

Microbial Deep Subsurface

How does it stack up regarding:
Biomass?
Metabolism?

Prokaryotes: The unseen majority

Whitman et al., 1998 PNAS

Table 5. Number and biomass of prokaryotes in the world

Environment	No. of prokaryotic cells, $\times 10^{28}$	Pg of C in prokaryotes*
Aquatic habitats	12	2.2
Oceanic subsurface	355	303
Soil	26	26
Terrestrial subsurface	25-250	22-215
Total	415-640	353-546

*Calculated as described in the text.

Pg = Petagram or 10^{15} grams

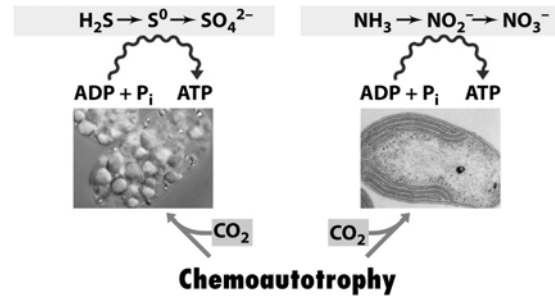
Prokaryotes: The unseen majority

Whitman et al., 1998 PNAS

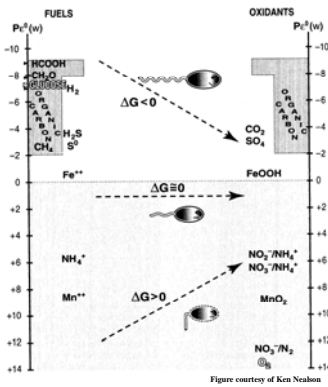
	Total C (Pg)	Total N (Pg)	Total P (Pg)
Plants:	560	12-20	1-2
Prokaryotes:	350-550	70-120	7-12

Take Home Message: Prokaryotes contain 60 to 100% the cellular carbon of all plants along with ~10x the N and P of plants!

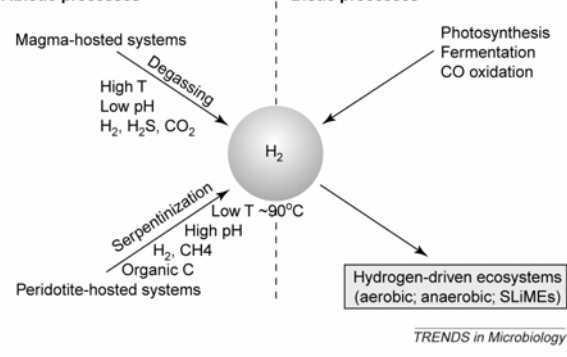
Chemolithotrophy

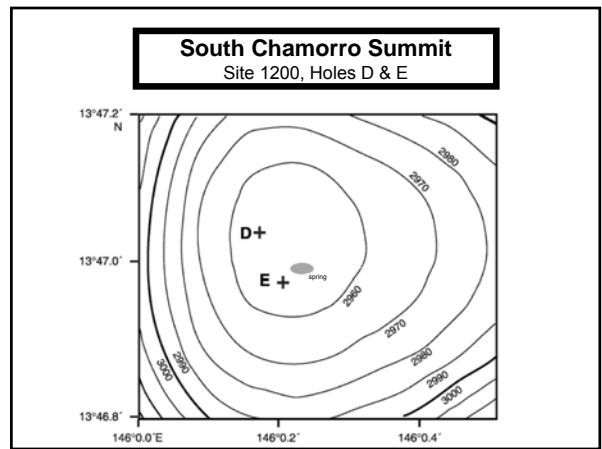
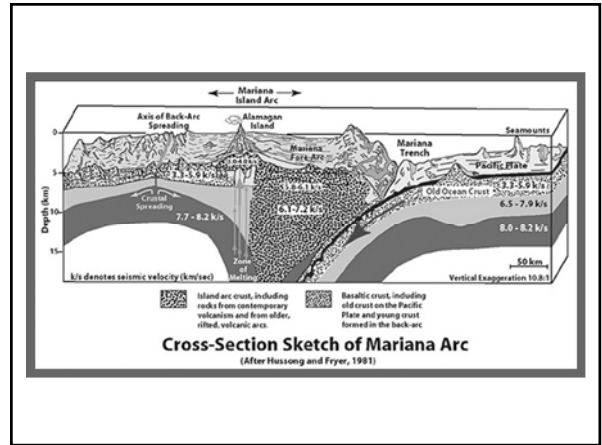
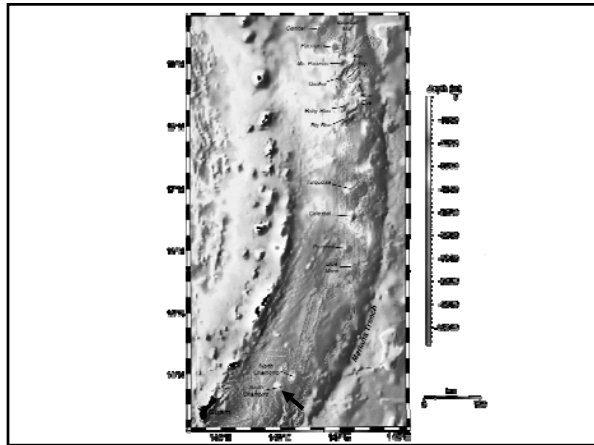
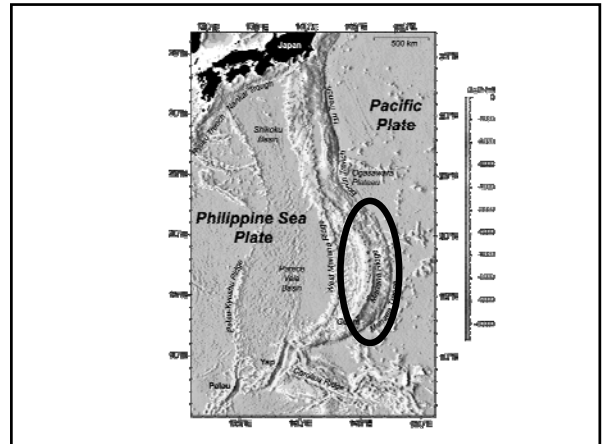
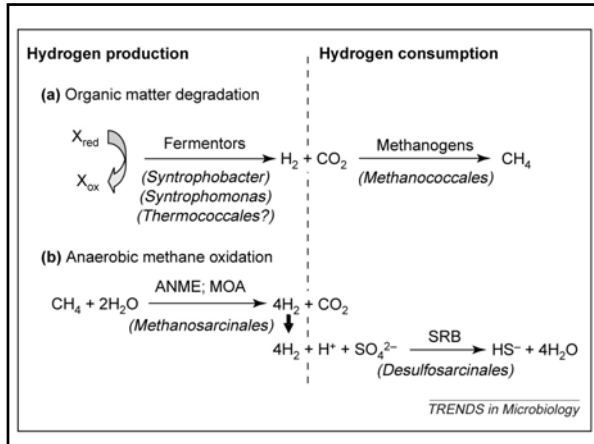


