluxes and budgets of sulphide and ammonia in the Black Sea anoxic layer. J Mar Syst 31: 203–216

Konovalov SK, Luther GW, Friederich GE, Nuzzio DB, Tebo BM, Murray JW, Oguz T, Glazer B, Trouwborst RE, Clement B, Murray KJ and Romanov AS (2003) Lateral injection of oxygen with the Bosporus plume – fingers of oxidizing potential in the Black Sea. Limnol Oceanogr 48: 2369–2376

Koopmans MP, Schouten S, Kohnen MEL and Sinninghe Damsté JS (1996a) Restricted utility of aryl isoprenoids as indicators for photic zone anoxia. Geochim Cosmochim Acta 60: 4873–4876

Koopmans MP, Köster J, van Kaam-Peters HME, Kenig F, Schouten S, Hartgers WA, de Leeuw JW and Sinninghe Damsté JS (1996b) Diagenetic and catagenic products of isorenieratene: Molecular indicators for photic zone anoxia. Geochim Cosmochim Acta 60: 4467–4496

Krubasik P and Sandmann G (2000) A carotenogenic gene cluster from *Brevibacterium linens* with novel lycopene cyclase genes involved in the synthesis of aromatic carotenoids. Mol Gen Genet 263: 423–432

Krügel H, Krubasik P, Weber K, Saluz HP and Sandmann G (1999) Functional analysis of genes from *Streptomyces griseus* involved in the synthesis of isorenieratene, a carotenoid with aromatic end groups, revealed a novel type of carotenoid desaturase. Biochim Biophys Acta 1439: 57–64

Kühl M and Jørgensen BB (1992) Spectral light measurements in microbenthic phototrophic communities with a fibre-optic microprobe coupled to a sensitive diode array detector. Limnol Oceanogr 37: 1813–1823

Kühl M, Lassen C and Jørgensen BB (1994) Light penetration and light intensity in sandy marine sediments measured with irradiance and scalar irradiance fiber-optic microprobes. Mar Ecol Prog Ser 105, 139–148

Kuypers MMM, Pancost RD, Nijenhuis IA and Sinninghe Damsté JS (2002) Enhanced productivity led to increased organic carbon burial in the euxinic North Atlantic basin during the late Cenomanian oceanic anoxic event. Paleoceanography 17: 1051 (doi:10.1029/2000PA000569)

Kuypers MMM, Lourens LJ, Rijpstra IC, Pancost RD, Nijenhuis IA and Sinninghe Damsté JS (2004) Orbital forcing of organic carbon burial in the proto-North Atlantic durign oceanic anoxic event 2. Earth Planet Sci Lett 228: 465–482

Lindahl T (1993) Instability and decay of the primary structure of DNA. Nature 362: 709–715

Manske AK, Glaeser J, Kuypers MMM and Overmann J (2005) Physiology and phylogeny of green sulfur bacteria forming a monospecific phototrophic assemblage at 100 m depth in the Black Sea. Appl Environ Microbiol 71: 8049–8060

Manske AK, Henßge U, Glaeser J, Overmann J (2008) Subfossil 16S rRNA gene sequences of green sulfur bacteria as biomarkers for photic zone anoxia in the ancient Black Sea. Appl Environ Microbiol (in press)

Martínez-Alonso M, Van Bleijswijk J, Gaju N and Muyzer G (2005) Diversity of anoxygenic phototrophic sulfur bacteria in the microbial mats of Ebro Delta: a combined morphological and molecular approach. FEMS Microbiol Ecol 52: 339–350

Menzel D, Hopmans EC, van Bergen PF, de Leeuw JW and Sinninghe Damsté JS (2002) Development of photic zone euxinia in the eastern Mediterranean basin during deposition of pliocene sapropels. Mar Geol 189: 215–226

Montesinos E, Guerrero R, Abella C and Esteve I (1983) Ecology and physiology of the competition for light between *Chlorobium limicola* and *Chlorobium phaeobacteroides* in natural habitats. Appl Environ Microbiol 46: 1007–1016

Murray JW, Jannasch HW, Honjo S, Anderson RF, Reeburgh WS, Top Z, Friederich GE, Codispoti LA and Izdar E (1989) Unexpected changes in the oxic/anoxic interface in the Black Sea. Nature 338: 411–413

Nicholson JAM, Stolz JF and Pierson BK (1987) Structure of a microbial mat at Great Sippewissett Marsh, Cape Cod, Massachusetts. FEMS Microbiol Ecol 45: 343–364

Okubo Y, Futamata H and Hiraishi A (2006) Characterization of phototrophic purple nonsulfur bacteria forming

colored microbial mats in a swine wastewater ditch. Appl Environ Microbiol 72: 6225–6233

