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Correspondence and requests for materials should be addressed to H.P.J. (e-mail: johnson@ocean.washington.edu).

Bacterial photosynthesis in surface waters of the open ocean

Z. S. Kolber*, C. L. Van Dover†, R. A. Niederman‡ & P. G. Falkowski*

* *Environmental Biophysics and Molecular Ecology Program, Institute of Marine and Coastal Sciences, Rutgers University, New Brunswick, New Jersey 08901-8521, USA*
† *Biology Department, College of William & Mary, Williamsburg, Virginia 23187, USA*
‡ *Department of Molecular Biology and Biochemistry, Rutgers University, Piscataway, New Jersey 08854-8082, USA*

The oxidation of the global ocean by cyanobacterial oxygenic photosynthesis, about 2,100 Myr ago[1], is presumed to have limited anoxygenic bacterial photosynthesis to oceanic regions that are both anoxic and illuminated[2,3]. The discovery of oxygen-requiring photosynthetic bacteria about 20 years ago[4] changed this notion, indicating that anoxygenic bacterial photosynthesis could persist under oxidizing conditions. However, the distribution of aerobic photosynthetic bacteria in the world oceans, their photosynthetic competence and their relationship to oxygenic photoautotrophs on global scales are unknown. Here we report the first biophysical evidence demonstrating that aerobic bacterial photosynthesis is widespread in tropical surface waters of the eastern Pacific Ocean and in temperate coastal waters of the northwestern Atlantic. Our results indicate that these organisms account for 2–5% of the photosynthetic electron transport in the upper ocean.

Aerobic phototrophic bacteria[4–7] are widely distributed in the α-subclass of Proteobacteria[8–11], indicating that they have evolved from several independent lines of purple photosynthetic bacteria. Nevertheless, these organisms are obligate aerobes, requiring molecular oxygen for growth and bacteriochlorophyll (bChl) *a* synthesis. Most of the known species are characterized by a wide variety and 'overabundance' of carotenoids[7,11]. In most species, these pigments do not appear to transfer excitation energy to reaction centres[12]. Furthermore, a relatively low cellular abundance of bChl *a* (refs 7, 11) indicates that the contribution of light to cellular energy demands is also low. Some species are potentially capable of anoxygenic photosynthesis, indicated by light-driven oxidation of the bacterial reaction centre P_{870} and cytochromes[13,14], or light-stimulated uptake of CO_2 (ref. 15). However, phototrophy has not been confirmed in most strains, and the potential advantage of phototrophy to non-photosynthetic bacteria in natural habitats has not been unequivocally demonstrated. Their lack of photoautotrophy and requirement for O_2 has led to the proposal that aerobic phototrophs represent an evolutionary link between purple photosynthetic bacteria and aerobic heterotrophs[8], or that they attained a stable, non-photoautotrophic phase while retaining redundant, non-functional photosynthetic machinery. It is plausible, however, that some of these organisms may have adapted their photosynthetic apparatus to aerobic conditions and maintain a competent photosynthetic electron transport with efficient photoheterotrophic metabolism.

Our search for photosynthetic aerobic phototrophs in the open ocean was stimulated by the report of the occurrence of such organisms in a black-smoker plume above a deep-sea vent area on Juan de Fuca Ridge[16]. It was proposed that light emanating from hydrothermal vents could potentially provide a habitat for photosynthetic life[17,18]. Using a newly developed infrared fast repetition rate (IRFRR) fluorometer, we searched for variable fluorescence transients as evidence of bacterial photosynthetic electron transport. We used water samples recovered from the hydrothermal vent area at 9° N with the deep-sea submersible, *Alvin*. We also surveyed a 1,000-km transect of the Pacific Ocean, and measured bacterial photosynthetic signals from waters off the coast of New Jersey.

