General Staining Methods

Fluorescent antibodies can be used as a cell tag

- Highly specific
- Making antibodies is time consuming and expensive

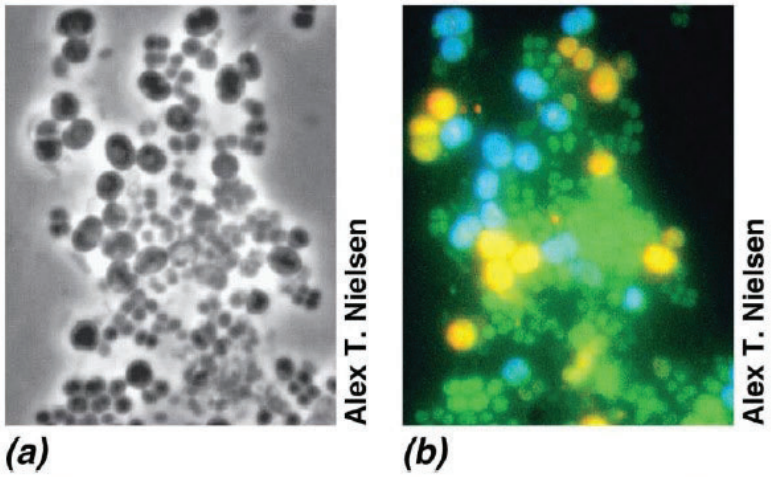
Green fluorescent protein can be genetically engineered into cells to make them autofluorescent

- Can be used to track bacteria
- Can act as a reporter gene

Fluorescent *In Situ* Hybridization (FISH)

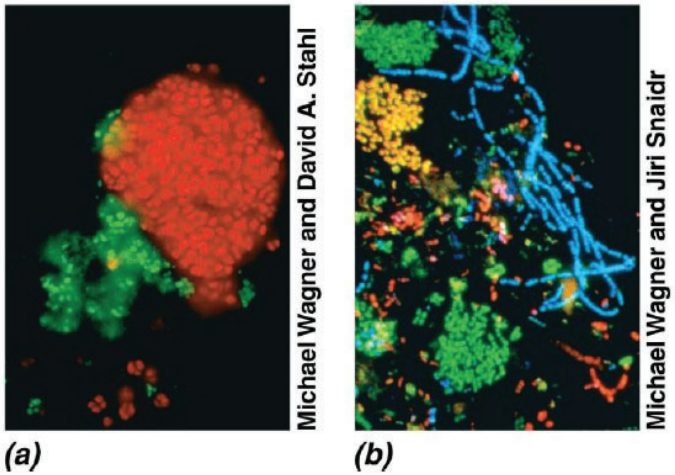
- Nucleic acid probe is DNA or RNA complementary to a sequence in a target gene or RNA
- FISH: fluorescent *in situ* hybridization
- Phylogenetics of microbial populations
- Used in microbial ecology, food industry, and clinical diagnostics
- CARD-FISH

Morphology and genetic diversity



The photomicrographs shown here, produced by (a) phase-contrast and (b) a technique called phylogenetic FISH, are of the same field of cells. Although the large oval cells are of a rather unusual morphology and size for prokaryotic cells and all look similar in phase-contrast microscopy, the phylogenetic stains reveal that there are two genetically distinct types (one stains yellow and one stains blue).

