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The SAR92 Clade: an Abundant Coastal Clade of Culturable Marine Bacteria Possessing Proteorhodopsin▽

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Proteorhodopsin (PR) is a protein that is abundant in marine bacterioplankton. PR is hypothesized to be a light-dependent proton pump, thus creating a proton gradient that can be used for energy production without electron transport. Currently, the only culture that has been reported to possesses PR is the highly abundant alphaproteobacterium "*Candidatus* Pelagibacter ubique" (SAR11 clade), but surprisingly, its growth in batch culture was not enhanced by light. Here, we present the first cultured gammaproteobacterium that possesses a PR gene. Genome sequencing and analysis of HTCC2207 showed that the PR gene is present as a lone transcriptional unit directly followed by an operon containing genes that are presumably involved in the synthesis of retinal, the chromophore of PR. Half-time decay times of different PR intermediates in native HTCC2207 cells ranged between 2 and 15 ms, and the absorbance maximum of PR was determined to be 528 nm. Proteorhodopsin was identified in three additional strains, using a specific PCR assay on other cultured members of the SAR92 clade. Phylogenetic analyses of the PR genes determined that they form a deeply rooting cluster not closely related to any PR genes recovered so far. Fluorescence in situ hybridization and RNA blots showed that the SAR92 clade reaches up to 10% of the total bacterial population in surface waters close to the Oregon coast and decreases over depth and distance from the shore. Although the growth of HTCC2207 is limited by the amount of available carbon that is present in the medium applied, these cultures do not grow at higher rates nor do they have higher growth yields when incubated under light.

Heterotrophic marine bacterioplankton play an important role in global nutrient cycling (4). Little is known about how heterotrophic, oligotrophic bacteria manage to thrive in low carbon concentrations of the open oceans. Recent metagenomic evidence from the Monterey Bay area (5) and the Sargasso Sea (29) showed that many marine bacteria possess genes for proteorhodopsin (PR), a protein homologous to the bacteriorhodopsin found among halophilic *Archaea* (19).

Like bacteriorhodopsin, PR was shown to be a light-dependent proton pump, thus raising the hypothesis that it can create an additional proton motive force without the cost of oxidizing carbon compounds (5). This would theoretically lead to a more effective use of organic carbon, which may exist at limiting concentrations in most marine environments (4).

Although the function of PR as a proton pump, as well as its expression in "*Candidatus* Pelagibacter ubique," was recently demonstrated, interestingly, no differences were detected between the growth rates and growth yields under light incubation conditions versus those under dark incubation conditions, raising speculations about the function of PR in marine bacteria (11).

In addition to its abundance, the diversity of PR genes in different marine ecosystems also seems to be extensive (6, 9, 25, 27, 29), indicating the importance of this protein to marine bacteria. Sabehi et al. estimated that about 13% of the bacteria in the photic zone of the Mediterranean Sea possess a proteorhodopsin gene (26). The expression of PR genes in seawater was demonstrated by transient expression studies of membrane preparations from Monterey Bay (6) as well as by a proteomics approach used in surface waters off the Oregon coast (11).

Most of the organisms harboring PR remain unidentified, but more and more evidence for important players accumulates. Lateral gene transfer most likely played an important role in the wide distribution of PR among different phylogenetic groups of marine prokaryotes (10). Recently, PR genes have been observed in the marine group II of *Euryarchaeota* (10), abundant archaea closely related to the order *Thermoplasmales*. The bacterial groups conclusively shown to have a PR gene include the most successful groups of proteobacteria in marine environments, the SAR86 clade of *Gammaproteobacteria*, where 16S rRNA genes were found together with a PR gene on bacterial artificial chromosome (BAC) clones (5, 25), and the SAR11 clade of *Alphaproteobacteria*, with the cultured representative, "*Candidatus* Pelagibacter ubique," whose genome sequence includes a PR gene (11). To date, "*Candidatus* Pelagibacter ubique" is the only published example of a marine bacterium in culture which has a PR gene.

In this study, we demonstrate that cultured members of the marine SAR92 clade of *Gammaproteobacteria* possess a PR gene. Genome sequencing of an isolate of this clade from the Oregon coast, HTCC2207, revealed a PR gene as well as an operon containing genes assumed necessary for the biosynthesis of retinal, the chromophore of the holoenzyme (20). The occurrence of PR in other isolates from the same cluster, the abundance of the SAR92 clade, the physical parameters of PR,

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and the effect of light on the growth behavior of HTCC2207 in carbon-limited media were investigated.

MATERIALS AND METHODS

Isolation and growth conditions of SAR92 strains. All strains presented in this study were isolated from surface waters 5 miles off the Newport, Oregon, hydroline (44°N; hydroline NH-5) using dilution-to-extinction culturing in low-nutrient medium as described previously (8). Cultures were grown in shaking incubators (Multitron, Laurel, MD) at 16°C and 60 rpm in capped 250-ml polycarbonate flasks containing 75 ml LNHM (22) as the medium. If stated, light was supplied as cool-white light at an intensity of 140 μmol m^{-2} s^{-1} for 12 h or 24 h per day.

Genome draft of HTCC2207. The genome of HTCC2207 was sequenced by the J. Craig Venter Institute (www.venterinstitute.org) as a part of the Moore Foundation Microbial Genome Sequencing Project (http://www.moore.org/microgenome). The annotated unclosed genome draft consists of seven contigs containing about 2,900 open reading frames and is publicly available in GenBank (accession number AAPI00000000, version GI:90333150).

