Cometabolic Biodegradation of 1,4-Dioxane by Psychrophilic Propanotrophs Enriched with a New Cluster of Group-6 Soluble Di-iron Monooxygenases

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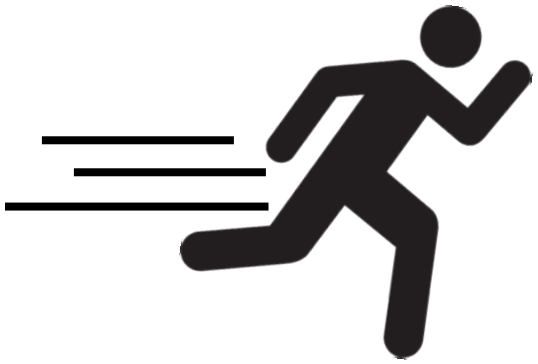
June 6, 2024

In Situ Bioremediation of 1,4-Dioxane

- Dioxane forms large dilute plumes

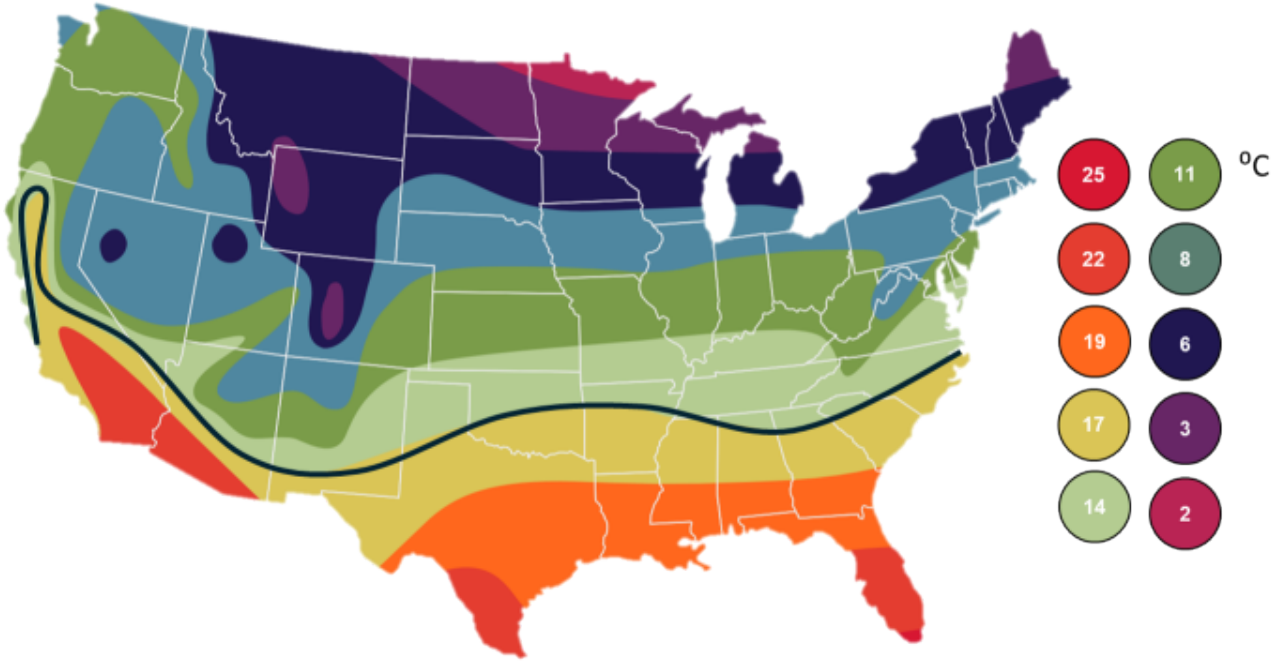


- Dioxane can migrate far and quickly due to chemical characteristics

- Low 10^{-6} lifetime cancer risk <0.35 µg/L

Why look for new bacteria?

- Most bacteria isolated to date have been isolated at mesophilic temperatures >20 ^{o}C
- Ground water temperature in the USA can range from 2 - 25 ^{o}C,
- Temperatures below the ideal growth temperature correlate with lower growth of bacteria and lower catalytic efficiency of enzymes



(modified from: https://www.powerequipmentdirect.com/manuals/8aac12f937dcfe115b55b6e2cc1fd03d.pdf)

Goals:

Enrichment

- Aquifer relevant temperature: 14 ^{o}C
- Dioxane removal
- Propane as the primary carbon source

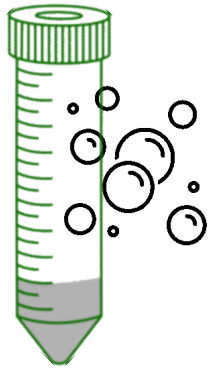
CAH Removal

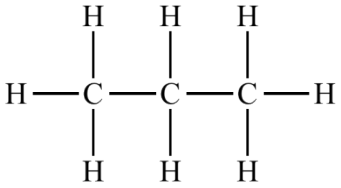
- Cooccurring with dioxane
- Inhibit dioxane biodegradation

SDIMOs

- Which ones are there?
- How many are there?
- What is their role?

