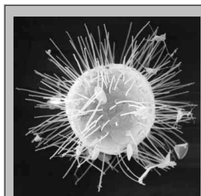
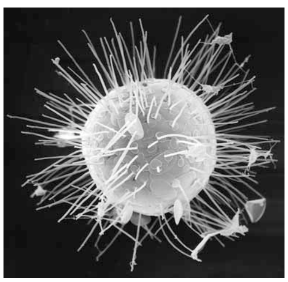


Overview of Marine Microbial Biology

Microbial Ecology
BSCI 464/MEES 698

Overview



SEM of a heliozoan, *Acanthocystis perpusilla*
Image: John van den Hoff

- The marine environment
- Methods of study
- Microbial communities
- The great plate count anomaly

The "Marine Environment"

Many diverse environments, niches

Coastal environment with fringing beaches, mangroves etc

Coral Reefs

Benthic sediments

Deep ocean: Depths >2,000 m over 60% of earth's surface. Mariana Trench 11,000 m.

Symbionts of marine invertebrates

We will focus on <u>water column microbes</u> and consider the "microbial loop" in euphotic zone (~150 m)

Sargasso Sea

Why are marine microbes important?

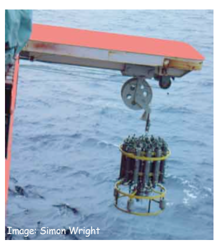
- Microorganisms comprise most of the living matter in the Ocean:
  - Food chain to krill, fish, whales and humans
  - Carbon cycle and the so-called microbial loop
- Marine microbes have major effects on the world's climate:
  - Uptake of CO_2 from the atmosphere; moderation of the global Greenhouse Effect
  - Ocean as carbon 'sinks'

How are field samples collected?

Ship based sampling

Samples are collected either with instruments deployed over the side of the ship or by collecting from a seawater supply line.

- Samples for depth profiles are collected with Rosette sampler: Niskin bottles and CTD (Conductivity, Temperature and Depth) system



CTD sampling rosette with Niskin bottles. Water samples are collected for various chemical analyses as well as analysis of microbiological components

How are field samples collected?

Ship based sampling

Samples are collected either with instruments deployed over the side of the ship or by collecting from a seawater supply line.

- Samples for depth profiles are collected with Rosette sampler: Niskin bottles and CTD (Conductivity, Temperature and Depth) system

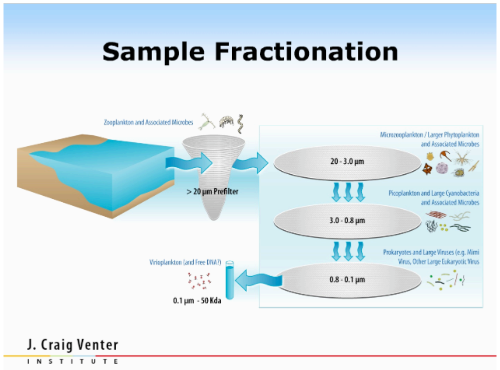
❖ Samples for phytoplankton pigment analysis are filtered onto glass fiber filters

❖ Bacteria and viruses are filtered onto black polycarbonate membrane filters, stained, counted by fluorescence microscopy

❖ Duplicate samples are preserved for light or electron microscopy

- Large phytoplankton collected with 20 micrometer mesh plankton nets

CTD sampling rosette with Niskin bottles. Water samples are collected for various chemical analyses as well as analysis of microbiological components



Sea ice sampling and land-based (Continental station) sampling

• Ice communities are sampled using a SIPRE ice corer or Jiffy drill. Alternatively, a partial core hole is drilled and the microbe-containing brine is allowed to drain into it.

Over-snow vehicles or small boats are used to get to Antarctic stations.

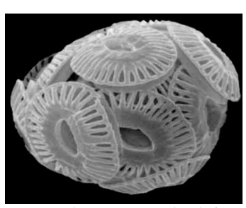
A Sipre-corer and part of the resultant sea-ice core
Image: Fiona Scott

Scientist cutting a hole in the sea-ice with a petrol-operated ice-drill
Image: Fiona Scott

How are microbes studied?

Traditional
* Light microscopy
* Electron Microsocopy
* Flow cytometry
* Pigment analysis
* Response to UV irradiation
* Culture

Molecular
* Phylogenetic analysis
* In situ detection (FISH)
* Sequencing
* DGGE, RFLP, ….