Overmann J (1997) Mahoney Lake: a case study of the ecological significance of phototrophic sulfur bacteria. Adv Microbial Ecol 15: 251–288

Overmann J (2001) Green sulfur bacteria. In Garrity JM (ed) Bergey's Manual of Systematic Bacteriology, Vol 1, pp 601–623. Williams and Wilkins, Baltimore

Overmann J (2006) The symbiosis between nonrelated bacteria in phototrophic consortia. Chapter II. In: Overmann J (ed) Molecular Basis of Symbiosis. Progress in Molecular Subcellular Biology, pp 21–37. Springer, Berlin, Heidelberg

Overmann J and Garcia-Pichel F (2000) The phototrophic way of life. In: Dworkin M, Falkow S, Rosenberg E, Schleifer K-H, Stackebrandt E (eds) The Prokaryotes: An Evolving Electronic Resource for the Microbiological Community, 3rd edition (latest update release 3.11, September 2002), Springer, New York, 2000 (http://ep.springer-ny.com:6336/contents/)

Overmann J and Manske A (2006) Anoxygenic phototrophic bacteria in the Black Sea chemocline. In: Neretin L (ed) Past and Present Water Column Anoxia. NATO ASI series, pp 543–561. Springer, Berlin

Overmann J and Pfennig N (1992) Buoyancy regulation and aggregate formation in *Amoebobacter purpureus* from Mahoney Lake. FEMS Microbiol Ecol 101: 67–79

Overmann J and Schubert K (2002) Phototrophic consortia: model systems for symbiotic interrelations between prokaryotes. Arch Microbiol 177: 201–208

Overmann J and Tuschak C (1997) Phylogeny and molecular fingerprinting of green sulfur bacteria. Arch Microbiol 167: 302–309

Overmann J, Beatty JT, Hall KJ, Pfennig N and Northcote TG (1991a) Characterization of a dense, purple sulfur bacterial layer in a meromictic salt lake. Limnol Oceanogr 36: 846–859

Overmann J, Lehmann S and Pfennig N (1991b) Gas vesicle formation and buoyancy regulation in *Pelodictyon phaeoclathratiforme* (Green sulfur bacteria). Arch Microbiol 157: 29–37

Overmann J, Cypionka H and Pfennig N (1992) An extremely low-light adapted phototrophic sulfur bacterium from the Black Sea. Limnol Oceanogr 37: 150–155

Overmann J, Beatty JT and Hall KJ (1994) Photosynthetic activity and population dynamics of *Amoebobacter purpureus* in a meromictic saline lake. FEMS Microbiol Ecol 15: 309–320

Overmann J, Beatty JT and Hall KJ (1996) Purple sulfur bacteria control the growth of aerobic heterotrophic bacterioplankton in a meromictic salt lake. Appl Environ Microbiol 62: 3251–3258

Overmann J, Coolen MJL and Tuschak C (1999a) Specific detection of different phylogenetic groups of chemocline bacteria based on PCR and denaturing gradient gel electrophoresis of 16S rRNA gene fragments. Arch Microbiol 172: 83–94

Overmann J, Hall KJ, Northcote TG and Beatty JT (1999b) Grazing of the copepod *Diaptomus connexus* on purple sulfur bacteria in a meromictic salt lake. Environ Microbiol 1: 213–222

Overmann J, Hall KJ, Northcote TG, Ebenhöh W, Chapman MA and Beatty JT (1999c) Structure of the aerobic food chain in a meromictic lake dominated by purple sulfur bacteria. Arch Hydrobiol 144: 127–156

Pancost RD, Freeman KH, Patzkowsky E, Wavrek DA and Collister JW (1998) Molecular indicators of redox and marine photoautotroph composition in the late Middle Ordovivian of Iowa, U.S.A. Org Geochem 29: 1649–1662

Pancost RD, Crawford N, Magness S, Turner A, Jenkyns HC and Maxwell JR (2004) Further evidence for the development of photic-zone euxinic conditions during Mesozoic oceanic anoxic events. J Geol Soc London 161: 353–364

Parkin TB and Brock TD (1980a) Photosynthetic bacterial production in lakes: the effects of light intensity. Limnol Oceanogr 25: 711–718

Parkin TB and Brock TD (1980b) The effects of light quality on the growth of phototrophic bacteria in lakes. Arch Microbiol 125: 19–27

Passier HF, Bosch H-J, Nijenhuis LJL, Böttcher ME, Leenders A, Sinninghe Damsté JS, de Lange GJ and de Leeuw JW (1999) Sulphidic Mediterranean surface waters during Pliocene sapropel formation. Nature 397: 146–149

