Environmental Biology of the Marine *Roseobacter* Lineage

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Abstract

The *Roseobacter* lineage is a phylogenetically coherent, physiologically heterogeneous group of α-Proteobacteria comprising up to 25% of marine microbial communities, especially in coastal and polar oceans, and it is the only lineage in which cultivated bacteria are closely related to environmental clones. Currently 41 subclusters are described, covering all major marine ecological niches (seawater, algal blooms, microbial mats, sediments, sea ice, marine invertebrates). Members of the *Roseobacter* lineage play an important role for the global carbon and sulfur cycle and the climate, since they have the trait of aerobic anoxygenic photosynthesis, oxidize the greenhouse gas carbon monoxide, and produce the climate-relevant gas dimethylsulfide through the degradation of algal osmolytes. Production of bioactive metabolites and quorum-sensing-regulated control of gene expression mediate their success in complex communities. Studies of representative isolates in culture, whole-genome sequencing, e.g., of *Silicibacter pomeroyi*, and the analysis of marine metagenome libraries have started to reveal the environmental biology of this important marine group.

Contents

INTRODUCTION

The unraveling of microbial communities in the world's oceans is one of the most fascinating chapters in microbial ecology. It was here where surveys of phylogenetic marker genes for the first time revealed a hidden world of microbes completely unknown to microbiologists at the time because these organisms had never been cultivated. Using 16S rRNA-based methods, global populations were discovered for many of these uncultivated marine clusters that showed clear patterns in time and space, making them ideal subjects to study ecological adaptations and evolution. Because of the huge volume of the oceans, all biochemical processes in the ocean are crucial for the global cycles of carbon, sulfur, and other elements. Since these biogeochemical cycles are driven by large, worldwide distributed populations of marine microbes that have been subjected to strong selective pressure over long evolutionary timespans, it is fundamentally scientifically important and mandatory from an environmental perspective to understand their ecology and physiology, particularly also in view of environmental threats such as global warming and pollution.

The so-called *Roseobacter* lineage has played a special role from the beginning because it was the only abundant marine group for which cultivated strains were available that were relatively closely related to cloned environmental sequences (18, 30, 35). Thus, interest in this group has been steadily increasing since the description of *Roseobacter denitrificans* (87), its first representative. This organism can generate metabolic energy from light using the ancient purple bacterial mechanism of anaerobic photosynthesis without production of oxygen (anaerobic anoxygenic photosynthesis, AnAnP); however, unlike purple bacteria, it is able to do this in the presence of oxygen (under aerobic conditions) and was therefore termed aerobic anoxygenic phototroph (AAnP) (9, 50, 113). Because the contribution of aerobic anoxygenic photosynthesis to the total energy budget of the cell is

small and only seen under ecologically relevant conditions, it was originally thought to be a trait of little or no adaptive significance. This perspective changed completely when bacteriochlorophyll a (Bchla) was found in the open ocean (56) and was estimated to contribute up to 10% to photosynthetic energy generation (55).

Today, approximately 1500 16S rRNA gene sequences from the *Roseobacter* lineage have been deposited in databases (18), making it the most extensively studied abundant marine group. Many new interesting physiological traits in addition to aerobic anoxygenic photosynthesis have been found, e.g., degradation of algal sulfur compounds, carbon monoxide (CO) oxidation, degradation of aromatics, reduction of trace metals, symbiotic and pathogenic relationships, production of bioactive secondary metabolites and quorum sensing. Thus, the *Roseobacter* lineage can now be viewed as a phylogenetically coherent but physiologically diverse group of abundant marine bacteria that inhabits a wide range of niches in the world's oceans, mainly but not exclusively in coastal zones and polar regions, including marine snow, phytoplankton, sea ice, hydrothermal vents, sediments, macroalgae, and various invertebrates. The combination of culture-independent surveys with work on representative isolates has resulted in a wealth of new and ecologically important findings for this group that will be reviewed below.

ABUNDANCE AND DIVERSITY IN MARINE ENVIRONMENTS

This topic has recently been covered in an excellent review by Buchan et al. (18). Their phylogenetic analysis provides a framework on which future work can be based. We briefly summarize the main points; more details and references can be found in Reference 18.

Abundance

The *Roseobacter* lineage is a group of marine bacteria and cloned environmental sequences with >89% identity of the 16S rRNA gene, comprising at present 17 genera, 36 described species, and hundreds of cloned environmental sequences and uncharacterized isolates (18). *Roseobacter* populations have been found in practically every oceanic habitat investigated, sometimes in large numbers. Reports on their relative abundance in the world's ocean vary, partly because of differences in methodology (e.g., frequency of *Roseobacter* isolates among cultivated strains; probe hybridization using group-, genus-, or subcluster-specific probes; frequency of clones in a PCR-generated clone library; quantitative PCR; most-probable-number counting coupled to PCR; frequency of 16S rRNA genes in BAC libraries; and shotgun clone libraries) and partly because of "true" differences between habitats and seasons. However, from the multitude of studies (18) it emerges that the *Roseobacter* lineage is one of the most abundant marine lineages that is especially dominant in coastal environments and the polar oceans. For example, in a bacterial artificial chromosome (BAC) library close to the coast of Monterey, California, *Silicibacter*-like sequences represented 21.1% (0-m depth) and 23.6% (80-m depth) of all 16S rRNA sequences present (24, 103), while they accounted for 3% in the Sargasso Sea shotgun clone library (69, 106), datasets largely free from PCR bias. The uncultivated *Roseobacter* subcluster DC5-80-3 accounted for 20% of all bacteria in the Southern Ocean on the basis of quantitative PCR (86).

Diversity

Buchan et al. (18) compiled all available 16S rRNA sequence information for *Roseobacter* organisms from the Ribosomal Database Project II release 9.22, the Sargasso Sea metagenomic library (106), and the Sapelo Island Microbial Observatory 16S rRNA database (**http://simo.marsci.uga.edu/**). From 1497 sequences longer than 1000 nt they constructed a comprehensive

AnAnP: anaerobic anoxygenic photosynthesis

AAnP: aerobic anoxygenic photosynthesis

Bacteriochlorophyll *a* (Bchla): photosynthetic pigment used by bacteria

Quorum sensing: density-dependent regulation of gene expression in bacteria by small chemical signaling molecules called autoinducers

BAC: bacterial artificial chromosome

Shotgun clone library: total DNA from an organism or a microbial community is digested into small fragments (~1.5 kbp), cloned into a standard cloning vector, and assembled after sequencing with high coverage using specially developed software

phylogenetic tree for the *Roseobacter* lineage (**Figure 1**). Using a sequence similarity greater than or equal to 99% as a criterion, 41 lineages became apparent, of which 13 represent major clusters with greater than 10 nonredundant members that are discussed briefly below.

DC5-80-3 is the largest of all clusters, consisting primarily of cloned environmental sequences, and the only cluster for which the global distribution has been systematically determined (86). Members of this group have been detected in surface waters (to a depth of 40 m) of temperate and polar oceans of both hemispheres, and to depths of 2300 m and 1000 m in the Arctic and Southern Oceans, respectively (86). On the basis of quantitative PCR, they were estimated to comprise 20% of all bacteria in the Southern Ocean (86), and around 5% in coastal clone libraries (39, 77).

OBULB and SPON are two related clusters comprised mainly of cultivated strains (~70%). Representatives of OBULB were found in coastal seawater samples, sediments, seagrass, and starfish. SPON isolates were retrieved from the oxic/anoxic interface in the Black Sea, coastal environments, open ocean, deep sea vents, and marine sponges. The OCT cluster has clone (55%) and isolate (45%) sequences in similar proportions, almost all of them derived from sea ice or polar environments. RGALL is well represented by cultivated strains (68% of all sequences), which were often isolated from eukaryotic marine organisms, e.g., the scallop *Pecten maximus*, the oyster *Ostrea edulis*, the fish *Scophthalmus maximus*, an egg from the squid *Loligo pealei*, dinoflagellates, and marine phytoplankton.

