APPLIED AND ENVIRONMENTAL MICROBIOLOGY, June 2002, p. 2972–2981 Vol. 68, No. 6
0099-2240/02/$04.00+0 DOI: 10.1128/AEM.68.6.2972–2981.2002

Experimental Study of Interactions between Purple and Green Sulfur Bacteria in Sandy Sediments Exposed to Illumination Deprived of Near-Infrared Wavelengths

Astrid Massé,[1] Olivier Pringault,[1,2]† and Rutger de Wit[1]*

Laboratoire d'Océanographie Biologique, CNRS-UMR 5805 Université Bordeaux 1, F-33120 Arcachon, France,[1] *and Microsensor Group, Max-Planck-Institut für Marine Mikrobiologie, D-28359 Bremen, Germany*[2]

Received 1 November 2001/Accepted 2 April 2002

Sedimentary biofilms of the green sulfur bacterium *Prosthecochloris aestuarii* strain CE 2404, the purple sulfur bacterium *Thiocapsa roseopersicina* strain 5811, and a mixed culture of both were cultured in fine sand (100- to 300-μm grain size) within counter gradients of oxygen and sulfide. The artificial sediments were exposed to illumination deprived of near-infrared light (NIR) by filtering out the wavelengths longer than 700 nm to simulate the critical light conditions in submerged aquatic sediments. A 16 h of visible light–8 h of dark regimen was used. We studied the effects of these light conditions on the metabolisms of and interactions between both species by comparing the single species biofilms with the mixed biofilm. The photosynthesis rates of *P. aestuarii* were shown to be highly limited by the imposed light conditions, because the sulfide photooxidation rates were strongly stimulated when NIR was added. *T. roseopersicina* performed both aerobic chemosynthesis and photosynthesis, but the photosynthesis rates were low and poorly stimulated by the addition of NIR. This species decreased the penetration depth of oxygen in the sediment by about 1 mm by actively respiring oxygen. This way, the strict anaerobe *P. aestuarii* was able to grow closer to the surface in the mixed culture. As a result, *P. aestuarii* benefited from the presence of *T. roseopersicina* in the mixed culture, which was reflected by an increase in the biomass. In contrast, the density of the latter species was almost completely unaffected by the interaction. Both species coexisted in a layer of the same depth in the mixed culture, and the ecological and evolutionary implications of coexistence are discussed.

Green sulfur bacteria (GSB) and purple sulfur bacteria (PSB) coexist in different aquatic ecosystems. The interactions between both groups and the ways in which these are influenced by light conditions have been studied in detail for the pelagic environment in both field studies and laboratory experimentation (13, 26, 41, 42, 43, 44). PSB and GSB also proliferate in the upper layers of different shallow aquatic sediments and form clear laminas in microbial mats (4, 27, 29, 30, 31). Because these laminas consist of microbial biomass at high densities adhering to the sand grains and developing at the oxic-anoxic interface, such systems can be considered biofilms (45). However, the anoxygenic photosynthetic biofilms growing on the sandy sediment are different from most of the commonly studied biofilms that grow on solid surfaces immersed in a flowing liquid. First, the depth layer of the phototrophic sulfur bacteria is open for mass transfer of solutes both at the top and at the bottom. The bacteria thus interact both with shallower and deeper sediment layers. Second, by growing in the sand below the sediment surface, the photosynthetic biofilm is protected from shear stress and therefore not directly subjected to sloughing and erosion. Many benthic phototrophic sulfur bacteria also produce extracellular polymeric substances (EPS), a feature typical of biofilm organisms (45). Considering these specific features, we will use the term sedimentary biofilms for the layers of benthic phototrophic sulfur bacteria growing in the sand.

Interactions between GSB and PSB are partially understood in terms of their different susceptibilities towards molecular oxygen. GSB are obligate anaerobes that use H_2S as the predominant electron donor for photolithoautotrophy. However, limited amounts of acetate can be photoassimilated together with H_2S. In contrast, many species of PSB survive and grow in the presence of molecular oxygen, which can be used as an electron acceptor for chemolithotrophy. The PSB *Thiocapsa roseopersicina*, which is characteristic of benthic environments, is an example par excellence. In the dark, this species shifts immediately to chemosynthesis when oxygen and a reduced sulfur compound are present. Oxygen inhibits photosynthetic pigment synthesis but does not directly inhibit photosynthesis in this species (8, 9). Therefore, oxic-anoxic alternations have a great impact on its physiology. Thus, it has been shown that the regular occurrence of short anoxic periods allow this species to synthesize sufficient bacteriochlorophyll (BChl) to maintain photosynthesis during the oxic light periods (8) but that photosynthesis and chemosynthesis may occur simultaneously in the light when the BChl content is very low (38).

It has been postulated that shallow benthic environments were the sites where life emerged on earth and where photosynthesis evolved early in evolutionary history within biofilms and stratified communities fossilized as stromatolites (39). GSB and most PSB have BChl *a* in their reaction centers, which are of different types. GSB possess a type 1 reaction center (RC1) with a low-potential iron-sulfur center as the

* Corresponding author. Mailing address: Laboratoire d'Océanographie Biologique, CNRS-UMR 5805 Université Bordeaux 1, 2 rue du Professeur Jolyet, F-33120 Arcachon, France. Phone: 33 5 56 22 39 09. Fax: 33 5 56 83 51 04. E-mail: r.de-wit@epoc.u-bordeaux.fr.

† Present address: Centre IRD de Nouméa, BP A5, 98848 Nouméa Cedex, New Caledonia.

electron acceptor, while PSB possess a type 2 reaction center (RC2) with a higher potential quinone as the electron acceptor. GSB and PSB have very different light-harvesting systems. Most PSB contain BChl *a* as antenna molecules associated with a whole range of different carotenoids. GSB contain chlorosomes with BChl *c* or *d* associated with chlorobactene (3, 5). The brown-colored pelagic GSB species are distinct because they contain BChl *e* associated with isorenieratene.

The light conditions in sediments are shaped by scattering and strong attenuation of UV and shorter visible wavelengths and are therefore proportionally enriched in red and near-infrared (NIR) light below the surface (15, 22, 29, 31). Therefore, the evolution of the different BChl's, which present a whole suite of complementary in vivo absorption peaks in the NIR, has been related to the evolutionary pressures in early benthic environments driving a niche differentiation for the use of NIR (30). In contrast, submerged environments have a very different light quality. Water strongly absorbs NIR: e.g., a 1-m water column attenuates NIR of wavelengths above 740 nm to below 1% of the surface value (18). Lower wavelengths are poorly absorbed by pure water. In addition, however, most natural waters contain dissolved organic compounds and nitrate that very strongly absorb blue and UV wavelengths (18). Thus, immersion has a profound effect on the light conditions in benthic microbial mats. In shallow benthic habitats, PSB and GSB are often exposed to fluctuations of water depth. Therefore, it is relevant for ecological and evolutionary studies to determine how water column heights put constraints on the growth of benthic PSB and GSB and how they affect the interactions among representative species.

The aim of the present study was to determine how NIR-depleted light affects the interactions between PSB and GSB in spatially ordered benthic sulfureta. We chose an experimental approach by growing representative species on sand in a gradient culture chamber, which has previously been called a benthic gradient chamber (BGC) (35). This device was designed to culture a biofilm of phototrophic sulfur bacteria on sand within experimentally controlled light and solute (i.e., oxygen and sulfide) gradients. This culture system provides a more realistic simulation of benthic growth conditions than do classical liquid cultures. A detailed analysis with microsensors allowed for study of the depth distribution of the bacterial populations in the sediment and quantification of the rates of sulfide oxidation and oxygen consumption and their variations in time and space (33, 35). *Prosthecochloris aestuarii* strain CE 2404 and *T. roseopersicina* strain 5811 (DSM 5653) (7) were selected for this study as representatives of GSB and PSB, respectively. Both species are commonly found in benthic environments. Interactions between species could be inferred by comparing the biofilms in the mixed culture with the respective axenic cultures under the same incubation conditions.

MATERIALS AND METHODS

Bacterial strains. Two strains of phototrophic microorganisms, the GSB *P. aestuarii* strain CE 2404 and the PSB *T. roseopersicina* strain 5811 (DSM 5653) (7), were cultured in a BGC under a 16 h of light–8 h of dark regimen of visible light. Both strains, which were from the culture collection of the Laboratory of Biological Oceanography (University of Bordeaux I, Arcachon, France), are nonmotile.