Pedrós-Alió C and Sala MM (1990) Microdistribution and diel vertical migration of flagellated vs. gas-vacuolate purple sulfur bacteria in a stratified water body. Limnol Oceanogr 35: 1637–1644

Permentier HP, Neerken S, Overmann J and Amesz J (2001) A bacteriochlorophyll *a* antenna complex from purple bacteria absorbing at 963 nm. Biochemistry 40: 5573–5578

Pfennig N (1978) General physiology and ecology of photosynthetic bacteria. In: Clayton RK and Sistrom WR (eds) The Photosynthetic Bacteria, pp 3–18. Plenum, New York

Pfennig N (1993) Reflections of a microbiologist, or how to learn from the microbes. Annu Rev Microbiol 47: 1–29

Pfennig N, Lünsdorf H, Süling J and Imhoff JF (1997) *Rhodospira trueperi* gen. nov., spec. nov., a new phototrophic Proteobacterium of the alpha group. Arch Microbiol 168: 39–45

Pierson B, Oesterle A and Murphy GL (1987) Pigments, light penetration, and photosynthetic activity in the multilayered microbial mats of Great Sippewissett Salt Marsh, Massachusetts. FEMS Microbiol Ecol 45: 365–376

Poinar HN, Höss M, Bada JL and Pääbo S (1996) Amino acid racemization and the preservation of ancient DNA. Science 272: 864–866

Repeta DJ (1993) A high resolution historical record of Holocene anoxygenic primary production in the Black Sea. Geochim Cosmochim Acta 57: 4337–4342

Repeta DJ, Simpson DJ, Jørgensen BB and Jannasch HW (1989) Evidence for anoxygenic photosynthesis from the distribution of bacterichlorophylls in the Black Sea. Nature 342: 69–72

Requejo AG, Allan J, Creaney S, Gray NR and Cole KS (1992) Aryl isoprenoids and aromatic carotenoids in Paleozoic rocks and oils from the Western Canada and Williston Basins. Org Geochem 19: 245–264

Rohling EJ and Hilgen FJ (1991) The eastern Mediterranean climate at times of sapropel formation: a review. Geol Mijnbouw 70: 253–264

Rohling EJ, Hopmans EC and Sinninghe Damsté JS (2006) Water column dynamics during the last interglacial anoxic event in the Mediterranean (sapropel S5). Paleoceanography 21: PA2018, doi:10.1029/2005PA001237

Romanowski G, Lorenz MG and Wackernagel W (1991) Adsorption of plasmid DNA to mineral surfaces and protection against Dnase I. Appl Environ Microbiol 57: 1057–1061

Ross DA and Degens ET (1974) In: Degens E and Ross D (eds), The Black Sea – Geology, Chemistry and Biology, pp 183–199 (Memoir No. 20. Am Ass. Petrol. Geol., Tulsa, Oklahoma)

Rossignol-Strick M (1985) Mediterranean Quaternary sapropels, an inmediate response of the African Monsoon to variation of insolation. Paleogeogr Paleoclimatol Paleoecol 49: 237–263

Sanchez O, van Gemerden and Mas J (1998) Acclimation of the photosynthetic response of *Chromatium vinosum* to light-limiting conditions. Arch Microbiol 170: 405–410

Schulz H-M, Sachsenhofer RF, Bechtel A, Polesny H and Wagner L (2002) The origin and hydrocarbon source rocks in the Austrian Molasse Basin (Eocene–Oligocene transition). Mar Petrol Geol 19: 683–709

Simons D-JH and Kenig F (2001) Molecular fossil constraints on the water column structure of the Cenomanian–Turonian Western Interior Seaway, USA. Palaeogr Palaeoclimatol Palaeoecol 169: 129–152

Sinninghe Damsté JS, Wakeham SG, Kohnen MEL, Hayes JM and de Leeuw JW (1993) A 6,000-year sedimentary molecular record of chemocline excursion in the Black Sea. Nature 362: 827–829

Sinninghe Damsté JS, Frewin NL, Kenig F and de Leeuw JW (1995) Molecular indictaors for palaeoenvironmental change in a Messinian evaporitic sequence (Vena del Gesso, Italy). I: Variations in extractable organic matter of ten cyclically deposited marl beds. Org Geochem 23: 471–483

Sorokin YuI (1970) Interrelations between sulphur and carbon turnover in meromictic lakes. Arch Hydrobiol 66: 391–446

Sorokin YuI (2002) The Black Sea. Ecology and oceanography. Backhuys, Leiden, The Netherlands