Within the detection limits of our instrument (about 10 pg bChl per l), we failed to detect photosynthetic electron transport in black-smoker plumes and chimneys. However, we consistently observed fluorescence kinetic transients at 880–905 nm in the surface waters, indicative of bacterial photosynthetic electron transport. Representative kinetic profiles of bacterial (880 nm) and phytoplanktonic (Chl-*a*-containing; 685 nm) origin are shown in Fig 1. Both kinetic transients were excited by radiation at 470 nm. At this wavelength, bacterial reaction centres display a small effective absorption cross-section (about 60 $Å^2$ compared with 250 $Å^2$ for the Chl-*a*-containing phytoplankton), a high quantum yield of primary charge separation (0.55–0.60) estimated from the change in variable fluorescence[19], a slow rate of overall photosynthetic electron transport and complete insensitivity to photo-inhibition. Assuming that the biomass of these organisms is, to first order, proportional to the amplitude of the measured fluorescence signal, we estimate a relative abundance of phototrophic bacteria about 1% of phytoplankton at 9° N.

Filtering surface seawater through a Whatman GF/F filter (approximate pore size of 0.8 μm) removed all of the 685-nm

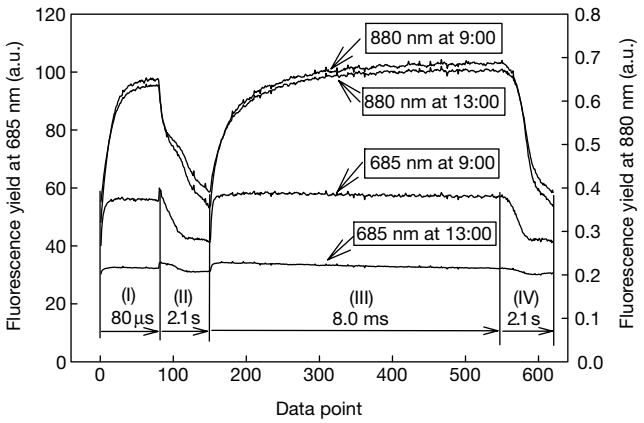

Figure 1 Typical fluorescence kinetic transients at 685 nm (planktonic photosynthesis) and 880 nm (bacterial photosynthesis). The fluorescence transients in Phase I are induced by 80 flashes of 0.4-μs duration at 1-μs intervals, resulting in reduction of the primary electron acceptor, Q_A within 20 (phytoplankton) to 60 (photosynthetic bacteria) μs. In Phase II, 70 flashes are applied at time intervals varied exponentially from 20 μs to 50 ms, resulting in a relaxation of the fluorescence signal with kinetics corresponding to the rate of electron transfer between Q_A^- and the quinone pool. In Phase III, 400 flashes are applied at 20–50-μs intervals causing reduction of the quinone pool. Phase IV is performed with the same protocol as in Phase II to measure the kinetics of electron transport from the quinone pool to the cytochrome *bc1* complex. The 880-nm transients displayed here are similar to those measured from laboratory cultures of *Rhodobacter sphaeroides* and *Rhodospirillum rubrum* (not shown).