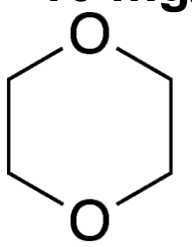
Initial Enrichment

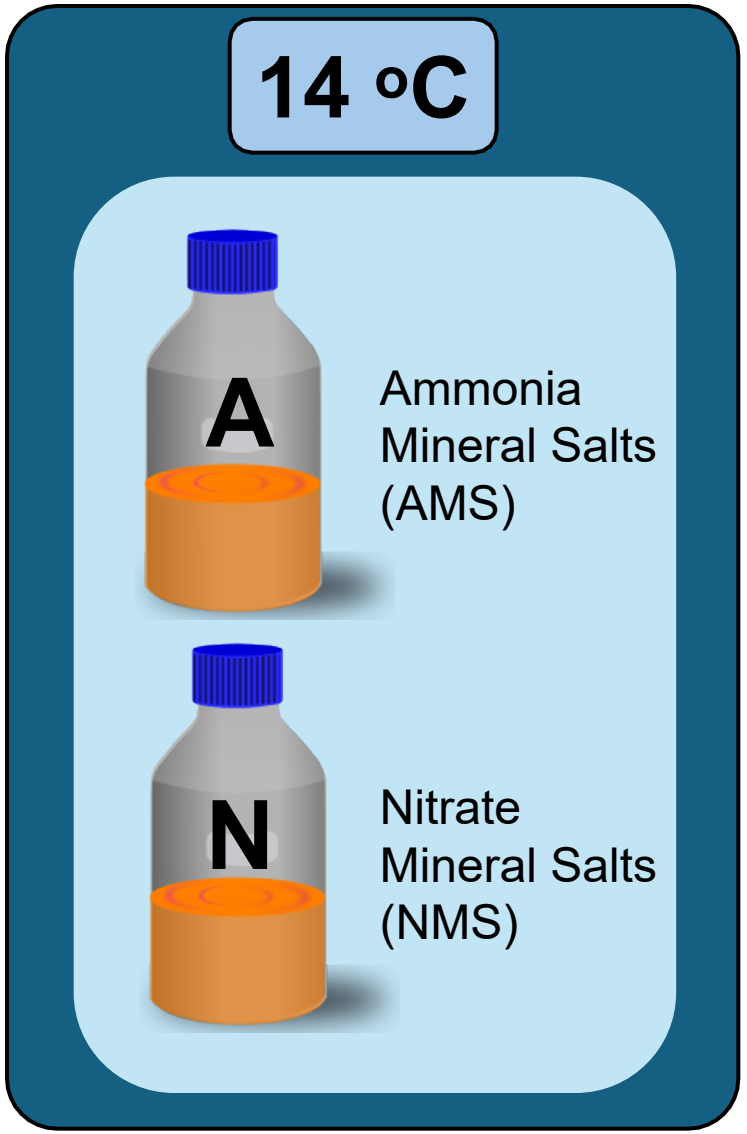




+20 mg/L

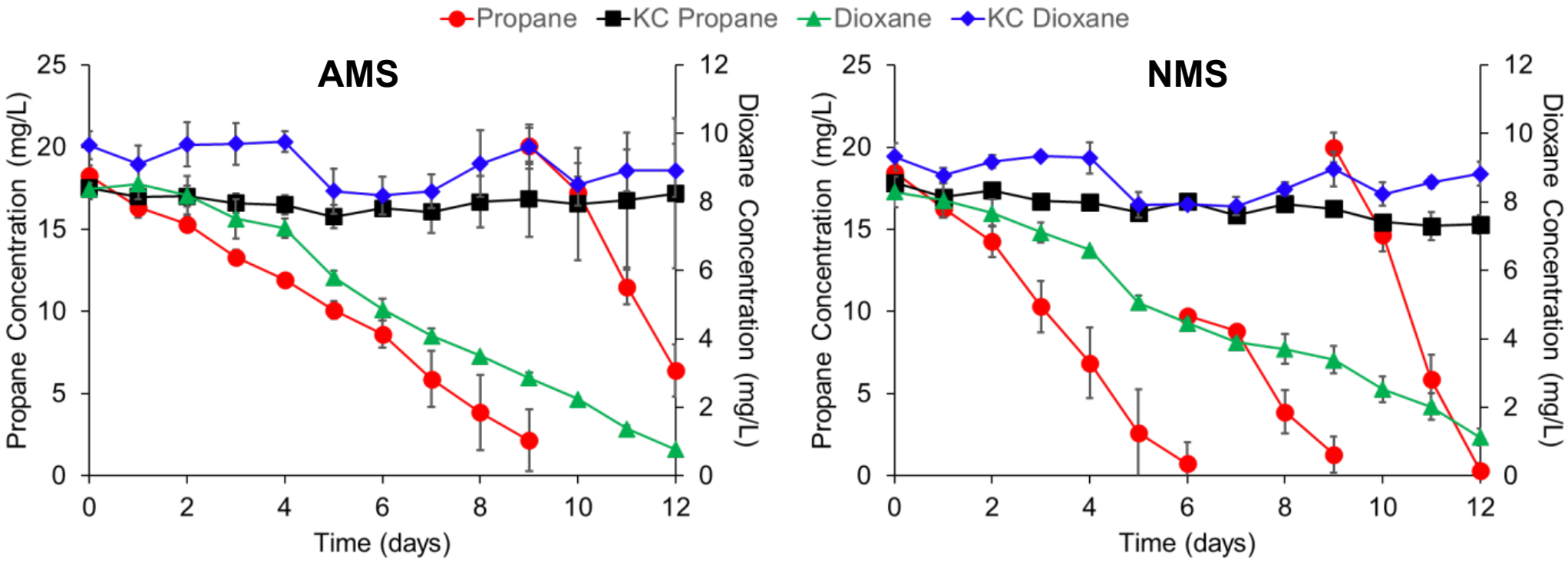
+10 mg/L





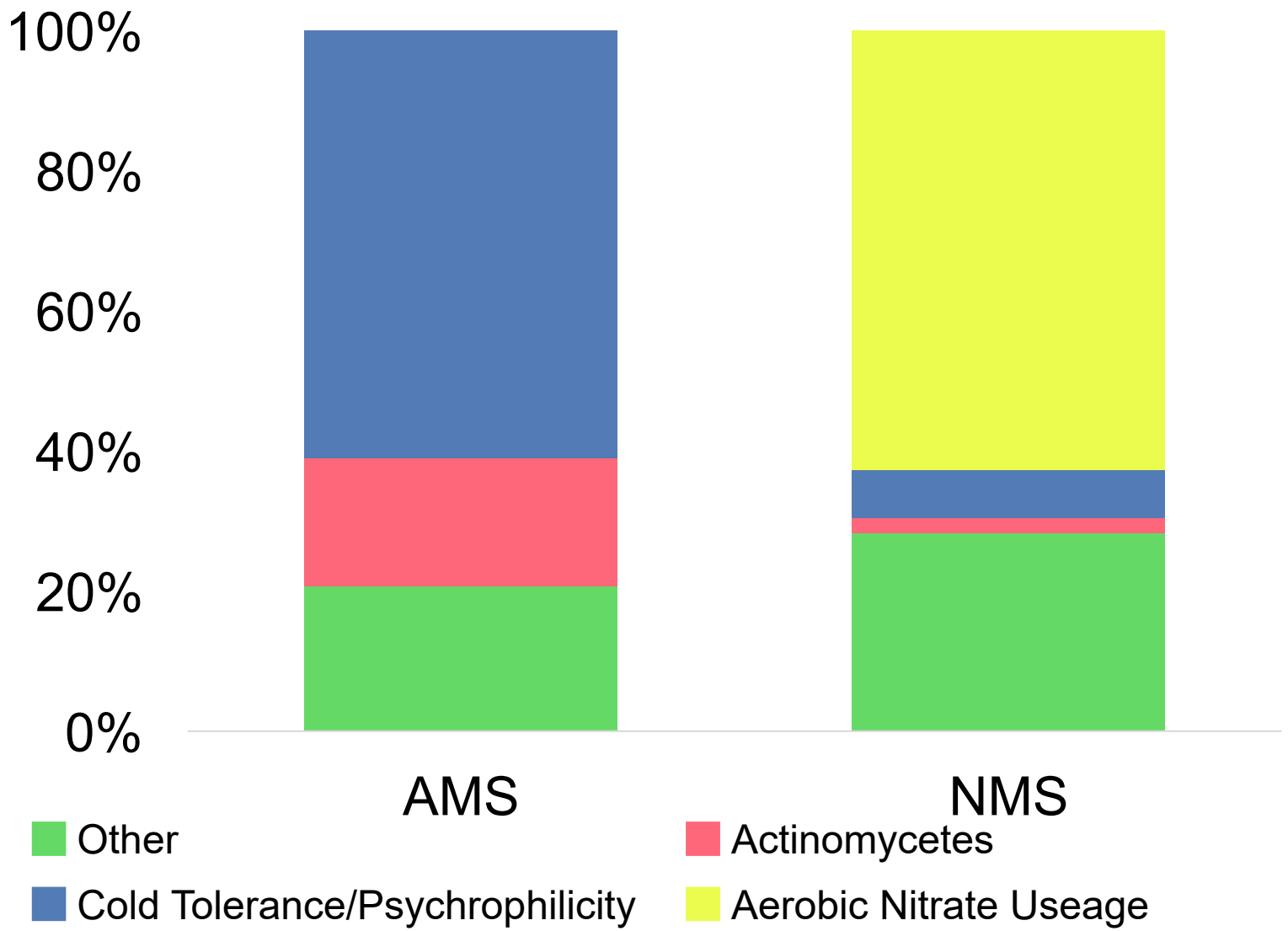
(https://www.generatorsource.com/blog/May-2021-(1)/Emergency-generators-keep-water-districts-ready-to.aspx)

Both consortia were capable of dioxane removal when amended with propane at 14 ºC



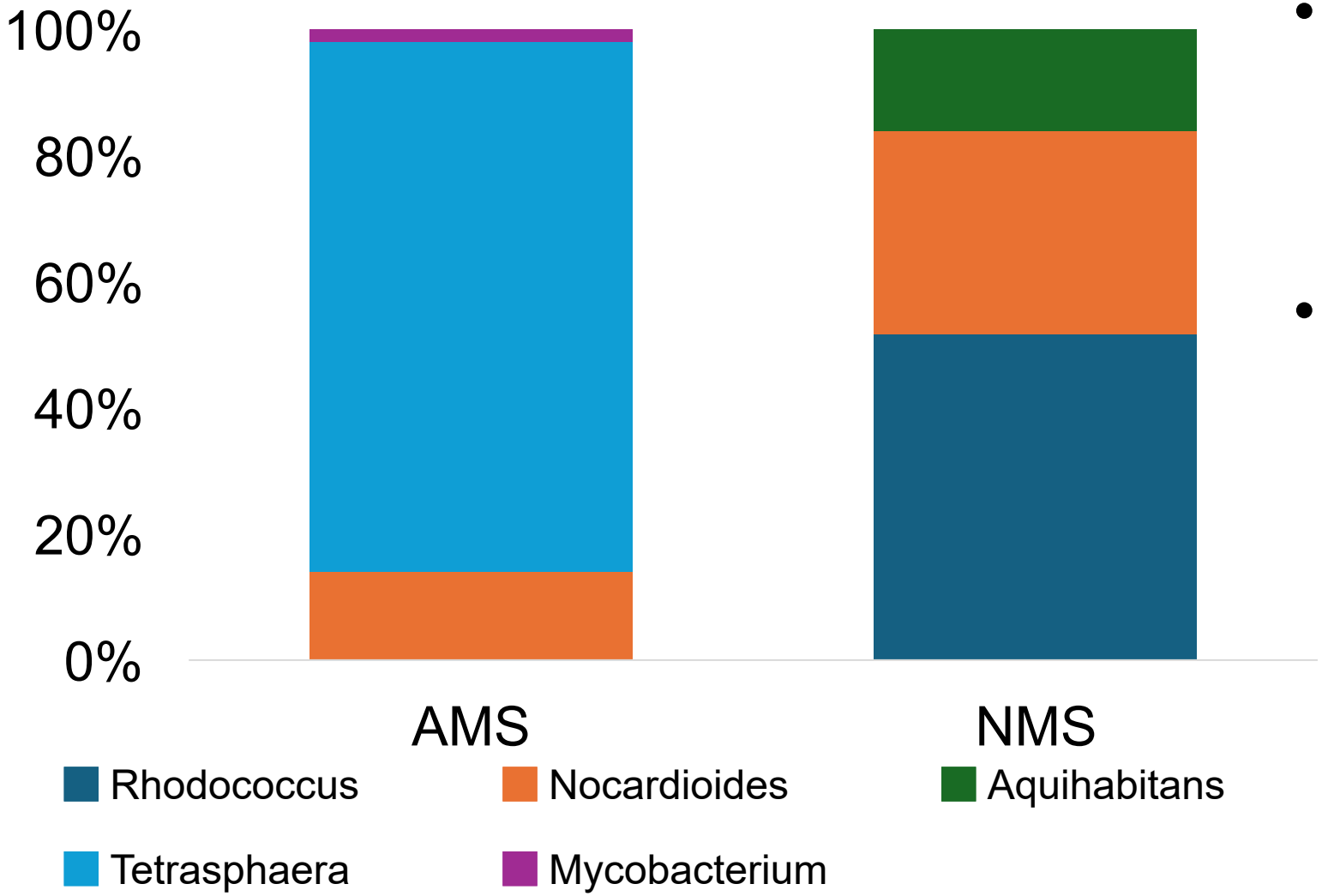
- Despite removing dioxane at similar rates the AMS consortia was more efficient dioxane in terms of propane concentration
  - 5.573±0.302 vs 4.152±0.092 mg dioxane/L/mg propane

Who’s present in our consortia?



