

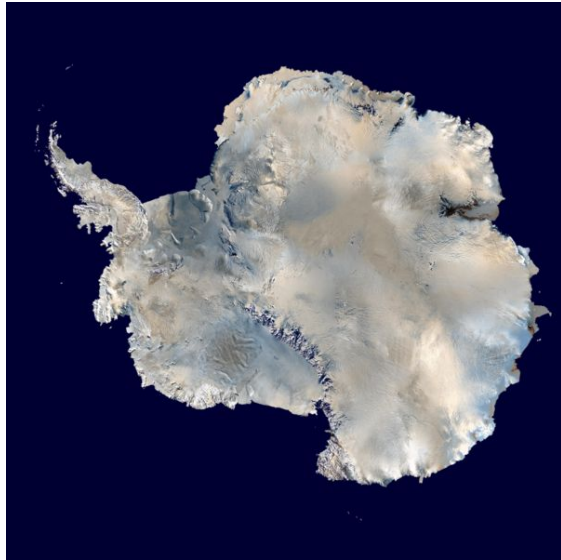
MODERN MICROBIOLOGICAL METHODS

Concepts: molecular, isotopic and chemical techniques in environmental microbiology, environmental “-omic” insights into microbial diversity and physiology, ecophysiology, modern microbially dominated environments, linking microbes and genes to biogeochemical cycles

Reading: DeLong review

Reading for in class discussion on Wednesday is posted (Valentine review and Milucka et al. 2012)

EXAMPLES OF MODERN MICROBIALLY-DOMINATED ENVIRONMENTS



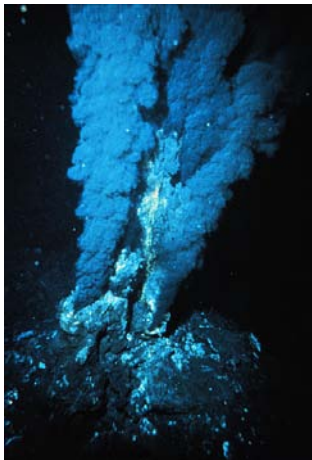
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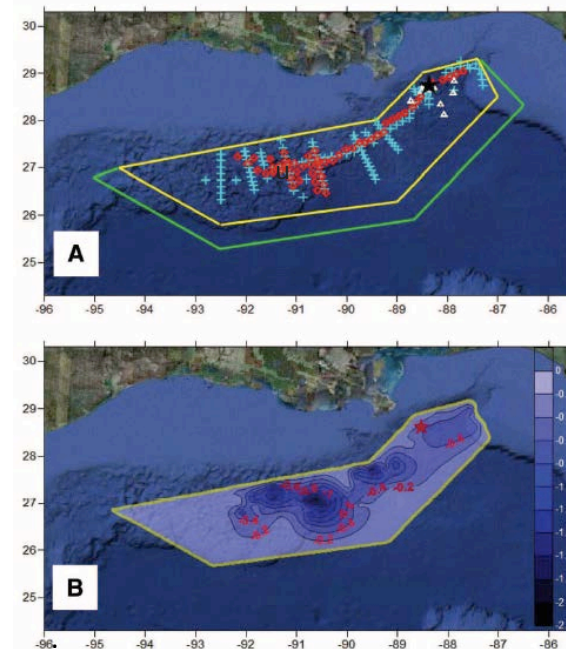
EXAMPLES OF MODERN MICROBIALLY-DOMINATED ENVIRONMENTS



Courtesy of [Robert Young](#) on flickr. CC-BY.

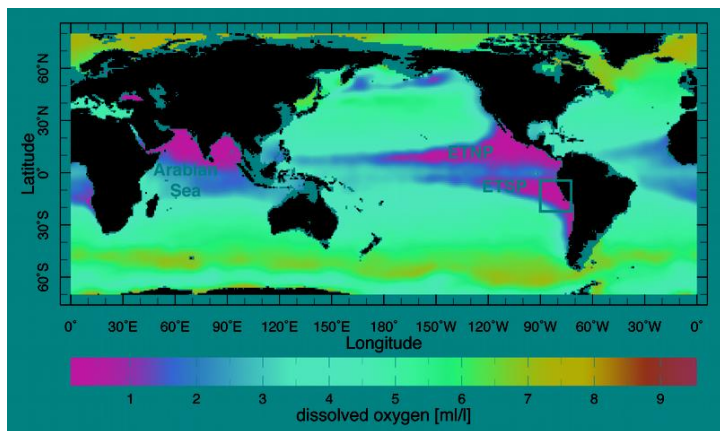


Courtesy of [Ebedgert](#) on wikipedia. Photograph is in the public domain.



Kessler et al., 2011

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Source: <http://iridl.ldeo.columbia.edu/>, IRI/LDEO Climate Data Library, Columbia

EXAMPLES OF MODERN MICROBIALLY-DOMINATED ENVIRONMENTS



GOES WEST



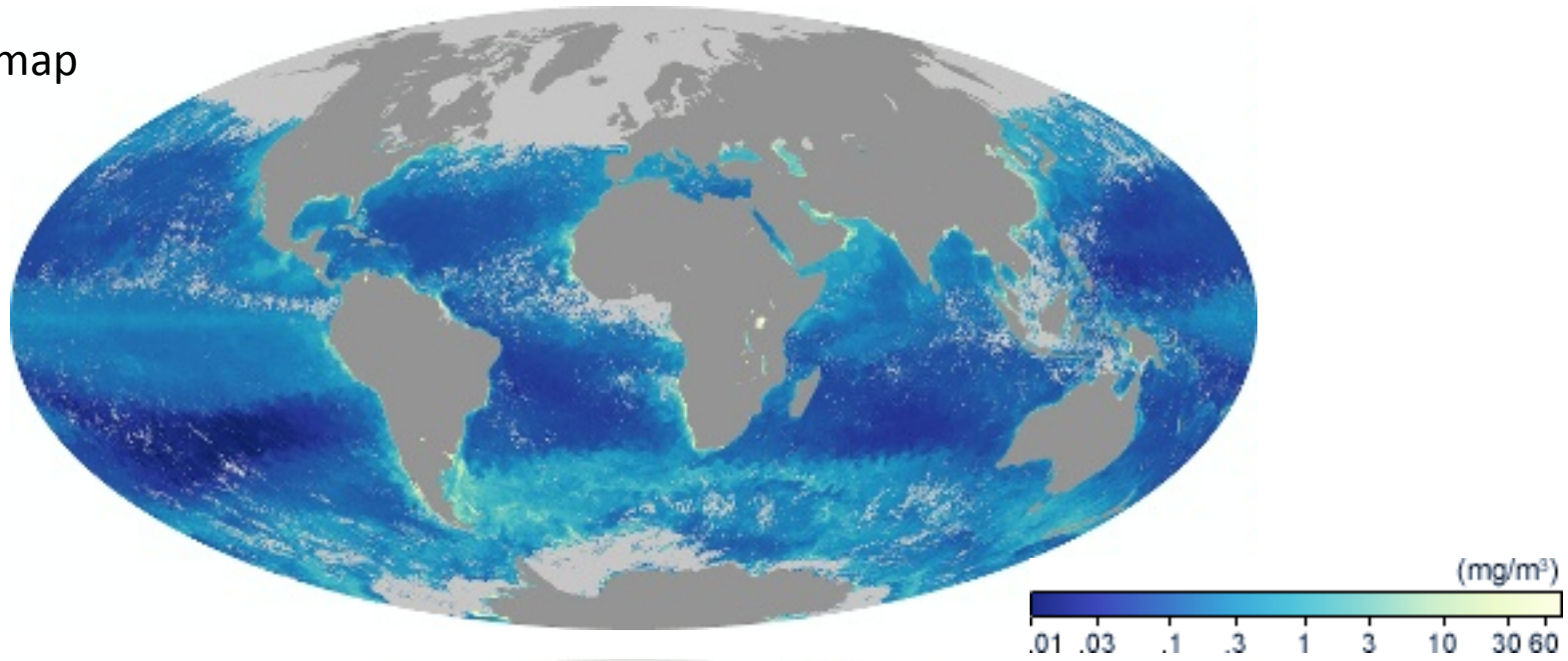
GOES EAST



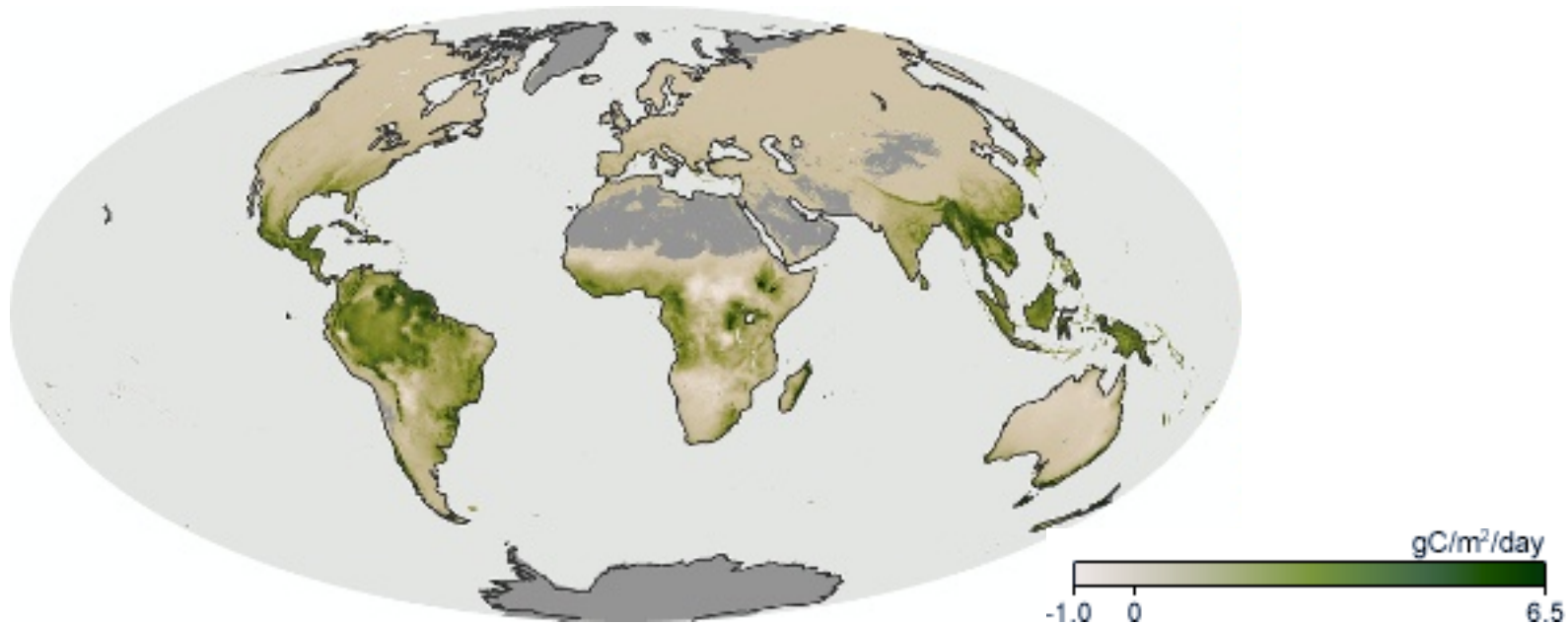
Courtesy of NASA. Image in the public domain.

Courtesy of National Oceanic and Atmospheric Administration. Photograph in the public domain.

Chlorophyll map



NPP map



Perspective

Prokaryotes: The unseen majority

William B. Whitman^{*†}, David C. Coleman[‡], and William J. Wiebe[§]

Departments of ^{*}Microbiology, [‡]Ecology, and [§]Marine Sciences, University of Georgia, Athens GA 30602

Table 2. Number of prokaryotes in soil

Ecosystem type [*]	Area, × 10 ¹² m ²	No. of cells, [†] × 10 ²⁷
Tropical rain forest	17.0	1.0
Tropical seasonal forest	7.5	0.5
Temperate evergreen forest	5.0	0.3
Temperate deciduous forest	7.0	0.4
Boreal forest	12.0	0.6
Woodland and shrubland	8.0	28.1
Savanna	15.0	52.7
Temperate grassland	9.0	31.6
Desert scrub	18.0	63.2
Cultivated land	14.0	49.1
Tundra and alpine	8.0	20.8
Swamps and marsh	2.0	7.3
Total	123.0	255.6

^{*}From ref. 73.