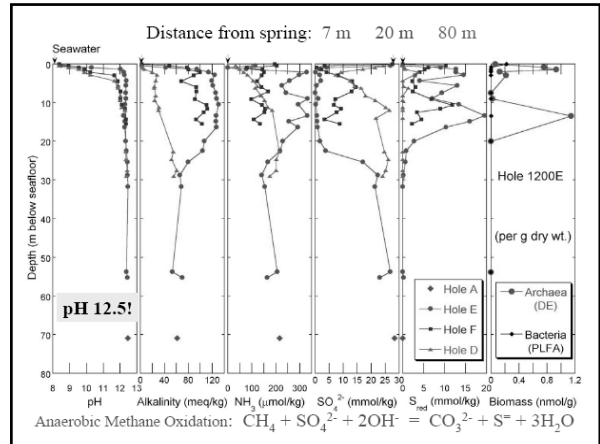
Microbial Metabolic Menu



Abiotic processes







Mariana Forearc
South Chamorro Summit
Site 1200, Hole E
55.3 meters below sea floor

Serpentinization and methane generation at high pH

$$2\text{Mg}_2\text{SiO}_4 + 3\text{H}_2\text{O} \rightarrow \text{Mg}_3\text{Si}_2\text{O}_5(\text{OH})_4 + \text{Mg}(\text{OH})_2$$

(*Peridotite) (water) (serpentine) (**brucite)

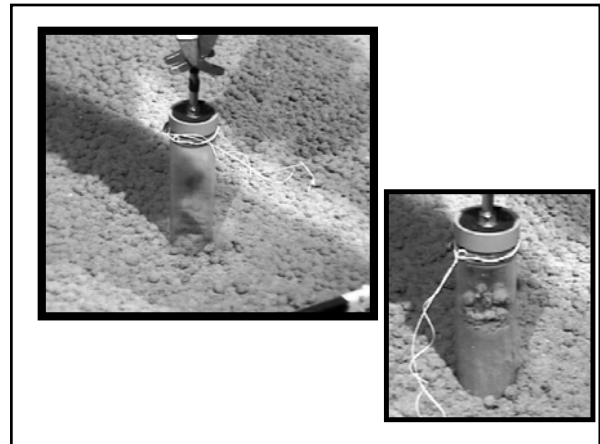
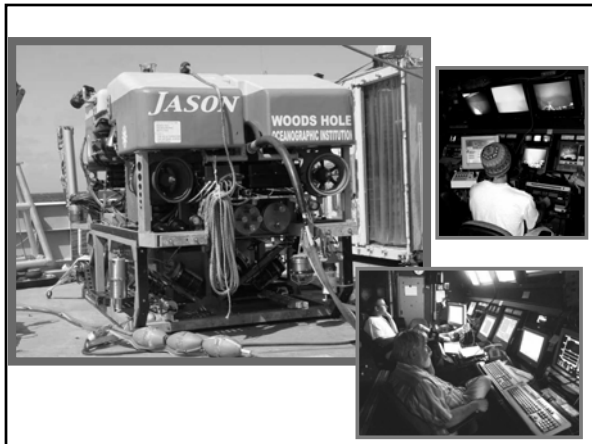
Methane production based on Fischer-Tropsch reaction:

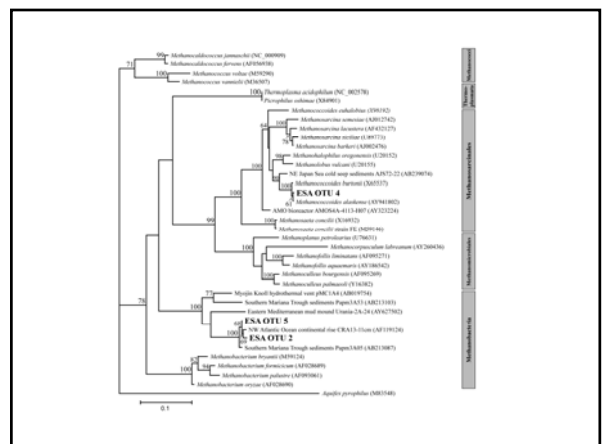
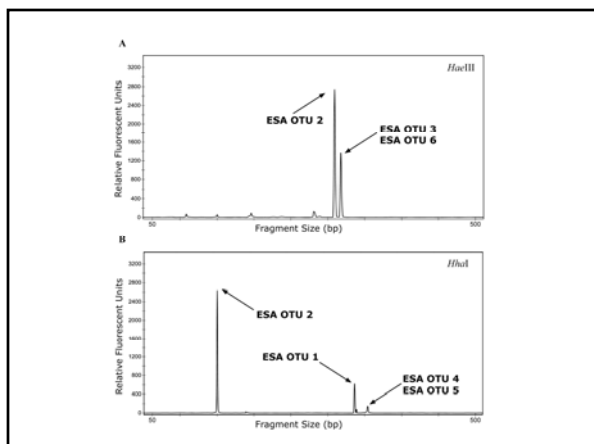
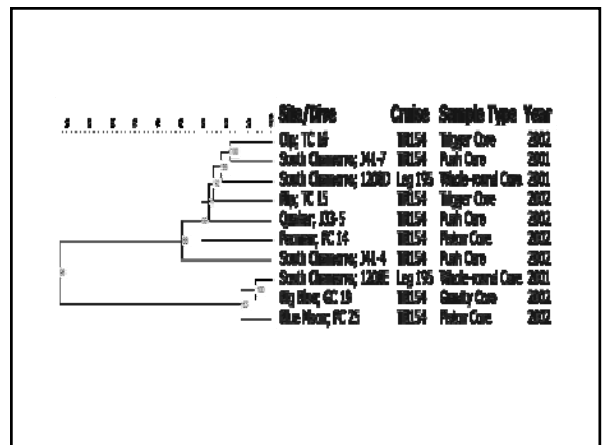
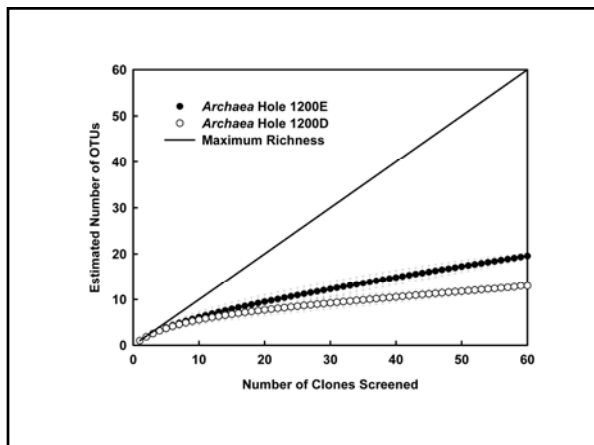
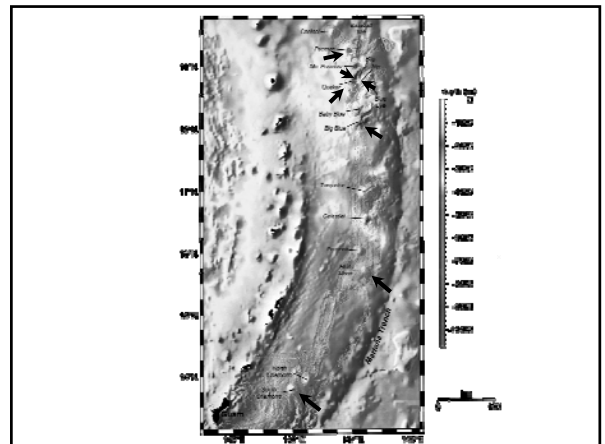
$$4\text{H}_2 + \text{CO}_3^{2-} \rightarrow \text{CH}_4 + \text{H}_2\text{O} + 2\text{OH}^-$$

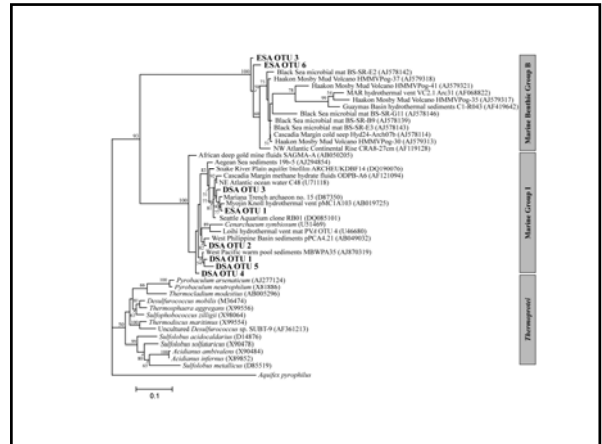
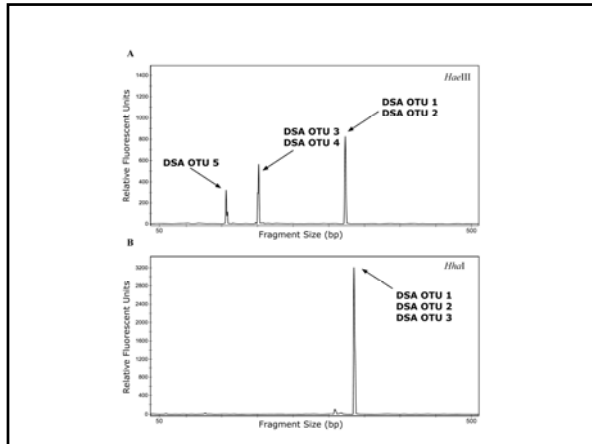
This reaction converts carbonate alkalinity to hydroxyl alkalinity, increasing surrounding pH