Proteorhodopsin PCR assay in isolates of the SAR92 clade. DNA was extracted from 200 μl of cultures in stationary phase, using a QIAGEN DNeasy kit (QIAGEN, Valencia, CA) following the manufacturer's instructions. DNA was eluted in 70 μl of sterile water and stored at −20°C. PCR primers were deduced from the PR gene sequence of HTCC2207 as follows: PR_2207_For 5′-ATGACAAACAACTTATCCGCTA-3′ and PR_2207_Rev 5′-TTACTCTGAATCAGCTACTGCTG-3′. Annealing temperature was determined empirically. Reaction mixtures (100 μl) were created using 1 μl extracted DNA as template, 200 nM of each primer, 200 μM of each deoxynucleoside triphosphate, 2.5 mM $MgCl_2$, 2 units of *Taq* polymerase (MBI Fermentas, Hanover, MD), and 1× of the respective buffer provided by the manufacturer. Routinely, reactions were amplified in a thermocycler (PTC 200; MJ Research, Ramsey, MI) with the following protocol: an initial denaturing step for 3 min at 94°C, 40 cycles of 30 sec at 94°C, 45 sec at 40°C, and 1 min at 72°C, followed by a final extension step for 5 min at 72°C. PCR amplicons were cleaned up using a QIAquick PCR purification kit (QUIAGEN, Valencia, CA) and sequenced with both of the above-mentioned primers at the CGRB Core Laboratories at Oregon State University. Sequences were checked, manually trimmed, and assembled using DNAStar (GATC Biotech, Constance, Germany).

Phylogenetic analysis of PR sequences. The PR nucleotide sequence of HTCC2207 was searched against the GenBank database and the Sargasso Sea metagenomic data set (29) using BLAST (1), and 100 best hits from each were retrieved. The nucleotide sequences were translated to amino acid sequences and aligned using ClustalW (www.clustalw.com) as implemented in ARB (www.arb-home.de). For rooting phylogenetic trees, rhodopsins of *Gloeobacter violaceus* and *Pyrocystis lunula* were included in the analysis. A filter of 248 unambiguously aligned amino acid positions was used for all further analyses. A phylogenetic tree was calculated using a maximum-parsimony program, ProtPars, of the PHYLIP package (24). Bootstrapping with 1,000 replicates was performed using SeqBoot as integrated in PHYLIP.

PCR and sequencing of 16S rRNA genes. PCR of 16S rRNA genes was performed according to a method described by Connon and Giovannoni (8). PCR products were cleaned and handled as described above. Sequencing was performed using primer 27Fb as described previously (8). Phylogenetic analysis of 16S rRNA genes was performed with ARB (www.arb-home.de), using a database of about 50,000 16S rRNA sequences. New sequences were added to the ARB database and aligned by using a Fast Aligner tool implemented in ARB; automatic alignments were checked and manually corrected where necessary. A phylogenetic tree was calculated by using fastDNAmL, a maximum-likelihood method implemented in ARB. The stability of the branching pattern was tested using the neighbor-joining and maximum-parsimony (DNAPARS) methods included in the PHYLIP package as implemented in ARB. The reproducibility of the branching pattern was confirmed by bootstrap analysis using the maximum-parsimony algorithm and the program SeqBoot from the PHYLIP package (1,000 replications). In all phylogenetic analyses, only those positions of the alignment that were identical in at least 50% of all sequences were used.

Probe design. An oligonucleotide probe for all SAR92 clade 16S rRNA sequences presented in this study was designed and checked for specificity by using the respective functions of ARB software. Probe SAR92_627 (5′-CAGACAGTTCTAACTGCAGTTCC-3′) had no mismatches with any members of the SAR92 clade (except for the original SAR92 sequence, which seems to be of minor quality and was omitted from the analysis) but did have at least three mismatches with all other sequences in the ARB database (ProbeMatch) and in GenBank (BLAST). The only exception was an unpublished clone that clustered together with *Teredinibacter* spp. sequences and had two mismatches. Fluorescence in situ hybridization (FISH) was performed as described in reference 3. For stringency controls, HTCC2207 cells were fixed for 30 min with 4% formaldehyde and filtered with polycarbonate membranes. A series of increasing formamide (FA) concentrations of 0%, 10%, 20%, 30%, and 35% was used in the hybridization buffer. While 0 to 20% FA resulted in strong hybridization signals, 30% and higher FA concentrations did not result in a specific signal. Controls performed using another strain of the oligotrophic marine *Gammaproteobacteria* group (HTCC2143 [7]), which contained three mismatches, did not result in a positive hybridization signal. The probe information has been submitted to probeBase (16; http://www.microbial-ecology.de/probebase).

FISH of bacterioplankton along the Oregon coast. Samples were collected along the Newport, Oregon, hydroline (44°N) in September 2004 as described previously (18). FISH analysis was performed as published previously (21). Briefly, 10-ml aliquots were fixed with 4% FA for 120 min and filtered onto 0.2-μm-pore-size polycarbonate membranes, and FISH was performed with probes EUB338 (2) and SAR92_627, using 20% FA as described above. Samples were hybridized for 120 min at 46°C followed by a washing step of 30 min at 48°C. Hybridized filters were stained with DAPI (4′,6′-diamidino-2-phenylindole), mounted on an objective slide, and embedded in low-fluorescence immersion oil. Counting was performed using an epifluorescence microscope equipped with a digital camera. At least 500 cells at random places were counted for each filter piece and dye.

RNA blotting. Samples were collected along the Newport, Oregon, hydroline (44°N) in May 2002 as described previously (18). Nucleic acids were isolated by cell lysis with proteinase K and sodium dodecyl sulfate, phenol-chloroform extraction, and cesium trifluoroacetate gradient centrifugation as described previously (13). Purified RNA was blotted and hybridized as described previously (30). Positive (HTCC2207) and negative (HTCC1062 and HTCC2143) control RNAs were processed and blotted similarly. Blots were probed with the general oligonucleotides 338RPL (17) and 1406R (15) and the SAR92-specific oligonucleotide SAR92_627.

