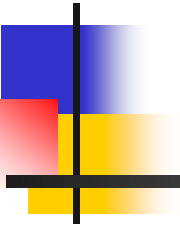


Anaerobic bacteria that dehalogenate  
chlorinated propanes: Insights into the  
roles played by *Dehalogenimonas* spp.



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# Acknowledgements

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- Coauthors

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- Trent Key
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- Other contributors

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- LA GBI
- DOE JGI
- ChunLab



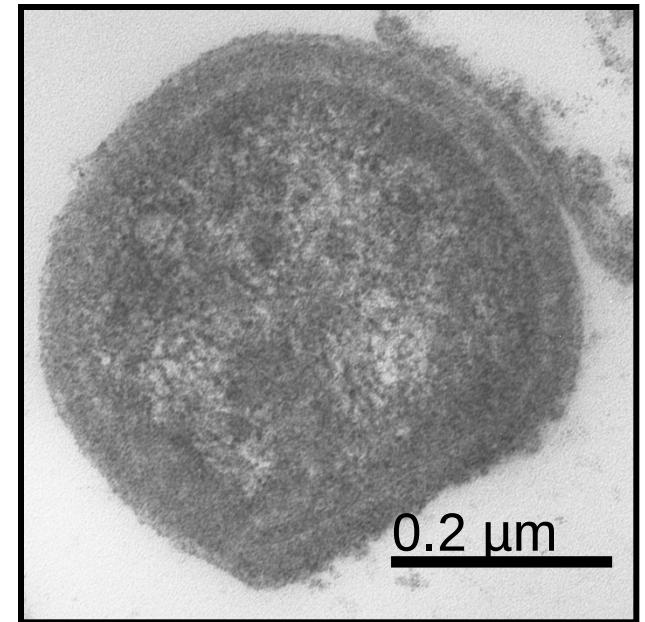
# Overview

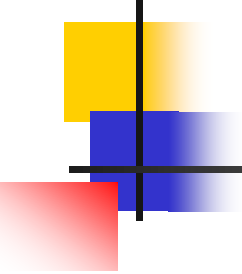
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- Characteristics of genus *Dehalogenimonas*
  - *D. lykanthroporepellens*
  - *D. alkenigignens*
- Groundwater monitoring
  - 16S rRNA gene based detection and quantification at a superfund site
- Significant findings

# *Dehalogenimonas lykanthroporepellens*

- Isolation: DNAPL contaminated groundwater (Yan *et al.*, 2009)
- Strictly anaerobic, mesophilic, Gram negative, non motile, non spore forming
- Electron donor: H<sub>2</sub>
- Electron acceptors: Polyhalogenated alkanes
- Formal taxonomic description (Moe *et al.*, 2009)



