

Alternative Electron Donor Utilization
in the Reductive Dechlorination
Processes by Organisms in the class
Dehalococcoidia: Dehalogenimonas Spp.

Trent Key and William Moe



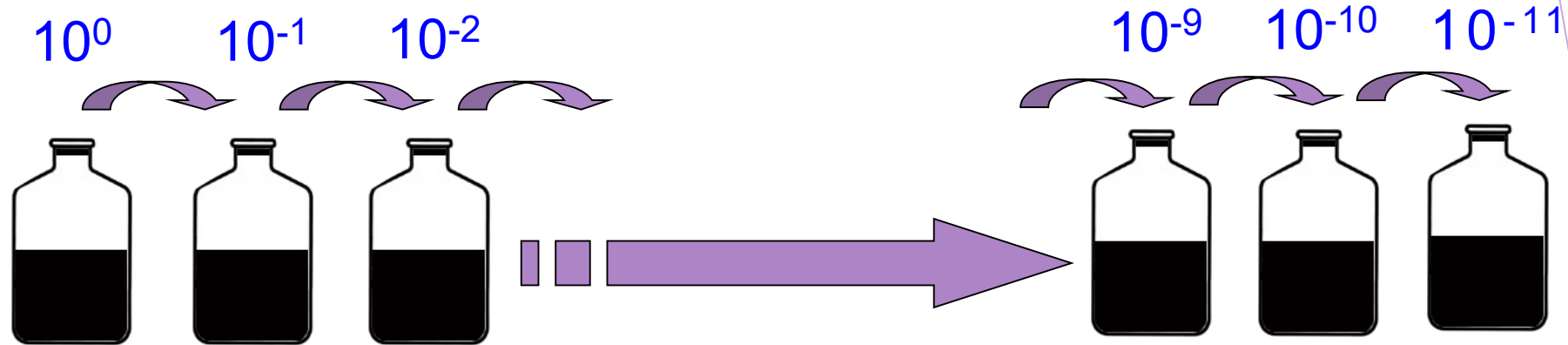
Overview

- ▶ Background and Isolation
- ▶ Electron Donor Utilization Experiments
- ▶ Genomic Analyses
- ▶ Conclusions

Background: Origin and Isolation

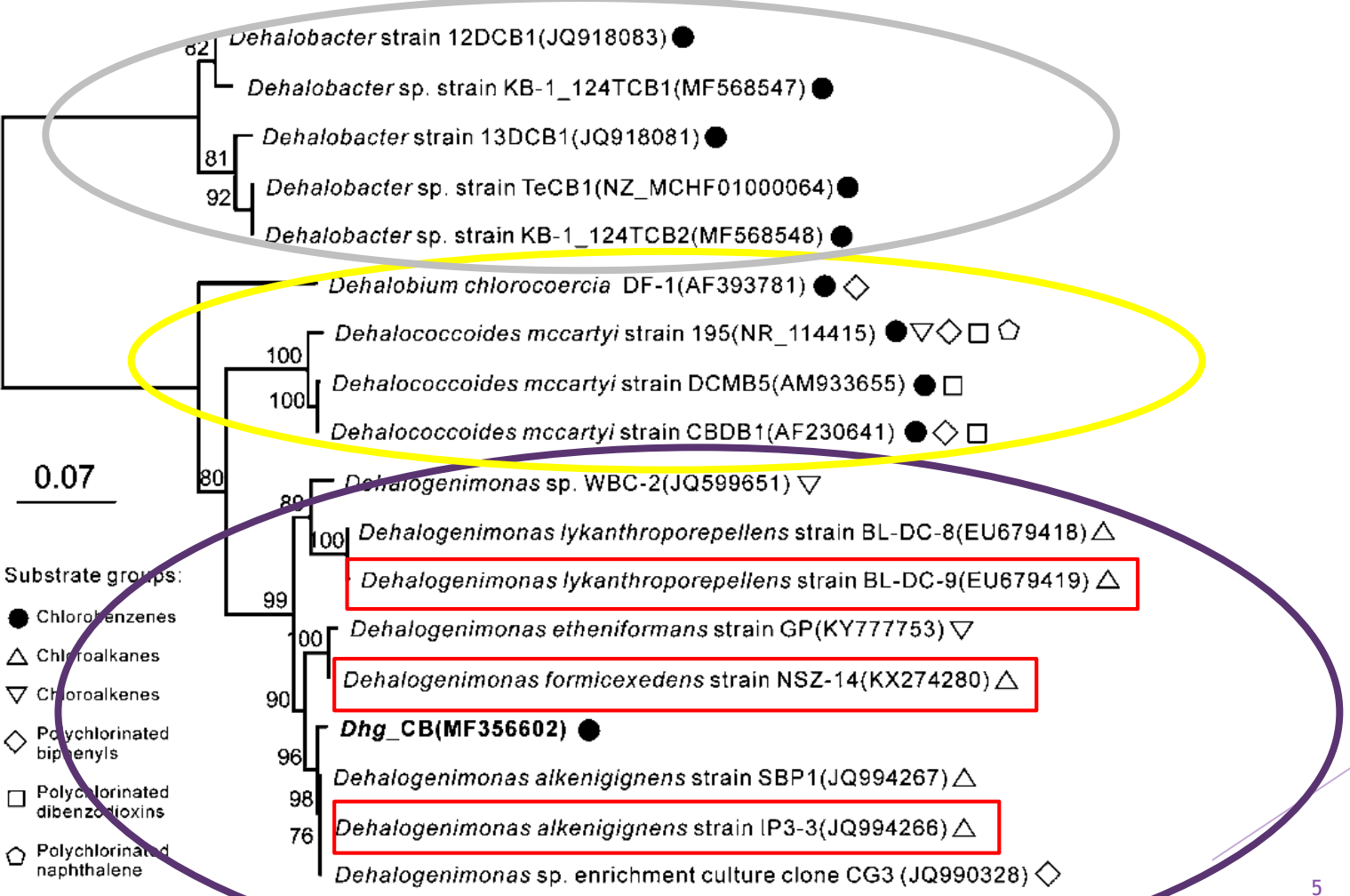
- ▶ Genus *Dehalogenimonas*
 - ▶ *D. lykanthroporepellens* (Moe *et al.*, 2009)
 - ▶ *D. alkenigignens* (Bowman *et al.*, 2013)
 - ▶ *D. formicexedens* (Key *et al.*, 2017)
- ▶ Both species isolated from Superfund Site in South Louisiana
 - ▶ Waste disposal from 1969 to 1980
 - ▶ Contaminants include
 - ▶ 1,2-DCA, 1,2-DCP, 1,1,2,2-TeCA, 1,1,2-TCA, VC