[†]For forest soils, the number of prokaryotes in the top 1 m was 4×10^7 cells per gram of soil, and in 1–8 m, it was 10^6 cells per gram of soil (16). For other soils, the number of prokaryotes in the top 1 m was 2×10^9 cells per gram of soil, and in 1–8 m, it was 10^8 cells per gram of soil (18). The boreal forest and tundra and alpine soils were only 1 m deep. A cubic meter of soil was taken as 1.3×10^6 g.

Table 3. Total number of prokaryotes in unconsolidated subsurface sediments

Depth interval, [*] m	Cells/cm ³ , × 10 ⁶	No. of cells, × 10 ²⁸		
		Deep oceans [†]	Continental shelf and slope [‡]	Coastal plains [§]
0.1	220.0	66.0	14.5	4.4
10	45.0	121.5	26.6	8.1
100	6.2	18.6	4.1	1.2
200	19.0	57.0	12.5	3.8
300	4.0	12.0	2.6	0.8
400	7.8		10.1	3.2
600	0.95		3.7	1.2
1,200	0.61		3.2	1.0
2,000	0.44		2.6	0.9
3,000	0.34			0.7
Total		275.1	79.9	25.3
Grand Total: $380 \times 10^{28} = 3.8 \times 10^{30}$				

^{*}Depth intervals are designated by the upper boundary. Thus, “0.1” represents 0.1–10 m and “3,000” represents 3,000–4,000.

[†]Corresponds to seismic layer I (23).

[‡]Corresponds to subcontinental sediments (23).

[§]Corresponds to geosyncline sediments of Mesocenozoic origin (23).

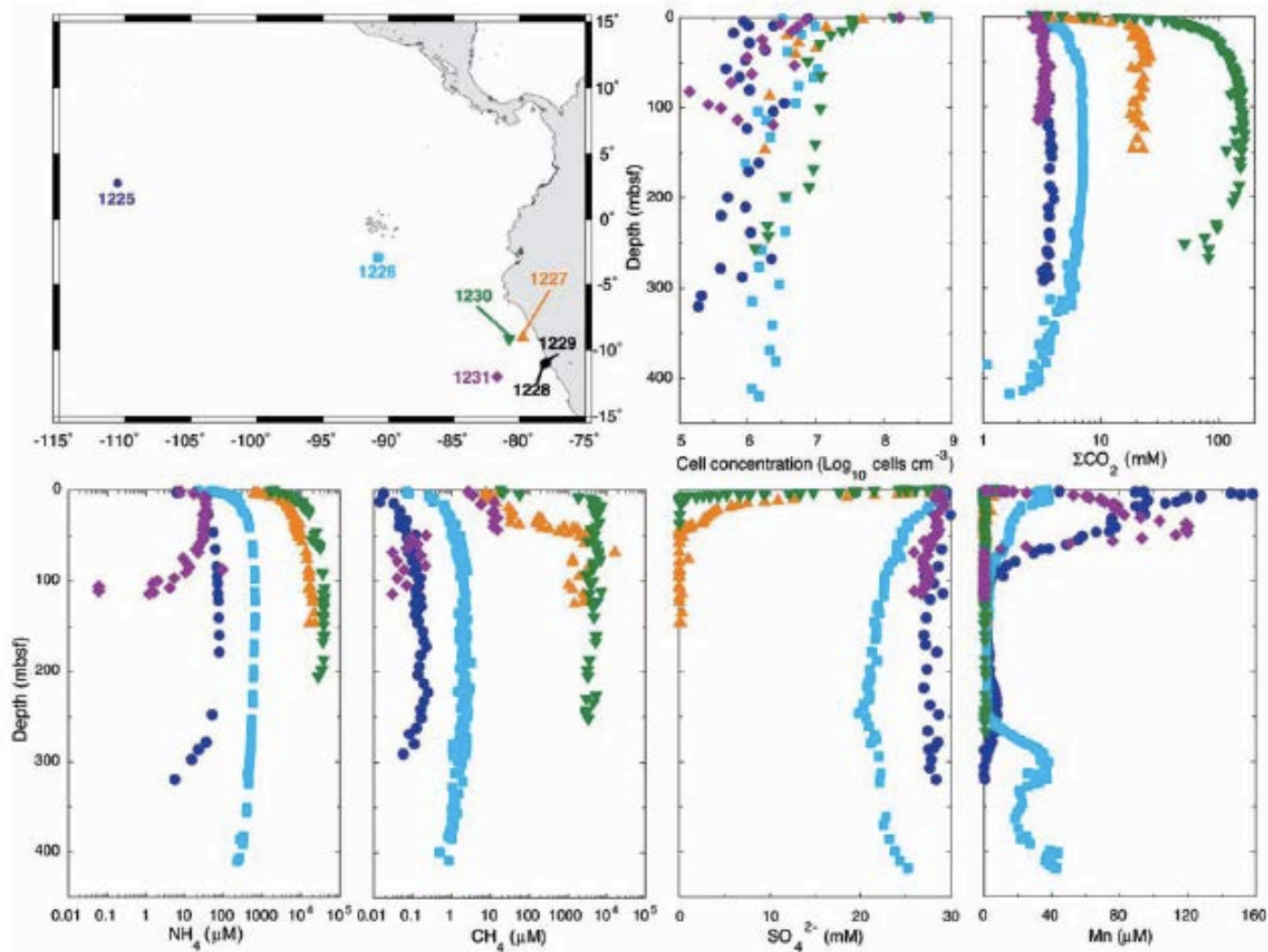
^{||}Calculated from the arithmetic averages.

^{||}Calculated by extrapolation of the formula of Parkes *et al.* (33).

Source: Whitman, William B., David C. Coleman, et al. "Prokaryotes: the Unseen Majority." *Proceedings of the National Academy of Sciences* 95, no. 12 (1998): 6578–83. Copyright National Academy of Sciences, U.S.A.

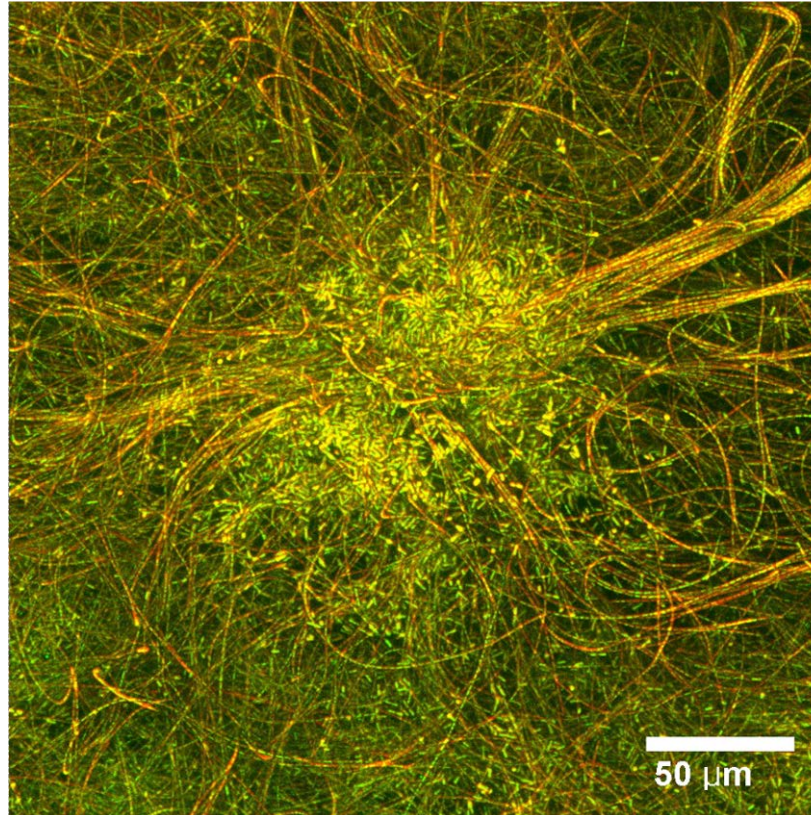
Step 0: geochemical observations

Distributions of Microbial Activities in Deep Subseafloor Sediments



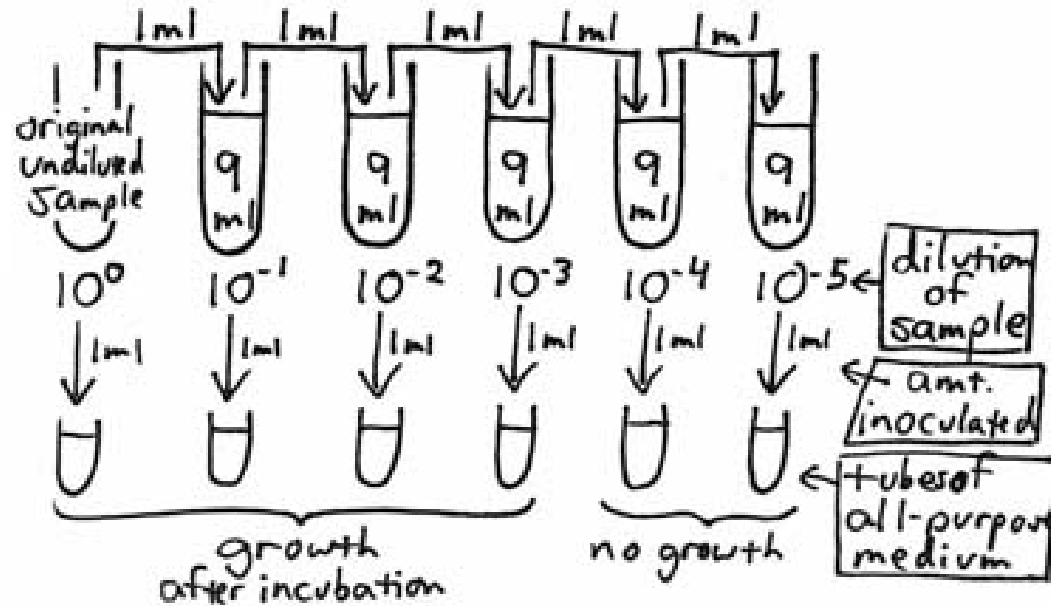
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Step 1: microscopy and morphological characterization



Courtesy of MDPI. CC-BY. Source: Figure 1C of Sim, M. S. et al. "[Oxygen-Dependent Morphogenesis of Modern Clumped Photosynthetic Mats and Implications for the Archean Stromatolite Record.](#)" *Geosciences* 2, no. 4 (2012): 235-59.

Step 2: enrichment, cultivation and isolation



<http://www.jlindquist.net/generalmicro/102dil3.html>

Courtesy of John Lindquist. Used with permission.