P. aestuarii strain CE 2404 is a spherical GSB with extrusions (prostheceae) that contains BChl *c* and carotenoids from the chlorobactene series as the major pigments. *P. aestuarii* also contains BChl *a*, which is associated with the baseplate of the chlorosome (16) and the Fenna-Matthews-Olson protein and is located in the reaction center. This strictly anaerobic strain grows photolithotrophically with H_2S and elemental sulfur as its electron donors. Thiosulfate and sulfite are not used as electron donors, and the latter compound is even inhibitory for growth (16).

T. roseopersicina strain 5811 (DSM 5653) was originally isolated from the Etang du Prévost (Palavas, France) and has been designated *T. roseopersicina* forma specialis, because it contains okenone instead of spirilloxanthin as the major carotenoid (7). We chose this okenone-containing strain, which is better adapted to grow below a water column than the spirilloxanthin-containing strain used in previous studies under visible light plus NIR (33). Cultures of the strain are purple-violet in color and exhibit in vivo absorption maxima at 370 nm (BChl *a*-Soret band), 520 nm (okenone), and 830 nm (LH2-BChl *a*). The cells are spherical (diameter, 1.2 to 3 μm) and able to grow photolithotrophically with sulfide, elemental sulfur, thiosulfate, or sulfite as the electron donor (7). Facultatively, it can also grow as a chemolithoautotroph with oxygen as the electron acceptor and sulfide, thiosulfate, or elemental sulfur as the electron donor (7). Hence, apart from the carotenoid pigmentation and in vivo absorption spectrum, it shares the major phenotypic traits with other *T. roseopersicina* isolates. A recent phylogenetic study based on 16S RNA sequence analysis showed that the strain belongs to the so-called *T. roseopersicina* group. Within this group, it clustered very closely together with several different spirilloxanthin-containing strains originally described as *T. roseopersicina*, although its phylogenetic distance from the type strain (DSM 217) was somewhat greater (14).

Culture conditions. *P. aestuarii* CE 2404 and *T. roseopersicina* 5811 were cultured in artificial sediments composed of fine sand of 100- to 300-μm grain size (Merck, Germany) with a BGC as described by Pringault et al. (35). Three different cultures were studied: *P. aestuarii* CE 2404 alone (P), *T. roseopersicina* 5811 alone (T), and a mixture of both organisms (PT). The BGC is composed of an artificial sediment core (internal diameter, 50 mm; length, 45 mm) sandwiched between an upper oxic and a lower anoxic chamber of 2,000 and 3,500 ml, respectively (35). The top centimeters of the sediment cores of cultures P and T were inoculated with 15 ml of liquid batch cultures in exponential phase of *P. aestuarii* strain CE 2404 and *T. roseopersicina* strain 5811, respectively. We also prepared a 1:1 mixture of portions from both axenic batch cultures in exponential phase under aseptic conditions. The top centimeter of the sediment core of culture PT was inoculated with 15 ml of this mixture. The culturing period was 5 weeks and was exactly the same for the three different cultures. However, the different cultures were set up sequentially, with a 1-week interval between successive cultures, in order to allow the time necessary for the detailed microsensor analyses and experiments with the biofilms after the 5-week incubation period (see below). The temperature was maintained at 20°C during the 5 weeks of culturing.

The BGC was illuminated from above by a collimated light beam of an incandescent lamp (100 W; E27, Sylvania reflector) by using a 16 h of light–8 h of dark regimen. A hot mirror filter (775FW82-50; Andover Corporation, Salem, N.H.) was placed in the light path (below the collimator) to filter out the infrared wavelengths. Compared with full-light conditions, the hot mirror filter did not affect the light transmission for the visible spectrum. Under the light conditions used for the culturing, the downwelling irradiance at the sediment surface was ca. 170 ± 10 μmol of photons $m^{-2}\ s^{-1}$ for 430 to 700 nm and ca. 10 μmol of photons $m^{-2}\ s^{-1}$ for the NIR part (700 to 900 nm).

Microsensor analyses. (i) Microelectrode measurements of solute concentrations. Oxygen concentration measurements were performed with a Clark type oxygen microelectrode with a guard cathode (36). Dissolved hydrogen sulfide (H_2S) concentrations were measured with an amperometric H_2S microelectrode (17). The pH was measured with a pH glass microelectrode (37) connected to a high-impedance millivoltmeter with a calomel electrode as a reference. Total dissolved sulfide (H_2S plus HS^- and S^{2-}), hereafter referred to as sulfide, was calculated from the dissolved H_2S concentration and the pH value (34). The three microelectrodes were mounted on a micromanipulator (World Precision Instruments, Berlin, Germany) at the same horizontal level; the distance between them was less than 2 mm. The optical and chemical microsensors were introduced from the side under a 150° zenith angle relative to the incident light in order to prevent shading of the measurement spots.

Prior to the measurements with the microsensors, we opened the BGC and replaced the upper oxic medium with a KH_2PO_4 buffer (100 mM, pH 7.2) amended with 30 g of NaCl $liter^{-1}$. This resulted in a constant pH throughout the biofilms, which improved the accuracy of the calculations of sulfide concentrations (H_2S plus HS^- and S^{2-}).

(ii) Microscale light measurements. Light measurements were performed with fiber-optic microprobes (scalar irradiance and field radiance microprobes) con-

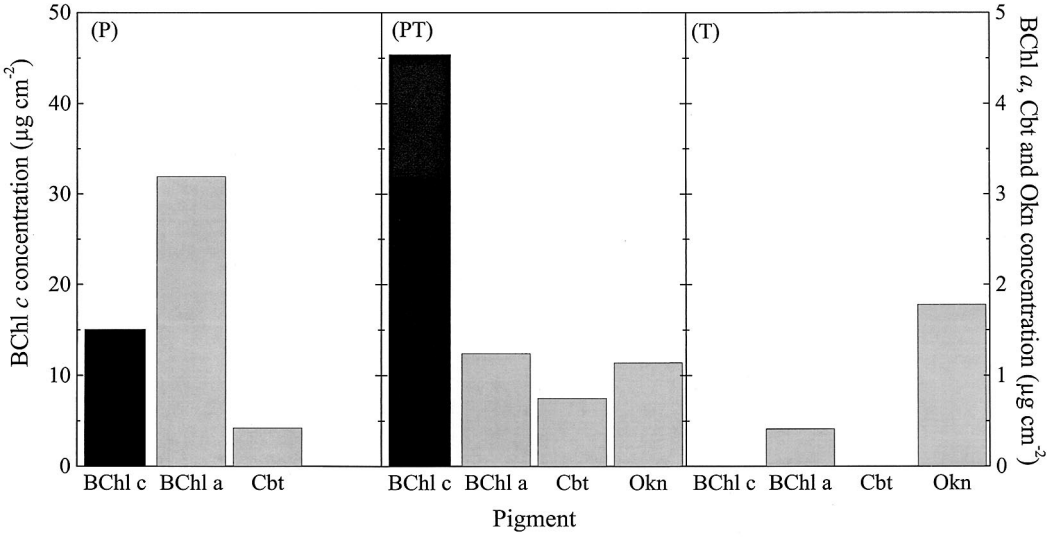

FIG. 1. Comparison of the BChl *c* (black bars), BChl *a*, chlorobactene (Cbt), and okenone (Okn) (gray bars) concentrations (in micrograms per square centimeter) in the axenic biofilm of *P. aestuarii* strain CE 2404 (P), the axenic biofilm of *T. roseopersicina* strain 5811 (T), and the mixed culture of both bacteria (PT). Note the different scales for the differently colored bars shown at right and left.

nected to a cooled optical spectral multichannel analyzer (22). The scalar irradiance was used to estimate the photon flux density available for photosynthesis, which was measured for both NIR-deprived-light (culturing conditions) and full-light conditions. The backscattered radiance was used to determine the depth distribution of major photopigments. The latter was measured under full-light conditions. Under full-light conditions, the hot mirror was removed for the period of time requested for the measurements. The measurement and calibration procedures have been described by Pringault et al. (34). The maxima of scalar irradiance and backscattered radiance were aligned with the sediment surface. The partial attenuation coefficient (K_D) for backscattered field radiance was defined as the rate of change of ln-transformed radiance values with depth (see references 18 and 34 for details).

