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REGULAR PAPER

Vertical distribution and characterization of aerobic phototrophic bacteria at the Juan de Fuca Ridge in the Pacific Ocean

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Abstract The vertical distribution of culturable anoxygenic phototrophic bacteria was investigated at five sites at or near the Juan de Fuca Ridge in the Pacific Ocean. Twelve similar strains of obligately aerobic phototrophic bacteria were isolated in pure culture, from depths ranging from 500 to 2,379 m below the surface. These strains appear morphologically, physiologically, biochemically, and phylogenetically similar to *Citromicrobium bathyomarinum* strain JF-1, a bacterium previously isolated from hydrothermal vent plume waters. Only one aerobic phototrophic strain was isolated from surface waters. This strain is morphologically and physiologically distinct from the strains isolated at deeper sampling locations, and phylogenetic analysis indicates that it is most closely related to the genus *Erythrobacter*. Phototrophs were cultivated from three water casts taken above vents but not from two casts taken away from active vent sites. No culturable anaerobic anoxygenic phototrophs were detected. The photosynthetic apparatus was investigated in strain JF-1 and contains light-harvesting I and reaction center complexes, which are functional under aerobic conditions.

Keywords Aerobic phototrophic bacteria · Anoxygenic photosynthesis · Bacteriochlorophyll · *Erythrobacter* · *Citromicrobium* · Juan de Fuca Ridge

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Introduction

Until the 1980s, anoxygenic bacterial phototrophy was thought to be confined to illuminated zones of anaerobic environments, such as anoxic lakes, and sulfide springs, as production of the bacterial type photosynthetic apparatus is generally inhibited under aerobic conditions (Pfennig 1978a). This belief led to a general assumption that anoxygenic phototrophy was unimportant in aerobic marine environments. However, the discovery of obligately aerobic bacteriochlorophyll (BChl) *a* producing bacteria in 1979 (Shiba et al. 1979; Shiba and Simidu 1982) indicated that anoxygenic phototrophy may be possible in a variety of aerobic environments. In recent years BChl-containing

aerobes have indeed been isolated from a number of unusual environments and characterized, including both marine and freshwater habitats, acid mine drainage sites, saline and soda lakes, and urban soils (Rathgeber et al. 2004; Yurkov and Csotonyi 2003). Obligately aerobic BChl *a* producing bacteria, commonly known as the aerobic phototrophic bacteria (APB) (Beatty 2002; Rathgeber et al. 2004), are a group of aerobic chemoorganoheterotrophs, possessing an anoxygenic photosynthetic apparatus, which functions only in the presence of oxygen (Rathgeber et al. 2004; Yurkov and Beatty 1998a). Photosynthesis is estimated to satisfy up to 20% of cellular energy requirements under illuminated aerobic conditions (Kolber et al. 2001; Yurkov and van Gemerden 1993b). If the expression of the photosynthetic apparatus increased in response to nutrient deprivation, it would allow the organism to generate a portion of its energy via facultative photoheterotrophy, so as to better compete with other heterotrophs when nutrients become limited.

Although anoxygenic photosynthesis has historically been considered to be unimportant in marine environments, much recent research has focused on the presence of APB in the ocean. Biophysical results based on a fluorescent transient at 880 nm ascribed to bacterial anoxygenic light driven electron transport indicate that BChl *a*-containing bacteria may contribute up to 2–5% of the total photosynthetic electron transport fluxes in the upper ocean (Kolber et al. 2000), and suggest that APB account for up to 11% of the total marine heterotrophic population in the oligotrophic upper ocean (Kolber et al. 2001). Reports on culturability of *Erythrobacter*-like (Koblizek et al. 2003) and *Roseobacter*-like bacteria (Oz et al. 2005) from a variety of marine surface waters including the Pacific, Atlantic, and Indian Oceans and the Mediterranean Sea support these data. However direct measurements of BChl by HPLC (Goericke 2002) and anoxygenic phototroph abundances calculated by infrared epifluorescence microscopy (IREM) and quantitative PCR (Schwalbach and Fuhrman 2005) indicate that APB account for much less of the total heterotrophic population in marine systems. These reports have focused primarily on anoxygenic phototrophy in the upper ocean. Vertical measurements of BChl *a* by HPLC and direct microscopic counts of BChl-containing cells by IREM indicate that APB numbers decrease rapidly below about 75 m depth in the oligotrophic Pacific Ocean (Kolber et al. 2001).

Perhaps the most unusual report was of the BChl-containing strain JF-1, later named *Citromicrobium bathyomarinum*, isolated from hydrothermal vent plume waters of the Juan de Fuca Ridge, in the Pacific Ocean (Yurkov and Beatty 1998b; Yurkov et al. 1999). It has been suggested that near infrared blackbody radiation emitted at deep ocean vents may provide sufficient energy to power phototrophic metabolism (Nisbet et al. 1995; Van Dover et al. 1996), and indeed the culture of an obligately anaerobic phototroph from effluent plume waters at the East Pacific Rise has recently been reported (Beatty et al. 2005). The publication of *C. bathyomarinum*, JF-1 not only stimulated active research on APB in open ocean surface waters (discussed above), but also raised a number of questions from the scientific community. Two major questions that needed answers were: (1) Was JF-1 endemic to black smoker plumes or distributed throughout the water column? (2) Was JF-1 able to use infrared light emitted by black smokers for photosynthesis?