Hydrogen and methane provide energy sources for deep sea chemotrophic microorganisms

* Peridotite: native rock consisting mostly of the minerals olivine and pyroxene
** Brucite: magnesium hydroxide, found in veins of serpentine







Marine Microbiology

How does it stack up regarding:
Diversity?
Metabolism?

Genetic diversity in Sargasso Sea bacterioplankton

Stephen J. Giovannoni, Theresa B. Britschgi,
Craig L. Moyer & Katharine G. Field

Department of Microbiology, Oregon State University, Corvallis,
Oregon 97331, USA

BACTERIOPHANKTON are recognized as important agents of biogeochemical change in marine ecosystems, yet relatively little is known about the species that make up these communities. Uncertainties about the genetic structure and diversity of natural bacterioplankton populations stem from the traditional difficulties associated with microbial cultivation techniques. Discrepancies between direct counts and plate counts are typically several orders of magnitude, raising doubts as to whether cultivated marine bacteria are actually representative of dominant planktonic species. We have phylogenetically analysed clone libraries of bacterial 16S ribosomal RNA genes amplified from natural populations of Sargasso Sea bacterioplankton by the polymerase chain reaction. The analysis indicates the presence of a novel microbial group, the SAR11 cluster, which appears to be a significant component of this oligotrophic bacterioplankton community. A second cluster of lineages related to the oxygenic phototrophs—cyanobacteria, prochlorophytes and chloroplasts—was also observed. However, some of the genes matched the small subunit rRNA sequences of cultivated marine cyanobacteria from similar habitats. The diversity of 16S rRNA genes observed within the clusters suggests that these bacterioplankton may be consortia of independent lineages sharing surprisingly distant common ancestors.

	Eubacterial	Eukaryotic	Archaeobacterial	SAR11	Hybrid
Sargasso Sea	81.0	19.0	0.0	0.0	0.0
Ferry Reach	66.8	13.7	0.0	0.0	0.0
Key Biscayne	74.8	20.4	0.0	0.0	0.0
Yagoua Head	85.2	6.7	0.0	0.0	0.0

Binding of the phylogenetic group-specific probes was determined relative to universal primer binding using arrays of known (RFLP ID 2.5, 2.5, 2.0, 3.0, 4.0, 5.0 ng) on nylon membranes. ³²P-DNA oligonucleotide probes were hybridized with 7.5 µg nucleic acid and 10⁶ cpm of a specific activity of between 1 × 10⁶–2 × 10⁶ cpm µg⁻¹. The amount of bound probe was measured using a gas scintillation counter (Beckman). The composition of the populations were determined from the binding of the oligonucleotide probes according to the following equation:

$$N_i = \frac{AC_i \times (R_i \times 10^6)^{-1} \times 10^6}{\sum_{j=1}^n (AC_j \times (R_j \times 10^6)^{-1} \times 10^6) + 1} \times 100$$

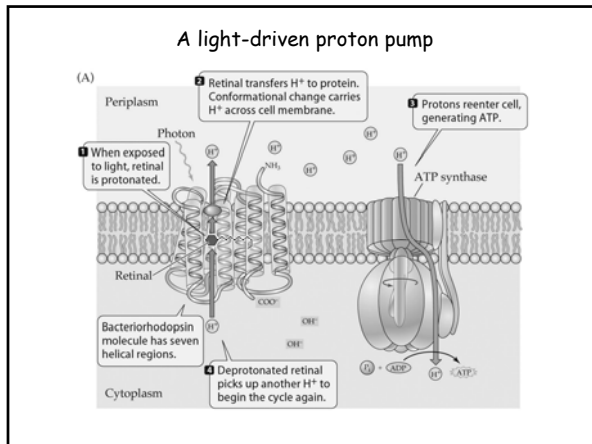
where N_i is the percentage of the population, AC_i is the amount of bound probe, R_i is the amount of probe, and n is the number of probes.

Bacterial Rhodopsin: Evidence for a New Type of Phototrophy in the Sea

Oded Bija, L. Aravind, Eugene V. Koonin,
Marcellino T. Szosák, Andrew Hadzi, Linh P. Nguyen,
Steven B. Jovanovich, Christian M. Gates, Robert A. Feldman,
John L. Spivey, Elena N. Spivey, Edward F. Delany

Extremely halophilic archaea contain retinal-binding integral membrane proteins called bacteriorhodopsins that function as light-driven proton pumps. So far, bacteriorhodopsins capable of generating a chemiosmotic membrane potential in response to light have been demonstrated only in halophilic archaea. We describe here a type of rhodopsin derived from bacteria that was discovered through genomic analysis of naturally occurring marine bacterioplankton. The bacterial rhodopsin was encoded in the genome of an uncultivated γ-proteobacterium and shared highest amino acid sequence similarity with archaeal rhodopsins. The protein was functionally expressed in *Escherichia coli* and found critical to form an active, light-driven proton pump. The new rhodopsin exhibited a photochemical reaction cycle with intermediates and kinetics characteristic of archaeal proton-pumping rhodopsins. Our results demonstrate that archaeal-like rhodopsins are broadly distributed among different taxa, including members of the domain Bacteria. Our data also indicate that a previously unsuspected mode of bacterially mediated light-driven energy generation may commonly occur in oceanic surface waters worldwide.