Isolation approach



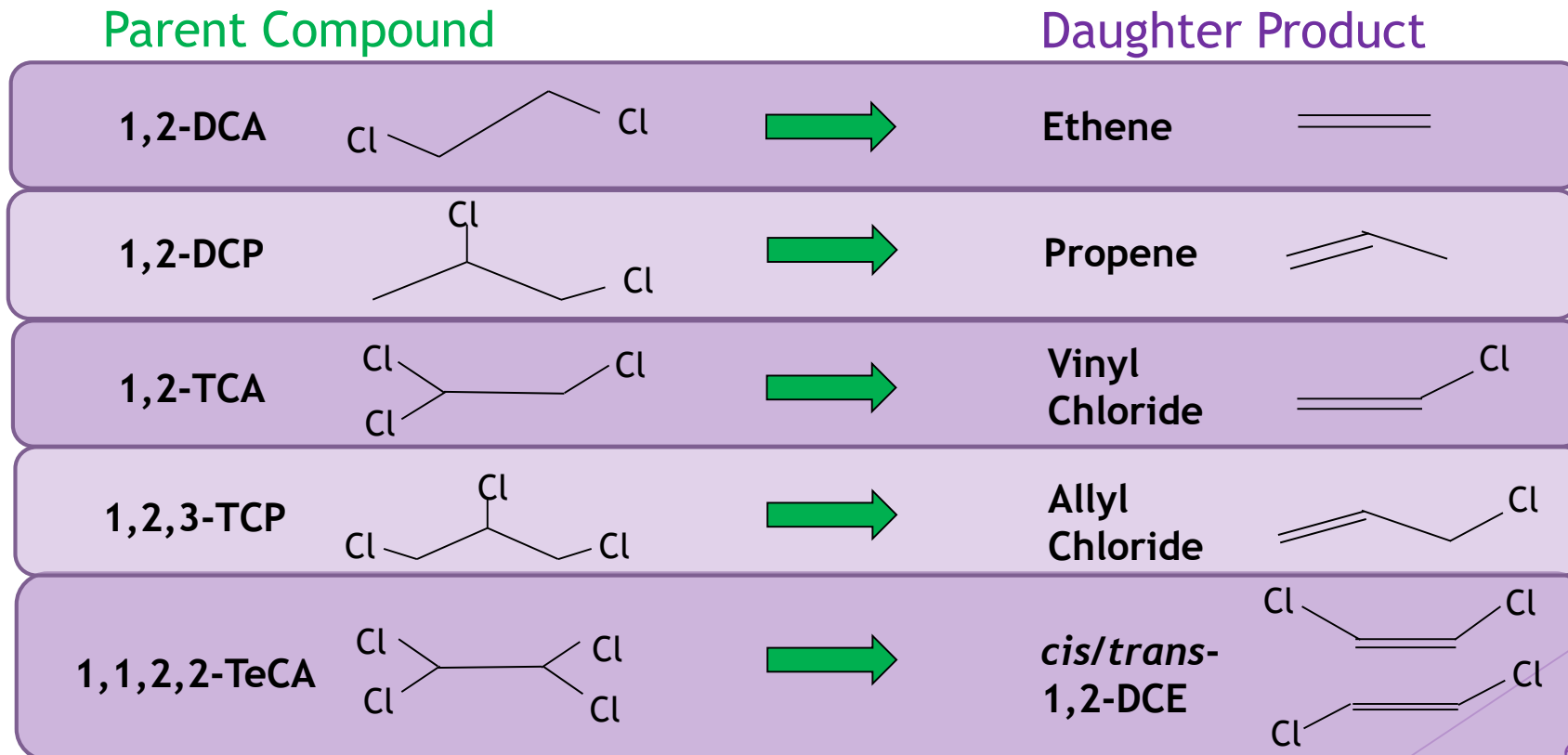
- Anaerobic, cysteine- and titanium-citrate reduced medium
- 1,1,2-TCA or 1,2-DCP as electron acceptor
- H_2 (10%, v/v) in gas headspace
- Ampicillin and vancomycin