Cloning and determination of absorption spectrum. Cloning of PR was performed with primers PR2207For (5′-ACCATGGGTACAAACAACTTATCCGCTAGC-3′) and PR2207Rev (5′-TTTCTCTGAATCAGCTACTGC-3′) as described previously (11). Attenuance spectra of proteorhodopsin were acquired from standard 1-cm cuvettes with a Cary 300 spectrophotometer (Varian Instruments), using membrane preparations of *Escherichia coli* cells expressing the cloned proteorhodopsin protein. The absorption spectra depicted in Fig. 2A were obtained by subtracting cubic baselines, determined by fitting the attenuance values from 350 to 375 nm and from 600 to 650 nm, which represent scattering and absorption, respectively, of the membrane suspensions, exclusive of proteorhodopsin.

Transient absorption spectra. Transient absorption spectra (Fig. 2B) of light-grown HTCC2207 cells were acquired with a laboratory-constructed apparatus. Each flash-induced transient absorption trace was obtained by averaging 48 acquisitions, one every 5 s. The excitation flash (10-mJ pulse) at 532 nm was supplied by a neodymium-yttrium aluminum garnet (Nd:YAG) laser (Quanta-Ray GCR-11; Spectra-Physics). The probe beams were supplied by a 30-W quartz-tungsten-halogen lamp (model HLX64265; Osram) shining through a Photon Technology monochromator (model 01-001). The samples were held in a 1-cm-path-length fused silica fluorometer cuvette (NSG Precision Cells) inserted into a temperature-controlled cuvette holder (Flash 200, Quantum Northwest). The probe beams transmitted through the samples were passed through a notch filter (model RNF-532.0; CVI Laser) and a Jobin-Yvon model HR-320 monochromator (Instruments, S. A.) and detected with a red-sensitive photomultiplier tube (model R928; Hamamatsu). Traces were acquired and stored on a digital oscilloscope (WaveSurfer 424; LeCroy). Polarizations of the excitation and probe beams were set to the "magic angle" orientation to eliminate absorption artifacts due to molecular motion.

RESULTS

Phylogeny of the SAR92 clade. The SAR92 clade is a monophyletic group of oligotrophic marine bacteria within *Gammaproteobacteria*, consisting almost exclusively of 16S rRNA clones and cultured organisms recovered from surface waters of the Oregon coast and the Sargasso Sea (Fig. 1) (7, 8, 12). The closest cultured relatives of the SAR92 clade are found within the genus *Microbulbifer* (14) but have more than 9% difference in the 16S rRNA gene sequence compared to that of

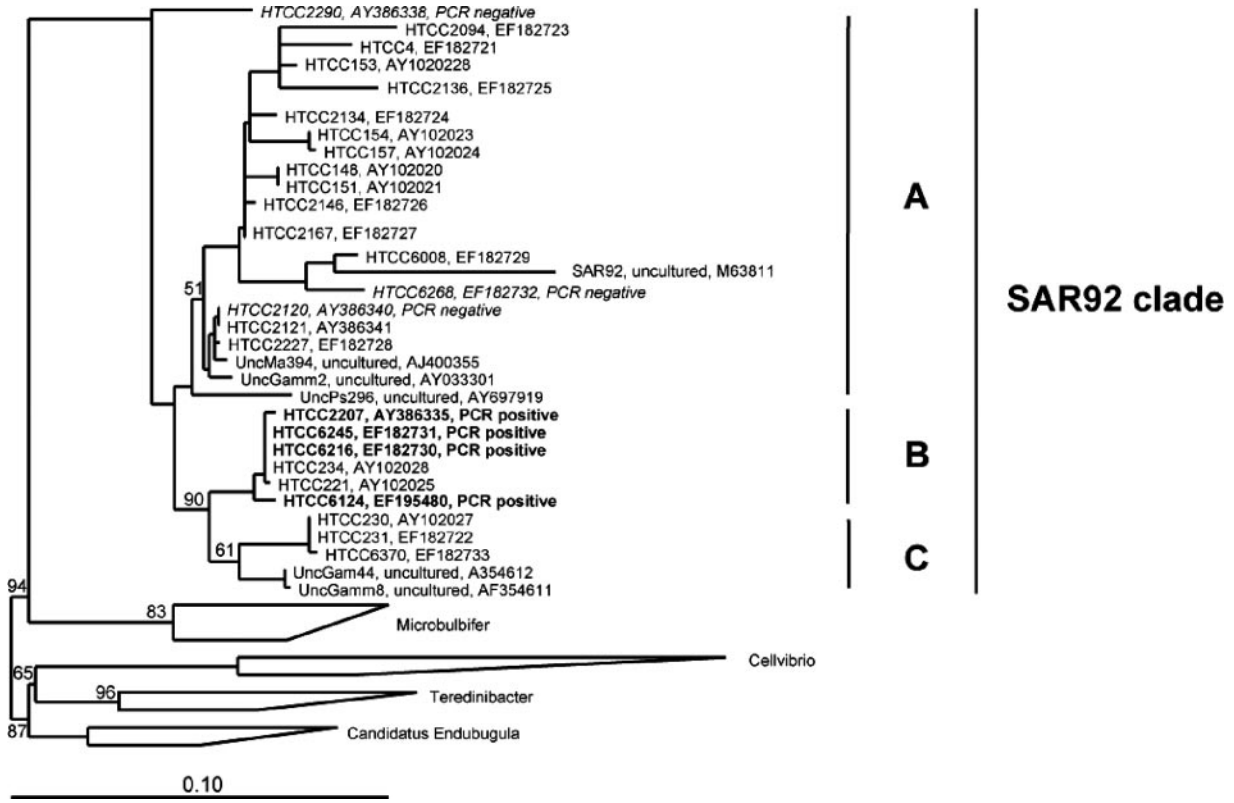

FIG. 1. Phylogenetic relationship of the SAR92 clade based on 16S rRNA gene sequences showing the positions of cultured strains (HTCC) among uncultured clones and closely related sequences. Strains with a proteorhodopsin gene are marked in bold, while strains that did not yield a PCR product in the PR assay are italicized. The tree is based on neighbor-joining analysis of the 16S rRNA gene sequences of all SAR92 sequences and a selection of reference organisms from the next-closest-related genera. Only those base positions that were identical in more than 50% of the aligned SAR92 sequences were included in the phylogenetic analyses. Bootstrap values over 50% are shown only when the respective branching order was supported in alternate phylogenetic analyses employing maximum-parsimony and maximum-likelihood algorithms. The bar represents 10 substitutions per 100 nucleotides.