FISH analysis of sewage sludge



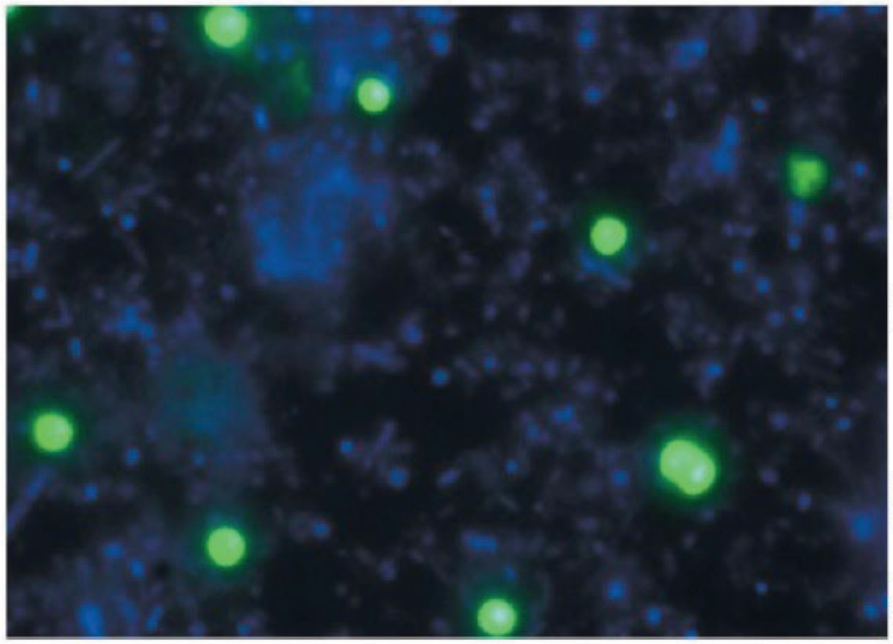
(a) Nitrifying bacteria. Red, ammonia-oxidizing bacteria; green, nitrite-oxidizing bacteria.
(b) Confocal laser scanning micrograph of a sewage sludge sample. The sample was treated with three phylogenetic FISH probes, each containing a fluorescent dye (green, red, or purple) that identifies a particular group of *Proteobacteria*. Green-, red- or purple-stained cells reacted with only a single probe; other cells reacted with multiple probes to give blue or yellow.

Fluorescent *In Situ* Hybridization (FISH)

CARD-FISH

- FISH can be used to measure gene expression in organisms in a natural sample
- A FISH method that enhances the signal is called catalyzed reporter deposition FISH (CARD-FISH)

