R E P O R T S

28. Run-off transcription procedures were as described (19). The znu transcript template was prepared in the same manner as the znta template in (19) and digested with Bgl II and Bbv I to make a 233-bp fragment. Different size templates were tested for Znt transcription assays and a transcript was always observed for znuCB but not znuA. Both promoters are active in vivo (16, 17). The znuCB promoter may be favored in vivo transcription because it is stronger than the znuA promoter.


39. F. W. Otten, unpublished data.

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Contribution of Aerobic Phototrophic Bacteria to the Carbon Cycle in the Ocean

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The vertical distribution of bacteriochlorophyll a, the numbers of infrared fluorescent cells, and the variable fluorescence signal at 880 nanometers wavelength, all indicate that photosynthetically competent anoxygenic phototrophic bacteria are abundant in the upper open ocean and comprise at least 11% of the total microbial community. These organisms are facultative phototrophs, metabolizing organic carbon when available, but are capable of photosynthetic light utilization when organic carbon is scarce. They are globally distributed in the euphotic zone and represent a hitherto unrecognized component of the microbial community whose presence appears to be critical to the cycling of both organic and inorganic carbon in the ocean.

Although closely related to purple photosynthetic bacteria, aerobic anoxygenic phototrophs (AAPs) are obligate aerobes, with unusually high concentrations of carotenoids (1–3), low cellular contents of bacteriochlorophyll a (BChl a) (4, 5), and while containing photosynthetic reaction centers (RC) and light harvesting complex I (LHI), they often lack LHII (6). Photosynthetic energy conversion has been confirmed in several species (5–8), but most known AAPs have been isolated from organic-rich environments (9–11).

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Although closely related to purple photosynthetic bacteria, aerobic anoxygenic phototrophs (AAPs) are obligate aerobes, with unusually high concentrations of carotenoids (1–3), low cellular contents of bacteriochlorophyll a (BChl a) (4, 5), and while containing photosynthetic reaction centers (RC) and light harvesting complex I (LHI), they often lack LHII (6). Photosynthetic energy conversion has been confirmed in several species (5–8), but most known AAPs have been isolated from organic-rich environments (9–11).
(Fig. 2B), indicate that the oceanic AAPs utilize carotenoids as an efficient, auxiliary photosynthetic pigment. These carotenoids harvest light between 460 and 550 nm (Fig. 3B), which penetrates relatively deeply into the water column, whereas infrared light in the 800- to 900-nm range is about 1000-fold attenuated owing to absorption by water (22).

The efficiency of excitation transfer from carotenoids to RC (defined as the ratio of quanta transferred to the RC to the quanta absorbed) measured on our isolates of AAPs (Fig. 2B) was relatively low, increasing from approximately 5 to 20% as the organic content in the growth medium decreased. From the IRFRR data acquired in natural AAP populations, we estimated the maximal cellular photosynthetic fluxes at about $1 \times 10^5$ J/mol of carbon in the euphotic zone. Assuming a specific growth rate of one cell per day, and a respiratory efficiency of $5 \times 10^5$ J/mol carbon, about 20% of the cellular energy requirement would be satisfied by photosynthetic electron transport.

Oceanic AAPs are capable of light-dependent CO$_2$ fixation (23) (Fig. 2C). Under saturating light, the rates of light-dependent CO$_2$ fixation in laboratory-grown isolates are relatively low, averaging at about 0.43 mol carbon/mol RC/s, with a maximum quantum yield of about 1% (mol carbon/mol quanta absorbed). These yields are about an order of magnitude lower than in phytoplankton, and comparable to that reported previously in *Erythrobacter* sp. (24). We estimate the daily cellular rates of CO$_2$ fixation at about 0.08 fmol of carbon, or 3% of the cellular carbon content. As the cells were grown in 2 to 20 mM organic medium, maintaining specific growth rates of four per day, CO$_2$ fixation contributed to about 1% of the total carbon anabolism. In the open ocean, where dissolved organic matter (DOM) is three orders of magnitude less abundant, the relative contribution of the CO$_2$ fixation may be significantly higher. Nevertheless, the potential contribution of AAPs to the oceanic carbon cycle is determined by their ability to supplement, or substitute respiration with the light-driven generation of ATP and reductants for carbon anabolism, preserving the existing organic carbon. Isolates of oceanic AAPs grown in 20 mM organic medium displayed high irradiance levels, and average $1 \times 10^5$ J/mol of carbon in the ephotic zone. Assuming a specific growth rate of one cell per day, and a respiratory efficiency of $5 \times 10^5$ J/mol carbon, about 20% of the cellular energy requirement would be satisfied by photosynthetic electron transport.