CHAB-I-5 is the only *Roseobacter* subcluster that presently contains exclusively cloned environmental sequences, more than half of which are derived from coastal seawater. CHAB-I-5 constitutes 20% of all 16S rRNA-containing clones of a Monterey Bay BAC clone library (23, 103).

NAC11-7 is dominated by cloned sequences (88%), most of them associated with phytoplankton blooms in coastal areas. The two isolates were obtained from coastal seawater on oligotrophic media (91).

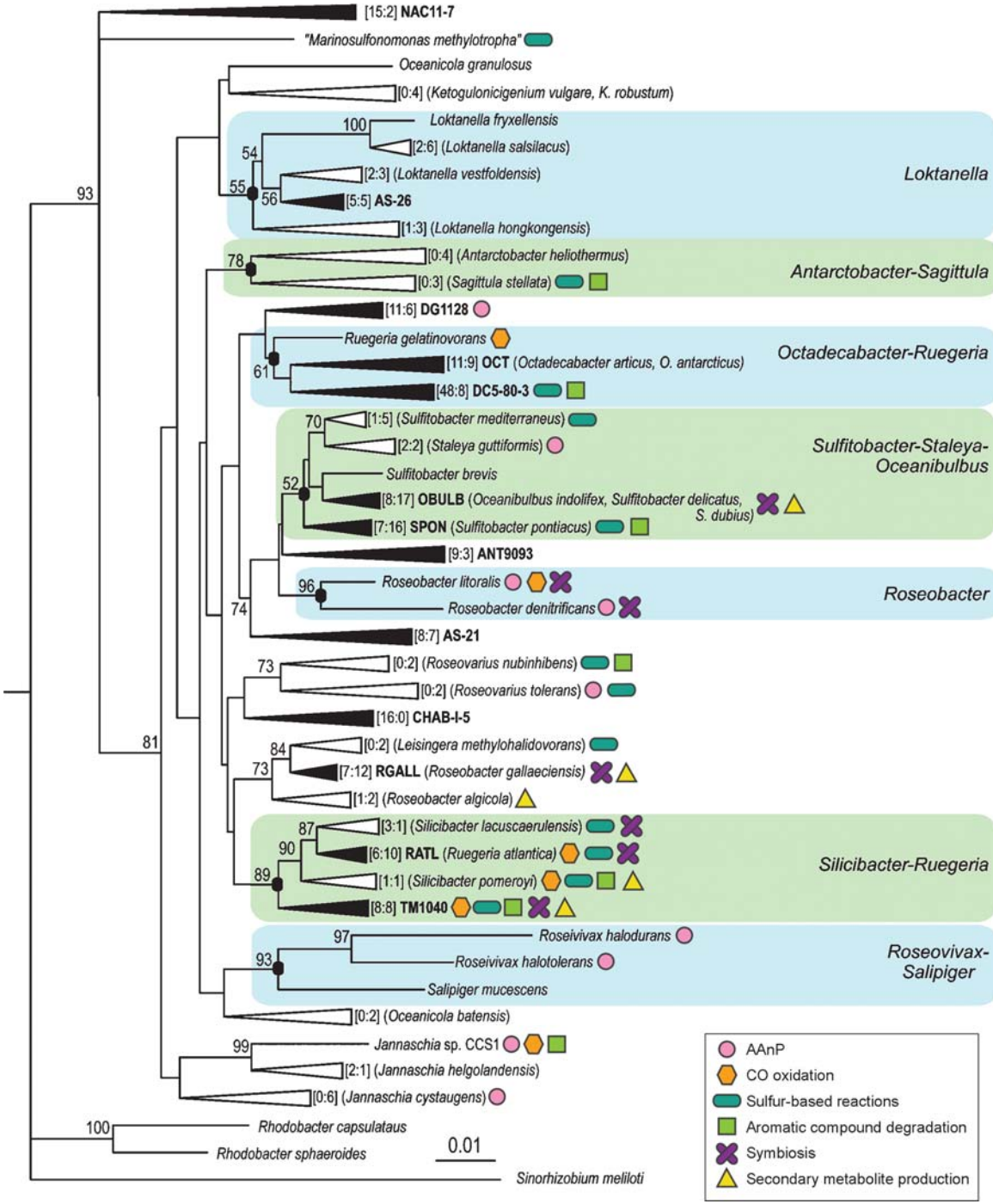
RATL and TM1040 are two clusters within the *Silicibacter-Ruegeria* superlineage, again composed of both isolates and clones. While RATL sequences are mostly from corals, TM1040 sequences are from coastal seawater or sediment.

Another cluster with a preference for a certain habitat is DG1128, which is well represented by sequences from macroalgae and phytoplankton. ANT9093 is a mixed cluster, containing sequences from polar sea ice, sediments, fish, and sponges, while clusters AS-21 and AS-26 are derived mainly from coastal water and sediment.

With the exception of CHAB-15, all clusters contain both cloned environmental sequences and cultivated strains, thus

Figure 1

The 41 major clusters of the *Roseobacter* lineage. The tree includes all currently described genera and the 13 major clusters described in the text. Filled triangles represent clusters of greater than or equal to 10 nonredundant members, and unfilled triangles represent clusters with fewer than 10 members. Described strains within each cluster are shown in parentheses. Robust phylogenetic lineages are indicated with filled ovals at branch nodes and vertical black lines. Numbers of clone and isolate sequences representing each cluster are provided in brackets ([number of clones:number of isolates]). Colored symbols represent evidence for the indicated physiologies. The tree is based on the sequences listed in Reference 18. *Sinorhizobium meliloti* (D14509) served as the outgroup. The tree is based on positions 92 to 1443 of the 16S rRNA gene (*Escherichia coli* numbering system). Prior to analysis, a filter was applied to the aligned sequences to exclude positions with a conservation less than 50%. The tree was constructed using Phylip and the neighbor-joining method. The bar represents Jukes-Cantor evolutionary distance. Bootstrap values greater than 50% are shown at branch nodes (100 iterations). Figure adapted, with permission, from Reference 18.



Proteorhodopsin: membrane-bound pigment that transforms light energy into a proton motive force across the membrane

confirming the original observation from the *Roseobacter* lineage, albeit at a much higher level of phylogenetic resolution. The level of ≥99% sequence similarity of the 16S rRNA gene within the clusters is usually regarded as a criterion for species identity. However, clusters were not homogeneous with respect to habitat (sources of clones and isolates) or physiology (e.g., AAnPs and most other metabolic traits are distributed throughout the tree) (**Figure 1**), although trends were sometimes apparent. Thus, a finer phylogenetic resolution is needed to determine evolutionary relationships within the *Roseobacter* lineage. Methods such as multilocus sequence typing of housekeeping genes or whole-genome sequence comparisons might be excellent tools for this.

Habitat-specific functional genetic islands, similar to pathogenicity islands or symbiotic islands (74), might be present in the genomes of these bacteria, which are transferred through lateral gene transfer. The photosynthesis gene cluster, comprising about 45 kb of genetic information and localized on a linear plasmids in some species (75), could be an example (3, 47, 71). The combination of habitat-specific functional genetic modules in the various representatives of the *Roseobacter* lineage might explain their physiological and ecological diversity on the one hand and phylogenetic coherence on the other. Hypotheses such as these will be testable by analyses of the upcoming genome sequences of this group.