Catalyzed reporter deposition FISH (CARD-FISH) labeling of *Archaea*

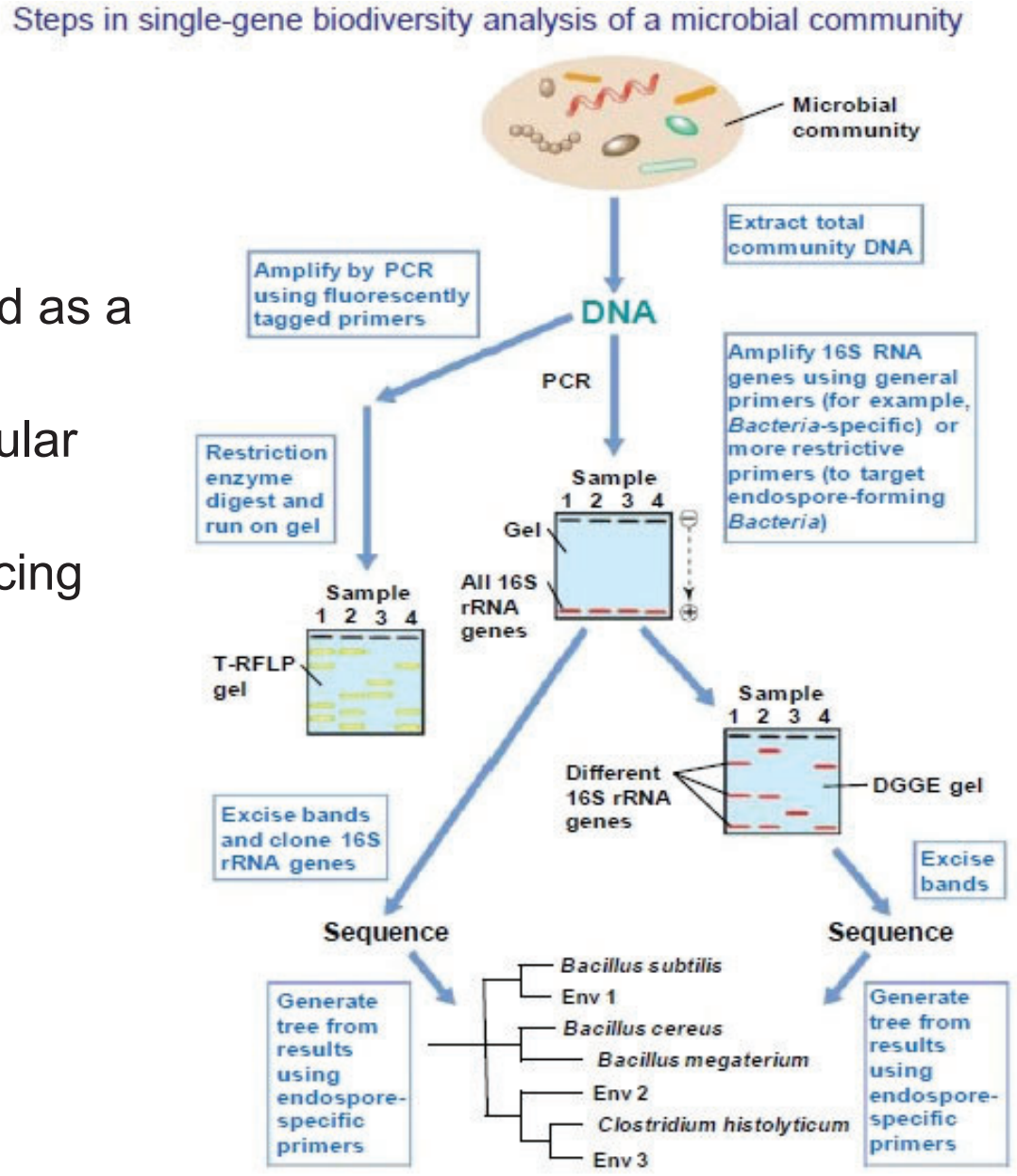


Michael Wagner and Marc Mussman

Archaeal cells in this preparation fluoresce intensely (green) relative to DAPI-stained cells (blue)

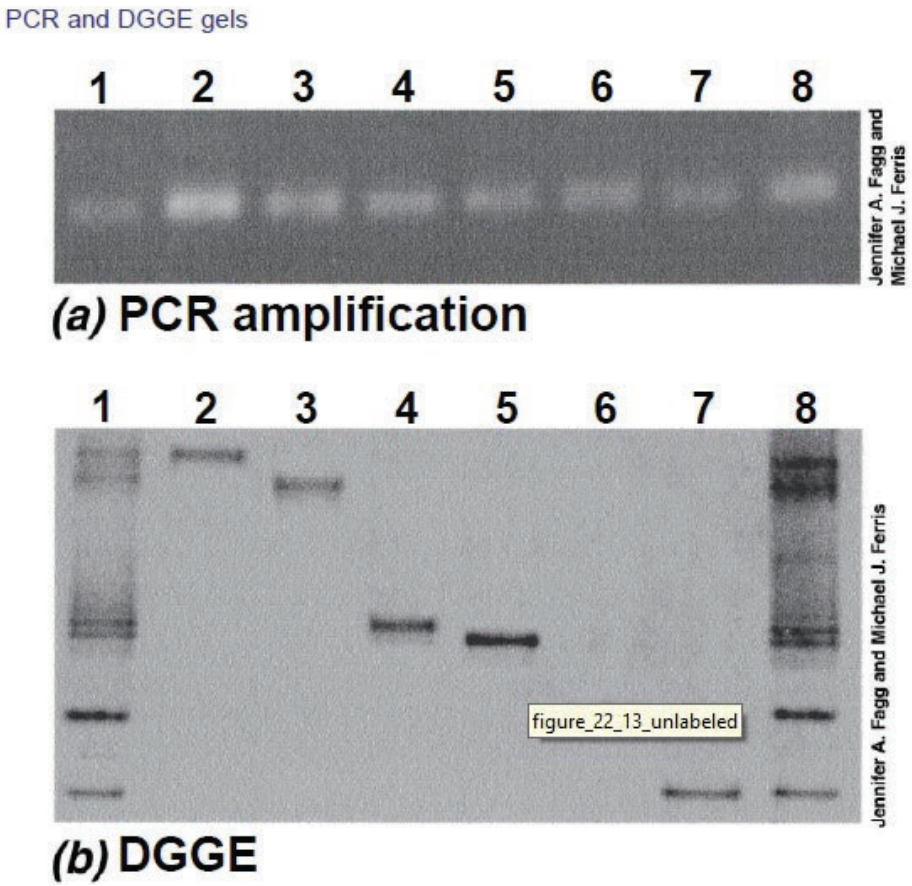
PCR Methods of Microbial Community Analysis

- Specific genes can be used as a measure of diversity
- Techniques used in molecular biodiversity studies
- DNA isolation and sequencing
- PCR
- Restriction enzyme digest
- Electrophoresis
- Molecular cloning