The Most Probable Number Method

Step 3: amplification of 16s rRNA sequences, clone libraries, DGGE

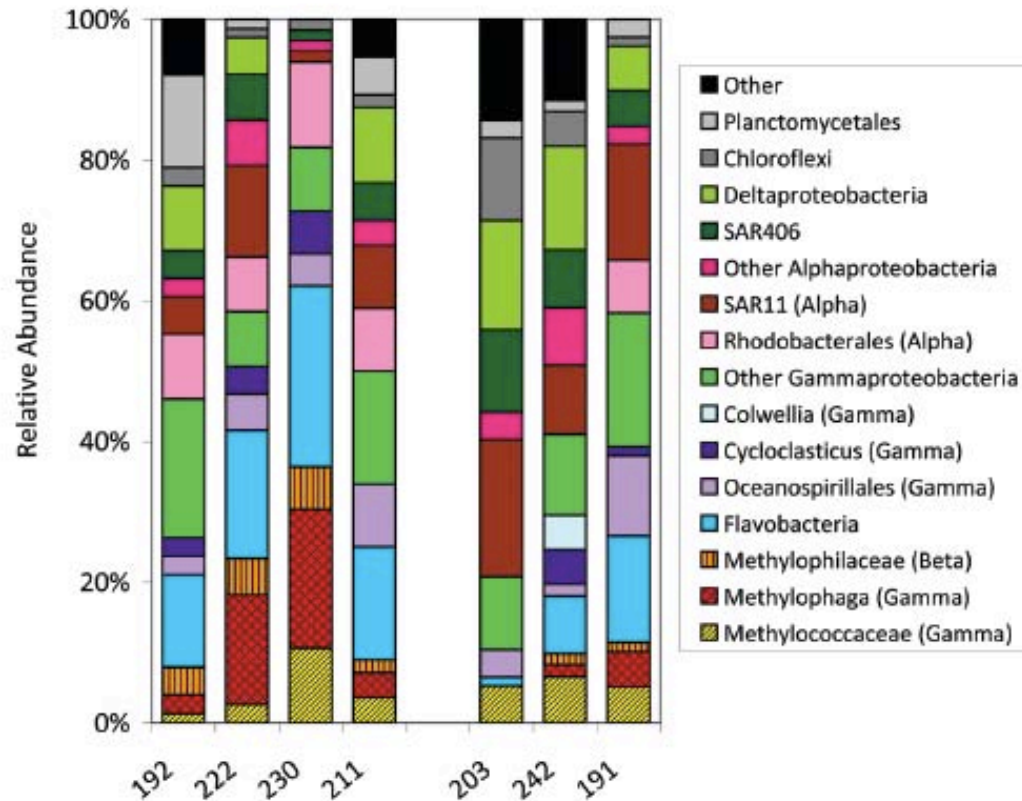
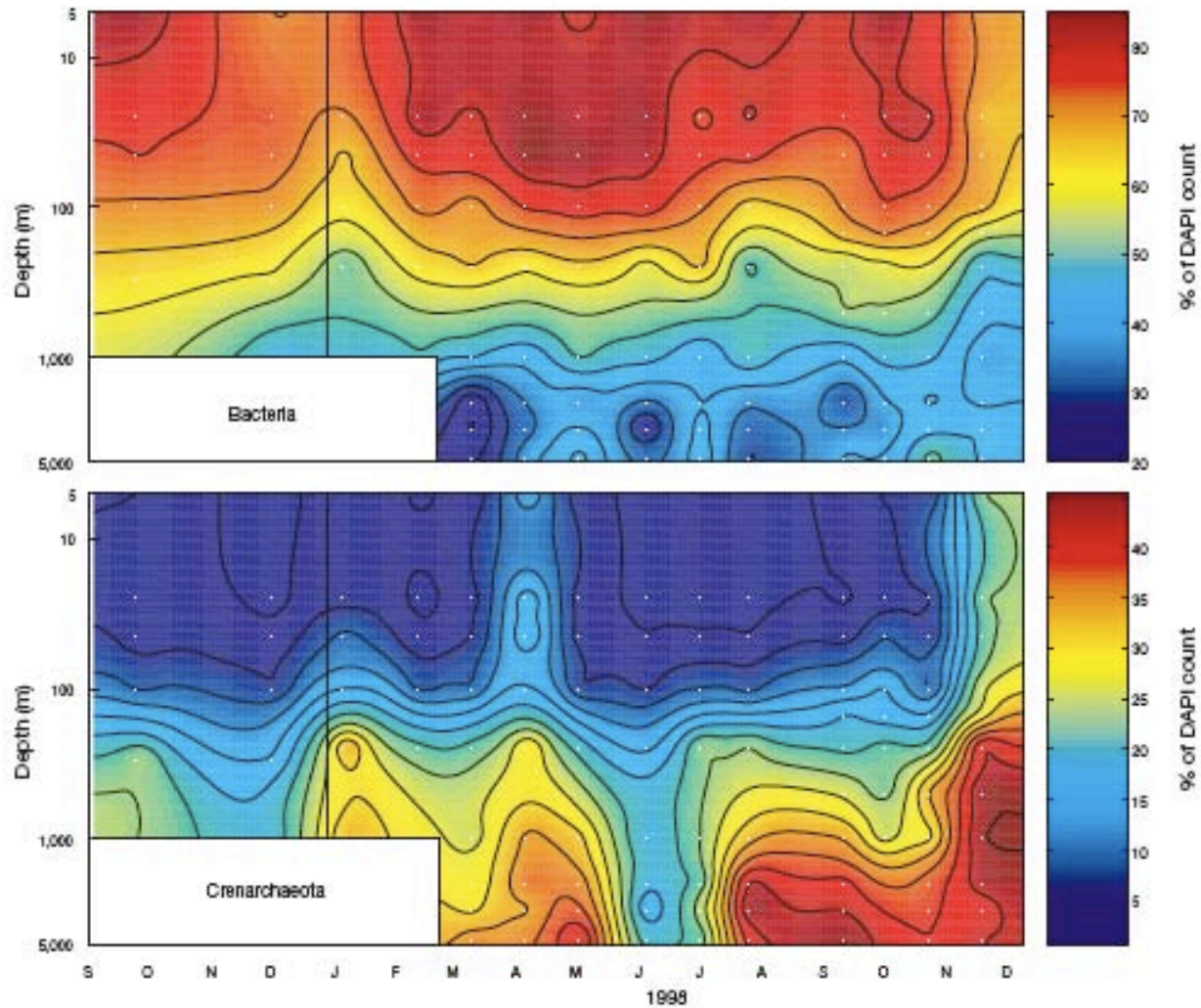


Fig. 2. Results from DNA surveys for bacterial 16S rRNA genes representing changes in community structure associated with oxidation of CH_4 in samples collected from 7 to 17 September 2010. Stations are shown from left to right in order of decreasing reductions in DO. Stations 192, 222, 230, and 211 had DO and fluorescence anomalies (integrated oxygen reductions of 1.1, 0.7, 0.5, and 0.1 mol m^{-2} , respectively), whereas stations 191, 242, and 203 did not (integrated oxygen reductions $< 0.00001 \text{ mol m}^{-2}$). Methylophages (*Methylococcaceae*, *Methylophaga*, and *Methylophilaceae*) are indicated by shading. The Other category includes groups observed at $< 5\%$ in all samples, predominately *Acidobacteria*, *Actinobacteria*, and *Verrucomicrobia*. $n = 56$ to 79 per station for a total of 492 samples.

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Step 4: fluorescent *in situ* hybridization (16s rRNA sequences)



Courtesy of Nature Publishing Group. Used with permission. Source: Karner, M.B et al. "[Archaeal Dominance in the Mesopelagic Zone of the Pacific Ocean.](#)" *Nature* 409, no. 6819 (2001): 507-10.

SAR11 – UBIQUITOUS MARINE BACTERIUM

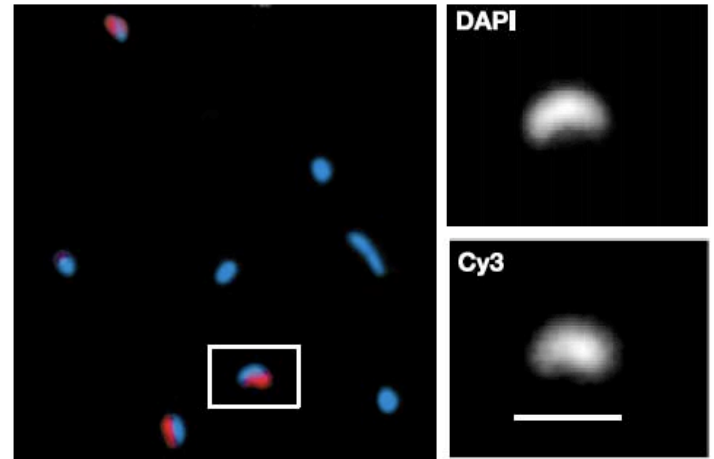
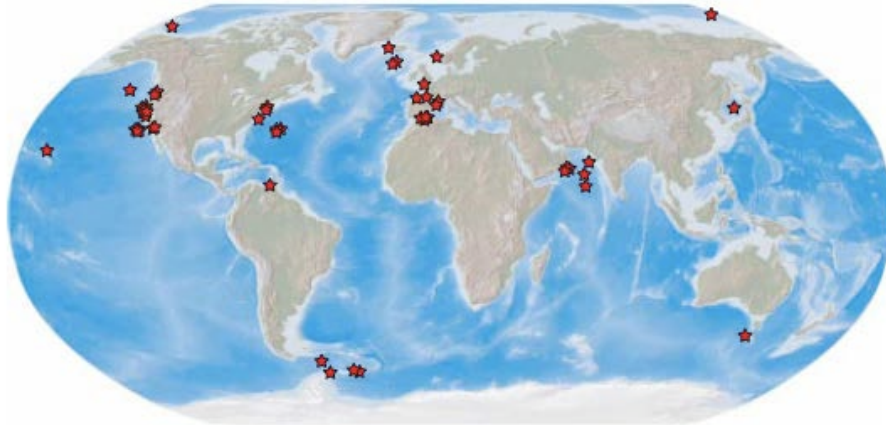
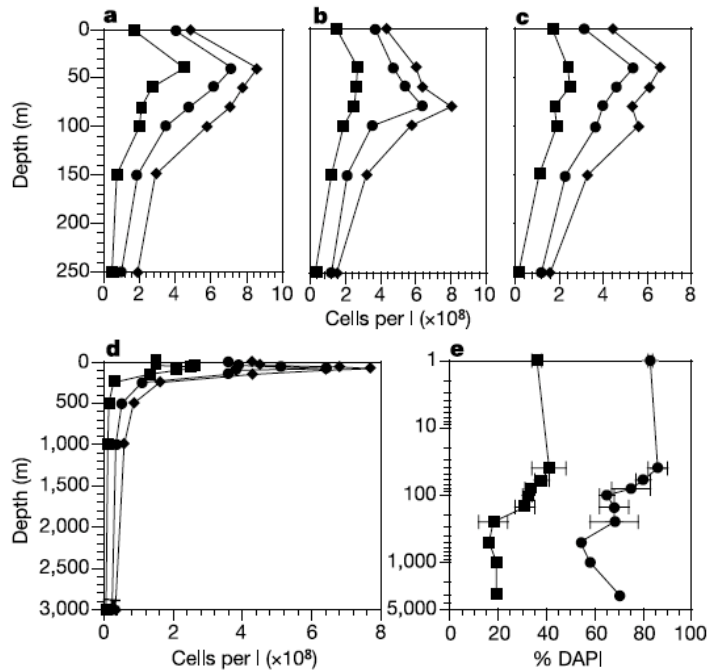
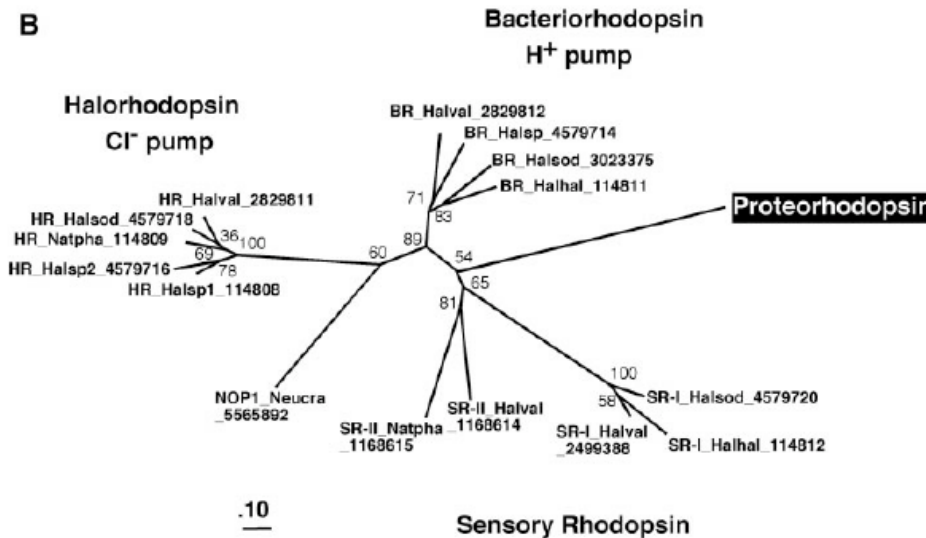
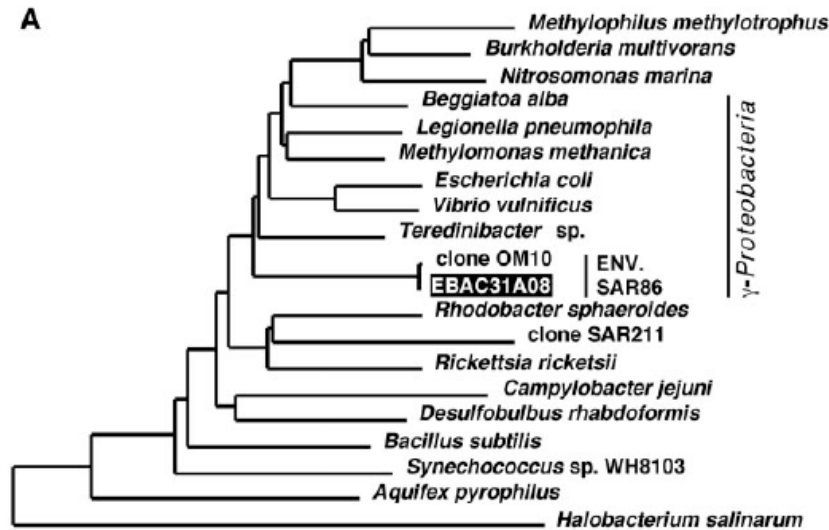


Figure 2 SAR11 fluorescence *in situ* hybridization image composite. Dual image overlay of DNA-containing cells stained with DAPI (blue) and the Cy3 probe (red). Cells emitting a signal for both DAPI and the Cy3 probe are both blue and red, and cells that did not hybridize to the set of SAR11 probes are blue. The identical fields of view in the DAPI- and Cy3-stained images show the characteristic size and curved rod morphology of a magnified SAR11 cell (white box). Scale bar, 1 μm .