Phylogenetic relationship based on 16S rRNA genes



Dehalorespiration

- ▶ *Dehalogenimonas spp.* dihaloelimination reactions



Electron acceptors for *Dehalogenimonas**

- ▶ 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

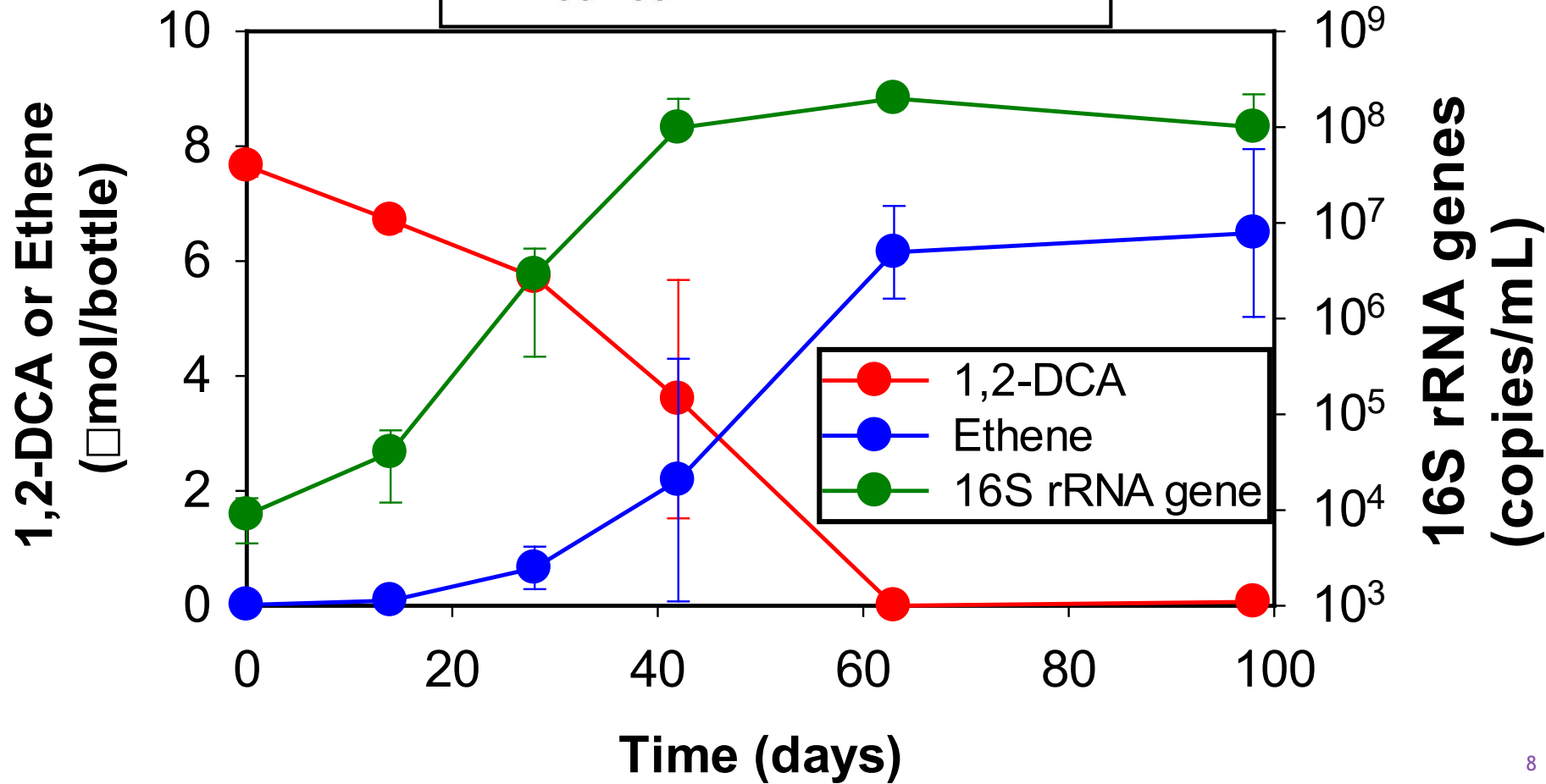
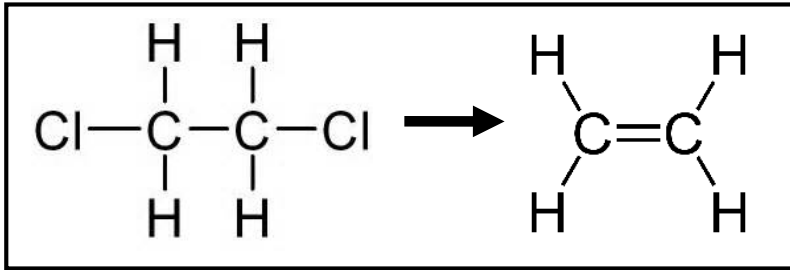
- ▶ Chlorinated alkenes