PCR Methods of Microbial Community Analysis

- DGGE: denaturing gradient gel electrophoresis separates genes of the same size based on differences in base sequence
- Denaturant is a mixture of urea and formamide
- Strands melt at different denaturant concentrations



PCR Methods of Microbial Community Analysis

T-RFLP: terminal restriction fragment length polymorphism

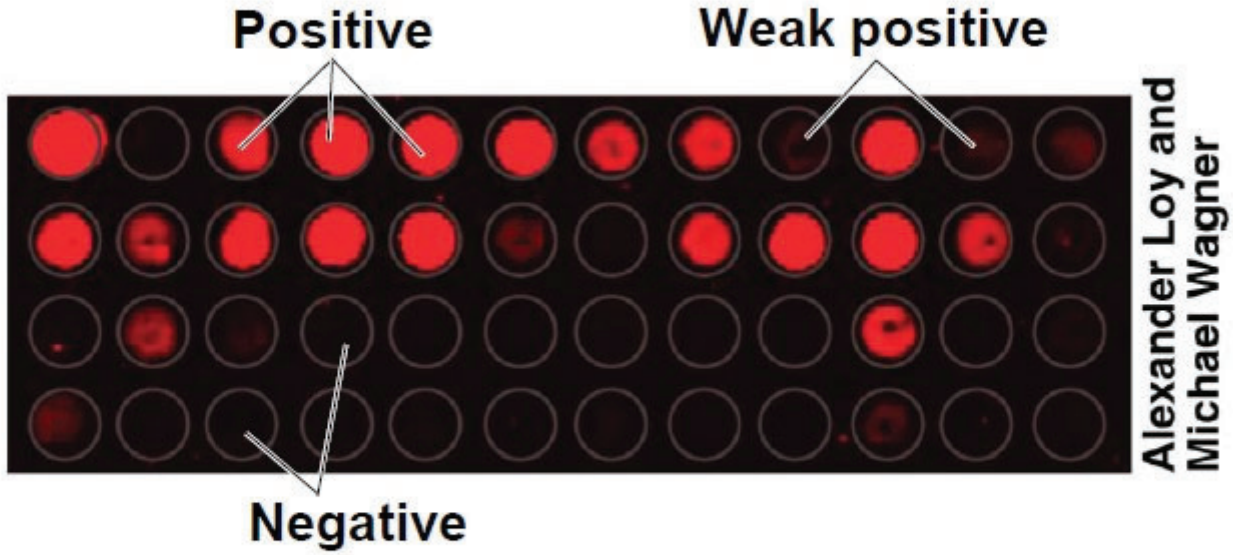
- Target gene is amplified by PCR
- Restriction enzymes are used to cut the PCR products
- ARISA: automated ribosomal intergenic spacer analysis
- Related to T-RFLP
- Uses DNA sequencing
- Results of PCR phylogenetic analyses
- Several phylogenetically distinct prokaryotes are present
- rRNA sequences differ from those of all known laboratory cultures
- Molecular methods conclude that less than 0.1% of bacteria have been cultured

Microarrays and Microbial Diversity: Phylochips

Phylochip: microarray that focuses on phylogenetic members of microbial community

- Circumvents time-consuming steps of DGGE and T-RFLP

Phylochip analysis of sulfate-reducing bacteria diversity



Each spot on the microarray shown has an oligonucleotide complementary to a sequence in the 16S rRNA of a different species of sulfate-reducing bacteria. After the microarray is hybridized with 16S rRNA genes PCR amplified from a microbial community and then fluorescently labeled, the presence or absence of each species is signaled by fluorescence (positive or weak positive) or nonfluorescence (negative), respectively.