Courtesy of Nature Publishing Group. Used with permission. Source: Morris, R.M. et al. "SAR11 Clade Dominates Ocean Surface Bacterioplankton Communities." *Nature* 420, no. 6917 (2002): 806-10.

Step 5: sequencing of environmental 16s rRNA + more

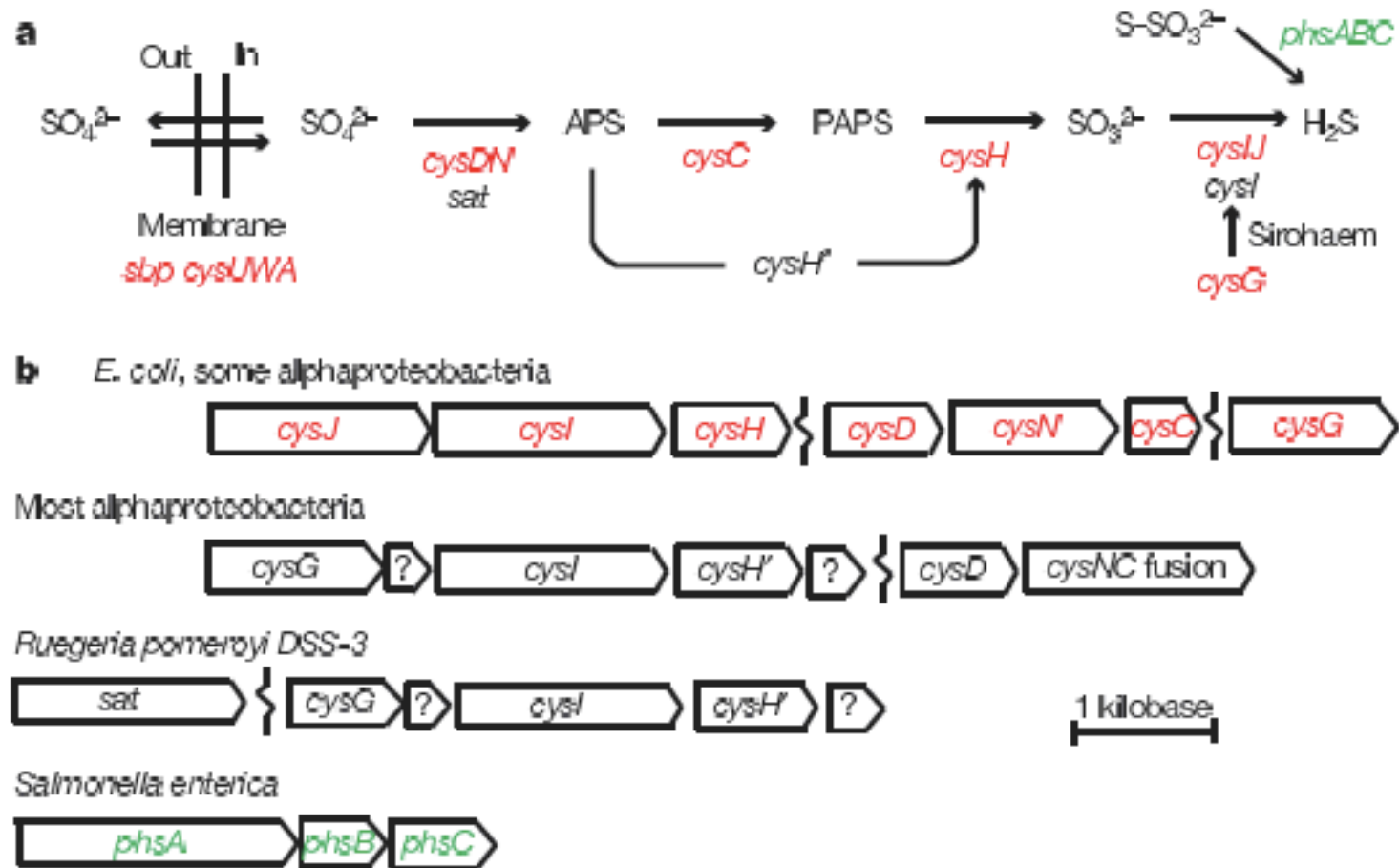


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Step 6: genome sequencing

SAR11 marine bacteria require exogenous reduced sulphur for growth

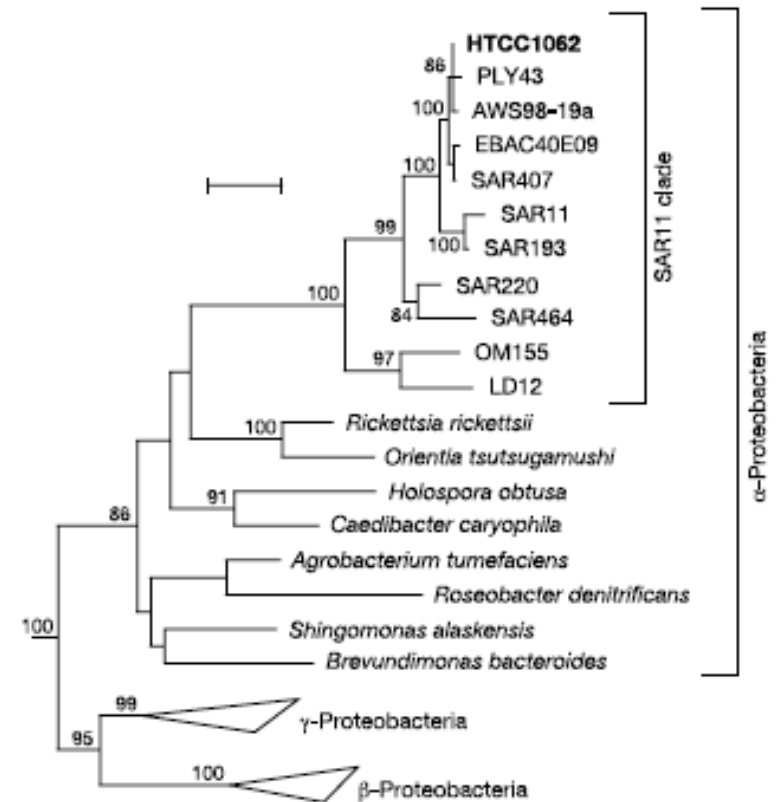
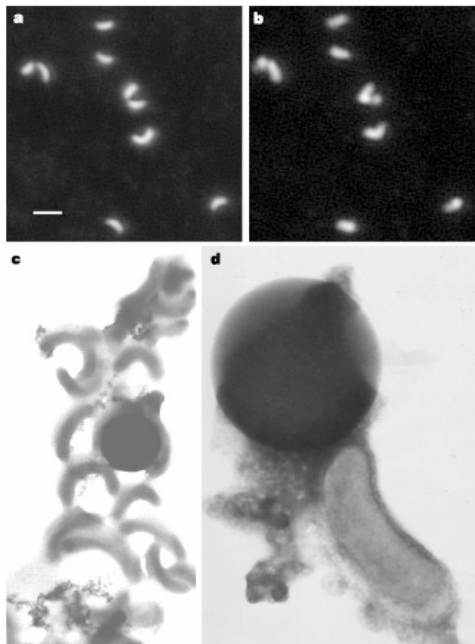
H. James Tripp¹, Joshua B. Kitner¹, Michael S. Schwalbach¹, John W. H. Dacey², Larry J. Wilhelm¹ & Stephen J. Giovannoni¹



Courtesy of Nature Publishing Group. Used with permission. Source: Tripp, H. J. et al. "SAR11 Marine Bacteria Require Exogenous Reduced Sulphur for Growth." *Nature* 452, no. 7188 (2008): 741-4.

Cultivation of the ubiquitous SAR11 marine bacterioplankton clade

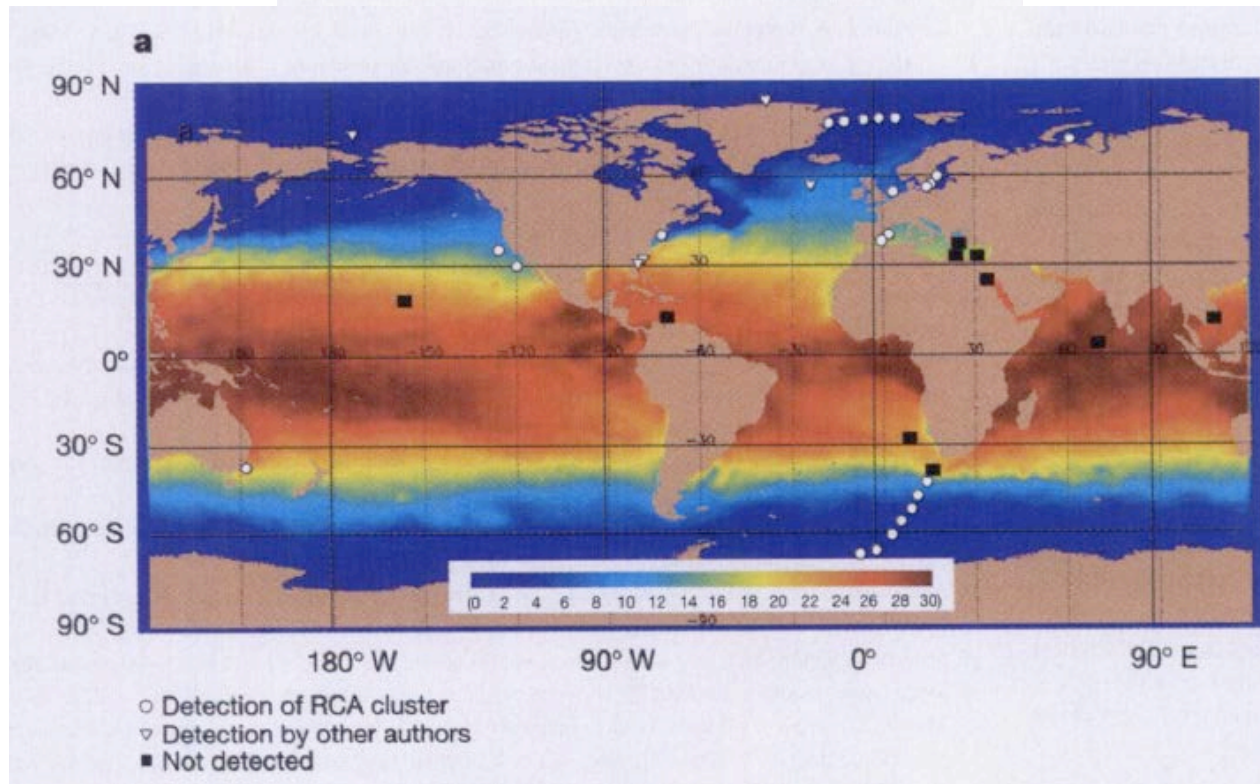
Michael S. Rappé, Stephanie A. Connon, Kevin L. Vergin
& Stephen J. Giovannoni



Courtesy of Nature Publishing Group. Used with permission. Source: Rappé, M.S. et al. "Cultivation of the Ubiquitous SAR11 Marine Bacterioplankton Clade." *Nature* 418, no. 6898 (2002): 630-3.