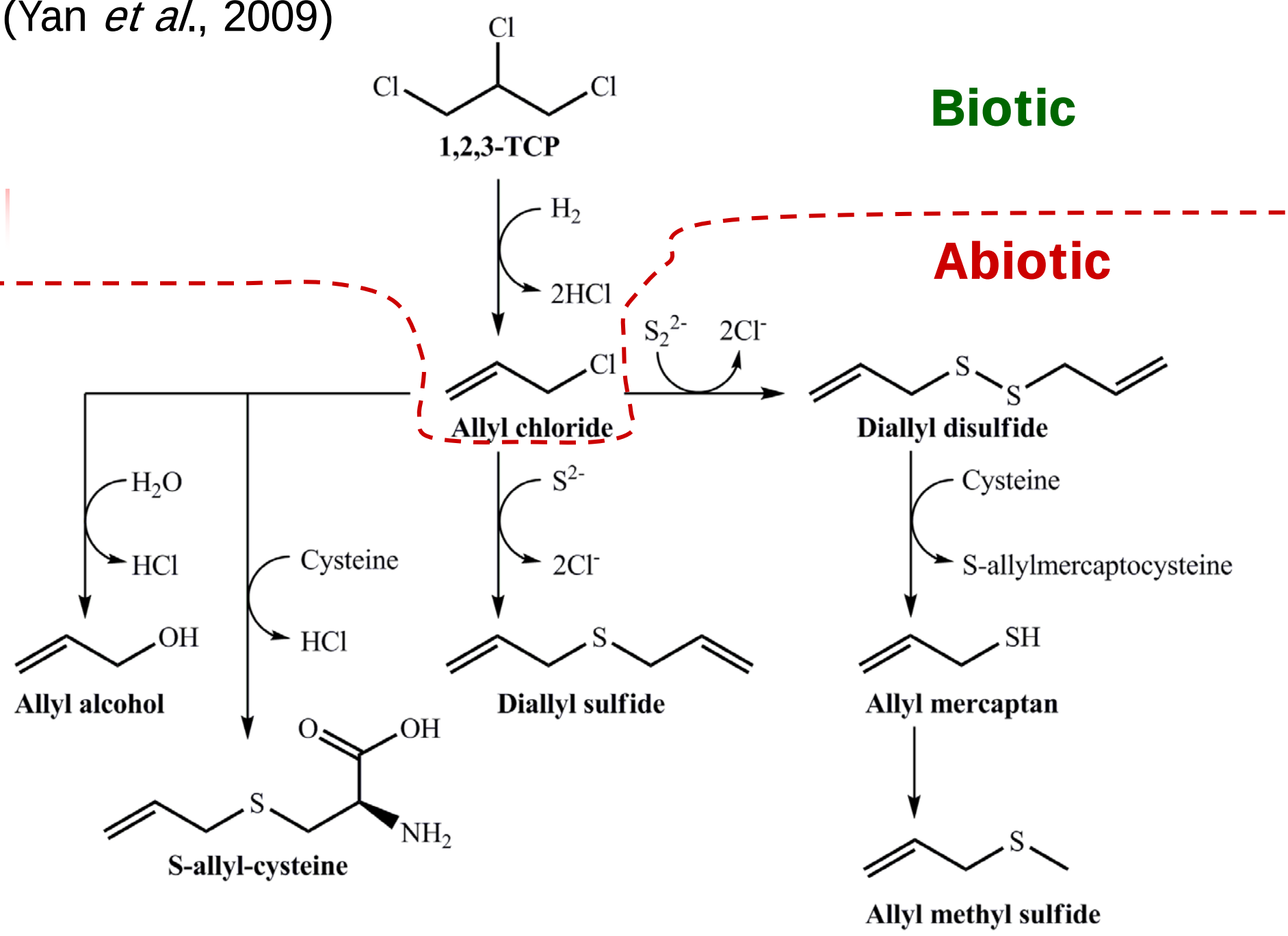


# *D. lykanthroporepellens*

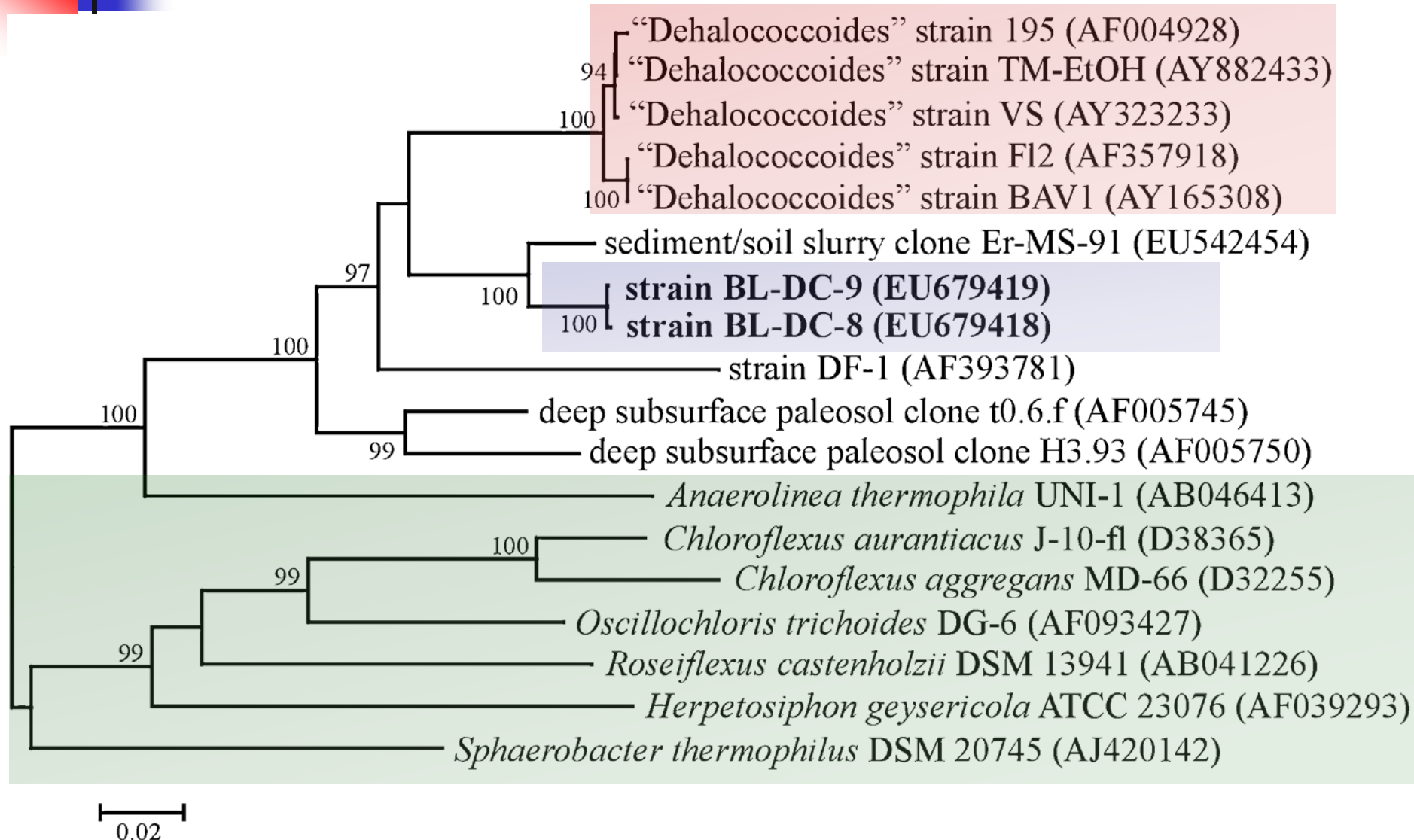
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- Dihaloelimination reactions
  - 1,2-Dichloroethane → Ethene
  - 1,2-Dichloropropane → Propene
  - 1,1,2-Trichloroethane → Vinyl chloride
  - 1,1,2,2-Tetrachloroethane → Dichloroethenes
  - 1,2,3-Trichloropropane → Allyl chloride →  
other compounds

(Yan *et al.*, 2009)



# *D. lykanthroporepellens*

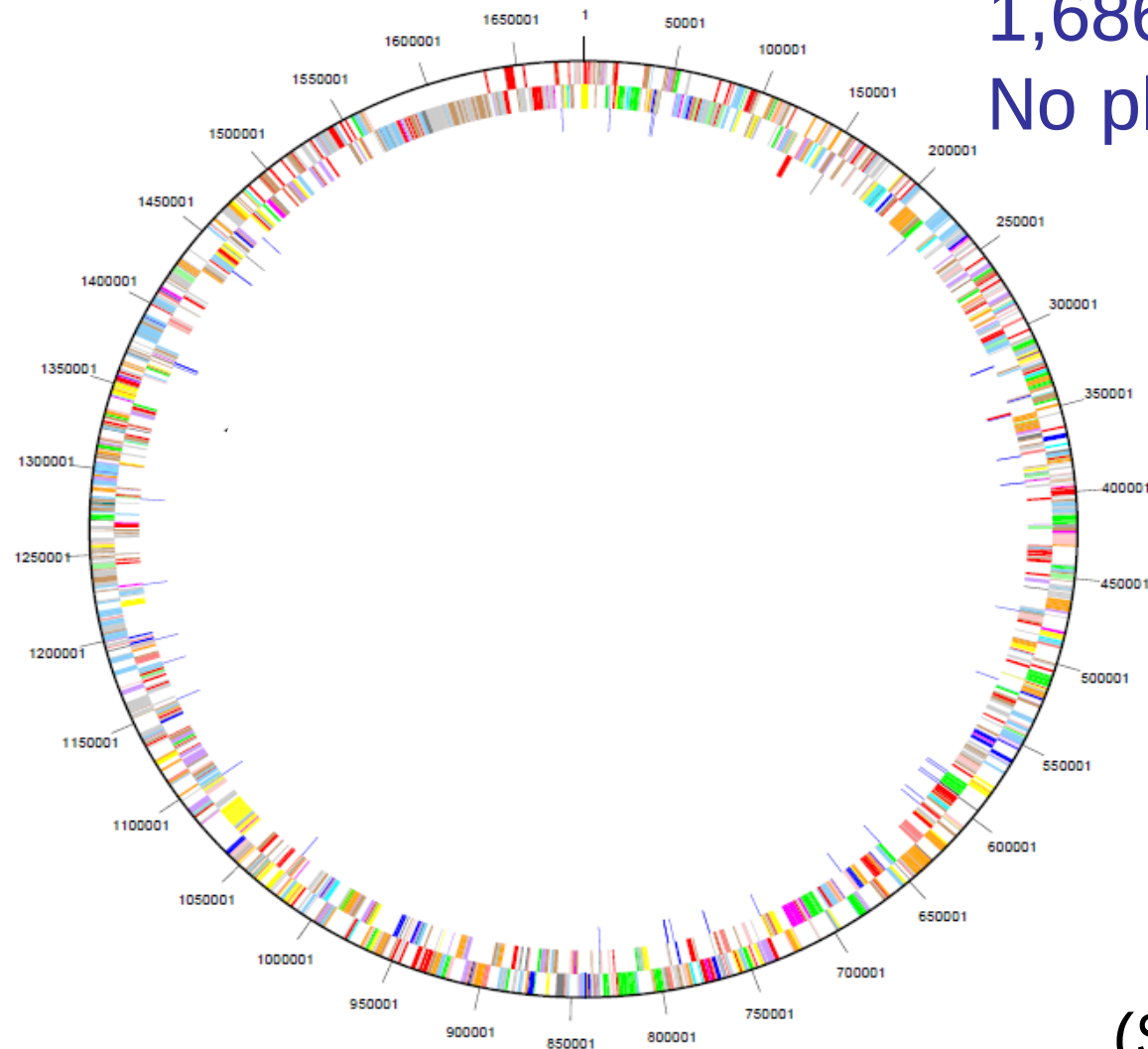


# Genome sequence of BL-DC-9<sup>T</sup>

1,686,510 bp chromosome  
No plasmids

~24 rdhA genes  
(17 “full length”)