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

- ▶ Chlorinated benzenes

- ▶ 1-Chlorobenzene
- ▶ 1,2-Dichlorobenzene

D. formicexedens NSZ-14^T grown on 1,2-DCA



Alternative Electron Donor Utilization

Experiment: Screening

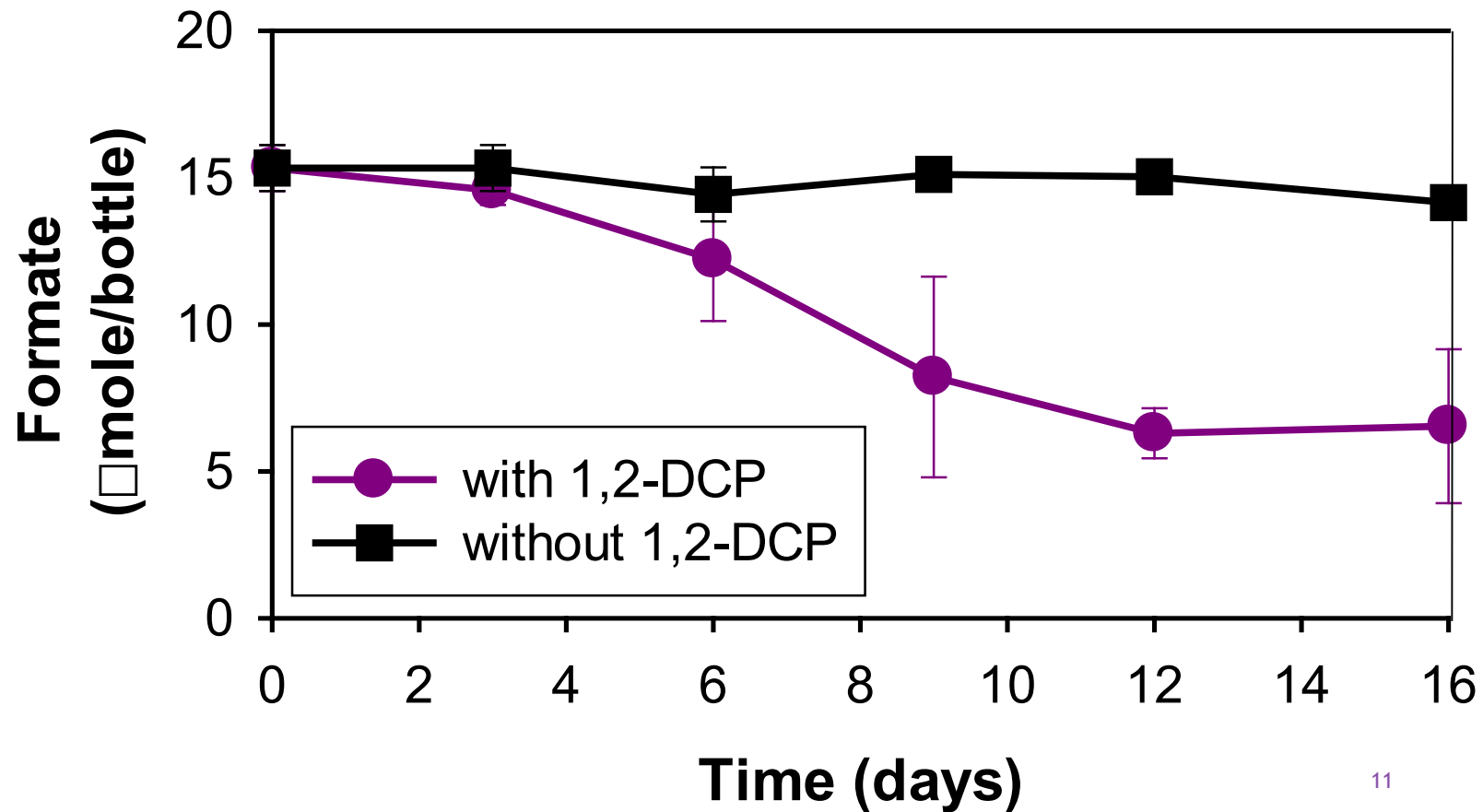
- ▶ Three *Dehalogenimonas* isolates in triplicate amended with 0.5 mM 1,2-DCP and oversupplied electron donor (5 mM)
 - ▶ Negative controls (uninoculated)
 - ▶ Positive controls (H₂ in headspace)
- ▶ Alternative electron donors/substrates evaluated:
 - ▶ Citrate, formate*, methyl ethyl ketone, propionate, pyruvate, starch, succinate, and yeast extract
- ▶ Analytical
 - ▶ GC for parent/daughter compound analysis

Alternative Electron Donor Utilization

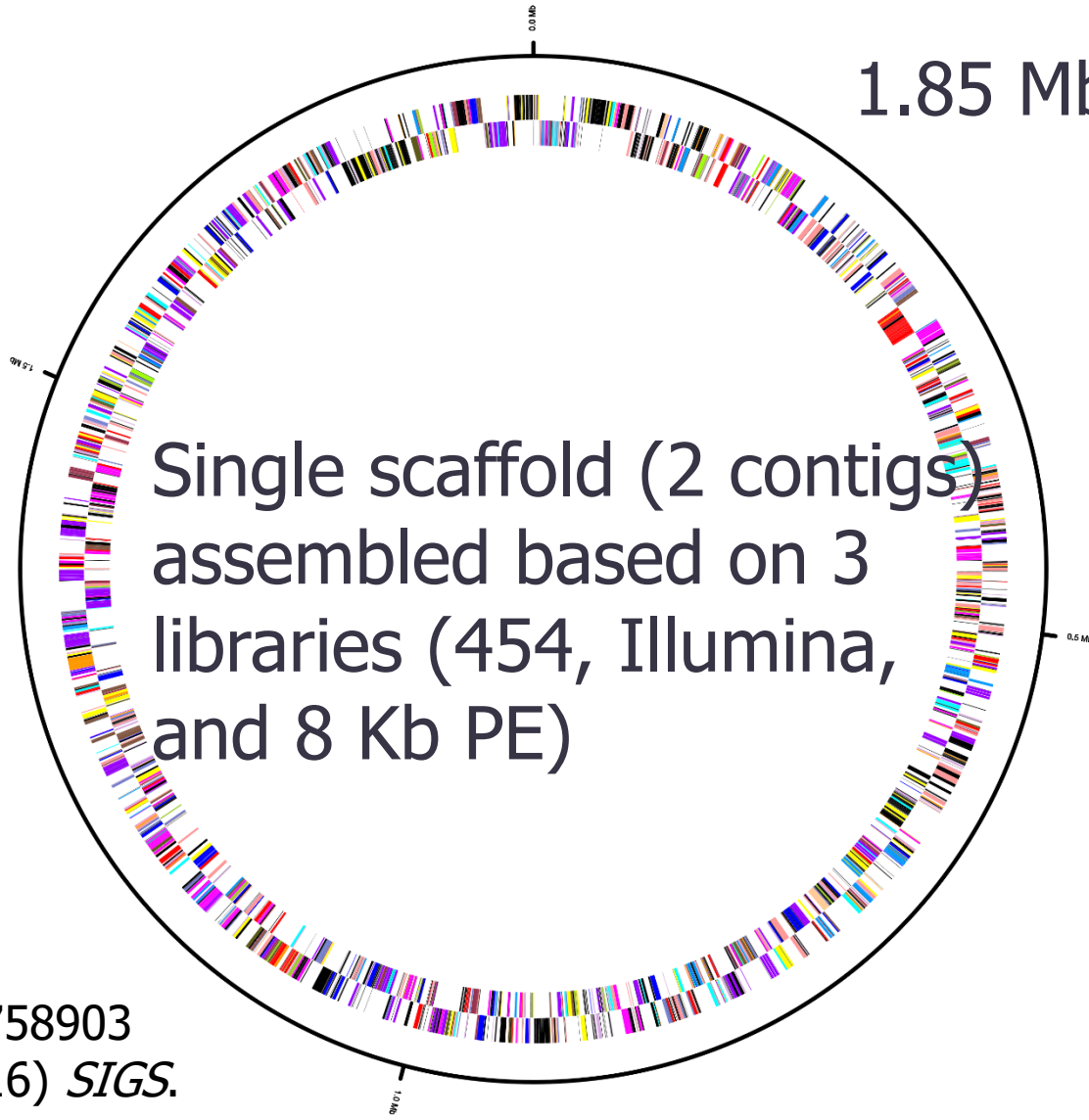
Experiment: Formate

- ▶ Three *Dehalogenimonas* isolates in triplicate amended with 0.5 mM 1,2-DCP and formate as electron donor (1.0 mM)
 - ▶ Negative controls (uninoculated)
 - ▶ Positive controls (H₂ in headspace)
- ▶ Analytical
 - ▶ GC for parent/daughter compounds
 - ▶ IC for formate
 - ▶ qPCR for enumeration