Proteorhodopsin in marine Bacteria and Archaea

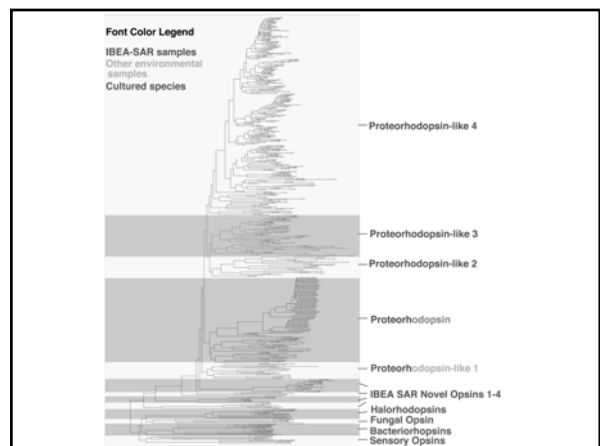
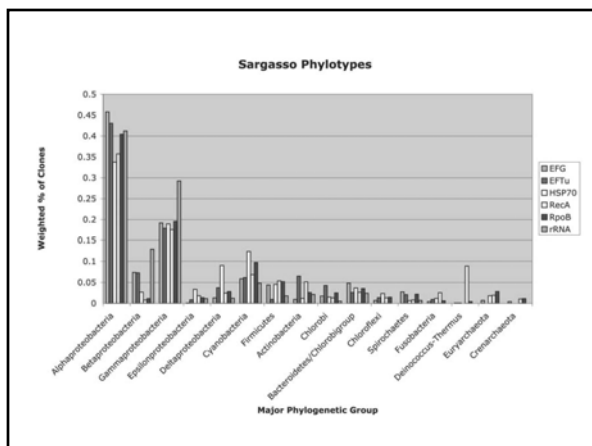
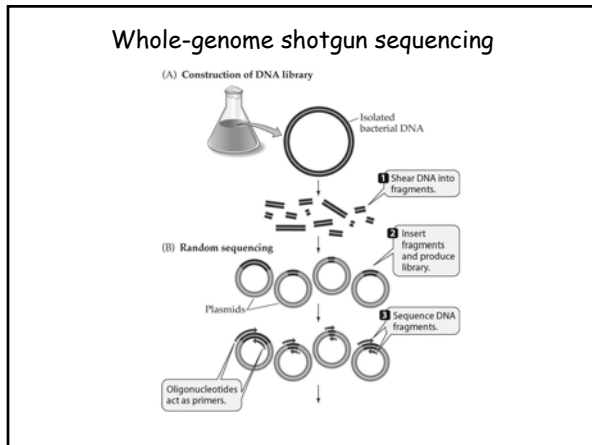


Environmental Genome Shotgun Sequencing of the Sargasso Sea

J. Craig Venter,^{1*} Karin Remington,¹ John F. Heidelberg,³ Aaron L. Halpern,² Doug Rusch,² Jonathan A. Eisen,² Dongying Wu,³ Ian Paulsen,³ Karen E. Nelson,³ William Nelson,³ Derrick E. Fouts,³ Samuel Levy,² Anthony H. Knap,⁶ Michael W. Lomas,⁶ Ken Nealson,³ Owen White,³ Jeremy Peterson,³ Jeff Hoffman,¹ Rachel Parsons,⁶ Holly Baden-Tillson,¹ Cynthia Pfannkoch,¹ Yu-Hui Rogers,⁴ Hamilton O. Smith¹

We have applied "whole-genome shotgun sequencing" to microbial populations collected en masse on tangential flow and impact filters from seawater samples collected from the Sargasso Sea near Bermuda. A total of 1,045 billion base pairs of nonredundant sequence was generated, annotated, and analyzed to elucidate the gene content, diversity, and relative abundance of the organisms within these environmental samples. These data are estimated to derive from at least 1800 genomic species based on sequence relatedness, including 148 previously unknown bacterial phylotypes. We have identified over 1.2 million previously unknown genes represented in these samples, including more than 782 new rhodopsin-like photoreceptors. Variation in species present and stoichiometry suggests substantial oceanic microbial diversity.

(Science, 2004)



Proteorhodopsin in the ubiquitous marine bacterium SAR11

Stephen J. Giovannoni,¹ Lisa Bibila,¹ Jang-Choon Cha,¹ Martha D. Stapels,¹ Russel Desiderio,¹ Kevin L. Vergin,¹ Michael S. Rappé,¹ Samuel Laney,² Lawrence J. Wilhelm,¹ H. James Tripp,¹ Eric J. Mathur¹ & Douglas F. Barotsky³

Proteorhodopsins are light-dependent proton pumps that are predicted to have an important role in the ecology of the ocean by supplying energy for microbial metabolism^{1,2}. Proteorhodopsin genes were first discovered through the cloning and sequencing of large genomic DNA fragments from seawater³. They were later shown to be widely distributed, phylogenetically diverse, and active in the ocean^{4,5}. Proteorhodopsin genes have not been found in cultured bacteria, and on the basis of environmental sequence data, it has not yet been possible to reconstruct the genomes of uncultured bacterial strains that have proteorhodopsin genes. Although the metabolic effect of proteorhodopsin is uncertain, they are thought to function in cells for which the primary mode of metabolism is the heterotrophic oxidation of dissolved organic carbon. Here we report that SAR11 strain HTCC1962 (‘Pelagibacter ubique’), the first cultured member of the extraordinarily abundant SAR11 clade, expresses a proteorhodopsin gene when cultured in oxidized seawater and in its natural environment, the ocean. The Pelagibacter proteorhodopsin functions as a light-dependent proton pump. The gene is expressed by cells grown in either diurnal light or in darkness, and there is a difference between the growth rates or cell yields of cultures grown in light or darkness.