This paper reports on the cultivability of APB from five sites at or near the Juan de Fuca Ridge in the Pacific Ocean. We show that pure cultures of strains phylogenetically related and phenotypically similar to *C. bathyomarinum* are readily obtainable from depths of 500 m and below, and that they possess a functional photosynthetic apparatus consisting of a reaction center (RC) and a single light harvesting (LH) I complex.

Materials and methods

Collection of samples

Water samples were collected over a 10-day period in the vicinity of the Juan de Fuca Ridge in the Pacific Ocean in July 2000. Samples were taken using 20 l Niskin bottles mounted on board a CTD Rosette sampler, from depths of 0, 500, 1,000, 1,500, 2,000 m and from 10 m above the sea floor at five different sites. Coordinate locations of the five sampling sites are given in Table 1. Fifty milliliter samples were removed from the Niskin bottles after cleaning the nozzle with 95% ethanol and flushing approximately 1 l of sample water through ethanol cleaned plastic tubing. Samples were immediately used to inoculate growth media.

Isolation

Serial dilutions were performed using a medium for purple non-sulfur bacteria modified from Pfennig (1978b) (PNS) containing in g l^{-1}: KH_2PO_4, 0.3; NH_4Cl, 0.3; KCl, 0.3; $CaCl_2$, 0.05; NaCl, 20.0; Na acetate, 1.0; malic acid, 0.3; yeast extract, 0.2; supplemented with 2 ml of a trace element solution (Drews 1983) and 2 ml of a vitamin solution (Yurkova et al. 2002), autoclaved at pH 5.9, and adjusted to pH 7.8–8.0 by addition of 0.5 N NaOH. Diluted samples were plated onto nutrient rich RO NaCl plates (Yurkov et al. 1999) for growth of copiotrophs and a nutrient poor modified CHU 10 medium (Gerloff et al. 1950) for growth of oligotrophic cyanobacteria, containing in g l^{-1}: $Ca(NO_3)_2$, 0.232; KH_2PO_4, 0.01; $MgSO_4$, 0.025; Na_2CO_3,

Table 1 CTD cast sampling locations

Sampling location	Lat.	Long.	Depth (m)	Description
1	47°57.0 N	129°05.8 W	2,164	Above Hulk[a], North Main Endeavour
2	47°57.0 N	129°05.8 W	2,176	Above Hulk, North Main Endeavour
3	47°57.0 N	129°08.5 W	2,375	Offset from Hulk
4	48°27.6 N	128°42.8 W	2,379	Above Middle Valley
5	48°34.3 N	129°58.4 W	>2,500	Open ocean

[a] Geological formation Hulk described in Delaney et al. (1992)

0.02; $NaSiO_3$, 0.044; $FeCl_3$, 0.0025, supplemented with 2 ml of the above trace element and vitamin solutions, autoclaved, and adjusted to pH 8.0 after sterilization. RO NaCl plates were incubated in the dark at room temperature for 20 days prior to enumeration. CHU 10 plates were incubated at room temperature in front of a window (daylight) for 20 days. After incubation plates were enumerated, and representative colonies were transferred into liquid RO NaCl media, and streaked repeatedly on RO NaCl plates until pure cultures were achieved.

Purified strains that formed pigmented colonies were grown at 30°C on agar plates overnight in the dark, and cells were resuspended in 150 µl 10 mM TRIS–HCl pH 7.8 and mixed with 450 µl of a 30% bovine serum albumin solution (ICN Biomedicals) to reduce light scattering. Absorbance spectra were recorded between 350 and 1,100 nm (Hitachi U-2010 spectrophotometer). The presence of a characteristic LH absorbance peak at approximately 870 nm was used to infer the presence of BChl *a*.

