Molecular Biogeochemistry Lecture 4

- Hopanoids
  - Different structures and known bacterial sources,
    - C30
    - C35
    - Composite
    - Unsaturated
    - Methylated
  - Biosynthesis
    - Squalene hopene cyclase
    - Beyond shc
    - Genes and taxonomic distribution of hopanoids
  - Function
    - Localization
    - Membrane permeability
    - Stress responses
    - Novel functions

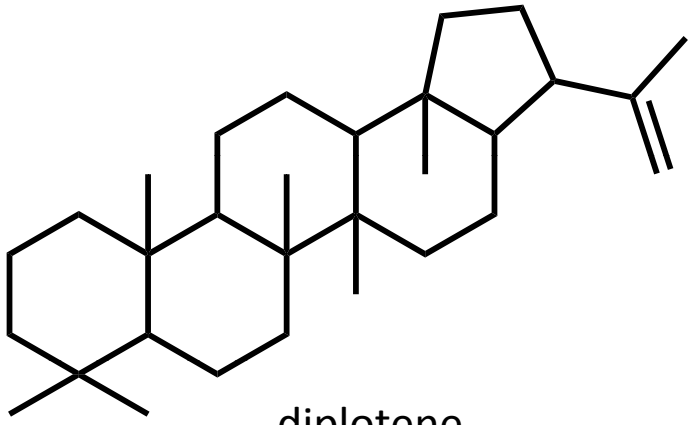
Hopanoids

- First recognized as a class of C_{30} pentacyclic triterpenes found in ferns, mosses and dammar resins

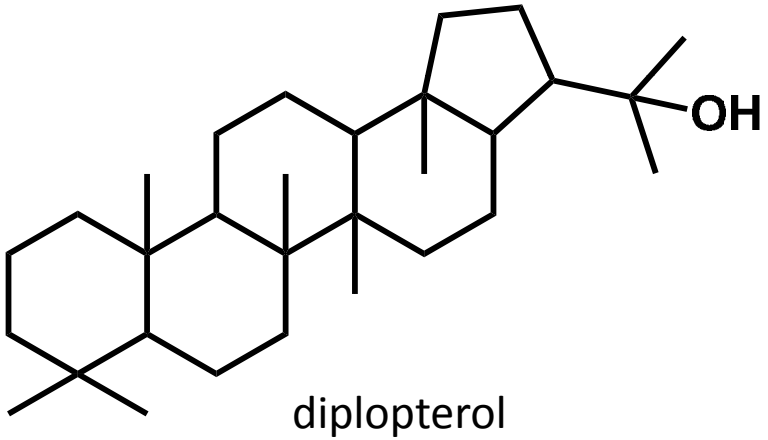
- 'Hopane' named after the Dipterocarp plant genus *Hopea*, itself after botanist John Hope

- Biosynthetic kinship to sterols, tetrahymanol & oleanoids, via squalene recognised in 60's

Hopanoid Structures: C_{30} Hopanoids



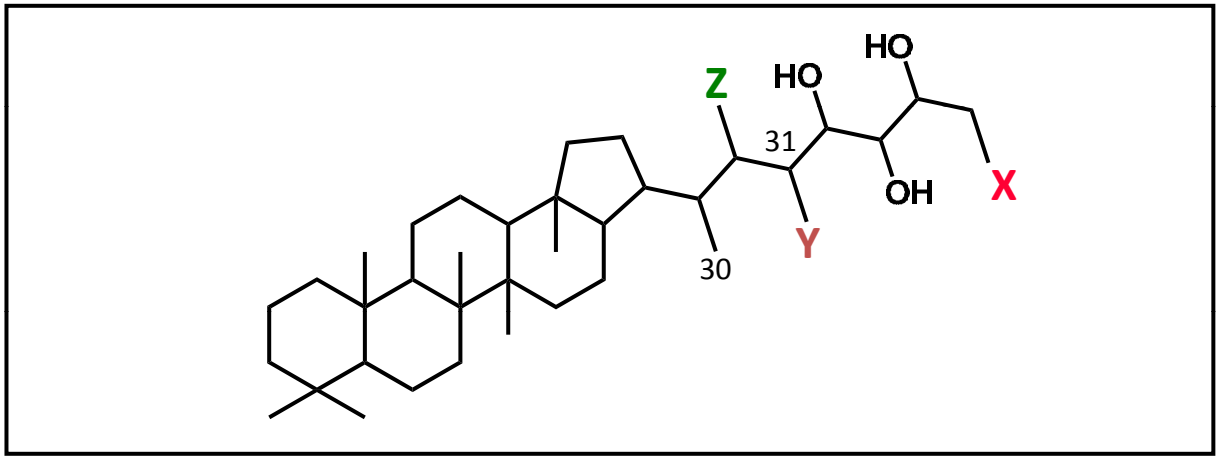
diplotene



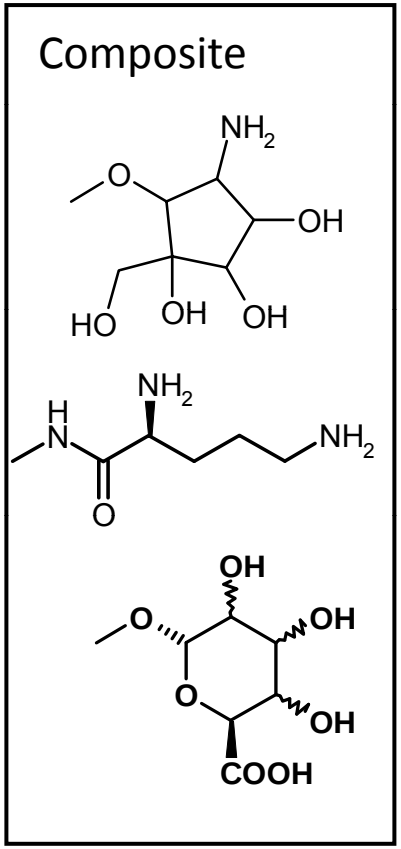
diplopterol

- Diploptene found in all hopanoid producing bacteria
- Diplopterol detected in most hopanoid producers
- Biosynthetic intermediates only?

Side Chain Variations



Composite



TETRA: X=OH, NH_2, composite; Y = Z = H

PENTA: X = OH, NH_2, composite; Y = OH, Z = H
X = OH, Y = H, Z = OH

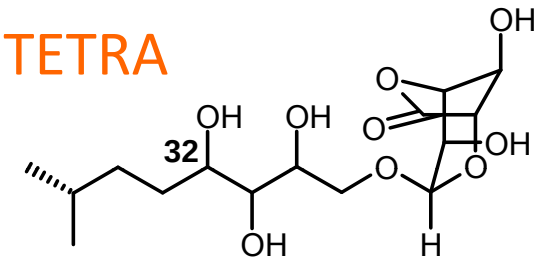
HEXA: X = NH_2; Y = Z = OH

Analysis Of Biohopanoids

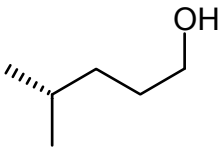
- Highly functionalized, amphiphillic
- Not amenable to conventional GC-MS
- Side chain cleavage (Rohmer et al., 1984)
  - Periodic acid/sodium borohydride
  - Product structure directly related to number and position of functional groups in side chain
- Specific nature of functional groups lost

Periodic Acid Oxidation

TETRA

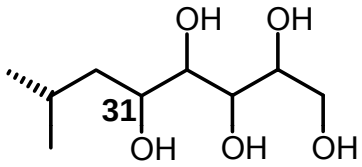


$H_5IO_6/NaBH_4$

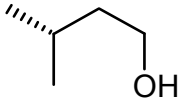


32-hopanol

PENTA

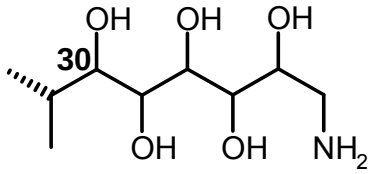


$H_5IO_6/NaBH_4$

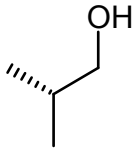


31-hopanol

HEXA



$H_5IO_6/NaBH_4$

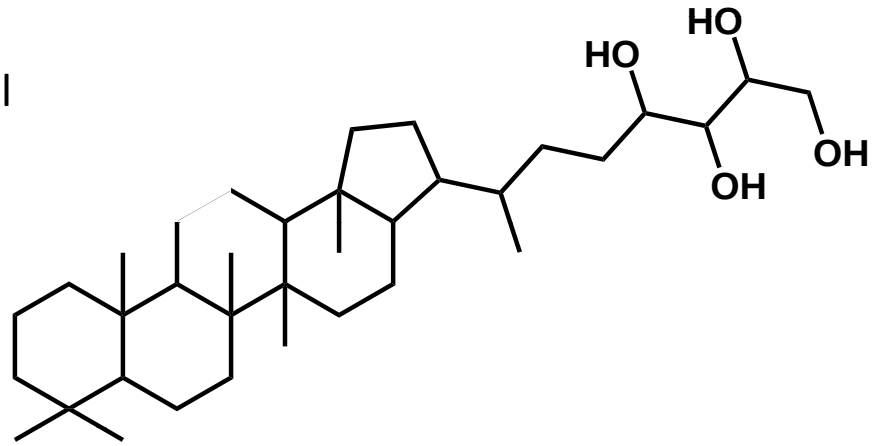


30-hopanol

Topic #1: How was analysis of functionalized (i.e. C_{35}) hopanoids improved? (Helen Talbot papers)

Hopanoid Structures: C_{35} Hopanoids

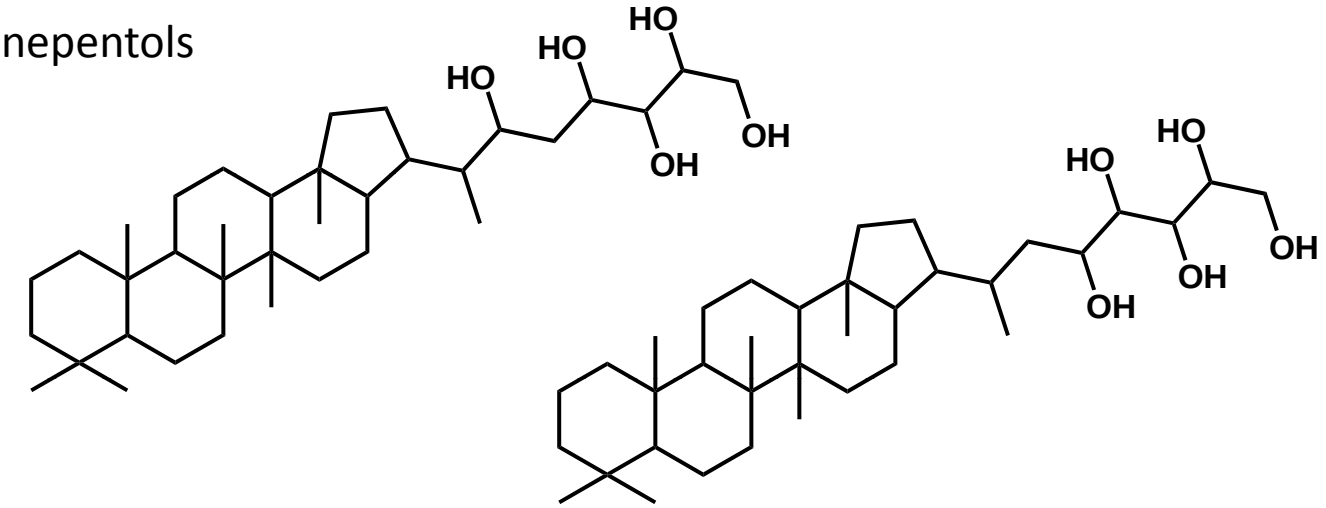
bacteriohopanetetrol



- Thought to be most common hopanoid produced
- Found in most but not all hopanoid producers:
  - Cyanobacteria
  - Gram-positive heterotrophs
  - Gram-negative heterotrophs
  - Obligte/Faculative methylotrophs
  - Purple nonsulfur bacteria
  - Sulfate reducing bacteria

Hopanoid Structures: C_{35} Hopanoids

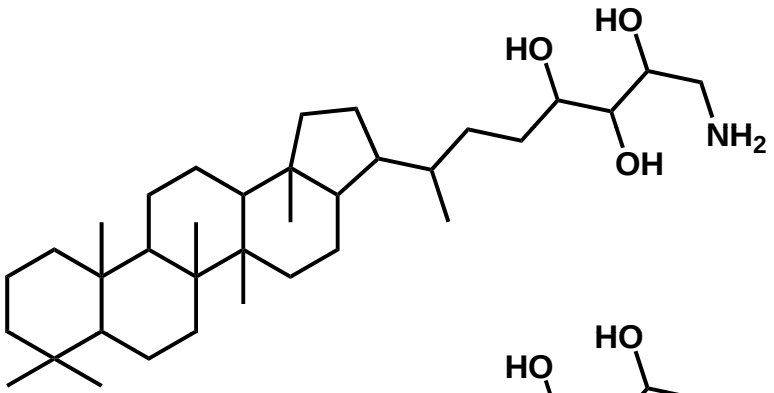
bacteriohopanepentols



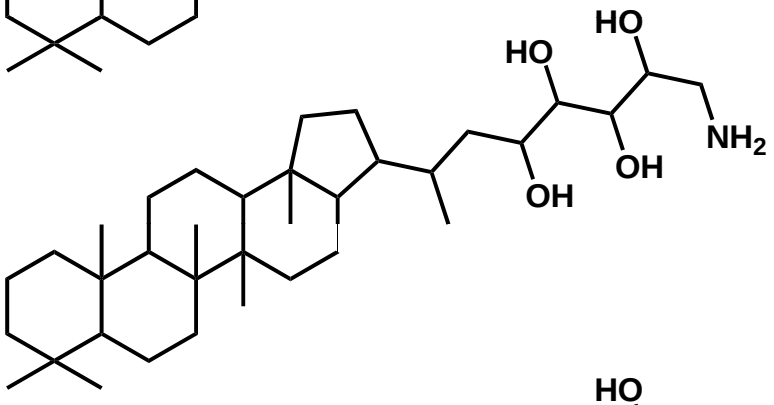
- C_{35} hopanoids with 5 hydroxyl groups (pentafunctionalized)
- Only observed in cyanobacteria