D. formicexedens NSZ-14^T formate consumption linked to dechlorination



D. alkenigignens IP3-3^T genome



1.85 Mbp genome

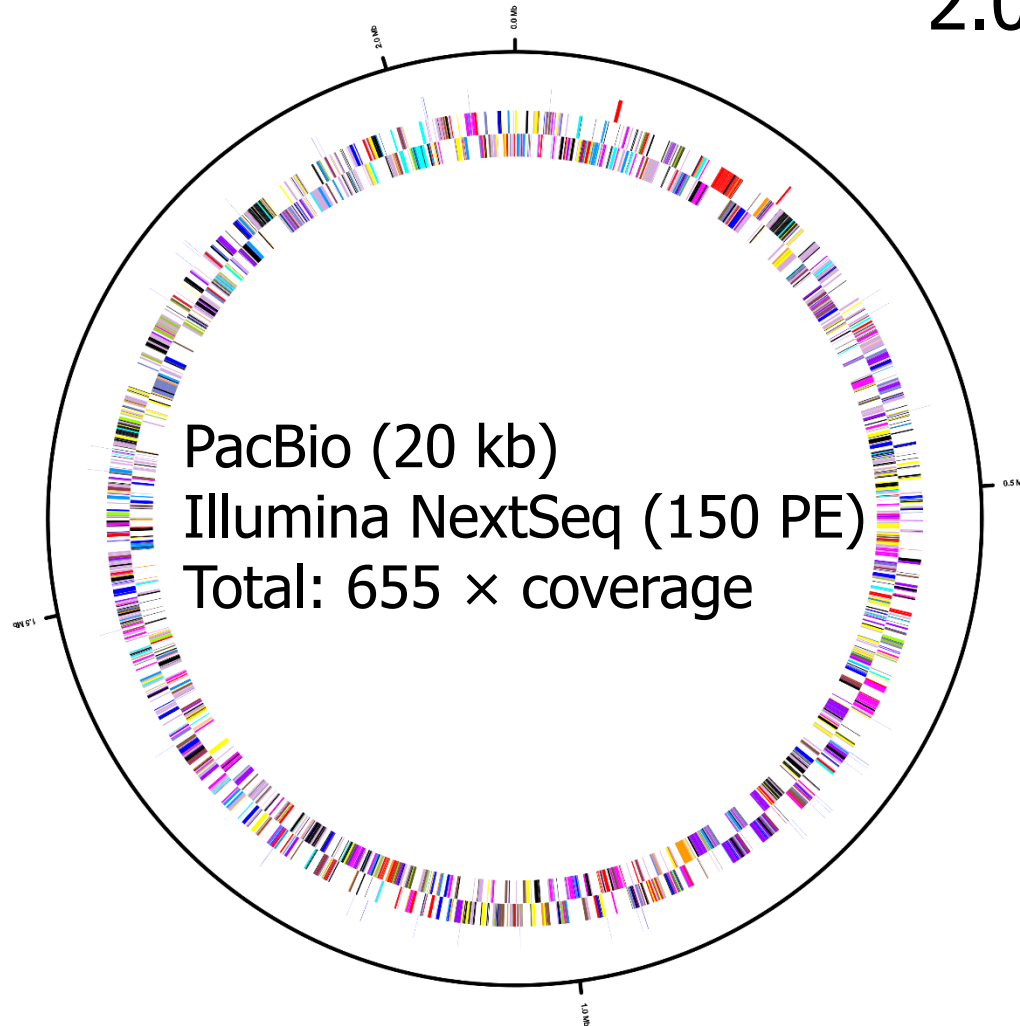
29 *rdhA* genes
(27 “full length”)

Most lack cognate
rdhB genes

Closest RdhA matches
in GenBank based
on PSIBLAST are from
Dhg, *Dhc*, and
uncultured bacteria

