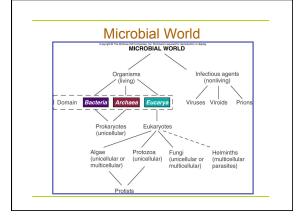
#### Microbial Diversity

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#### Contents

- Microbial diversity
  - Morphological, structural, metabolic, genetic
- Microbial organisms classifications
  - Definition of microbial species
- Why we care about microbial diversity



#### Microbial Diversity

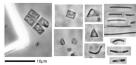
- Morphological diversity:
  - bacilli, cocci, and spirals are 3 common shapes
  - filamentous form & pleiomorphic forms
  - many varieties of size, ranging from submicroscopic up to a few bacteria that can be seen with the naked eye.
- Metabolic diversity:
  - heterotrophs vs autotrophs
  - fermentation vs respiration (aerobic and anaerobic).
- Structural diversity
  - gram-positive vs gram-negative bacteria
  - presence or absence of walls
  - external appendages
  - endospores
- Genetic diversity
  - 16S rRNA or entire genome?

#### Microscopic Methods for Assessing Microbial Diversity

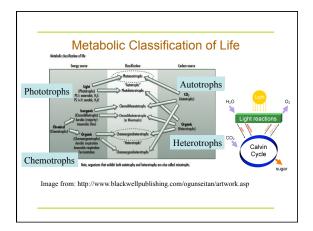
- Microorganisms are generally 5-100 times smaller than the perceptive limits of the human eve
- Microscopes are the traditional instruments used for microbial diversity assessment
- They remain indispensable tools for exploring the morphological, physiological, and genetic diversity present in microbial communities

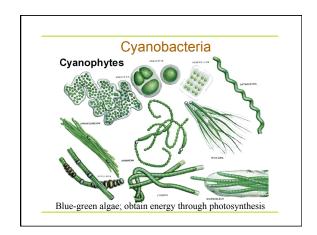
### Objectives for Microscopic Analysis in Microbial Diversity Assessment

- Microbial cell morphological types
  - Spherical, rod-like, and spiral (Coccus, Bacillus, and Spirillum); square-shaped (*Haloarcula*, "saltbox"; 1980); triangular (*H. Japonica*; 1995)



Artwork from http://www.blackwellpublishing.com/ogunseitan/artwork.asp





#### Molecular and Genomic Methods

- Molecules used for diversity estimate
  - Proteins
  - DNA
  - Fatty acids (lipids)
  - Carbohydrates

#### Signature Lipid Biomarkers

- Branched chain fatty acids with 9-20 atoms are particularly useful in differentiating phylogenetic groups.
- PLFA (phospholipid fatty acids) taxonomic markers
  - Monoenoic PLFAs indicates Gram-negative bacteria and cyanobacteria
  - Terminally branched chain fatty acids (TBCFA) are characteristic of Gram-positive bacteria.
- Lipids in Archaea represents a distinctive feature of this group of microbes, and they are involved in their survival in extreme environments.

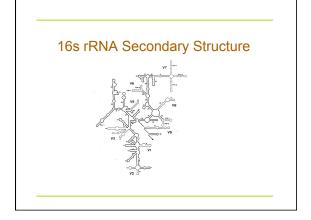
#### **Protein Profiles**

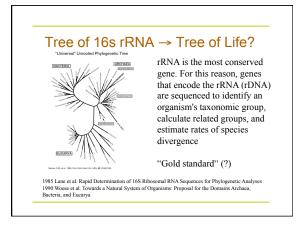
- Protein-based phylogenetic reconstruction is likely to produce more robust measurements of diversity than other non-coding sequences
- Traditionally focus on several phylogenetically conserved molecules, cytochromes, protein elongation factors, ATPases, etc
- 31 phylogenetic markers defined by Ciccarelli et al. Ref: Science, 311(5765):1283-1287, 2006)

#### 16s rRNA

- rRNA: Ribosome RNA; S in 16S represents Svedberg units
- Ribosome is composed of two subunits, named based on how fast they sediment when subject to centrifugation
  - prokaryotic
     70S
     50S (5S, 23S)
     30S (16S)
     eukaryotic
     80S
     60S (5S, 5.8S, 28S)
     40S (18S)







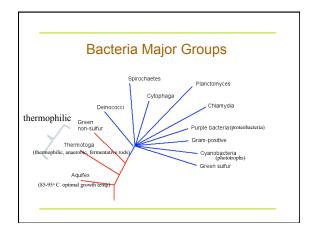
#### "What is a bacterial species?"

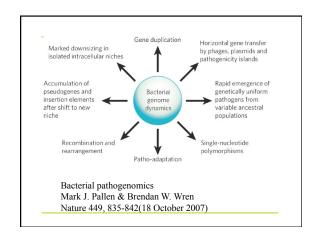
- On the genetic level, it is well accepted that two isolates are part of the same species if their 16S rRNA genes share at least 98% identity (or 97%?)
- "while many people had felt that genomics would clarify the species concept in prokaryotes, it has actually done the exact opposite and made it harder to define" (W. Ford Doolittle)
- recombination and gene transfer between a variety of prokaryotic organisms
- The concept of a bacterial species appears to take different forms depending on the scientific perspective
  - Escherichia coli and Shigella species are significantly
  - different based on genomic and clinical examinations

    But they are the same species based on 16sRNA

#### The Concept of Species

- Reproductive isolation (however, morphology is always the first pass method)
- Species we can be sure about; all higher taxonomic levels we can argue about
- Common ancestor (for the genome as a set): Phylogenies





# Applications of 16S rRNA Sequencing

- Bacterial identification
- Study of the community structure (regarding species diversity/distribution) of an environmental community
- Sequencing of 16S rRNA hyper-variable regions (instead of entire sequences) for identification of species
  - 454 sequencers (can not cover entire 16S rRNA sequences, which are ~1500 nt).

#### Microbial Diversity and Geography

- Biogeochemical interactions
  - biogeochemical cycle is a circuit or pathway by which a chemical element or molecule moves through both biotic ("bio-") and abiotic ("geo-") compartments of an ecosystem
- The "biocartography" collaborative project
  - Develop a geographically referenced database containing microbiological and geochemical data that can be used to support predictive models of biogeochemical interactions, and to improve access to biotechnologically important organisms

## Microbial Diversity and Global Environmental Issues

- Of what use is microbial diversity?
  - Biodiversity <-> environmental parameters
  - Using assessments of microbial diversity to detect environmental change
  - Use such knowledge in the best service of ecosystem restoration

#### Estimating the magnitude of microbial diversity

Louindaing the magnitude of microbi	a. a
Number of bacteriophages on Earth	10 <sup>31</sup>
Number of microbes on Earth	5 × 10 <sup>30</sup>
Number of stars in the universe	7 × 10 <sup>21</sup>
Number of microbes in all humans	6 × 10 <sup>23</sup>
Number of humans	6 × 10 <sup>9</sup>
Number of microbial cells in one human gut	10 <sup>14</sup>
Number of human cells in one human	1013
Number of microbial genes in one human gut	3 × 10 <sup>6</sup>
Number of genes in the human genome	2.5 × 10 <sup>4</sup>
Combined length of all bacteriophages on Earth	10 <sup>8</sup> Ly
Diameter of the Milky Way	10 <sup>5</sup> Ly

Fifteen years of microbial genomics: meeting the challenges and fulfilling the dream Nature Biotechnology 27, 627 - 632 (2009)