7 rdhB genes  
(1 orphan)

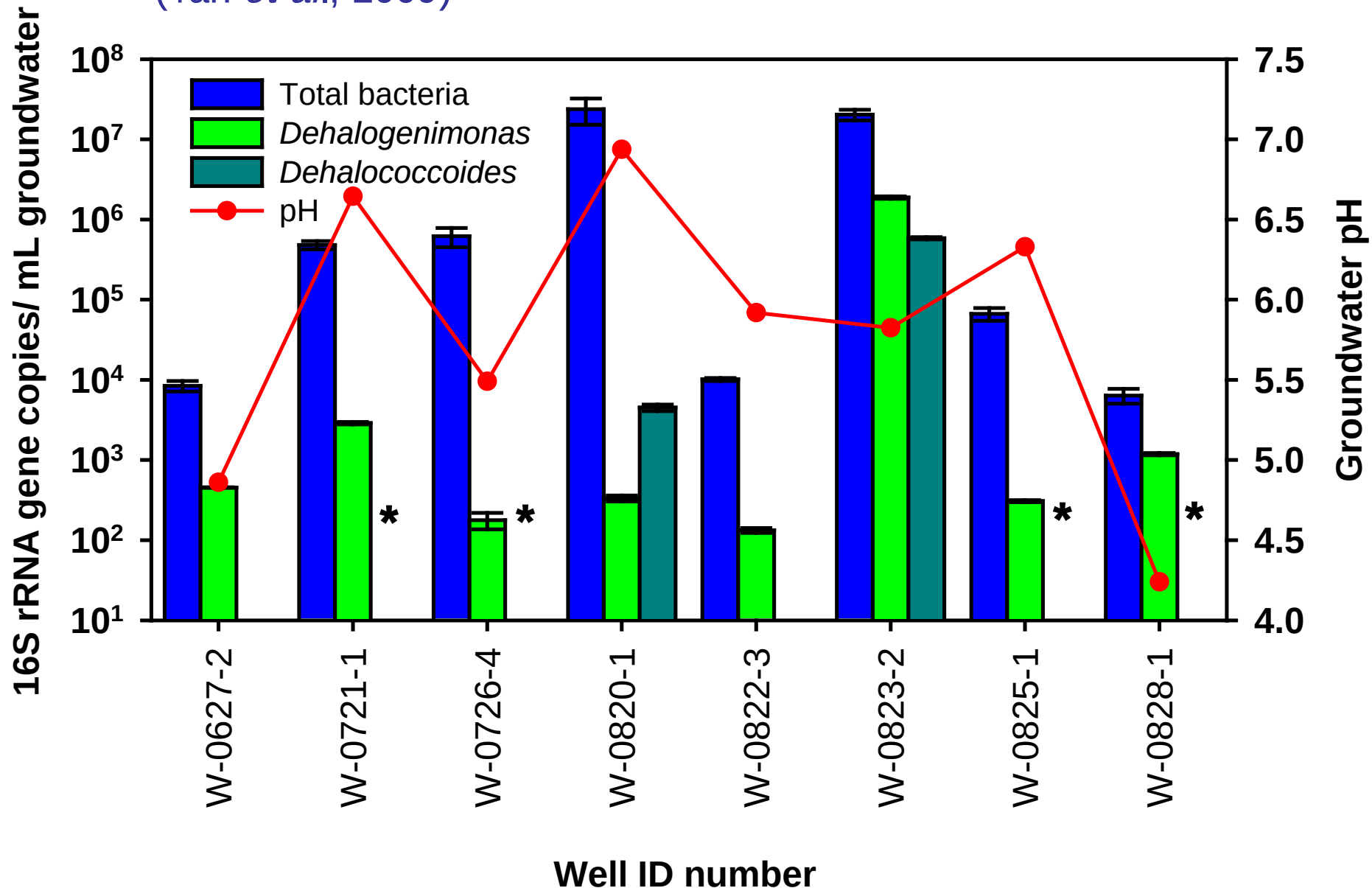


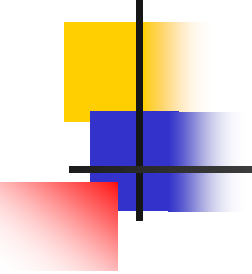
(Sidaramappa *et al.*, 2012)



# Data from PPI Brooklawn studies

(Yan *et al.*, 2009)



