Phylogeny of Bacterial Lipids

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**Figure 2** Phylogenetic tree illustrating the major lineages (phyla) of the domain Bacteria. **Wedges shown in black are the 12 original phyla**, as described by Woese (112), **in white are the 14 phyla with cultivated representatives** recognized since 1987, and **in gray are the 26 candidate phyla** that contain no known cultivated representatives. This evolutionary distance dendogram was constructed by the comparative analysis of over 600 nearly full-length 16S ribosomal RNA gene sequences using the ARB sequence analysis software package (65), selected from a larger database of over 12,000 sequences. A modified version of the “Lane mask” was employed in this analysis (55), along with the Olsen evolutionary distance correction and neighbor-joining tree-building algorithm. Horizontal wedge distances indicate the degree of divergence within a given phylum. The scale bar corresponds to 0.05 changes per nucleotide position. Phylum names are designated by selecting the first applicable option out of the following: (a) their convention in *Bergey’s Manual of Systematic Bacteriology*, if it exists (36); (b) the first described representative genus within the phylum if it has cultivated representatives; (c) the first label given to a candidate phylum if previously published; or (d) the first clones or environment where the first clones were retrieved, for previously unnamed candidate phyla.
Outline

• Fatty Acids
• Hopanoids
• Pigment Biomarkers
• Porphyrins
• Special Bacterial Lipids
• Steroids?
• Note on Isotopes
• Papers
Fatty Acids

- Higher even-number saturated fatty acids ($C_{12-24}$) are a significant contribution to total lipid content
- $C_{16}$ is the most common fatty acid in bacteria
  - $C_{18}$, $C_{14}$ and $C_{12}$ are also common
- $C_{20-28}$ are less common
  - except in extremophiles
- Odd numbered compounds $C_{15}$ and $C_{17}$ have been reported in bacteria
Fatty Acids

\[ \text{C}_{16} \]

\[ \text{C}_{18} \]

\[ \text{C}_{14} \]

\[ \text{C}_{12} \]
Fatty Acids

• Unsaturated fatty acids are also important components to the total lipid content of bacteria

• Most common are octadecanoic acid and hexadecanoic acid
  – Occasionally, but not always, oleic acid and palmitoleic acid – specific placement of double bond

• Less common, though still present, are unsaturated carbon compounds with $C_{10-14}$ and $C_{20-28}$
Fatty Acids

- Oleic Acid
- Palmitoleic Acid
Fatty Acids

- Hydroxy, branched chain and cyclopropane acids have been identified in small amounts in few groups of bacteria
  - Mid-chain monomethyl alkanes are produced by cyanobacteria
Hopanoids

- $C_{30}$ compounds found in diverse bacterial lineages
- Some hopanes are produced by eukaryotes (plants) but $C_{31}$ to $C_{35}$ (homohopanes) are exclusively bacterial and originate from bacteriohopanetetrol and other polyfunctional $C_{35}$ hopanoids
Squalene

- In bacteria, squalene-hopene cyclase is used to turn this compound into generic $C_{30}$ pentacyclic triterpenoid hydrocarbon skeleton
- Can be highly associated with certain archaea
Hopanoids

- General $C_{30}$ hydrocarbon structure
- Biological stereochemistry $\beta\beta(22R)$
- Skeleton preserved with some rearrangements in fossil hydrocarbons
Hopanoids

- Diplopterol
- Synthesized by aerobic bacteria, including methanotrophic species
  - Evidence for anaerobic synthesis
- Can be cyclized directly from squalene (no O₂ required)
Hopanoids

- Bacteriohopanepolyol - BHP
- Functional hopanoids in Bacteria are often in the form of BHP
- X,Y,Z can be substituted with other –OH groups, sugars, amino acids or other functionalized units
- Side chain carbons are derived from ribose
Hopanoids

- 2α-Me Hopane
- Biomarkers for Cyanobacteria (prochlorophytes)
Hopanoids

- 3β-Me Hopane
- Biomarkers for microaerophilic proteobacteria (methylootrophs, methanotrophs and acetic acid bacteria)
Hopanoids

- $\text{C}_{30}$ hydrocarbon structure with $\Delta^6$ and/or $\Delta^{11}$
- Acetic acid bacteria
Hopanoids

- 28,30-bisnorhopane (BNH) and 25,28,30-trisnorhopane (TNH) are believed to originate from chemooautotrophic bacteria growing at the oxic-anoxic interface.
  - *Beggiatoa*?
  - *Thioploca*?
Hopanoids

- Isoarborinol and arborinone
- Initially believed to be derived solely from certain families of angiosperms, but identification of derivatives identified in samples predating the evolution of angiosperms indicate a possible bacterial (algal?) source
Pigment Biomarkers

- Okenone/Okenane
- Exclusively known from the planktonic species of *Chromatiaceae* (purple sulfur bacteria)
Pigment Biomarkers

- Chlorobactene/Chlorobactane
- Biomarker for planktonic as well as benthic mat-forming green pigmented species of *Chlorobiaceae* (green sulfur bacteria)
Pigment Biomarkers