Agar deeps were prepared using three media types. PNS (given above) supplemented with 5 ml l^{-1} of a filter sterilized 0.3 mM cysteine solution and 5 ml l^{-1} of a 0.3 mM methionine solution. A purple sulfur bacteria medium (PS) containing in g l^{-1}: KH_2PO_4, 0.3; NH_4Cl, 0.3; $CaCl_2$, 0.05; NaCl, 20.0, and 2 ml each of the above trace element and vitamin solutions, adjusted to pH 5.9 before autoclaving. After sterilization the following were added in ml l^{-1}: 0.3 mM cysteine, 5; 0.3 mM methionine, 5; 10% $NaHCO_3$, 20; 10% Na_2S, 3.5; and the pH was adjusted to 7.8–7.9. A pyruvate mineral salts (PMS) medium containing in g l^{-1}: EDTA, 0.1; $MgSO_4$, 0.2; $CaCl_2$, 0.075; NH_4Cl, 1.0; K_2HPO_4, 0.9; KH_2PO_4, 0.6; Na pyruvate, 2.2; NaCl, 20.0; yeast extract, 0.1; with 2 ml each of the above trace element and vitamin solutions, autoclaved at pH 6.8. Following sterilization, 5 ml l^{-1} each of 0.3 mM cysteine and 0.3 mM methionine were added and the pH was adjusted to 7.5–7.8. The media were mixed 2:1 with molten 2% agar containing 20.0 g l^{-1} NaCl, to achieve a final concentration of 0.67% agar. Deeps were allowed to cool to about 45°C before inoculation with 1 ml of diluted samples, and then closed with a rubber stopper to exclude oxygen. After solidification, agar deeps were stored on ice until return to the lab (5–15 days) where they were incubated at 30°C in an illuminated incubator (light intensity of $\sim$30 µE m^{-2} s^{-1}).

Physiology and biochemistry

Physiological and biochemical tests were performed as previously described (Yurkov and van Gemerden 1993a; Yurkov et al. 1994b). Photoheterotrophic growth under anaerobic conditions was tested in screw cap tubes containing RO NaCl, PNS, or PS media (Rathgeber et al. 2005).

Pigments were extracted in acetone:methanol (7:2) and total carotenoid content was determined using 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 increased to 100% acetone over 15 min. Carotenoids were detected with the UV-VIS detector set to scan from 350 to 600 nm.

Microscopy

Morphology and cytology were examined in log-phase cultures grown in liquid RO NaCl medium under dark aerobic conditions at 30°C, by phase contrast (Zeiss Axioskop 2) and electron microscopy (Hitachi H7600 TEM or Zeiss EM 10C TEM). Negative stains were performed by treating cells with 1.0% aqueous uranyl acetate. For thin sections, the bacteria were embedded in Epon after fixation with 2.5% glutaraldehyde and 1.0% osmium tetroxide (Kellenberger et al. 1958).

Phylogenetic analysis

Extraction of genomic DNA, PCR amplification of 16S rRNA gene segments, and sequence analyses were performed as previously reported (Rainey et al. 1996). Sequences were aligned using a sequence alignment editor (Bioedit). Phylogenetic trees were constructed using the algorithms contained in TREECON for Windows, and bootstrap values were determined using the TREECON package.

Photosynthetic apparatus

The methods of membrane and LH-RC complex isolation by sucrose density gradient centrifugation were previously described (Yurkov et al. 1993, 1994a). Cells were grown in a minimal glucose medium containing in g l^{-1}: $MgSO_4$, KH_2PO_4, 0.3; NH_4Cl, 0.3; KCl, 0.3; $CaCl_2$, 0.05; NaCl, 20.0; glucose, 1; yeast extract, 0.05, adjusted to pH 5.5, and incubated at 28°C with shaking in the dark. The photosynthetic electron transport study and titrations of the RC primary electron donor and primary electron acceptor were performed as previously described (Yurkov et al. 1998a, b; Alric 2005).

Results and discussion

Isolation

To answer the question about endemism of *C. bathyomarinum*, water samples were collected in the vicinity of the Juan de Fuca Ridge from different depths including the surface (see Materials and methods). One hundred sixty-three strains of yellow, bright yellow, citron-yellow, peach, pink, and red pigmented, aerobic heterotrophic bacteria (representative of all pigmented morphotypes present on RO NaCl and CHU 10 plates) were isolated in pure culture and screened for the presence of BChl. Of these 163 strains, 13 strains similar in color, bright-yellow to citron-yellow, were found to produce BChl *a*. Table 2 describes sampling location and depth for each strain.

Table 2 Isolates of aerobic BChl *a*-containing bacteria

Strain	Depth (m)	Site[a]	Medium	Dilution[b]
C6	1,500	1	CHU 10	10^{-1}
C7	1,500	1	CHU 10	10^{-1}
C46	1,500	1	CHU 10	10^{-1}
C8	2,164	1	CHU 10	10^{-1}
N25	2,164	1	RO	10^{-1}
C12	500	2	CHU 10	10^{-1}
C14	500	2	CHU 10	10^{-1}
N34	500	2	RO	10^{-1}
N78	1,000	2	RO	10^{-1}
C23[c]	Surface	4	CHU 10	10^{-1}
C26	1,500	4	CHU 10	10^{-2}
N56	1,500	4	RO	10^{-1}
N48	2,379	4	RO	10^{-1}

[a] See Table 1 for site location

[b] Dilution at which strain was isolated

[c] All strains form citron-yellow colonies, except C23, which is bright-yellow

Interestingly, BChl *a*-containing strains were found throughout the water column at sites directly above deep ocean hydrothermal vent fields, although no such strains were found at sites offset from the vents (Tables 1 and 2). Isolates of APB were obtained from all depths tested, and although only one strain originated from surface waters, it should be noted that total heterotroph numbers were far higher (5–10×) in samples taken from the surface than from deep waters. This may have directly influenced our ability to culture APB using these methods. Heterotrophs could out-compete APB on the relatively rich medium and thus overcrowd agar plates, preventing development of slower growing APB colonies. Additionally, production of antagonistic compounds by several marine bacteria including some *Pseudoalteromonas* species has been reported (Holmstrom and Kjelleberg 1999). Presumably colonies that produce antimicrobial agents, grown on an agar surface, would inhibit the growth of other bacterial species, including APB.