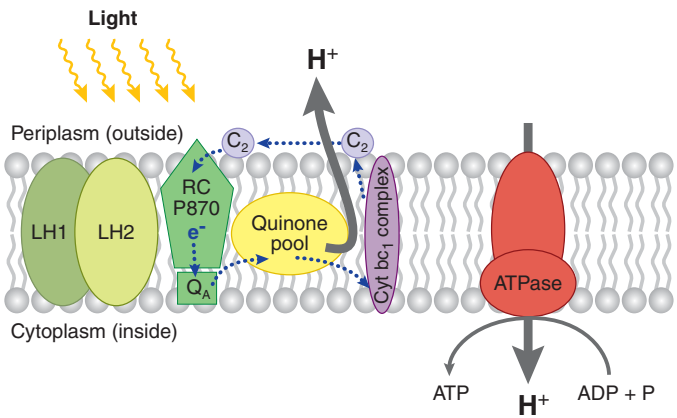


Figure 2

Scheme of the arrangement of protein complexes in the photosynthetic membrane of anoxygenic bacteria and the flow of electrons during cyclic photophosphorylation. Light transforms the reaction center Bchla (P870) into a strong electron donor. Electrons flow via the primary electron acceptor, Q_A, into the quinone pool to the cytochrome bc_1 complex and are transported back to P870 by the soluble cytochrome c_2. In the process, a proton gradient is established across the cell membrane. By the ATPase membrane complex, ADP is phosphorylated to ATP at the expense of proton motive force. Light-harvesting complex (LH), consisting of carotenoids, bacteriochlorophyll, and proteins (also called antenna complex); RC P870, reaction center with the special pair of bacteriochlorophyll molecules engaged in the primary electron transfer event, and the pufL and pufM proteins, which determine its exact stereochemistry. Q, quinone; Cyt bc_1, cytochrome bc_1 complex; c_2, cytochrome c_2. Based on Reference 62a.

AEROBIC ANOXYGENIC PHOTOTROPHY

Definition

Light is the one virtually unlimited energy source in the upper layers of the ocean. Oxygenic phototrophic bacteria, i.e., *Synechococcus* and *Prochlorococcus* species, grow autotrophically using light and CO_2 as sole sources of energy and carbon, respectively. Their important role for the ecology of the oceans and the global fixation of CO_2 is well established, and they occur as huge vertically stratified populations that have evolved into genetically distinct ecotypes adapted to the light regime and nutrient conditions of their respective ocean habitat.

Recently, two other types of light-driven energy generation have been shown to be of major importance in the ocean, namely AAnP and proteorhodopsin-based phototrophy. Both are based on membrane-bound pigments and accessory proteins and use light energy to pump protons out of the cell, thus transforming light into an electrochemical potential across the membrane. The resulting proton motive force in turn is used for the synthesis of ATP from ADP (**Figure 2**). Both AAnP- and proteorhodopsin-based phototrophy are not coupled directly to the fixation of CO_2 (and therefore do not represent photosynthesis in the classical sense), but supply

additional energy to the cell, reducing the need for respiratory oxidation of organic substrates.

The term aerobic anoxygenic photosynthesis, which literally means "photosynthesis performed in the presence of oxygen (aerobic) without generation of oxygen (anoxygenic)," is misleading because no light-driven synthesis of organic carbon (photosynthesis) is carried out. However, this term is used in this review because it is established in the scientific literature (9, 50, 113). While proteorhodopsin has not been detected in *Roseobacter* bacteria until now, AAnP is found in many of its representatives and is discussed below.

Ecological Importance

In 1979, aerobic pink-pigmented bacteria containing Bchla were discovered in the sediments of the Tokyo Bay by Shiba et al. (89) and were later described as *Erythrobacter* (88) and *Roseobacter* species (87). In the following years, many more Bchla-containing bacteria were isolated that occurred in marine, aquatic, and terrestrial habitats, and even in the depth of the Atlantic Ocean in the black smoker waters of the Juan Fuca Ridge (112). Most of the Bchla-containing bacteria belonged to the α-Proteobacteria, but one β-Proteobacterium, *Roseateles depolymerans*, was also found (102). Table S1 (follow the Supplemental Material link from the Annual Reviews home page at **http://www.annualreviews.org**) shows a list of current validly described Proteobacteria containing Bchla. However, many more Bchla-containing organisms, both α- and β-Proteobacteria, remain to be discovered (9). Within the *Roseobacter* lineage, AAnP is scattered throughout the tree (**Figure 1**) (3). At present, AAnP is found in 9 of the 41 *Roseobacter* lineages. Aerobic anoxygenic phototrophs were reviewed by Yurkov, Beatty, and coworkers (6, 78, 113).

Interestingly, it was the search for Bchla in the Juan Fuca Ridge using in situ measurements that triggered the discovery of the widespread distribution of Bchla in the world's oceans (56) and then led to the estimation that Bchla-containing organisms constituted 10% or more of total bacterial cells in seawater (55). This value was later shown by quantification of Bchla and the *puf LM* genes to be about one order of magnitude too high (33, 85). However, counts of Bchla-containing organisms were diverse, being highest in the Norwegian Sea (2.2% of all bacteria), Chesapeake Bay (10.7%), and Crane Neck, Long Island Sound, New York (18.7%), and around 1% or lower in the open ocean (85). This is in accordance with the Sargasso Sea shotgun clone library, where only 17 new *pufM* sequences from 1.2 million genes were found (85, 106). Thus, AAnP bacteria may be especially adapted to conditions arising during algal blooms, coastal pollution, or in cold seas.

pufL: L-protein of the photosynthesis reaction center

Anoxygenic Photosynthesis

Anaerobic anoxygenic photosynthesis (AnAnP) is the most ancient form of photosynthesis, functioning only in the absence of oxygen. AnAnP bacteria grow photoautotrophically or photoorganotrophically using light as an energy source, reduced inorganic compounds (e.g., H_2S) for the generation of reducing power [NAD(P)H], and CO_2 or organic substrates for the synthesis of biomass. Because of the spectacular colors caused by their carotenoids, AnAnPs are collectively called purple bacteria. They are believed to be the ancestors of the Proteobacteria, and all species isolated so far belong to either the α-, β-, or γ-Proteobacteria. Some are facultative anaerobes, i.e., in the presence of oxygen, photosynthesis is switched off completely and energy is generated from organic substrates by respiratory electron transfer, such as in *Rhodobacter sphaeroides*, which is related most closely to the *Roseobacter* lineage.

Structure of photosynthetic membrane and cyclic photophosphorylation. AnAnP is one of the best-studied membrane processes and has served as a model to

understand the primary electron transfer events during photosynthesis. Energy is generated through a light-driven cyclic photophosphorylation process brought about by membrane-bound pigment-protein complexes that consist of different units spatially arranged in a highly complex and completely defined fashion to set up the proton motive force: (*a*) the so-called reaction center (RC) P870, with the special pair of bacteriochlorophyll molecules engaged in the primary energy transformation event, and the pufL and pufM proteins, which determine its exact stereochemistry; (*b*) light-harvesting complex 1 (LH1) and (*c*) light harvesting complex 2 (LH2), also called antennae complexes, which consist of Bchla, carotenoids, and accessory proteins, and serve to channel light energy to the RC and protect it from oxidative damage; and (*d*) the cytochrome bc_1 complex, which is shared with the oxidative respiratory electron chain.

An adjacent ATPase membrane complex synthesizes ATP at the expense of proton motive force (**Figure 2**). The RC P870 has a low electrochemical potential. Light energy transforms it into an activated state with a high electrochemical potential. Subsequently, electrons flow across a series of electron carriers back to P870, which returns to its original state and is then able to take up new light energy. In the process, ATP is generated through cyclic photophosphorylation by the ATPase complex.