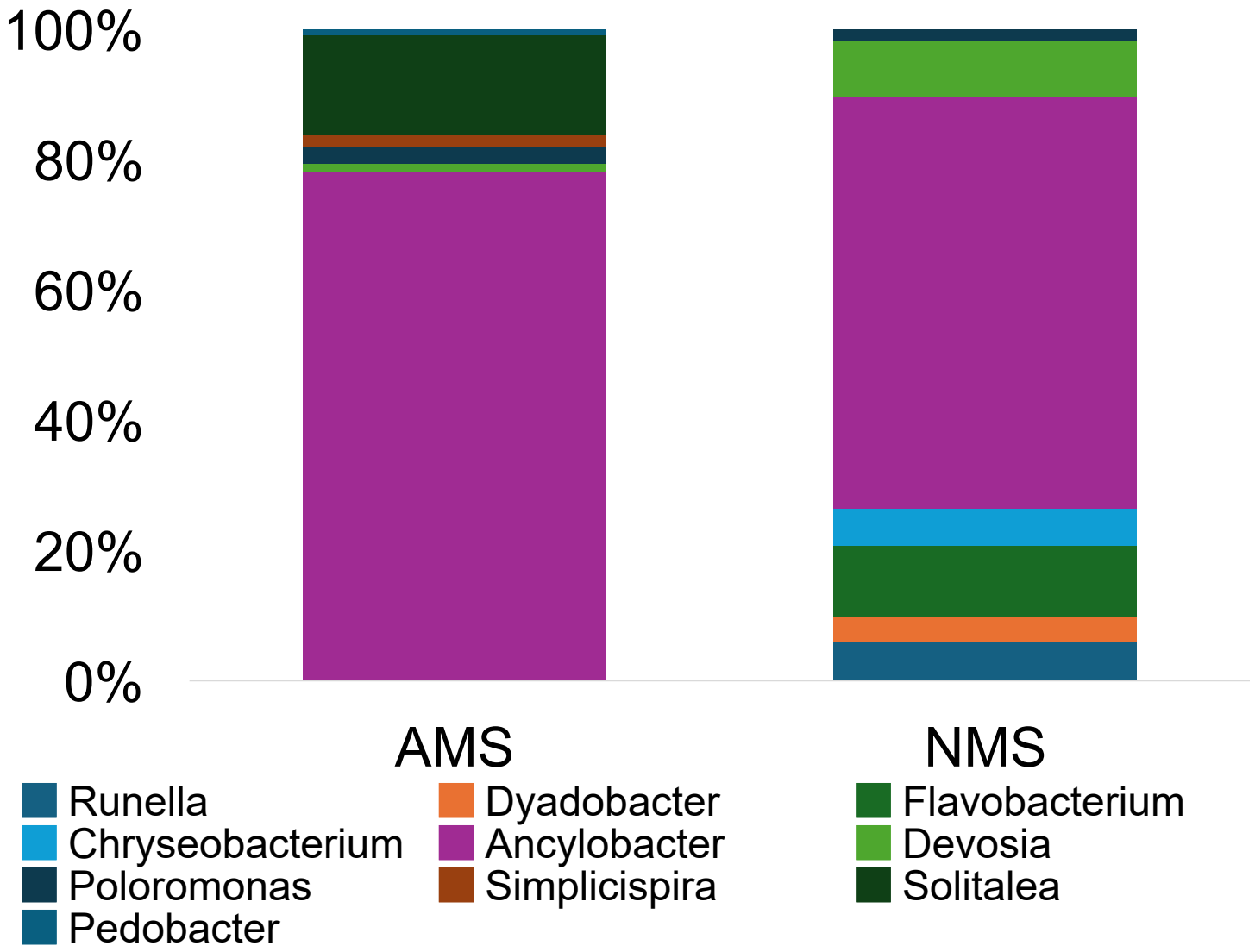
- 16S rRNA amplicon sequencing was performed on the V3-V4 variable using Illumina
- Highlights genera level presence

Actinomycetes



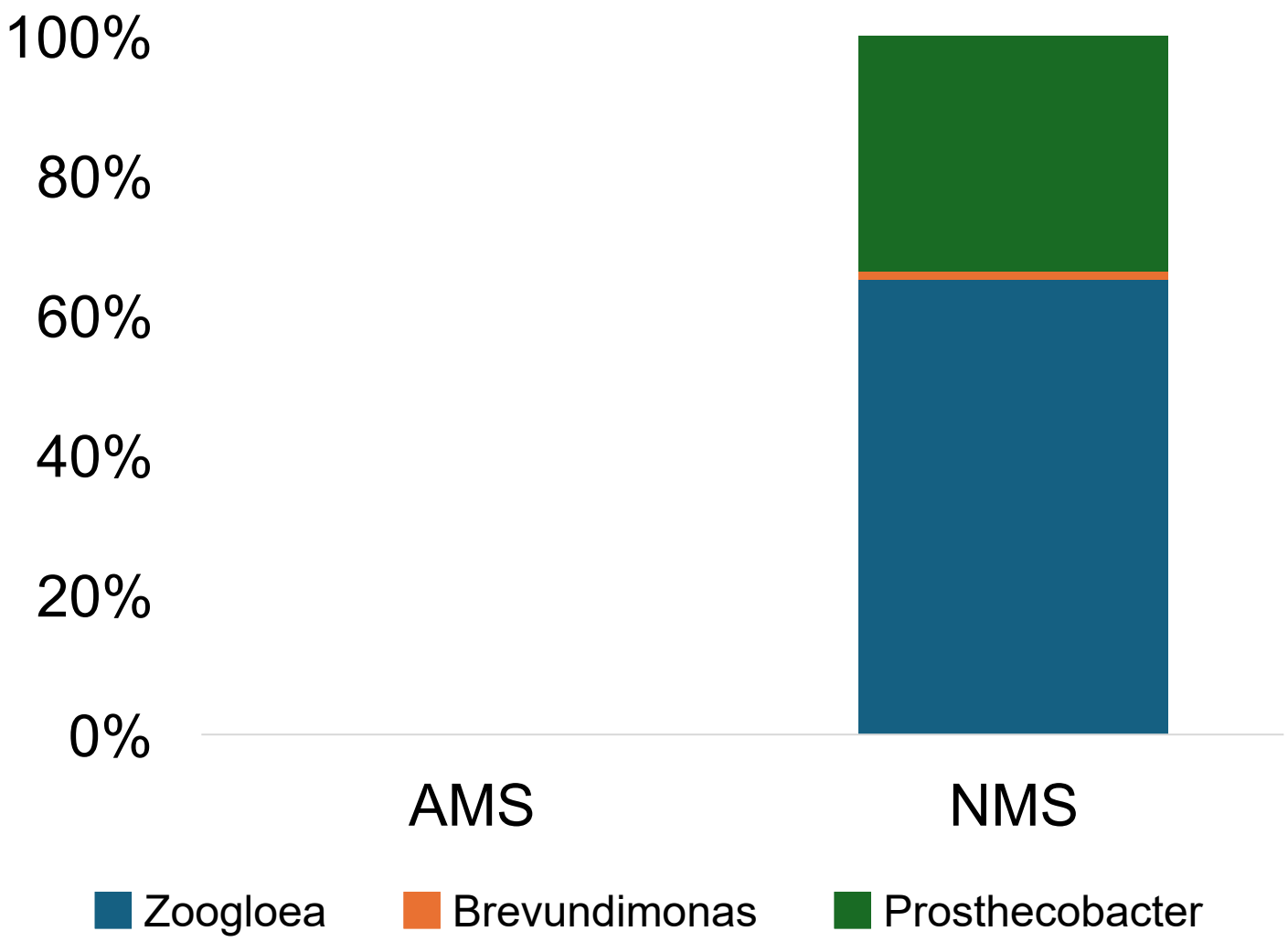
- Actinomycetes have been found to harbor dioxane degrading bacteria and SDIMOs
- Particularly Mycobacterium (PH-06) and Rhodococcus (Ruber s. 219) are associated with known dioxane degraders