Hopanoid Structures: C_{35} Hopanoids

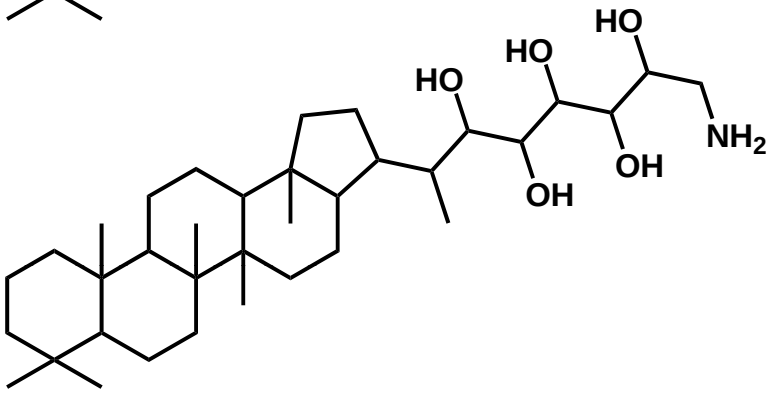
Aminohopanoids



aminobacteriohopanetriol



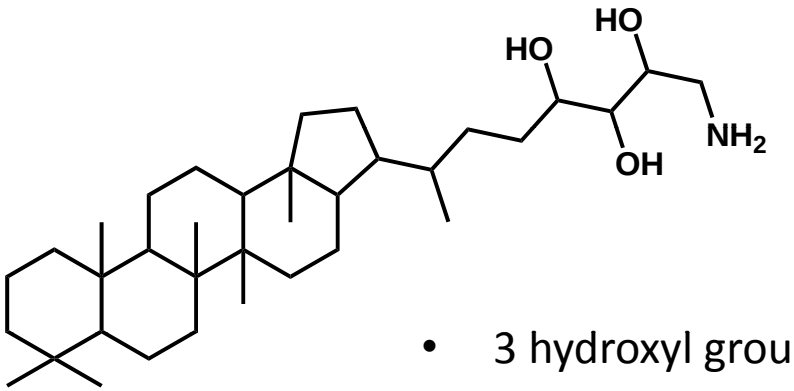
aminobacteriohopanetetrol



aminobacteriohopanepentol

Hopanoid Structures: C_{35} Hopanoids

Aminohopanoids

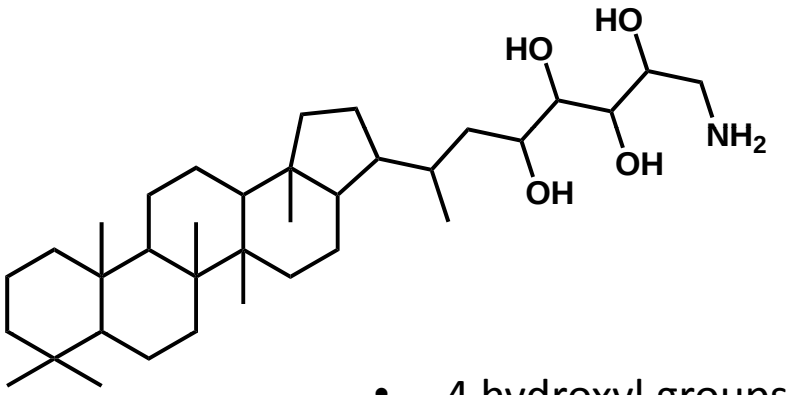


aminobacteriohopanetriol

- 3 hydroxyl groups with amino group at C-35
  - Type I/II methylotrophs
  - Nitrogen fixing bacteria
    - *Beijerinckia*
  - Purple nonsulfur bacteria
    - *Rhodopseudomonas*
  - Actinobacteria
    - *Streptomyces*

Hopanoid Structures: C_{35} Hopanoids

Aminohopanoids

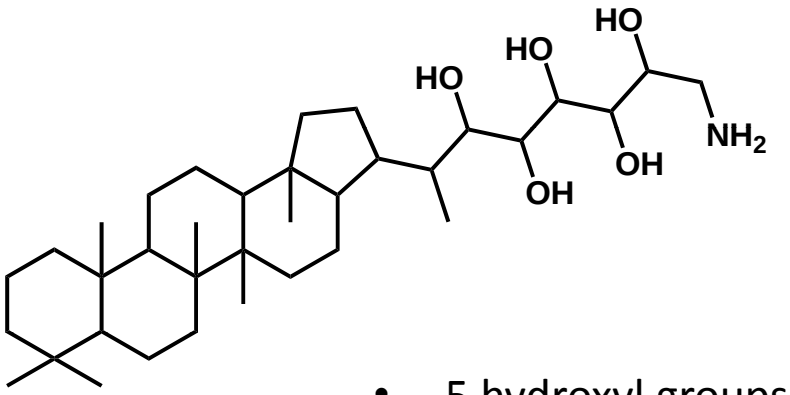


aminobacteriohopanetetrol

- 4 hydroxyl groups with amino group at C-35
- Type I, X, II methanotrophs
  - *Methylosinus*
    - Type II facultative
  - *Methylococcus*
    - Type X obligate
  - *Methylomonas*
    - Type I facultative

Hopanoid Structures: C_{35} Hopanoids

Aminohopanoids



aminobacteriohopanepentol

- 5 hydroxyl groups with amino group at C-35
- Type X methanotrophs
  - *Methylococcus*
    - Type X obligate
  - *Methylocaldum*
    - Type I/X obligate

Methylotroph vs Methanotroph

- Type I, X, II methylotrophs
  - **Methylotrophs**: aerobic bacteria use C-1 compounds as carbon and energy source
  - **Methanotrophs**: subset of methylotrophs; can use methane as carbon and energy source
  - obligate and/or facultative

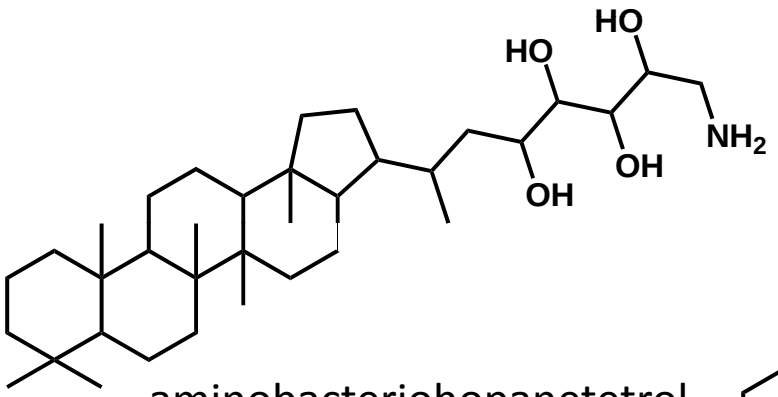
Type distinction made primarily on how they assimilate formaldehyde:

- Type II
  - Serine pathway
  - All α-Proteobacteria
- Type I
  - ribulose monophosphate (RuMP) pathway
  - β-Proteobacteria (no CH_4 oxidation)
  - γ-Proteobacteria (CH_4 oxidation)
- Type X
  - subset of Type I
  - RuMP + some serine pathway enzymes
  - Can grow at higher temperatures
  - Usually have a higher G + C content
  - γ-Proteobacteria

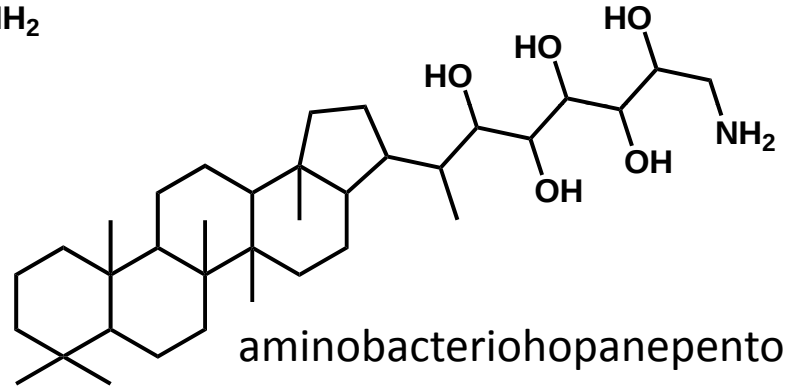
Hanson and Hanson, Microbiological Reviews, 1996: p. 439-471

Aminohopanoids as proxies for aerobic methanotrophy?

- Biogenic methane from methanogenesis is severely ^{13}C depleted; reflected in biomass from **methanotrophs**
- Aminohopanoid identification coupled with stable isotopic analysis good indicator of methanotrophy in given **modern** environment



aminobacteriohopanetetrol



aminobacteriohopanepentol

Unexpected occurrence of hopanoids at gas seeps in the Black Sea

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(returned to author for revision 20 August 2002)

Occurrence of unusual steroids and hopanoids derived from aerobic methanotrophs at an active marine mud volcano

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Available online 22 November 2007

Aerobic methanotrophy in the oxic-anoxic transition zone of the Black Sea water column

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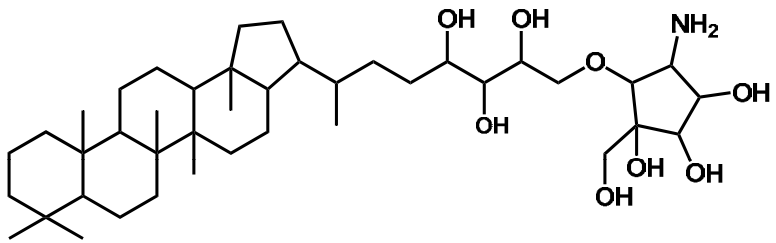
Received 8 February 2006; received in revised form 17 AAugust 2006; accepted 30 August 2006
Available online 30 October 2006

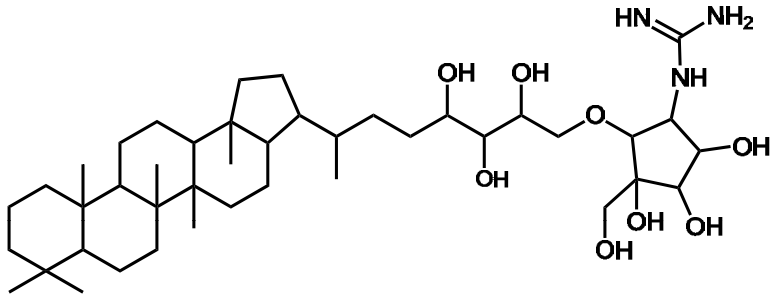
Hopanoid Structures: Composite Hopanoids

- Hydroxyl or amino group at C-35 linked to diverse complex moieties



bacteriohopanetetrol cyclitol ether

- Cyanobacteria
- α-proteobacteria
  - Acetic acid bacteria
  - Type II methylotrophs
- β-proteobacteria
  - *Burkholderia*
- γ-proteobacteria
  - *Azotobacter*

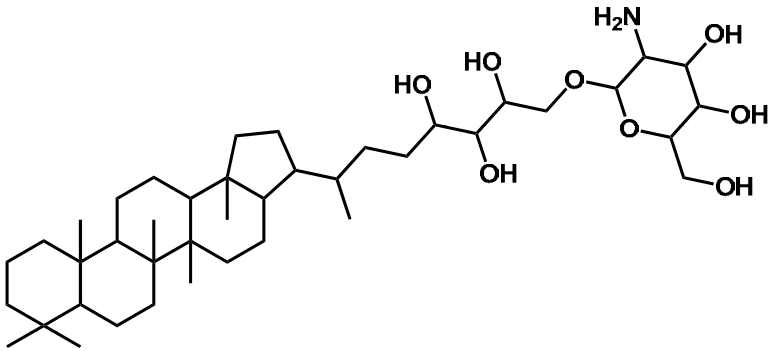


guanidine substituted bacteriohopanetetrol cyclitol ether

- *Methylobacterium organophilum*
  - α-proteobacteria
  - Type II methylotroph

Hopanoid Structures: Composite Hopanoids

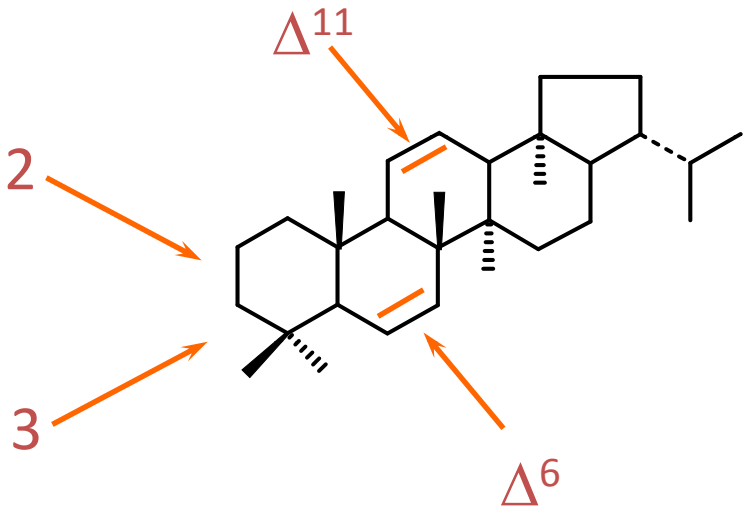
- Hydroxyl or amino group at C-35 linked to diverse complex moieties