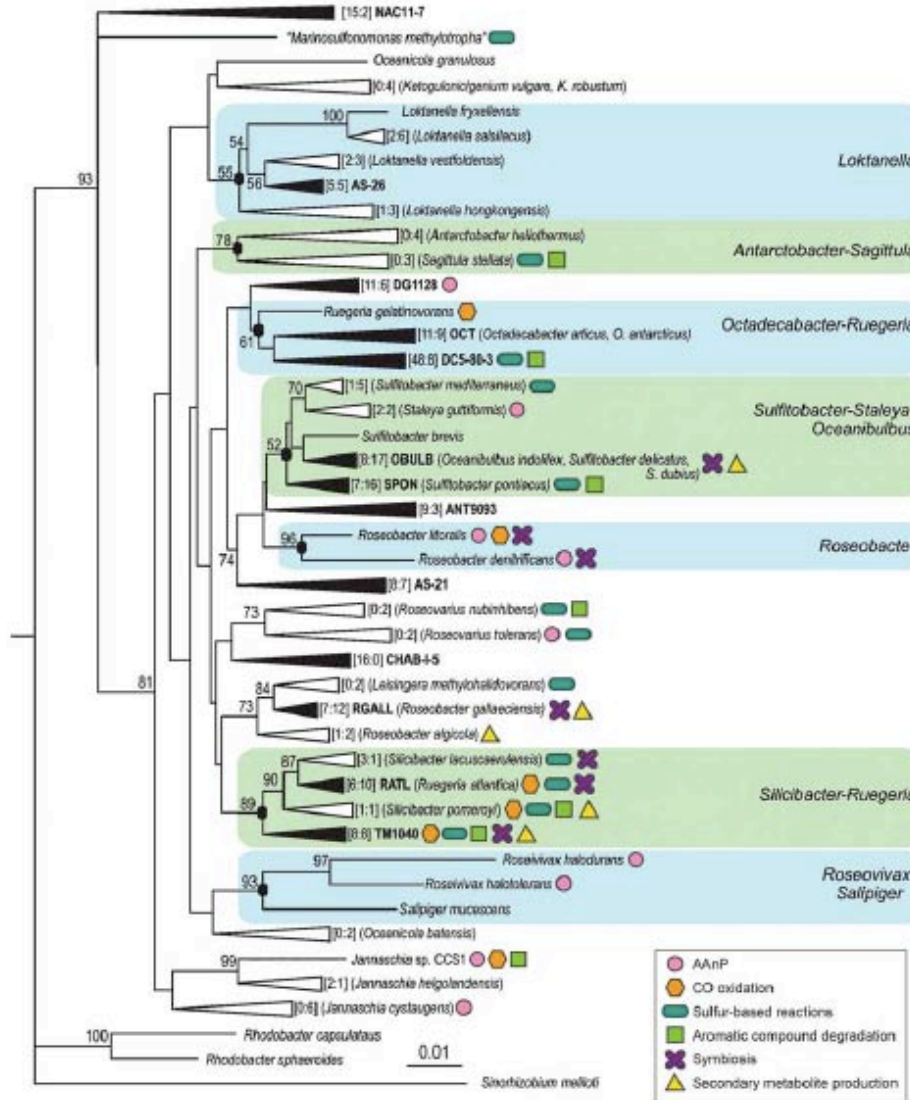
A newly discovered *Roseobacter* cluster in temperate and polar oceans

Natascha Selje*, Meinhard Simon & Thorsten Brinkhoff



Courtesy of Nature Publishing Group. Used with permission. Source: Selje, N. et al. "A Newly Discovered *Roseobacter* Cluster in Temperate and Polar Oceans." *Nature* 427, no. 6973 (2004): 445-8.

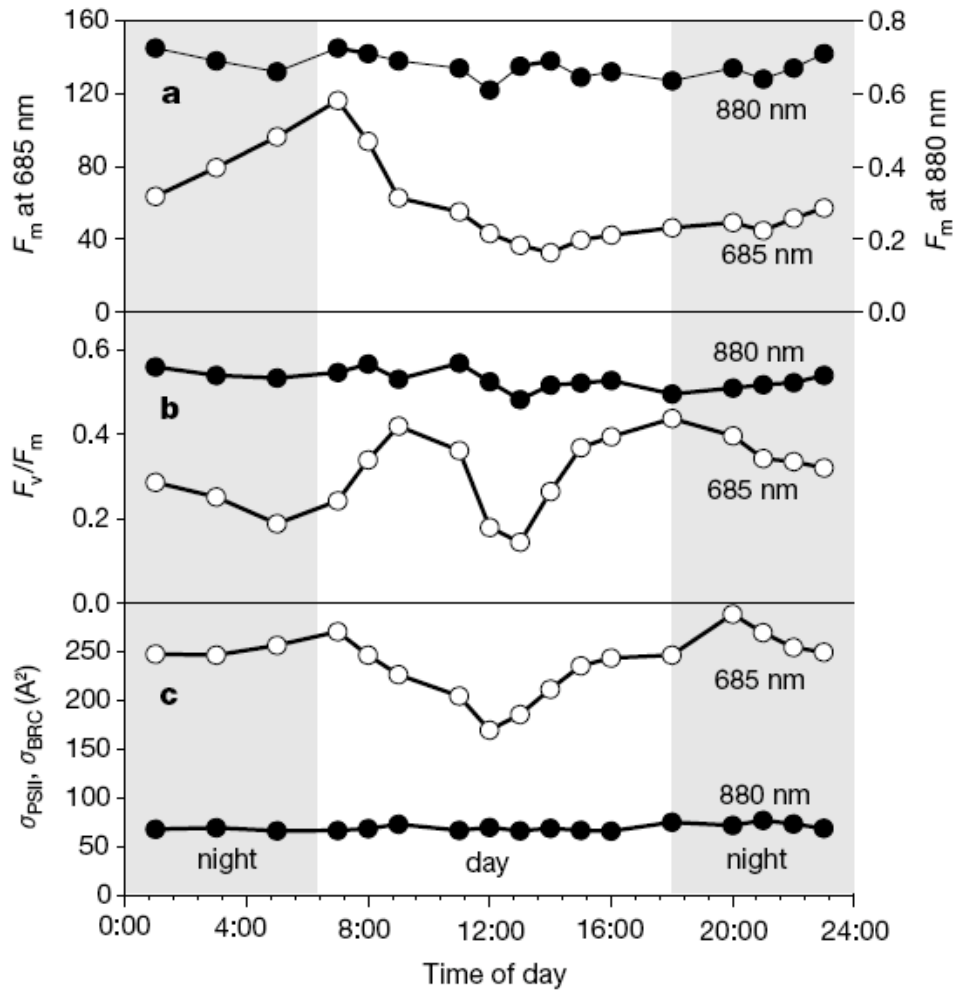
Step 8: physiological and genomic diversity



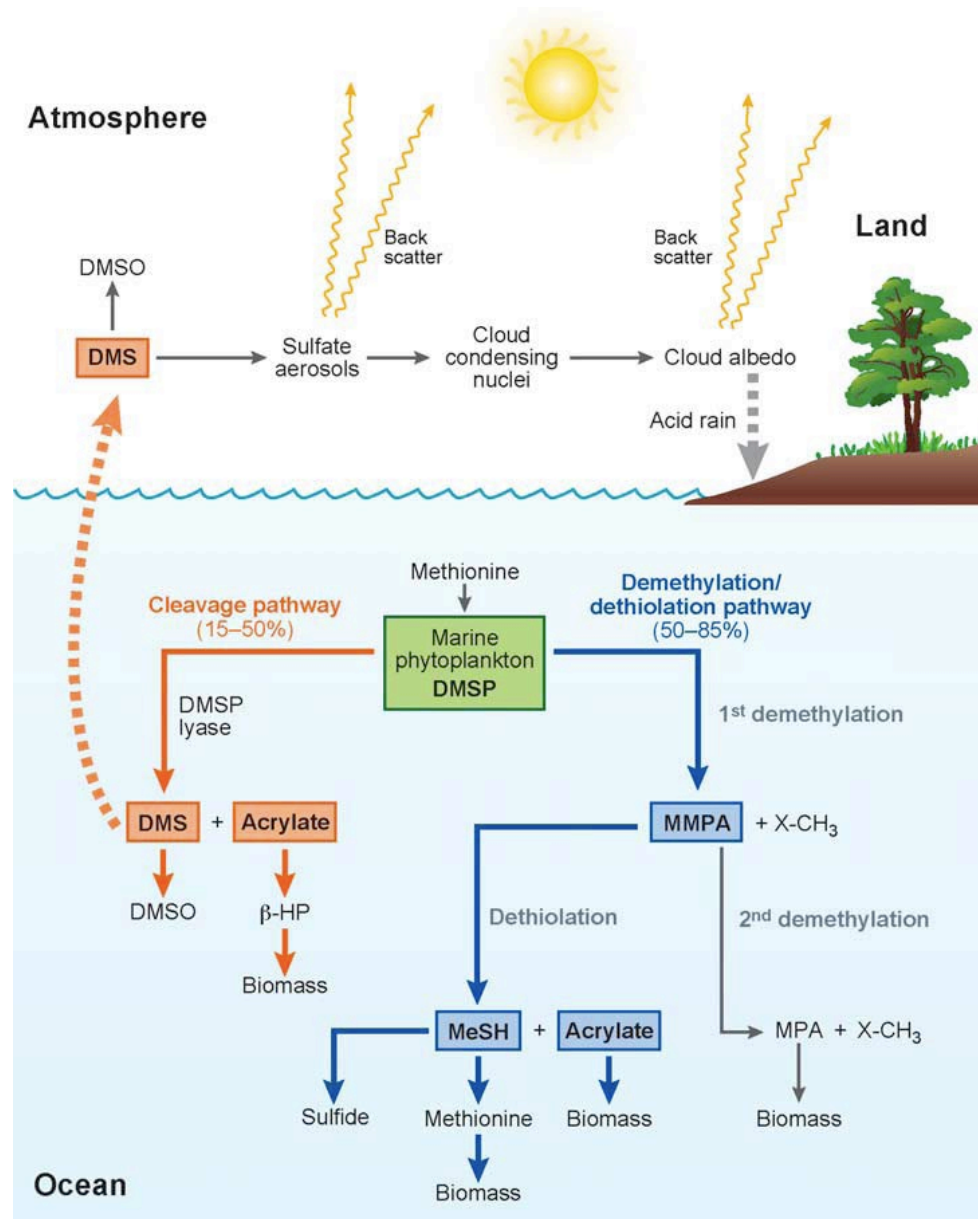
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Bacterial photosynthesis in surface waters of the open ocean

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



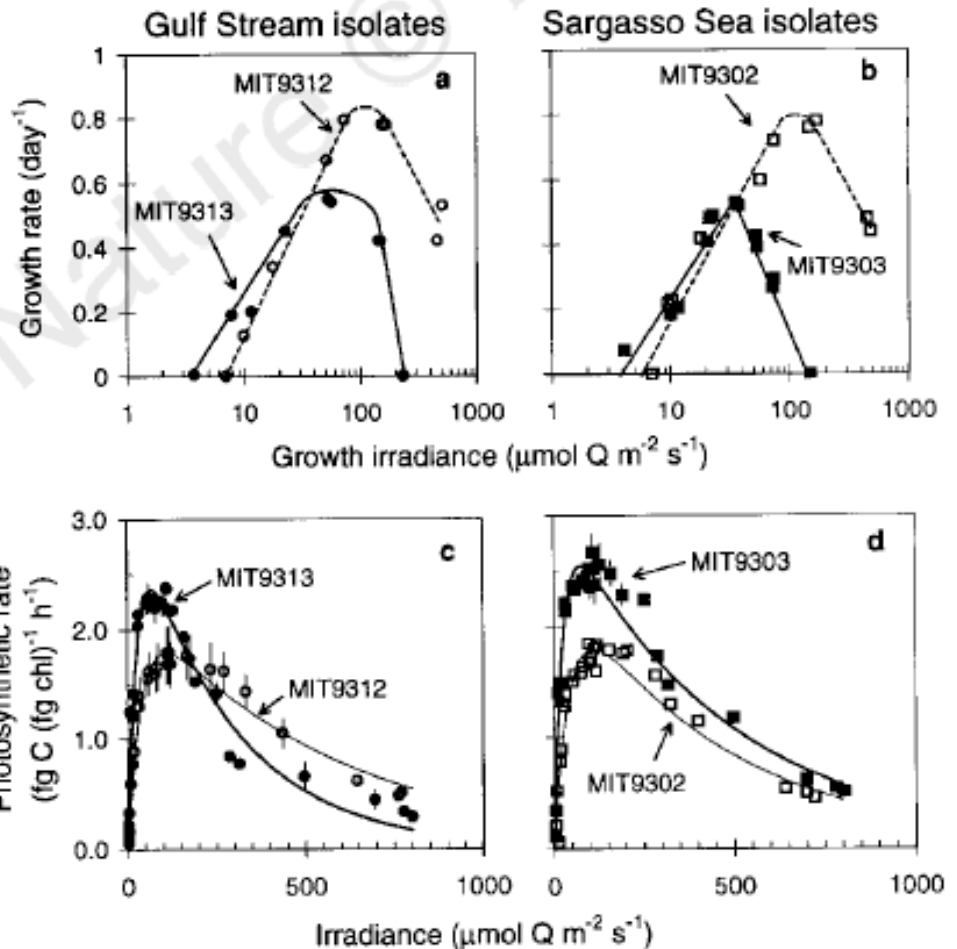
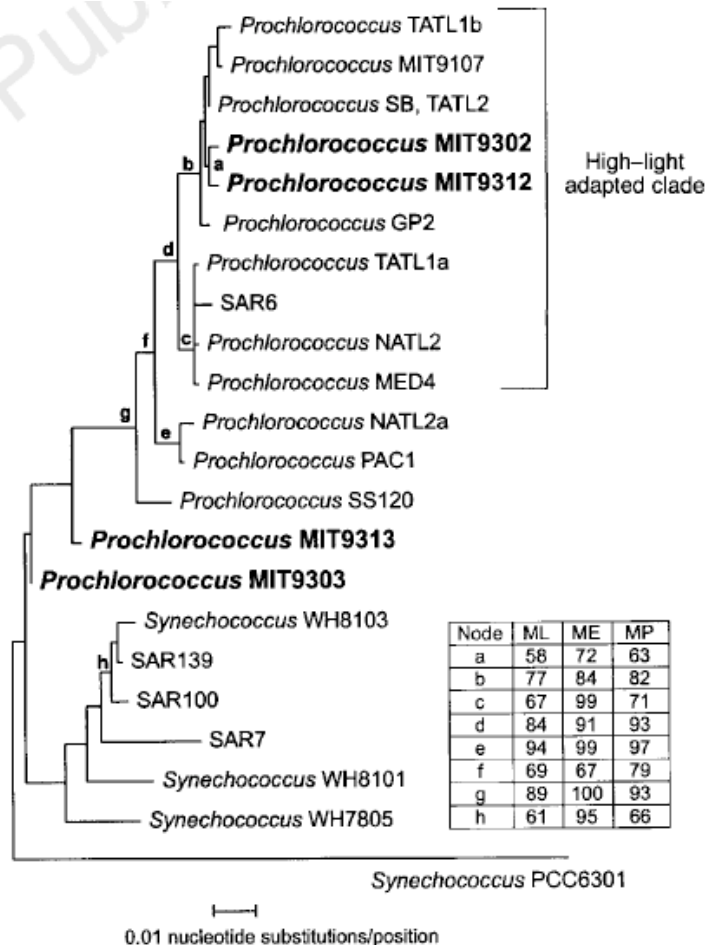
Courtesy of Nature Publishing Group. Used with permission. Source: Figure 2 from Kolber, Z.S. et al. "Bacterial Photosynthesis in Surface Waters of the Open Ocean." *Nature* 407, no. 6801 (2000): 177-9.