Oxygenic photosynthesis. By contrast, oxygenic phototrophs (e.g., cyanobacteria and chloroplasts of green plants) have a two-stage, interconnected light reaction using photosystem I (P700) and the preceding photosystem II (P680) that serves to split water into protons and oxygen. Electrons travel from P680 to P700, generating ATP by noncyclic photophosphorylation. However, under anaerobic conditions many algae and some cyanobacteria are able to carry out cyclic photophosphorylation using only photosystem I, thus they photosynthesize anoxygenically as do purple bacteria, showing the close evolutionary relationship between oxygenic and anoxygenic photosynthesis.

pufM: M-protein of the photosynthesis reaction center

RC: reaction center

LH: light harvesting complex

Rubisco: ribulose bisphosphate carboxylase, key enzyme of the Calvin cycle, the series of reactions by which most photosynthetic organisms convert CO_2 to organic molecules

Anaplerotic metabolism: replenishment of tricarboxylic acid cycle intermediates during growth, mainly by carboxylation of pyruvate or phosphoenolpyruvate

Aerobic Anoxygenic Photosynthesis

Most *Roseobacter*-lineage AAnP bacteria are obligate aerobes, but some [e.g., *Roseobacter litoralis* (87) and *Dinoroseobacter shibae* (11)] are able to use nitrate as a terminal electron acceptor and thus are facultative anaerobes. They rely on the respiratory electron chain for energy generation and biomass synthesis, deriving only little additional energy from light-driven ATP synthesis. The Calvin cycle, including its key enzyme Rubisco needed for autotrophic CO_2 fixation, is not present. Low levels of light-induced CO_2 fixation, which have sometimes been observed, are consistent with anaplerotic metabolism common in heterotrophic bacteria (55, 113). The photosynthetic gene cluster of *Roseobacter denitrificans* is highly homologous to that of *Rhodobacter sphaeroides* (approximately 45 kb). Detailed comparisons of these organisms are reviewed by Yurkov & Beatty (113) and can briefly be summarized as follows.

Pigments. Spheroidenone is thus far the only carotenoid detected in *Roseobacter* AAnP bacteria; it is also present in *Rhodobacter* species. Other AAnPs, however, show a range of different carotenoids (113). Their main function is to protect the cell from oxidative damage. All AAnP bacteria use Bchla. The synthesis of Bchla is completely inhibited even by low-light intensities. The amount of Bchla produced in the dark is generally about 10% or less than that produced by AnAnP bacteria, and in some species it depends strongly on nutrient composition, salt concentration, and unknown stress factors (10, 12, 101). Synthesis of Bchla can even be transiently and completely suppressed, as in *Roseovarius tolerans* (3, 58). This finding may indicate that Bchla is produced as an adaptive response to specific conditions relevant to the natural habitat of the organism.

Structure of photosynthetic membranes. No specialized photosynthetic membranes have been found so far in *Roseobacter* AAnP bacteria, presumably because of the low concentrations of Bchla present. The structural diversity of the peripheral antenna complexes is large in AAnP bacteria, and some show unusual light adsorption characteristics. However, the photosynthetic membrane complex of *Rhodobacter* and of *Roseobacter* is virtually indistinguishable.

Functioning of the reaction center. RC complexes from several AAnP bacteria were isolated, and their absorption spectra were found to be similar to those of AnAnP bacteria and functional in terms of a cyclic electron transfer system (113). No electron transfer occurred under anaerobic conditions. It was suggested that the midpoint potential of the quinone Q_A, the primary electron acceptor of the RC, might be too high; therefore this Q_A is in the reduced state (dihydroquinol) under anaerobic conditions and cannot accept an electron unless an oxidant such as O_2 transforms it into the quinone form, which is capable of acting as an electron acceptor.

Regulation. The anoxygenic photosystems of purple bacteria and *Roseobacter* organisms, which are virtually indistinguishable with respect to structure and functioning, operate in complementary mode without overlap: Low-light intensities completely stop Bchla synthesis in AAnP bacteria, whereas high-light intensities merely inhibit Bchla synthesis in AnAnP bacteria. All cyclic electron transport ceases in the presence of oxygen in AnAnPs and in the absence of oxygen in AAnPs.

In summary, AAnP bacteria have a completely functional photosynthetic machinery of the purple bacterial type that is operated under conditions contrary to those for which it originally evolved. Despite the detailed knowledge about RCs and electron transfer mechanisms in purple bacteria and AAnP bacteria, the rate-limiting step for the process of aerobic anoxygenic photosynthesis is not completely understood. Oxidative stress may be one of the key problems limiting the extent to which anoxygenic photosynthesis is feasible in an aerobic cell environment. Another problem might be the electrochemical potential of intermediate electron carriers in the photosynthetic membrane complex. Solving this problem might contribute toward a deeper understanding of the evolution of oxygenic photosynthesis.

Adaptive Advantage of Aerobic Anoxygenic Photosynthesis

The contribution of aerobic anoxygenic photosynthesis toward biomass yield has been demonstrated by chemostat cultures grown in regular diurnal day/night cycles (113; H. Biebl, unpublished data). Bchla is only synthesized during the night; its decrease during the day is consistent with dilution by growth in the absence of synthesis. Protein concentration increases during the day and is always higher under day/night illumination cycles than in constant darkness (or constant light). These data could mean that the energy generation by Bchla, which provides the cell with ATP for active transport, nutrient uptake, movement, and biosynthesis, is balanced against oxidative stress by Bchla, which is strongest during the day. Thus, Bchla-driven photophosphorylation must be maintained at a low level, but it has an adaptive advantage in the ocean under stable light conditions and nutrient limitation, because every ATP molecule generated by photophosphorylation reduces the need for organic carbon compounds. It seems likely, however, that nutrient limitation and other factors important in the natural habitat need to be reproduced in the laboratory much more realistically to demonstrate this.

Interesting parallels can be drawn to proteorhodopsin, a light-driven proton pump that is highly expressed in the oligotrophic ubiquitous marine picoplankton bacterium *Pelagibacter ubique* SAR11, where it covers about 20% of the cell surface (29). However, no consistent increase in growth yield could be

observed in light (29). Thus, either the "right" conditions have not yet been found or it is not growth yield that is increased in light, but other traits relevant in the ocean, e.g., long-term resistance against starvation (101).

Carboxydotrophic: able to gain energy from the oxidation of carbon monoxide

CARBON MONOXIDE OXIDATION

Carbon monoxide (CO) is one of the most important chemical reactants in the troposphere; it affects the fate of the greenhouse gases ozone and methane by removing the major atmospheric oxidizing agent, hydroxyl radical (80). CO is produced by natural and anthropogenic sources (burning of fossil fuels and biomass, oxidation of atmospheric hydrocarbons by sunlight, release from ocean and vegetation), which account for an emission of 12–14 $\times$ 10^8 tons CO per year (48). However, repeated measurements have shown that concentrations of CO in the atmosphere are relatively constant, ranging from 0.06 to 0.15 ppm (48), indicating that it is steadily consumed.

High concentrations of CO are toxic for animals because CO binds irreversibly to the heme iron of hemoproteins, resulting in hypoxia and death (83). However, numerous bacteria can use CO as a source of carbon and energy. CO oxidation has been shown under both aerobic (64) and anaerobic conditions (95, 96). However, most studies focused on a small number of bacteria that grow autotrophically on CO using external electron acceptors such as sulfate or CO_2. In the environment, however, bacteria that do not grow chemoautotrophically but use CO at ambient concentrations to supplement their carbon and energy budget may be more abundant. Aerobic-root-associated CO consumption by carboxydotrophic bacteria plays a large role in wetlands (80). Terrestrial microbes remove as much as 15% of the annual flux of CO to the atmosphere, but the populations involved in situ are poorly known (19).

Two operons for CO oxidation were found in the genome of *S. pomeroyi* (69) that belonged to the two different *coxL* clusters BMS and OMP, respectively (53). Enzymes from the BMS cluster have a relatively low affinity to CO, whereas those in the OMP cluster have a higher affinity. The presence of both genes in the same organism indicates a possible adaptive advantage for optimal enzyme kinetics under a range of CO concentrations (53). The frequency of *coxL* genes in the environmental Sargasso Sea shotgun library was 7.1%, while the *Roseobacter* lineage was represented at approximately 3% of all 16S rRNA genes (69). Thus, other marine phyla must also play a role for CO oxidation in the ocean.