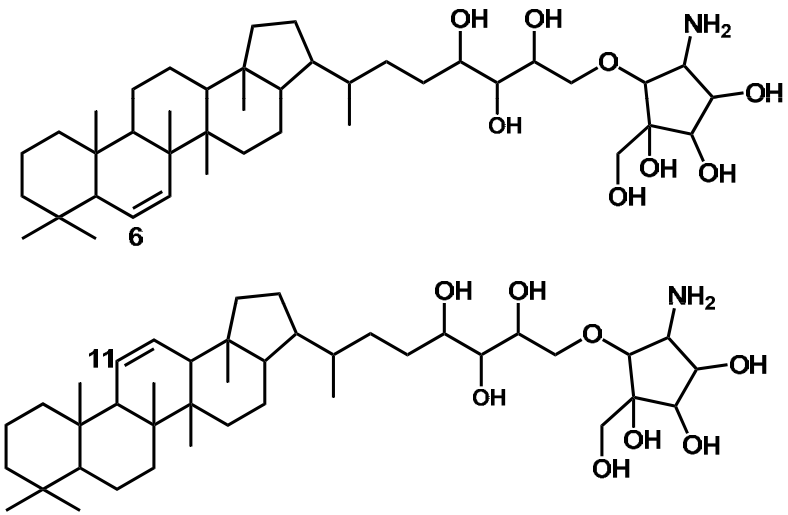
bacteriohopanetetrol glycoside

- Cyanobacteria
- α-proteobacteria
  - Acetic acid bacteria
  - Type II methylotrophs
  - *Zymomonas mobilis*
- β-proteobacteria
  - *Burkholderia*

Ring Variations



Hopanoid Structures: Unsaturated hopanoids

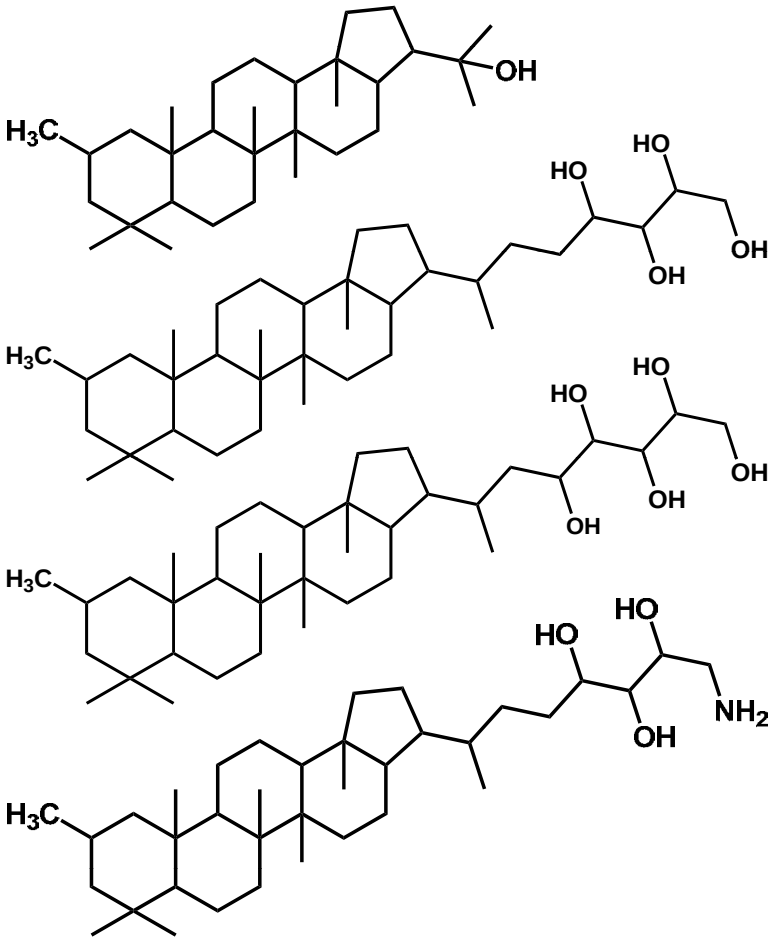


Unsaturated bacteriohopanetetrol cyclitol ethers

- Acetic acid bacteria most abundant producers
  - Also produce $\Delta^6\Delta^{11}$ double unsaturation
- Recently discovered in *Burkholderia*
- Other unsaturated BHPs found in small amounts in
  - Cyanobacteria
  - *Methylosinus*
  - *Methylocaldum*

Hopanoid Structures: Methylated hopanoids

- Methylation at C-2



- Cyanobacteria
  - Produce all of these structures
- α-Proteobacteria
  - Only methylate some of these structures
  - Varies between bacterial classes
  - *Rhodopseudomonas*
  - *Bradyrhizobium*
  - *Methylobacterium*
  - *Beijerinckia*

2-Methylhopanoids as biomarkers for cyanobacterial oxygenic photosynthesis

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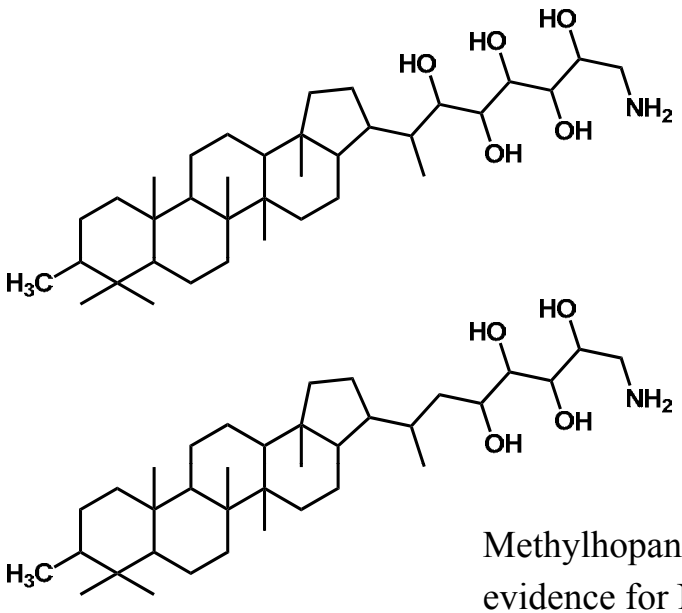
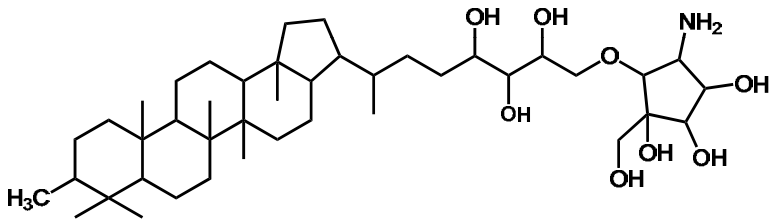
Hopanoid Structures: Methylated hopanoids

- Methylation at C-3



- Acetic acid bacteria
- **Type I and Type X methanotrophs**



Methylhopane biomarker hydrocarbons in Hamersley Province sediments provide evidence for Neoarchean aerobiosis

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Late Archean molecular fossils from the Transvaal Supergroup record the antiquity of microbial diversity and aerobiosis

Jacob R. Waldbauer[a], Laura S. Sherman[b,1], Dawn Y. Sumner[c], Roger E. Summons [b,*]

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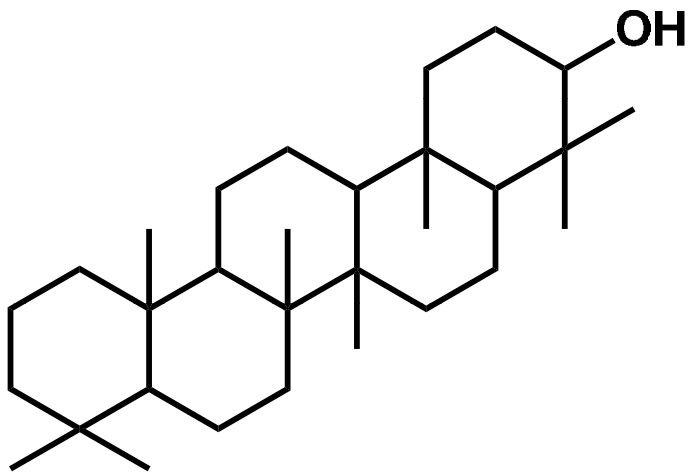
Isotopic Signature of 3-Methylhopanoids

Distribution and C-isotopic fractionation in hopanoids of *M. capsulatus* as function of growth stage