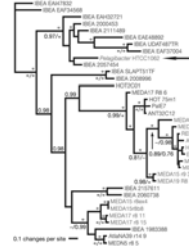


Figure 1 | Phylogenetic relationships between proteorhodopsin amino acid sequences. Shown is the relationship between the Pelagibacter strain HTCC1962 proteorhodopsin amino acid sequence and selected representatives of proteorhodopsin genes cloned from seawater DNA. The sequences most similar to Pelagibacter proteorhodopsin are included in the tree. Green text indicates proteorhodopsin genes encoding a histidine residue at position 105. Blue text indicates a glutamine residue at this position. The tree was rooted with the sequences of *Amidibacter* from *Chlorobacterium* and *Paracoccus* family. Numbers above nodes are posterior probabilities; numbers below nodes are bootstrap booting values and neighbor-joining booting values (separated by a slash). Asterisks indicate a value of 1.0.

(Nature, 2005)

Genome Streamlining in a Cosmopolitan Oceanic Bacterium

Stephen J. Giovannoni,¹ H. James Tripp,¹ Scott Given,² Mircea Podor,² Kevin L. Vergin,¹ Damon Baptista,¹ Lisa Bibila,¹ Jonathan Eide,¹ Toby M. Richardson,² Michiel Noordewij,² Michael S. Rappé,¹ Jay M. Short,² James C. Carrington,² Eric J. Mathur³

The SAR11 clade consists of very small, heterotrophic marine *α*-proteobacteria that are found throughout the ocean, where they account for abundant cells in all microbial cells. *Pelagibacter ubique*, the first cultured member of this clade, has the smallest genome and encodes the smallest number of predicted open reading frames known for a free-living microorganism. In contrast to parasitic bacteria and archaea with small genomes, *P. ubique* has complete biosynthetic pathways for all 20 amino acids and all but a few cofactors. *P. ubique* has no penicillins, tetracyclins, tetracycline-resistance elements, or other, few parasites, and the shortest intergenic spacers yet observed for any cell.

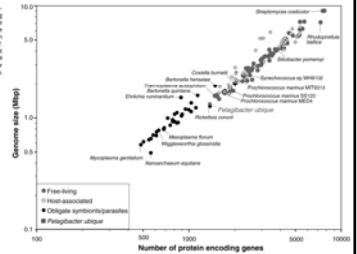


Fig. 1. Number of predicted protein-encoding genes versus genome size for 200 different published genomes from bacteria and archaea. *P. ubique* has the smallest number of genes (1754 open reading frames) for any free-living organism.

(Science, 2005)

Abundance and diversity of microbial life in ocean crust

Cara M. Santelli,^{1,2} Beth N. Orcutt,¹ Erin Banning,¹ Wolfgang Bach^{1,3}, Craig L. Moyer¹, Mitchell L. Sogin¹, Hubert Staudigel¹ & Katrina J. Edwards^{1,3}

Oceanic lithosphere exposed at the sea floor undergoes seawater-rock alteration reactions involving the oxidation and hydration of glassy basalt. Basalt alteration reactions are theoretically capable of supplying sufficient energy for chemolithoautotrophic growth^{1,2}. Such reactions have been shown to generate microbial biomass in the laboratory³, but field-based support for the existence of microbes that are supported by basalt alteration is lacking. Here, using quantitative polymerase chain reaction, *in situ* hybridization and microscopy, we demonstrate that prokaryotic cell abundances on sea floor-exposed basalts are 3–4 orders of magnitude greater than in overlying deep sea water. Phylogenetic analyses of basaltic lavas from the East Pacific Rise (9° N) and around Hawaii reveal that the basalt-hosted biosphere harbours high bacterial community richness and that community membership is shared between these sites. We demonstrate that alteration reactions fuel chemolithoautotrophic microorganisms, which constitute a trophic base of the basalt habitat, with important implications for deep-sea carbon cycling and chemical exchange between basalt and sea water.

(Nature, 2008)

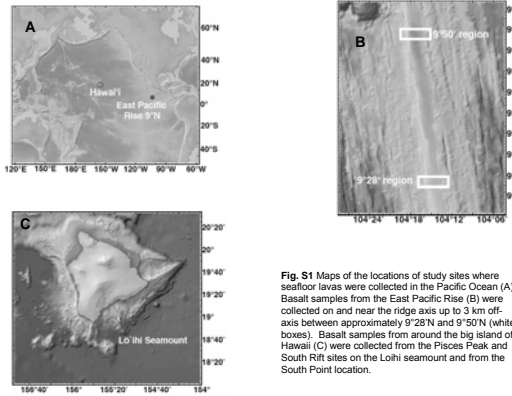


Fig. S1 Maps of the locations of study sites where seafloor lavas were collected in the Pacific Ocean (A). Basalt samples from the East Pacific Rise (B) were collected on and near the ridge axis up to 3 km off-axis between approximately 9°28'N and 9°50'N (white boxes). Basalt samples from around the big island of Hawaii (C) were collected from the Pisces Peak and South Rift sites on the Loihi seamount and from the South Point location.