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Physiology and molecular phylogeny of coexisting *Prochlorococcus* ecotypes

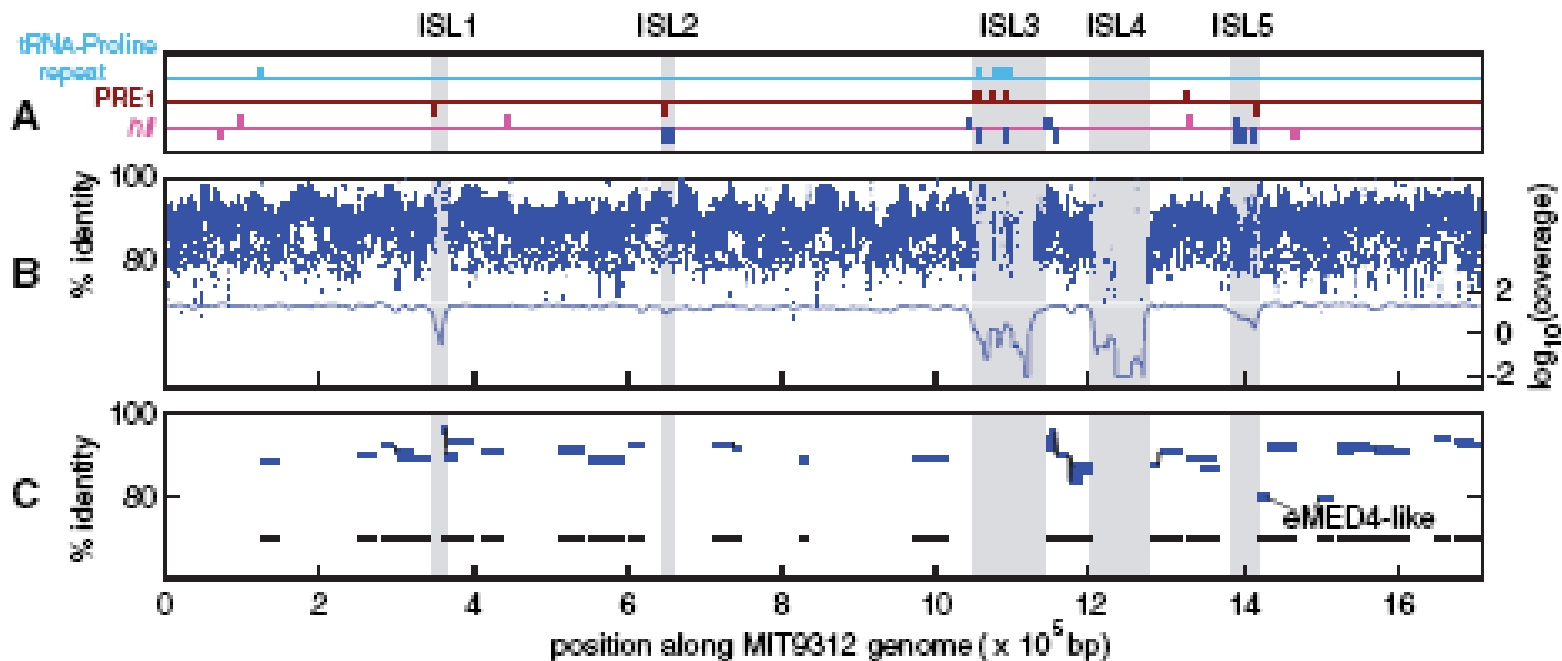
Lisa R. Moore*†, Gabrielle Rocap*†‡§ & Sallie W. Chisholm†‡



Courtesy of Nature Publishing Group. Used with permission. Source: Moore, L. R et al. "Physiology and Molecular Phylogeny of Coexisting *Prochlorococcus* Ecotypes." *Nature* 393, no. 6684 (1998): 464-7.

Genomic Islands and the Ecology and Evolution of *Prochlorococcus*

Maureen L. Coleman,¹ Matthew B. Sullivan,¹ Adam C. Martiny,¹ Claudia Steglich,^{1*}
Kerrie Barry,² Edward F. DeLong,¹ Sallie W. Chisholm^{1†}

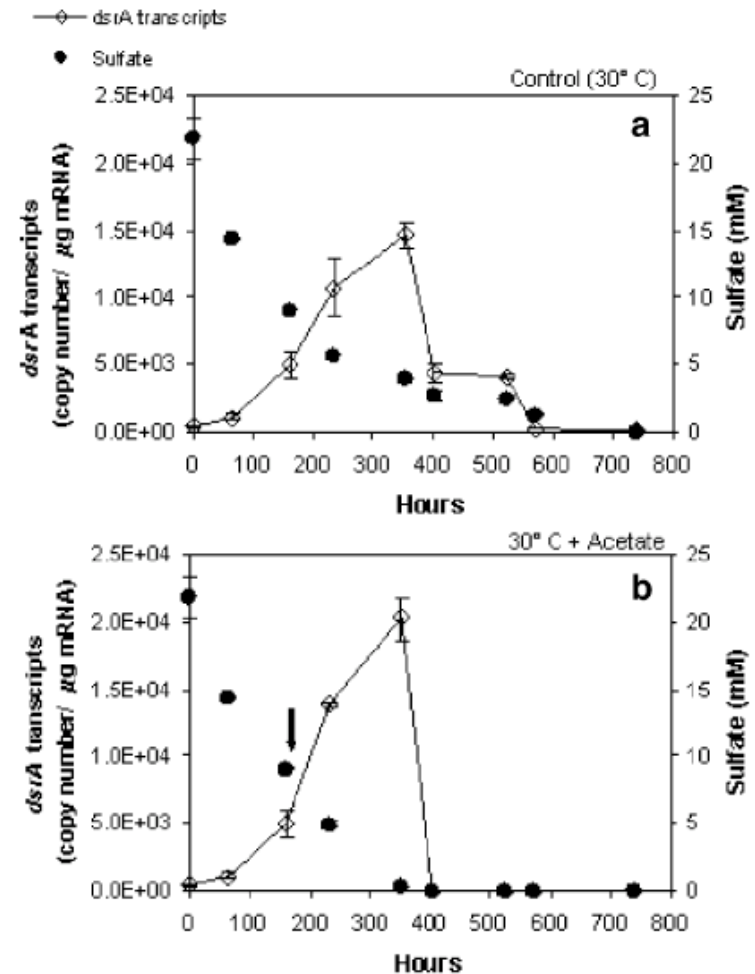


Stable core genome and variable genomic islands: phenotypic differences

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Source: Coleman, M.L. et al. "Genomic Islands and the Ecology and Evolution of *Prochlorococcus*." *Science* 311, no. 5768 (2006): 1768-70.

Quantifying Expression of a Dissimilatory (bi)Sulfite Reductase Gene in Petroleum-Contaminated Marine Harbor Sediments

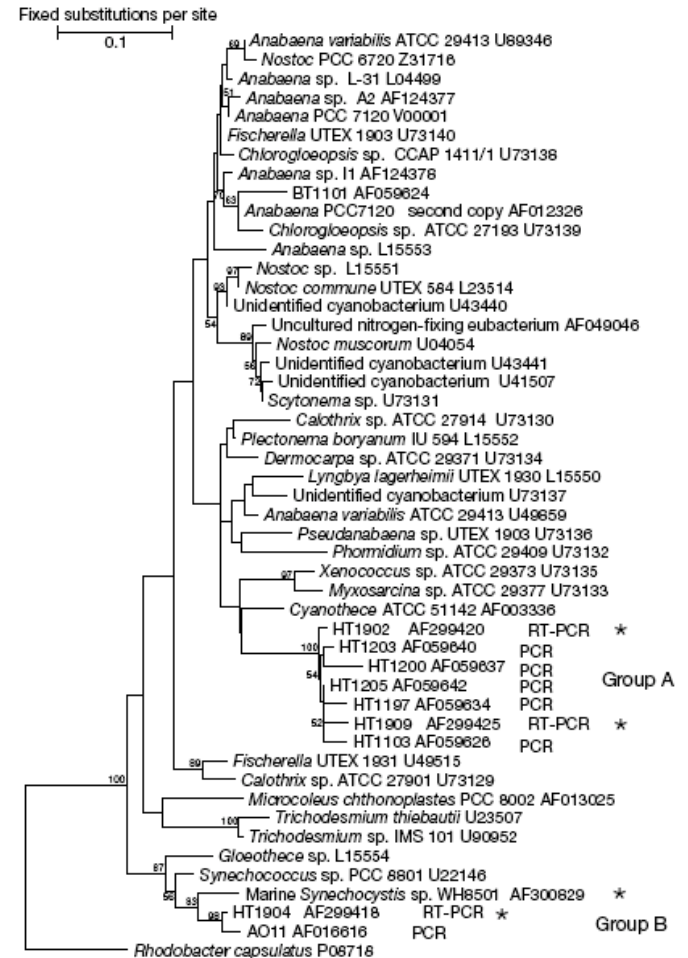
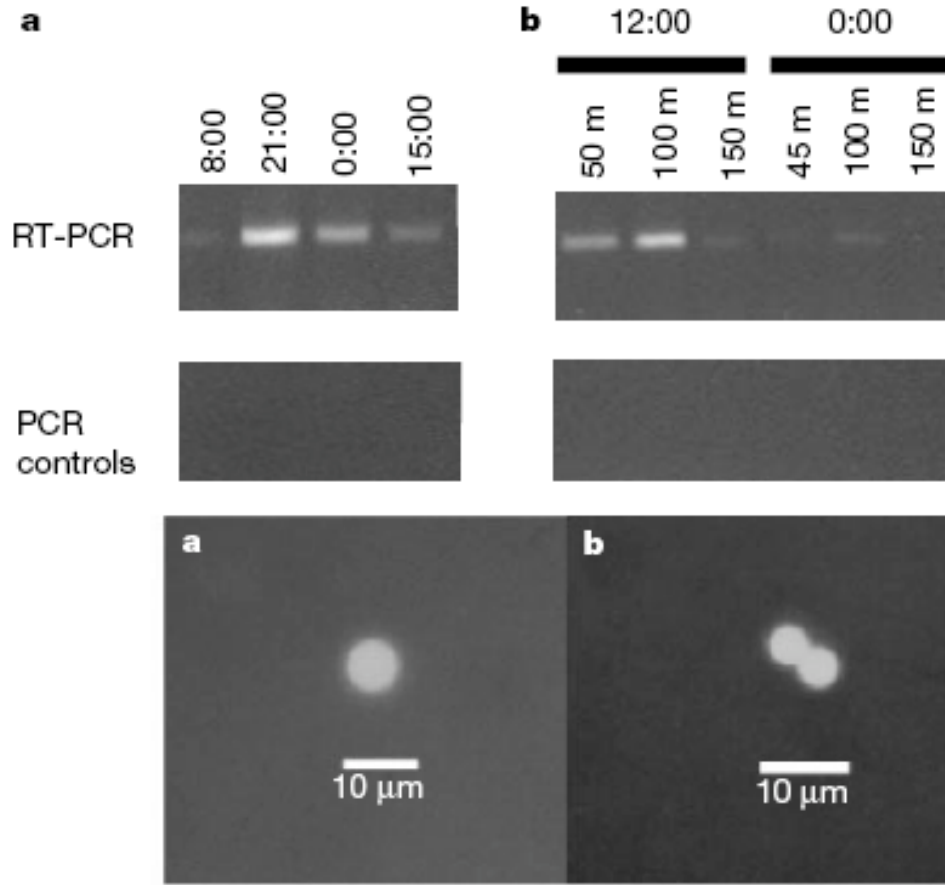
Kuk-Jeong Chin • Manju L. Sharma •
Lyndsey A. Russell • Kathleen R. O'Neill •
Derek R. Lovley



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Source: Chin, K. et al. "Quantifying Expression of a Dissimilatory (bi)Sulfite Reductase Gene in Petroleum-Contaminated Marine Harbor Sediments." *Microbial Ecology* 55, no. 3 (2008): 489-99.