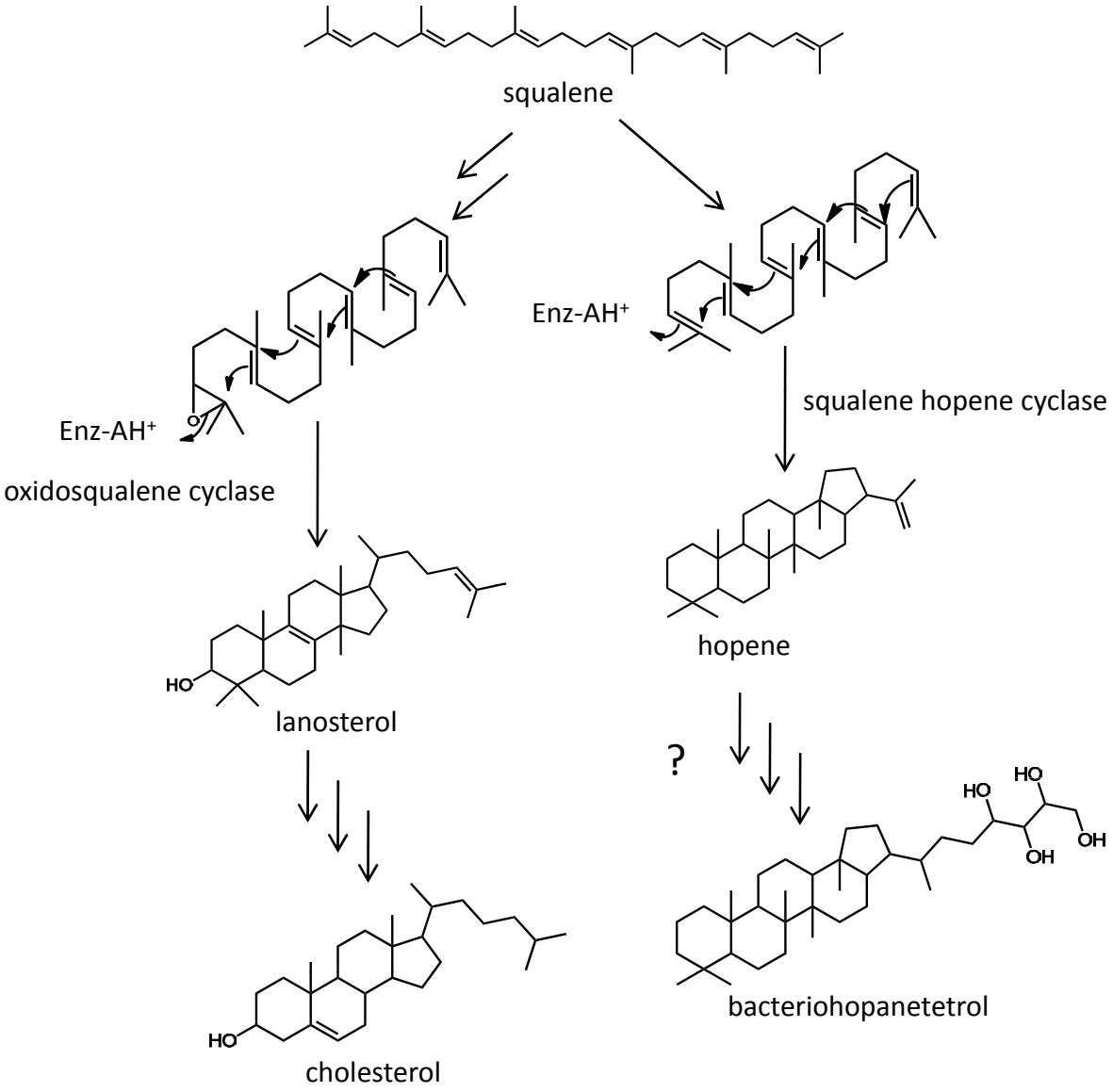
Summons et al., GCA, 1994

Tetrahymanol

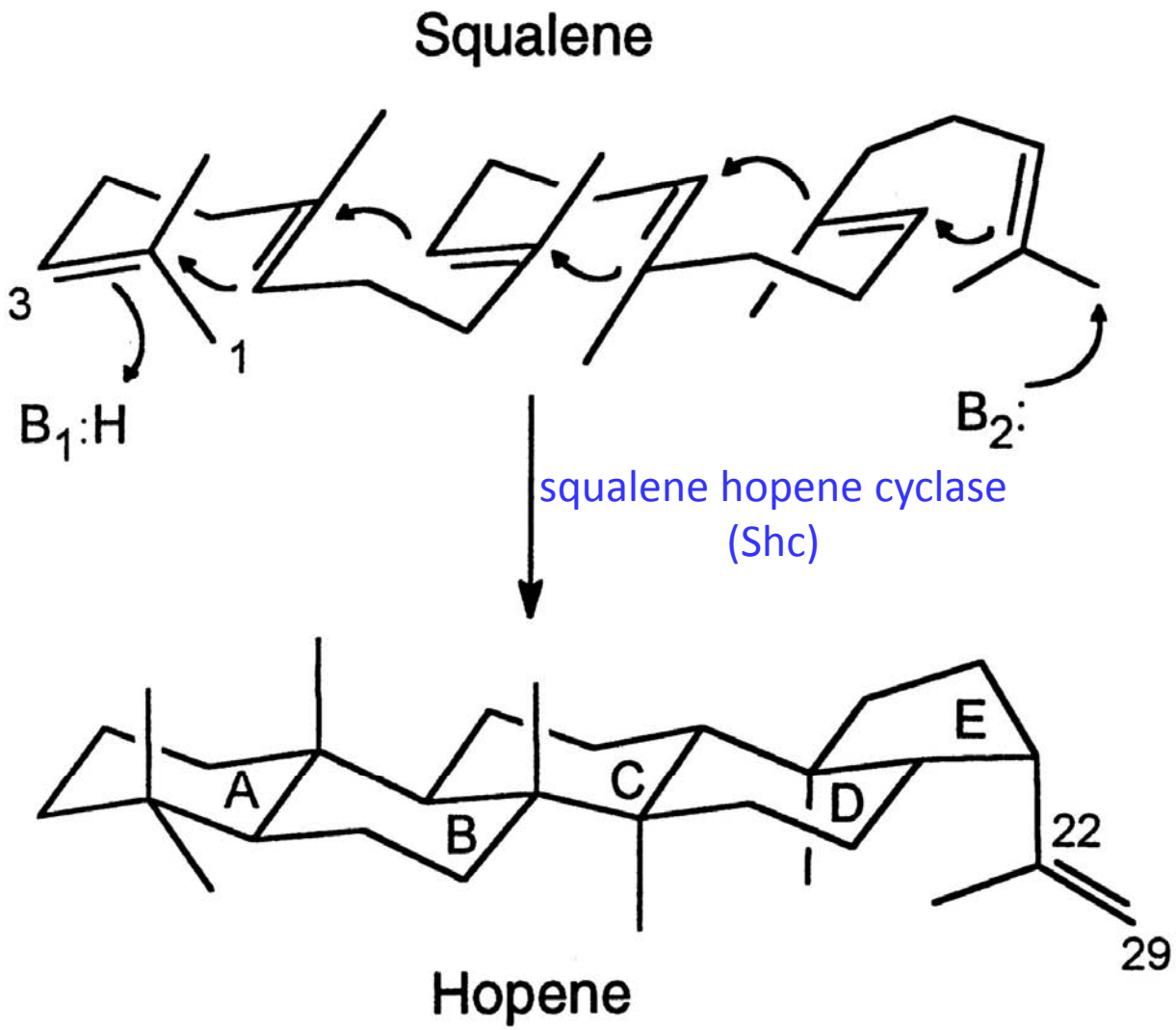


- Not a hopanoid
- Discovered in ciliated protozoan *Tetrahymena pyriformis*
- Also found in
  - Other ciliates
  - An anaerobic rumen fungus: *Piromonas communis*
  - A fern: *Oleandra wallicii*
  - Two α-Proteobacteria:
    - *Rhodopseudomonas palustris*
    - *Bradyrhizobium japonicum*
- Proposed to function as sterol

Hopanoid and Sterol Biosynthesis



Hopanoid Biosynthesis

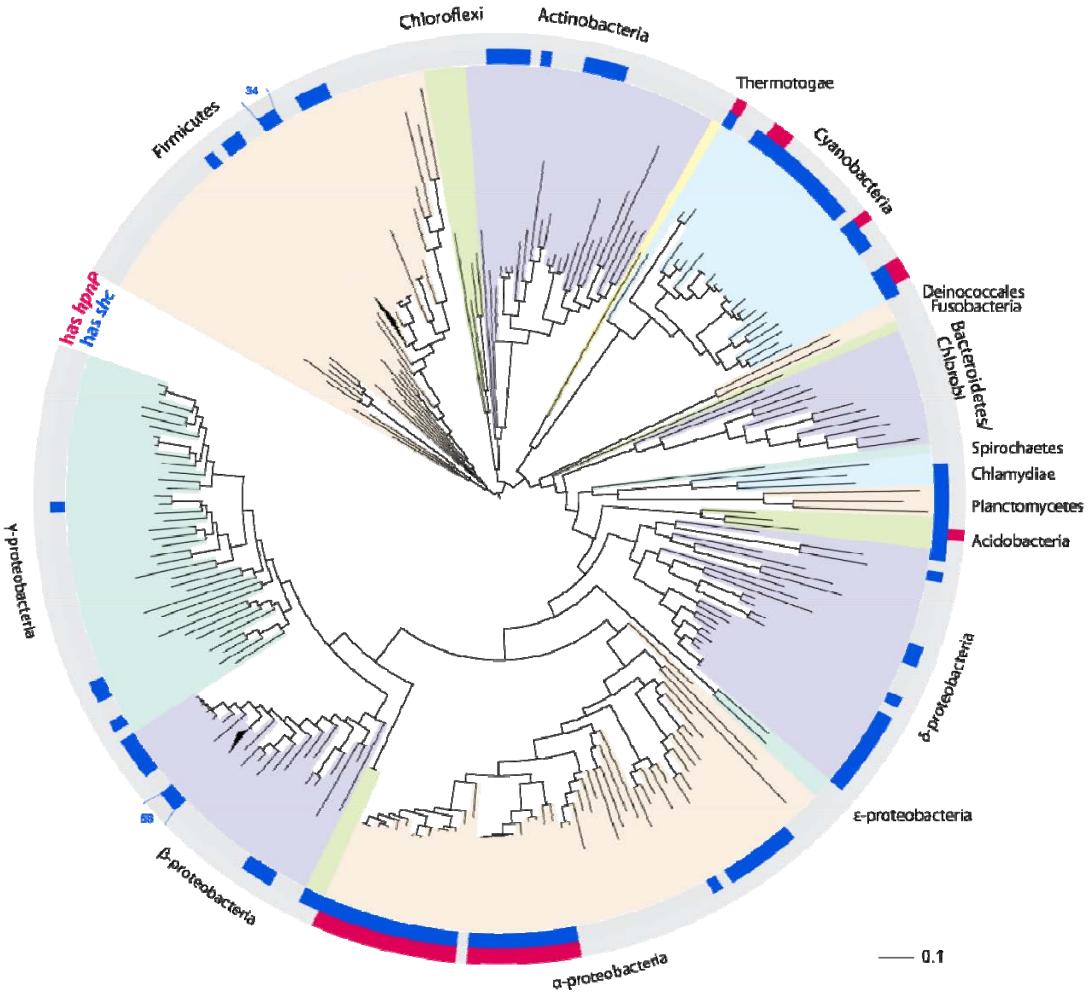


Squalene hopene cyclase

- Most well studied hopanoid biosynthesis protein
- Purified from 7 organisms
- Crystal structure from *A. acidocaldarius*
- Catalyzes one of the most complex enzymatic one-step reaction
- Shc can generate several minor hydrocarbons in vitro
  - Diplopterol (1) viewed as a side product
  - Tetrahymanol (2) catalyzed from squalene not by Shc but Stc
- Loose substrate specificity (Table 2)
  - More so than the oxidosqualene cyclase

Loose substrate specificity of Shc

Phylogenetic analysis of Shc



- Phylogenetic tree of bacterial species
- Blue bar = presence of Shc
- Before genome analysis of Shc, it was thought about 50% of bacteria made hopanoids
  - Lipid surveys of approx. 90 strains
- BLAST analysis of Shc shows that is only about 10%
- Shc found in
  - Firmicutes
  - Actinobacteria
  - Thermotogae
  - Cyanobacteria
  - Planctomycetes
  - Acidobactria
  - Proteobacteria
    - δ, α, β, γ
    - Not ε

Welander PV, et al. PNAS, 107: p. 8537-8542

Evolutionary link between Shc and Osc?

Hypotheses for the origin and early evolution of triterpenoid cyclases

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Hopanoid Biosynthesis: Beyond Shc

HpnH: Generating the Adenosyl hopane Intermediate

HpnH: Generating the Adenosyl hopane Intermediate

- The *hpnH* gene identified in *Methylobacterium extorquens*
- HpnH annotated as a radical SAM protein
- Transfers adenosine ribose to diploptene to from adenosyl hopane
  - Mechanism not experimentally verified
- BLAST analysis of HpnH shows that all Shc containing genomes contain this protein
- Leads to idea that all hopanoid producers can make functionalized hopanoids (i.e., C_{35} hopanoids)
- Questions the use of adenosyl hopane as a biomarker for soil bacteria – just an intermediate produced by all hopanoid producing bacteria

HpnG: Removal of adenine

HpnG: Removal of adenine

- The *hpnG* gene also first identified in *Methylobacterium extorquens*
- HpnG annotated as a nucleoside hydrolase
- Removes adenine nucleotide to form ribosyl hopane
  - Mechanism not experimentally verified
- BLAST analysis of HpnG inconclusive
  - High similarity to other nucleosides not involved in hopanoid biosynthesis
  - Presumably all Shc and HpnH containing genomes would have this protein as well

Conversion of ribosyl hopane to formyl hopane

- Equilibrium between open and closed form of ribose
- Hypothesis is that no enzyme needed to catalyze this step

HpnO: Addition of amino group

HpnO: Addition of amino group

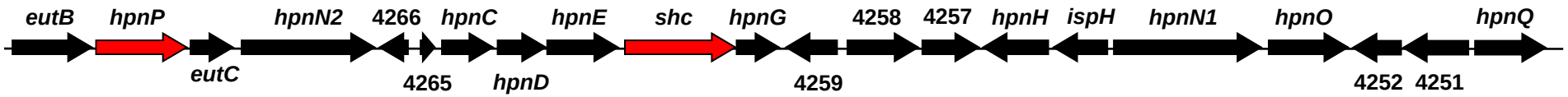
- The *hpnO* gene identified in *Rhodopseudomonas palustris*
- HpnO annotated as an aminotransferase
- Presumably adds amine group to formyl hopane
  - Mechanism not experimentally verified
- BLAST analysis of HpnO shows limited number of aminohopanoid producers
  - Confirms presence in strains known to make aminohoapnoids
  - Demonstrates potentially new aminohopanoids producers

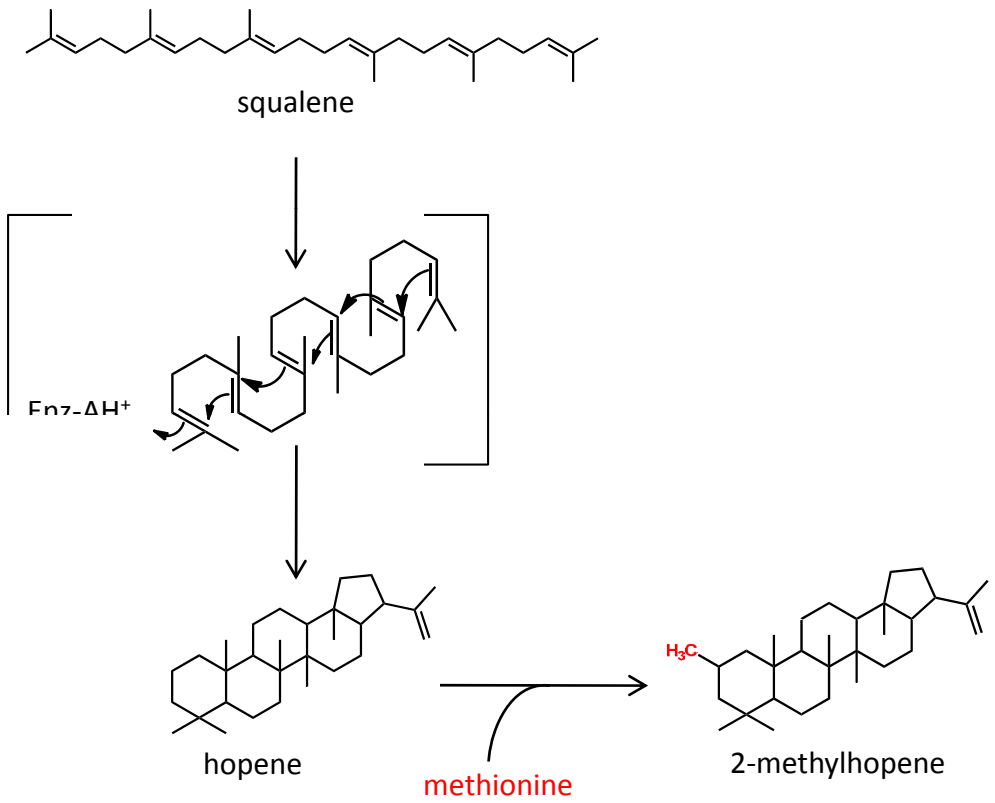
HpnO: Phylogeny

Unknown biosynthetic steps

- Gene specifically needed to produce bacteriohopanetetrol not discovered yet
- No composite hopanoid biosynthesis genes known

