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 MICROBIAL DOMINATED ENVIROMENTS

Courtesy of NASA. Photograph in the public domain.

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Courtesy of NASA. Photograph in the public domain.
EXAMPLES OF MODERN MICROBIALLY-DOMINATED ENVIRONMENTS

Courtesy of Robert Young on flickr. CC-BY.

EXAMPLES OF MODERN MICROBIALLY-DOMINATED ENVIRONMENTS

Courtesy of NASA. Image in the public domain.

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

http://earthobservatory.nasa.gov/GlobalMaps/view.php?d1=MY1DMM_CHLORA&d2=MOD17A2_M_PSN#

NPP map

Courtesy of NASA Earth Observatory. Figures in the public domain.
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

<table>
<thead>
<tr>
<th>Ecosystem type</th>
<th>Area, × 10^{12} m^2</th>
<th>No. of cells, × 10^{27}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tropical rain forest</td>
<td>17.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Tropical seasonal forest</td>
<td>7.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Temperate evergreen forest</td>
<td>5.0</td>
<td>0.3</td>
</tr>
<tr>
<td>Temperate deciduous forest</td>
<td>7.0</td>
<td>0.4</td>
</tr>
<tr>
<td>Boreal forest</td>
<td>12.0</td>
<td>0.6</td>
</tr>
<tr>
<td>Woodland and shrubland</td>
<td>8.0</td>
<td>28.1</td>
</tr>
<tr>
<td>Savanna</td>
<td>15.0</td>
<td>52.7</td>
</tr>
<tr>
<td>Temperate grassland</td>
<td>9.0</td>
<td>31.6</td>
</tr>
<tr>
<td>Desert scrub</td>
<td>18.0</td>
<td>63.2</td>
</tr>
<tr>
<td>Cultivated land</td>
<td>14.0</td>
<td>49.1</td>
</tr>
<tr>
<td>Tundra and alpine</td>
<td>8.0</td>
<td>20.8</td>
</tr>
<tr>
<td>Swamps and marsh</td>
<td>2.0</td>
<td>7.3</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>123.0</strong></td>
<td><strong>255.6</strong></td>
</tr>
</tbody>
</table>

*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

<table>
<thead>
<tr>
<th>Depth interval, m</th>
<th>Cells/cm^3, × 10^6</th>
<th>Deep oceans†</th>
<th>Continental shelf and slope‡</th>
<th>Coastal plains§</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>220.0†</td>
<td>66.0</td>
<td>14.5</td>
<td>4.4</td>
</tr>
<tr>
<td>10</td>
<td>45.0†</td>
<td>121.5</td>
<td>26.6</td>
<td>8.1</td>
</tr>
<tr>
<td>100</td>
<td>6.2†</td>
<td>18.6</td>
<td>4.1</td>
<td>1.2</td>
</tr>
<tr>
<td>200</td>
<td>19.0†</td>
<td>57.0</td>
<td>12.5</td>
<td>3.8</td>
</tr>
<tr>
<td>300</td>
<td>4.0†</td>
<td>12.0</td>
<td>2.6</td>
<td>0.8</td>
</tr>
<tr>
<td>400</td>
<td>7.8†</td>
<td>10.1</td>
<td>3.2</td>
<td></td>
</tr>
<tr>
<td>600</td>
<td>0.95§</td>
<td>3.7</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>1,200</td>
<td>0.61§</td>
<td>3.2</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>2,000</td>
<td>0.44§</td>
<td>2.6</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>3,000</td>
<td>0.34§</td>
<td>0.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>275.1</strong></td>
<td><strong>79.9</strong></td>
<td><strong>25.3</strong></td>
<td></td>
</tr>
</tbody>
</table>

*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).

Step 0: geochemical observations

Distributions of Microbial Activities in Deep Subseafloor Sediments

Step 1: microscopy and morphological characterization

![Microscopy Image]

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

![Graph showing bacterial 16S rRNA genes](image)

**Fig. 2.** Results from DNA surveys for bacterial 16S rRNA genes representing changes in community structure associated with oxidation of CH₄ 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 mol m⁻²). Methylo trophs (*Methylococcaeae, 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)

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.
Step 5: sequencing of environmental 16s rRNA + more

A

Bacteriorhodopsin

B

H+ pump

Sensory Rhodopsin

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Step 6: genome sequencing

SAR11 marine bacteria require exogenous reduced sulphur for growth

H. James Tripp, Joshua B. Kitner, Michael S. Schwalbach, John W. H. Dacey, Larry J. Wilhelm & Stephen J. Giovannoni

![Diagram of the sulphur metabolism in SAR11 and other bacteria]

Step 7: isolation (if lucky)

Cultivation of the ubiquitous SAR11 marine bacterioplankton clade

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

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

Natascha Selje*, Meinhard Simon & Thorsten Brinkhoff

Step 8: physiological and genomic diversity
Bacterial photosynthesis in surface waters of the open ocean

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

a

\[ F_m \text{ at } 685 \text{ nm} \]

\[ F_m \text{ at } 880 \text{ nm} \]

\[ F_v/F_m \]

\[ \Phi_{PSII} \text{ or } q_{PSII} (\%\text{)} \]

\[ 0 \text{:00} \quad 4:00 \quad 8:00 \quad 12:00 \quad 16:00 \quad 20:00 \quad 24:00 \]

Time of day

Physiology and molecular phylogeny of coexisting *Prochlorococcus* ecotypes

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

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**Gulf Stream isolates**

![Gulf Stream isolates graph](image)

**Sargasso Sea isolates**

![Sargasso Sea isolates graph](image)

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**Node ML ME MP**

<p>| | | | |</p>
<table>
<thead>
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<td>100</td>
<td>93</td>
</tr>
<tr>
<td>h</td>
<td>61</td>
<td>95</td>
<td>86</td>
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</tbody>
</table>

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0.01 nucleotide substitutions/position

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Stable core genome and variable genomic islands: phenotypic differences

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Step 9: diversity/transcription of individual genes

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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Step 10: diversity/transcription of the whole genome

**Genome-wide analysis of diel gene expression in the unicellular N$_2$-fixing cyanobacterium *Crocosphaera watsonii* WH 8501**

Tuo Shi$^1$, Irina Ilikchyan, Sophie Rabouille$^2$ and Jonathan P Zehr

*Department of Ocean Sciences, University of California, Santa Cruz, CA, USA*

![Gene expression heat map](image)

Step 11: environmental transcriptomics

![Diagram of environmental transcriptomics process]

Figure 2: Transcriptome sequencing protocol for marine microbial assemblages. Cells are collected and processed to produce genomic DNA, or cDNA from total RNA. 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 *Crocosphaera watsonii*

Mak A. Saito1, Erin M. Bertrand4, Stephanie Dutkiewicz4, Vladimir V. Bulygin2, Dawn M. Moran8, Fanny M. Monteiro5, Michael J. Follows9, Frederica W. Valois5, and John B. Waterbury5

![Graph showing proteomic analysis](image)

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