(iii) Estimation of fluxes and rates. The 5-week cultivation period following inoculation allowed the formation of the different biofilms growing in the sand. After this period, the light conditions and light-dark regimen were maintained as used for the culturing. The depth distributions of metabolic processes and fluxes were inferred from steady-state oxygen and sulfide profiles performed under light conditions used during the culturing at the end of the 16-h light and 8-h dark periods, respectively. The oxygen influx into the reaction zone was calculated from the oxygen profile in the diffusion boundary layer (37). The sulfide flux into the reaction zone was calculated from the linear part of the total sulfide profile below the reaction zone according to Fick's first diffusion law as applied to the sediment (2). The reaction rates were inferred from the parabolic shape of the solute profiles in the reaction zone, assuming that rates were zero order (28). The sedimentary diffusion coefficients and the porosity of the sand have been determined previously (32). Secondly, short light-dark cycles of 30 min of light—30 min of dark were applied to estimate the sulfide photooxidation activity (see below). NIR-depleted-light conditions (same as those used for culturing) were compared with full-light conditions (visible light amended with NIR). Finally, the biofilm was harvested by subcoring (cores of 24-mm diameter). The cores were frozen and stored at −80°C for further analysis of pigment contents.

The experiment with short light-dark cycles (30 min of light–30 min of dark) had been designed to study the sulfide dynamics upon a shift from dark to light. The H_2S concentration and pH were monitored at a given depth during one light-dark cycle, and the procedure was repeated sequentially at different depths until the entire zone of interest had been scanned. At all depths, we checked that the sulfide concentrations returned to the same value after completion of the full light-dark cycle. Hence, for a given depth, the sulfide concentration was the same for each dark-to-light transition event. By combining the time series measured sequentially at the different depths, we obtained the transient sulfide profiles. From this information, we calculated the sulfide photooxidation activity in the biofilm by using Fick's second diffusion law, as described previously (24, 32). We compared the effect of NIR-deprived light (culture conditions) with that of full light on the sulfide photooxidation activity. Under full-light conditions, the hot mirror was removed for the period of time requested for the measurements.

Pigment analysis. The pigment compositions of the different biofilms were studied by high-performance liquid chromatography (HPLC). The sediment core with the biofilm was extracted from the BGC, frozen, and stored at −80°C. The superficial sediment comprising the biofilm (width, 1.5 cm) was homogenized. Pigments were extracted in 5 ml of acetone (100%, 4°C) by sonicating for 30 s while flushing with N_2 and stored at −30°C for 3 h. Extracts were separated from the sediment by centrifugation (3,000 × *g*, 10 min; Seli, Toulouse, France). The material was extracted three times, pooled, and stored overnight under N_2 flush at −80°C. The liquid chromatography system used in this study was described by Lemaire et al. (23). Since authentic standards were not available, the pigment contents were quantified from the peak area assuming an extinction coefficient of 86 liters g^{-1} cm^{-1} at 440 nm for BChl *c* (40), 84.1 liters g^{-1} cm^{-1} at 770 nm for BChl *a* (11), 304 liters g^{-1} cm^{-1} at 440 nm for chlorobactene (12), and 232 liters g^{-1} cm^{-1} at 440 nm for okenone (12).

RESULTS

Description of the biofilms obtained after 5 weeks of culturing in the BGC. (i) Bacterial density. The pigment contents of the different biofilm cultures are shown in Fig. 1. The BChl *c* concentration in the mixed biofilm (PT) was threefold higher than that in the axenic *P. aestuarii* biofilm (P), i.e., 45.4 and 15.1 μg cm^{-2}, respectively. In addition, the level of chlorobactene was higher in the mixed PT biofilm than in the P biofilm, but the difference was twofold smaller (0.75 and 0.42 μg cm^{-2}, respectively). Both pigments indicated that the density of *P. aestuarii* was more important in the biofilm when this species was cultured together with *T. roseopersicina* (PT) than when cultured alone (P). In contrast, for *T. roseopersicina* 5811, the okenone content in the mixed PT biofilm was smaller than that found in the T biofilm, i.e., 1.1 and 1.78 μg cm^{-2}, respectively. BChl *a* was synthesized by both *T. roseopersicina* 5811 and *P. aestuarii.* In the mixed culture (PT), the content of this compound was 1.24 μg cm^{-2}, which, surprisingly, was lower than that in the axenic culture of *P. aestuarii* (P) (3.19 μg cm^{-2}), but was, as expected, higher than the BChl *a* concen-

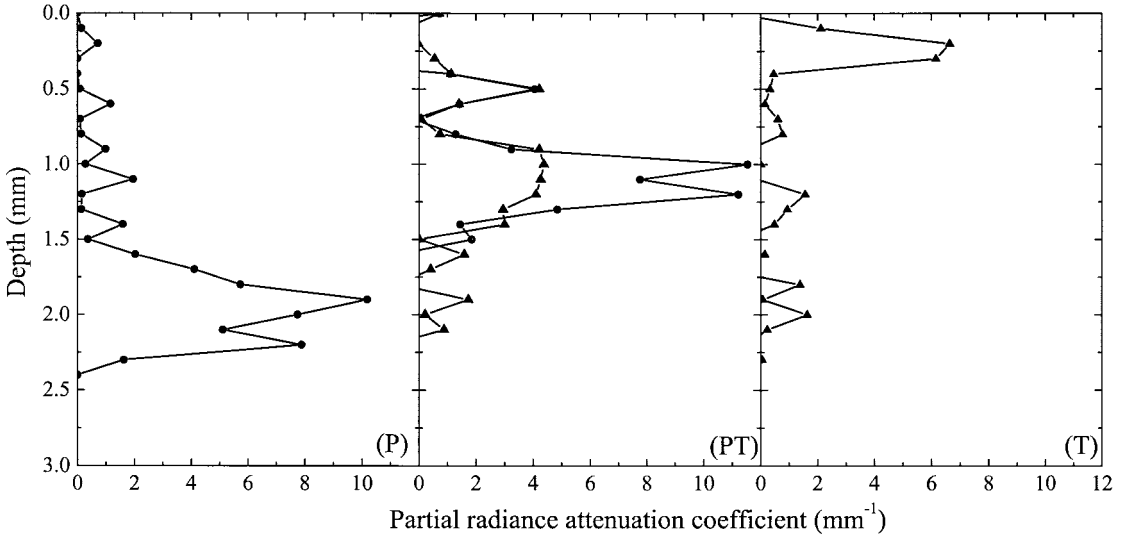

FIG. 2. Comparison of the depth distributions of *P. aestuarii* strain CE 2404 and *T. roseopersicina* strain 5811 in the axenic biofilms of *P. aestuarii* (P) and *T. roseopersicina* (T) and the mixed culture of both bacteria (PT). The depth distributions have been inferred from the partial radiance attenuation for 750 nm (●) and 830 nm (▲) (see text) as proxies for the depth distributions of *P. aestuarii* and *T. roseopersicina*, respectively.

tration in the axenic culture of *T. roseopersicina* 5811 (T) (0.41 μg cm^{-2}). The BChl *c*-to-BChl *a* molar ratio in the P culture was ca. 5:1, which is low for *P. aestuarii*, for which typical ratios of 50:1 have been observed in batch cultures (A. Massé and R. de Wit, unpublished data). Assuming that for *T. roseopersicina* 5811 the ratio of BChl *a* to okenone was the same in the T and PT cultures, we can calculate by subtraction the amount of BChl *a* attributed to *P. aestuarii* in the PT culture. This indicates that the BChl *c*-to-BChl *a* molar ratio was close to 50:1 for *P. aestuarii* in the PT culture.

(ii) Depth distribution of bacteria in the biofilms. In order to characterize the depth distribution of the bacterial strains in the sediments, we measured the depth profiles of backscattered radiance and calculated their partial attenuation coefficients, i.e., K_D, for photons of 750 and 830 nm. Values of 750 and 830 nm correspond to the in vivo absorption maxima of the Q_y band of BChl *c* of *P. aestuarii* and the BChl *a*-containing LH2-complex of *T. roseopersicina* 5811, respectively. The abiotic attenuation by the sand, measured in an unpopulated artificial sediment core, was approximately 1 mm^{-1}. This background value has been subtracted from the values measured in the different biofilm cultures, and the partial attenuation coefficients are shown in Fig. 2. Hence, the partial attenuation coefficient at 750 nm can be used as a proxy for *P. aestuarii* density, and its corresponding value at 830 nm can be used as a proxy for *T. roseopersicina* 5811 density.

