Environmental Microbiology

Methods in Microbial Ecology

prepared by Prof. Bulent Icgen

Culture-Dependent Analyses of Microbial Communities

- Enrichment
- Isolation

Enrichment

- Isolation

The separation of individual organisms from the mixed community

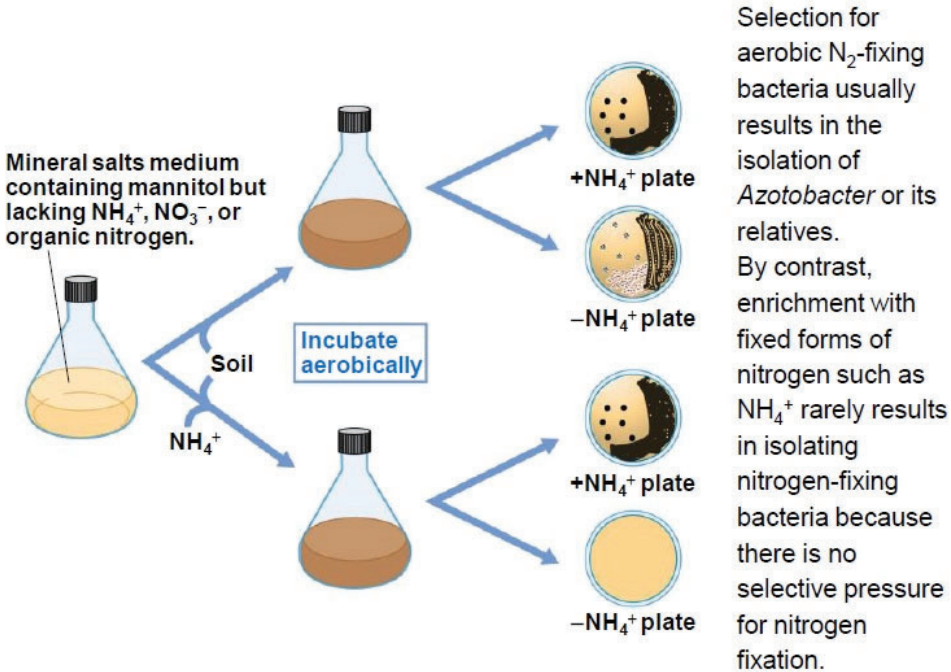
- Enrichment Cultures

Select for desired organisms through manipulation of medium and incubation conditions

- Inocula (singular Inoculum)

The sample from which microorganisms will be isolated

The isolation of *Azotobacter*.



Selection for aerobic N_2-fixing bacteria usually results in the isolation of *Azotobacter* or its relatives. By contrast, enrichment with fixed forms of nitrogen such as NH_4^+ rarely results in isolating nitrogen-fixing bacteria because there is no selective pressure for nitrogen fixation.

Enrichment

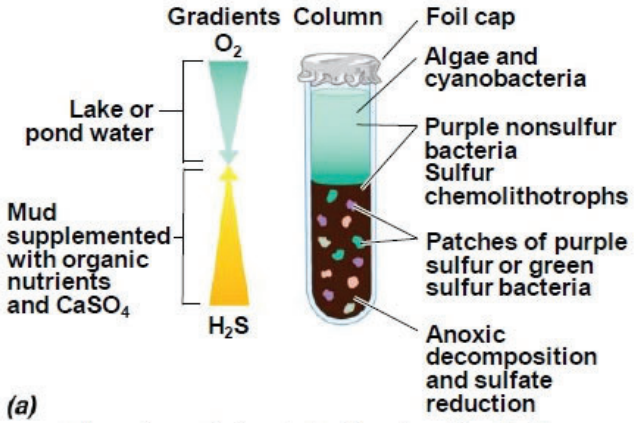
Enrichment Cultures

- Can prove the presence of an organism in a habitat
- Cannot prove an organism does not inhabit an environment
- The ability to isolate an organism from an environment says nothing about its ecological significance

Enrichment

The Winogradsky Column

- An artificial microbial ecosystem
- Serves as a long-term source of bacteria for enrichment cultures
- Named for Sergei Winogradsky
- First used in late 19th century to study soil microorganisms



(a) The column is incubated in a location that receives subdued sunlight. Anoxic decomposition leading to sulfate reduction creates the gradient of H_2S.

(b) Photo of Winogradsky columns that have remained anoxic up to the top; each column had a bloom of a different phototrophic bacterium.