the SAR92 clade, indicating that they represent a potential new genus within *Gammaproteobacteria* (Fig. 1). Dilution-to-extinction high-throughput culturing in medium based on natural seawater led to the isolation of several strains of that clade from the Oregon coast (7, 8 and Stingl and Giovannoni, unpublished results) which differ by up to 12% in their 16S rRNA genes (Fig. 1). Based on the phylogenetic analysis, the SAR92 clade can be divided into three subclusters (Fig. 1).

PR gene and genes responsible for the biosynthesis of retinal in HTCC2207. Initial genome sequencing of HTCC2207 resulted in a genome draft consisting of seven contigs with a total length of 2.6 Mbp and contained 2,390 open reading frames (Mellbye et al., submitted). The genome had one copy of PR upstream of genes annotated as *idsA*, *crtIBY*, *blh*, and *fni*, all of which encode proteins hypothesized to be involved in the biosynthesis of retinal, the chromophore of PR. Operon prediction using MolQuest (SoftBerry, Inc., Mt. Kisco, NY) software indicated that the PR is a lone transcriptional unit, whereas the genes for the biosynthesis of retinal are organized in an operon. The translated PR protein has 229 amino acids, including Asp102 and Glu113, which are hypothesized to act as proton acceptor and donor, respectively, of the protein (5). Position 105, which has been demonstrated to affect absorbance wavelength (6), is occupied in the SAR92 PRs by a lysine, indicating maximal absorbance in green light. This prediction was checked by cloning and overexpressing the gene in *E. coli.* The absorption maximum was 528 nm (Fig. 2A), determined by subtractive measurements of induced and noninduced *E. coli* cell membrane preparations carrying the PR gene.

PR PCR assay of other isolates from the SAR92 clade. Published primer sets for PR (26, 27) would not amplify the PR gene of HTCC2207 and did not yield products with other, unsequenced members of the SAR92 clade (data not shown). There were no closely related PR sequences in the databases, so that to check for PR in other isolates of the SAR92 clade, primers were deduced from the PR gene of HTCC2207. PCR was performed with low stringency to accommodate for possible mismatches in the primer region. Three additional strains possessing PR were identified (HTCC6124, HTCC6216, and HTCC6245; Fig. 1). These strains were closely related to HTCC2207 with 16S sequence similarities of 99 to 100% and clustered together with HTCC2207 in subcluster B. No PCR products were obtained for more distantly related strains of the SAR92 clade subcluster A (Fig. 1). The PR genes recovered in this study differed by 0.3 to 5% in nucleotide sequence, resulting in amino acid substitution at three positions. A phylogenetic analysis of the SAR92 PRs showed that they formed a