Unicellular cyanobacteria fix N₂ in the subtropical North Pacific Ocean

Jonathan P. Zehr^{*,} John B. Waterbury^{†,} Patricia J. Turner^{*,}
Joseph P. Montoya^{‡,} Enoma Omoregie^{*,} Grieg F. Steward^{*,}
Andrew Hansen[§] & David M. Karl[§]

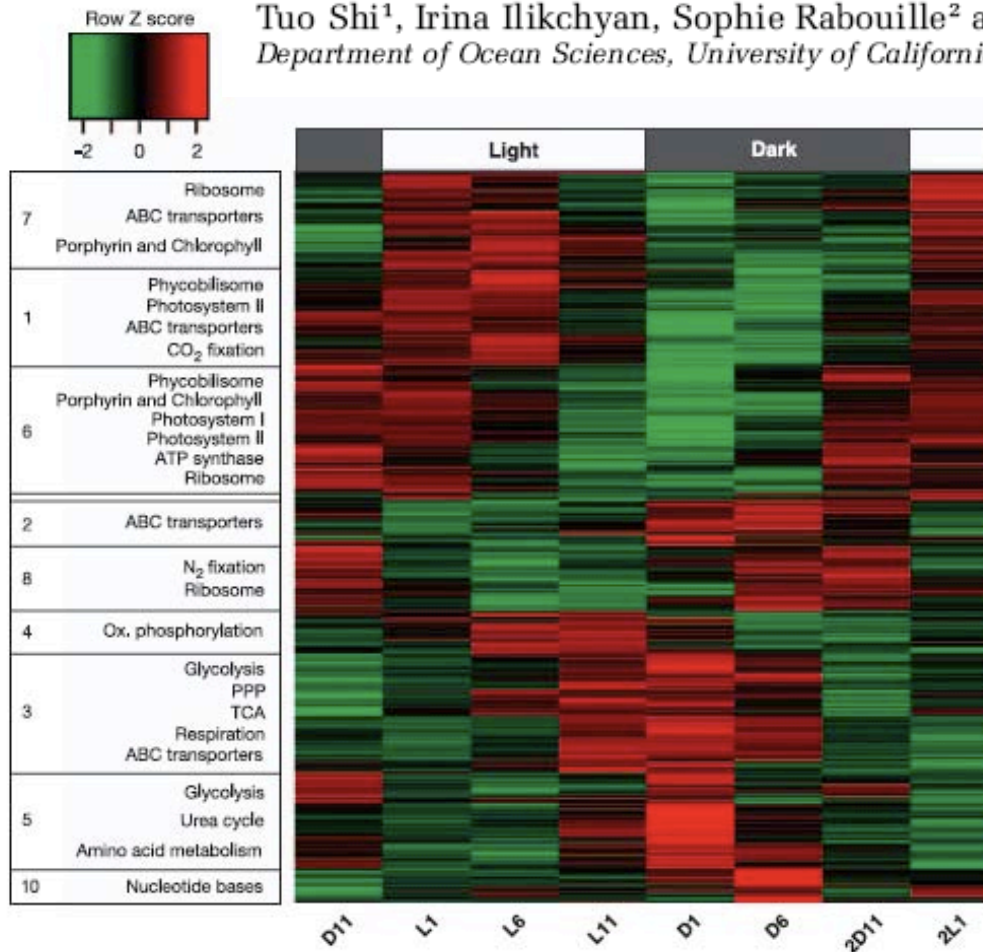


Courtesy of Nature Publishing Group. Used with permission. Source: Zehr, J.P. et al. "Unicellular Cyanobacteria fix N₂ in the Subtropical North Pacific Ocean." *Nature* 412, no. 6847 (2001): 635-8.

Step 10: diversity/transcription of the whole genome

Genome-wide analysis of diel gene expression in the unicellular N₂-fixing cyanobacterium *Crocospaera watsonii* WH 8501

Tuo Shi¹, Irina Ilikchyan, Sophie Rabouille² and Jonathan P Zehr
 Department of Ocean Sciences, University of California, Santa Cruz, CA, USA



Courtesy of International Society for Microbial Ecology. Used with permission. Source: Shi, T. et al. "Genome-Wide Analysis of Diel Gene Expression in the Unicellular N₂-fixing Cyanobacterium *Crocospaera Watsonii* WH 8501." *The ISME Journal* 4, no. 5 (2010): 621-32.

Step 11: environmental transcriptomics

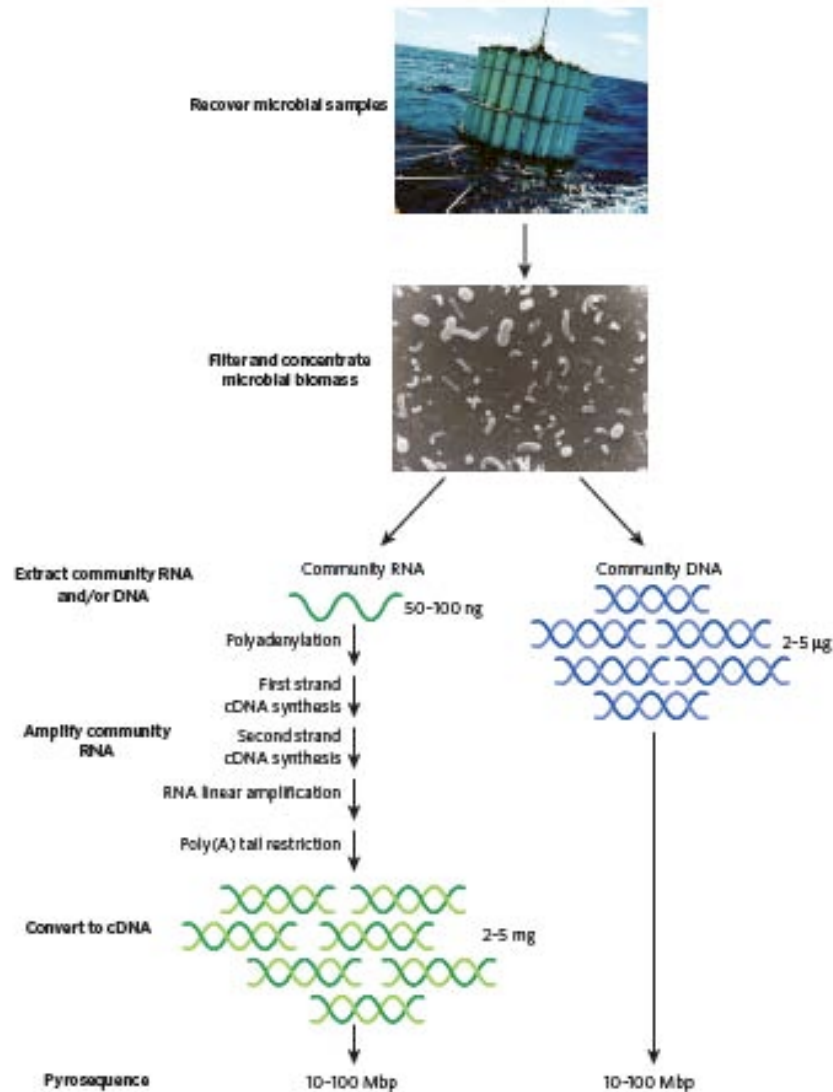
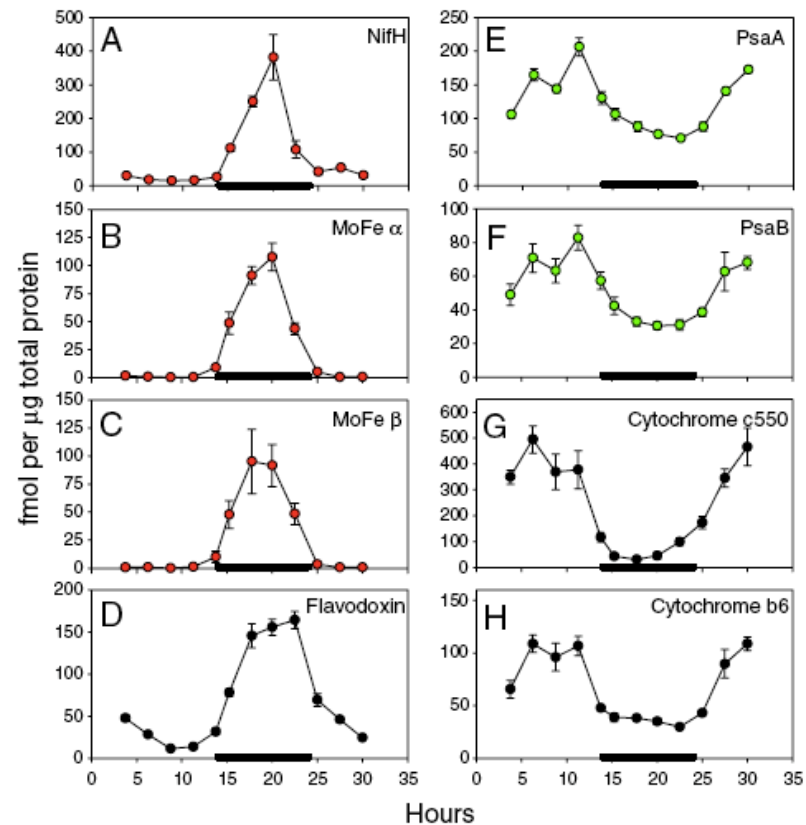
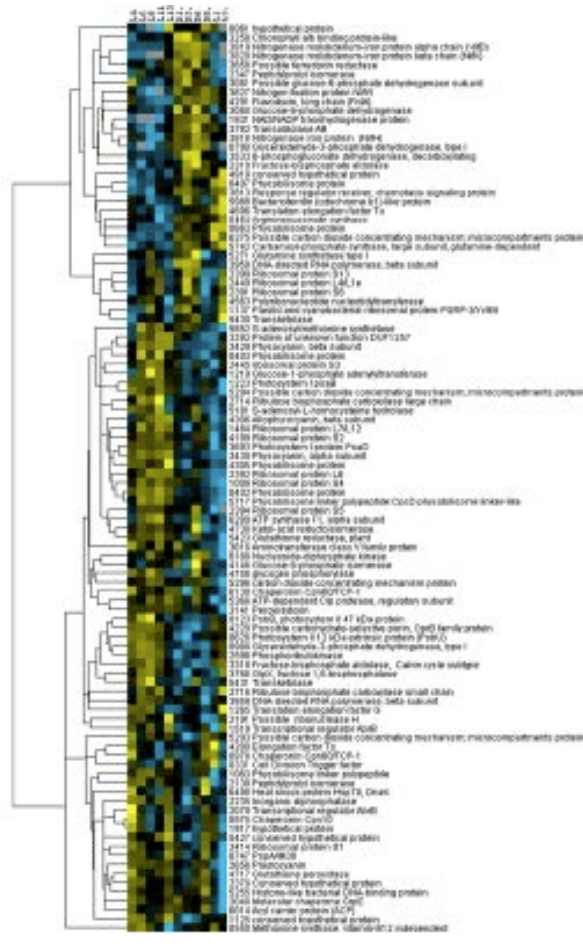


Figure 2 | Transcriptome sequencing protocol for marine microbial assemblages. Cells are collected and processed to produce genomic DNA, or cDNA from total RNA⁴; samples for RNA extraction are collected in smaller volumes (less than 1 litre) and filtered as rapidly as possible (about 10 min). After RNA amplification and conversion to cDNA, cDNA and genomic DNA from the same assemblage are sequenced and compared.

