Today

- OUT/Ecotype
- Quiz on Thurs
- Finish methods…!!

What is a microbial species

- Unit of ecology= species
- What is a species
  - Biological species
  - Evolutionary species
  - Ecotype
  - Microbial spp
  - BOTTOM LINE- define your UNIT

Microbial spp defn

- Physiology/DNA hybridization etc.
- Small subunit (16S) rRNA- Woese 1987
- ITS (AEM 66:5448-5456)
- Genomes changing our views
- Ecotype/phylotype/OTU
  - “A subspecies, or breed, that has adapted to its local environment and as a result is distinctive from other members of the species, but can still successfully interbreed with other members of the species.”

Speciation

- Speciation occurs as a result of evolutionary processes
- Allopatric (Geographic) speciation
- Sympatric speciation
- Adapative radiation (adapt to fit a new environment – new energy/carbon source)
- Biological species (Ernst Mayr)
  - Reproductive isolation
  - Limited to bisexual organisms
- Evolutionary species better?
- Ecotype/phylotype/otu
  - IJSEM 47:1145. “Evolutionary genetic theory predicts that in the bacterial world, each sequence similarity cluster should correspond to an ecologically distinct population”

Molecular phylogenetic analysis

- Decide what sequence /gene to examine
- Get sequence
- Align sequences so that “homologous” residues juxtaposed
- Structural superimposition
- Phylogenetic analysis
  - Signature analysis
  - Tree building
    - Distance methods
    - Maximum likelihood
    - Maximum parsimony
Tree methods

- Choose an outgroup
- Choose a representative tree
- A tree is a MODEL/hypothesis not FACT!!
- Distance
  - Count the number of differences between pairs of sequences; this is some measure of “evolutionary distance” that separates the organisms
- Maximum parsimony
  - Simpler hypotheses are preferable—simplicity underestimates though
- Maximum likelihood
  - Incorporates models of evolutionary change,

Other random thoughts

- Lateral gene transfer
- # of genes/genome
- Clock-like behaviour?-- time

Molecular ecological methods

- Cloning, DGGE, TRFLPs
- Looking for expression of genes (nitrogenase (nif), ammonia oxidation (amo), Rubisco)
- FISH
- Review papers: DeLong 2001 Current Opinion in Microbiol. 4:290-95 Microbial seascapes revisited
Sorting genes

- Cloning
- DGGE
- TRFLP

FISH

Examples…

- Barns et al., PNAS91:1609
- Hugenholtz et al
- Ward et al. MMBR62:1353
- Nature 409:6 “unexpected diversity of small euks”
- Nat 417:137 “Euk diversity in Spain’s River of Fire”
- Cultivating the uncultured, PNAS 99:15681

Other related

- In situ PCR (RT-PCR)
- Laser/optical tweezers

Metagenomics

- Tyson et al paper to discuss
### Activity measurements

- Colormetric approaches
  - e.g. amylase, protease, chitinase, dehydrogenase
- Gas Chromatography (e.g. N fixation)
- Radioisotopes
  - Specific activity
  - Killed controls
- Productivity
- Microelectrodes

### N-fixation measurements

- Indirect measurement with C2H2 (acetylene)
- Produce ethylene (measure with GC)
- Direct measurement with 15N

### Radioisotope methods

- Activity, heterotrophic potential
- Productivity

### Stable isotopes

- Carbon (13/12), oxygen, sulfur (32/34), iron
- Measurement
- Track food chains (e.g. deep-sea vents)
- \[ \frac{^{13}\text{C}/^{12}\text{C} \text{ sample- } ^{13}\text{C}/^{12}\text{C std}}{^{13}\text{C}/^{12}\text{C std}} \times 1000 \] (std PeeDee rock formation)
Stable isotope and molecular methods


TOF-SIMS and FISH

Methane-Consuming Archaea Revealed by Directly Coupled Isotopic and Phylogenetic Analysis Victoria J. Orphan, Christopher H. House, Kai-Uwe Hinrichs, Kevin D. McKeegan, and Edward F. DeLong Science 20 July 2001; 293: 484-487

Other?

- E.g. Lipids
  - Schouten and others… looking for archaeal lipids
  - Ladderane lipids of anammox microbes

Volts vs Ag/AgCl

In situ cyclic voltammograms above a vent chimney 1000 mV/sec

Current (µA)