Cold Tolerance and Psychrophilicity



- These genera have been found to house species capable of growing between 4 and 14 ^{o}C

Aerobic Nitrate Usage



- Despite the same seed sludge, the microbial communities are different
  - Likely due to the difference in nitrogen source
- *Zoogloea* is also responsible for the key physical difference among the consortia, large flocs which were only present in the NMS

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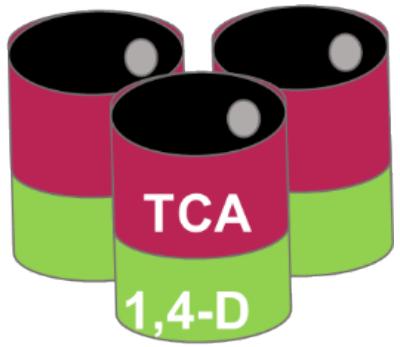
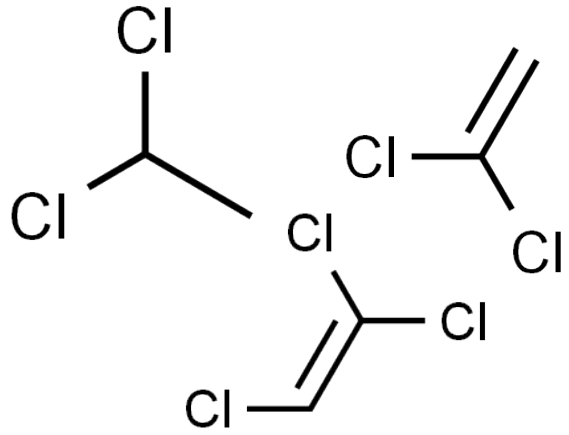
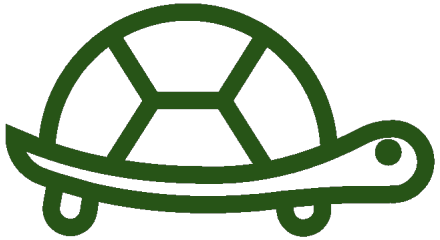
CAHs Cooccur With Dioxane



- Dioxane was mainly used as a stabilizer for 1,1,1-TCA



- Dioxane contamination often cooccurs with other CAHs and their degradation products



- CAHs are highly inhibitory to dioxane biodegradation

Both Consortia can tackle key CAHs

CAHs	AMS Consortia	NMS Consortia
1,1-DCE	95.09 ± 31.14	113.61 ± 39.96
1,2-c-DCE	37.90 ± 3.10	77.60 ± 13.90
TCE	58.96 ± 30.71	13.78 ± 4.73
VC	68.01 ± 5.40	81.10 ± 4.20
1,2-DCA	31.70 ± 4.10	31.50 ± 2.30
1,1-DCA	10.70 ± 2.00	11.00 ± 1.50
TCA	-	-
1,2-t-DCE	-	-

(nmol/h/mg protein)

- Three of the most inhibitory CAHs for dioxane removal
  - 1,1-DCE, 1,2-c-DCE, and TCE

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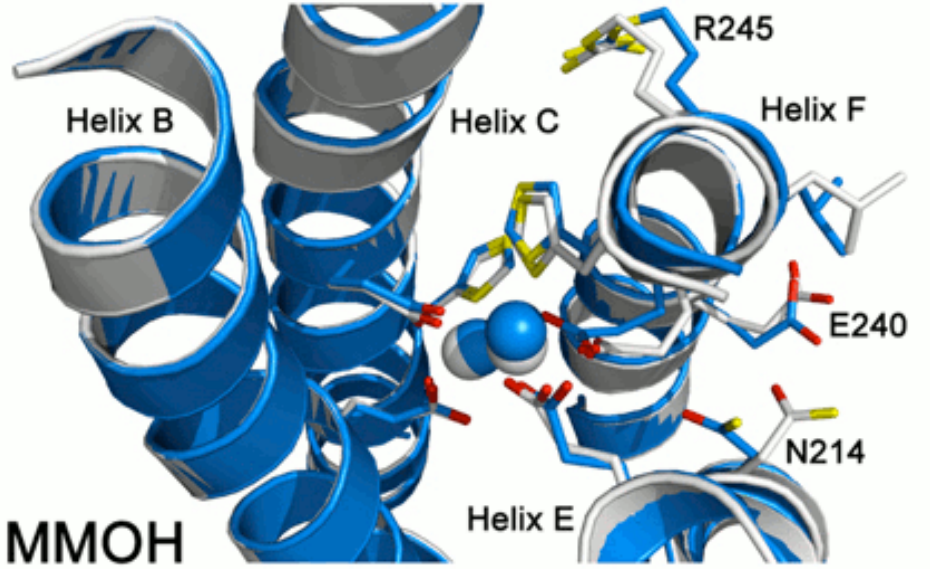
Soluble Diiron Monooxygenase?

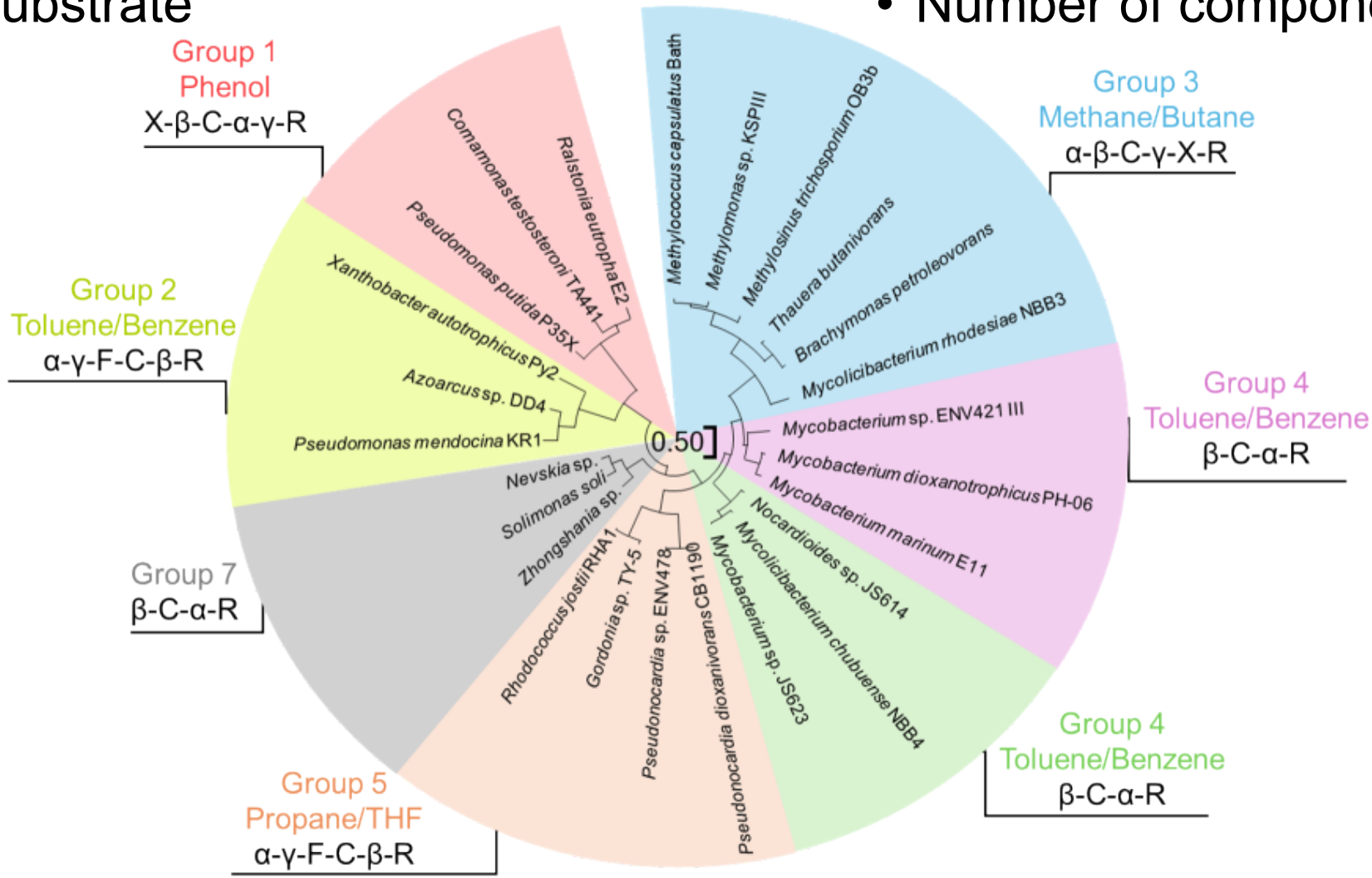
- Soluble diiron monooxygenases (SDIMOs) are multicomponent bacterial enzymes found across multiple phyla
- Non-heme diiron center that incorporates one atom from O_2 into many substrates:
  - Alkanes, alkenes, aromatic hydrocarbons, and chlorinated solvents, among others including environmental pollutants like dioxane