Anaerobic agar deeps were incubated for 30 days at 30°C under continuous illumination, resulting in the growth of non-pigmented colonies. No pigmented phototrophic colonies were detected in the three media tested, which however does not definitively confirm their absence in samples, as the majority of species in any given environment are frequently considered to be uncultivable.

Phenotypic and physiological properties

To recognize the taxonomical relationship of new strains to *C. bathyomarinum*, 13 aerobic yellow BChl *a* producing strains were isolated in pure culture and subjected to further characterization. All had similar absorbance characteristics with an in vivo BChl peak at 865–867 nm indicative of a photosynthetic LH I complex, and a peak at 801–802 nm indicative of a RC. The spectral properties of these strains correlate well with that of *C. bathyomarinum* JF-1 which also has a relatively small amount of BChl giving rise to peaks at 867 and 800 nm (Yurkov et al. 1999). The magnitude of the absorbance peaks attributed to BChl in new isolates is extremely small in comparison to that typically seen for APB (Fig. 1). Comparative absorbance spectra of strain C8 and *Erythrobacter litoralis*, strain T4, show that deep ocean BChl-containing organisms produce far less photosynthetic units than the most commonly cultured APB (Fig. 1).

Absorbance spectra also show peaks attributed to the presence of carotenoids at 434, 457–458, and 487 nm in all strains (Fig. 1) again in accordance with those of *C. bathyomarinum* (Yurkov et al. 1999). Analysis by HPLC revealed the major carotenoid present in 12 isolated strains and JF-1 to be erythroxanthin sulfate. Interestingly strain C23, the only isolate from surface waters, possessed

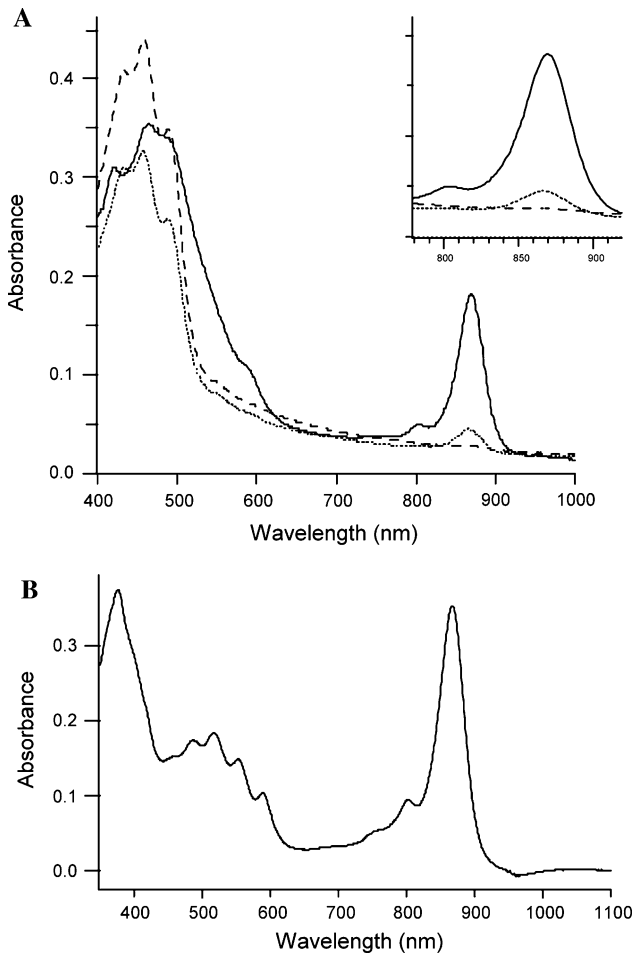

Fig. 1 Absorption spectra of (**A**) strain JF-1 grown in rich organic medium (dashed line) and in minimal glucose medium at pH 5.5 (dotted line) show a peak at about 865 nm indicating the presence of BChl incorporated into a light-harvesting I complex. The spectrum of *E. litoralis* strain T4 (solid line) is given for comparison. Inset shows the 800–900 nm region in greater detail. (**B**) Partially purified light-harvesting-reaction center complexes of strain C8 (see text)

caloxanthin sulfate as the major carotenoid, even though the spectral characteristics are identical to that of the deep ocean strains.