In the axenic culture of *P. aestuarii* (Fig. 2, panel P), the biofilm was located at a depth between 1.5 and 2.5 mm, with a density peak at a depth of 2 mm (K_D = 10 mm^{-1}). In the axenic culture of *T. roseopersicina* 5811 (Fig. 2, panel T), the biofilm was located at a depth between 0 and 1 mm, with a density peak at a depth of 0.2 mm (K_D = 6.5 mm^{-1}). In the mixed culture (PT), pigmented cells of both bacteria coexisted in the same depth horizon of the sediment core, i.e., from depths of 0.25 to 1.5 mm (Fig. 2, panel PT). Minor attenuation peaks reflecting the presence of both organisms were observed at a depth of ca. 0.5 mm. The major peaks were observed deeper in the biofilm, at a depth of ca. 1 mm, with K_Ds of 11.5 and 4.4 mm^{-1} for 750 and 830 nm, respectively.

(iii) Steady-state oxygen and sulfide profiles. Steady-state profiles of O_2 and H_2S measured in the different biofilms at the end of the 8-h dark and 16-h light periods, respectively, are depicted in Fig. 3. In all cultures, the oxygen profiles remained rather constant throughout a diel cycle, because only minor differences were observed between oxygen profiles measured at the ends of the light and dark periods. The oxygen penetration depth in the sediment was 0.5 and 0.6 to 0.7 mm in the PT and T cultures, respectively. In contrast, oxygen penetrated down to depths of 1.3 to 1.5 mm in the sediments of the P culture. Thus, the presence of *T. roseopersicina* 5811 in the biofilm resulted in a much shallower oxygen penetration depth. This latter bacterium is capable of respiring oxygen and growing as a facultative aerobic chemolithotroph (7, 9).

In the mixed culture (PT), oxygen and sulfide coexisted between the surface and a depth of 0.5 mm at the end of the dark period. The concentrations of both coexisting compounds were low. This suggests a high turnover rate for these compounds, implying that a chemosynthetic reaction was taking place. This was attributed to *T. roseopersicina* 5811 and was confirmed by the O_2 uptake rate of approximately 0.084 μmol of O_2 cm^{-2} h^{-1} measured at the end of the dark period in the reaction zone of the mixed culture (PT) (Table 1). The stoichiometry, which was 1 H_2S to 1.1 O_2, indicated incomplete oxidation of sulfide, probably resulting in a mixture of SO_4^{2-}, S, and other intermediate oxidation products. Such intermediate products may have served as electron donors during the light period, when the sulfide front shifted downwards to a depth of 0.8 mm due to photosynthetic sulfide oxidation by the phototrophic bacteria. As a result, at the end of the light period, H_2S and O_2 did not coexist and the depth zone between 0.5 and 0.8 mm was characterized by the absence of oxygen and sulfide.

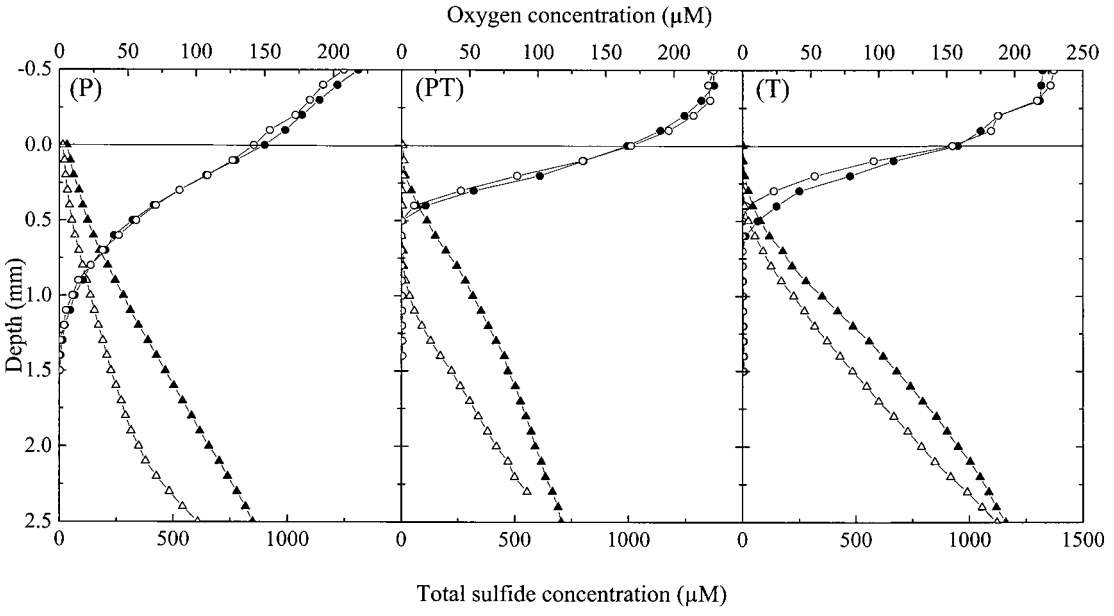

FIG. 3. Comparison of sulfide (triangles) and oxygen (circles) profiles in the axenic biofilms of *P. aestuarii* strain CE 2404 (P) and *T. roseopersicina* strain 5811 (T) and the mixed culture of both bacteria (PT) cultured in the BGC under visible light (16 h of light–8 h of dark). ▲ and ● represent the situation at the end of the 8-h dark period; △ and ○ represent the situation at the end of the 16-h light period.

In the axenic culture of *P. aestuarii* (P), oxygen and sulfide coexisted from the surface down to a depth of 1.3 mm at the end of the dark period. In contrast with the PT culture, the oxygen coexistence zone was thus six times broader and both compounds coexisted in higher concentrations. This reflected a much lower turnover rate of both compounds, which can be explained by abiotic reaction kinetics (25). During the light period, the coexistence front of oxygen and sulfide shifted downwards to a depth of 1.5 mm. Between 1.5 and 2.5 mm in depth, the steady-state sulfide profile at the end of the light period was concave, which reflected photosynthetic sulfide oxidation by the biofilm.

In the axenic culture of *T. roseopersicina* 5811 (T), a coexistence zone of oxygen and sulfide was persistent but shifted during the diel cycle. The oxygen profiles were similar at the end of the dark and light periods, and the maximum oxygen penetration depth was located at 0.6 mm. At the end of the dark period, the sulfide was observed at a depth of 0.25 mm, whereas it shifted downwards to 0.5 mm during the light period. The high turnover rates of oxygen and sulfide measured at the end of the dark period in the reaction zones, i.e., 0.129 μmol of O_2 cm^{-2} h^{-1} and 0.132 μmol of sulfide cm^{-2} h^{-1}, indicated chemosynthesis by *T. roseopersicina* 5811, pointing to an incomplete oxidation of sulfide (Table 1). The minor shift of the sulfide profile during the light period indicated that photosynthetic sulfide consumption by *T. roseopersicina* 5811 was low. However, this is perhaps an underestimation of photosynthesis, because it cannot be excluded that a part of the sulfide oxidation shifted from chemosynthesis to photosynthesis upon the transition from dark to light.