Environmental Genomics and Related Methods

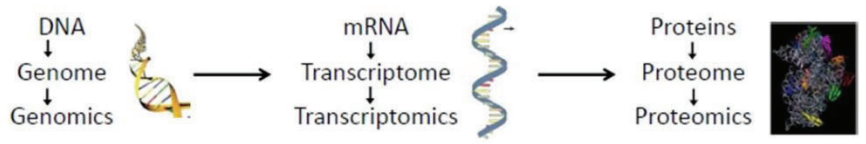
Environmental genomics (metagenomics)

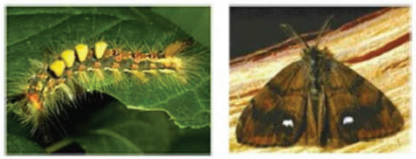
- DNA is cloned from microbial community and sequenced
- Detects as many genes as possible
- Yields picture of gene pool in environment
- Can detect genes that are not amplified by current PCR primers
- Powerful tool for assessing the phylogenetic and metabolic diversity of an environment

"-Omics"

"**-omics** informally refers to a field of study in biology ending in *-omics*, such as **genomics**, **proteomics** or **metabolomics**. The related suffix **-ome** is used to address the objects of study of such fields, such as the **genome**, **proteome** or **metabolome**...."

www. en.wikipedia.org



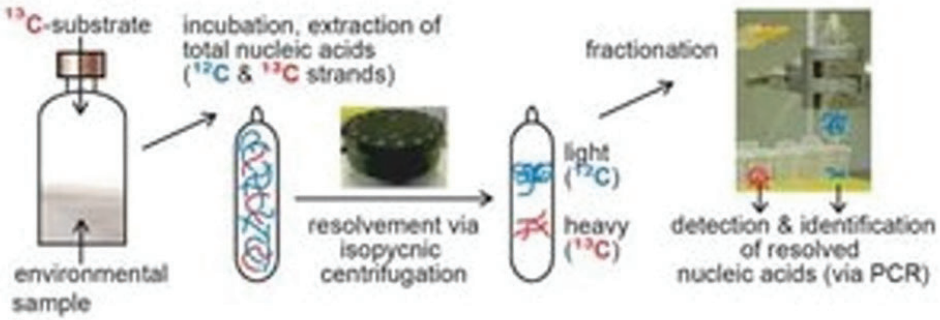


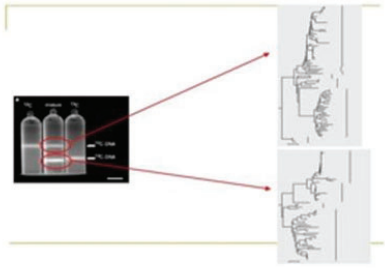
Same GENOME
but
different PROTEOME

Measuring Microbial Activities in Nature

- Chemical Assays, Radioisotopic Methods, and Microelectrodes
- Stable Isotopes
- Linking Specific Genes and Functions to Specific Organisms

Stable Isotope Probing (SIP)