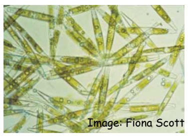
Scanning electron microscopy (SEM) of *Emiliania huxleyi*, a Southern Ocean coccoithophorid
Image: Fiona Scott

Microbial primary producers

Phytoplankton

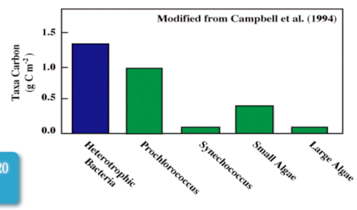
- Single-celled algae or prokaryotes
- Photosynthesis
- Size from <1 - 100 micrometres
- Three size classes: picoplankton (<2 µm), nanoplankton (2 - 20 µm) and microplankton (> 20 µm)
- At least 360 different species of eukaryotic phytoplankton identified in Antarctic waters, many of which can swim

[Protists is the general term for single celled eukaryotic organisms, including algae (phytoplankton) and protozoa (zooplankton). Each liter of surface water from the Southern Ocean can contain from 0.5 million to 60 million protists.]

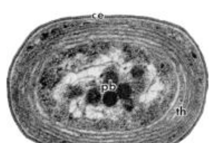


Light micrograph of the common sea-ice diatom *Nitzschia stellata*. Cells in a star-shaped pattern, colored bright green from cellular chlorophyll

Relative abundance of *Prochlorococcus* and heterotrophic Bacteria in oligotrophic systems



Prochlorococcus marinus SS120
Genome Project Home Page



(0.5-0.7µm)

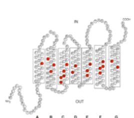
Bacterial rhodospsin and phototrophy
light-driven energy production in the world's oceans

Beja et al. 2000. Bacterial rhodopsin: evidence for a new type of phototrophy in the sea. Science 289:1902-6

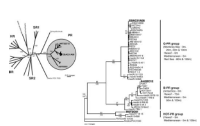
de la Torre et al. 2003. Proteorhodopsin genes are distributed among divergent marine bacterial taxa. Proc. Natl. Acad. Sci. USA. 100(22):12830-5.

Sabehi et al. 2005. New insights into metabolic properties of marine bacteria encoding proteorhodopsins. PLoS Biol. 3(8):e273. Epub

"proteorhodopsin-exploiting bacteria account for 13% of microorganisms in the photic zone"



Secondary structure of proteorhodopsin



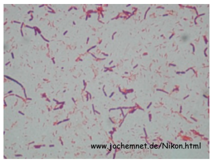
Phylogenetic analysis of archaeal and bacterial rhodopsin family amino acid sequences

Microbial secondary producers

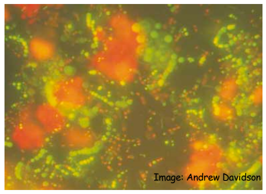
Bacteria and Archaea are abundant in the Ocean. Typically ~ 10^6 cells/ml of seawater

They are vital components of the microbial community, breaking down particulate matter (cells and detritus), releasing nutrients for use by other organisms

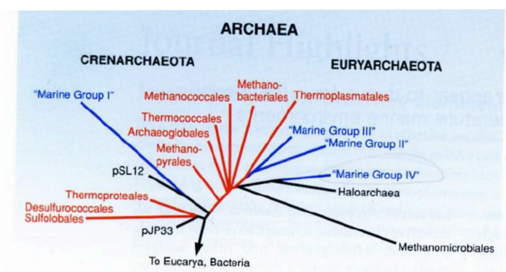
They also take up dissolved organic matter, converting it to cell mass, and making it available to grazers



Gram-staining of bacteria isolated from the Gulf Stream off the South Florida coast. Long gram-positive rods in chains in violet, gram-negative rods in red.



Fluorescence microscope image of the natural marine bacterial community selectively stained to show live bacteria as yellow/green and dead bacteria as red. Large red blobs are phytoplankton.