D. formicexedens NSZ-14^T genome

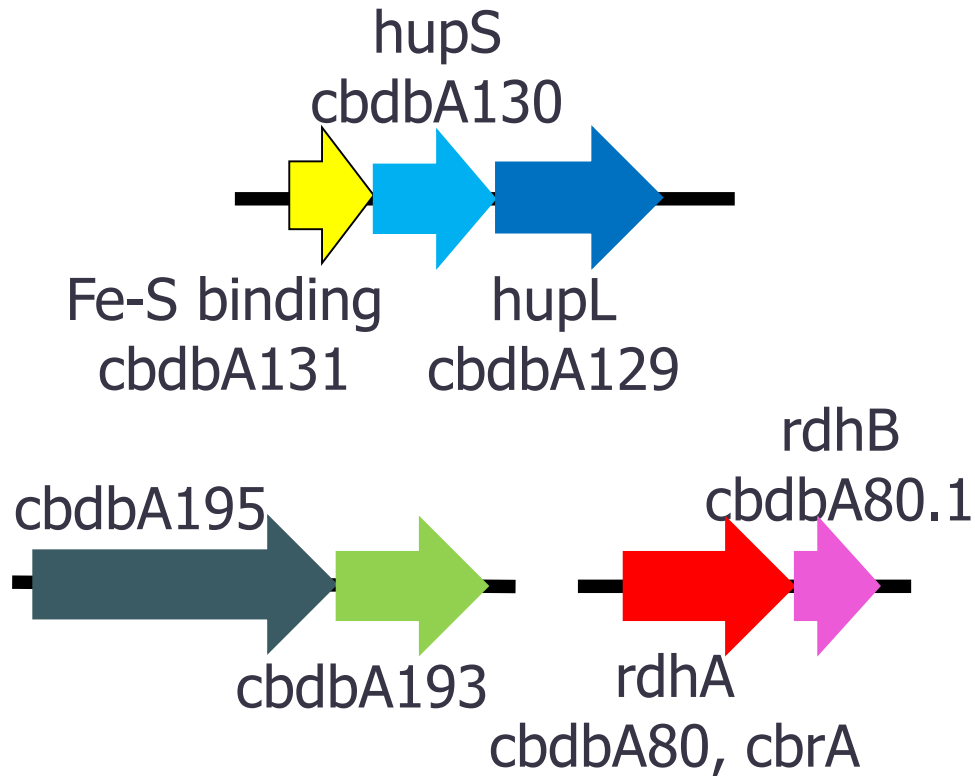
2.09 Mbp genome



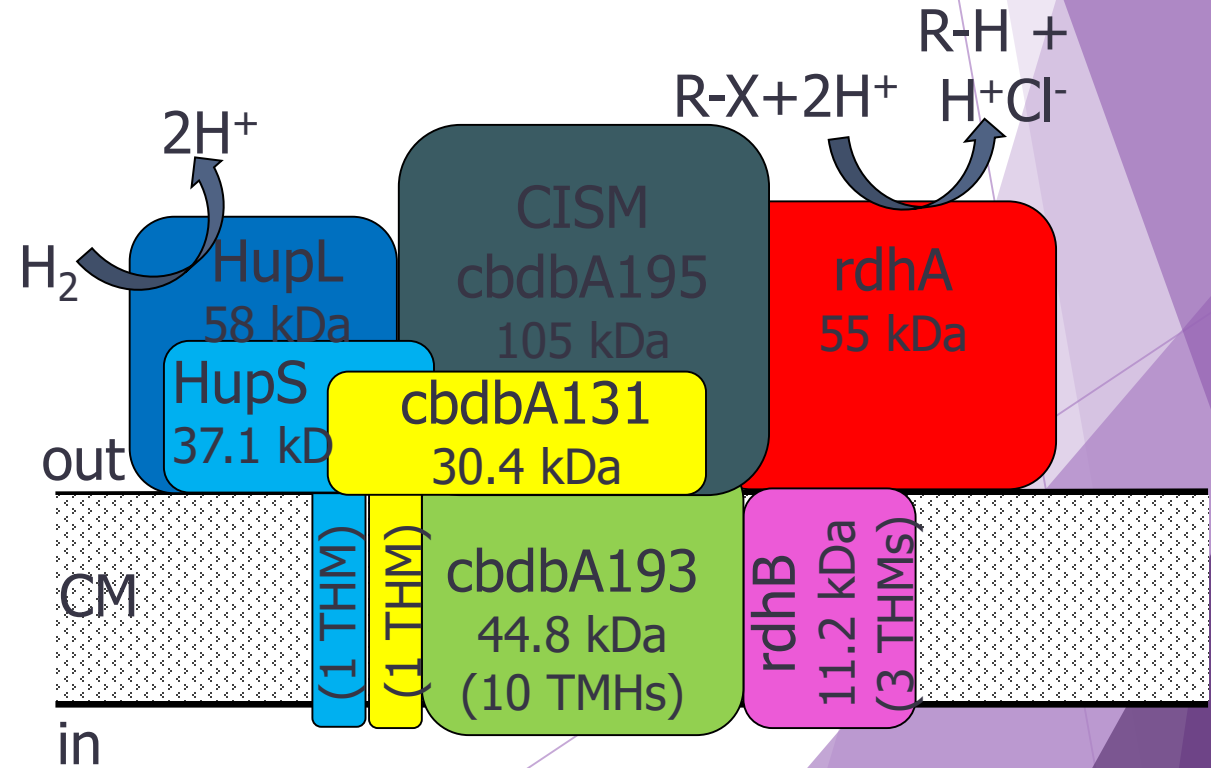
25 *rdhA* genes
(23 “full length”)

8 *rdhB* genes
(1 or 2 orphans)

Multi-protein reductive dehalogenase complex in *Dehalococcoides mccartyi* CBDB1



CISM=complex iron-sulfur molybdoenzyme (formerly "FDH")



Kublik *et al.* (2016), *Environ. Microbiol.* 18(9):3044-3056.

Genes annotated as encoding formate dehydrogenases

(aligned starting at pos 189 in Dform_00419)