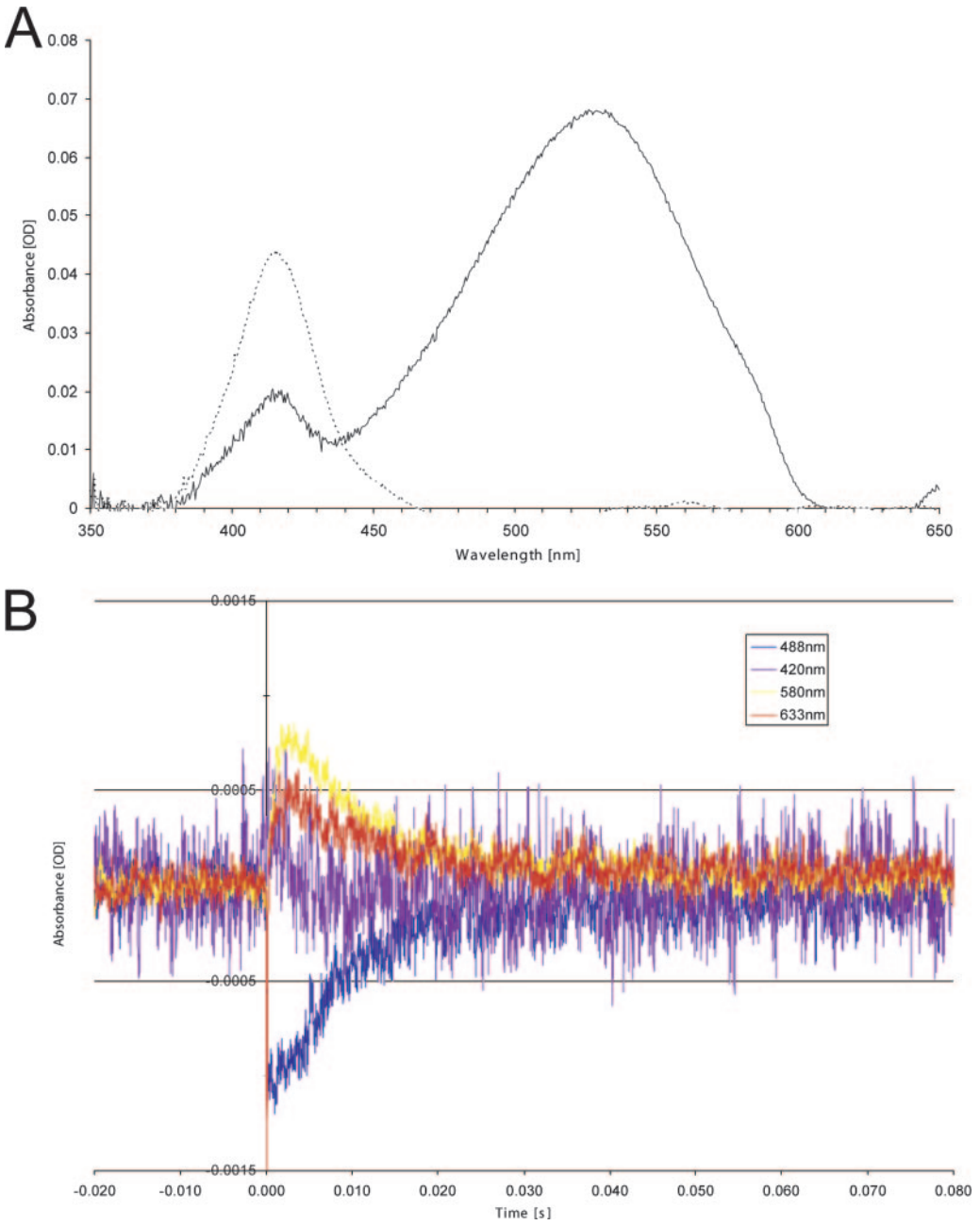

FIG. 2. (A) Absorption spectrum of induced (solid line) versus noninduced (dotted line) *E. coli* cell membranes carrying the proteorhodopsin of HTCC2207. The absorbance maximum was determined to be 528 nm. (B) Transient absorption measurements of light-grown HTCC2207 cells showing the fast decay times of PR intermediates at 420 nm, 488 nm, 580 nm, and 633 nm.

new cluster, not closely related to any of the PR genes recovered so far (Fig. 3). A clustering of the SAR92 sequences with environmental sequences as well as the alphaproteobacterial BAC clone H2C01, as indicated by the maximum-parsimony tree shown in Fig. 3, was not corroborated using maximum-likelihood and neighbor-joining algorithms (data not shown). The closest PR sequences in public databases were 68% identical in their amino acid sequences and were retrieved from the Mediterranean Sea and the Sargasso Sea.

Abundance of the SAR92 clade. The abundance of the SAR92 clade along a transect off the Oregon coast was investigated by FISH and RNA dot blots. Results of both methods, performed with samples collected at different seasons and in different years, were in good agreement and showed that this clade can comprise up to 10% of the total bacterial population (Table 1). The abundance was highest in samples collected from surface waters 4 to 10 miles off the coast but decreased in deeper waters and farther than 10 miles from the coast (Table 1), reaching background levels at 127 miles off the Oregon coast.

Transient absorption measurements. The 1/*e* decay time of the 488-nm transient trace, which to a good approximation is