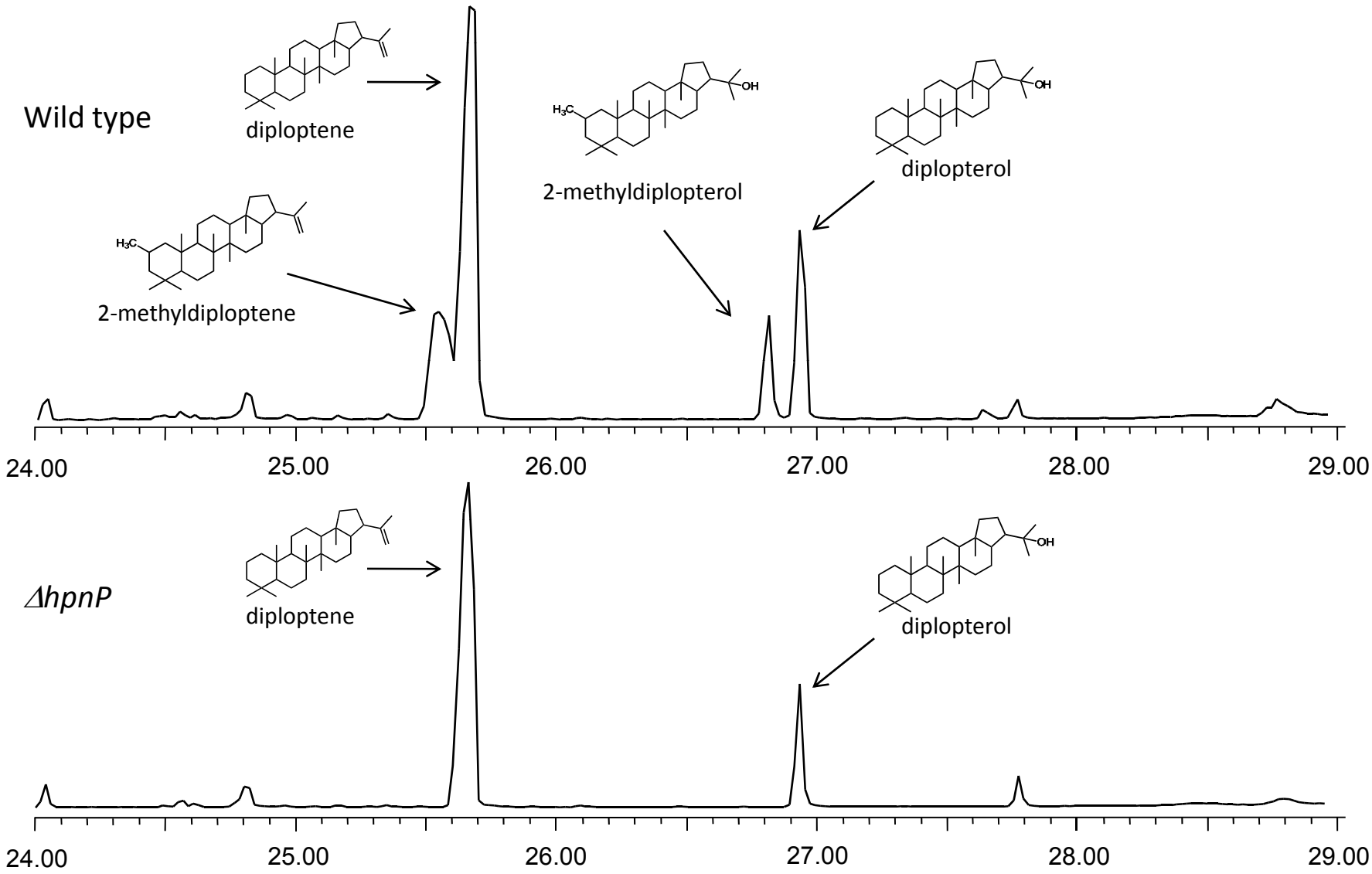
Methylation at C-2: *R. palustris* Experiments





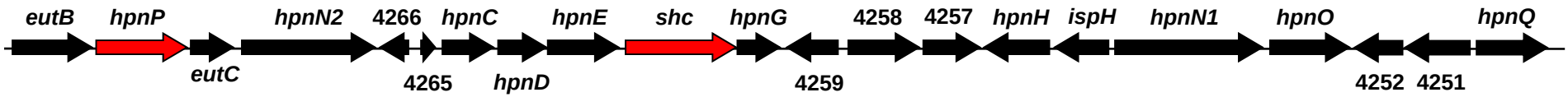
Welander PV, et al. PNAS, 107: p. 8537-8542

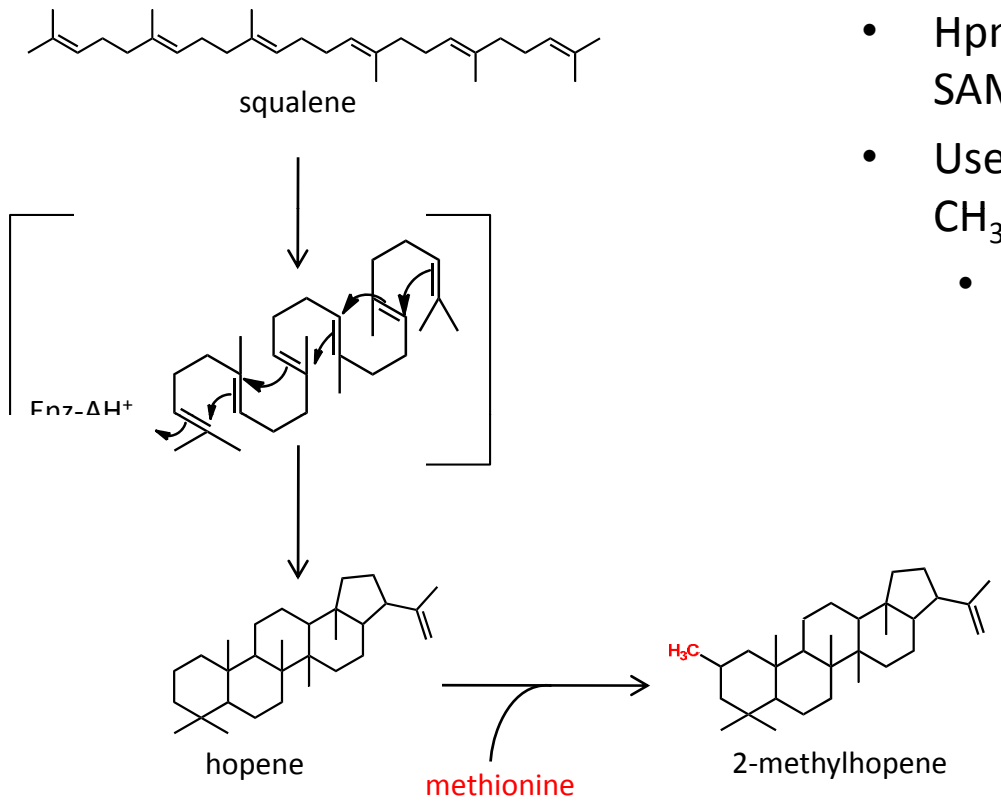
hpnP encodes for the C-2 methylase



Welander PV, et al. PNAS, 107: p. 8537-8542

HpnP: Methylation at C-2

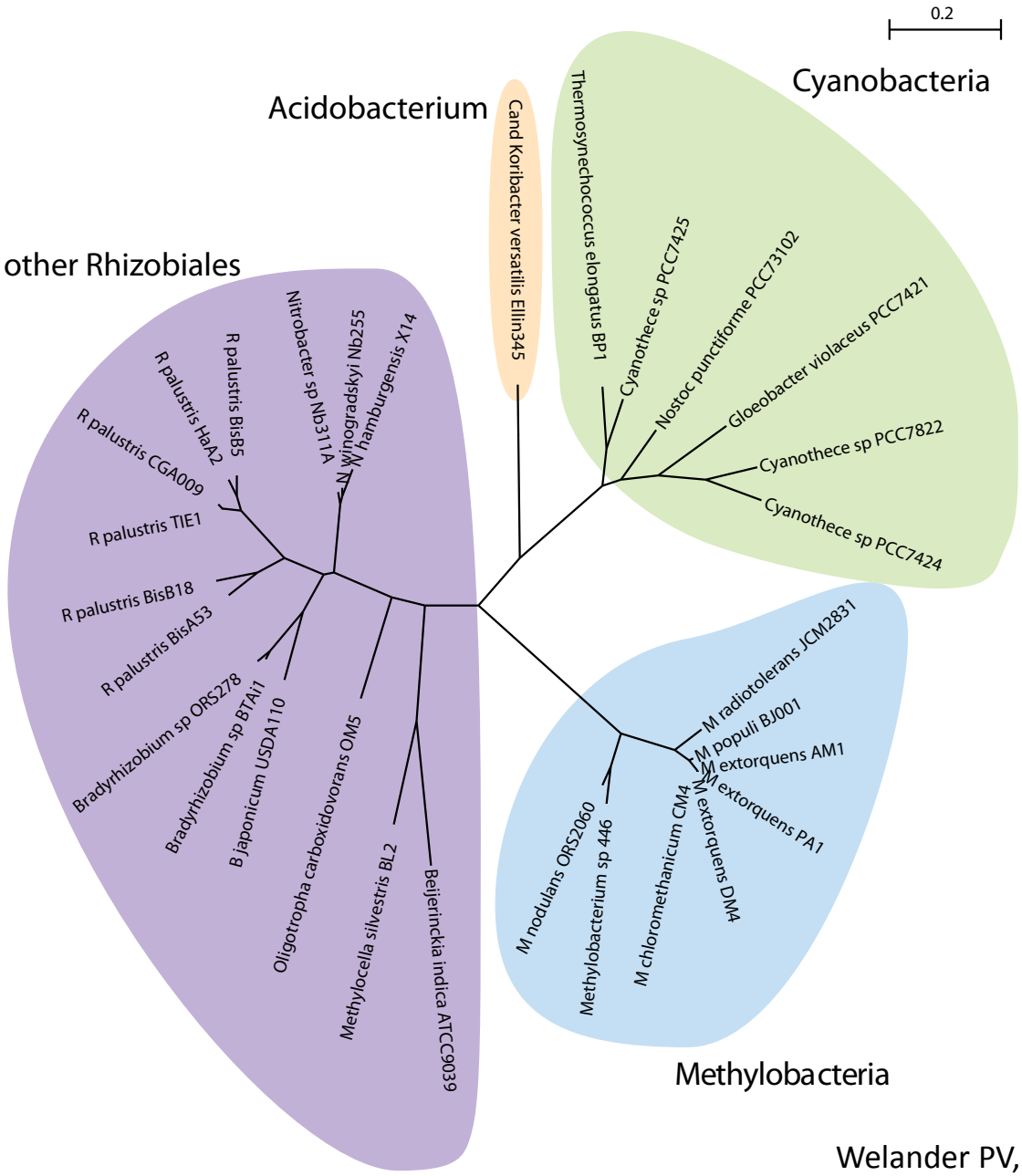




- HpnP annotated as a B-12 binding radical SAM
- Uses S-adenosylmethionine radical to add CH_3 to C-2
  - Mechanism not experimentally verified

Welander PV, et al. PNAS, 107: p. 8537-8542

Phylogenetic analysis of the HpnP methylase



Welander PV, et al. PNAS, 107: p. 8537-8542

Topic 4: Are 2-methylhopanes good biomarkers for cyanobacteria and/or O_2-photosynthesis?

PNAS

Identification of a methylase required for 2-methylhopanoid production and implications for the interpretation of sedimentary hopanes

Paula V. Welander[a,1], Maureen L. Coleman[a,1], Alex L. Sessions[b], Roger E. Summons[c], and Dianne K. Newman[a,c,d,2]

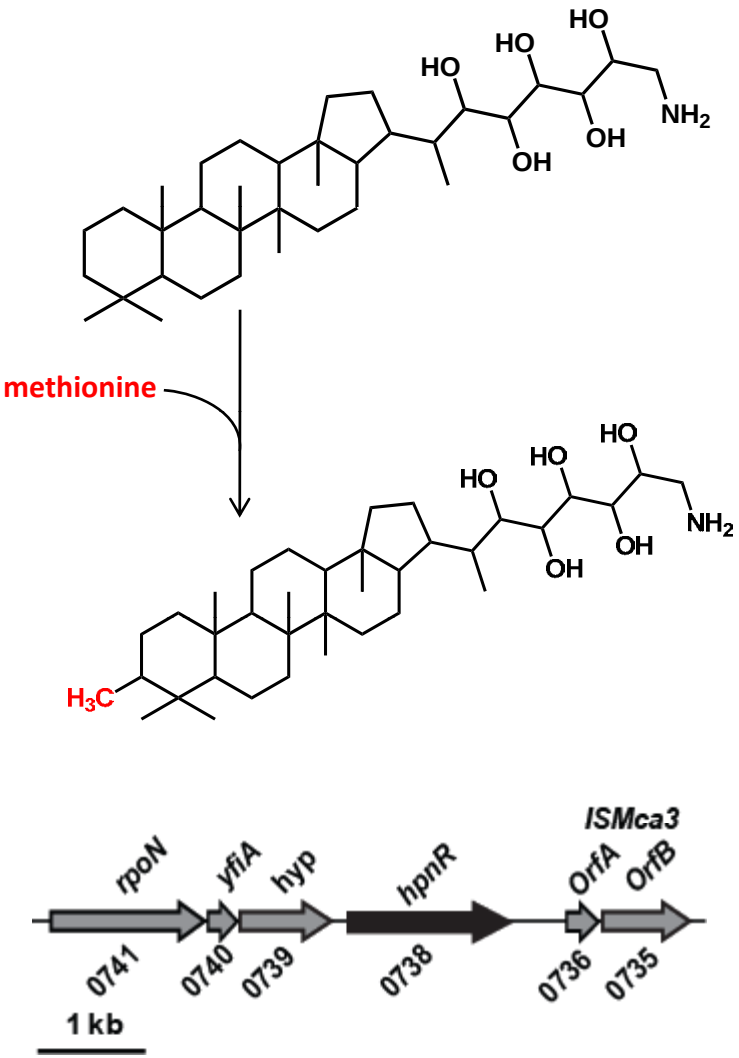
[a]Department of Biology, Massachusetts Institute of Technology, 77 Massachusetts Avenue, 68-380, Cambridge, MA 02139; [b]Division of Geological and Planetary Sciences, California Institute of Technology, Pasadena, MC100-23, 1200 East California Boulevard, Pasadena, CA 91125; [c]Department of Earth, Atmospheric and Planetary Science, Massachusetts Institute of Technology, 77 Massachusetts Avenue, E25-633, Cambridge, MA 02139; and [d]Howard Hughes Medical Institute, 77 Massachusetts Avenue, 68-171, Cambridge, MA 02139