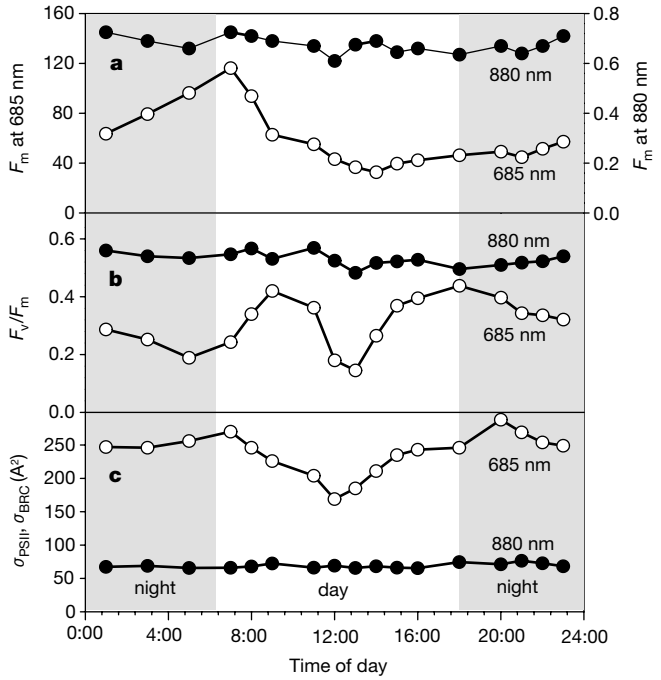

Figure 2 Diel cycle in bacterial photosynthesis (filled symbols) and planktonic photosynthesis (open symbols). **a**, Daily changes in the fluorescence yield indicating strong non-photochemical quenching in planktonic photosynthesis around local noon. **b**, Variations in F_v/F_m displaying a midday photoinhibition and midnight cell division in planktonic photosynthesis. **c**, Changes in the effective absorption cross-section. The diel cycle is absent in bacterial photosynthesis.

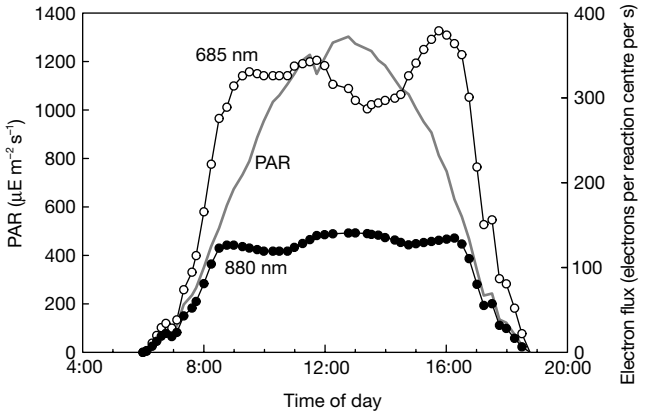

Figure 3 Reaction-centre-normalized photosynthetic electron fluxes in phytoplankton (open symbols) and aerobic phototrophs (filled symbols) calculated from Equation 1 under clear sky, and photosynthetically available radiation (PAR) conditions (continuous line). The saturation level of planktonic photosynthesis is about three times higher than in photosynthetic bacteria owing to faster electron turnover time. Also, the photosynthetic rates under subsaturating irradiance are higher in the planktonic photosynthesis owing to a much bigger effective absorption cross-section. The high saturation levels in planktonic photosynthesis are realized in the middle of the day despite strong photoinhibition (see Fig. 2a).

signal, but retained about 20% of the original 880-nm fluorescence intensity. Filtration through a 0.22-μm Nucleopore filter removed both signals. These results indicate that the majority of the phototrophic bacteria were about 0.2–0.8 μm. In samples taken off the coast of New Jersey in December 1999, only the bacterial (880-nm) fluorescence transients were observed in water passing through a 1-μm Nucleopore filter. When cultured on agar plates enriched with minimal F/2 media, the 1-μm filtrate produced individual colonies which, on transfer to liquid media, produced exclusively bacterial fluorescence transients. In all of these samples, we observed efficient excitation energy transfer from the carotenoids to bChl. The effective absorption cross-sections at 730 and 795 nm were, respectively, 15 and 3% of that at 470 nm. Our observations indicate a high concentration of carotenoid pigments, well coupled with the reaction centre antenna, but probably a paucity of either light-harvesting complexes I or II. Such a pigment organization is consistent with high light environments in the upper ocean.