Schematic tree showing the relationships of the four major groups of planktonic marine Archaea relative to cultivated groups (Delong, ASM News 2003)

Cold Crenarchaea isolated: autotrophic ammonia oxidizing marine archaea. (Nature Stahl 2005)

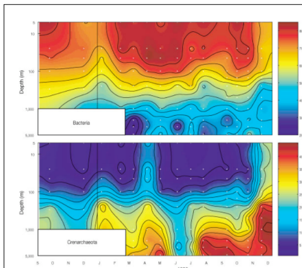


Figure 1 Contour plots of relative abundances with depth of bacteria and crenarchaeota at the Hawai'i Ocean Time-series station. Contour lines are percentages of bacteria and pelagic crenarchaeota as compared with total microbial abundance at each depth. Total cell abundance was assessed using DAPI. Bacteria and archaea were enumerated using whole-cell rRNA targeted FISH with fluorescein-labelled polynucleotide probes.

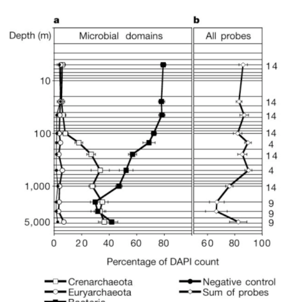
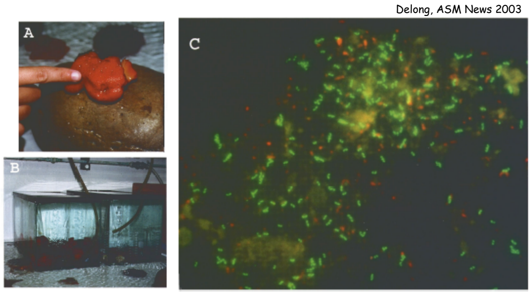


Figure 2 Mean annual depth profiles of microbial domains in the North Pacific. Percentages of bacteria and archaea as compared to total microbial abundance at each depth. a, Depth profiles for bacteria (solid squares), pelagic crenarchaeota (open squares), pelagic euryarchaeota (open circles), and a non-specific control probe ('negative', solid circles). b, Depth profile of the sum of relative abundances of bacteria, pelagic crenarchaeota and pelagic euryarchaeota (open diamonds).

(Markus et al., Nature 2001)

The marine sponge *Axinella mexicana* and its archaeal symbiont, *Cenarchaeum symbiosum*

Delong, ASM News 2003



C. Fish experiment showing *C. symbiosum* population (green) present in the sponge tissues

C. Symbosium genome is sequenced -Hallam et al. PNAS 2006

Microorganisms of the Oceans

Primary producers

- Eukaryotes: phototrophic phytoplancton (algae)
- Prokaryotes
  - Bacteria; Cyanobacteria (*Prochlorococcus*)

Secondary producers

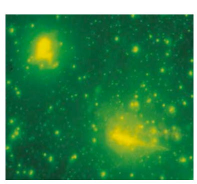
- Prokaryotes
  - Heterotrophic bacteria (SAR11)
  - Archaea; cold crenarchaeota

Agents of death

Marine microbes are subject to attack by a range of organisms with voracious appetites and a variety of feeding strategies

Viruses - inject their DNA or RNA and take over the cell metabolism of the host resulting in viral multiplication and eventual cell lysis

Viruses are the most abundant biological agents in seawater
Antarctic waters: from 1 to 4 million particles/ml
Viruses may be important in controlling the abundance and composition of microbial communities in the Ocean



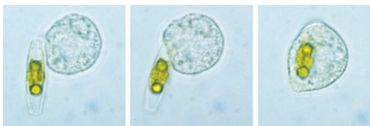
SYBR Green 1 stained filter showing two highly fluorescent protist cells (yellow), many less fluorescent bacteria (green/yellow) and large numbers of very small viruses (pale green)

Agents of death

Protozoa are single celled animals that consume phytoplankton, bacteria and organic matter

Their respiration releases much of the carbon dioxide incorporated by phytoplankton.

However they also help remove CO_2 from the atmosphere by converting their microscopic food into their own cell mass, making it available for higher levels of the food web whose bodies and faecal pellets sink into the deep ocean.