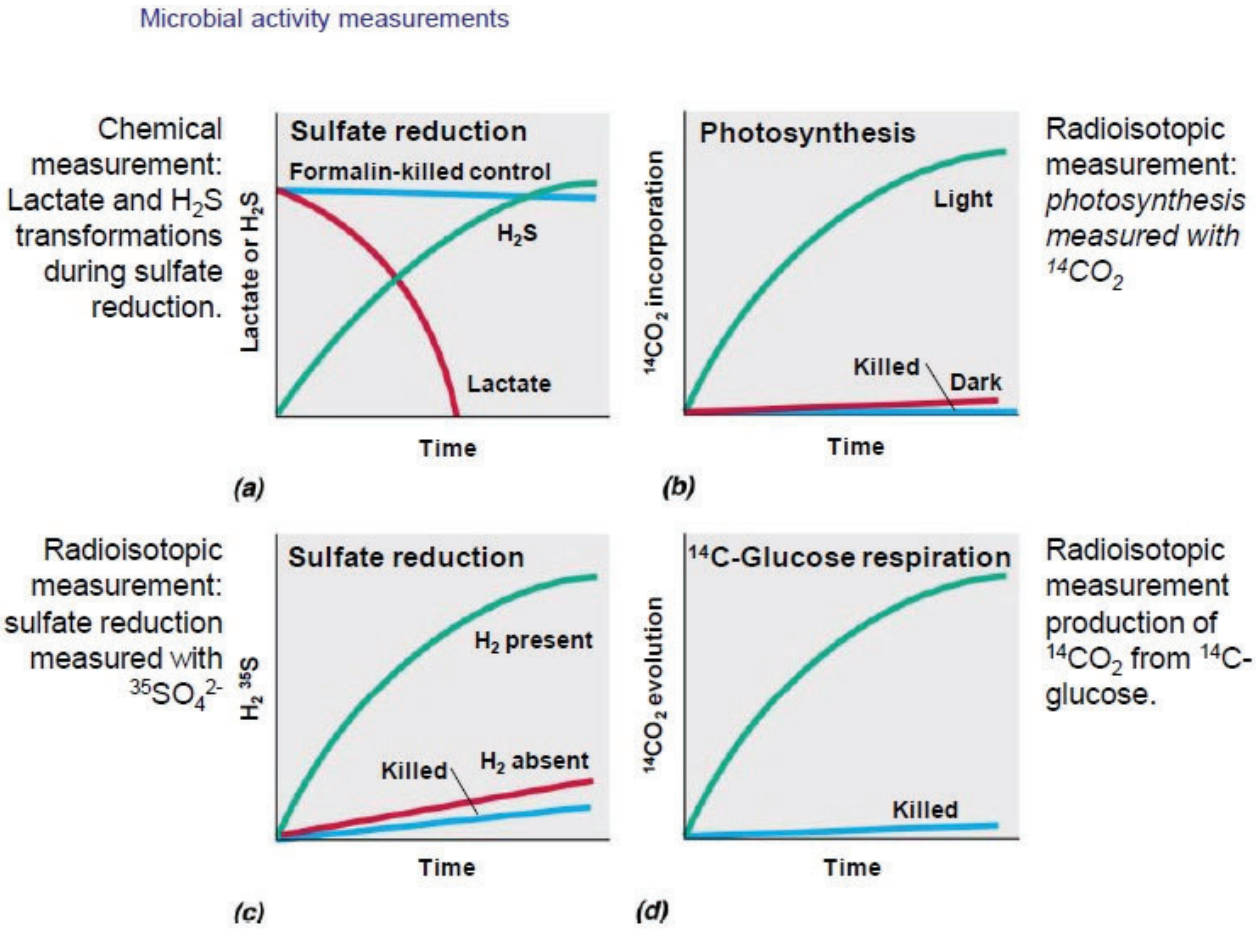


Who is there and who is active?

SIP facility

Chemical Assays, Radioisotopes, & Microelectrodes

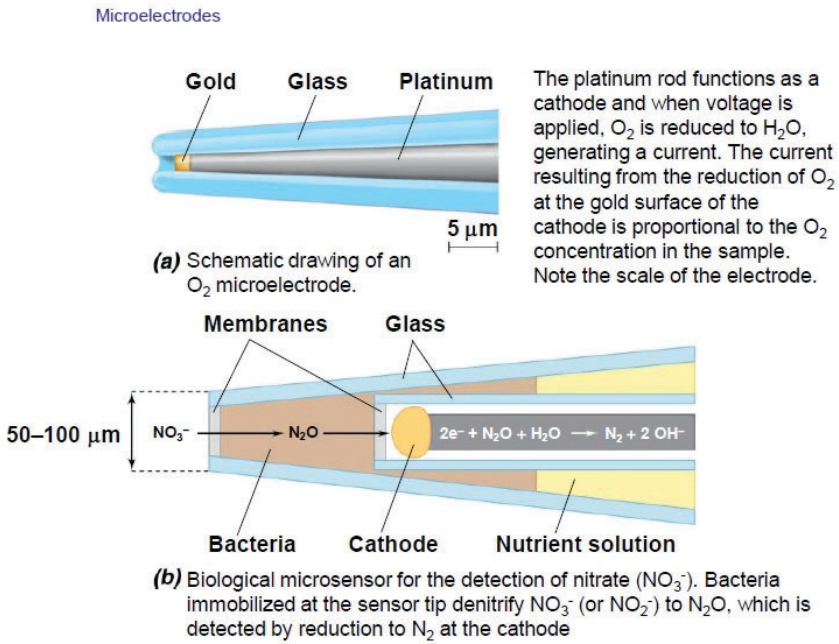
- In many studies, direct chemical measurements are sufficient
- Higher sensitivity can be achieved with radioisotopes
- Proper killed cell controls must be used