Four of the strains (C6, C8, C23, C46), as well as *C. bathyomarinum*, JF-1 were tested for their ability to utilize organic substrates, and hydrolyze certain macromolecules, and for their tolerance to high levels of NaCl (Table 3). All strains were tolerant to a wide range of salinity with robust growth occurring from 0% to 8% NaCl. Reduced growth occurred up to 14% NaCl for C23, and up to 16% for the other strains. The new isolates utilized a somewhat greater variety of organic substrates than JF-1 (Yurkov et al. 1999). Strains C6, C8, C23, and C46 grew heterotrophically on acetate, pyruvate, glutamate, butyrate, glucose, and ethanol as sole source of carbon. Additionally C23 is capable of growth on succinate. All strains hydrolyze gelatine and Tween 60, as does JF-1; additionally C23 and C46 exhibit weak hydrolysis of starch.

None of the strains grew under anaerobic conditions in the presence or absence of light. BChl *a* was produced only under dark aerobic conditions as revealed by characteristic absorbance in the 800 and 870 nm regions, which is typical for APB (Yurkov and Beatty 1998a).

Morphology and cytology were examined in three selected strains (C6, C8, and C46). Cells of each strain were similar, consisting of short (0.6 × 1.6 μm) to long (0.6 × 2.4 μm) Gram-negative, motile rods, which occurred singly or in rosette-like formations (Fig. 2A), and often formed Y-shaped cells (Fig. 2B). The cytoplasmic membrane was visible, but no intracytoplasmic membranes (ICM) of the type usually implicated to hold the photosynthetic apparatus in typical anaerobic anoxygenic phototrophs were detected. The absence or poor development of ICM is characteristic of APB, and it is suggested that the poorly developed photosynthetic apparatus is located directly in the cytoplasmic membrane (Yurkov and Beatty 1998a). No inclusions or storage materials were observed. The mode of cell division varies as budding (Fig. 2C), binary, and trinary fissions (Fig. 2B) were detected. As in JF-1, negative stains show the production of as yet unknown cell connective materials (Fig. 2D), and reveal the presence of one or more polar to sub-polar flagella.

Phylogenetic analysis

To determine phylogenetic relationships and to confirm a genospecies identity with *C. bathyomarinum*, genomic DNA was extracted from eight BChl *a*-containing strains. Analysis of almost complete (1,391 nucleotides) 16S rRNA genes shows that seven deep ocean strains (C6, C8, C14, C46, N25, N48, N78) are closely related to *C. bathyomarinum*, JF-1 (99.7–99.8% sequence similarity). They form a distinct grouping with strain JF-1 supported by 100% bootstrap confidence, which confirms their identity as members of this species (Fig. 3). Strain C23, the lone phototrophic isolate from surface waters, is more distantly related to *C. bathyomarinum*, falling within the *Erythrobacter–Porphyrobacter–Erythromicrobium* cluster of the class *Alphaproteobacteria*, related most closely to *E. litoralis* (96.9%). This suggests that C23 is a new species within the genus *Erythrobacter*.

Photosynthetic apparatus

As stated in the introduction, the capability of *C. bathyomarinum* to use infrared light emitted by black smokers and its photosynthetic competence in general were at the center

Table 3 Comparative physiology of deep ocean aerobic phototrophs

Test	Strain				
	JF-1	C6	C8	C23	C46
Growth at NaCl %					
0	++	++	++	++	++
2	++	++	++	++	++
4	++	++	++	++	++
6	++	++	++	++	++
8	++	++	++	++	++
10	+	++	+	+	+
12	+	+	+	+	+
14	+	+	+	W	+
16	W	W	W	−	W
20	−	−	−	−	−
Organic sources					
Acetate	W	+	+	+	+
Pyruvate	−	+	+	+	+
Glutamate	+	+	+	+	+
Butyrate	+	++	++	++	++
Malate	−	−	−	−	−
Lactate	−	−	−	−	−
Citrate	−	−	−	−	−
Succinate	−	−	−	+	−
Formate	−	−	−	−	−
Glucose	W	++	++	++	++
Fructose	−	−	−	−	−
Ethanol	−	+	+	+	+
Methanol	−	−	−	−	−
Yeast extract	+	++	++	++	++
Hydrolysis of					
Gelatin	+	+	++	+	+
Starch	−	−	W	−	W
Tween 60	+	+	++	W	+
Major carotenoid pigment	Erythroxanthin sulfate	Erythroxanthin sulfate	Erythroxanthin sulfate	Caloxanthin sulfate	Erythroxanthin sulfate

+, Positive; −, negative; W, weak positive

of polemic discussions. To solve this puzzle, we selected the type strain of the species, JF-1, and the newly isolated C8 for study of the photosynthetic apparatus.

Because production of BChl in JF-1 was increased when cells were grown in a minimal glucose medium at pH 5.5 (Fig. 1A), these conditions were used for all further experiments. The increased production of BChl observed in minimal media agrees well with the idea that APB use photosynthesis as an auxiliary energy source under nutrient-deprived (oligotrophic) conditions, allowing the organism to satisfy a portion of its energetic requirements using light energy and thus allowing it to better compete with non-phototrophic heterotrophs.