Analysis of sulfide oxidation within the biofilms measured during short light-dark cycles. In order to estimate the effect of light quality on the sulfide photooxidation, series of transient profiles were measured under NIR-deprived light, as used during the culturing, and under light amended with NIR (see Materials and Methods). These transient profiles are de-

TABLE 1. O_2 respiration, H_2S oxidation, and oxygen and sulfide diffusive fluxes

Biofilm	Substrate	Reaction zone (cm)	Zero-order rate (μmol cm^{-3} h^{-1})	Integrated rate in reaction zone (μmol cm^{-2} h^{-1})	Diffusive flux toward reaction zone (μmol cm^{-2} h^{-1})
T. roseopersicina (T), end of dark	Oxygen	0–0.07	1.84	0.129	0.140
	Sulfide	0–0.09	1.47	0.132	0.126
T. roseopersicina (T), end of light	Oxygen	0–0.06	3.05	0.183	0.138
	Sulfide	0.02–0.09	1.46	0.102	0.099
Coculture (PT), end of dark	Oxygen	0–0.05	1.67	0.084	0.109
	Sulfide	0–0.04	1.87	0.075	0.069
Coculture (PT), end of light	Oxygen	0–0.05	1.54	0.077	0.093
	Sulfide	0.08–0.13	1.21	0.061	0.077

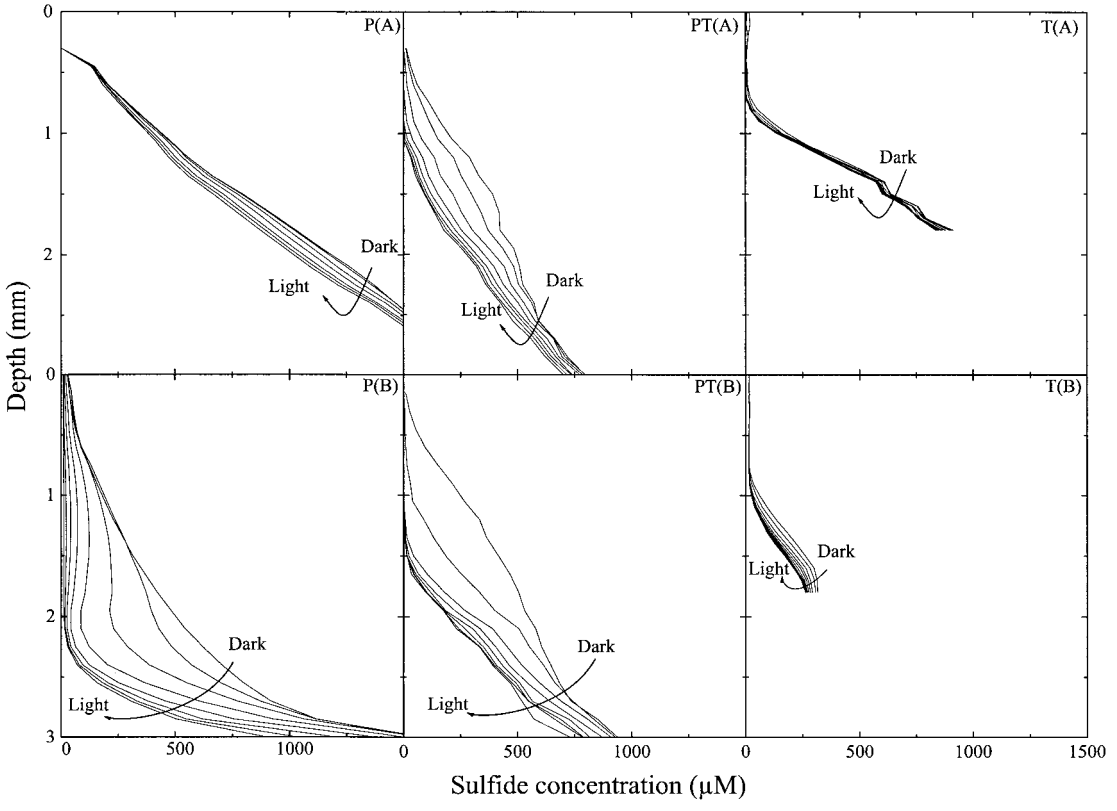

FIG. 4. Effect of short light-dark cycles (30 min of light–30 min of dark) on sulfide profiles in the axenic biofilms of *P. aestuarii* strain CE 2404 (P) and *T. roseopersicina* strain 5811 (T) and the mixed culture of both bacteria (PT). The transient profiles have been obtained from sequential monitoring at different depths (see Materials and Methods). The profiles are given for selected depths and represent the transition from dark to light. The time interval between two successive profiles is 2 min. (A) Visible light (culture conditions); (B) full light (visible light enriched with NIR).

picted in Fig. 4. Under NIR-deprived light, the transient profiles indicated that the sulfide photooxidation rates in both axenic cultures, P and T, were lower than the sulfide photooxidation rates in the mixed culture, PT. In contrast, when NIR was added, the most pronounced sulfide dynamics were observed in the axenic culture of *P. aestuarii* (P), where the average sulfide photooxidation rate was fivefold higher than under NIR-deprived light (6.37 and 1.22 μmol of sulfide cm^{-3} h^{-1}, respectively). For the mixed culture (PT), sulfide dynamics were also enhanced (1.73 and 2.99 μmol of sulfide cm^{-3} h^{-1} under NIR-deprived-light and full-light conditions, respectively), but this was not as pronounced as in the P culture. The smallest effect of NIR addition on sulfide dynamics was observed in the axenic culture of the purple strain *T. roseopersicina* 5811 (T), implying that light quantity and quality had little effect on the sulfide photooxidation activity: the activity was still very low and almost equal to the activity measured under the culturing light conditions. This indicates that sulfide photooxidation in the axenic culture of *T. roseopersicina* 5811 occurred but did so at very low rates and was poorly affected by the light quality.

Effect of the different biofilms on the light availability within the sand. Figure 5 shows the values of the scalar irradiance as a function of depth in the sediment for the different biofilm cultures. The values have been integrated for the visible, which has been defined as photosynthetically active radiation for oxygenic photosynthesis (PAR) (actually from 430 to 700 nm) and for NIR (700 to 850 nm). The results for PAR are presented only for NIR-deprived-light conditions, whereas the results for NIR are presented for both NIR-deprived-light and full-light conditions. The fact that the scalar irradiance at the sediment surface in the P culture was more than 150% higher than the incident downwelling irradiance (461 and 180 μmol of photons m^{-2} s^{-1}, respectively) indicated that the top sediment layer was a highly light-scattering environment. Similar observations have been reported for wet sand (21), which is in agreement with the fact that *P. aestuarii* developed at depths below 1.5 mm in the sand of the P culture. In contrast, in the T and PT cultures, we observed lower scalar irradiance values at the sediment surface than in the P culture, despite the same incident downwelling irradiance. Thus, the top layer in the T and PT cultures scattered light to a lesser extent than did wet sand, which indicated that the presence of the organisms, and noticeably of *T. roseopersicina*, reduced the scattering properties of the sand. The sedimentary attenuation of scalar irradiance was strongest in the PT and T cultures. The strongest attenuation was between 0 and 0.5 mm in the T culture and between 0.5 and 1.5 mm in the PT culture, values which correspond to the location of the pigment-containing biofilms in both cultures (Fig. 2). The light penetration was deepest in the

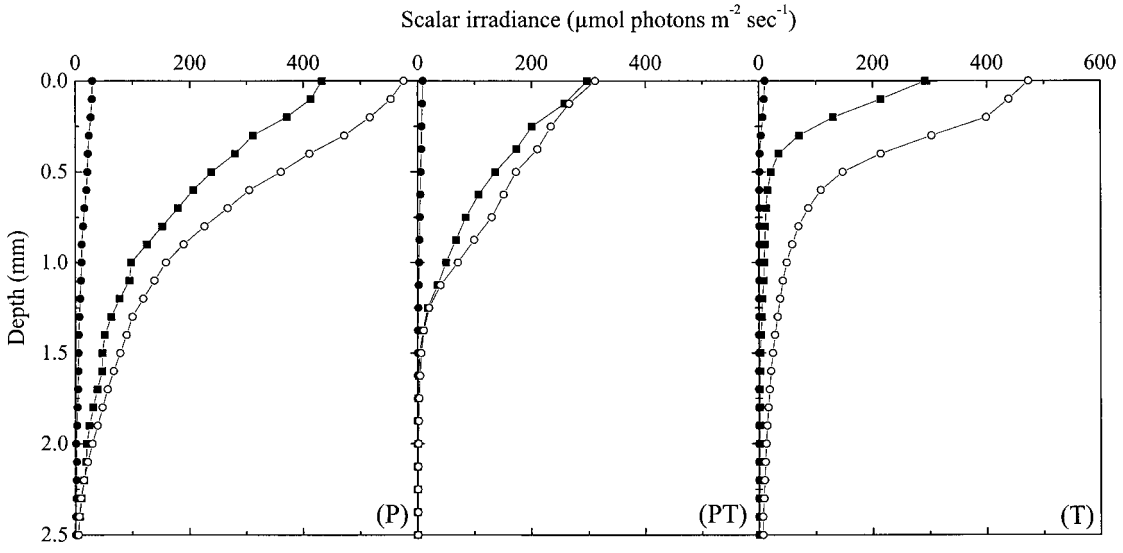

FIG. 5. Distribution of PAR (■) and NIR radiation (●) (micromoles of photons per square meter per second) measured under culture conditions and NIR radiation measured under full-light conditions (○) at different depths within the axenic biofilms of *P. aestuarii* strain CE 2404 (P) and *T. roseopersicina* strain 5811 (T) and the mixed biofilm of both bacteria (PT).