(https://lippardlab.mit.edu/research/mono/bacterial-multicomponent-monooxygenases-diiron-modeling)

SDIMO Grouping

- Component Arrangement
- Key substrate
- Identity
- Number of components



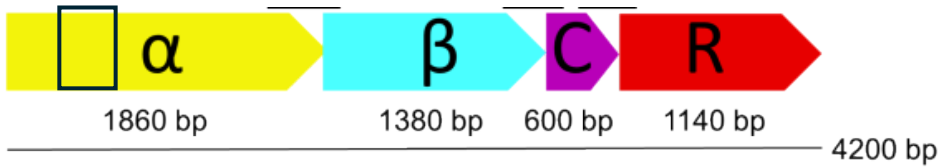
α – hydroxylase
β – hydroxylase
γ – hydroxylase
C – coupling protein
R – reductase
F – ferredoxin component
X – unknown function

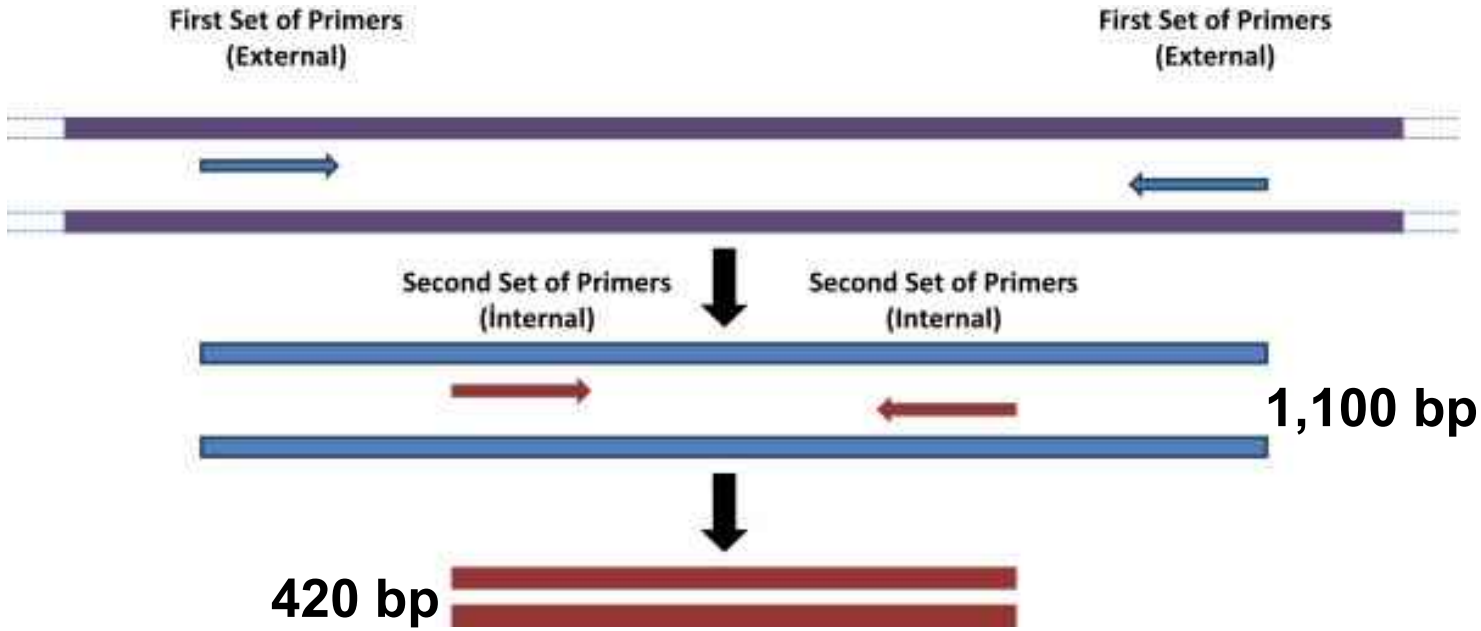
Group-6 SDIMOs

- Can initiate dioxane biodegradation
- Propane induces the same enzyme
- Capable of overcoming many inhibitory chlorinated solvents
- Widespread

What SDIMOs are present?

- SDIMOs were targeted using degenerate nested PCR
  - Primers were designed by Coleman - target the highly conserved region around the diiron centers across all SDIMO groups