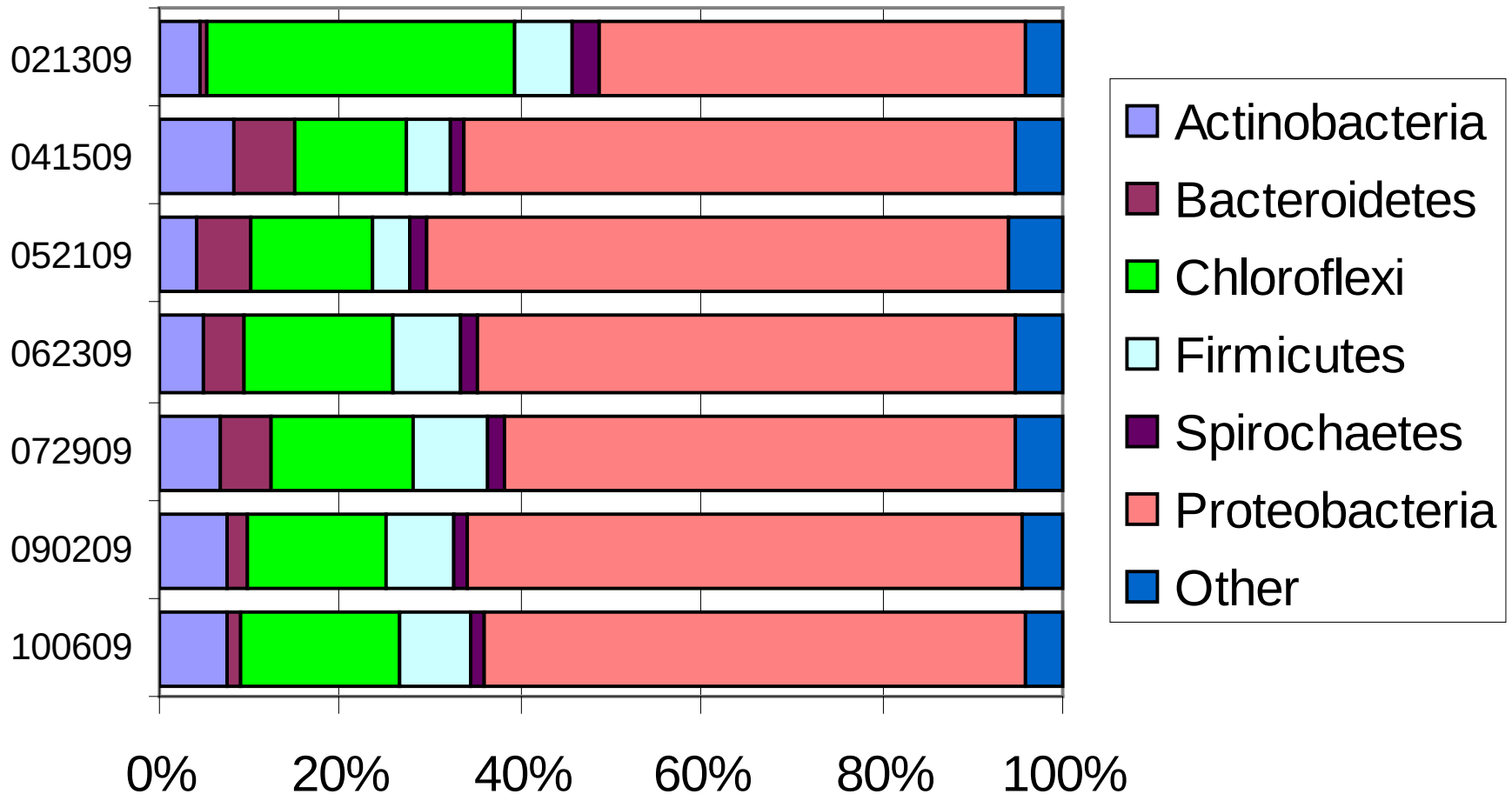


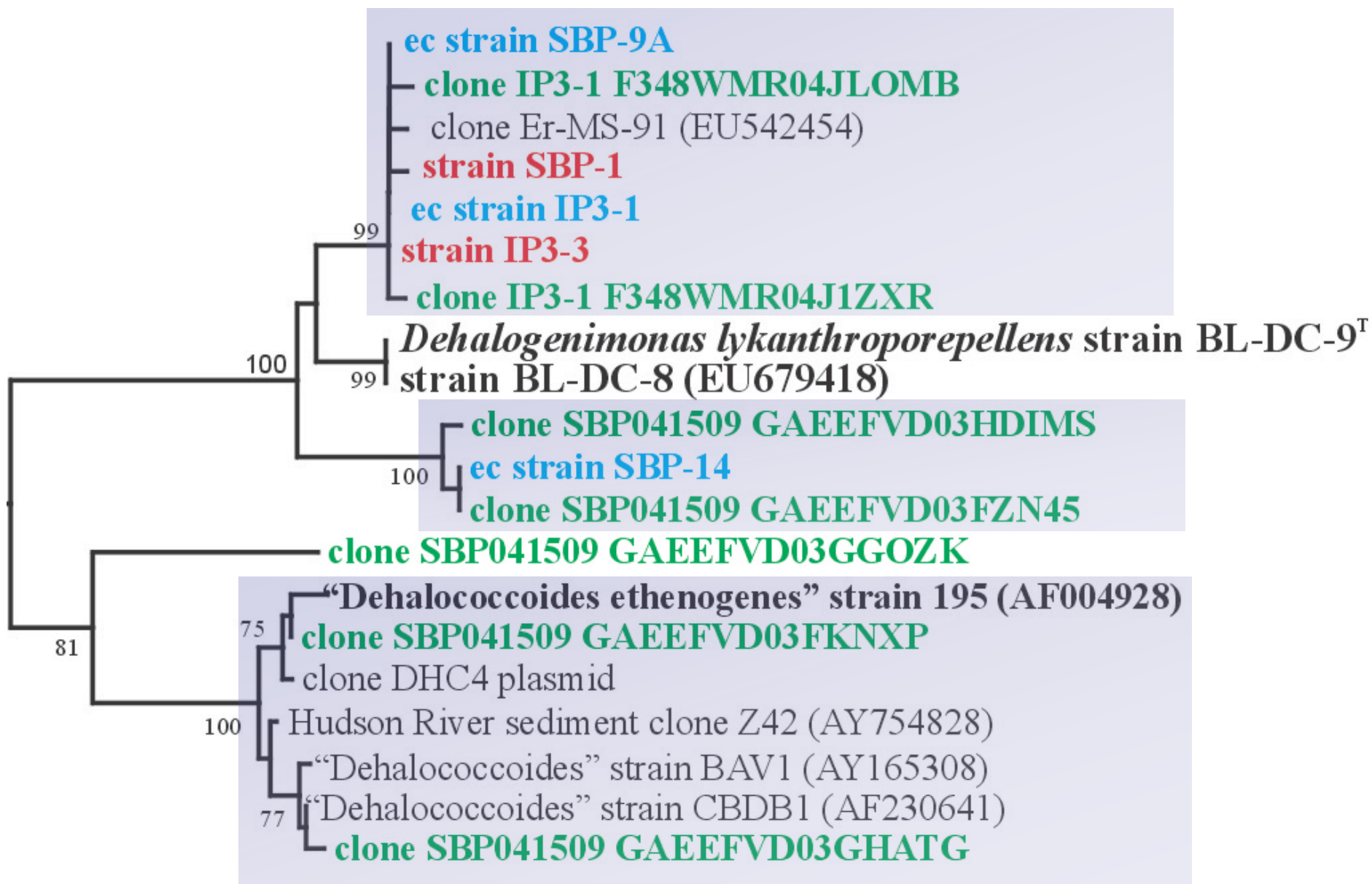
# PPI Scenic Site Plume

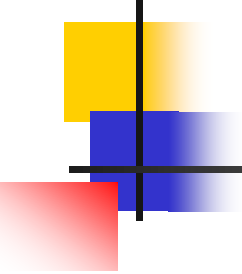
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- Groundwater contaminants: mixture of chlorinated alkanes and alkenes
- Enrichment cultures exhibited reductive dechlorination (1,2-DCA, 1,2-DCP, 1,1,2-TCA, TCE)
- 16S rRNA gene libraries (pyrosequencing)
  - >165,000 sequences