To confirm the organization of the photosynthetic apparatus in *C. bathyomarinum*, photosynthetic units from strains JF-1 and C8 were isolated after disruption of cells using a French press, treatment of photosynthetic membranes with detergent, and subsequent sucrose density gradient centrifugation. Fractions containing the LH-RC complex were further purified by anion exchange chromatography. Absorbance spectra of purified fractions confirmed the presence of a LH I complex with a major absorbance peak at 867 nm and a photosynthetic reaction center peak at 801 nm (Fig. 1B). No evidence of a LH II complex was found.

To gauge the ability of *C. bathyomarinum* to carry out photosynthetic electron transport and thus produce cellular energy from light, we examined photoinduced electron transfer in JF-1 whole cells, photosynthetic membrane fragments, and purified LH-RC complexes. Although photochemistry was clearly observed in both whole cells and membrane fragments, the signal strength was weak.

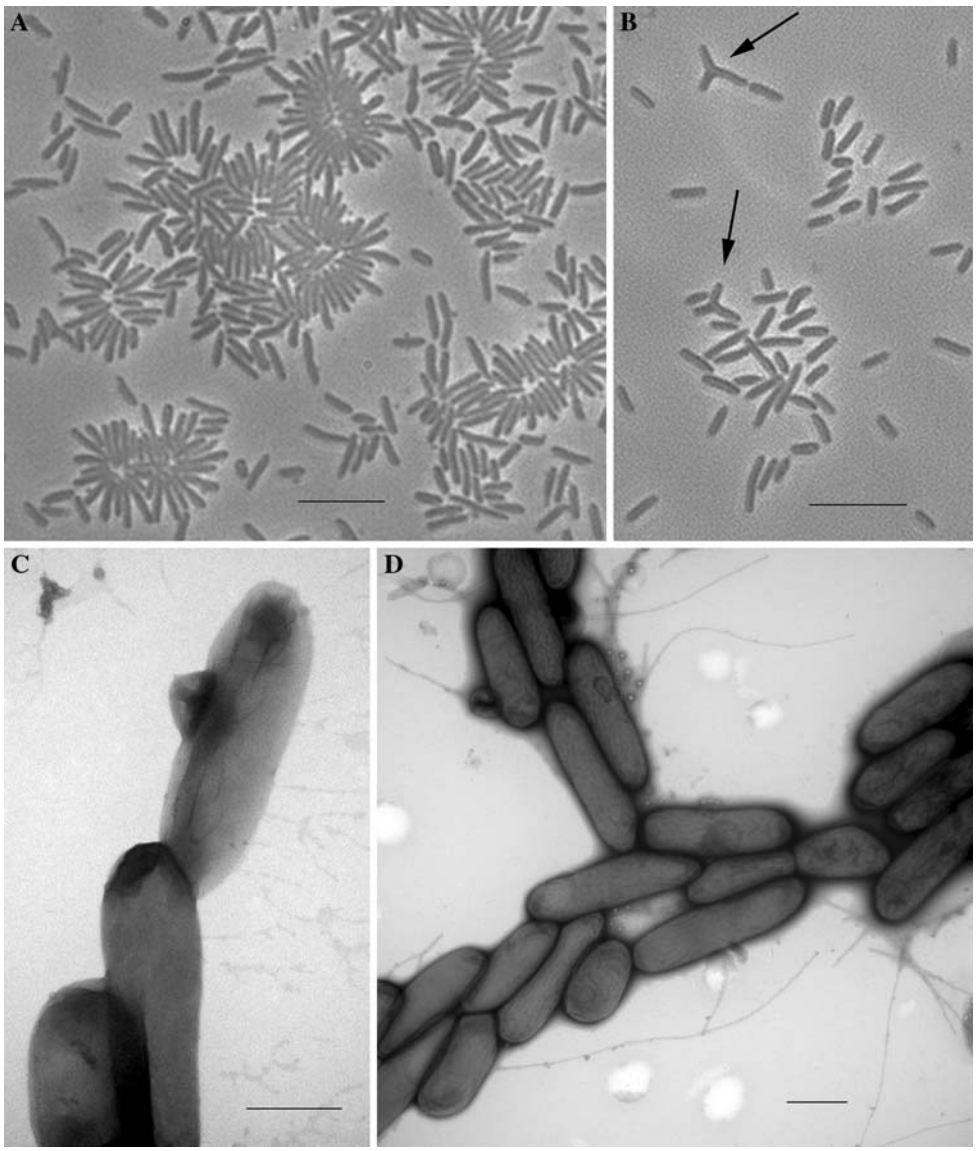

Fig. 2 Phase contrast microscopy of strain C8 shows (**A**) the formation of interesting rosette-like conglomerates of cells, and (**B**) pleomorphism, characteristic of the deep ocean *C. bathyomarinum*, including the production of "Y-cells" (indicated by arrows). Electron microscopic negative stains of (**C**) strain C6 show replication by budding, and (**D**) connective materials produced by strain C46. Bars: (**A**, **B**) 5 μm, (**C**, **D**) 500 nm