When PCR primers targeting the *coxL* gene coding for CO dehydrogenase were used, it became clear that the ability to oxidize CO is much more widely distributed than previously thought (53), as it is present in numerous species of α-, β-, and γ-Proteobacteria, including *S. pomeroyi* (69), several clusters of the *Roseobacter* lineage (18), and the closely related marine α-Proteobacterium genus *Stappia*. Further studies based on total DNA extracts from volcanic deposits suggested that the ability to oxidize CO is also present in members of the classes *Actinobacteria* and *Firmicutes* and in novel organisms that have not yet been identified (25, 26).

While none of the *Roseobacter* organisms studied to date uses the Calvin cycle to synthesize biomass, anaplerotic CO_2 fixation has been described during heterotrophic growth (113). CO oxidation may improve this process by providing an additional intracellular pool of CO_2 to the microbes. Both aerobic CO oxidation and aerobic anoxygenic phototrophy, known previously as an autotrophic way of life restricted to rare and extreme habitats, are used as additional modes of generating energy in the *Roseobacter* lineage. Interestingly, an ε-Proteobacterium enriched from a North Sea sediment can grow in an atmosphere of 100% CO or microaerobically and is able to couple the oxidation of CO to the reduction of reduced sulfur compounds (49). Although the result of an enrichment procedure and thus probably not representative

of natural microbial populations, this type of metabolism might be a model for *Roseobacter* metabolic networks that might also link CO oxidation and sulfur metabolism.

ROLE OF THE *ROSEOBACTER* LINEAGE FOR SULFUR CYCLING IN THE OCEAN

At least 12 of the 41 major *Roseobacter* lineages are involved in sulfur transformation reactions (18). Most important from an ecological point of view is the degradation of the algal osmolyte dimethylsulfoniopropionate (DMSP) through both the cleavage and the demethylation/dethiolation pathways, yielding the climate-relevant gas dimethylsulfide (DMS) as well as carbon and sulfur compounds for incorporation into microbial biomass (**Figure 3**). In addition, sulfur-based lithoheterotrophy has been discovered in several *Roseobacter* strains (98, 99) and the genome of *S. pomeroyi* (69). *Roseobacter* strains are also involved in transformations of DMS (35), methanethiol, methanesulfonate (46), and dimethyl sulfoxide (DMSO) (70).

Source and Biogeochemical Importance of DMSP and DMS

DMSP is a sulfonium compound produced in high concentrations as an osmoprotectant from methionine by marine micro- and macroalgae and halophytic plants (111). It is released into the ocean water by leakage, death, or grazing, and it is probably one of the most important sulfur and carbon sources of marine bacteria. One of its degradation products is the volatile DMS, which is universally present in seawater and emitted at a significant rate into the atmosphere (61). DMS was identified as the missing gaseous compound needed to enable the steady-state flow of sulfur between terrestrial and marine environments, making DMS emissions a key step in the global sulfur cycle and a shuttle between sea and land (61, 111). In the atmosphere, DMS is oxidized to acidic aerosol sulfates, which serve as "cloud condensing nuclei" and absorb incoming radiation before falling to earth as acid rain (111). Model calculations have shown that a reduction in the amount of sunlight that reaches the earth's surface caused by a change in DMS concentration could theoretically decrease the global mean temperature (110, 111).

The total annual flux of biogenic DMS from the ocean has been estimated to be 13 to 37 Tg (51, 111), at least one order of magnitude higher than the total annual flux from all other sources, including soils, plants, salt marshes, and freshwater swamps. This amount is even more remarkable because between 50% (111) and 85% (115) of the released DSMP is demethylated, and a fraction of DMS is oxidized by bacteria in the water column before it reaches the atmosphere (52, 111).

DMSP: dimethylsulfoniopropionate

DMS: dimethylsulfide

Degradation of DMSP

Because DMSP is the main source or DMS, its degradation has been studied extensively. There are two principal degradation routes (70) (**Figure 3**): (*a*) the cleavage pathway by a lyase enzyme to form DMS and acrylate, which is used by both eukaryotic algae and many bacteria, and (*b*) the demethylation/dethiolation pathway, which is found only in bacteria and results in the formation of MMPA (3-methylmercaptopropionate) and a methyl compound. MMPA can be degraded further either by the "double demethylation pathway" to form MPA (3-mercaptopropionate) and a methyl compound, or by dethiolation, resulting in methanethiol (MeSH) and acrylate. MeSH can be further metabolized to sulfide and sulfate or it can serve as a precursor for the synthesis of the sulfur-containing amino acid methionine, thus closing the circle.

The two different routes of DMSP degradation have fundamentally different biogeochemical implications. The cleavage pathway results in DMS production, increases the formation of cloud condensing nuclei, and

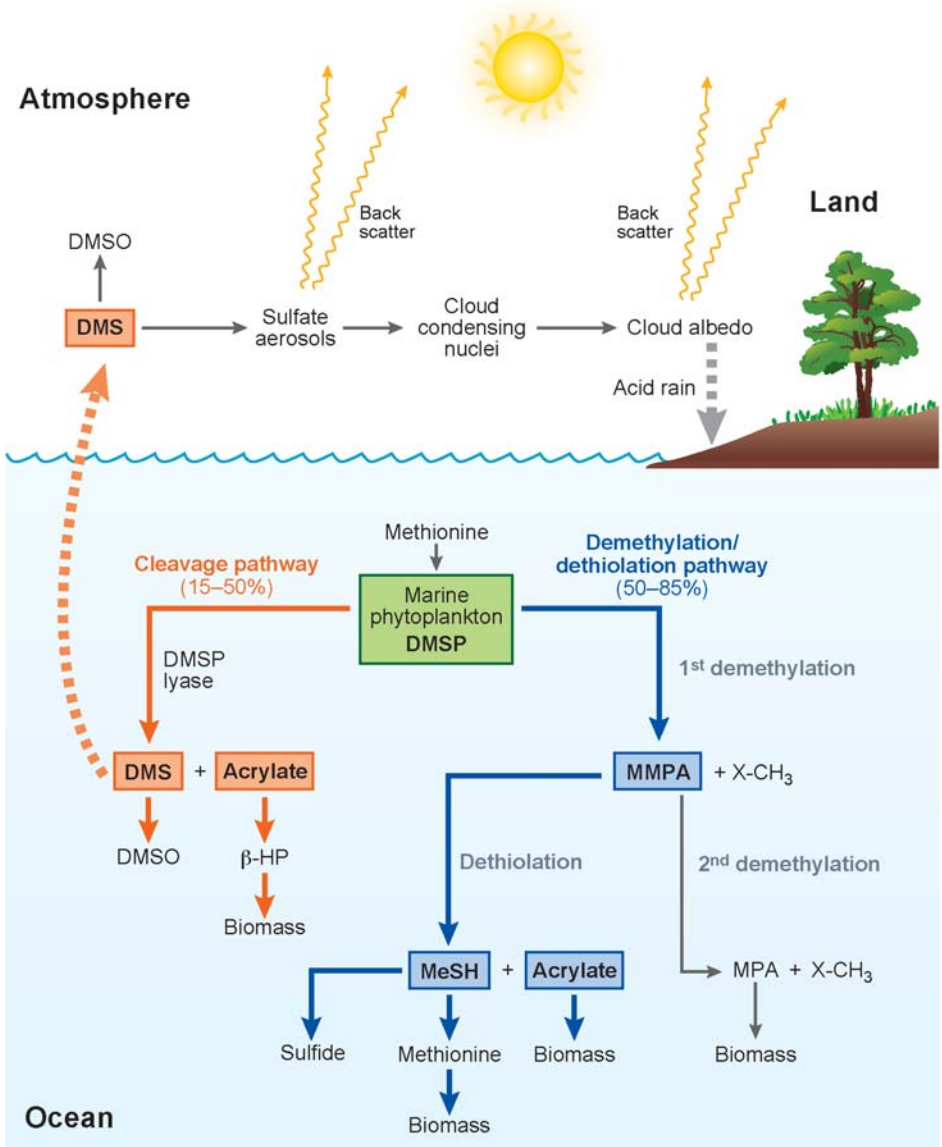


Figure 3

Role of *Roseobacter* lineage bacteria for the global sulfur cycle. DMS, dimethylsulfide; DMSP, dimethylsulfoniopropionate; MMPA, 3-methyl-propionate; MeSH, methanethiol; X-CH_3, unidentified molecule with a terminal methyl group; MPA, 3-mercaptopropionate; β-HP, β-hydrocypropionate; DMSO, dimethyl sulfoxide. Based on References 70 and 111 and references therein. The two pathways present in *Roseobacter* organisms are blue (demethylation/dethiolation pathway) and orange (cleavage pathway). See text for further explanation.