# 16S rRNA gene libraries







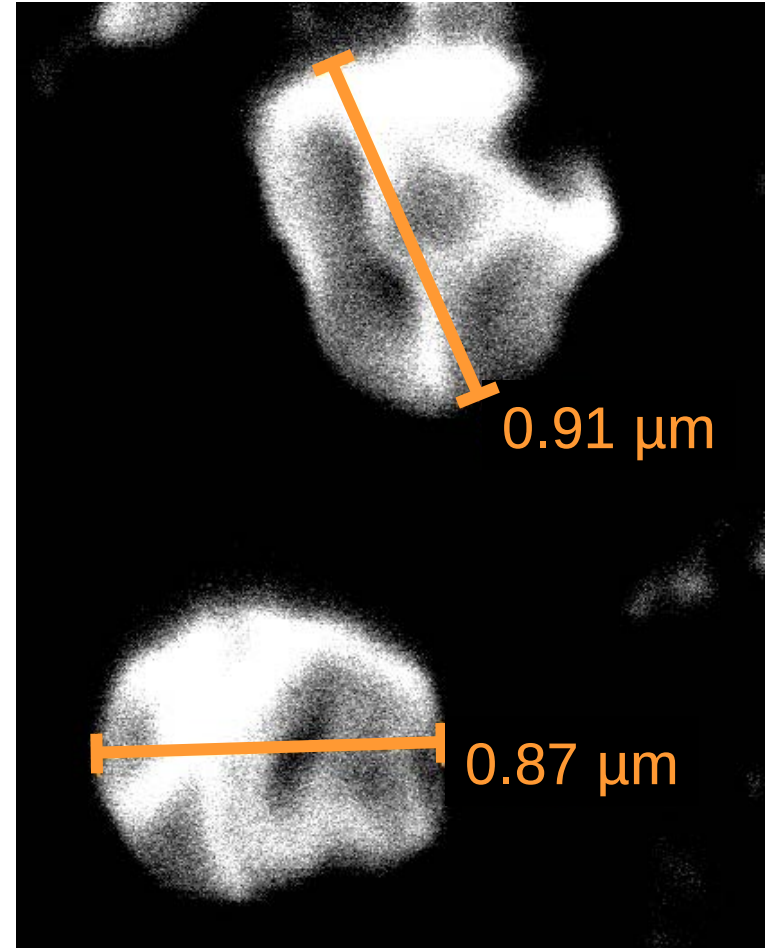
# Isolation approach

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- Dilution-to-extinction
- Chlorinated solvents as electron acceptors
- H<sub>2</sub> as potential electron donor
- Titanium citrate as reducing agent
- Terminal positives identified via gas chromatography

# *Dehalogenimonas alkenigignens*

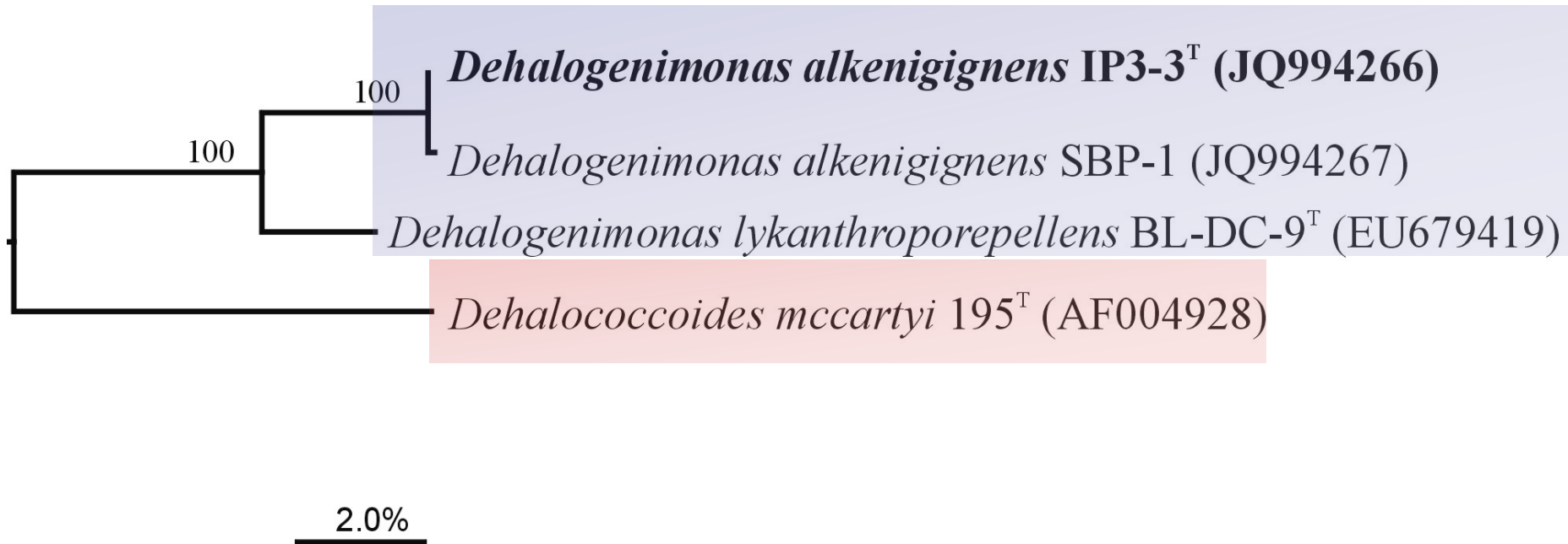
- 96% 16S rRNA gene sequence identity with *D. lykanthroporepellens*
- Strictly anaerobic
- Small, irregular cocci
- H<sub>2</sub> as electron donor
- Resistant to antibiotics (ampicillin, vancomycin)