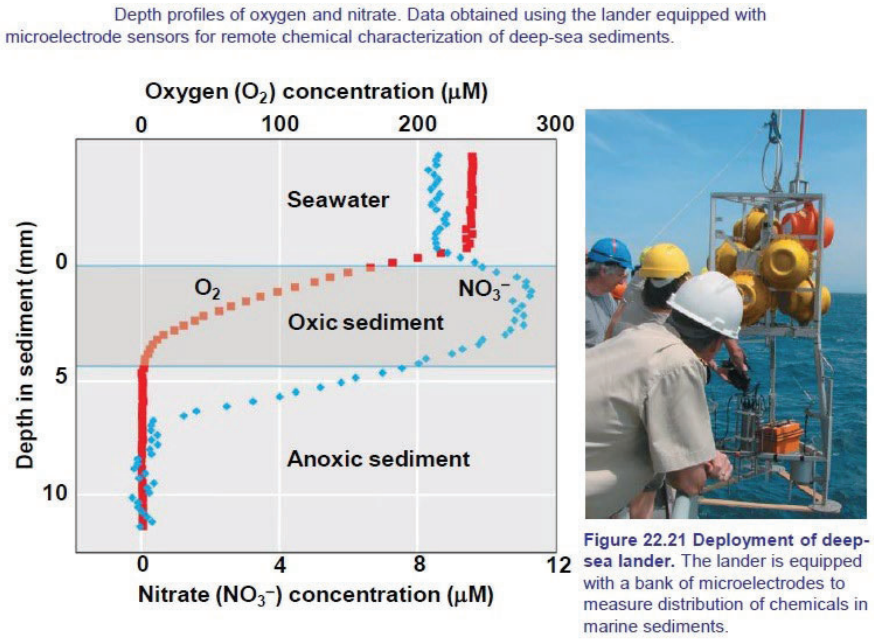


Chemical Assays, Radioisotopes, & Microelectrodes

Microelectrodes

- Can measure a wide range of activity
- pH, oxygen, CO2, and others can be measured
- Small glass electrodes, quite fragile
- Electrodes are carefully inserted into the habitat (e.g., microbial mats)
- Measurements taken every 50–100mm

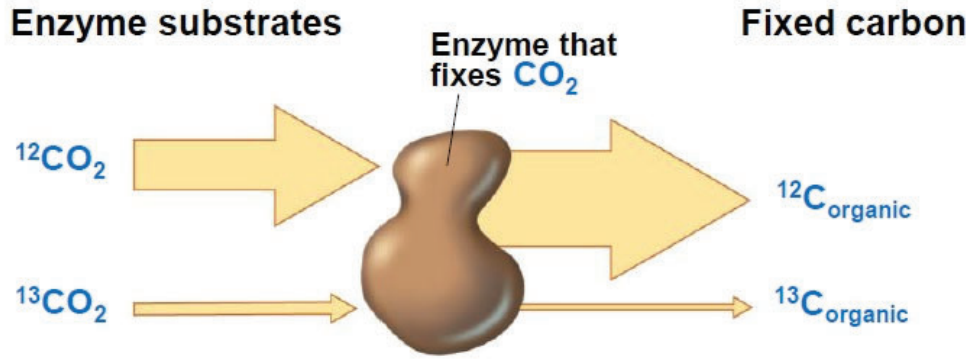




Stable Isotopes

- Nonradioactive isotopes of an element
- Used to study microbial transformations in nature
- Isotope fractionation
- Carbon and sulfur are commonly used
- Lighter isotope is incorporated preferentially over heavy isotope
- Indicative of biotic processes
- Isotopic composition reveals its past biology (e.g., carbon in plants and petroleum)
- The activity of sulfate-reducing bacteria is easy to recognize from their fractionation of sulfur in sulfides