Fig. 2. (A) HPLC chromatogram of fluorescent pigments from a surface sample (2 m depth) collected at station C354-004. Excitation was at 365 nm, emission at 780 nm, with 20-nm slits. These wavelengths were chosen to maximize the signal from BCHla, while minimizing the signal from the more abundant pigments, Chla and Chlb. (Inset) Fluorescence emission spectrum of the peak eluting at 16.7 min in (A). Excitation was at 365 nm and slits were 20 nm. Virtually identical retention times and fluorescence emission spectra were recorded for BCHla derived from *R. sphaeroides*, and isolate NAP1 obtained from the coastal North Atlantic Ocean. Lettuce extract served as standards for Chla and Chlb. (B) Fluorescence excitation spectra recorded at 875 nm (thick gray line) and absorption spectra (black line), both measured on whole cells of NPP1 isolate from Northeastern Pacific Ocean. Quantum efficiency of excitation transfer from carotenoids to the reaction center (broken line) was about 20%. (C) P versus I curves of carbon fixation (23) and IRFRR based estimates of the photosynthetic electron transport rates (12) in NAP1 isolate obtained from the coastal North Atlantic Ocean. The continuous line represents a numerical fit of carbon fixation data to a hyperbolic model, $y = a_0 + a_1 x/(a_2 + x)$, where $y$ is the CO$_2$ fixed, and $x$ is the irradiance.
Numerically, AAPs constitute approximately 11.3 ± 1.7% of the total microbial community in the euphotic zone in the northwestern Pacific Ocean at 48°N, 128°W (Figs. 1C and 3C). The BChl/Chla ratio at this location, about 0.8%, increased to as much as 10% in the oligotrophic waters of the Eastern Pacific Ocean at 14°N, 104°W (J2). Such a dramatic change in the BChl/Chla ratio almost certainly reflects an increase in the relative abundance of AAPs within the microbial community. Thus, the calculated 11.3% of the total cell count at 14°N, 104°W may be greatly exceeded in the oligotrophic ocean, and the globally averaged BChl/Chla ratio may be as high as 5 to 10%.

We isolated 10 AAP strains from the Coastal North Atlantic Ocean, Northeastern Pacific Ocean, Equatorial Pacific Ocean, Southern Ocean, and Mediterranean Sea (25). After 8 to 10 days incubation on organic-poor agar plates in the dark, they all formed small pink colonies, on replating with the same medium frequently segregated into purple and yellow isolates.

When grown in an organic-poor, autotrophic liquid medium, all the isolates displayed IRFRR fluorescence transients characteristic of photosynthetic electron transport, similar to that measured in the natural water samples. When transferred to an organic-rich medium, the purple isolates displayed a 40% decline in the amplitude of the IRFRR fluorescence transient within 24 hours, whereas the yellow isolates lost this signal completely, showing no accumulation of BChla and RC despite sufficient concentration, but are also capable of controlling the expression of their photosynthetic apparatus; i.e., they are facultative phototrophs, switching to a mostly heterotrophic metabolism under organic-rich conditions, where photosynthesis presumably offers less of an advantage. We observed this behavior within a dense particle layer in the upper portion of the euphotic zone, where we detected a strong fluorescence signal at 880 nm, but with unusually low amplitude of the variable fluorescence.