P culture sediments. The attenuation of scalar irradiance was particularly low at depths between 0 and 1.5 mm, because in this area, the sand was not populated by *P. aestuarii* (Fig. 2). There was, however, a marked increase of scalar irradiance attenuation at depths between 1.5 and 2.5 mm in the biofilm of *P. aestuarii*. The light conditions at the peak of the *P. aestuarii* biomass were remarkably similar in the PT and P cultures, i.e., 17.6 μmol of PAR photons m^{-2} s^{-1} plus 1.2 μmol of NIR photons m^{-2} s^{-1} at a depth of 1 mm for PT and 20.1 μmol of PAR photons m^{-2} s^{-1} plus 1.2 μmol of NIR photons m^{-2} s^{-1} at a depth of 2 mm for P.

DISCUSSION

Interactions between *P. aestuarii* and *T. roseopersicina*. In the present work, we experimentally simulated the constraining light conditions, i.e., the depletion of NIR, which exist in submerged sediments, and we studied the effect of these drastic conditions on the interactions between PSB and GSB in benthic biofilms. Using these conditions, we found significantly higher biomarker pigment concentrations specific for *P. aestuarii* in the PT than in the P culture (Fig. 1), indicating that *P. aestuarii* clearly benefited from the presence of *T. roseopersicina* 5811 in the artificial sulfidic sandy sediment. The strictly anaerobic character of *P. aestuarii* was confirmed by the fact that this species proliferated in the sediment below the oxygen penetration depth. In contrast, the steady-state profiles and short light-dark shift experiments performed in the axenic culture T clearly reflected that *T. roseopersicina* performed chemolithotrophy close to the surface while simultaneously a low rate of photosynthesis was detected in the light (Fig. 3 and 4 and Table 1).

The main effect of *T. roseopersicina* 5811 in the PT culture was to decrease the oxygen penetration depth by respiring oxygen for chemosynthesis; i.e., the oxygen penetration depths were 1.5 and 0.5 mm in the P and PT cultures, respectively. As a result, *P. aestuarii* could grow closer to the surface in the PT than in the P culture, where it initially benefited from higher scalar irradiance values. Thus, the oxygen respiration by *T. roseopersicina* 5811 calculated in the PT culture, i.e., 0.13 μmol of O_2 cm^{-2} h^{-1} (Table 1), exerted a positive interaction effect on *P. aestuarii* in the spatially ordered photosynthetic biofilm. After a 5-week incubation period, this was reflected by the depth distribution of pigmented cells in the respective biofilms (Fig. 2). By that period, the light conditions at the peak of the *P. aestuarii* biomass were remarkably similar, i.e., 17.6 μmol of PAR photons m^{-2} s^{-1} plus 1.2 μmol of NIR photons m^{-2} s^{-1} at a depth of 1 mm and 20.1 μmol of PAR photons m^{-2} s^{-1} plus 1.2 μmol of NIR photons m^{-2} s^{-1} at a depth of 2 mm in the PT and P cultures, respectively (Fig. 5).

When cultured together with *P. aestuarii*, we can assume that *T. roseopersicina* escapes the competition for light by switching from photolithotrophy to chemolithotrophy, thus competing for sulfide only. However, in addition, light use in the visible spectrum is complementary for both strains, because the absorption maxima are 440 and 520 nm for *P. aestuarii* and *T. roseopersicina* 5811, respectively. The okenone content was only slightly lower in the PT than in the T culture, indicating that competition with *P. aestuarii* for sulfide exerted virtually no effect on *T. roseopersicina* 5811. In a previous study, we showed that the use of NIR-deprived light on an axenic biofilm culture of *P. aestuarii* in the BGC resulted in limitation by light rather than by sulfide (24). Therefore, the competition for sulfide between *P. aestuarii* and *T. roseopersicina* was of minor importance under the light conditions studied.

Interestingly, we observed that the presence of microorganisms, and noticeably of *T. roseopersicina*, reduced the scattering properties of the top sand layer. *T. roseopersicina* is a coccoid bacterium that forms a capsule comprising EPS. Single cells and microcolonies of this species strongly adhere to the sand grains. EPS coatings of sand grains change the reflective index at the water-solid interface, which seems a likely explanation

for the observed phenomenon. Strong light scattering in sediments enhances the availability of photons for photosynthesis (20, 21, 22). Thus, it can be argued that by reducing light scattering, *T. roseopersicina* 5811 also exerted a slightly negative effect on *P. aestuarii*. However, such a slightly negative effect was largely overruled by the positive interaction due to oxygen consumption by *T. roseopersicina* in the upper layers of the PT sediment, which allowed *P. aestuarii* to grow closer to the surface.

Critical evaluation of backscattered irradiance attenuation as a proxy for pigment distribution. Backscattered radiance profiles at wavelengths corresponding to in vivo absorption by photosynthetic pigments have been commonly used to trace photosynthetic microorganisms at high spatial resolution in biofilms and microbial mats (10, 19, 20, 22, 32, 33, 34). This approach implies that the radiance attenuation coefficient can be used as a proxy for the density of the pigment-containing cells (34). In this study, we measured the pigment contents of the total biofilm by HPLC, which gave us the opportunity to critically compare the extracted pigment content with the depth-integrated bacterial biomass estimated from the corresponding radiance attenuation coefficient. In this specific case, such a comparison was facilitated by the fact that all pigments except BChl *a* originated from a single bacterial strain.

The increase of the concentration of BChl *c* and chlorobactene in the PT culture compared to that in the P culture (Fig. 1) indicates that the density of the green bacterium was higher when the latter was cultivated in coculture with *T. roseopersicina*. This was confirmed by an increase in the depth-integrated partial attenuation coefficient specific for BChl *c*, i.e., $\int K_D\, dz = 0.50$ and 0.45 (z corresponds to the sediment depth) as measured in the PT and P cultures, respectively. However, it is surprising that the increase in the depth-integrated partial attenuation coefficient in the PT culture did not match the increase in the BChl *c* concentration. Thus, it seems that the partial attenuation coefficient of 750-nm radiance in the PT culture was low in light of the BChl *c* content. This points to an underestimation by the methodological procedures and/or a mechanism previously observed and described as the package effect (1, 18). The package effect can be due to spatial arrangements of pigments within cells but probably also to spatial arrangements of cells in a densely packed biofilm. In the present study, we can suppose that this phenomenon was particularly important in the PT culture, where very high concentrations of BChl *c* were measured. In addition, a possible underestimation of the radiance attenuation coefficient is not unlikely for the lower part of the biofilm, for which this value was calculated from backscattered radiance values that were close to the detection limit. In the P culture, for example, it was thus inferred that the biomass was extremely low at depths below 2.4 mm. This was surprising, because photosynthesis was detected at a depth of 2.5 mm, particularly when NIR was added. However, in axenic biofilms in general, there is good agreement between the depth of the biomass peak and the maximal photosynthetic activity inferred from the different methods (this study and references 24, 32, and 34).

For *T. roseopersicina* 5811, the comparison between the okenone content and the K_D at 520 nm was problematic, because the backscattered radiance profile for this wavelength was noisy. Therefore, we chose to trace the depth distribution of this strain from the K_D at 830 nm, which is the maximum of its LH2 complexes. Only in the T culture may this value be directly compared to its BChl *a* content, because in the PT culture, BChl *a* also included contributions from *P. aestuarii* (with an evaluated contribution of 0.98 μg of BChl *a* cm^{-2}; see Results). In the PT culture, the okenone concentration was slightly lower than that in the T culture (1.1 and 1.8 μg cm^{-2}, respectively). The depth-integrated partial attenuation coefficient at 830 nm measured in the PT culture was similar to the depth-integrated partial attenuation coefficient measured in the T culture, i.e., $\int K_D\, dz = 0.24$ and 0.20 in the PT and T cultures, respectively. In the PT culture, the K_D peaked at a depth between 0.9 and 1.3 mm both for 750 and 830 nm, indicating that *P. aestuarii* clearly coexisted with *T. roseopersicina* 5811 in this depth layer.