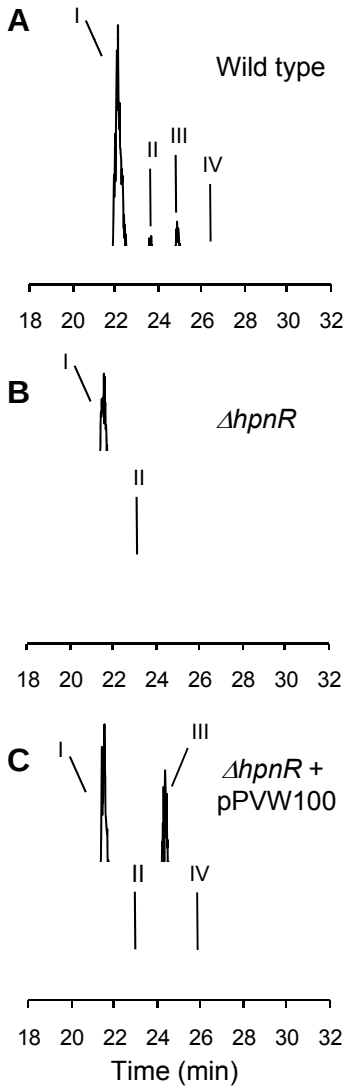
Edited by John M. Hayes, Woods Hole Oceanographic Institution, Berkeley, CA, and approved January 27, 2010 (received for review November 10, 2009)

The rise of atmospheric oxygen has driven environmental change and biological evolution throughout much of Earth's history and

Sedimentary hopanes are biomarkers whose distribution has been studied extensively. These hydrocarbon molecules are re-

Methylation at C-3: *M. capsulatus* experiments

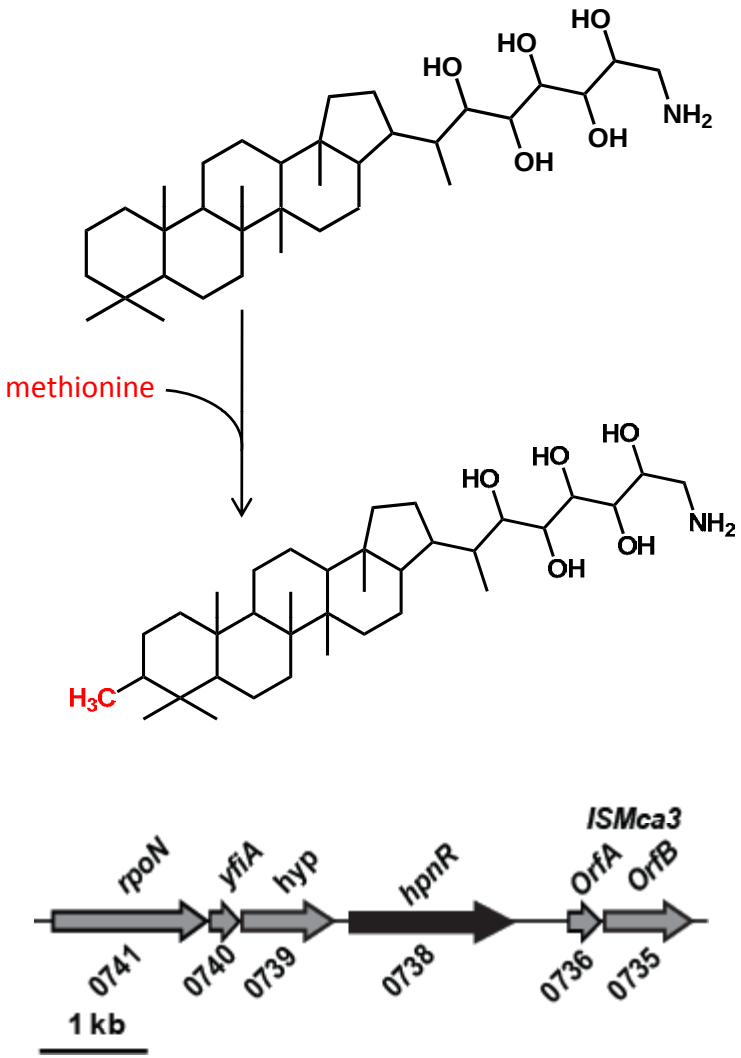




I and II: desmethyl aminohopanoids

III and IV: C-3 methylated aminohopanoids

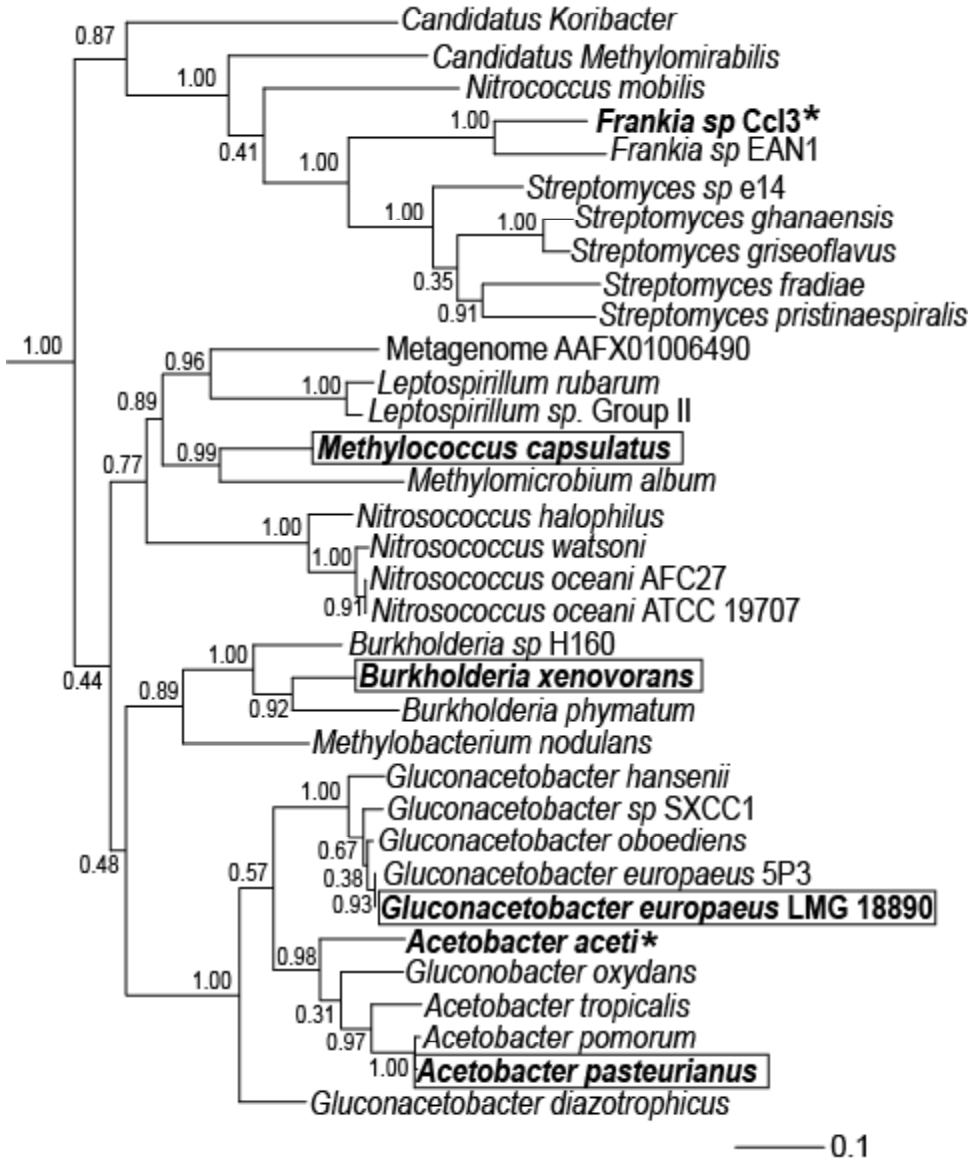
Methylation at C-3



- HpnR also annotated as a B-12 binding radical SAM
- Uses S-adenosylmethionine radical to add CH_3 to C-3
  - Mechanism not experimentally verified
- Very low sequence identity to HpnP
  - Although share the B-12 binding and radical SAM motifs
  - Raises evolutionary questions about the similarity of these two methylations

Topic 5: What is the C-2 and C-3 methylation mechanism proposed earlier in the literature? How is it different from the use of radical SAM chemistry?

Phylogenetic analysis of the HpnR methylase



- Very few bacteria with HpnR in their genomes have been tested for hopanoid production
- Two have been tested (*) and no 3-methylhopanoids reported.
- If HpnR is correlated to 3-methylhopanoid production in other organisms:
  - Expands diversity of 3-methylhoapnoid producers beyond methanotrophs and acetic acid bacteria
  - Actionbacteria
  - α, γ, and β-Proteobacteria
  - Nitrospirae
  - Acidobacteria
  - Unclassified organism

Functional Role of Hopanoids?

Proc. Natl. Acad. Sci. USA
Vol. 76, No. 2, pp. 847–851, February 1979
Evolution

Molecular evolution of biomembranes: Structural equivalents and phylogenetic precursors of sterols

(triterpenes/tetraterpenes/prokaryotes/pre-aerobic evolution)

MICHEL ROHMER*†, PIERRETTE BOUVIER*†, AND GUY OURISSON*‡§

Rohmer & Ourisson, 1976

Rohmer et al., 1979

Kannenberg & Poralla, 1980

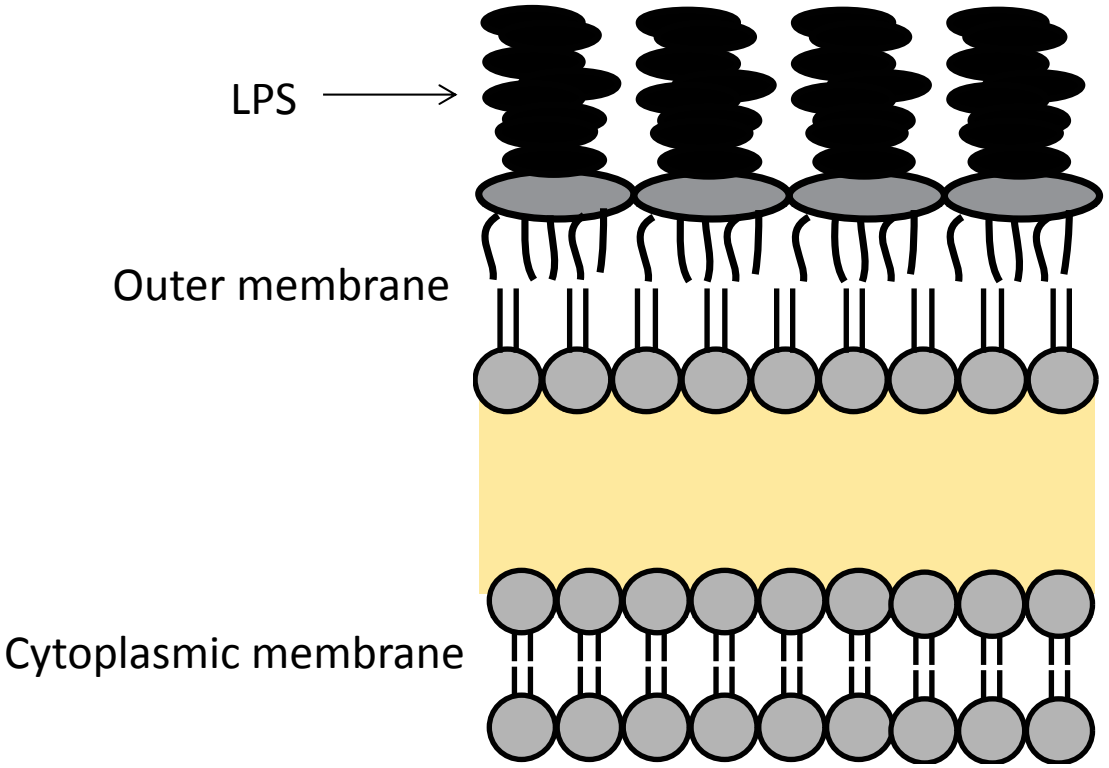
Many lines of evidence show an association of hopanoids with cellular membranes

But majority were in vitro studies. What about in vivo studies?