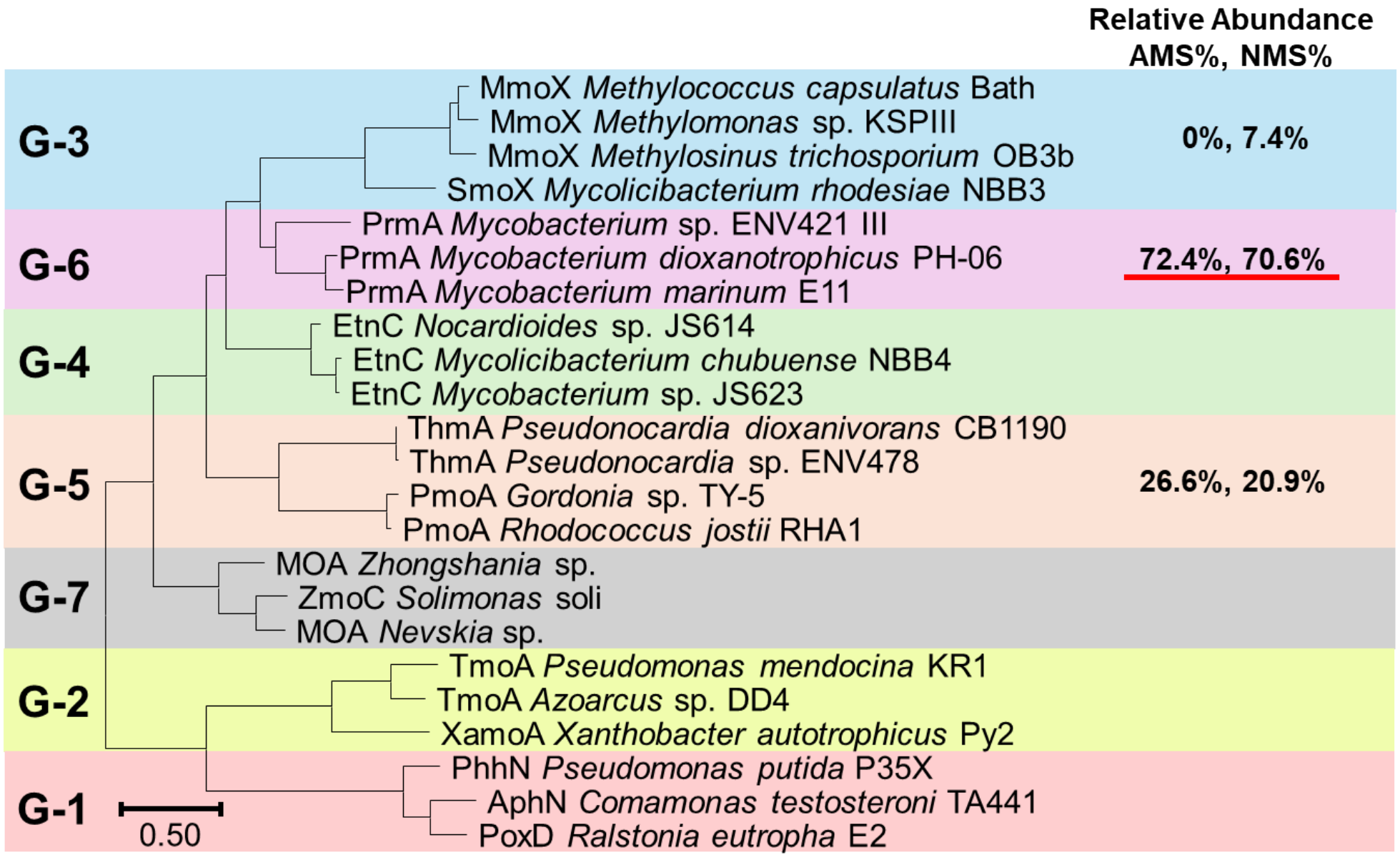




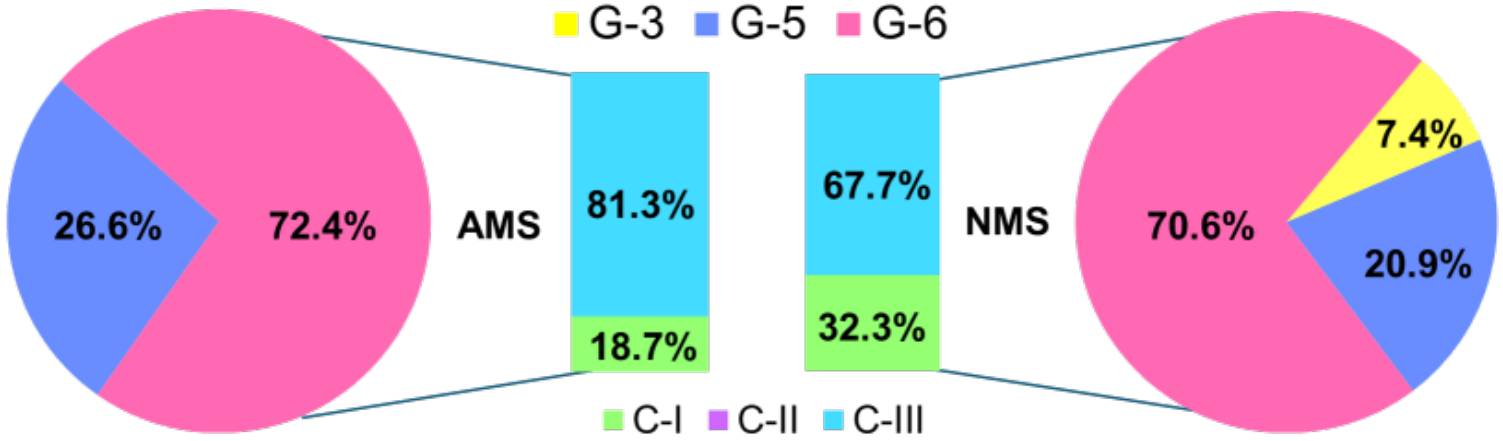
- Amplicons were sent off for Illumina sequencing

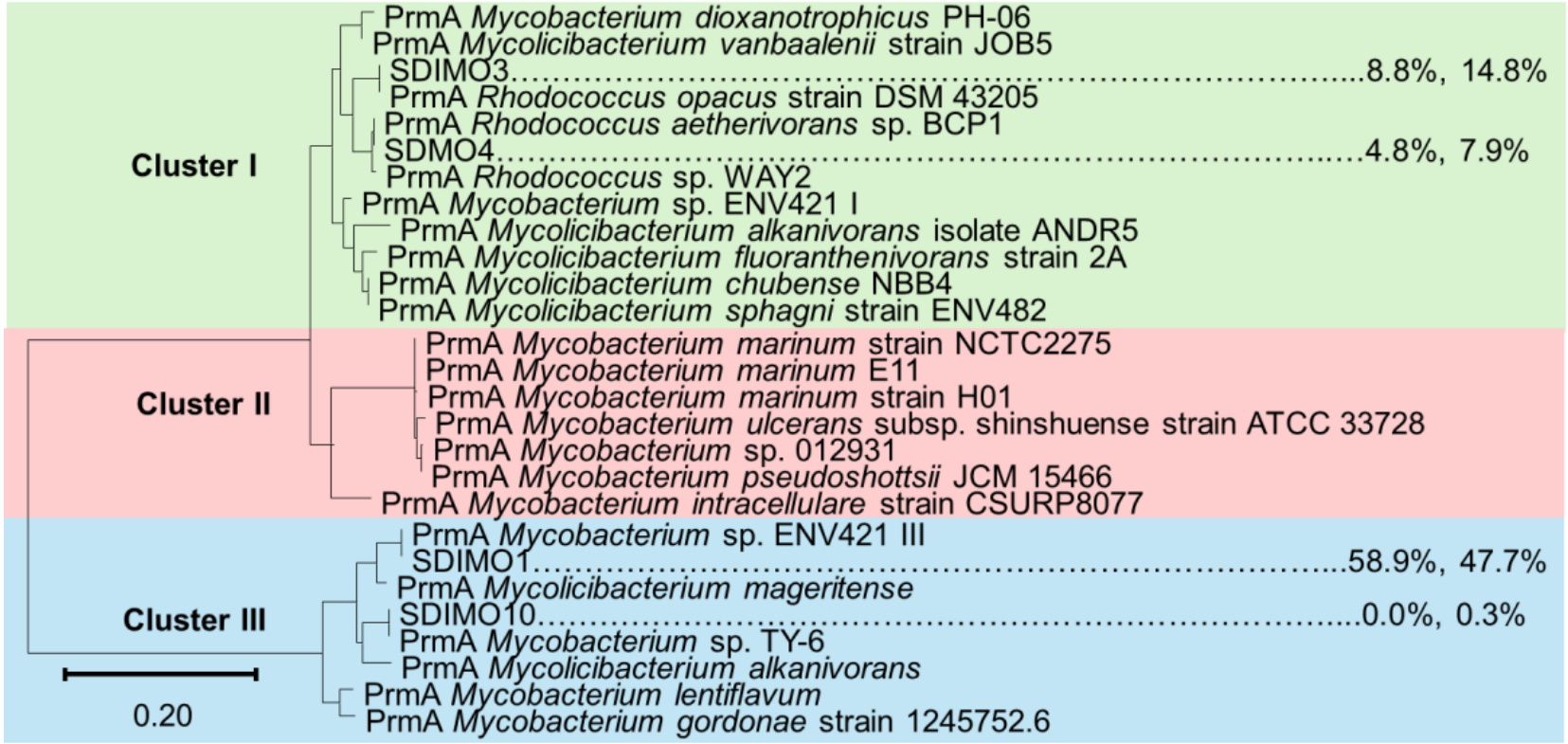
(doi:10.1111/j.1462-2920.2006.01015.x, doi.org/10.1016/B978-0-12-814264-6.00014-1)

SDIMOs were detected belonging to three groups



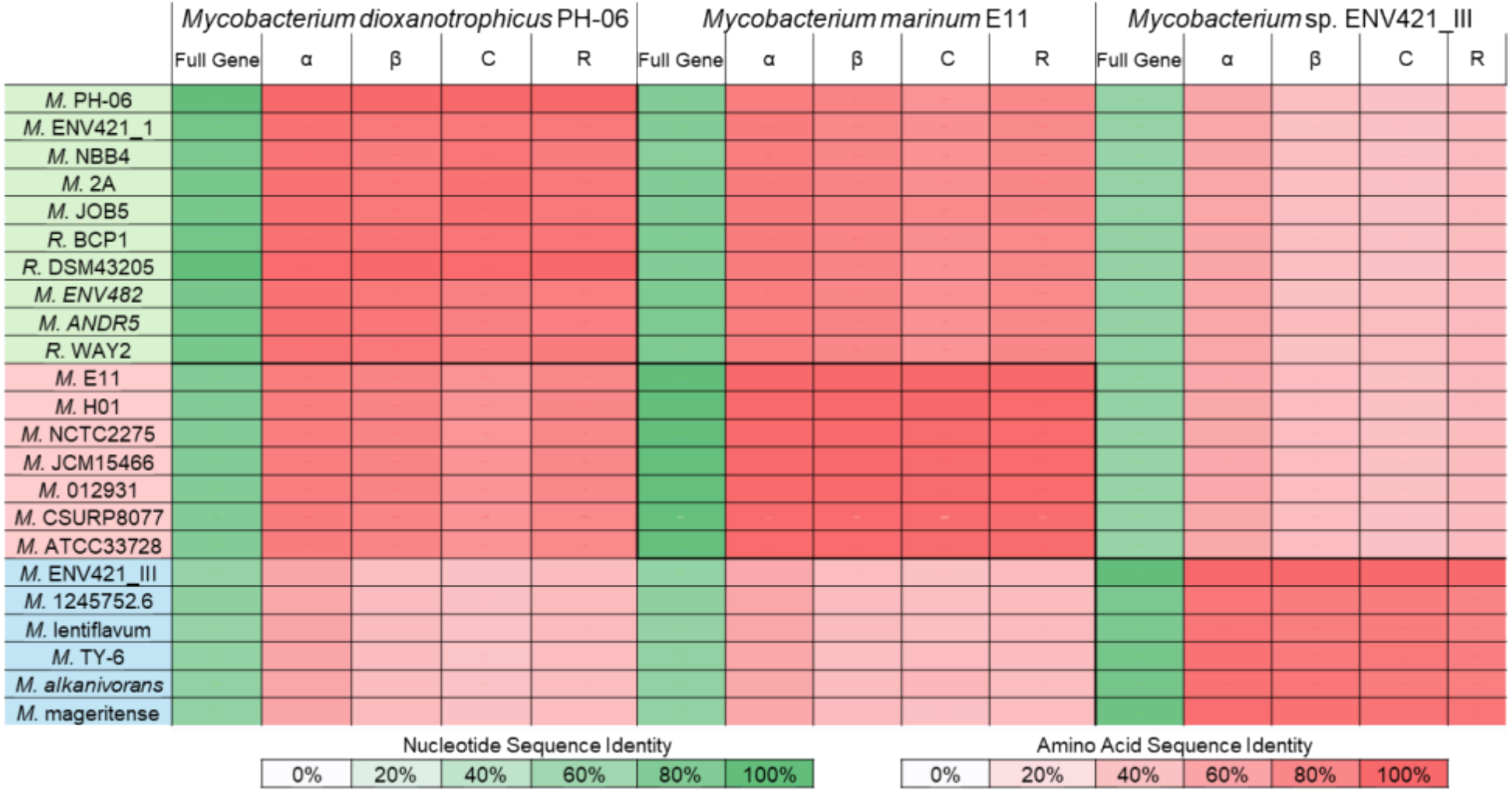
Group-6 – Propane Monooxygenase





- G6 PRM have been found to degrade dioxane, propane and cVOCs, which in combination with the high relevant abundance highlight them as key enzymes in these consortia
- Of particular interest is the clustering we observe in the phylogenetic tree, this is of high interest as it is at the amino acid level in the subunit which houses the catalytic diiron center
- To date most studies have been performed on the archetypic G6 PH-06 from the phylogenetically distinct Cluster I