This is likely due to the great amounts of yellow carotenoids, absorbing robustly in the blue light region, and the relatively low quantity of photosynthetic units produced by JF-1. Light-induced absorbance changes recorded in whole cells of JF-1, taken under aerobic conditions (Fig. 4), showed absorbance troughs at both 555 and 605 nm, 50 μs after an excitation flash, indicating that part of P^+ has already been reduced by a cytochrome (cyt) at this time (Yurkov et al. 1998b). This trough at 555 nm shifted to 552 nm by 7 ms after excitation. Since absorbance spectra measured in membrane fractions or purified LH-RC complexes have the same trough at 555 nm after 50 μs but with no detectable shift to shorter wavelength on a longer time scale, we propose that the absorbance changes observed in whole cells are attributed to the oxidation of the RC-bound cyt, followed by its reduction by a soluble cyt *c* acting as secondary electron donor (Yurkov et al. 1998b). This RC-bound cyt displays unusual activity concerning the re-reduction of the RC primary electron donor, presenting a halftime of about 500 μs (Fig. 4), which is very slow for a re-reduction by a RC-bound tetraheme cyt, which usually occurs within a few microseconds (Shopes et al. 1987). Once oxidized, the rate of re-reduction for the soluble cytochrome (presumably by the cyt bc_1 complex) displays a halftime of about 80 ms, indicating that under aerobic conditions JF-1 is indeed capable of photosynthetic cyclic electron transfer. The rate of this cyclic electron transfer is slow but is comparable to the rate of cyclic electron transfer found in some anoxygenic phototrophs (Shopes et al. 1987). Under anaerobic conditions the case is different. No light-induced absorbance changes are observed after an excitation flash, meaning that the electron carriers did not undergo redox changes and photosynthetic electron transfer did not occur. From this set of experiments we can

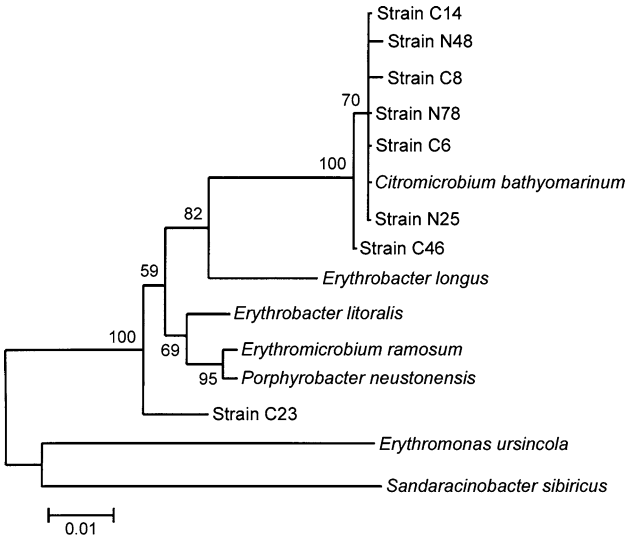

Fig. 3 Unrooted phylogenetic tree showing the position of eight isolated strains among the most closely related aerobic phototrophic species, within the α-4 subclass of the *Proteobacteria*. Bootstrap values, based on 500 resamplings, are indicated. Bar, 1 substitution per 100 sequence positions

conclude that *C. bathyomarinum* can be photosynthetically active if infrared light is available only under aerobic conditions.

To understand why photosynthetic electron transfer occurs only under aerobic conditions the redox potentials of the RC primary electron donor (P/P^+) and primary electron acceptor (Q_a/Q_a^-) were titrated in membrane fractions. To this end we have measured the light-induced absorption changes at 605 nm as a function of redox potential (Fig. 5). The midpoint redox potential of the Q_a/Q_a^- was found to be +80 mV versus a standard hydrogen electrode and the midpoint potential of the P/P^+ was around +470 mV. The positive signal detected at 100 ns for flash excitation for redox potentials lower than 0 mV is consistent with the decay of a carotenoid triplet state (Alric 2005). Previously it was demonstrated that the photosynthetic electron transfer system of other APB such as *Roseobacter denitrificans*, *E. litoralis*, *Erythromicrobium ursincola*, *Sandaracinobacter sibiricus*, and *Roseococcus thiosulfatophilus* is inoperative in anaerobic cells, presumably due to the high midpoint potential of Q_a (Yurkov and Beatty 1998a). This rather high midpoint potential of Q_a explains why there is no charge separation under anaerobic conditions and, thus, may be one of the reasons why *C. bathyomarinum*, as well as other APB, is incapable of anaerobic photosynthetic growth.