Phylogenetic analysis of the 16S ribosomal RNA gene (16S rDNA) (26) from a North Atlantic isolate (NAP1) placed it in the *Erythrobacter-Citromicrobium-Porphyrobacter* cluster within the α-4 subclass of the Proteobacteria (3), which forms a relatively isolated group with respect to other AAPs (27). The 16S rDNA sequence of NAP1 showed high sequence similarity with those of *Erythrobacter longus* and *Erythrobacter litoralis* (98.2 and 97.9%, respectively), suggesting that this isolate belongs to the *Erythrobacter* genus. A preliminary characterization of a purple isolate from the Northeastern Pacific Ocean (NPP1), based on both the restriction fragment length polymorphism (RFLP) and sequence of the 16S rDNA, revealed that this isolate is closely related to NAP1.

Our data indicate that, like cyanobacteria, AAPs are ubiquitous in the euphotic zone of the open ocean. We have observed IRFRR fluorescence transients characteristic of bacterial photosynthesis in every ocean surface water sample analyzed to date. All of these water samples yielded isolates displaying similar fluorescence characteristics. The isolates from the Northeastern Pacific and coastal North Atlantic Oceans, (the most extensively characterized) are morphologically, biophysically and phylogenetically similar. Oceanic AAPs occupy a uniform environment characterized by relatively low concentrations of DOM, and are exposed to high irradiance levels. They are photosynthetically competent in situ and utilize carotenoids as a major LH pigment. All are readily cultivated on organic-poor media. These common features suggest that oceanic AAPs are probably represented by a relatively uniform, widespread clade.

We speculate that the phylogenetically related, yet phenotypically diverse aerobic anoxygenic photosynthetic bacteria, discovered in a variety of ecological niches over the last 20 years, may have spicated from a common ancestral oceanic AAP. Possible evolutionary adaptations may have ranged from a permanent loss of photosynthetic activity to the development of a regulatory mechanism that controls the level of expression of the photosynthetic apparatus in response to nutrient concentrations.

The close correlation between AAPs and oceanic phototrophs in the euphotic zone indicates that they coexist in a tightly linked nutrient cycle. Because AAPs are unable to utilize water as an electron donor, they most likely rely on exudants produced by oceanic photoautotrophs to supply reductants. AAPs will use the available DOM if present at sufficient concentration, but are also capable of photosynthetic CO2 fixation under DOM-deficient conditions. Their photosynthetic efficiency and spectral light utilization is similar to that of the oceanic phototrophs, further explaining their co-occurrence with phototplankton in the water column. These facultative photoheterotrophs coexist with oxygenic photoautotrophs, contributing to a light-controlled component of microbial carbon and redox cycle, the details of which are presently unknown.

References and Notes
14. We occupied five stations over a 14-day period in the Northeast Pacific off the Washington and Oregon coasts in July 2000 onboard the RV Atlantis.
15. Water samples (1 liter) were filtered through GF/F filters. Filters were extracted briefly in 100% acetone, then water was added to yield 1 ml of 80% acetone. Pigments were extracted for 6 to 8 hours at ~20°C and debris removed by centrifugation at 14,000g for 10 min. 100 μl of extract was injected into an Agilent Technologies HPLC Model 1100 equipped with a Microsorb-MV (Rainin Instruments) C18 column (4.6 × 250 mm). The initial mobile phase was 75% acetone for 3 min and then 100% acetone over 15 min. The fluorescence detector was equipped with a Hamamatsu R928 photomultiplier tube, which proved to be crucial for detection of BChla. Excitation was set at 365 nm, emission: 780 nm, and slits were 20 nm. These wavelengths maximized signals from BChla and minimized those from the more abundant pigments, Chla and Chlb. Acetone extracts from lettuce and R. sp. served as standards. An extinction coefficient ε770 = 60 was used for BChla [J. Oelze, Methods Microbiol. 18, 257 (1985)].
16. Water samples of 10 ml were stained with 0.1 mg/ml 4′,6-diamidino-2-phenylindole (DAPI) [K. G. Porter, Y. S. Feigl, Limnol. Oceanogr. 25, 943 (1980)] and cells were collected by filtration through 0.2 μm pore size polycarbonate membranes. DAPI-stained particles in five fields (field size 1.4 × 104 μm2) were enumerated using epifluorescence with the following filter set: excitation 330 to 390 nm, emission 440 to 490 nm, beam splitter 400 to 430 nm. infrared fluorescent cells, diagnostic of the presence of Bchl a, were counted from five fields (field size 3.3 × 104 μm2) using the following filter set: excitation 350 to 550 nm, emission >550 nm, beam splitter 550 nm, and viewed with an IR-sensitive CCD camera. The five values obtained for each were averaged and multiplied by appropriate factors to yield cell concentrations in the original samples.
21. Cells were grown in liquid autotrophic medium enriched with 0.2 g of yeast extract and 0.1 g of peptone per liter, to a concentration of about 106.
Nitrogen Fixation by Symbiotic and Free-Living Spirochetes