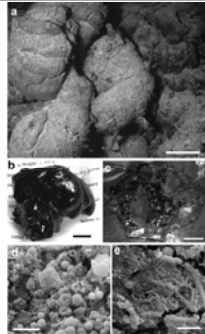


Figure 1 | A highly diverse and abundant epi- and endo-lithic microbial community exists on basaltic lavas from the East Pacific Rise. a, The East Pacific Rise at 9° N is characterized by lava flows, such as pillow basalts shown here, directly exposed at the seafloor. Scale bar, 40 cm. b, c, Photographs showing the range of volcanic samples used in this study from fresh and glassy (b) to more altered and oxide coated (c). Scale bars, 2 cm (b) and 4 cm (c). d, e, SEM images of different presumed cellular morphologies, such as coccoidal (d) and filamentous (e) structures that were observed on ferromanganese oxide encrusted and Fe-oxide-coated samples. Scale bars, 5 μm.

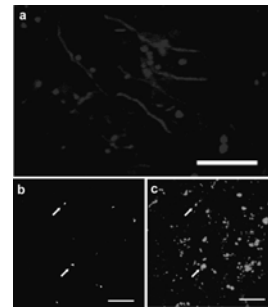


Figure 2 | CARD-FISH analyses. a, b, c, Confocal laser scanning micrographs depicting CARD-FISH on lava surfaces from the EPR. Prokaryotic cells were hybridized with either probe EUB338(I-II) mix (a) or probe ARCH915 (b) to target Bacteria, or Archaea respectively. A variety of bacterial cell morphologies such as filaments, cocci, and rods were confirmed with hybridizations (a). Side-by-side comparisons of Archaea (b) versus total cells (c) reveals that Archaea account for only a small portion of the total cells. Total cells were identified with the general DNA stain SYBR Green. The arrows point to some cells that overlap in each frame. Scale bars, 10 μm (a) and 20 μm (b, c).

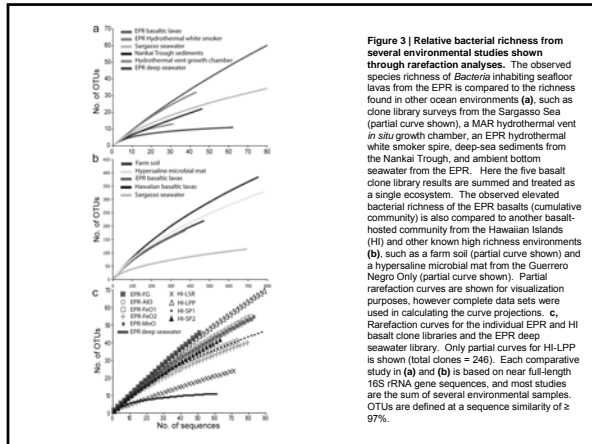


Figure 3 | Relative bacterial richness from several environmental studies shown through rarefaction analyses. The observed species richness of *Bacteria* inhabiting seafloor lavas from the EPR is compared to the richness found in other ocean environments (a), such as clone library surveys from the Sargasso Sea (partial curve shown), a MAAR hydrothermal vent in situ growth chamber, an EPR hydrothermal white smoker spire, deep-sea sediments from the Nankai Trough, and ambient bottom seawater from the EPR. Here the five basalt clone library results are summed and treated as a single ecosystem. The observed elevated bacterial richness of the EPR basalts (cumulative community) is also compared to another basalt-hosted community from the Hawaiian Islands (HI) and other known high richness environments (b), such as a farm soil (partial curve shown) and a hypersaline microbial mat from the Guerrero Negro Only (partial curve shown). Partial rarefaction curves are shown for visualization purposes, however complete data sets were used in calculating the curve projections. c. Rarefaction curves for the individual EPR and HI basalt clone libraries and the EPR deep seawater library. Only partial curves for HI-LPP are shown (total clones = 246). Each comparative study in (a) and (b) is based on near full-length 16S rRNA gene sequences, and most studies are the sum of several environmental samples. OTUs are defined at a sequence similarity of $\geq 97\%$.