In conclusion, it appears that while backscattered radiance attenuation is a powerful method to detect the depth horizon of the biomass peak of phototrophic microorganisms in a biofilm, its conversion to pigment content or biomass is problematic. Hence, future studies requiring high-spatial-resolution biomass depth profiles in biofilms need to include other techniques. Unfortunately, confocal laser microscopy cannot be easily applied in sandy layers of several millimeters in thickness, and the coring and slicing of fine sand is difficult to achieve at the desired depth resolution.

Ecological aspects and evolutionary implications. The present work shows the complexity of interactions in spatially ordered microbial ecosystems, such as photosynthetic biofilms and microbial mats, that cannot be easily studied by classical liquid batch cultures. We reported that *P. aestuarii* and *T. roseopersicina* 5811 formed biofilms in artificial sulfidic sandy sediments under critical light conditions (NIR-deprived light) which are characteristic for submerged sediment. In the mixed culture (PT), both strains coexisted in the same depth horizon (0.8 to 1.4 mm) and *P. aestuarii* benefited from the interaction with *T. roseopersicina.* Natural environments are often exposed to fluctuating water levels and consequently to very variable light conditions. We have studied the effect of the sudden addition of NIR on photosynthesis (Fig. 4B) and on the light distribution (Fig. 5) in the biofilms that were pregrown under NIR-deprived light. It was shown that the photosynthesis rates were strongly enhanced in the P and PT cultures but only to a lesser extent in the T culture. This indicates that *P. aestuarii* was strongly limited by light under the culture conditions (24) while this was less stringent for *T. roseopersicina.* Thus, *P. aestuarii* may immediately take advantage of improved light conditions when the water height decreases or sediments emerge. However, during such an event, the biofilm can shift from being limited by light to being limited by the diffusive sulfide supply, and the competition for sulfide thus becomes important. Indeed, single-species and mixed biofilms of *P. aestuarii* and *T. roseopersicina* grown under NIR-rich light were limited by the diffusive sulfide supply rather than by light (33, 34). Under these conditions, the *P. aestuarii* biomass was reduced due to competition with *T. roseopersicina.* Collectively, these studies show that GSB and PSB may coexist in benthic biofilms under different light conditions and that the distribution of GSB is constrained by the oxygen penetration depth. The backscattered radiance profiles indicated that PSB and GSB coexisted in the same depth layer in both mixed biofilms

cultured so far (this study and reference 33). The possibility of a coexistence zone of PSB and GSB without any clear vertical stratification in the sediment appears to be in disagreement with usual observations and statements, which report that PSB and GSB communities are well stratified in benthic environments (6, 27, 29, 31). Recently, a study combining microscope spectrometry and fluorometry was performed to identify the vertical distribution of phototrophs in an artificial cyanobacterial mat (19). It revealed a coexistence layer of BChl *c* and BChl *a* at depths between 3.8 and 4.0 mm, below the oxygenic zone, thus indicating the presence of GSB and PSB in the same depth horizon. Thus, we can assume that PSB and GSB may coexist in benthic environments. We realize that when green and purple laminas are visible to the naked eye (27), a certain degree of overlap of GSB and PSB is likely. The coexistence of PSB and GSB was probably even more widespread early in evolutionary history, when the occurrence of GSB was less constrained due to the absence of oxygen. It has been postulated that shallow benthic sediments were sites where life emerged on earth (39). A recent study of comparative genomics of different genes involved in photosynthesis showed that while the RC2-containing PSB were the most ancient group, the RC1-containing anoxygenic phototrophs (heliobacteria and GSB) preceded oxygenic phototrophs, as well. A heterologous fusion model has been put forward to explain the evolution of oxygenic photosynthesis based on the recruitment of genes from the different groups of anoxygenic phototrophs (46). The coexistence of PSB and GSB in microbial mats and photosynthetic biofilms, as we observed in our experimental studies, becomes very interesting in light of this hypothesis. Conceivably, the coexistence of high densities of PSB and GSB in the same depth layer of photosynthetic biofilms may provide a very likely occasion for lateral gene transfer as invoked by the heterologous fusion model.

ACKNOWLEDGMENTS

This work was supported by a grant from the Deutscher Akademischer Austauschdienst (DAAD) and a Ph.D. fellowship from the Aquitaine Council. HPLC equipment was acquired with the support of the Aquitaine Council. Olivier Pringault was supported by a postdoctoral stipend from the Max Planck Society. Part of this study was financed by the European Union Biotechnology Program through the project MICROMAT, microbial diversity of microbial mats in Antarctica (contract no. BIO4-CT98-0040).

Astrid Massé thanks Bo Barker Jørgensen and Dirk de Beer for the opportunity to stay at the Max-Planck-Institut für Marine Mikrobiologie (Bremen, Germany) during a 3-month period. Special thanks are due to Gaby Eickert, Anja Eggers, Vera Hübner, and Mathilde Schapira for the microsensor construction and the pigment extraction work.