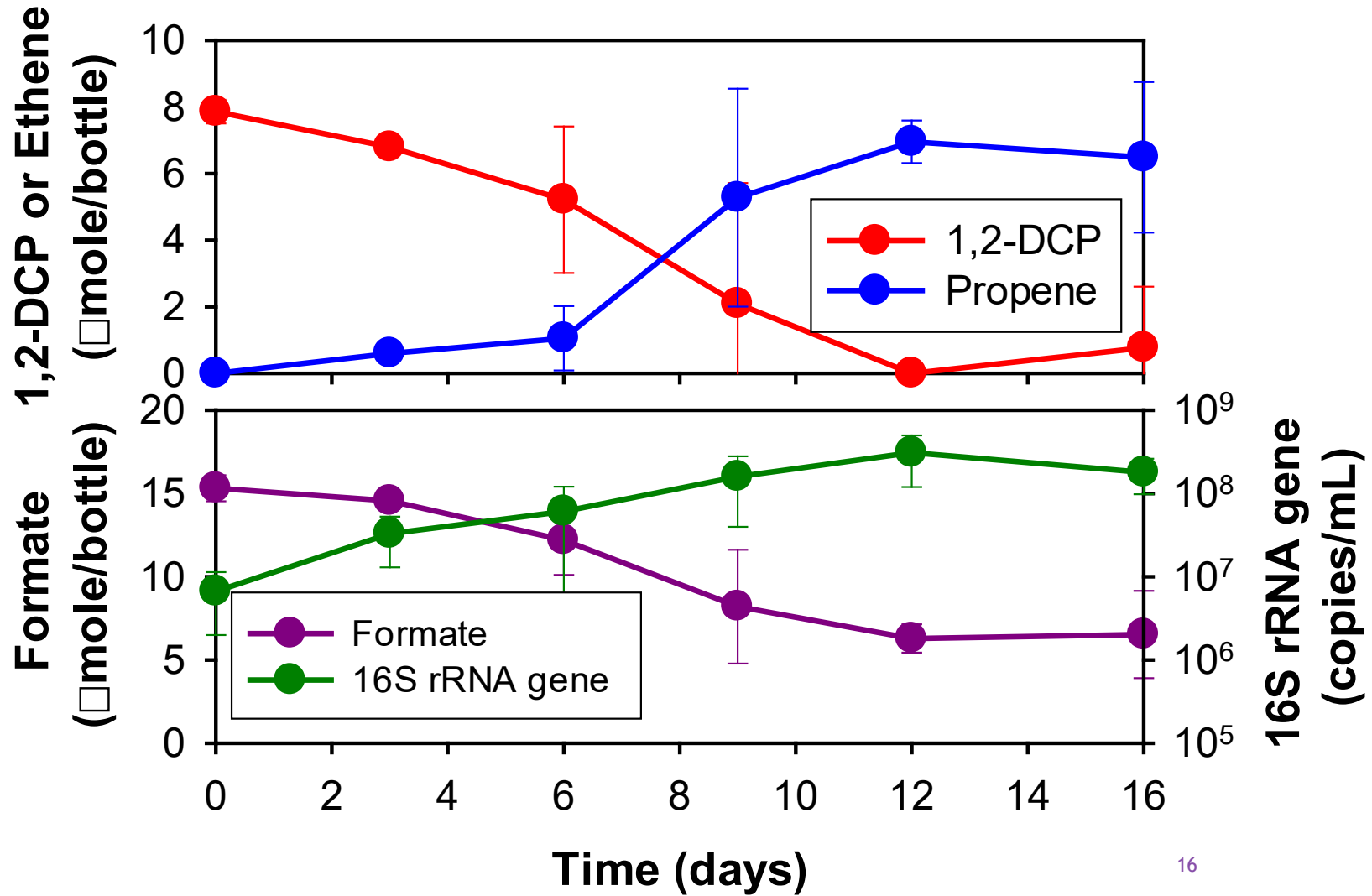
| | |
|---|---------------------|
| Strain NSZ-14 ^T (Dform_00419, 1,069 aa) | QARLUHSSTVASLAESFG |
| <i>D. lykanthroporepellens</i> BL-DC-9 ^T (BK009976, 1,027 aa) | QARLUHSSTVASLAESFG |
| <i>D. alkenigignens</i> IP3-3 ^T (DEALK_19115, 1,029 aa) | QARLUHSSTVASLAESFG |
| ^a <i>Dehalococcoides mccartyi</i> 195 ^T (DET0187, 993 aa) | QARLS TASSLEALAASFG |
| <i>Desulfomonile tiedjei</i> DSM 6799 ^T (Desti_2315, 1,010aa) | QARIUHSATVAALAESFG |