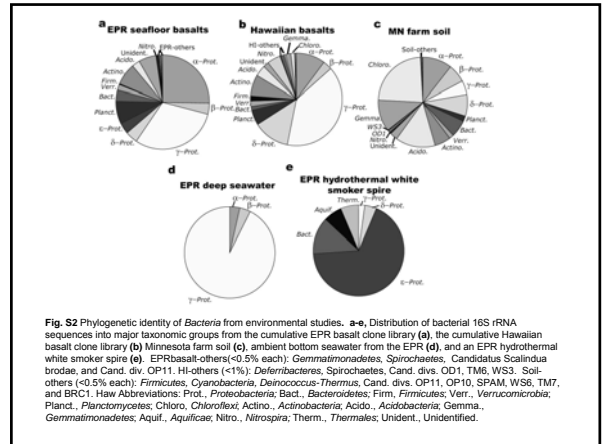


Fig. S2 Phylogenetic identity of *Bacteria* from environmental studies. a-e, Distribution of bacterial 16S rRNA sequences into major taxonomic groups from the cumulative EPR basalt clone library (a), the cumulative Hawaiian basalt clone library (b) Minnesota farm soil (c), ambient bottom seawater from the EPR (d), and an EPR hydrothermal white smoker spire (e). EPR-basalt-others (<0.5% each): Gemmatimonadetes, Spirochaetes, Candidatus Scalindia brodae, and Cand. div. OP11. HI-others (<1%): Deferribacteres, Spirochaetes, Cand. divs. OD1, TM6, WS3. Soil-others (<0.5% each): Firmicutes, Cyanobacteria, Deinococcus-Thermus, Cand. divs. OP11, OP10, SPAM, WS6, TM7, and BR1. Haw Abbreviations: Prot., Proteobacteria; Bact., Bacteroidetes; Firm., Firmicutes; Verr., Verrucomicrobi; Plant., Planctomycetes; Chloro., Chloroflexi; Actino., Actinobacteria; Acido., Acidobacteria; Gemma., Gemmatimonadetes; Aquif., Aquificae; Nitro., Nitrospira; Therm., Thermales; Unident., Unidentified.

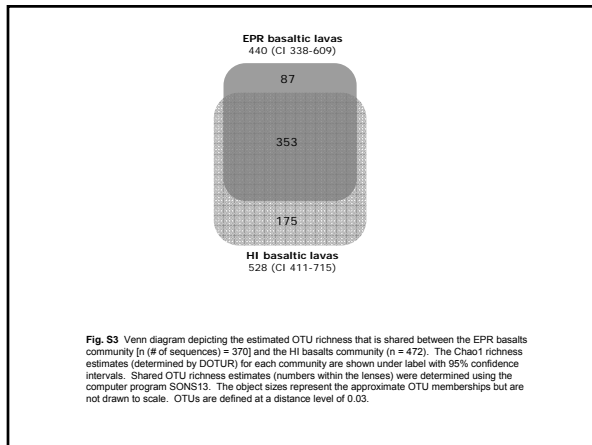


Fig. S3 Venn diagram depicting the estimated OTU richness that is shared between the EPR basaltic lavas community (n (# of sequences) = 440) and the HI basaltic community (n = 528). The Chao1 richness estimates (determined by DOTUR) for each community are shown under label with 95% confidence intervals. Shared OTU richness estimates (numbers within the lenses) were determined using the computer program SONS13. The object sizes represent the approximate OTU memberships but are not drawn to scale. OTUs are defined at a distance level of 0.03.

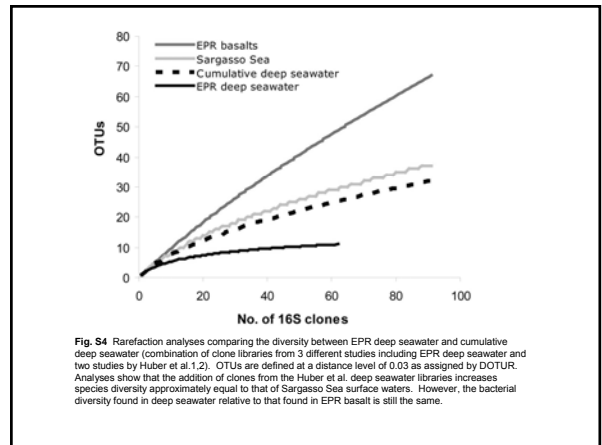


Fig. S4 Rarefaction analyses comparing the diversity between EPR deep seawater and cumulative deep seawater (combination of clone libraries from 3 different studies including EPR deep seawater and two studies by Huber et al. 1,2). OTUs are defined at a distance level of 0.03 as assigned by DOTUR. Analyses show that the addition of clones from the Huber et al. deep seawater libraries increases species diversity approximately equal to that of Sargasso Sea surface waters. However, the bacterial diversity found in deep seawater relative to that found in EPR basalt is still the same.

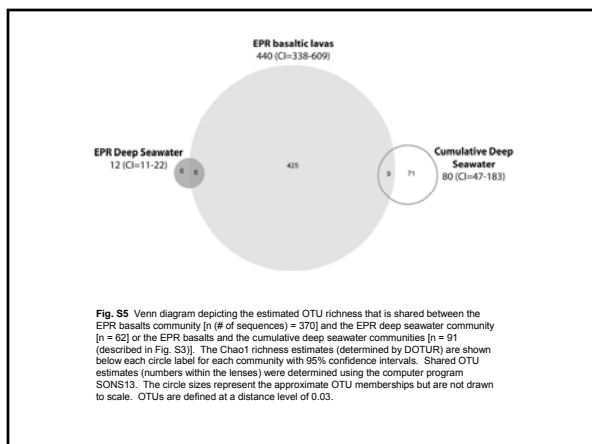


Fig. S5 Venn diagram depicting the estimated OTU richness that is shared between the EPR basalts community (n (# of sequences) = 440) and the EPR deep seawater community (n = 12) or the EPR basalts and the cumulative deep seawater communities (n = 91) (described in Fig. S3)). The Chao1 richness estimates (determined by DOTUR) are shown below each circle label for each community with 95% confidence intervals. Shared OTU estimates (numbers within the lenses) were determined using the computer program SONS13. The circle sizes represent the approximate OTU memberships but are not drawn to scale. OTUs are defined at a distance level of 0.03.