REFERENCES

1. **Amesz, J., L. N. M. Duysens, and D. C. Brandt.** 1961. Methods for measuring and correcting the absorption spectrum of scattering suspensions. J. Theoret. Biol. **1:**59–74.
2. **Berner, R. J.** 1980. Early diagenesis: a theoretical approach. Princeton University Press, Princeton, N.J.
3. **Blankenship, R. E., J. M. Olson, and M. Miller.** 1995. Antenna complexes from green photosynthetic bacteria, p. 399–435. *In* R. E. Blankenship, M. T. Madigan, and C. E. Bauer (ed.), Advances in photosynthesis, vol. 2: anoxygenic photosynthetic bacteria. Kluwer Academic Publishers, Dordrecht, The Netherlands.
4. **Bustillos-Guzmán, J., D. López-Cortés, F. Hernandez, and I. Murillo.** 2000. Pigment signatures with an anoxic coastal zone: Bahia Concepcion, Gulf of California. J. Exp. Mar. Biol. Ecol. **249:**77–88.
5. **Caple, M. B., H. Chow, and C. E. Strouse.** 1978. Photosynthetic pigments of green sulfur bacteria. J. Biol. Chem. **253:**6730–6737.
6. **Caumette, P.** 1989. Ecology and general physiology of anoxygenic phototrophic bacteria in benthic environments, p. 283–304. *In* Y. Cohen and E. Rosenberg (ed.), Microbial mats: physiological ecology of benthic microbial communities. American Society for Microbiology, Washington, D.C.
7. **Caumette, P., C. Schmidt, H. Biebl, and N. Pfennig.** 1985. Characterization of a *Thiocapsa* strain containing okenone as major carotenoid. Syst. Appl. Microbiol. **6:**132–136.
8. **De Wit, R., and H. van Gemerden.** 1990. Growth and metabolism of the purple sulfur bacterium *Thiocapsa roseopersicina* under combined light/dark and oxic/anoxic regimens. Arch. Microbiol. **154:**459–464.
9. **De Wit, R., and H. van Gemerden.** 1987. Chemolithotrophic growth of the phototrophic sulfur bacterium *Thiocapsa roseopersicina*. FEMS Microbiol. Ecol. **45:**117–126.
10. **Fenchel, T., and M. Kühl.** 2000. Artificial cyanobacterial mats: growth, structure and vertical zonation patterns. Microb. Ecol. **40:**85–93.
11. **Fenchel, T.** 1998. Formation of laminated microbial mats in the absence of benthic fauna. Aquat. Microb. Ecol. **14:** 235–240.
12. **Foppen, F. H.** 1971. Tables for the identification of carotenoid pigments. Chromatogr. Rev. **14:**133–298.
13. **Garcia-Gil, L. J., E. Vicente, A. Camacho, C. M. Borrego, X. Vila, X. P. Cristina, and J. Rodriguez-Gonzalez.** 1999. Vertical distribution of photosynthetic sulphur bacteria linked to saline gradients in lake 'El Tobar' (Cuenca, Spain). Aquat. Microb. Ecol. **20:**299–303.
14. **Guyoneaud, R., J. Süling, R. Petri, R. Matheron, P. Caumette, N. Pfennig, and J. F. Imhoff.** 1998. Taxonomic rearrangements of the genera *Thiocapsa* and *Amoebobacter* on the basis of 16 S rDNA sequence analyses, and description of *Thiolamprovum* gen. nov. Int. J. Syst. Bacteriol. **48:**957–964.
15. **Hoffmann, C.** 1949. Über die Durchlässigkeit dünner Sandschichten für Licht. Planta **36:**48–56.
16. **Imhoff, J. F.** 1995. Taxonomy and physiology of phototrophic purple bacteria and green sulfur bacteria, p. 1–15. *In* R. E. Blankenship, M. T. Madigan, and C. E. Bauer (ed.), Advances in photosynthesis, vol. 2: anoxygenic photosynthetic bacteria. Kluwer Academic Publishers, Dordrecht, The Netherlands.
17. **Jeroschewski, P., C. Steuckart, and M. Kühl.** 1996. An amperometric microsensor for the determination of H_2S in aquatic environments. Anal. Chem. **24:**4351–4357.
18. **Kirk, J. T. O.** 1994. Light and photosynthesis in aquatic ecosystems, 2nd ed. Cambridge University Press, Cambridge, United Kingdom.
19. **Kühl, M., and T. Fenchel.** 2000. Bio-optical characteristics and the vertical distribution of photosynthetic pigments and photosynthesis in an artificial cyanobacterial mat. Microb. Ecol. **40:**94–103.
20. **Kühl, M., and B. B. Jørgensen.** 1994. The light field of microbenthic communities: radiance distribution and microscale optics of sandy coastal sediments. Limnol. Oceanogr. **39:**1368–1398.
21. **Kühl, M., and B. B. Jørgensen.** 1994. Light penetration and light intensity in sandy marine sediments measured with irradiance and scalar irradiance fiber-optic microprobes. Mar. Ecol. Prog. Ser. **105:**139–148.
22. **Kühl, M., and B. B. Jørgensen.** 1992. Spectral light measurements in microbenthic phototrophic communities with a fiber-optic microprobe coupled to a sensitive diode array detector. Limnol. Oceanogr. **37:**1813–1823.
23. **Lemaire, E., G. Abril, R. de Wit, and H. Etcheber.** Distribution of phytoplankton pigments in nine European estuaries and implications for an estuarine typology. Biogeochemistry, in press.
24. **Massé, A., O. Pringault, and R. de Wit.** Effect of light quality on sulfide photo-oxidation and growth in an artificial biofilm of the green sulfur bacterium *Prosthecochloris aestuarii*. Photosynth. Res., in press.
25. **Millero, F. J.** 1986. The thermodynamics and kinetics of the hydrogen sulfide system in natural waters. Mar. Chem. **18:**121–147.
26. **Montesinos, E., R. Guerrero, C. Abella, and I. Esteve.** 1983. Ecology and physiology of the competition for light between *Chlorobium limicola* and *Chlorobium phaeobacteroides* in natural habitats. Appl. Environ. Microbiol. **46:**1007–1116.
27. **Nicholson, J. A., J. F. Stolz, and B. K. Pierson.** 1987. Structure of microbial mat at Great Sippewissett Marsh, Cape Cod, Massachusetts. FEMS Microbiol. Ecol. **45:**343–364.
28. **Nielsen, L. P., P. B. Cristensen, N. P. Revsbech, and J. Sorensen.** 1990. Denitrification and oxygen respiration in biofilms studied with a microsensor for nitrous oxida and oxygen. Microb. Ecol. **19:**63–72.
29. **Pierson, B. K., V. M. Sands, and J. L. Frederick.** 1990. Spectral irradiance and distribution of pigments in a highly layered marine microbial mat. Appl. Environ. Microbiol. **56:**2327–2340.
30. **Pierson, B. K., and J. M. Olson.** 1989. Evolution of photosynthesis in anoxygenic photosynthetic prokaryotes, p. 402–427. *In* Y. Cohen and E. Rosenberg (ed.), Microbial mats: physiological ecology of benthic microbial communities. American Society for Microbiology, Washington, D.C.
31. **Pierson, B. K., A. Oesterle, and G. L. Murphy.** 1987. Pigments, light penetration, and photosynthesis activity in the multi-layered microbial mats of Great Sippewissett Salt Marsh, Massachusetts. FEMS Microbiol. Ecol. **45:** 365–376.
32. **Pringault, O., E. Epping, R. Guyoneaud, A. Khalili, and M. Kühl.** 1999. Dynamics of anoxygenic photosynthesis in an experimental green sulfur bacteria biofilm. Environ. Microbiol. **1:**295–307.

33. **Pringault, O., R. de Wit, and M. Kühl.** 1999. A microsensor study of the interaction between purple sulfur and green sulfur bacteria in experimental benthic gradients. Microbiol. Ecol. **37:**173–184.
34. **Pringault, O., M. Kühl, R. de Wit, and P. Caumette.** 1998. Growth of green sulphur bacteria in experimental benthic oxygen, sulphide, pH and light gradients. Microbiology **144:**1051–1061.
35. **Pringault, O., R. de Wit, and P. Caumette.** 1996. A benthic gradient chamber for culturing phototrophic sulfur bacteria on reconstituted sediments. FEMS Microbiol. Ecol. **20:**237–250.
36. **Revsbech, N. P.** 1989. An oxygen microsensor with a guard cathode. Limnol. Oceanogr. **34:**472–476.
37. **Revsbech, N. P., and B. B. Jørgensen.** 1986. Micro-electrodes. Their use in microbial ecology. Adv. Microbiol. Ecol. **9:**293–352.
38. **Schaub, B. E. M., and H. van Gemerden.** 1994. Simultaneous phototrophic and chemotrophic growth in the purple sulfur bacterium *Thiocapsa roseopersicina* M1. FEMS Microbiol. Ecol. **13:**185–196.
39. **Schopf, J. W., and M. R. Walter.** 1982. Origin and early evolution of cyanobacteria: the geological evidence, p. 543–564. *In* N. G. Carr and B. A. Whitton (ed.), The biology of the cyanobacteria. Blackwell, Oxford, United Kingdom.
40. **Stal, L. J., H. van Gemerden, and W. E. Krumbein.** 1984. The simultaneous assay of chlorophyll and bacteriochlorophyll in natural microbial communities. J. Microbiol. Methods **2:**295–306.
41. **van Gemerden, H., and J. Mas.** 1995. Ecology of phototrophic sulfur bacteria, p. 49–85. *In* R. E. Blankenship, M. T. Madigan, and C. E. Bauer (ed.), Advances in photosynthesis, vol. 2: anoxygenic photosynthetic bacteria. Kluwer Academic Publishers, Dordrecht, The Netherlands.
42. **Vila, X., C. A. Abella, J. B. Figueras, and J. P. Hurley.** 1998. Vertical models of phototrophic bacterial distribution in the metalimnetic microbial communities of several freshwater North-American kettle lakes. FEMS Microbiol. Ecol. **25:**287–299.
43. **Vila, X., M. Dokulil, L. J. Garcia-Gil, C. A. Abella, C. M. Borrego, and L. Bañeras.** 1996. Composition and distribution of phototrophic bacterioplankton in the deep communities of several central European lakes: the role of light quality. Arch. Hydrobiol. Spec. Issues Adv. Limnol. **48:**183–196.
44. **Vila, X., and C. A. Abella.** 1994. Effects of light quality on the physiology and the ecology of planktonic green sulfur bacteria in lakes. Photosynth. Res. **41:**53–65.
45. **Wimpenny, J.** 2000. An overview of biofilms as functional communities, p. 1–24. *In* D. G. Allison, P. Gilbert, H. M. Lappin-Scott, and M. Wilson (ed.), Community structure and co-operation in biofilms. Cambridge University Press, Cambridge, United Kingdom.
46. **Xiong, J., I. Kazuhito, and C. E. Bauer.** 1998. Tracking molecular evolution of photosynthesis by characterization of a major photosynthesis gene cluster from *Heliobacillus mobilis*. Proc. Natl. Acad. Sci. USA **95:**14851–14856.