counteracts the process of global warming. The demethylation/dethiolation pathway results in uptake of both carbon- and sulfur-containing moieties of DSMP into microbial cells and thus increases their residence time in biomass.

Role of the *Roseobacter* Lineage for DMSP Degradation

Roseobacter strains have been among the first aerobic bacteria isolated that grow on DMSP (34, 35, 59). They are the only bacteria known to have both the degradation and the demethylation/dethiolation pathway, sometimes within the same organism (65, 70). They exhibit a positive chemotactic response toward DMSP degradation products (66). The genome sequence of *S. pomeroyi* revealed no fewer than five uptake systems for glycine betaine and/or DMSP (OpuA and OpuD) (69). Thus, *Roseobacter* organisms are optimally adapted to algal blooms (36), where DMSP is released locally in large, varying quantities.

Transformation of Inorganic Sulfur Compounds

Roseobacter strains have frequently been found to transform sulfite and thiosulfate to sulfate under aerobic conditions. *Sulfitobacter pontiacus*, from the oxic/anoxic interface of the Black Sea (97) has a sulfite reductase activity that is almost as high as that of autotrophic inorganic sulfur reducers (35). While none is known to grow lithoautotrophically, thiosulfate enhances the growth yield of many strains, sometimes by 45% (69), again confirming that these bacteria use all energy sources available to them without being specialized to a single one. Accordingly, *sox* genes have been found in the genome of *S. pomeroyi* (69) and also with a high prevalence (10%) in the Sargasso Sea metagenome library (10%) (69). In the marine environment, reduced sulfur compounds can occur at the interface between aerobic and anaerobic environments in stratified ecosystems (e.g., in the Black Sea) or in anaerobic microniches (e.g., biofilms on planktonic invertebrates or marine snow particles), or they can be the products of DMSP degradation.

MeSH: methanethiol

Metagenome: total genomic content of all organisms in an environmental sample, extracted as total DNA and cloned into various types of vectors

EUKARYOTIC HOSTS: MUTUALISM, SYMBIOSIS, AND PATHOGENESIS

Symbiotic relationships between bacteria and eukaryotic hosts are a fascinating aspect of natural products biochemistry. For example, the most promising drug from the sea, bryostatin, was isolated from the bryozoan *Bugula neritica* and is currently in phase II clinical trials for several cancer types. It is a polyketide that is produced by the as yet uncultured symbiont "Candidatus *Endobugula sertula*" (40, 73).

Roseobacter strains are closely associated with diverse marine eukaryotes, e.g., marine red and green macroalgae, marsh grass, diatoms, bryozoa, corals, oysters, squid, and dinoflagellates. They are the dominant microorganisms in the reproductive glands of certain cephalopods and the phycosphere of *Pfiesteria* dinoflagellates. In many of these associations the precise nature of the interaction has not been determined. In some cases, evidence has been found for a coevolution between host and microorganism (5).

Dinoflagellate Toxins

More than 4000 living and fossil species of dinoflagellates are known. Only about 25 of these produce polyketide toxins of large structural complexity (79). The largest nonpeptidic, nonpolymeric natural product described to date, maitotoxin, is produced by the dinoflagellate *Gymnodinium toxicus*, and the only natural products possessing five-, six-, seven-, eight-, and nine-membered rings in the same molecule are the polyethers brevetoxin A and ciguatoxin, produced by the dinoflagellates *Gymnodinium breve* and *G. toxicus*, respectively

Polyketide synthase (PKS): enzyme complex that adds acetate moieties to the growing carbon backbone of natural compounds of the polyketide type

(79). These toxins are highly potent sodium channel blockers (79).

Dinoflagellates are responsible for alga blooms known as red tides. Food chain accumulation of their toxins was identified as the reason for paralytic shellfish poisoning and fish kills (27). It was further suggested that the associated bacteria might be the true producers of the toxins rather than the algae (54, 90). Dinoflagellates live in close association with intracellular and extracellular bacteria, which can be found in all life stages of the alga in the nucleus, the cytoplasma, and on the cell surface.

Initially, Kodama et al. (54) reported that after curing cultures of *Protogonyaulax* (later renamed *Alexandrium*) *tamarense* from bacteria using antibiotics, intracellular bacteria could be obtained by breaking the cells, and these bacteria produced small amounts of saxitoxins (paralytic shellfish toxins). The bacteria, first named *Moraxella* sp., were later identified as strains belonging to the *Roseobacter* lineage (38, 57, 76). Silva (90) isolated an intracellular bacterium from *A. tamarense* that, when inoculated into nontoxic algal cultures of other dinoflagellates, induced toxin production, and concluded that the bacteria, which were not toxic in pure culture, caused the toxicity of the algae by interfering with algal metabolism. Gallacher et al. (28) initially described that 40% to 60% of the bacterial isolates from cultures of toxic *Alexandrium* spp. produced paralytic shellfish toxin (saxitoxin) at high concentrations. Many of these bacteria belonged to the *Roseobacter* lineage (44).

These initial findings could not be confirmed by other authors (22). Lu and coworkers (62) isolated both intra- and extracellular bacteria from the toxic dinoflagellate *Alexandrium minutum* T1 and found that none of them produced toxins, and the toxicity of the dinoflagellate culture was even higher in the absence of bacteria. Other groups also observed that the dinoflagellates produced the same or only slightly changed toxins if cultured axenically (104, 109). Gallacher's group later showed that two toxin-producing strains of dinoflagellates (*Alexandrium lusitanicum* and *A. tamarense*) exhibited the same toxin profile with and without bacteria (43). Martins et al. (63) demonstrated that earlier results regarding toxin-producing *Pseudomonas* strains were false positives caused by cellular and medium components in the raw extracts.

In 2005, the polyketide origin of most dinoflagellate toxins and their biochemical synthesis had been firmly established by feeding experiments (reviewed in Reference 68). The ability to produce toxin was shown to be inherited in a Mendelian way, indicating that it is located with the dinoflagellate rather than the associated bacteria. Moreover, the presence of type I polyketide synthases (PKS) has been demonstrated in toxin-producing dinoflagellates [the okadaic acid producers *Prorocentrum lima* and *P. hoffmanianum*; *Karenia brevis* (formerly *Gymnodinium breve*), a producer of brevetoxin; *Amphidinium operculatum*; and several others], whereas no type II PKS, characteristic of bacteria, were detected (93, 94). However, some doubts still remain. It could not be excluded that at least some of the identified PKS genes might originate from the accompanying bacteria, and the amplified and sequenced fragments were so small that their phylogenetic affiliation could not be established (93, 94). Studies on whole-genome gene expression profiling in *K. brevis* should finally clarify this question in the near future (60). For now, it must be summarized that no toxin-producing bacterial strain has been isolated from a dinoflagellate so far, and there is strong evidence that the associated bacteria do not produce toxins but may be involved in other interactions with the host.