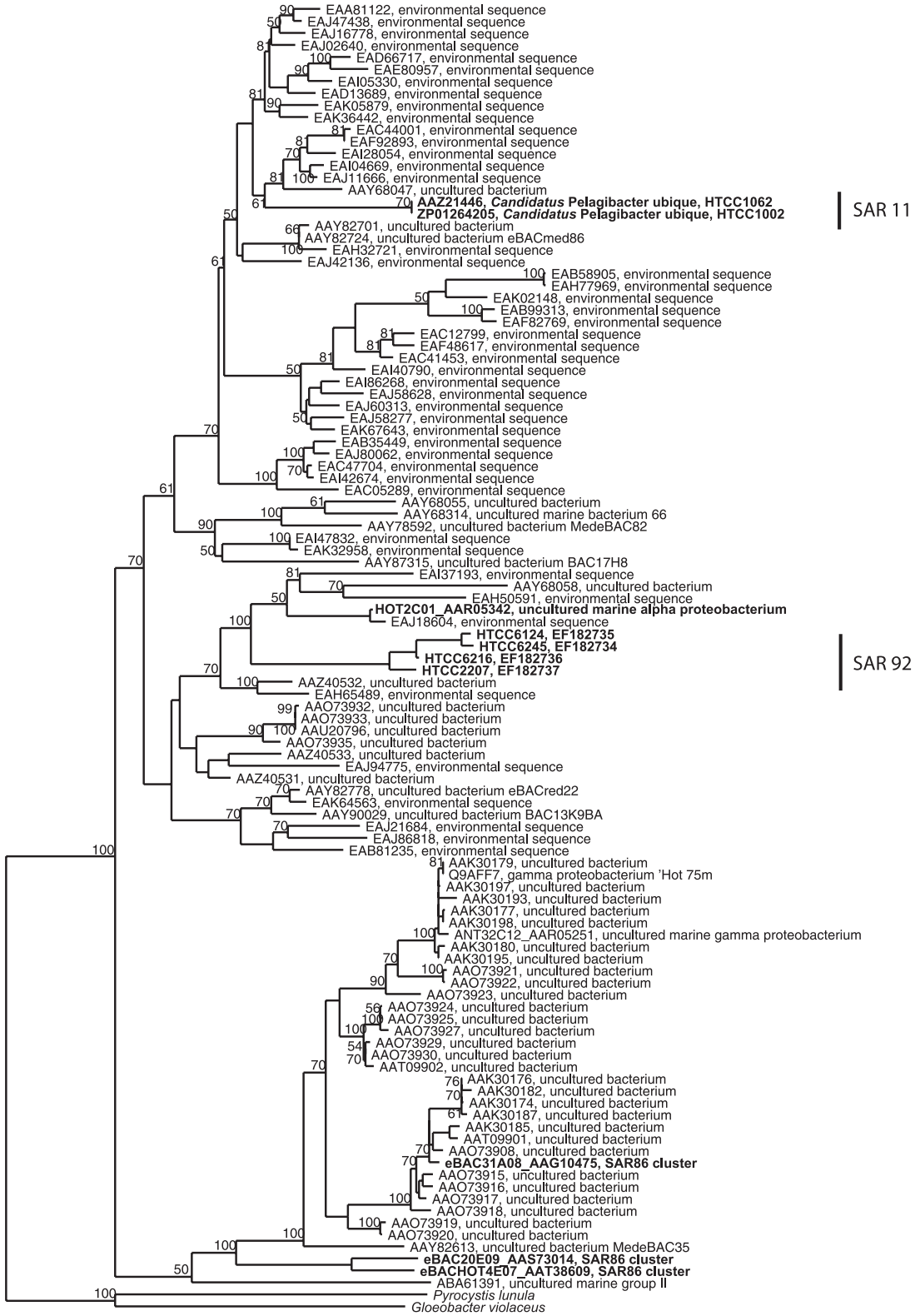

FIG. 3. Maximum-parsimony tree showing the phylogenetic position of PR genes of cultured bacteria (SAR92 and SAR11) relative to that of PR genes of bacteria with known 16S rRNA gene sequences (bold) and environmental sequences. Bootstrap values over 50% (SeqBoot, 1,000 replications) are shown.

TABLE 1. Abundance of the SAR92 clade along a transect off the Newport, Oregon, hydroline as determined by RNA blotting and FISH[a]

Hydroline location[b]	Depth (m)[c]	RNA blot (% of total RNA)[d]	FISH fluorescence counts by:	
			EUB probe (%)	DAPI staining (%)
NH-1	25	ND	0.3	0.2
NH-4	5	ND	10.4	6.5
NH-4	20	ND	7.3	5.0
NH-10	15	9.0	ND	ND
NH-25	15	5.1	ND	ND
NH-35	15	1.7	ND	ND
NH-35	250	1.2	ND	ND
NH-55	500	0.5	ND	ND
NH-65	15	0.9	ND	ND
NH-65	500	0.7	ND	ND
NH-85	15	2.8	ND	ND
NH-85	500	1.1	ND	ND
NH-127	5	ND	0.2	0.1
NH-127	15	0.7	ND	ND
NH-127	50	ND	1.0	0.6
NH-127	500	1.9[e]	0.1	0.1
HTCC1062 control	NA	1.1	ND	ND
HTCC2143 control	NA	0.7	ND	ND

[a] All RNA samples were collected during May 2002. All samples for FISH were collected during September 2004. ND, not determined.
[b] The hydroline (NH) number indicates distance from the shore in miles.
[c] NA, not applicable.
[d] Calculated by comparison to RNA from a pure culture of HTCC2207.
[e] Low RNA yield in sample.

indicative of the regeneration time of ground-state proteorhodopsin after depletion due to absorption of photons from the 532-nm flash beam, was reported to be 13 ms for PR in light-grown SAR11 cells (11). However, more recent results under more carefully controlled conditions using an incandescent source for probe beams have determined this value to be about 30 ms (R. A. Desiderio and S. Sowell, unpublished results). Transient absorption measurements of light-grown HTCC2207 cells showed a 1/*e* decay time of 13 ms at 488 nm (Fig. 2B), about half that of SAR11. In addition, the trace at 420 nm shows the creation and decay of a short-lived intermediate, and the traces at 580 nm and 633 nm show the creation and decay of a longer-lived intermediate(s), as has been observed for proteorhodopsin in SAR11 cells (U. Stingl et al., unpublished results) and for recombinant proteorhodopsin (5).

Growth curves of HTCC2207 in response to light. To investigate the influence of light on the growth and yield rates of HTCC2207, triplicate flasks were grown under dark, constant light, and diurnal light conditions (12 h light, 12 h dark). There were no significant differences between the different conditions, neither in growth rate nor in yield (Fig. 4). Cells grown in the same medium amended with glucose (1 μM to 5 μM) showed increasing cell yields but also no differences between light and dark incubations. Addition of 1 μM retinal to the cultures did not affect cell growth (data not shown).

DISCUSSION

Proteorhodopsin phototrophy deduced from the genome of HTCC2207. Retinal is the chromophore of rhodopsin holoenzymes. The genome of HTCC2207 most likely includes all genes necessary for the biosynthesis of retinal. The gene arrangement with PR and an adjacent operon including *crtIBY* is similar to that found with BAC clones of the SAR86 group and other uncultured *Gammaproteobacteria* (25). In the alphaproteobacterium "*Candidatus* Pelagibacter ubique" (SAR11 clade), a similar gene arrangement with *crtIBY* and *blh* is present, but the PR gene is at a different location (11). In *Halobacterium* spp., the *crtIBY* genes (phytoene desaturase, phytoene synthase, and lycopene cyclase, respectively) catalyze β-carotene synthesis from phytoene. *blh* was shown to cleave β-carotene to retinal (26). Additionally present in the same operon is an *isdA* homolog, which is probably responsible for the synthesis of geranyl diphosphate, as well as *fni*, an isopentenyl pyrophosphate (IPP) isomerase which was shown to enhance IPP formation. The genome draft of HTCC2207, similar to that of "*Candidatus* Pelagibacter ubique," did not contain known genes for CO_2 fixation, indicating a heterotrophic metabolism.