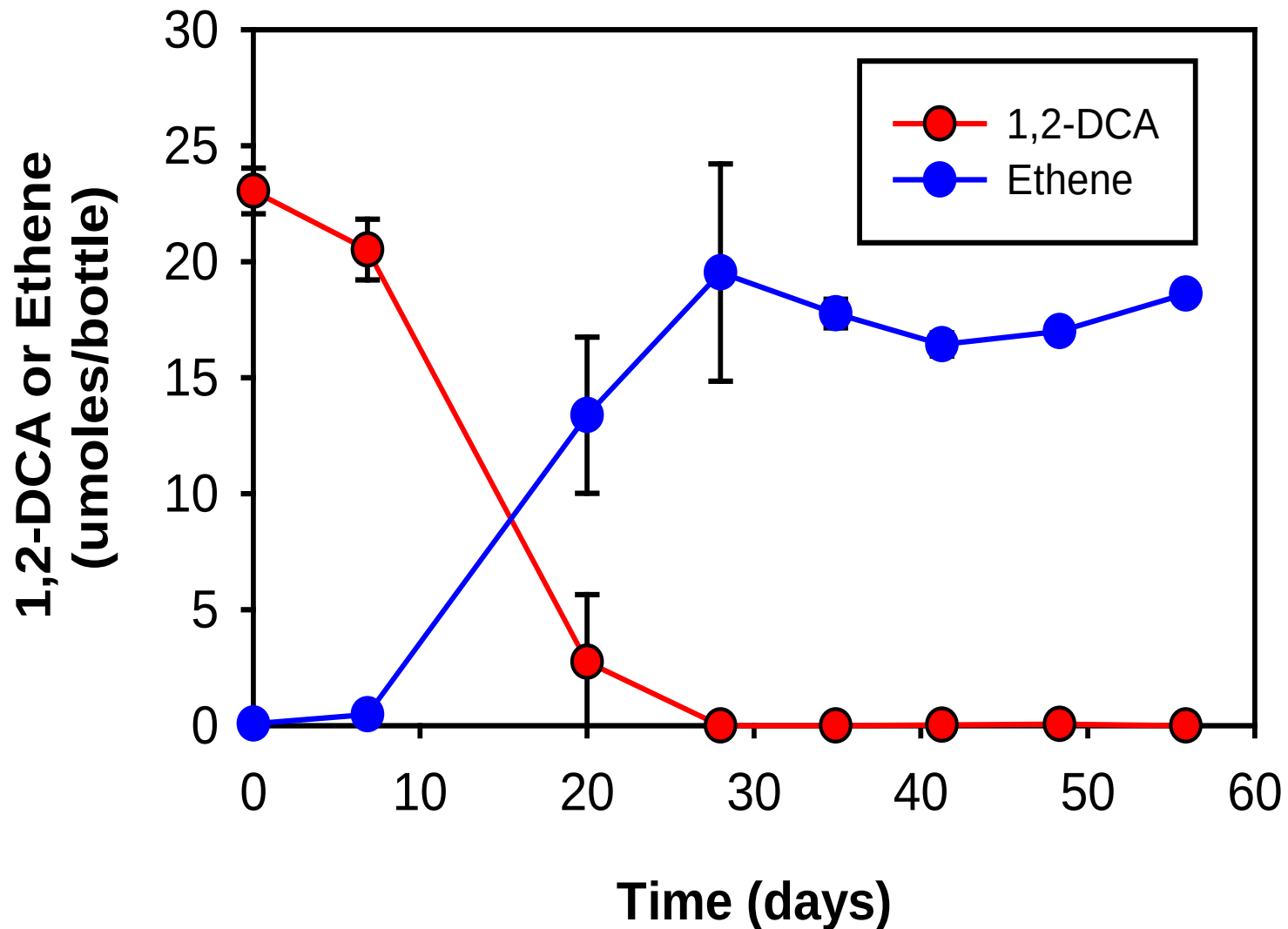
(Bowman *et al.*, 2013)



# Phylogenetic relationship based on 16S rRNA gene sequences

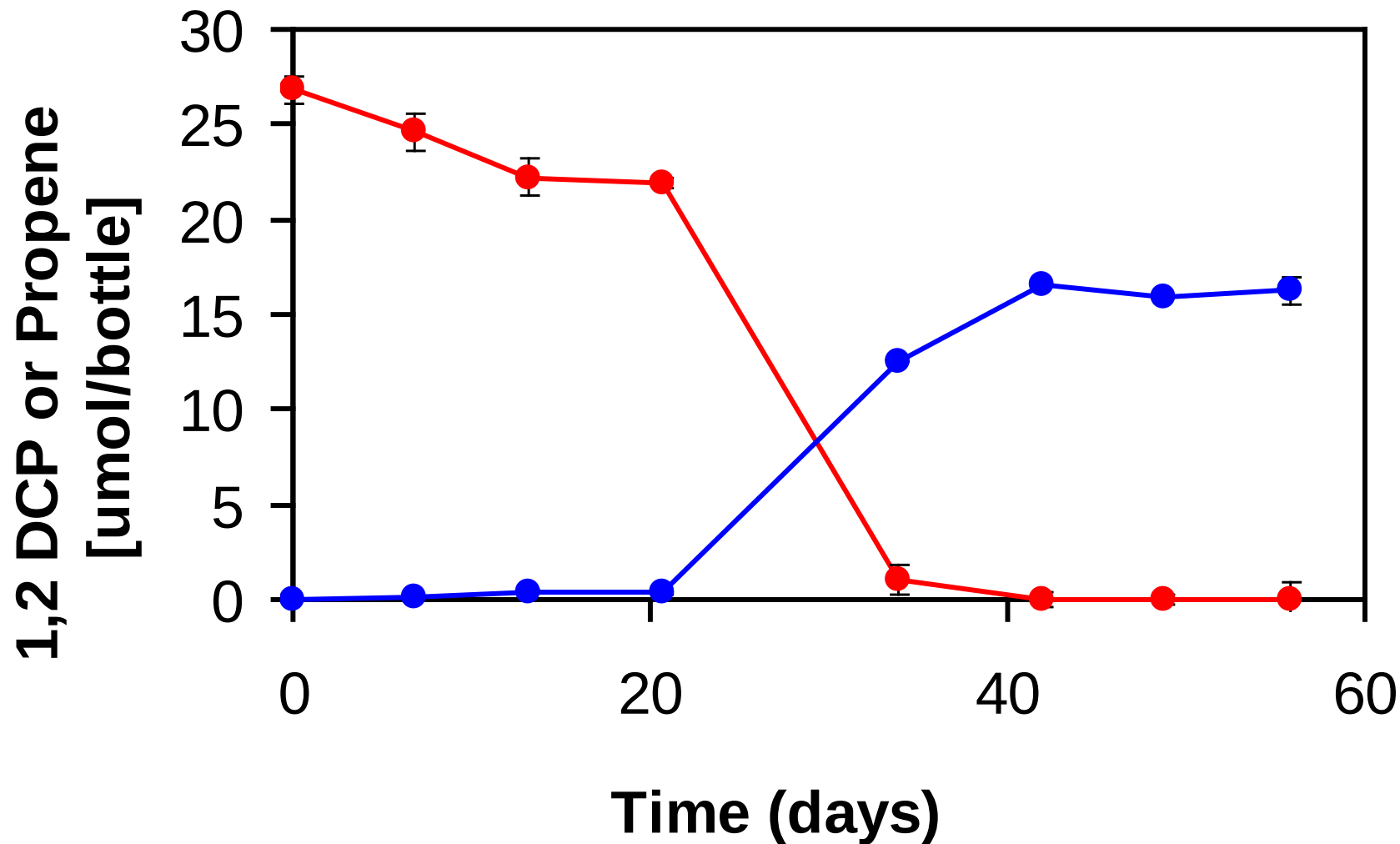


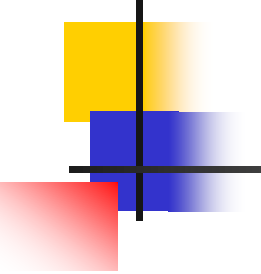
# IP3-3<sup>T</sup> grown on 1,2-DCA





# IP3-3<sup>T</sup> grown on 1,2-DCP





# Electron acceptors

- Vicinally halogenated alkanes

- 1,2-Dichloroethane
- 1,2-Dichloropropane
- 1,1,2,2-Tetrachloroethane
- 1,1,2-Trichloroethane
- 1,2,3-Trichloropropane