Hopanoid localization in *Nostoc punctiforme*

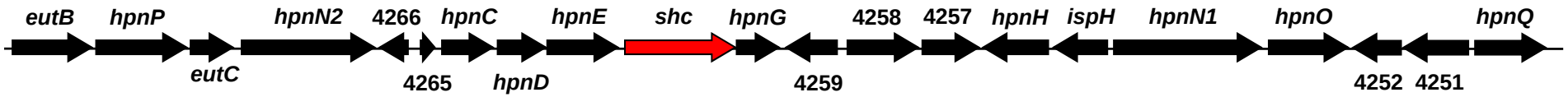
- Hopanoids localize to the outer membrane; none to the cytoplasmic
  - Also observed in *M. capsulatus*
- Akinetes are resting state structures that do not do oxygenic photosynthesis
- Functional role not involved in oxygenic photosynthesis

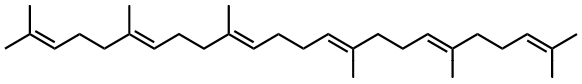
Outer membrane versus cytoplasmic membrane



- Gram-negative bacteria have outer membrane in addition to cytoplasmic membrane
- Studies are finding that hopanoids localize to this membrane
- Hopanoid membrane studies were all done in cytoplasmic membrane models
  - Do they apply in vivo?
  - Currently no in vitro system available to model the outer membrane

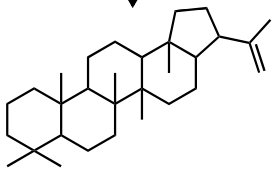
Other in vivo studies: *R. palustris shc* mutant





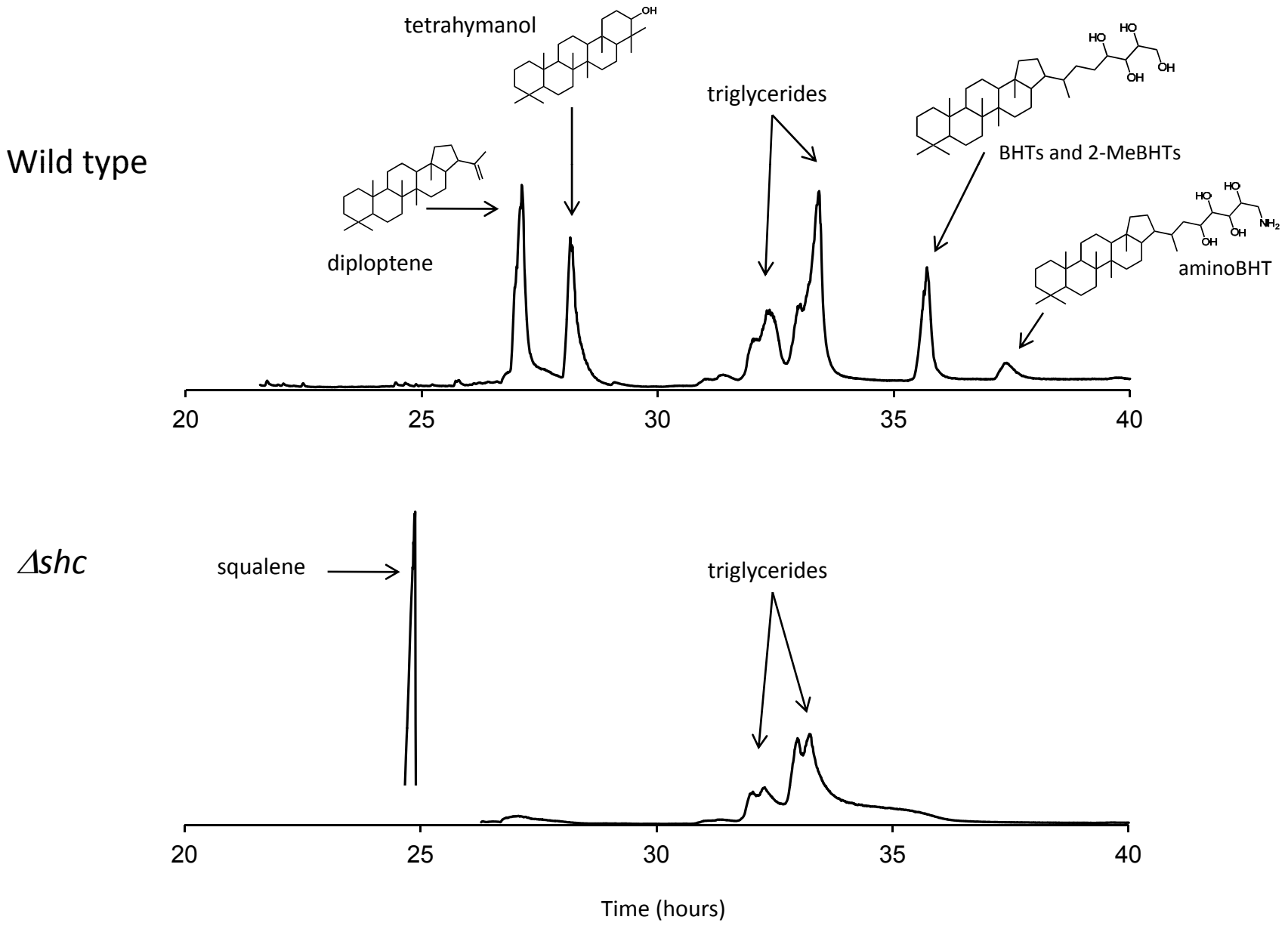
squalene

squalene hopene cyclase (Shc)

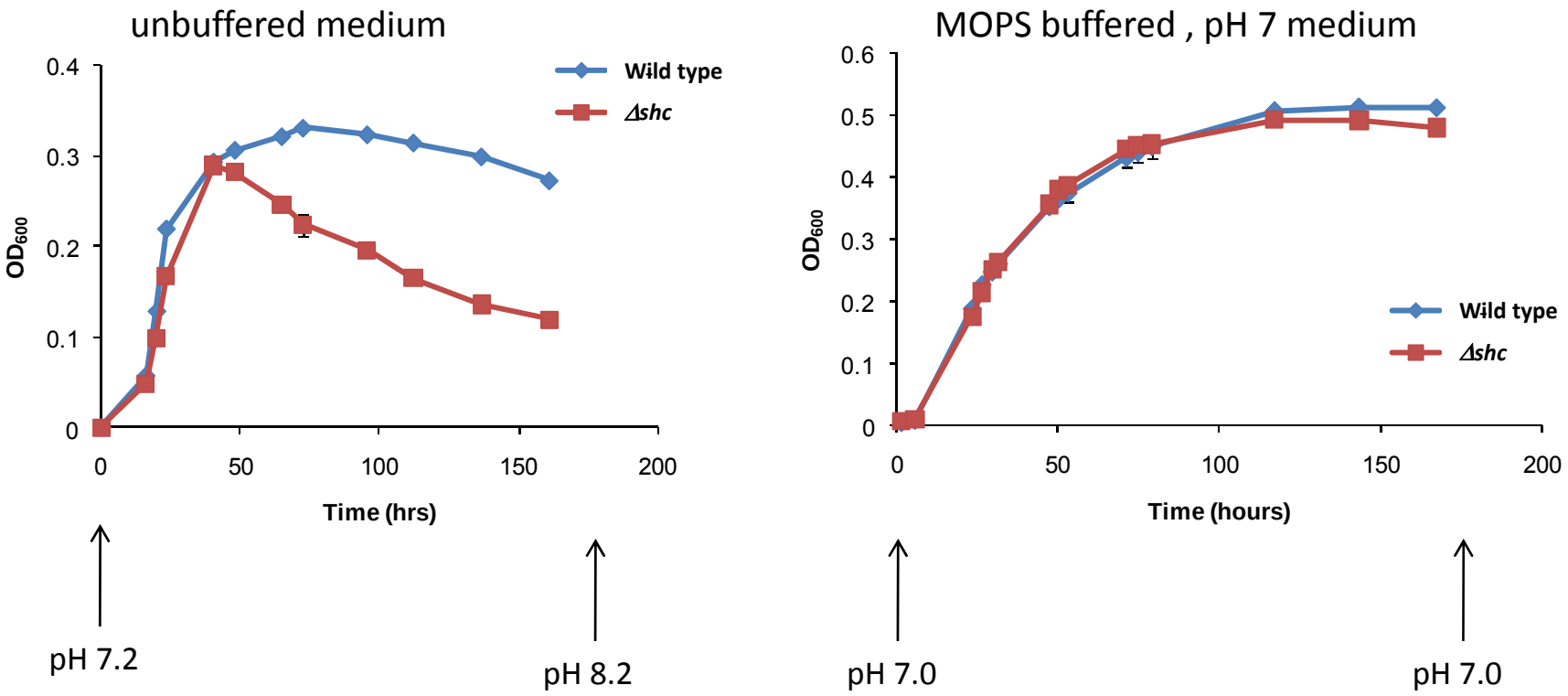


hopene

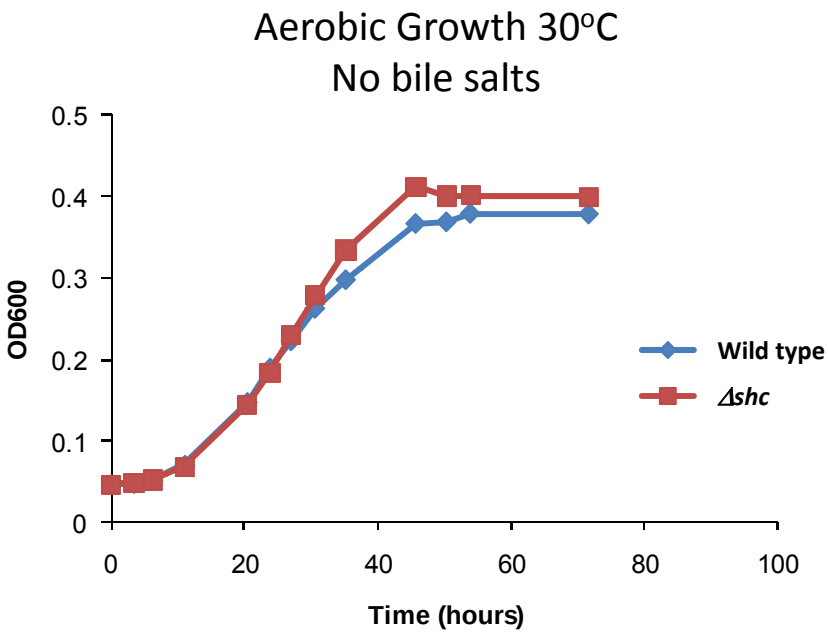
Δshc strain no longer produces hopanoids

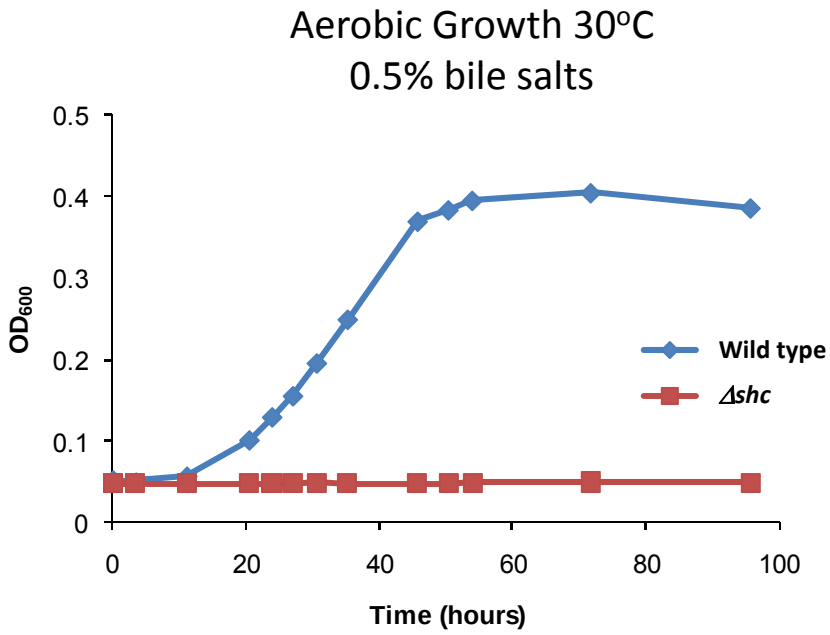


Sensitivity of hopanoid mutant to pH

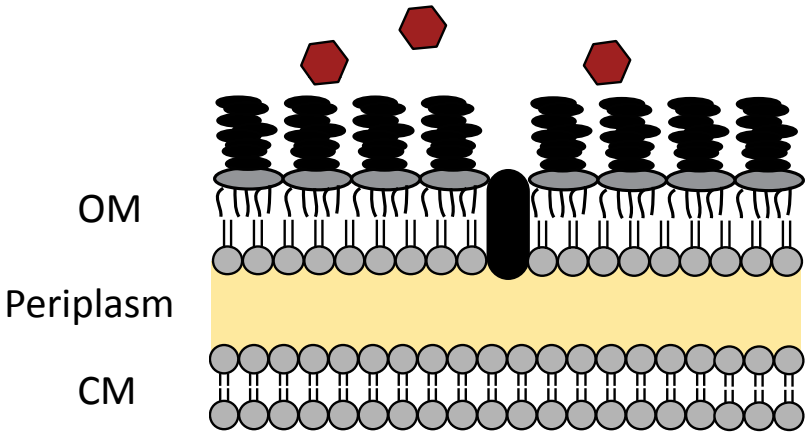


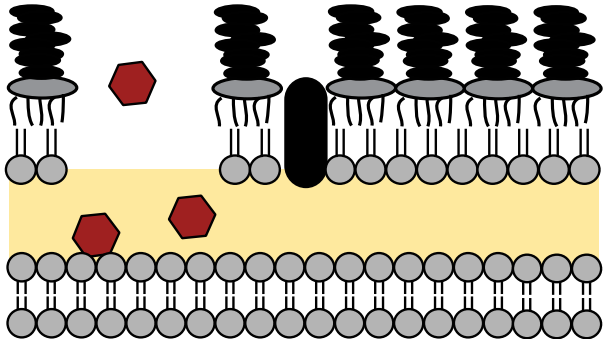
Membrane integrity of the *Δshc* mutant is compromised