A colourless protozoan cell nearing, and then ingesting a small green diatom.
Image: Fiona Scott

Agents of death

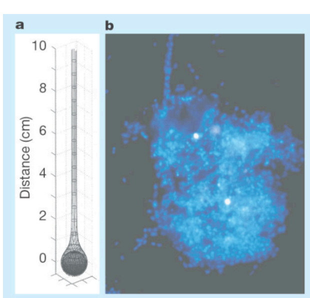
Marine microbes are subject to attack by a range of organisms with voracious appetites and a variety of feeding strategies

Viruses
- most abundant biological agents in seawater

Zooplankton

- Protozoa
  - dinoflagellates
  - ciliates
- Mesoplankton
  - copepods
  - krill

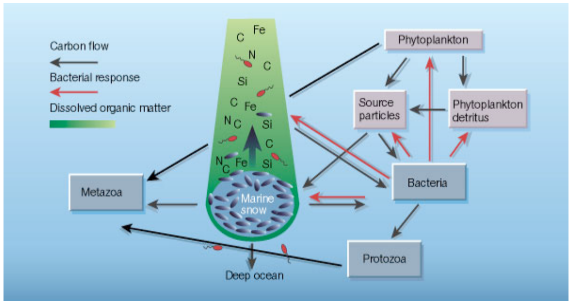
Marine Snow



(b) Marine snow is intensively colonized by bacteria (~10^9 bacteria/ml) stained blue with fluorochrome DAPI and photographed in an epifluorescence microscope. Size of organic particle aggregate is 37 mm X 27 mm
(a) Model by Kiørboe and Jackson: formation of a plume of dissolved organic matter streaming behind a sinking particle of marine snow. The plume is formed by the enzymatic dissolution of the snow particle by colonizing bacteria.

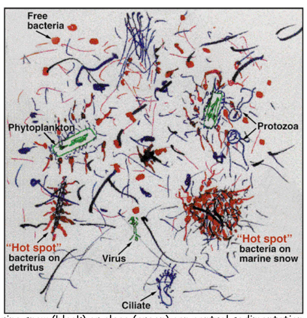
(Azam and Long, Nature 2001)

Carbon fluxes in the ocean involving marine snow



(Azam and Long, Nature 2001)

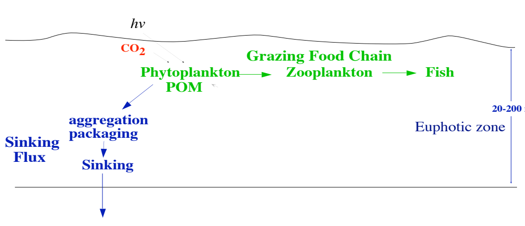
Micro-structure of microbial communities in the Sea



Bacteria (red) acting on marine snow (black) or algae (green) can control sedimentation and primary productivity; diverse microniches (hotspots) can support high bacterial diversity.
Seawater is an organic matter continuum, a gel of tangled polymers with embedded strings, sheets, and bundles of fibrils and particles, including living organisms as "hotspots."

(Azam, Science 98)

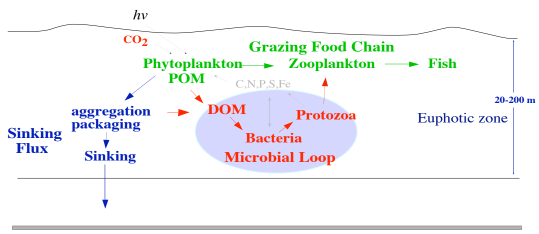
Food web pathways



POM: particulate organic matter

Adapted from Azam 1998 Science

Food web pathways



POM: particulate organic matter
DOM: dissolved organic matter

Adapted from Azam 1998 Science

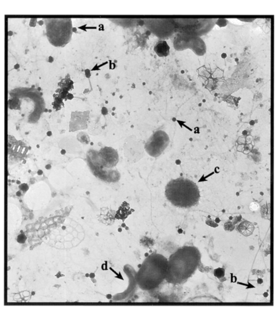
Viruses in marine ecosystems

- Most common biological agent (10 billion per liter)
- Infect all organisms, including bacteria and phytoplankton
- Harmful algal bloom control?
- Influence biogeochemical and ecological processes

  Biodiversity and species distributions: bacteria and algae

  Genetic transfer

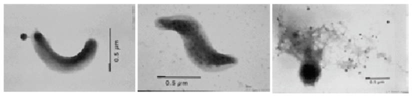
  Nutrient cycling [futile loop]



Transmission electron micrograph of an unfiltered Chesapeake Bay water sample (magnification, ca. X36,000). a, short-tailed or nontailed virus-like particle; b, tailed virus-like particle; c, bacterium, coccal morphotype; d, bacterium, vibrio morphotype.