Steensgaard DB, Wackerbarth H, Hildebrandt P and Holzwarth AR (2000) Diastereoselective control of bacteriochlorophyll *e* aggregation. 3^1-S-BChl *e* is essential for the formation of chlorosome-like aggregates. J Phys Chem B 104: 10379–10386

Thar R and Kühl M (2001) Motility of *Marichromatium gracile* in response to light, oxygen, and sulfide. Appl Environ Microbiol 67: 5410–5419

Tonolla M, Demarta A, Peduzzi S, Hahn D and Peduzzi R (2000) In situ analysis of sulfate-reducing bacteria related to *Desulfocapsa thiozymogenes* in the chemocline of meromictic Lake Cadagno (Switzerland). Appl Environ Microbiol 66: 820–824

Tonolla M, Peduzzi R and Hahn D (2005) Long-term population dynamics of phototrophic sulfur bacteria in the chemocline of Lake Cadagno, Switzerland. Appl Environ Microbiol 71: 3544–3550

Tuschak C, Glaeser J and Overmann J (1999) Specific detection of green sulfur bacteria by in situ-hybridization with a fluorescently labeled oligonucleotide probe. Arch Microbiol 171: 265–272

van den Ende FP, Laverman AM and van Gemerden H (1996) Coexistence of aerobic chemotrophic and anaerobiv phototrophic sulfur bacteria under oxygen limitation. FEMS Microbiol Ecol 19: 141–151

Van Dover CL, Reynolds GT, Chave AD and Ryson JA (1996) Light at deep-sea hydrothermal vents. Geophys Res Lett 23: 2049–2052

van Gemerden H and Mas J (1995) Ecology of phototrophic sulfur bacteria. In: Blankenship RE, Madigan MT and Bauer CE (eds) Anoxygenic Photosynthetic Bacteria, Advances in Photosynthesis, Vol II, pp 49–85. Kluwer Academic, Dordrecht, The Netherlands

van Gemerden H, Tughan CS, De Wit R and Herbert RA (1989) Laminated microbial ecosystems on sheltered beaches in Scapa Flow, Orkney Islands. FEMS Microbiol Ecol 62: 87–102

van Niel (1931) On the morphology and physiology of the purple and green sulphur bacteria. Arch Microbiol 3: 1–112

van Rossum B-J, Steensgaard DB, Mulder FM, Boender GJ, Schaffner K, Holzwarth AR and de Groot HJM (2001) A refinded model of the chlorosomal antennae of the green sulfur bacterium *Chlorobium tepidum* from proton chemical shift constraints obtained with high-field 2-D and 3-D MAS NMR dipolar correlation spectroscopy. Biochemistry 40: 1587–1595

Vetriani C, Tran HV and Kerkhof LJ (2003) Fingerprinting microbial assemblages from the oxic/anoxic chemocline of the Black Sea. Appl Environ Microbiol 69: 6481–6488

Vila X, Guyoneaud R, Cristina XP, Figueras JB and Abella CA (2002) Green sulfur bacteria from hypersaline Chiprana Lake (Monegros, Spain): habitat description and phylogenetic relationship of isolated strains. Photosynth Res 71: 165–172

Vogl K, Glaeser J, Pfannes KR, Wanner G and Overmann J (2006) *Chlorobium chlorochromatii* sp. nov., a symbiotic green sulfur bacterium isolated from the phototrophic

consortium "*Chlorochromatium aggregatum*". Arch Microbiol 185: 363–372

Wagner T, Sinninghe Damsté JS, Hofmann P and Beckmann B (2005) Euxinia and primary production in Late Cretaceous eastern equatorial Atlantic surface waters fostered orbitally driven formation of marine black shales. Palaeoceanography 19:PA3009

Wakeham SG, Sinnighe Damsté JS, Kohnen MEL and DeLeeuw JW (1995) Organic sulfur compounds formed during early diagenesis in Black Sea sediments. Geochim Cosmochim Acta 59: 521–533

Warthmann R, Cypionka H and Pfennig N (1992) Photoproduction of H_2 from acetate by syntrophic cocultures of green sulfur bacteria and sulfur-reducing bacteria. Arch Microbiol 157: 343–348

Willerslev E, Hansen AJ, and Poinar HN (2004) Isolation of nucleic acids and cultures from fossil ice and permafrost. TRENDS Ecol Evol 19: 141–147

Winogradsky S (1887) Über Schwefelbakterien. Bot Ztg 45: 489–508

Zuber H and Cogdell RJ (1995) Structure and organization of purple bacterial antenna complexes. In: Blankenship RE, Madigan MT and Bauer CE (eds) Anoxygenic Photosynthetic Bacteria, pp 315–348. Kluwer Academic, Dordrecht, The Netherlands