Occurrence and abundance of the SAR92 clade. 16S rDNA sequences of the SAR92 clade were among the first to be recovered from marine bacteria (12). Surprisingly, during the next 16 years of research and extensive sequencing of 16S rRNA genes from marine environments (23), only few clones of this clade were reported. Furthermore, no closely related sequences to either the PR genes or the 16S rRNA genes of the SAR92 clade were found in the recent Sargasso Sea metagenomics library (29). To investigate the abundance of SAR92 along a transect off the Oregon coast, we designed a SAR92 clade-

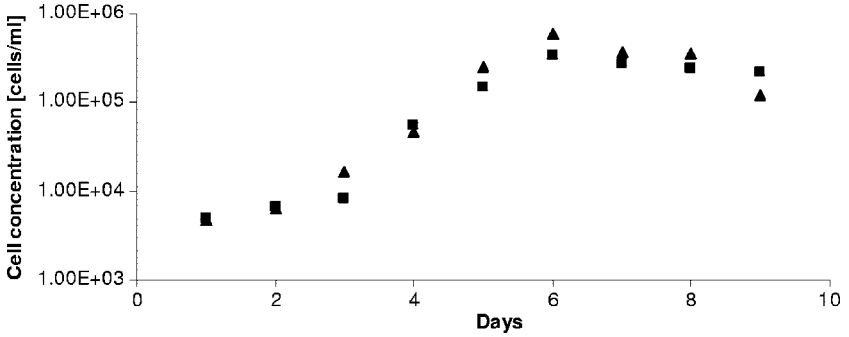

FIG. 4. Influence of light on the growth behavior of HTCC2207. Means for triplicate flasks of light-grown cells (▲) and dark-grown cells (■) are shown. Standard deviations were below 5% and were omitted for clarity. Light was supplied as cool-white light at an intensity of 140 μmol m^{-2} s^{-1} for 12 h per day. The addition of 1 μM retinal did not enhance either growth rate or growth yield.

specific oligonucleotide probe and performed FISH as well as RNA dot blots using samples collected at the same location over different seasons and different years. The results were in good agreement and showed that the SAR92 clade can comprise up to 10% of the total bacterial population in these environments. Interestingly, the peak of abundance correlates with the relatively high nutrient concentrations found in an upwelling region off the Oregon coast. In the lower-nutrient regions farther off the coast, the abundance of the SAR92 was low, close to the limit of detection. This preference for higher-nutrient conditions might explain the low abundance in the very-low-nutrient gyre present in the Sargasso Sea. In addition, the higher counts at surface layers compared to those in deeper waters and the spectral tuning of the PR toward green light are in good agreement.

Function of PR for the cells. Although the very fast half-time of decay times of the PR intermediates implies a proton-pumping activity, light did not enhance either the growth yield or the growth rate of HTCC2207 in batch culture. This result is similar to that found for "*Candidatus* Pelagibacter ubique" (11) as well as for mesocosm experiments in which light did not promote the growth of members of either the SAR92 clade or the SAR11 clade (28).

In contrast to "*Candidatus* Pelagibacter ubique," the growth of HTCC2207 as presented in this study is carbon limited. Therefore, a proton-pumping activity of PR should result in higher growth yields under light incubation. Although we did not see an effect of light on PR-containing strains, we still believe that PR is working as a proton pump in nature. One explanation for the negative results could lie in the fact that, although no external carbon compounds were supplied in the medium, the cells already grew at a maximum growth rate (μmax) and the proposed additive effect of proteorhodopsin was, therefore, not visible. To circumvent this problem, experiments in continuous cultures at low growth rates should be performed to address the function and benefit of proteorhodopsin phototrophy in pure cultures and in the ocean. Future studies should also include elucidating the expression pattern of PR during growth, because a visible effect from the growth curve can only be expected when the protein is actually expressed during exponential growth. An alternative function for proteorhodopsin could be in helping the cell to maintain a proton gradient during starvation times, which might be seen in high expression rates in stationary phase compared to exponential phase.