(Wommack and Colwell, 2000)

Viral infection of marine microbes

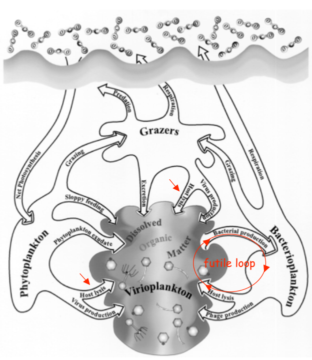


Significantly affect community structure

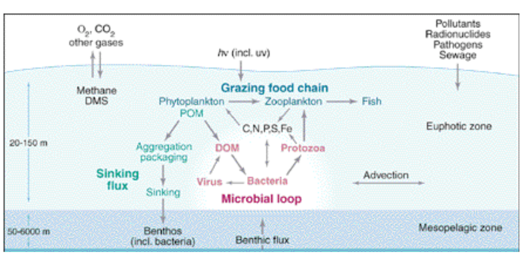
Responsible for futile carbon cycle
(25% increase in respiration)

Steward et al. 1996 MEPS. 131:287-300

Viruses and the microbial loop: potential role of viral infection and lysis in the production of DOM in aquatic ecosystems



(Wommack and Colwell, 2000)



Modern view of the pelagic food web, emphasizing the microbial loop as a major path for organic matter flux. Competition between the three main flux paths--grazing food chain, microbial loop, and sinking--significantly affects oceanic carbon cycle and productivity. DOM, dissolved organic matter; DMS, dimethylsulfide.

(Azam, Science 2003)

Summary of Microbial processes (1)

Photosynthesis: Phytoplankton absorb CO_2 and harness the energy of sunlight to manufacture sugars and other cell components, releasing oxygen. The sunlight is absorbed by chlorophylls and carotenoids, which can be used to identify the various groups of phytoplankton in the water.

Respiration: All organisms (from microbes to whales) oxidize intracellular carbon reserves to produce the energy necessary for cell function (growth, movement, chemical metabolism). This process of respiration releases to the atmosphere much of the CO_2 taken up by phytoplankton.

Feeding: The ocean has been likened to a vast very dilute jelly, containing a continuum of matter ranging from small molecules to large aggregate. Microbes are able to consume matter throughout this size range, changing the kind and size of these compounds. This alters the availability of these food sources to other organisms. Bacteria release enzymes that convert complex matter to simple molecules that can be absorbed across their cell membrane. Protozoa have various means of consuming cells and can graze on a large range of particles from molecules to cells larger than themselves. All protists are grazed by crustaceans and other zooplankton.

Microbial loop: The processes discussed above operate simultaneously in a microbial community, whose collective metabolism is called the microbial loop. Most of the carbon in the marine ecosystem is cycled through this loop, strongly influencing the quality, quantity and size distribution of food available to higher organisms.

Summary of Microbial processes (2)

Aggregation: There are several processes by which particles can aggregate. Particulate and dissolved organic matter can spontaneously aggregate in seawater, a process aided by mixing. Grazing protozoa and higher organisms repackage matter into faecal pellets. Mucilage produced by algae provides a substrate that can be colonized by other cells. Aggregates support a rich and diverse microbial community within which the close proximity of organisms enhances recycling of matter via the microbial loop. Such aggregates are often called marine snow. Marine snow particle containing mucilage and detritus as well as many motile and non-motile cells.

Sedimentation: There is a continuous 'rain' of particles from the sunlit upper waters to the ocean depths where there is insufficient light for photosynthesis and hence respiration rules. Much of the matter is recycled en route. Sedimentation to the deep ocean is the principal global process by which CO_2 is biologically removed from the atmosphere for geological time scales.

Succession: The composition and abundance of marine microbes varies greatly due to physical and environmental factors including, light, temperature, salinity, depth, nutrient concentrations, the nature, extent and persistence of sea ice, the depth and speed of vertical mixing of the water column, and grazing pressure.

Oligotrophy and Culturability

0.1-1% of cells from oligotrophic marine waters will grow on Marine Agar 2216

"The Great Plate Count Anomaly"

Marine waters are very low in nutrients: Lab media >1000x carbon conc.

Marine bacterial cells often very small-ultramicrobacteria

Oligotrophic prokaryotes: "evolutionarily adapted to exploit ecological niches characterized by low substrate concentrations and low energy fluxes" Semenov (1991). Can be obligate or facultative.