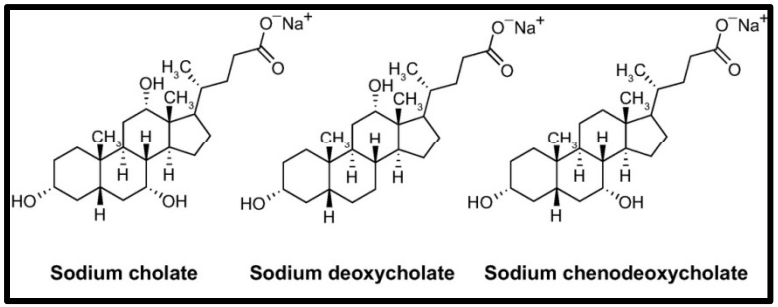




Sensitivity to bile salts is indicative of a permeable OM

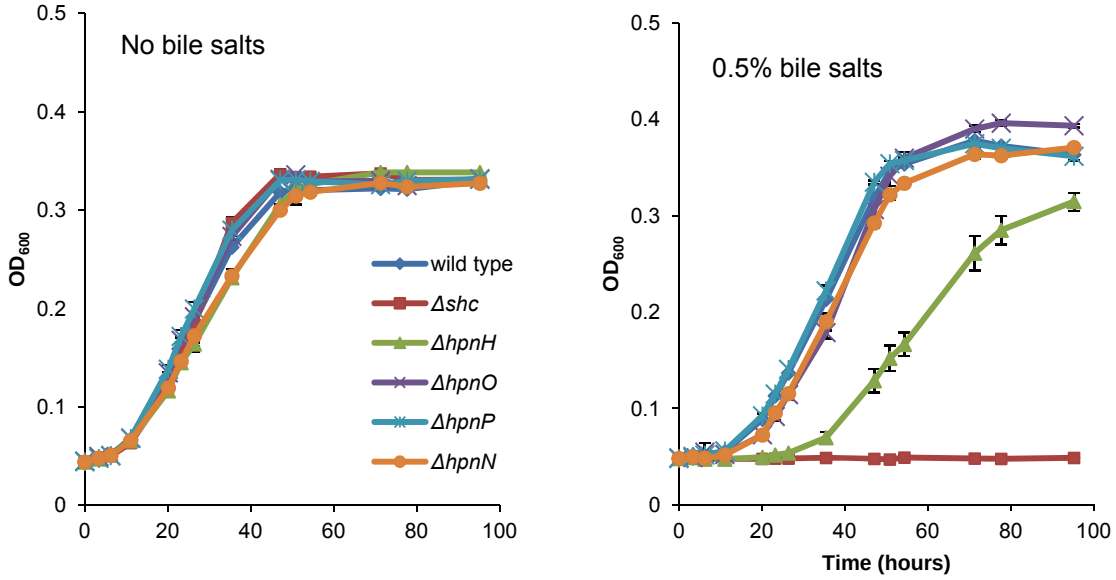






Membrane integrity of other hopanoids mutants is NOT compromised

Does this indicate a novel function for amino and methylated hopanoids?



ΔhpnH: Only C_{30} hopanoids produced

ΔhpnO: No amino hopanoid production

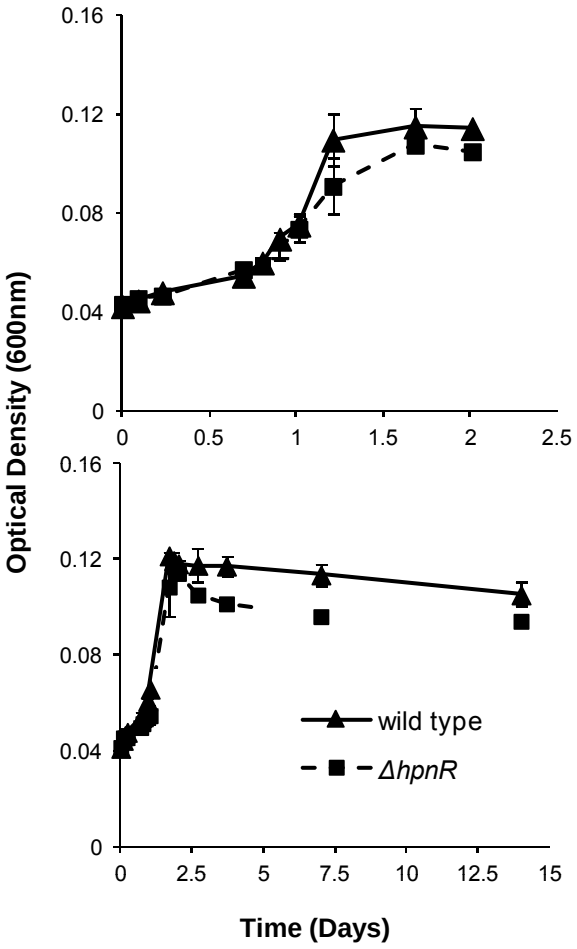
ΔhpnP: No methylated hopanoid production

ΔhpnN: No hopanoids in outer membrane

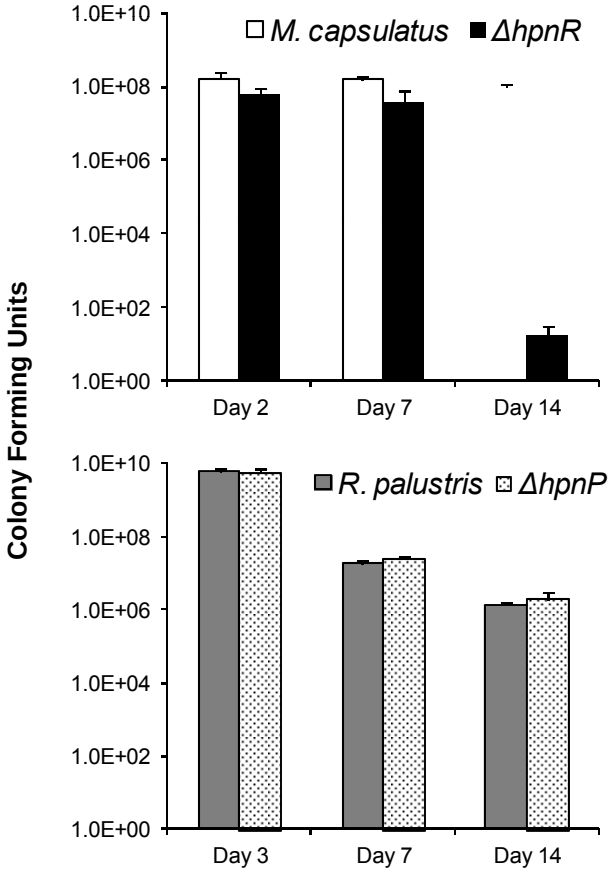
Functional role for 3-methylhopanoids in stationary phase survival?

Growth experiments with *M. capsulatus hpnR* mutant

Growth over time at 37°C

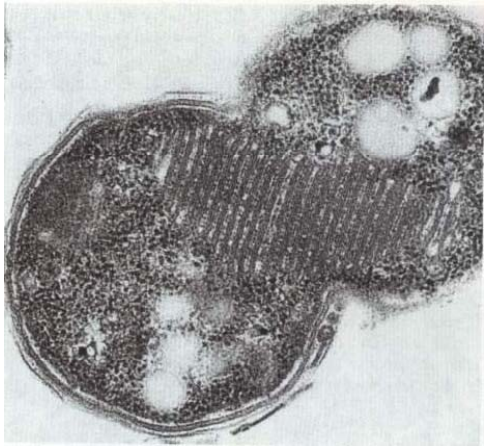


Cell survival assay

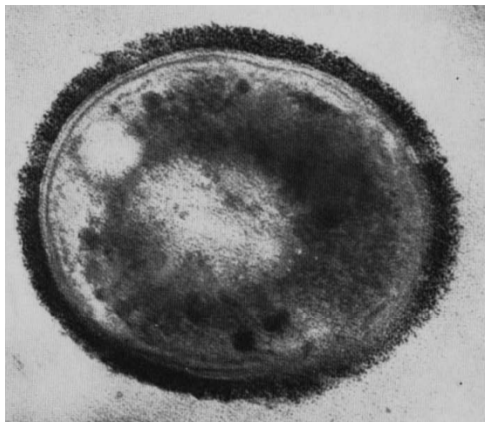


Functional role for 3-methylhopanoids in stationary phase survival?

Growth experiments with *M. capsulatus hpnR* mutant

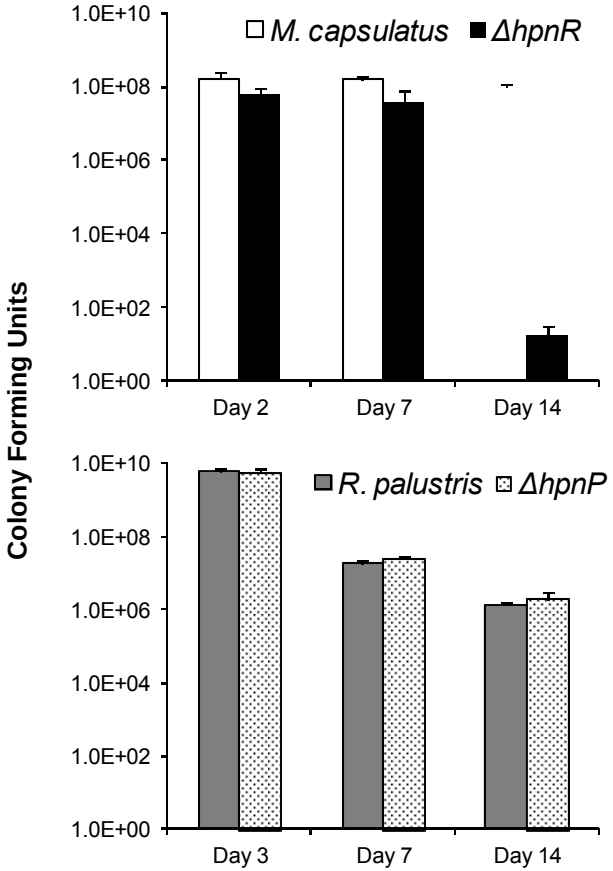


3-methyls?

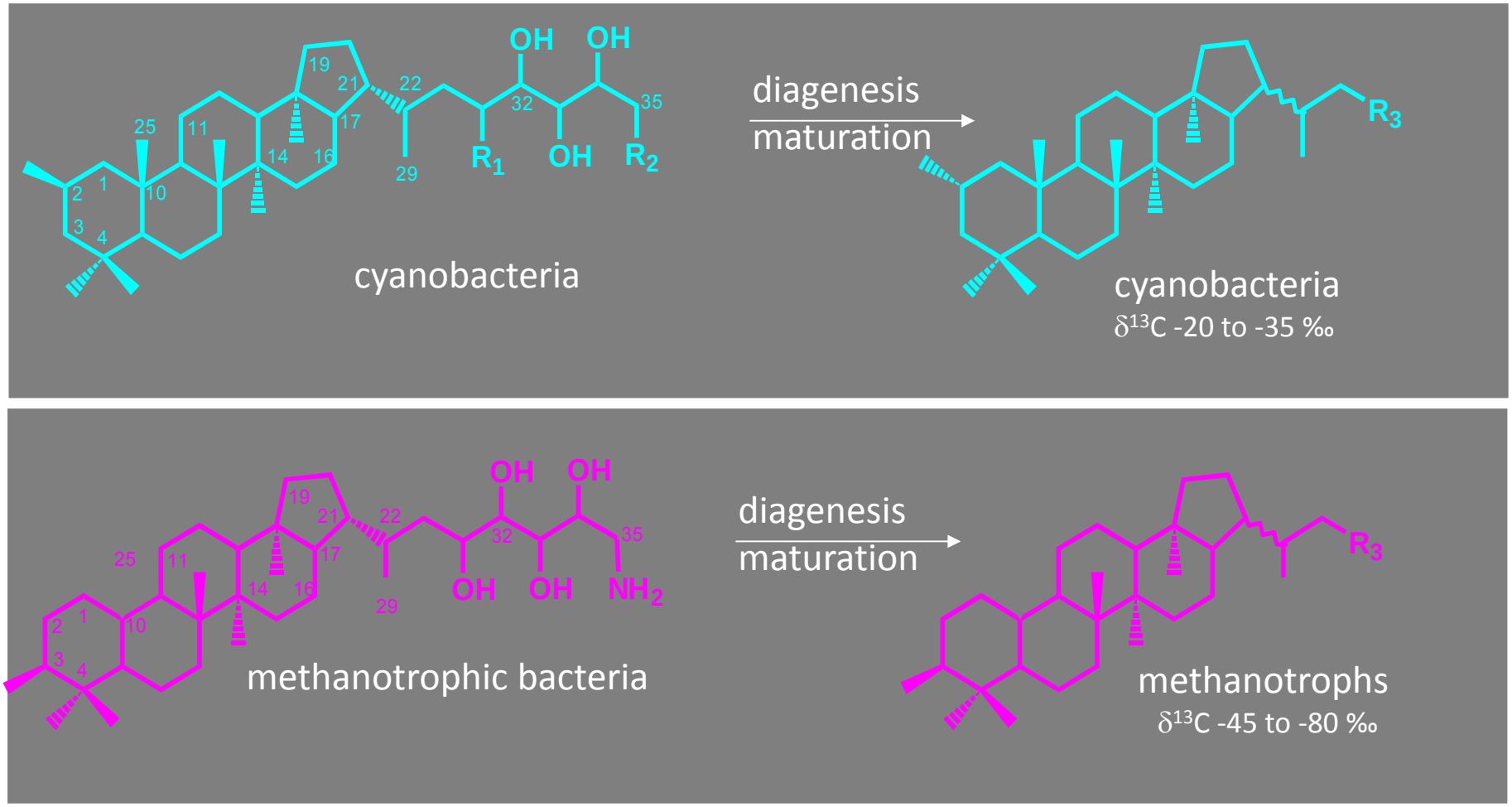


Methylococcus cyst

Cell survival assay



Diagnostic Bacteriohopanes?



- Will the physiological and biochemical data reveal that certain hopanes are better proxies for microbial processes rather than a specific bacterial group?

12.158 Molecular Biogeochemistry

Fall 2011