Mechanism of isotopic fractionation with carbon as an example

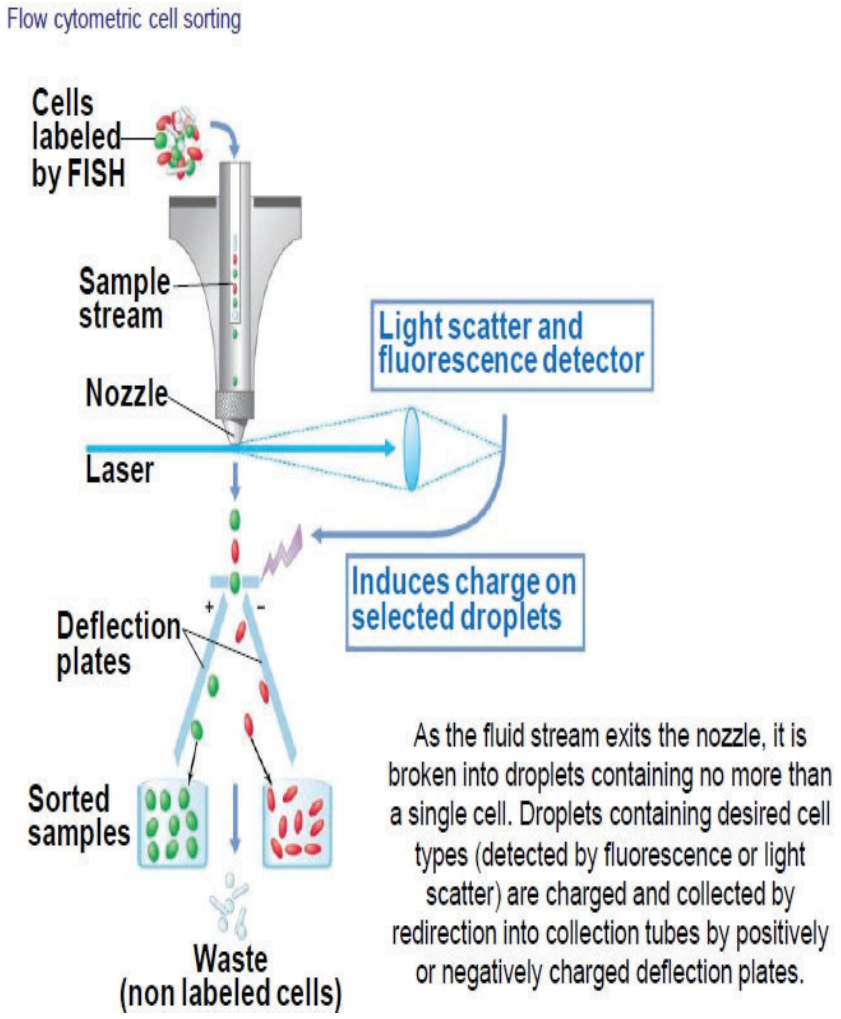


Enzymes that fix CO_2 preferentially fix the lighter isotope (^{12}C). This results in fixed carbon being enriched in ^{12}C and depleted in ^{13}C relative to the starting substrate. The size of the arrows indicates the relative abundance of each isotope of carbon.

Linking Specific Genes and Functions to Specific Organisms

Flow cytometry and multiparametric analysis

- Natural communities contain large populations
- Flow cytometer examines specific cell parameters very fast
- Cell size
- Cell shape
- Fluorescence
- Parameters can be combined and analyzed (multiparametric analysis)



Linking Specific Genes and Functions to Specific Organisms

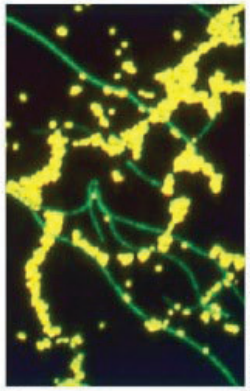
- Radioisotopes used as measures of microbial activity in a microscopic technique called microautoradiography (MAR)
- Radioisotopes can also be used with FISH
- FISH microautoradiography (FISH-MAR)
- Combines phylogeny with activity of cells

Fluorescent in situ hybridization (FISH) combined with microautoradiography (MAR).

Michael Wagner, Kilian Stöcker, and Holger Daims

An uncultured filamentous cell belonging to the *Gammaproteobacteria* is shown to be an autotroph (as revealed by MAR-measured uptake of $^{14}CO_2$).

(a)

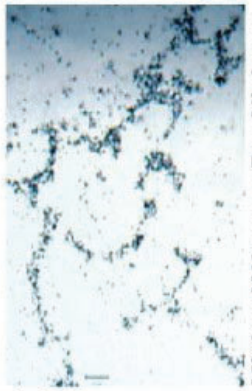


Michael Wagner, Per Nielsen, and Natuscka Lee

Uptake of ^{14}C-glucose by a mixed culture of *Escherichia coli* (yellow cells) and *Herpetosiphon aurantiacus* (filamentous, green cells).

(b)

MAR of the same field of cells shown in (b). Incorporated radioactivity exposes the film and shows that glucose was assimilated mainly by cells of *E. coli*



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(c)