Oligotrophy in the Marine Environment

Concs of low molecular weight C compounds ~ nanomolar range

Too low to support bacterial growth?

Starvation-survival response. Feast or famine conditions?

Ultramicrobacteria

Pass through 0.2 μm filter

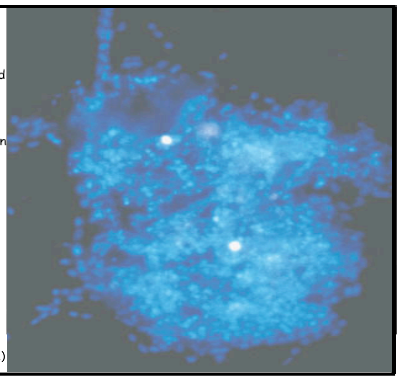
Can determine biomass with light scatter measurements using flow cytometry

Organism	Mean dry mass (pg/cell)	Volume (μm^3)
Oligotroph	0.03	0.26
E. coli	0.38	1.13

Feast: particle-attached

"Refueling station":
Transient state between
growth and non-growth

Solubilization



(Azam and Long, Nature 2001)

Copiotrophs vs Oligotrophs

Copiotrophs-rapid degradation of easily-utilized material, then
Form resting-stage cells
r-strategists

Oligotrophs-well adapted to low nutrient conditions, grow slowly,
capture low levels of nutrients
K-strategists

Oligotrophic isolates: a contradiction in terms?

Sphingomonas alaskensis

First species of ultramicrobacteria to be isolated

Numerically dominant isolates from Resurrection Bay, Alaska and North Sea (Button and colleagues)

Isolated by extinction dilution method in filtered autoclaved seawater

Ultramicrosize of 0.03 to 0.07 μm^3 maintained with C concs between 0.8 and 800 mg l^{-1}. Dimensions of 0.3 μm x 1.0 μm

Grows from above 45°C to below 5°C: possible cosmopolitan distribution

α–Proteobacterium

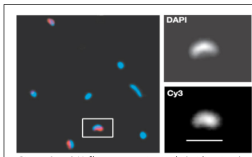
Cultivating the Uncultivable

The culturing of SAR11

About 25% of all cells in seawater belong to SAR11 group (α-proteobacterium)

Very active:
may be responsible for as much as 10% of the nutrient recycling on earth

SAR11 and three other representatives of new groups cultured in 2002



SAR11 clade dominates Ocean Surface Bacterioplankton Communities

Figure 2 SAR11 fluorescence in situ hybridization. Dual image overlay of DNA-containing cells stained with DAPI (blue) and the SAR11 Cy3 probe (red). Both DAPI- and Cy3-stained images show the characteristic size and curved rod morphology of a magnified SAR11 cell (white box). Scale bar, 1μm.

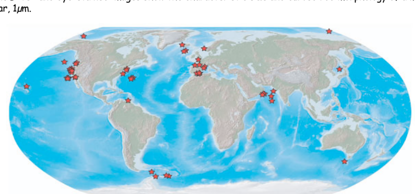


Figure 1 Distribution of the SAR11 clade in the world's oceans. Red marks indicate locations where SAR11 ribosomal RNA genes have been detected using molecular techniques.

(Morris et al., Nature 2002)

SAR11 probe counts

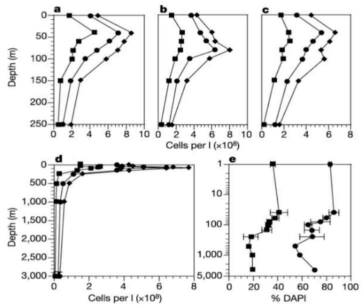


Figure 3 SAR11 probe counts, bacterial probe counts and direct cell counts (DAPI staining particles) in the northwestern Sargasso Sea. a–d, SAR11 clade (squares), Bacteria (circles) and DAPI (diamonds) counts. e, A transect composite shows the mean abundance values by depth for SAR11 clade and bacterial cell counts as percentages of direct cell counts (DAPI staining particles)

(Morris et al., Nature 2002)

Success of High-Throughput Culturing - HTC

Direct count of the inoculum by fluorescence microscopy

↓

Dilute inoculum to 1-5 cells/ml; fill 48-well microtiter plate with 1 ml per well