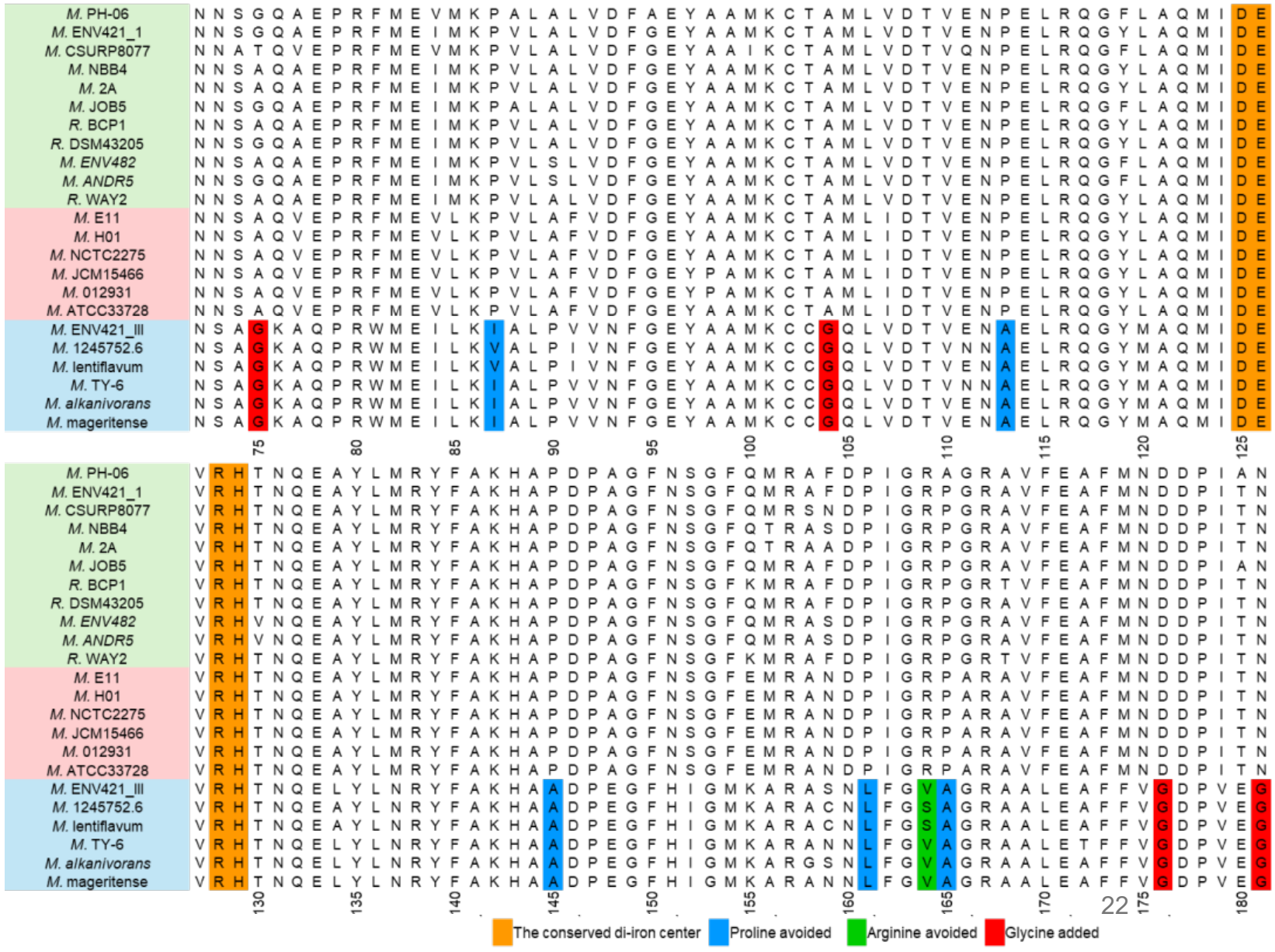
Is this unique to the alpha subunit?



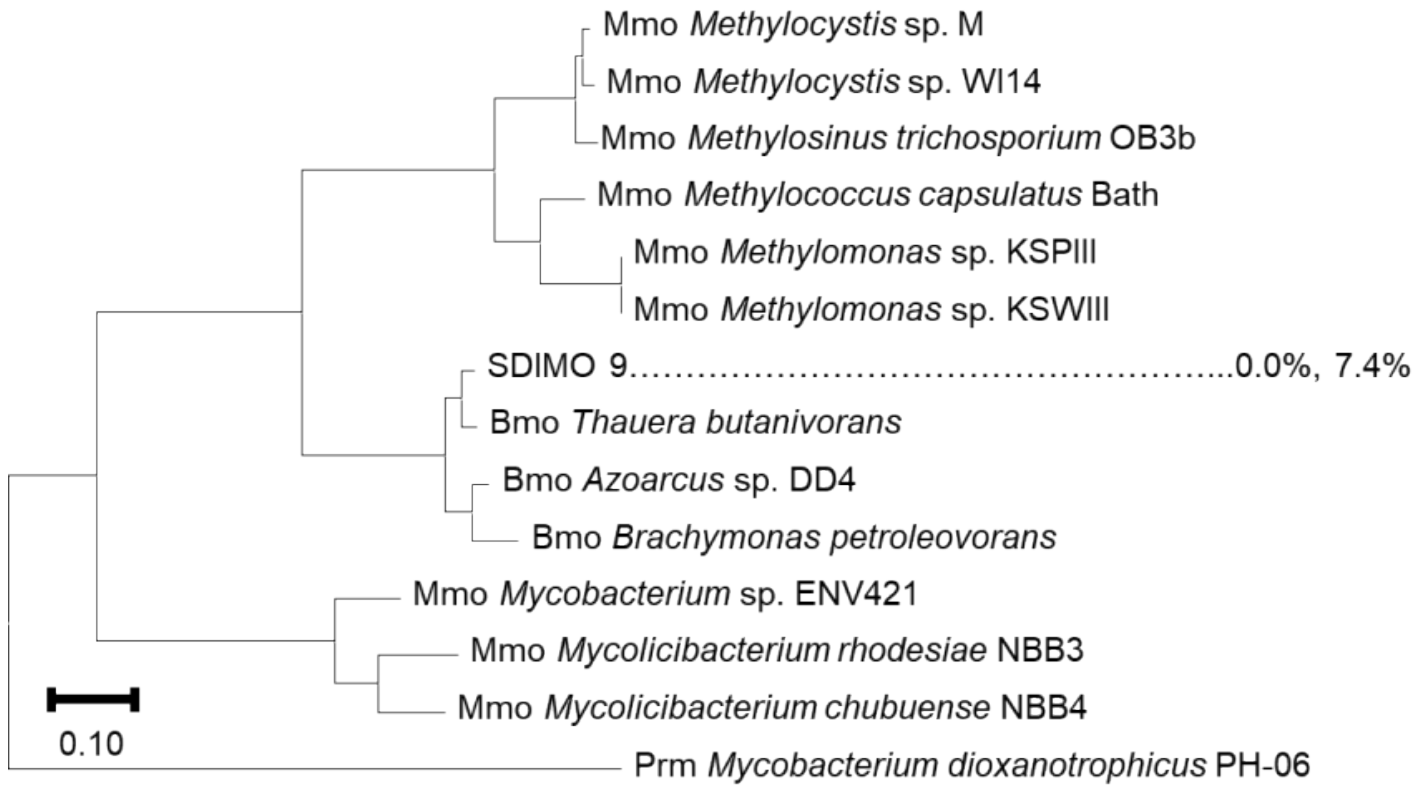
- High intracluster identity - >85%, >76% and >81% in β, C, and R subunits
- Intercluster identity is high between clusters I and II, than with III (>79%, >70% and >77% vs <46%, <47%, and <45%) in β, C, and R subunits