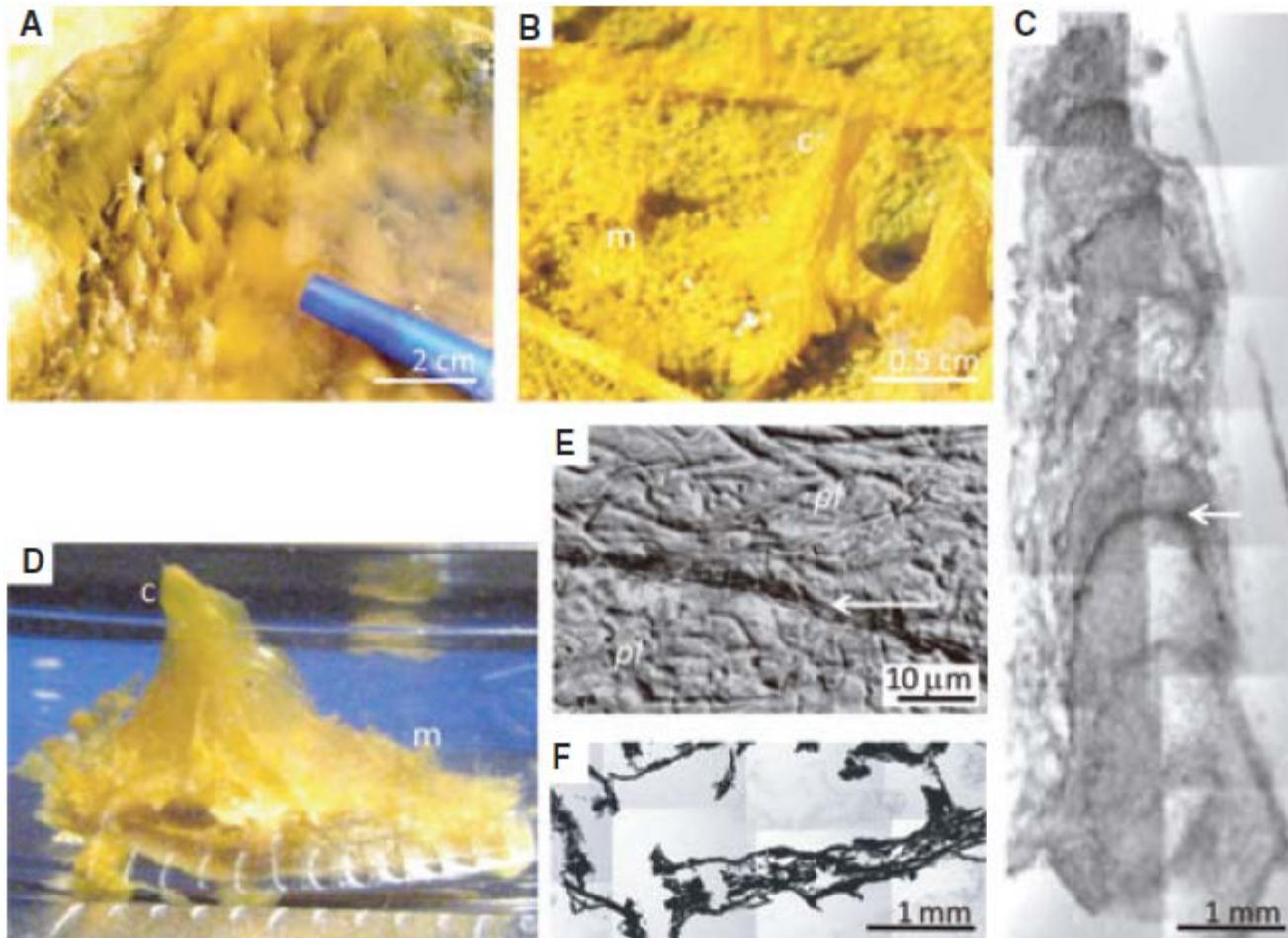
Step 12: proteomics

Iron conservation by reduction of metalloenzyme inventories in the marine diazotroph *Crocospaera watsonii*

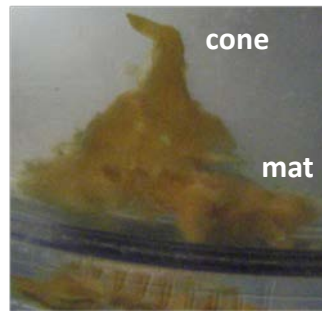
Mak A. Saito^{a,1}, Erin M. Bertrand^a, Stephanie Dutkiewicz^b, Vladimir V. Bulygin^{a,2}, Dawn M. Moran^a, Fanny M. Monteiro^b, Michael J. Follows^b, Frederica W. Valois^c, and John B. Waterbury^c



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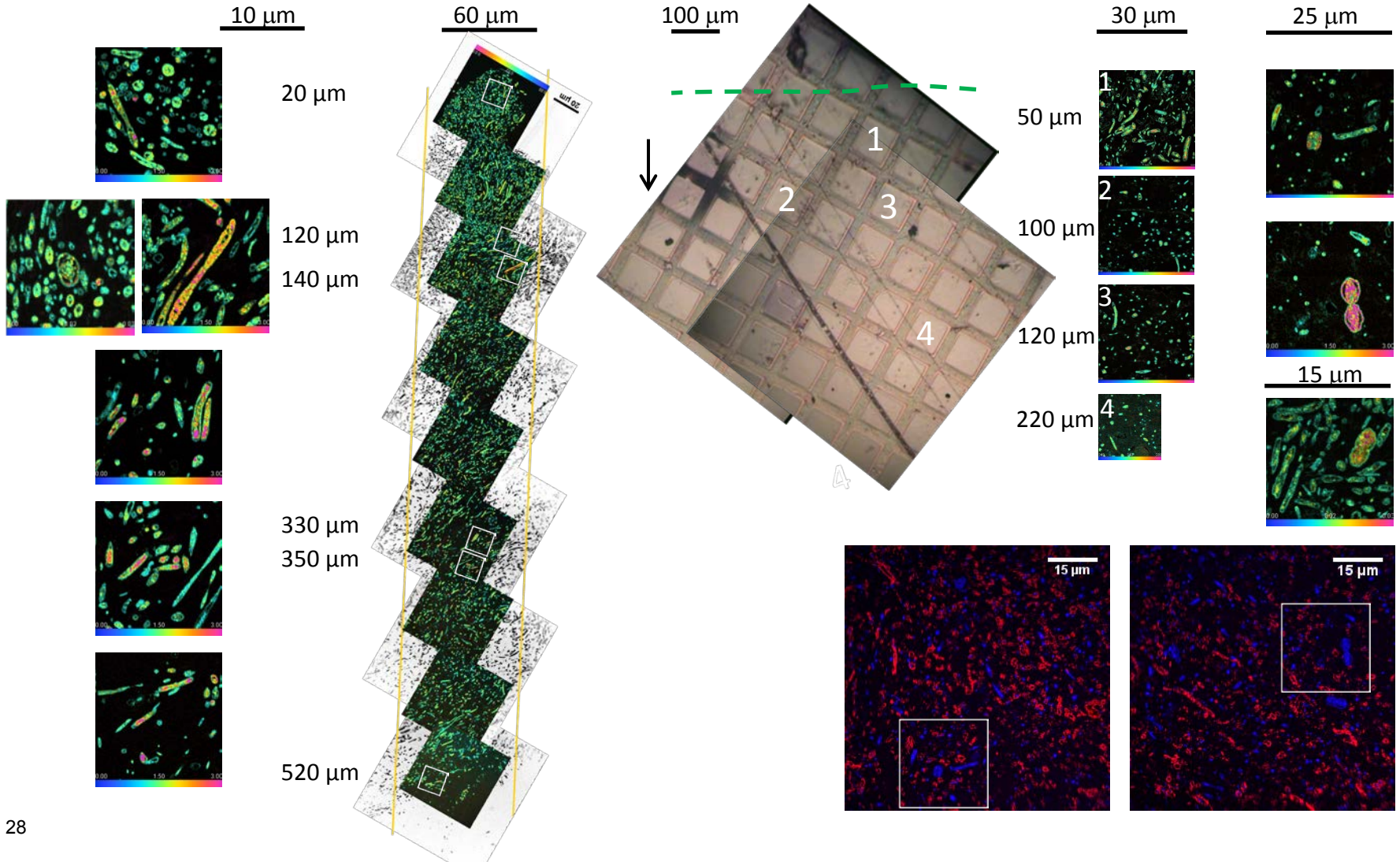


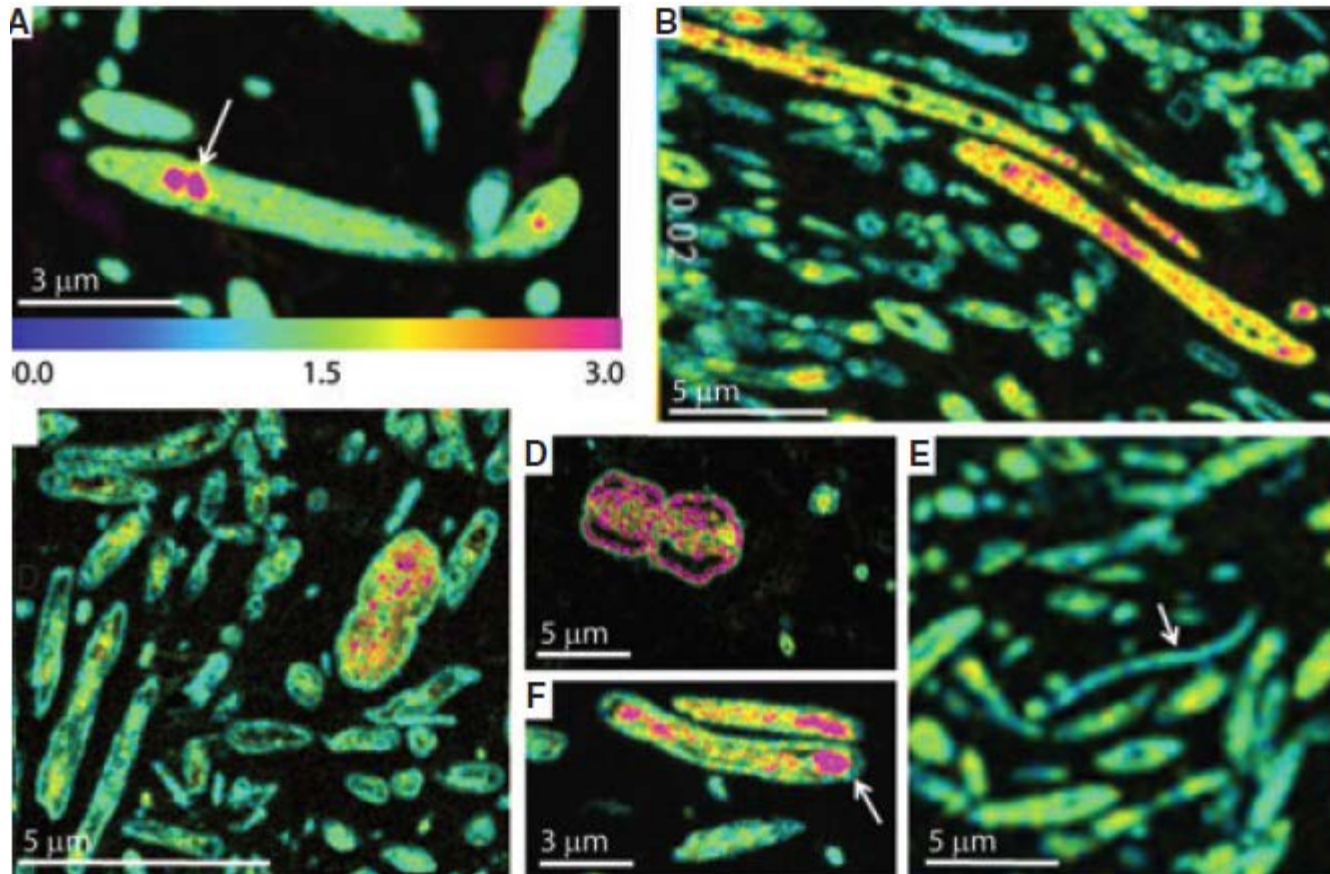
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 Source: Bosak, T. et al. "Cyanobacterial Diversity and Activity in Modern Conical Microbialites." *Geobiology* 10, no. 5 (2012): 384-401.



CONE

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