Incubate

Extinction culturing
3 orders of magnitude less C

↓

Array 200 µl onto a 48-sector manifold, stain and count

Screen for growth; epifluorescence

↓

Confirm growth (1,000 to 1,000,000 cells per ml)

↙ ↓ ↘

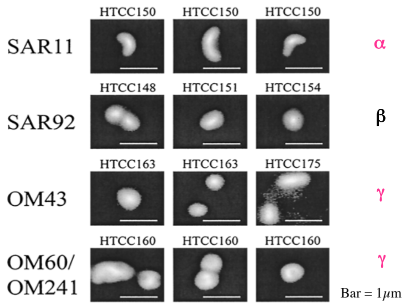
PCR, 16S rRNA seq ID | Transfer | Store

Connon and Giovannoni, AEM 2002

High-Throughput Culturing Results

- 2500 extinction cultures from 11 samples
- 3 years
- Up to 14% cells collected were cultured versus <0.1%
- 4 unique cell lineages, previously uncultured marine proteobacteria

DAPI images of HTCC isolates



Connon and Giovannoni, 2002

SAR11 characterization

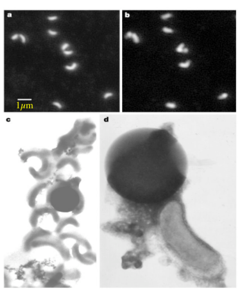


Figure 1 Photomicrographs of a culture of SAR11 clade isolate HTCC1062

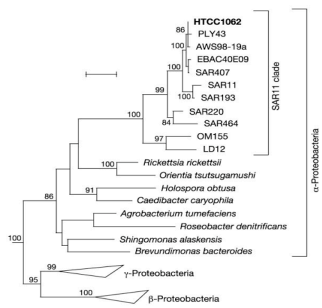


Figure 2 Phylogenetic relationships between strain HTCC1062 and representatives of the SAR11 clade and α-Proteobacteria inferred from 16S rRNA gene sequence comparisons.

Rappe et al., Nature 2002

Major advantages to culturing

Makes genomics/proteomics easier

Test hypotheses from genome sequencing

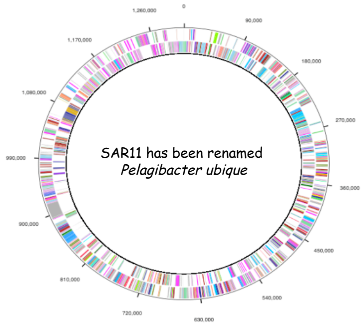
Study physiology, effects of nutrient limitation, different environmental conditions

Access biodiversity for biotech applications

SAR86 also abundant, not yet cultured.

Try different light conditions etc.

SAR11 is the dominant microorganism in the ocean surface. Sequencing of the 1.3Mb genome of *Pelagibacter* by Diversa Corp. Sequence published in Science in 2005 (Presentation 14-Sept)



The data from this NSF-sponsored project will be used to understand how SAR11 contributes to geochemical cycles. Also, because SAR11 is one of the smallest cells known, the data will be useful for understanding the basic architecture of cells and genomes.

Sciencexpress Research Article

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,[3] 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,[5] 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. Smith[1]

[1]The Institute for Biological Energy Alternatives, [2]The Center for the Advancement of Genomics, 1901 Research Boulevard, Rockville, MD 20850, USA. [3]The Institute for Genomic Research, 9712 Medical Center Drive, Rockville, MD 20850, USA. [4]The J. Craig Venter Science Foundation Joint Technology Center, 5 Research Place, Rockville, MD 20850, USA. [5]University of Southern California, 223 Science Hall, Los Angeles, CA 90089–0740, USA. [6]Bermuda Biological Station for Research, Inc., 17 Biological Lane, St George GE 01, Bermuda.