U=selenocysteine

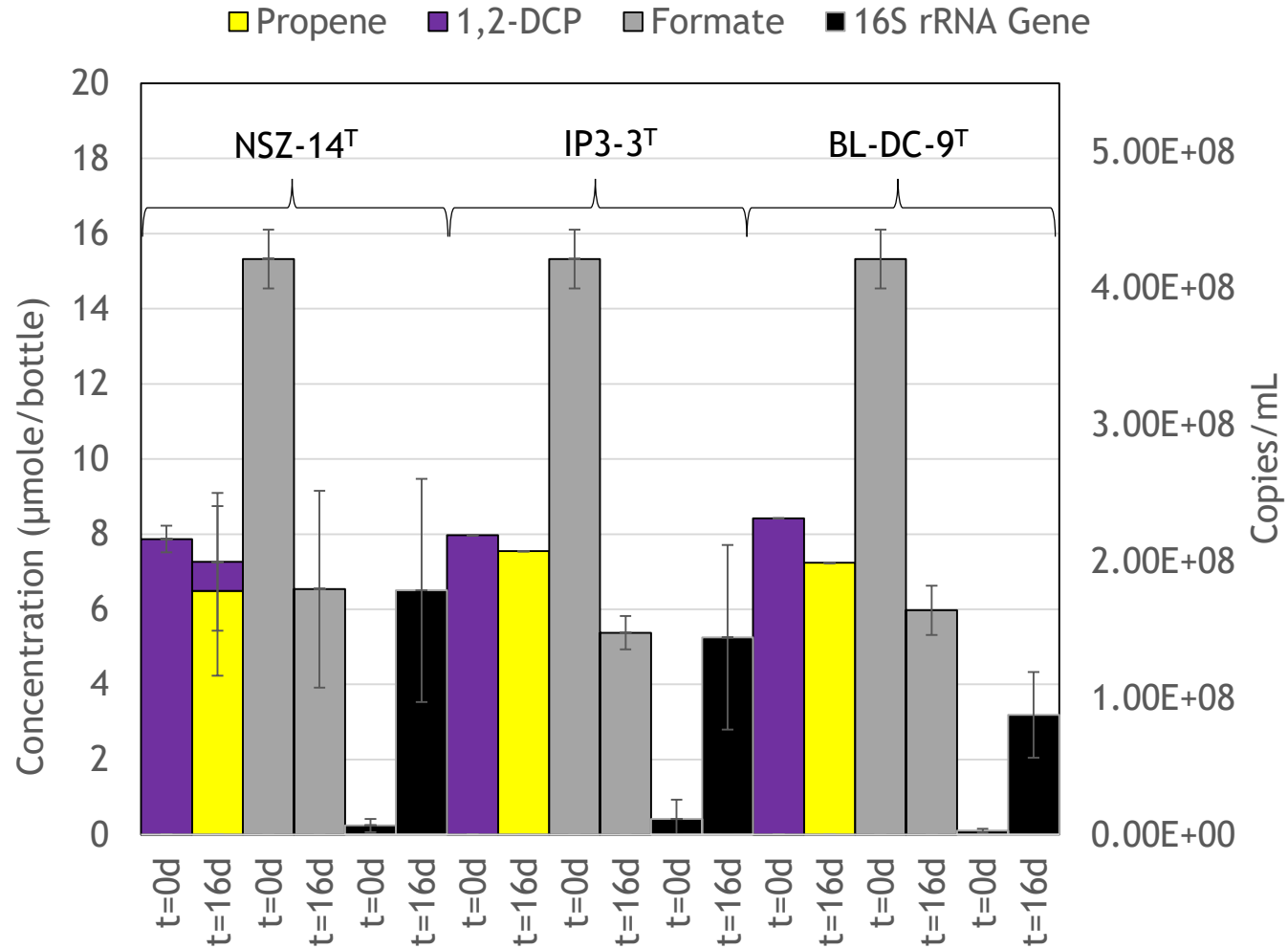
S=serine

^a*Dehalococcoides mccartyi* strains identical in the comparison region shown: 11a (CY91_04325), 11a5 (Dm11a5_0194), BAV1 (DehaBAV1_0165), BTF08 (btf_128), CBDB1 (cbdbA195), CG4 (X793_00865), CG5 (X794_00795), DCMB5 (dcmb¹⁵_191), GT (DehalGT_0247), IBARAKI (IBK_0232), MB (DA01_02820), and SG1 (WP_034376812)

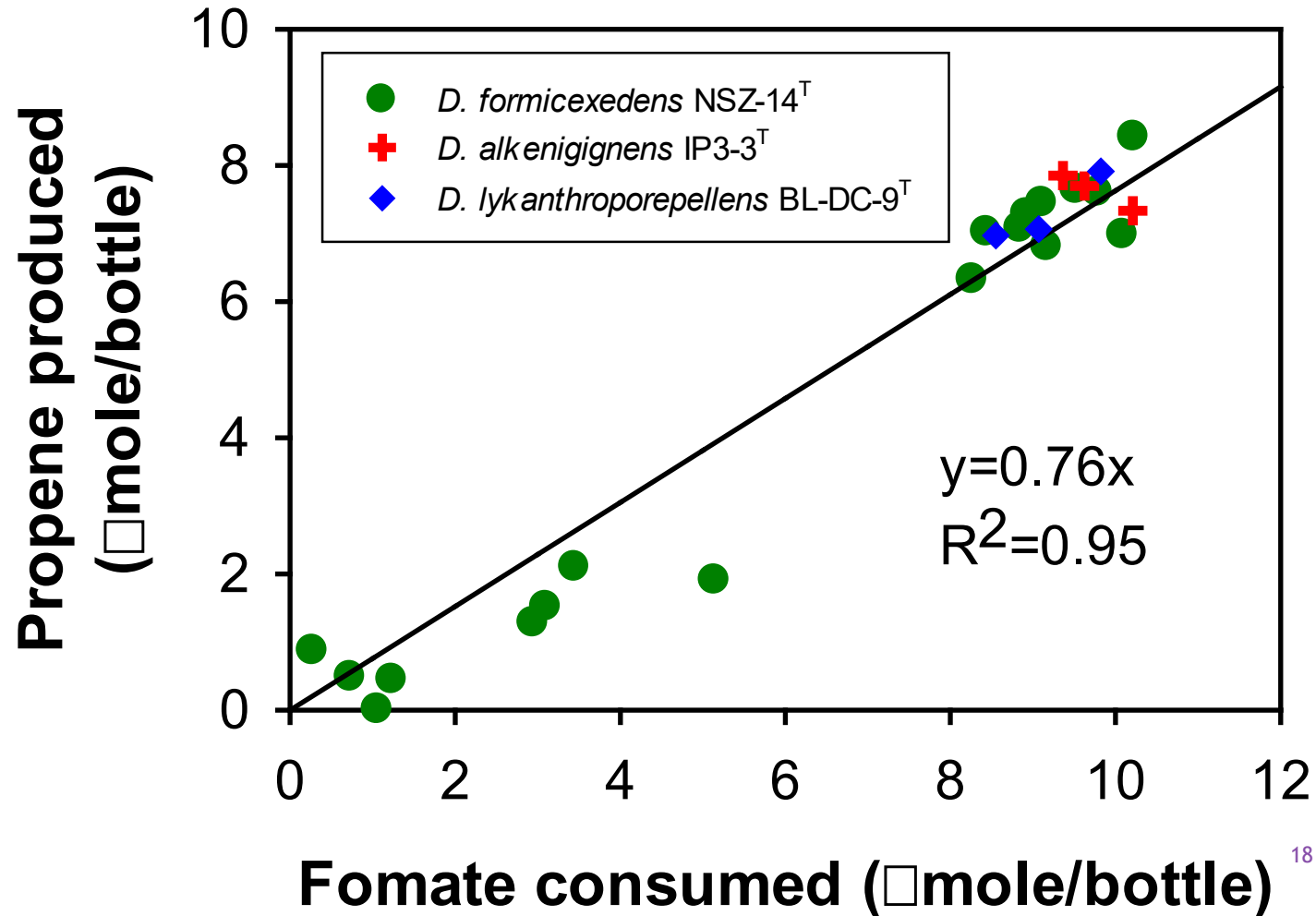
Formate as electron donor



Formate as electron donor



Formate utilization during 1,2-DCP dechlorination



Conclusions

- ▶ There is considerable phylogenetic diversity among reductively dehalogenating members of phylum *Chloroflexi*
- ▶ *Dehalogenimonas* spp. can use H₂ and **formate** as electron donors for dechlorination
- ▶ Field scale tests area needed to further evaluate practical use of formate as a biostimulation amendment (source zones?)
- ▶ There is much more work to do regarding functional characterization of genes

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- ▶ Other contributors
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Questions?