1. **Methods in Microbial Ecology**
   Chapter 22
2. **22.1 Culture Dependant Microbial Community Analysis**
   - *Isolation*
     - separation of individual organisms from mixed community
   - *Enrichment Cultures*
     - Select desired organisms by manipulation of medium & incubation conditions
3. **Some Enrichment Culture Methods**
4. **22.1 Enrichment and Isolation**
   - Enrichment Cultures
     - Can prove presence of an organism in a habitat
     - Cannot prove an organism does not inhabit environment
   - isolation does not indicate ecological significance
   - *Winogradsky Column*
     - artificial microbial ecosystem
     - long-term source of bacteria for enrichment cultures
     - Named for *Sergei Winogradsky*
5. **Some Enrichment Culture Methods**
6. **Some Enrichment Culture Methods**
7. **22.1 Enrichment and Isolation**
   - *Enrichment Bias*
     - Microorganisms cultured in lab are frequently only minor components of the microbial ecosystem
       - 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*
8. **22.2 Isolation in Pure Culture**
   - *Pure cultures*
     - streak plate, agar shake, or liquid dilution
   - *Agar dilution tubes*
     - mixed cultures diluted in molten agar
     - Useful for purifying anaerobic organisms
   - *Most-probable number technique*
     - Serial 10X dilutions of inocula in a liquid media
     - estimate number of microorganisms in food, wastewater, and other samples
9. **22.2 Isolation in Pure Culture**
   - *Laser tweezers*
     - Isolating slow-growing bacteria from mixed cultures
10. **22.3 General Staining Methods**
    - Fluorescent staining using *DAPI* or *acridine orange (AO)*
      - nonspecific and stain nucleic acids
      - Cannot differentiate between live and dead cells
        - DAPI - bright blue
        - *acridine orange (AO)* - orange or greenish-orange
11. **22.3 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 nonspecific staining in environmental samples

12 **22.3 General Staining Methods**

- *Fluorescent antibodies*
  - Highly specific
  - Making antibodies is time consuming and expensive
- *Green fluorescent protein*
  - genetically engineered into cells to make them *autofluorescent*
  - Can be used to track bacteria
  - Can act as a reporter gene

13 **22.4 FISH**

- *Nucleic acid probe*
  - DNA or RNA complimentary to a sequence in a target gene or RNA
- *FISH*: fluorescent *in-situ* hybridization
  - Phylogenetics of microbial populations (rRNA)
  - microbial ecology, food industry, & clinical diagnostics
  - *IRST-FISH* & *CARD-FISH*
    - Gene expression in natural populations & slow growing cells

14 **22.6 Environmental Genomics**

- *Environmental Genomics (metagenomics)*
  - DNA is cloned from microbial community and sequenced
  - detect as many genes as possible
  - All genes in a sample can be detected
  - Yields picture of gene pool in environment
  - Powerful tool for assessing the phylogenetic and metabolic diversity of an environment

15 **22.7 Chemical Assays, Radioisotopes, & Microelectrodes**

- direct chemical measurements are sufficient
  - Higher sensitivity with radioisotopes
    - Proper *killed cell* controls must be used

16 **22.7 Chemical Assays, Radioisotopes, & Microelectrodes**

- *Microelectrodes*
  - Can measure a wide range of activity
  - pH, oxygen, CO_2, and others can be measured
  - Small glass electrodes, quite fragile
  - Electrodes are carefully inserted into the habitat (microbial mats)