Phytoplankton in the surface waters undergo a strong diel cycle in variable fluorescence. The diel pattern is manifested in variations in photochemical quantum efficiency (the F_v/F_m ratio), the maximum fluorescence yield (F_m) and the effective absorption cross-section of photosystem (PS) II (σ_{PSII}; Fig. 2). The midday minimum in the cycle of F_v/F_m is indicative of photoinhibition or 'downregulation' of PSII photochemistry owing to overexcitation by light, leading to the formation of singlet oxygen species and damage of PSII reaction centres[20]. The night-time minimum correlates with cell division in phytoplankton[21] or respiratory reduction of the plastoquinone pool[22]. In contrast, the F_v/F_m ratio from phototrophic bacteria remained consistently high over the diel cycle, implying a lack of photoinhibition and no diel coherence in cell division (at least as reflected by photochemistry).

Using the measured photosynthetic parameters, we calculated the photosynthetically driven electron fluxes, P_e^{RC}, in both the phytoplankton and aerobic phototrophs under ambient irradiance, E, as follows[23]:

$$P_e^{RC} = E\,\sigma_{PS}\,F_v/F_m \quad \text{for} \quad E < E_s \tag{1}$$

$$P_e^{RC} = E_s\,\sigma_{PS}\,F_v/F_m \quad \text{for} \quad E \geqslant E_s \tag{2}$$

where P_e^{RC} is the reaction-centre-normalized electron flux, E_s is the saturation irradiance, σ_{PS} is the effective absorption cross-section of the respective photosystem, and F_v/F_m represents the quantum yield of the primary charge separation. The results presented in Fig. 3 indicate that the photosynthetic electron fluxes in both phytoplankton and aerobic phototrophs at the surface are saturated for most of the day under clear skies, but the saturation level of bacterial electron flux is about three times lower than that of phytoplankton. The difference is partially due to the slower photosynthetic electron transport rate in aerobic phototrophs (about 11 ms per electron compared with 3 ms in phytoplankton). At subsaturating irradiances, bacterial photosynthesis is also lower as a result of the smaller effective absorption cross-section. Interestingly, the ratio of cross-section to turnover rate in phytoplankton and bacteria is highly conserved, resulting in balanced light use and electron transport in both of these organisms[24].

Although bacterial photosynthesis has a consistently high yield of charge separation (Fig. 2b), the overall photosynthetic efficiency of the bacterial reaction centre is about three times lower than that of the PSII/PSI reaction centre over the entire range of natural solar irradiance. However, the lower photosynthetic efficiency does not explain the much lower abundance of these organisms. We do not know the primary electron donor for these organisms, however, dissolved organic matter (DOM) is a likely source of reductant. If so, the disproportionate abundance of the phytoplankton compared with the phototrophic bacteria in the upper ocean could be due to the availability of their electron source (water), as compared with a relatively dilute pool of DOM, and the inability of these bacteria to grow photoautotrophically.

To explore the spatial relationship between bacterial and photoplankton distribution, we measured their fluorescence signatures along a 1,000-km transect from 9.4° N to 19° N (Fig. 4a). The distribution of these two phototrophs showed a correlation over the first 200 km; however, the relative abundance of the aerobic phototrophs increased from 1% to 10% within a small distance. Chl *a* distributions, obtained from ocean colour analyses of SeaWiFS satellite images, correspond with our fluorescence-based estimates