Enrichment

Enrichment bias

- Microorganisms cultured in the lab are frequently only minor components of the microbial ecosystem

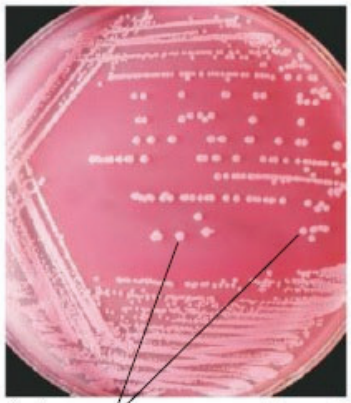
Reason: the nutrients available in the lab culture are typically much higher than in nature

- Dilution of inoculum is performed to eliminate rapidly growing, but quantitatively insignificant, weed species

Isolation

- Pure cultures contain a single kind of microorganism
- Can be obtained by streak plate, agar shake, or liquid dilution
- Agar dilution tubes are mixed cultures diluted in molten agar
- Useful for purifying anaerobic organisms

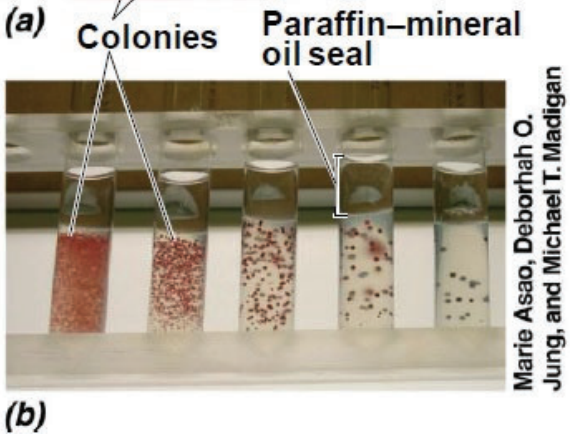
Pure culture methods



James Shapiro

(a)

- Organisms that form distinct colonies on plates are usually easy to purify.



Marie Asao, Deborhah O. Jung, and Michael T. Madigan

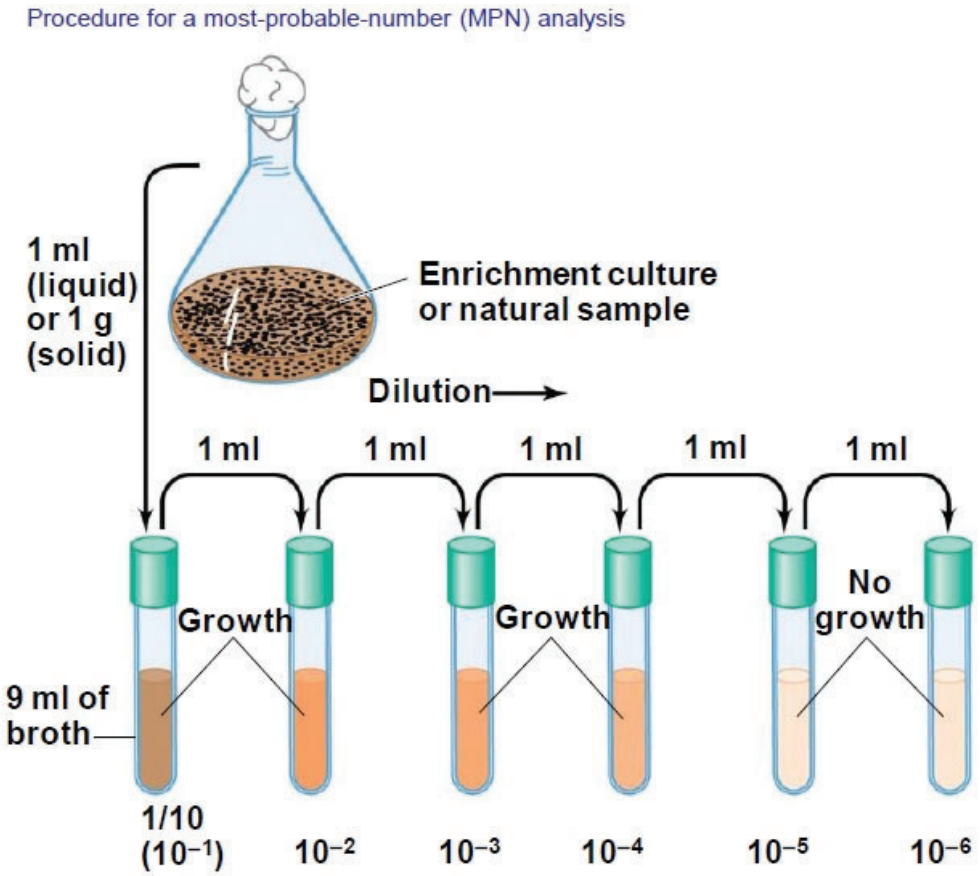
(b)

- Colonies of phototrophic purple bacteria in agar dilution tubes; the molten agar was cooled to app. 45°C before inoculation.
- A dilution series was established from left to right, eventually yielding well-isolated colonies.
- The tubes were sealed with a 1:1 mixture of sterile paraffin and mineral oil to maintain anaerobiosis