Mutualistic Interactions between *Roseobacter* Strains and Marine Algae

Roseobacter organisms can represent a large percentage of the associated bacteria of planktonic algae and dinoflagellates in particular (36). For example, Alavi and coworkers (1)

showed that 50% of the clones obtained from *Pfiesteria* culture medium belonged to the *Roseobacter* lineage, and fluorescent in situ hybridization (FISH) and confocal laser scanning microscopy (CFLSM) studies showed bacteria in close association with the cell surface and within the *Pfiesteria* cells. Several types of interactions have been suggested.

1. Degradation of the algal osmolyte DMSP. *Roseobacter* strains associated with *Pfiesteria* cultures degrade DMSP, which reaches intracellular concentrations up to 500 μM and is released upon cell death or aging (65, 111). Moreover, chemotaxis of *Roseobacter* strains toward DMSP degradation products was demonstrated (65).
2. Epibiosis to obtain both light and nutrients, required for AAnP bacteria. The large number of AAnPs that could be isolated from the surface of toxin-producing *Alexandrium* sp. and *Prorocentrum* sp. (3, 10, 12) may indicate that the dinoflagellates served as ferries transporting bacteria to depths sufficiently illuminated for photosynthesis and also provided locally high concentrations of dissolved organic matter.
3. *Roseobacter* strains degrade or transform dinoflagellate toxins (92), thus confirming earlier findings that in the presence of bacteria less toxin is produced (62).
4. Production of algal-lytic compounds occurs in *Roseobacter* strains (4). In such a way, they might recruit large amounts of nutrients and affect the population dynamics of algal blooms.
5. Alavi et al. (2) have revealed a special relationship between a *Roseobacter* isolate living in association with the dinoflagellate *Pfiesteria piscicida* and its prey algae, *Rhodomonas*. For reasons not understood, the presence of the *Roseobacter* strain enhances the predation rate of the dinoflagellate on *Rhodomonas*, resulting in better growth.

Thus, *Roseobacter* strains might have an important role for controlling toxicity and duration of algal blooms by the mechanisms listed above.

Roseobacter Pathogens

Juvenile oyster disease (JOD) refers to a syndrome resulting in seasonal mortalities of hatchery-produced juvenile *Crassostrea virginica* raised in the northeastern United States (13). Mortalities can be up to 90% of total production in some years. Infected animals are heavily colonized by *Roseovarius crassostreae* (14). Interestingly, an α-Proteobacterium from the alpha-2 subgroup, *Stappia stellulata*, also colonizes *Crassostrea* larvae and seems to prevent infection by *R. crassostreae*, thus having a probiotic effect (13). The same or a similar strain of *Roseovarius* was also found associated with two diseases of scleractinean corals in the Caribbean, e.g., white plague (72) and black band disease (20).

Roseobacter Probiotics

Aquaculture is vulnerable to opportunistic pathogenic bacteria that may spread rapidly through the dense population, causing >99% mortality. Rearing units for larvae of the turbot, a marine flatfish (*Scophthalmus maximus*), were used as sources for indigenous bacterial antagonists that might have the potential to be used as probiotic strains (41, 42). After screening 8500 colonies for antibacterial activity against the fish pathogens *Vibrio anguillarum*, *V. splendidus*, and *Pseudoalteromonas* sp., 15 *Roseobacter* strains were found that were strongly inhibitory against all three pathogens and did not cause mortality in the turbot larvae (41). Strain 27-4 was 99.1% identical to *Roseobacter gallaeciensis* (81). Cell extracts of *R. gallaeciensis* had already been used earlier as a probiotic to increase the survival of scallop larvae (82).

Over the course of one year, the natural abundance and diversity of *Roseobacter* potential probiotics was then investigated in turbot rearing units by screening 19,000 colonies (from tank walls, tank water, and larvae) for

inhibition of the fish pathogen *V. anguillarum* (42). One hundred thirty-two antagonistic *Roseobacter* isolates were found, which fell into several phylogenetic clusters, including the previously isolated *R. gallaeciensis*–related isolate 27-4 (42).

AHL: acylated homoserine lactone

Autoinducer-2: furanosyl-borate-diester synthesized by the widely distributed *luxS* gene-encoded autoinducer-2 synthase and potentially acting as a universal bacterial signaling molecule

PRODUCTION OF ANTIBIOTICS AND QUORUM SENSING

Antibiotics

Two antibiotics have been isolated from *Roseobacter* strains: tryptantrin from *Oceanibulbus indolifex* (107) and thiotropocin from the *R. gallaeciensis*–related isolates T5 (15) and 27-4 (16). Interestingly, the production of thiotropocin was only found under static growth conditions, when the bacteria formed star-shaped aggregates (16). Many *Roseobacter* strains are good biofilm formers, and they were among the earliest colonizers of marine surfaces (21), where the production of an antibiotic might represent an important competitive advantage. Thiotropocin is a sulfur-containing antibiotic and may be derived from the metabolism of DSMP. A number of sulfur-containing indole derivatives and other secondary metabolites whose functions remain to be elucidated were also found in *Roseobacter* strains (107).

Quorum Sensing

The synthesis of thiotropocin was correlated with cell density, indicative of quorum-sensing regulation (15, 16) (see sidebar). Indeed, C10-OH-HSL was identified in culture supernatants of strain 27-4 (16), an acylated homoserine lactone (AHL) previously discovered in the plant pathogen *Pseudomonas fluorescens* and the human pathogen *Burkholderia pseudomallei*.

Production of AHLs has been detected in many members of the *Roseobacter* lineage (37, 108). Functional *LuxI* and *LuxR* homologs were found in the genome of *S. pomeroyi* (69). *Roseobacter* strains typically produce mixtures of AHLs with chain lengths from 8 to 18 carbon atoms and one or two unsaturations; some of these compounds are new, and some are similar to those of the *Rhizobiales*, an order of symbiotic soil bacteria also belonging to the α-proteobacteria (108). Regulation of gene expression in relation to cell density can be expected to be most important if the bacteria live in close proximity in confined spaces, e.g., as intracellular bacteria, within body cavities of the host, or in biofilms on surfaces of suspended particles, dinoflagellates, marine invertebrates, or macroalgae, all modes of life that are common for *Roseobacter* organisms. Traits known to be regulated by quorum sensing in other Proteobacteria are host-symbiont and host-pathogen interactions, the production of polysaccharides, gene transfer, formation of biofilms, and the production of antibiotics and toxins, in other words, most of the specific adaptations of the organisms to their respective habitat and lifestyle with the exception of primary energy-yielding metabolism. Moreover, AHLs may be involved in the regulation of culturability (17). The *Roseobacter* group is exploiting many diverse ecological niches and has a relatively large genome (69, 75), rather than being adapted exclusively to the oligotrophic ocean like SAR11, whose streamlined genome does not contain any signs of quorum-sensing-related signaling (32). Thus, complex regulatory mechanisms can be expected.

Cyclic dipeptides (diketopiperazines) were repeatedly isolated from *Roseobacter* isolates (67, 107). Their biological role is not yet clear. Although some have been reported to act as quorum-quenching compounds in high concentrations (45), most cyclic dipeptides seem to be remains of the culture medium (108). No *luxS* homologs have been found in the genomes of α-Proteobacteria (100, 105) until now, indicating that autoinducer-2-related signaling is absent or restricted to the "listening" component in these organisms. For more information on secondary metabolites isolated from *Roseobacter* strains please refer to Figure S1 (follow the Supplemental

Material link from the Annual Reviews home page at **http://www.annualreviews.org**).