- Other chlorinated alkanes

- 1-Chloropropane
- 2-Chloropropane
- 1,1-Dichloroethane
- 1,1,1-Trichloroethane

- Chloromethanes

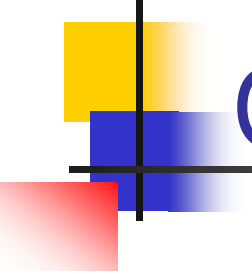
- Dichloromethane
- Trichloromethane
- Tetrachloromethane

- Chlorinated alkenes

- Tetrachloroethene
- Trichloroethene
- *cis*-1,2-Dichloroethene
- *trans*-1,2-Dichloroethene
- Vinyl chloride

- Chlorinated benzenes

- 1-Chlorobenzene
- 1,2-Dichlorobenzene



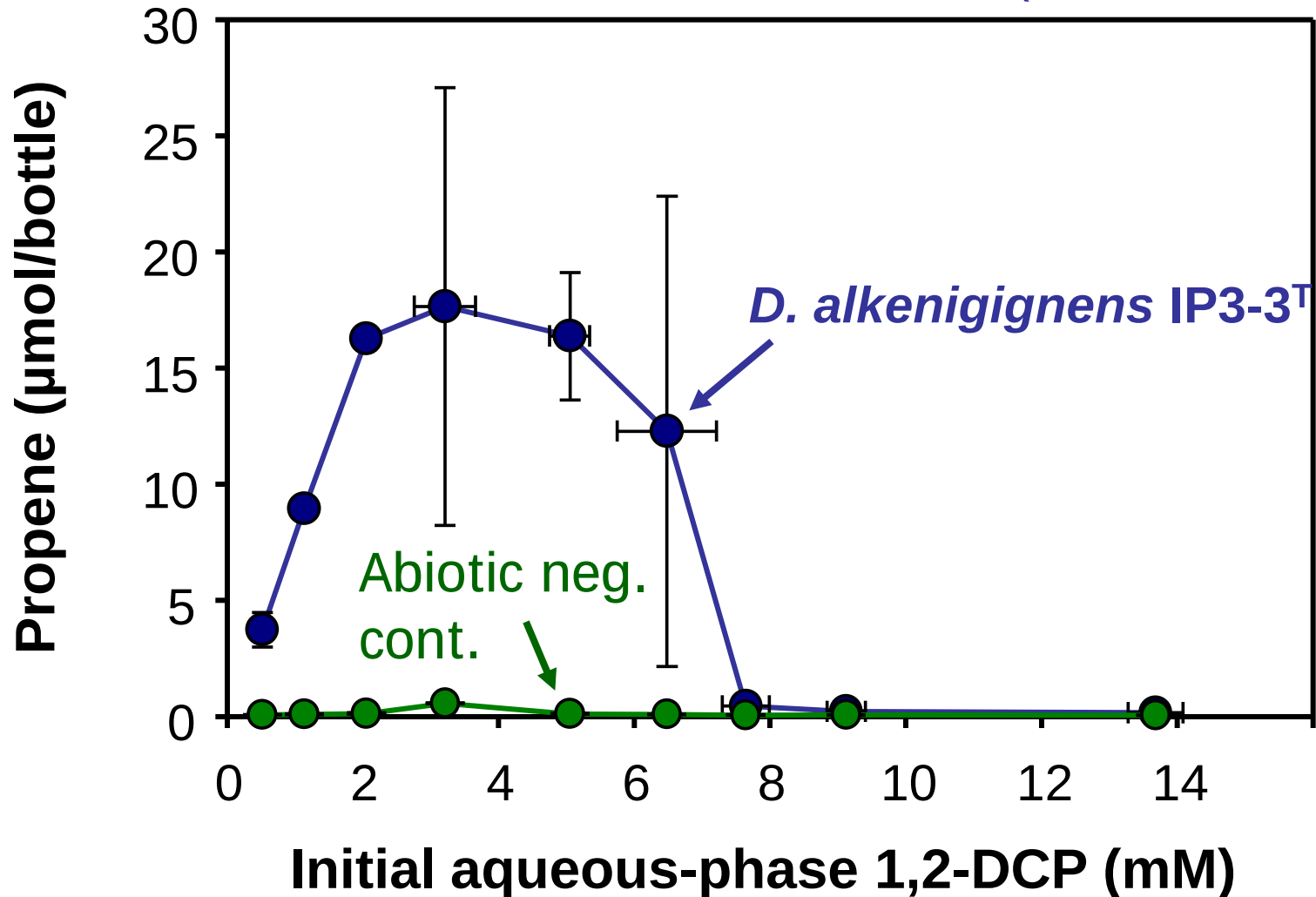
# Growth conditions (laboratory)

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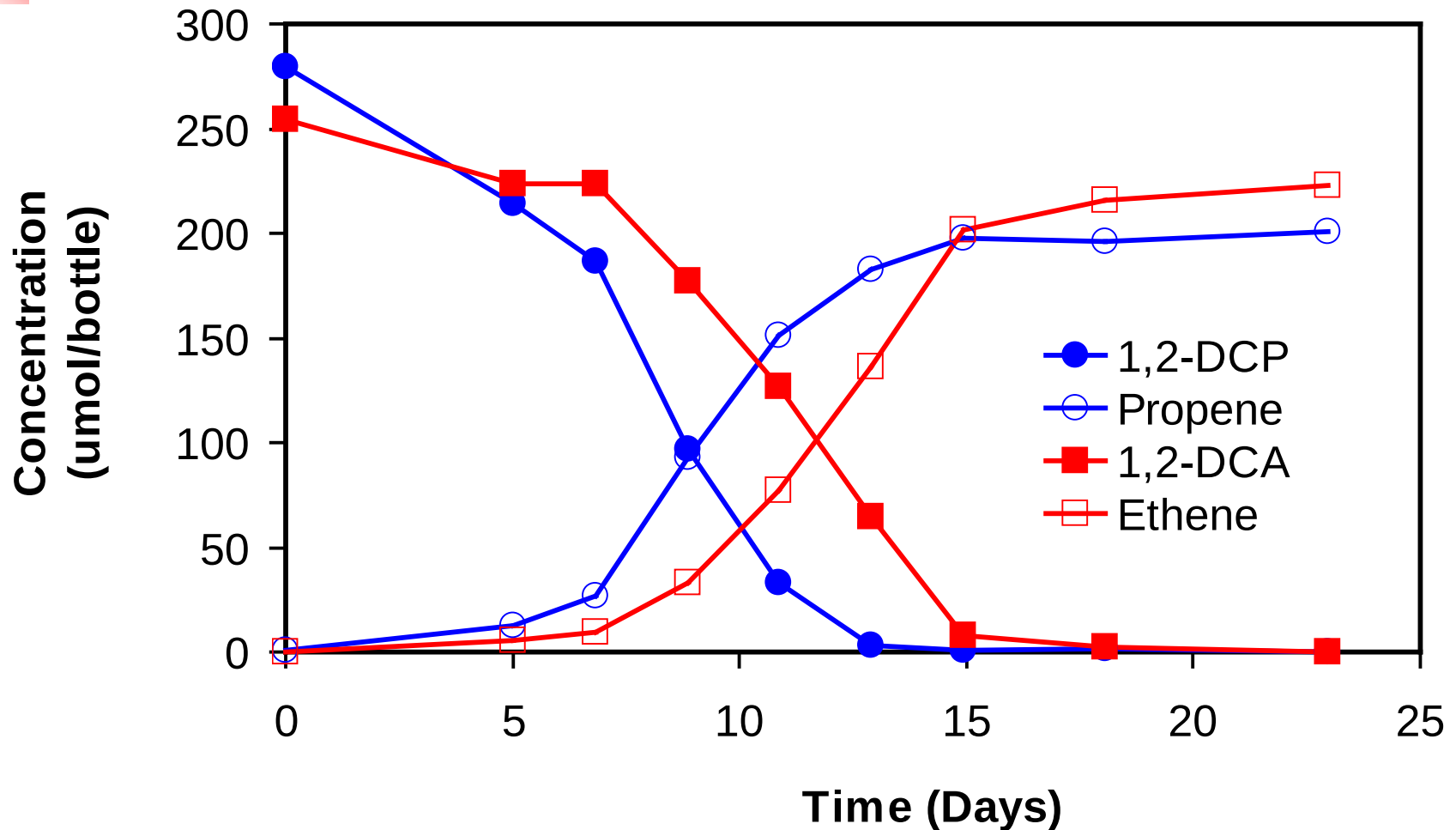
- Temperature range: 18-42°C (optimum 30-34°C)
- pH range 6-8 (optimum 6.5-7.5)
- Salinity (m/v):  $\leq 1\%$