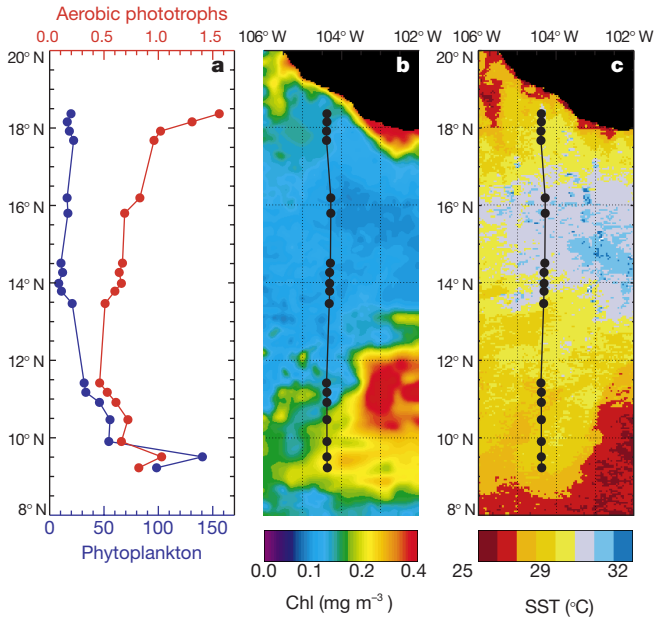

Figure 4 Spatial distribution of photosynthetic bacteria and oxygenic phytoplankton in the Pacific Ocean along 104° W in November 1999. **a**, The relative concentrations of phytoplankton (blue symbols) and aerobic phototrophs (red symbols) based on fluorescence emission signals at 685 nm and 880 nm, respectively. **b**, Spatial distribution of SeaWiFS-retrieved chlorophyll from the first week of December 1999 along the cruise track. **c**, The corresponding SST image. A relatively tight correlation between the phytoplankton and aerobic phototrophs (at about 100:1 ratio) is maintained in the high-Chl region, but the relative abundance of the aerobic phototrophs increases by a factor of ten in the low-Chl region.

of the phytoplankton concentration (Fig. 4b). The sea surface temperature (SST) data from the same period (Fig. 4c) indicate a close relationship between the physical structure of the water masses and the biological signals. Measured from natural cell populations, these signals characterize photosynthetic properties of phytoplankon/bacterial communities, each potentially composed of several different species.

Although observed in many different geographical areas, the occurrence of marine aerobic phototrophs has been assumed to be limited to small, organic-rich ecological niches such as beach sands[4], tropical high tidal zones[25], mature cyanobacterial mats[9], surfaces of green seaweeds (but not the seawater)[4] and deep-sea hydrothermal vents (but not the surface waters directly above)[26]. Our results indicate that phototrophic bacteria are not limited to nutrient-rich, sheltered environments, but are distributed in surface waters throughout the oceans of the world. Their abundance, relative to oxygenic phototrophs, appears to increase in oligotrophic waters, possibly reflecting the competitive advantage over non-phototrophic bacteria whose abundance is controlled solely by the concentration and quality of DOM.

The bacterial photosynthetic energy fluxes have important implications for global carbon cycling. Even though the described aerobic phototrophs may not fix inorganic carbon, the generation of ATP by photosynthesis should reduce their respiratory energy requirements in assimilating the DOM into bacterial biomass[27,28]. This will both reduce the amount of carbon dioxide released by heterotrophic respiratory metabolism, and increase the amount of DOM assimilated into cell material. □

Methods

Measurements of photosynthetic parameters

Photosynthetic parameters of phytoplankton and photosynthetic bacteria were measured using IRFRR fluorometry. This method is based on FRR[19] and modified to use excitation light at 470 nm (30 nm bandwidth, 6 W cm^{-2} optical power), 730 nm (50 nm bandwidth, 160 mW optical power) and 795 nm (3 nm bandwidth, 8 W cm^{-2} optical power), and to measure the fluorescence transients at emission wavelengths selected from 680 nm to 950 nm using an IR-sensitive avalanche diode. The fluorescence transients were digitized at 10 MHz sampling rate and processed to calculate the effective absorption cross-section of the photosynthetic apparatus, the quantum yield of charge separation and the rates of the photosynthetic electron transport[19].

Sample handling

Water samples from the hydrothermal vents site were recovered using a pair of 2 litre Niskin bottles mounted on the sample basket of the deep-sea submersible, *Alvin*. A total of 18 water samples from 9 dives were recovered and processed within 2–4 h from sample acquisition. Surface water samples were recovered using a small bucket and were processed within 10 min from sample collection. Both the raw samples (effective instrument sensitivity of 4 ng bChl per l) and filter-concentrated water samples (effective sensitivity of 10 pg bChl per l) were processed.

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Correspondence and requests for materials should be addressed to P.F. (e-mail: falko@imcs.rutgers.edu).