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Spirochetes from termite hindguts and freshwater sediments possessed homologs of a nitrogenase gene (nifH) and exhibited nitrogenase activity, a previously unrecognized metabolic capability in spirochetes. Fixation of 15-dinitrogen was demonstrated with termite gut Treponema ZAS-9 and free-living Spirochaeta aurantia. Homologs of nifH were also present in human oral and bovine ruminal treponemes. Results implicate spirochetes in the nitrogen nutrition of termites, whose food is typically low in nitrogen, and in global nitrogen cycling. These results also proffer spirochetes as a likely origin of certain nifHs observed in termite guts and other environments that were not previously attributable to known microbes.

Termites are important terrestrial decomposers of Earth’s major form of biomass: lignocellulosic plant material and residues derived from it, e.g., humus (1). However, the carbon-rich but typically nitrogen-poor character of the termite diet has led many species into symbiotic interactions with gut microbes to augment their nitrogen economy. These interactions include the recycling of excretory (uric acid) nitrogen and the acquisition of new nitrogen through N2 fixation (2). In wood-feeding termites, whose food may contain as little as 0.05% nitrogen (dry weight basis), N2 fixation can supply up to 60% of the nitrogen in termite biomass (3). Unfortunately, our understanding of N2-fixing microbes in termites is meager: only a few strains have been isolated (4). Recently, the first pure cultures of these forms were obtained (9). Isolated strains ZAS-1, ZAS-2, and ZAS-9 were also phylogenetically affiliated with the treponemes (Fig. 1), and all three strains produced acetate as a major fermentation product (10). ZAS-1 and ZAS-2 could make acetate from H2 + CO2, a mode of energy-yielding metabolism previously unknown in the phylum Spirochaetes (11). Hence, they are important to the nutrition of termites, which use microbially produced acetate as a major carbon and energy source (2). Having these spirochetes in culture prompted us to examine whether they might also fix N2 and thereby contribute to termite nitrogen economy as well. To do this, we examined their genomic DNA for the presence of nifH (12) and their ability to fix N2 (13). Two nifH homologs were found in each termite gut treponeme. nifH homologs were also found in the bovine ruminal treponeme, Treponema bryanti, the human oral treponemes, Treponema denticola and Treponema pectinovorum; and the free-living spirochetes, Spirochaeta aurantia, Spirochaeta zuelzerae, and Spirochaeta stenooestra. The deduced amino acid sequence of each NifH had motifs typically present in the nitrogenase iron-protein, including conserved cysteine positions at (Klebsiella pneumonia) numbering 86, 98, and 133 (19, for nifH clones obtained with the IGK forward primer for polymerase chain reaction (PCR) and an arginine at position 101, which is a site for reversible inactivation by adenosine diphosphate–ribosylation in some bacteria (14)). However, the NifHs were phylogenetically diverse and not congruent with spirochete phylogeny based on 16S rRNA sequences, which groups all spirochetes in a single phylum. This lack of congruence extended to multiple NifH homologs in the same spirochete (Fig. 2).