# Strain IP3-3<sup>T</sup> with high concentrations of 1,2-DCP

(Maness *et al.*, 2012)

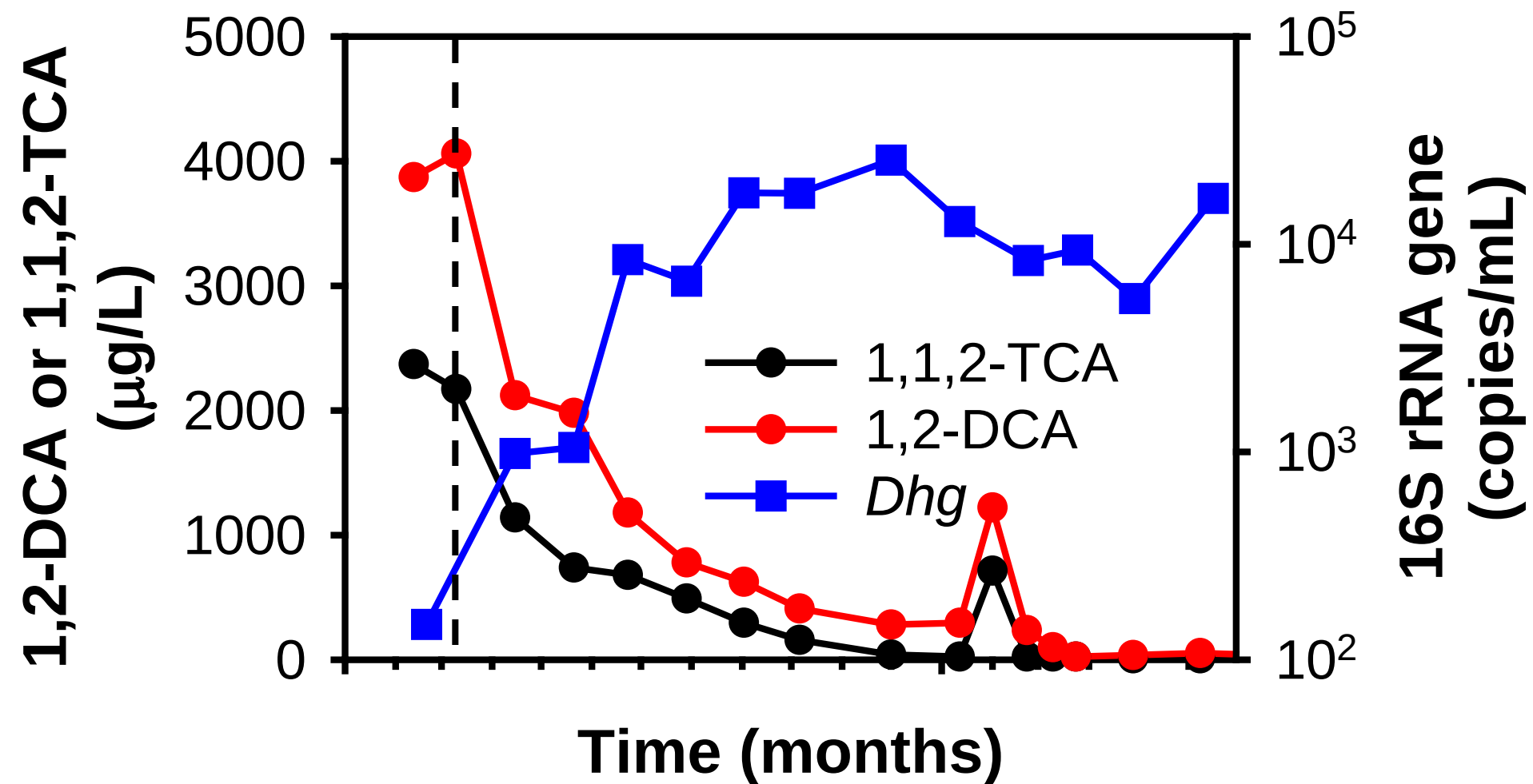


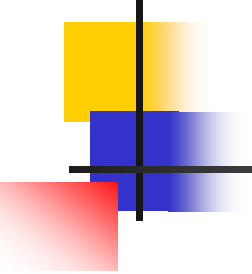
# Contaminant Mixtures (0.5 mM each 1,2-DCA and 1,2-DCP)



(Dillehay *et al.*, in press)

# Biostimulation: Field study

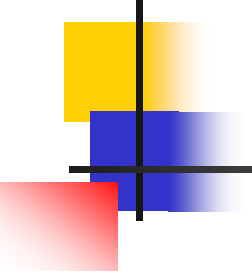




# Significant findings

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- There is considerable phylogenetic diversity among reductively dehalogenating members of phylum *Chloroflexi*
- *Dehalogenimonas* spp. can dehalogenate polychlorinated alkanes, even at high concentrations and in the presence of mixtures



# Significant findings

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- *Dehalogenimonas* spp. appear to respond to biostimulation as a remediation strategy in a manner similar to *Dehalococcoides* and other dechlorinators