Amino Acid Alignment Reveals Psychrophilic Character of G6 Cluster III

- 55 residues up and down stream of the diiron active site
- High conservation across all clusters
- Several key changes are observed in CIII related to psychrophilicity
  - Proline avoidance
  - Arginine avoidance
  - Glycine Inclusion

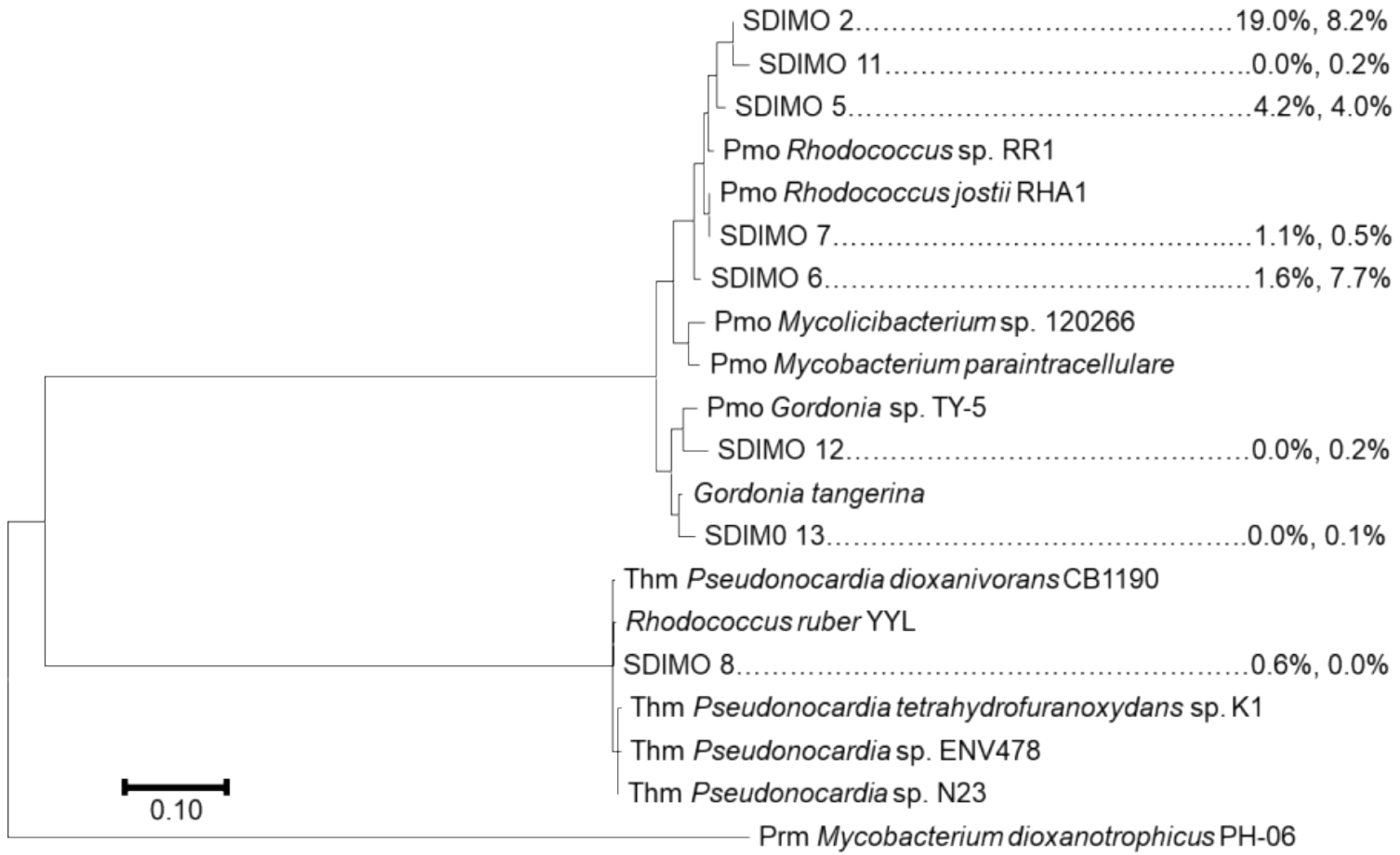


Group 3 – Butane monooxygenase



- SDIMO9 is 96.9% identical to BmoA in *Thauera butanivorans*
  - Can consume linear alkanes of 3 - 6 carbons
  - Can degrade several cVOCs
    - 1,1-DCE, 1,2-c-DCE, 1,1,1-TCA, VC, 1,1-DCA, 1,2-DCA

Group 5 – Propane and Tetrahydrofuran monooxygenase

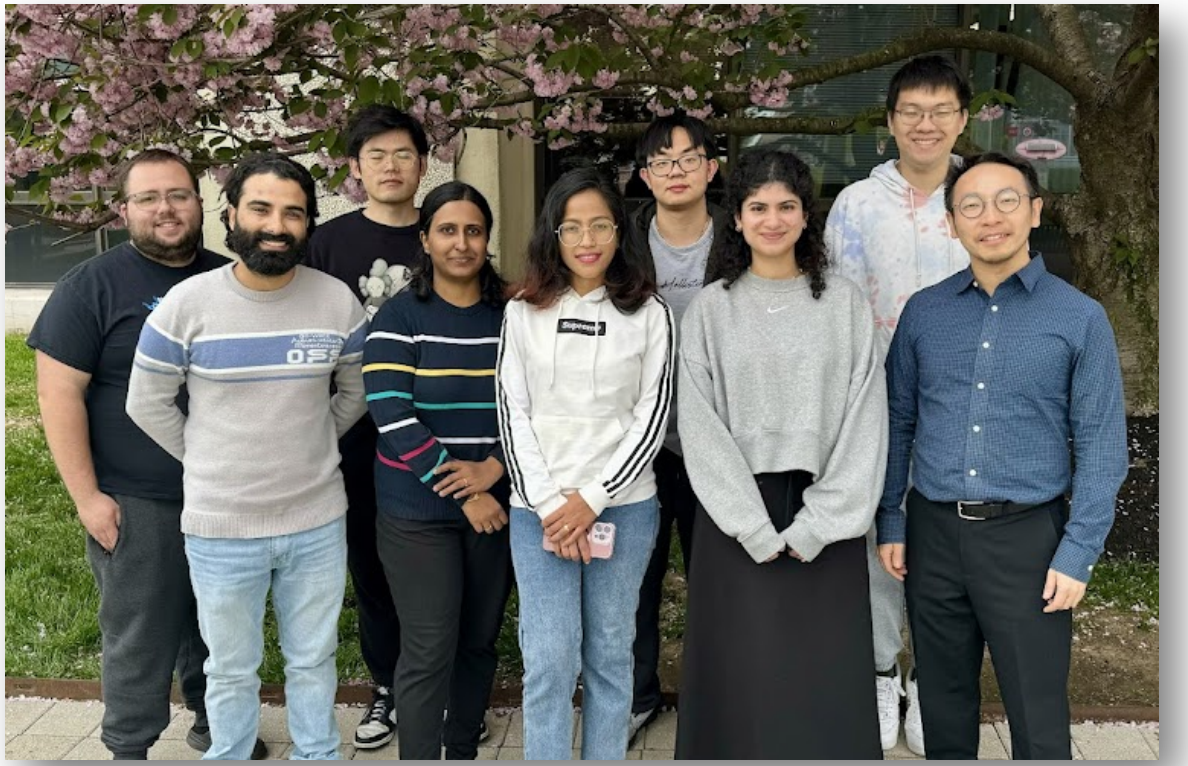


- A majority of detected G5 SDIMOs were PMOs known to degrade propane but not dioxane
  - Some PMOs like the one in *Azoarcus* DD4 have been found to degrade cVOCs like VC
- SDIMO8 is highly identical to ThmA in *P.* CB1190
  - THM have been found to be highly specific to cyclic ethers and alkanes
  - Likely involved in dioxane degradation but not propane or cVOCs

Takeaways

- Two consortia were enriched capable of removing dioxane and key CAHs when amended with propane at 14 ^{o}C
- Key SDIMOs were detected in Groups 3, 5 and 6
  - Group 6 was the dominant SIDMO group likely involved in propane consumption, dioxane removal, and CAH removal
- Group 6 shows three distinct subclusters, with divergences at the amino acid level around that active site which are likely functional (psychrophilicity), as well as in other subunits
  - M. PH-06 has been thoroughly explored of cluster I, leaves room for the exploration of other clusters

Acknowledgments:



- My advisor: Dr. Mengyan Li
- Dr. Daiyong Deng & Dr. Fei Li
- My lab mates
- My friends and family
- Collaborators

NSF

USGS

NJIT New Jersey Institute of Technology

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