In summary, we show that strains of *C. bathyomarinum* are readily cultured from deep ocean waters above the Juan de Fuca Ridge in the Pacific Ocean. The inability to successfully culture APB from deep ocean waters at sites

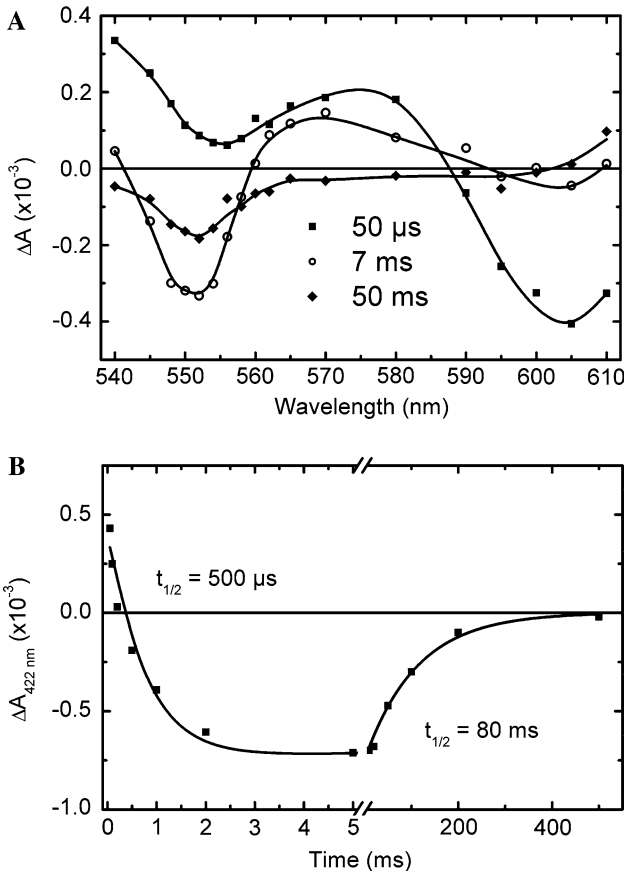

Fig. 4 (**A**) Flash-induced absorbance spectra of JF-1 whole cells measured under aerobic conditions. The trough observed at 555 nm, 50 μs after excitation, is attributed to photooxidation of a RC-bound cytochrome. The shift to 552 nm observed at 7 ms is attributed to the oxidation of a soluble cytochrome, indicating light-induced cyclic electron transfer. ΔA, change in absorbance. (**B**) A kinetic of electron transfer demonstrates re-reduction of the primary electron donor and subsequent re-reduction of the bound cytochrome. $\Delta A_{422\ nm}$, change in absorbance measured at 422 nm

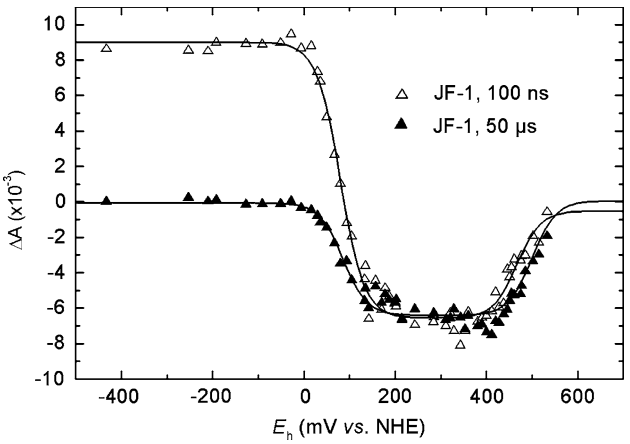

Fig. 5 Redox titration of the reaction center primary electron donor (P/P^+) and primary electron acceptor (Q_a/Q_a^-) performed on membrane fraction of strain JF-1. ΔA, light-induced absorbance changes were measured at 605 nm. NHE, standard hydrogen electrode

offset from hydrothermal vents remains a mystery. Possibly nutrients ejected in vent effluents rise through the water column, thus supporting a different microflora in those particular locations. The presence in deep ocean waters and absence of *Citromicrobium*-like APB in surface waters despite the detection of *Erythrobacter*-like strains suggest that the habitat of *C. bathyomarinum* may be endemic to the lightless deep ocean. However some factors, including competition by other heterotrophs and overcrowding of nutrient rich agar plates by the abundance of microbial cells in surface waters, might account for our inability to isolate *C. bathyomarinum* from those samples. *C. bathyomarinum* is shown to use infrared light for photosynthetic electron transfer under laboratory conditions; however, its ability to grow phototrophically at deep ocean vents remains undetermined.

The only APB that we isolated from surface waters, strain C23, shares several physiological properties with *C. bathyomarinum*; however it has a different pigment composition and is phylogenetically distinct, appearing more closely related to *Erythrobacter*, a genus also isolated from marine surface environments. The low levels of BChl produced by C23 (as well as all strains of *C. bathyomarinum*), and the effects of culture conditions on BChl production, suggest that identification of APB from the Pacific Ocean should include a thorough analysis of pigments lest strains producing low or variable amounts of BChl be overlooked.

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