GENOMES AND METAGENOMES

The genome sizes of *S. pomeroyi* and *Roseobacter litoralis* are 4.6 and 4.7 Mp, respectively, including extrachromosomal elements (69, 75). These sizes are typical of free-living bacteria with a versatile mode of life. At the moment, only the genome of *S. pomeroyi* has been fully sequenced (69), revealing a number of adaptations to the marine environment, including the CO oxidation pathway, the ability to reduce inorganic sulfur compounds, two AHL synthases, and the multitude of ABC transporter systems.

Extrachromosomal elements are common in *Roseobacter* organisms. Large circular megaplasmids, linear plasmids, and small circular plasmids have all been found in phototrophic *Roseobacter* strains (75). For example, *Dinoroseobacter shibae* harbors no fewer than seven linear plasmids, together comprising 860 kbp of genetic information (75). Plasmids, however, are not restricted to phototrophs: *S. pomeroyi* has a megaplasmid 591 kbp in length (69). Two large cryptic plasmids have been found in a *Ruegeria* strain (114). All linear plasmids known today are conjugative, thus horizontal gene transfer can be expected to play a large role in shuffling adaptive information between *Roseobacter* populations. Theoretically, some of the linear plasmids could also represent lysogenic phages at a certain point in their life cycle, thus opening up additional routes for gene transfer and population control.

Cultivation-independent information beyond the 16S rRNA sequence is accumulating at a fast rate. Large insert BAC and fosmid libraries allow researchers to clone genome fragments up to 200 kb, theoretically representing 5% to 10% of a small bacterial genome (23). These libraries have been screened for biochemical traits coupled to 16S rRNA phylogenetic tags and led to the discovery of proteorhodopsin phototrophy in the sea (7, 8, 84). Shotgun libraries of total community DNA are also beginning to be available, starting with the Sargasso Sea metagenome library (106), which represents an inventory of 1.2 million pooled microbial genes without the bias of PCR amplification (31). It is expected that similar inventories will become available from practically all marine habitats in the upper zone of the ocean in the near future. The Sargasso Sea, an extreme and oligotrophic environment, has always been regarded as a habitat with a low diversity of microbes. However, from the shotgun library 1500 different species were estimated to be present (106), which made it impossible to reliably assemble reasonable amounts of genomic DNA (23). Thus, genome sequences of ecologically

QUORUM SENSING

Many bacteria constantly secrete small, self-produced signaling compounds (autoinducers) into the medium. These are sensed by neighboring cells and, once a threshold concentration (the quorum) has been reached, induce or repress transcription through specific regulatory mechanisms. Thus, expression of adaptive phenotypes is coordinated in the whole population in a density-dependent manner. This type of cell-cell communication, also termed quorum sensing, was discovered in *Vibrio fischeri*, in which it regulates the transition between the free-living low-density lifestyle and the high-density symbiotic mode of life. Quorum sensing in Proteobacteria is mediated through AHL autoinducers, which are produced by a *luxI*-type synthetase and regulate transcription through binding a *luxR*-type regulatory protein. The length and modification of the acyl side chain determine the species specificity of the signaling mechanism. Most work on quorum sensing has focused on human pathogens, e.g., *Pseudomonas aeruginosa*, and little is known about its role in environmentally important bacteria. AHLs discovered in the *Roseobacter* lineage have side chains from 8 to 18 carbon atoms in length, the longest detected to date. They regulate biofilm formation and the production of antibiotics and thus may play an important role for the transition between the planktonic and the biofilm modes of life.

abundant marine bacteria are urgently needed as a reference (**http://www.moore.org/**).

SUMMARY AND OUTLOOK

Marine microbiology, always at the forefront of microbial ecology, is advancing at a breathtaking rate through sequencing of whole genomes of ecologically important cultivated strains, metagenome analyses, and the ecological, physiological, and genetic experiments that are based on these data. The *Roseobacter* group is abundant in diverse marine habitats and involved in global biogeochemical cycles (e.g., sulfur and carbon cycle) and interactions with marine plants and animals, with important implications for marine ecology and the global climate. The *Roseobacter* lineage is unique because cultivated and not-yet-cultivated organisms are closely related and a mosaic of physiological traits is present at the subspecies level. Over the next two years, about 40 full genome sequences will become available for the *Roseobacter* group, more than for any other lineage of microbes of environmental importance. Many of the sequenced strains are representative of ecologically important *Roseobacter* lineages and will be subjected to the powerful methods of functional genomics investigations. On this basis it should be possible to elucidate the specific adaptations of *Roseobacter* organisms to their respective ecological niches in the ocean and understand the mechanisms controlling culturability in this important marine lineage.

SUMMARY POINTS

1. The *Roseobacter* lineage is a phylogenetically coherent group of marine α-Proteobacteria. About 1500 16S rRNA gene sequences are available in public databases, which form 41 subclusters with sequence similarities of ≥99%. Cultivated representatives of the group are closely related to not-yet-cultivated clones.
2. The *Roseobacter* lineage is one of the most abundant marine groups. Its abundance in seawater is highest in coastal zones and polar oceans (up to 25% of the total microbial community). Representatives have been found in all major marine habitats investigated. They are often associated with algal blooms, form biofilms on marine surfaces, and are known as symbionts and pathogens of marine invertebrates.
3. Aerobic anoxygenic photosynthesis (AAnP), a way of generating additional metabolic energy through the transformation of light energy into ATP, is present in many members of the group and is thought to play a large role for the marine carbon cycle. It may represent an adaptation to diurnal light regimes in an oligotrophic environment.
4. CO is an important greenhouse gas which is present in the ocean at low concentrations. It is oxidized by *Roseobacter* strains as a way of gaining additional energy, thus reducing the emission of CO to the atmosphere.
5. The degradation of the algal osmolyte DMSP through both the cleavage and the demethylation/dethiolation pathways is a prominent feature of the *Roseobacter* group and one of the reasons for their close association with algal blooms, affecting the global production of the climate-relevant gas DMS. The *Roseobacter* group is also involved in numerous other sulfur transformation reactions, including oxidation of inorganic sulfur compounds.
6. Antibiotics and other bioactive secondary metabolites are produced by some *Roseobacter* strains; they may be involved in the probiotic and pathogenic effects observed.

7. Members of the *Roseobacter* lineage have a complex genome structure. Megaplasmids and linear plasmids, which facilitate horizontal gene transfer and the adaptation of the organism to its ecological niche, are often found.
8. Mixtures of long-chain AHLs are produced by many species of the *Roseobacter* lineage, indicative of intra- and possibly interspecies cell-cell communication; they may regulate the production of antibiotics and the biofilm mode of growth.

FUTURE ISSUES

1. At present, many different reasons for the observed unculturability of the majority of environmental bacteria are being discussed, from the "viable but not culturable state" induced by starvation, to the fact that types and concentrations of carbon, sulfur, and nitrogen sources and the incubation conditions for bacteria in the laboratory (either high or no oxygen and light, vigorous shaking, high temperature) are artificial. The analysis of cultivated strains within most of the subclusters of the *Roseobacter* lineage should allow researchers to develop concepts for the cultivation of the 60% not-yet-cultivated diversity within this lineage.
2. *Roseobacter* strains combine several ways of generating energy, e.g., AAnP, sulfide, and CO oxidation, degradation of DMSP. Based on fully sequenced genomes, analyses of the cell proteome and transcriptome at ecologically relevant conditions will reveal metabolic networks of connected pathways and the role of quorum-sensing control of gene expression. Thus, the mechanisms controlling the contribution of the *Roseobacter* lineage to the global carbon and sulfur cycle can be analyzed.
3. The upcoming large number of fully sequenced genomes in the *Roseobacter* lineage will allow researchers to study the adaptive radiation and evolution of this group far beyond the 16S rRNA gene-based phylogeny. Long-term evolution should be reflected in the phylogeny of conserved core genes of the α-Proteobacteria, and short-term adaptive radiation into ecotypes might be based on adaptive islands shuffled between its members through horizontal gene transfer.

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Contents