Isolation

Most-probable-number technique

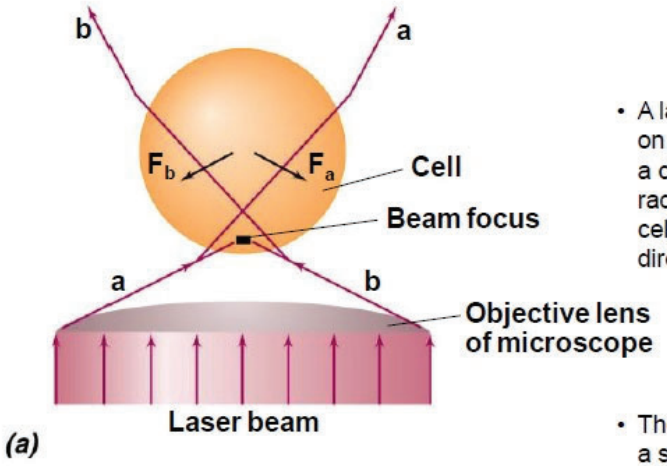
- Serial 10X dilutions of inocula in a liquid media
- Used to estimate number of microorganisms in food, wastewater and other samples



Isolation

- Axenic culture (grown under sterile conditions) can be verified by
- Microscopy
- Observation of colony characteristics
- Tests of the culture for growth in other media
- Laser tweezers are useful for isolating slow-growing bacteria from mixed cultures

The laser tweezers for the isolation of single cells



- A laser beam strongly focused on a very small object such as a cell creates downward radiation forces that allow the cell to be dragged in any direction.
- The laser beam can lock onto a single cell present in a mixture in a capillary tube and drag the optically trapped cell away from the other cells.
- Once the desired cell is far enough away from the other cells, the capillary is severed and the cell is flushed into a tube of sterile medium.

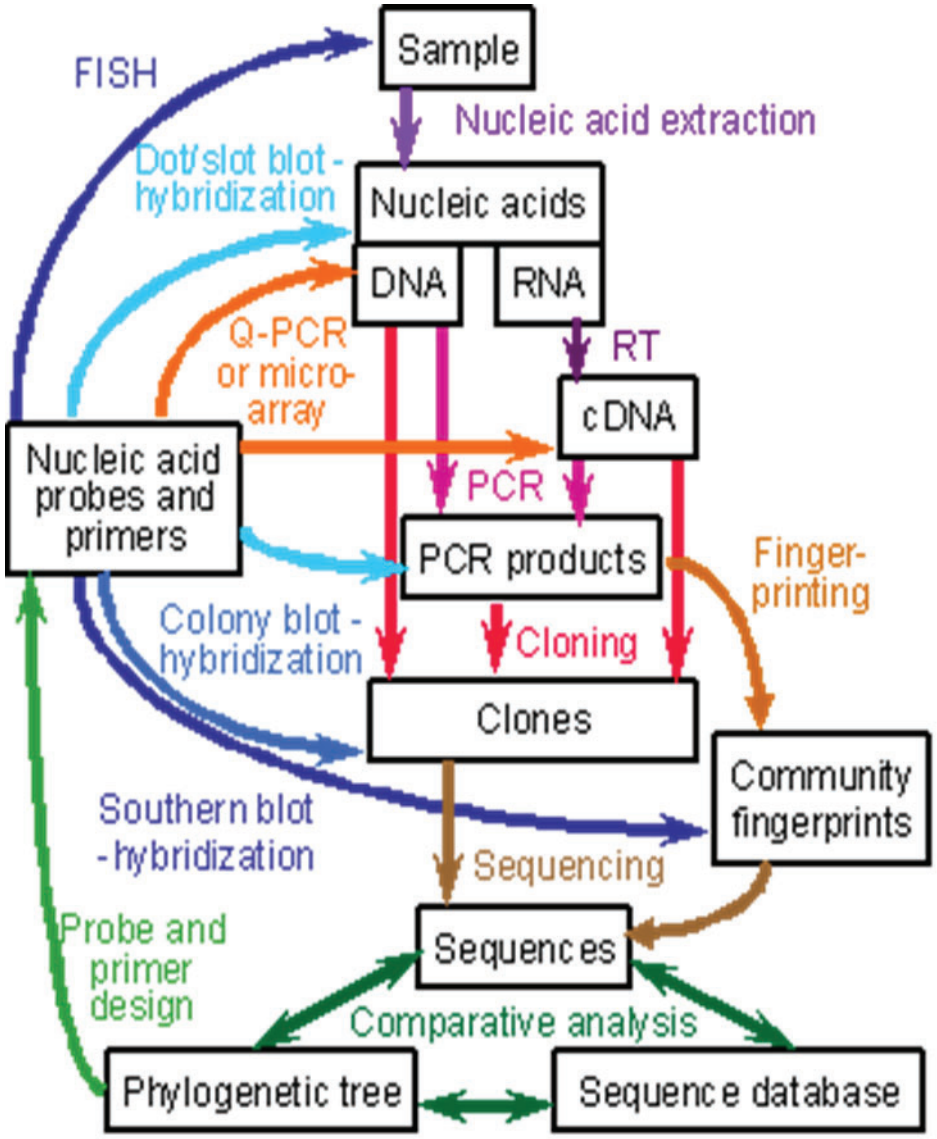
Isolation

Flow cytometry

- Uses lasers
- Suspended cultures passed through specialized detector
- Cells separated based on fluorescence