- Isorenieratene/Isorenieratane
- Isorenieratane and (β -) isorenieratane are biomarkers for brown pigmented species of Chlorobiaceae
Pigment Biomarkers

• Aryl isoprenoids are products of pigment diagenesis
• 2,3,6- substituted (derived from isorenieratane and/or chlorobactane)
Porphyrrins

• Bacteriochlorophyll
  – Present in purple sulfur bacteria (a,b), green sulfur bacteria (c,d,e), chloroflexi (c) and heliobacteria (g)
  – c and d as chlorins with one reduced pyrrole ring and the rest as bacteriochlorin with two

• Some bacteria also use chlorophyll
Porphyrrins

B Chl a – purple sulfur bacteria

B Chl c – green sulfur bacteria
Porphyryns

- Cytochrome P450
  - Enzyme used in electron transport chain
Porphyrrins

- Maleimides
- Derive from porphyrins
- R-groups can aid in identification of original compound
  - Often considered biomarkers for bacteriochlorophylls c/d/e in green sulfur bacteria (or chlorophyll a in phytoplankton)
Pristane/Phytane

• Derived from phytol (phytol) in chlorophyll
  – Oxic conditions $\rightarrow$ pristane
  – Suboxic conditions $\rightarrow$ phytane

• Careful – can also be derived from other sources
Farnesane

- Similar to pristane/phytane, farnesane is derived from farnesyl as a side chain of bacteriochlorophyll c
  - Green sulfur bacteria
Ladderanes

- Lipids specific for bacteria that perform anammox
  - planctomycetes
- Found primarily in anammoxosome as a barrier to diffusion of hydrazine

Van Niftrik et al. 2004
Tetrahymananol/Gammacerane

- Tetrahymananol long thought to be a biomarker for bacterial-consuming protozoans
  - Discovered in bacteria, along with a series of related methylated compounds
- Gammacerane is fossil skeleton
Steroids?

• Characteristic of Eukarya; limited proven steroid-producing bacteria
  – Pathways are incomplete
  – May be LGT from Eukarya

• Reports of sterols in cyanobacterial samples
  – Reported in taxonomically diverse cyanobacteria
  – May be contamination

Summons et al. 2006
• Cholesterol (left) and lathosterol (right)
  – Common sterols in Eukaryotes and most complete sterol in bacterial species, respectively

Summons et al. 2006
Steroids?

- Comparison of total ion chromatograms and selected ion chromatograms showing differences between possible contaminated cyanobacterial samples and species grown in specified conditions (only sterol is ergosterol from growth media)

Summons et al. 2006
A Note on Isotopes

- As with all organisms, the $\delta^{13}C$ value of organic compounds (such as lipids) in bacteria reflects the $\delta^{13}C$ of the compounds that they consume
  - Low $\delta^{13}C$ values (-45 to -80‰) common in methanotrophs
  - Hopanes with $\delta^{13}C \sim -20$ to $-35‰$ common in cyanobacteria
- Unlike Archaea, many Bacteria consume a range of organic compounds and tagging of specific carbons can be used to determine biosynthetic pathways for lipids
  - Two pathways for isoprenoid synthesis were identified
  - Fatty acids and isoprenoids can be produced from different pathways and therefore have different bulk $\delta^{13}C$ values
- However, different growth conditions have also been shown to produce different $\delta^{13}C$ values for organisms
Papers!
Rohmer et al. 1984

• Hopanoids are phylogenetic sterol ancestors
• BHPs have never been identified in eukaryotes, even in those with hopanoids, must be “prokaryotic metabolites”
• Hopanoid content in bacteria is same order of magnitude as sterol content of eukarya
• Half the strains do not contain detectable hopanoids, but cannot derive taxonomic conclusions from distributions
Pancost et al. 2000

• Fatty acids and methylhopanoids of methanotrophic bacteria not observed, only bishomohopanol (nonspecific bacterial biomarker); $\delta^{13}C$ values and geochemical evidence are inconsistent with aerobic methanotrophy by bacteria

• $\delta^{13}C$ values and biomarker evidence suggest a grouping in this area of methotrophy, sulfate-reduction and chemoorganotrophy in a consortium including bacterial acetogens and sulfate-reducers

• Hopanoids are generated at depth under anoxic conditions
‘Geological’ configuration of αβ-hopanoids can be produced by living organisms at methane seeps – but not seen in surrounding sediments.

However, the extreme location indicates this will not disrupt the use of this compound as part of a maturity parameter.

δ¹³C values indicate these organisms incorporate methane into biomass.

Location (in anoxic Black Sea gas seeps) indicates that anaerobic bacteria can produce hopanoids in anoxic environments.
Harvey et al. 2006

• During incubations (generally):
  – Fatty acid concentrations increased
  – Shifts in fatty acid distribution were substrate-specific
  – A number of site-specific shifts were also observed

• Major bacterial groups show overlap in metabolic capacities – important when using fatty acids to describe community distribution

• The production of certain types of fatty acids shows in response to different substrates is the same across many phylogenetic groups