REFERENCES

1. **Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman.** 1990. Basic local alignment search tool. J. Mol. Biol. **215:**403–410.
2. **Amann, R. I., B. J. Binder, R. J. Olson, S. W. Chisholm, R. Devereux, and D. A. Stahl.** 1990. Combination of 16S rRNA-targeted oligonucleotide probes with flow cytometry for analyzing mixed microbial populations. Appl. Environ. Microbiol. **56:**1919–1925.
3. **Amann, R. I., L. Krumholz, and D. A. Stahl.** 1990. Fluorescent-oligonucleotide probing of whole cells for determinative, phylogenetic, and environmental studies in microbiology. J. Bacteriol. **172:**762–770.
4. **Arrigo, K. R.** 2005. Marine microorganisms and global nutrient cycles. Nature **437:**349–355.
5. **Beja, O., L. Aravind, E. V. Koonin, M. T. Suzuki, A. Hadd, L. P. Nguyen, S. B. Jovanovich, C. M. Gates, R. A. Feldman, J. L. Spudich, E. N. Spudich, and E. F. DeLong.** 2000. Bacterial rhodopsin: evidence for a new type of phototrophy in the sea. Science **289:**1902–1906.
6. **Beja, O., E. N. Spudich, J. L. Spudich, M. Leclerc, and E. F. DeLong.** 2001. Proteorhodopsin phototrophy in the ocean. Nature **411:**786–789.
7. **Cho, J.-C., and S. J. Giovannoni.** 2004. Cultivation and growth characteristics of a diverse group of oligotrophic marine *Gammaproteobacteria*. Appl. Environ. Microbiol. **70:**432–440.
8. **Connon, S. A., and S. J. Giovannoni.** 2002. High-throughput methods for culturing microorganisms in very-low-nutrient media yield diverse new marine isolates. Appl. Environ. Microbiol. **68:**3878–3885.
9. **de la Torre, J. R., L. M. Christianson, O. Beja, M. T. Suzuki, D. M. Karl, J. Heidelberg, and E. F. DeLong.** 2003. Proteorhodopsin genes are distributed among divergent marine bacterial taxa. Proc. Natl. Acad. Sci. USA **100:**12830–12835.
10. **Frigaard, N. U., A. Martinez, T. J. Mincer, and E. F. DeLong.** 2006. Proteorhodopsin lateral gene transfer between marine planktonic Bacteria and Archaea. Nature **439:**847–850.
11. **Giovannoni, S. J., L. Bibbs, J. C. Cho, M. D. Stapels, R. Desiderio, K. L. Vergin, M. S. Rappe, S. Laney, L. J. Wilhelm, H. J. Tripp, E. J. Mathur, and D. F. Barofsky.** 2005. Proteorhodopsin in the ubiquitous marine bacterium SAR11. Nature **438:**82–85.
12. **Giovannoni, S. J., T. B. Britschgi, C. L. Moyer, and K. G. Field.** 1990. Genetic diversity in Sargasso Sea bacterioplankton. Nature **345:**60–63.
13. **Giovannoni, S. J., E. F. DeLong, T. M. Schmidt, and N. R. Pace.** 1990. Tangential flow filtration and preliminary phylogenetic analysis of marine picoplankton. Appl. Environ. Microbiol. **56:**2572–2575.
14. **González, J. M., F. Mayer, M. A. Moran, R. E. Hodson, and W. B. Whitman.** 1997. *Microbulbifer hydrolyticus* gen. nov., sp. nov., and *Marinobacterium georgiense* gen. nov., sp. nov., two marine bacteria from a lignin-rich pulp mill waste enrichment community. Int. J. Syst. Bacteriol. **47:**369–376.
15. **Lane, D. J., A. P. Harrison, Jr., D. Stahl, B. Pace, S. J. Giovannoni, G. J. Olsen, and N. R. Pace.** 1992. Evolutionary relationships among sulfur- and iron-oxidizing eubacteria. J. Bacteriol. **174:**269–278.
16. **Loy, A., M. Horn, and M. Wagner.** 2003. probeBase: an online resource for rRNA-targeted oligonucleotide probes. Nucleic Acids Res. **31:**514–516.
17. **Morris, R. M., M. S. Rappe, S. A. Connon, K. L. Vergin, W. A. Siebold, C. A. Carlson, and S. J. Giovannoni.** 2002. SAR11 clade dominates ocean surface bacterioplankton communities. Nature **420:**806–810.
18. **Morris, R. M., M. S. Rappé, E. Urbach, S. A. Connon, and S. J. Giovannoni.** 2004. Prevalence of the *Chloroflexi*-related SAR202 bacterioplankton cluster throughout the mesopelagic zone and deep ocean. Appl. Environ. Microbiol. **70:**2836–2842.
19. **Oesterhelt, D., and W. Stoeckenius.** 1971. Rhodopsin-like protein from the purple membrane of Halobacterium halobium. Nat. New Biol. **233:**149–152.
20. **Peck, R. F., E. A. Johnson, and M. P. Krebs.** 2002. Identification of a lycopene β-cyclase required for bacteriorhodopsin biogenesis in the archaeon *Halobacterium salinarum*. J. Bacteriol. **184:**2889–2897.
21. **Pernthaler, J., F. O. Glockner, W. Schonhuber, and R. Amann.** 2001. Fluorescence in situ hybridization (FISH) with rRNA-targeted oligonucleotide probes. Methods Microbiol. **30:**207–226.
22. **Rappe, M. S., S. A. Connon, K. L. Vergin, and S. J. Giovannoni.** 2002. Cultivation of the ubiquitous SAR11 marine bacterioplankton clade. Nature **418:**630–633.
23. **Rappe, M. S., and S. J. Giovannoni.** 2003. The uncultured microbial majority. Annu. Rev. Microbiol. **57:**369–394.
24. **Retief, J. D.** 2000. Phylogenetic analysis using PHYLIP. Methods Mol. Biol. **132:**243–258.
25. **Sabehi, G., O. Beja, M. T. Suzuki, C. M. Preston, and E. F. DeLong.** 2004. Different SAR86 subgroups harbour divergent proteorhodopsins. Environ. Microbiol. **6:**903–910.
26. **Sabehi, G., A. Loy, K. H. Jung, R. Partha, J. L. Spudich, T. Isaacson, J. Hirschberg, M. Wagner, and O. Beja.** 2005. New insights into metabolic properties of marine bacteria encoding proteorhodopsins. PLoS Biol. **3:**e273.
27. **Sabehi, G., R. Massana, J. P. Bielawski, M. Rosenberg, E. F. Delong, and O. Beja.** 2003. Novel proteorhodopsin variants from the Mediterranean and Red Seas. Environ. Microbiol. **5:**842–849.
28. **Schwalbach, M. S., M. Brown, and J. A. Fuhrman.** 2005. Impact of light on marine bacterioplankton community structure. Aquat. Microb. Ecol. **39:**235–245.
29. **Venter, J. C., K. Remington, J. F. Heidelberg, A. L. Halpern, D. Rusch, J. A. Eisen, D. Wu, I. Paulsen, K. E. Nelson, W. Nelson, D. E. Fouts, S. Levy, A. H. Knap, M. W. Lomas, K. Nealson, O. White, J. Peterson, J. Hoffman, R. Parsons, H. Baden-Tillson, C. Pfannkoch, Y. H. Rogers, and H. O. Smith.** 2004. Environmental genome shotgun sequencing of the Sargasso Sea. Science **304:**66–74.
30. **Wright, T. D., K. L. Vergin, P. W. Boyd, and S. J. Giovannoni.** 1997. A novel δ-subdivision proteobacterial lineage from the lower ocean surface layer. Appl. Environ. Microbiol. **63:**1441–1448.