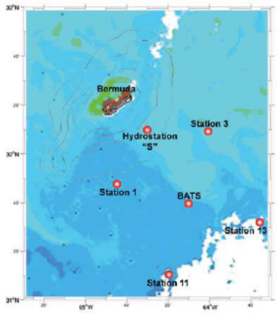
Published Online March 4, 2004
Science DOI: 10.1126/science.1093857

Environmental Genome Shotgun Sequencing of the Sargasso Sea

- whole genome shotgun sequencing
- sea water samples filtered on tangential flow and impact filters
- 1.045 billion basepairs of non-redundant sequence
- data estimated to derive from < 1800 genomic species based on sequence relatedness, including 148 novel bacterial phylotypes

(Venter et al., 2004)

Sargasso Sea near Bermuda

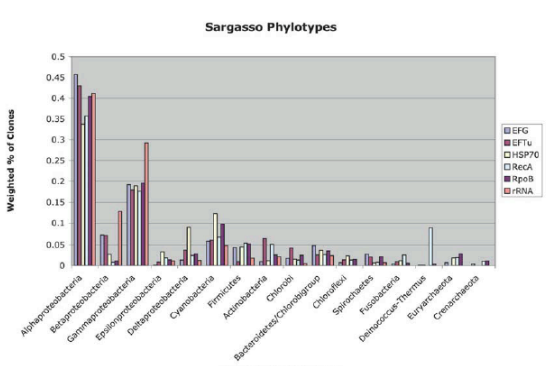


(Venter et al., 2004)

Environmental Genome Shotgun Sequencing of the Sargasso Sea

- whole genome shotgun sequencing
- sea water samples filtered on tangential flow and impact filters
- 1.045 billion basepairs of non-redundant sequence
- data estimated to derive from < 1800 genomic species based on sequence relatedness, including 148 novel bacterial phylotypes

(Venter et al., 2004)

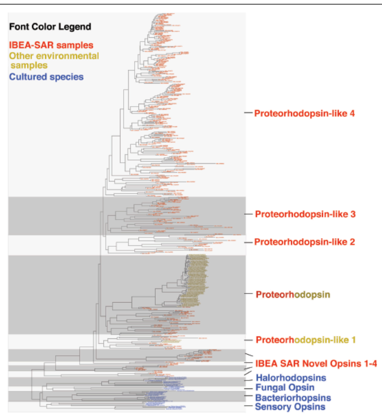


(Venter et al., 2004)

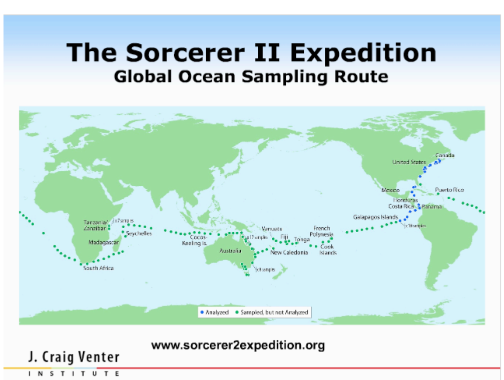
Environmental Genome Shotgun Sequencing of the Sargasso Sea

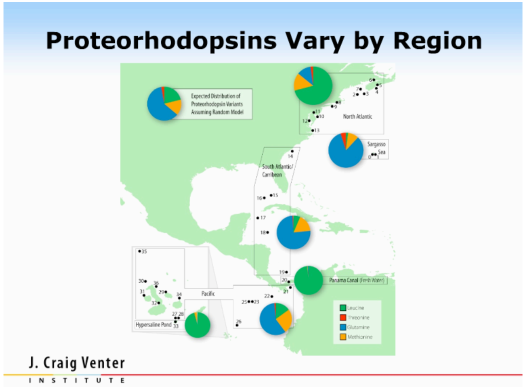
- whole genome shotgun sequencing
- sea water samples filtered on tangential flow and impact filters
- 1,045 billion base pairs of non-redundant sequence
- data estimated to derive from < 1800 genomic species based on sequence relatedness, including 148 novel bacterial phylotypes
- < 1.2 million new genes were identified including more than 782 new rhodopsin-like photoreceptors

(Venter et al., 2004)



(Venter et al., 2004)





http://plos.cnpg.com/lsca/webinar/venter/20070306/index.html

Global Ocean Sampling Expedition

- whole genome shotgun sequencing (Sanger)
- sea water samples filtered on tangential flow and impact filters (0.1 - 0.8 μM); 41 sites
- 5.9 billion base pairs of non-redundant sequence (x5 Sargasso Sea); 6.3 Gbp total (x2 size of human genome)
- 85% of the assembled sequence and 57% of the un-assembled data is unique at a 98% sequence identity cutoff
- 5 to 6 millions genes identified including more than 2,674 putative rhodopsin-like photoreceptors

Data suggests a vast oceanic microbial diversity of which only a very small fraction has been captured so far

(PLOS Biology 2007)