Culture-Independent Analyses of Microbial Communities

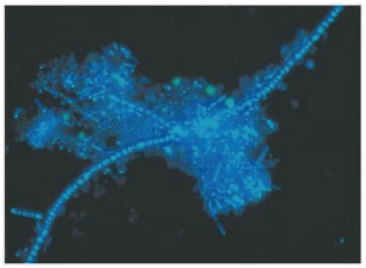
- General Staining Methods
- Fluorescent *In Situ* Hybridization (FISH)
- PCR Methods of Microbial Community Analysis
- Microarrays and Microbial Diversity: Phylochips
- Environmental Genomics and Related Methods



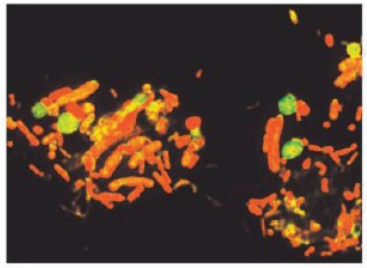
General Staining Methods

- Fluorescent staining using DAPI or acridine orange (AO)
- DAPI-stained cells fluoresce bright blue
- AO-stained cells fluoresce orange or greenish-orange
- DAPI and AO fluoresce under UV light
- DAPI and AO are used for the enumeration of microorganisms in samples
- DAPI and AO are nonspecific and stain nucleic acids
- Cannot differentiate between live and dead cells

Nonspecific fluorescent stains

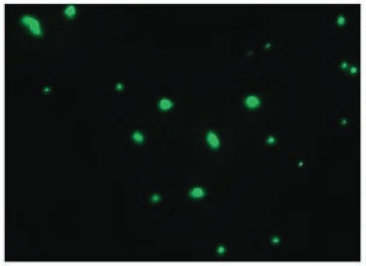


Marc Mussman and Michael Wagner



Marc Mussman and Michael Wagner

(a) DAPI and (b) acridine orange (AO) staining showing microbial communities inhabiting activated sludge in a municipal wastewater treatment plant. With acridine orange, cells containing low RNA levels stain green.



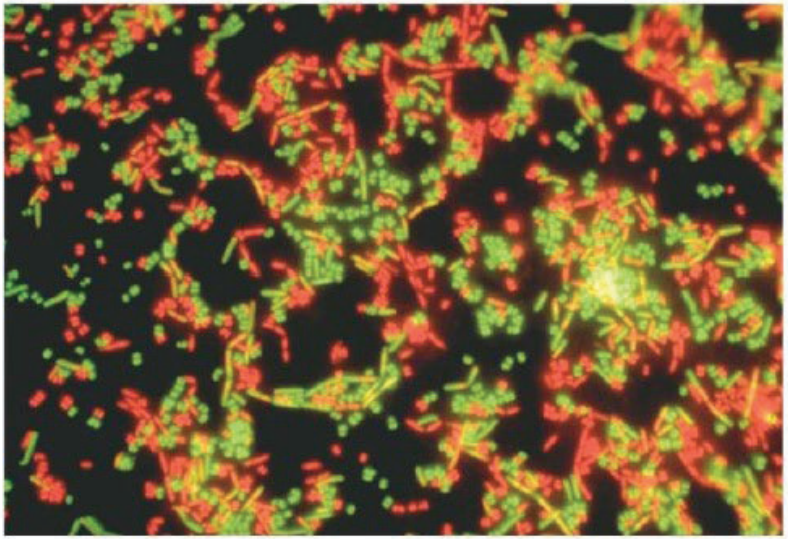
Willm Martins-Habenna

(c) SYBR Green–stained sample of Puget Sound (Washington, USA) surface water showing green-fluorescing bacterial cells. The large cells near the center of the field are 0.8–1.0 m in diameter

General Staining Methods

Viability stains: differentiate between live and dead cells

- Two dyes are used
- Based on integrity of cell membrane
- Green cells are live
- Red cells are dead
- Can have issues with nonspecific staining in environmental samples



Live (green) and dead (red) cells of *Micrococcus luteus* (cocci) and *Bacillus cereus* (rods) stained by the LIVE/DEAD BacLight Bacterial Viability Stain