Genome-Enabled Molecular Tools for Reductive Dehalogenation

- A Shift in Paradigm for Bioremediation -

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Genome-Enabled Molecular Tools for Reductive Dehalogenation

Chloroethenes, including vinyl chloride, are widespread groundwater pollutants. A comparative genomic analysis revealed that the vinyl chloride reductase operon, vcrABC, of Dehalococcoides sp. strain VS is embedded in a horizontally-acquired genomic island that integrated at the single-copy gene ssrA. The high similarity between vcrABC sequences from diverse Dehalococcoides is quantitatively consistent with recent horizontal acquisition driven by ~100 years of industrial pollution with chlorinated ethenes. Moreover, most rdh genes relevant for bioremediation appear to be predominantly acquired by Dehalococcoides via this mechanism. This talk will discuss the implications of these finding for development and use of molecular tools for monitoring and predicting in situ remediation of chloroethenes via anaerobic reductive dehalogenases.
GENOME-ENABLED MOLECULAR TOOLS FOR REDUCTIVE DEHALOGENATION

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The ‘old’ paradigm

Bioremediation is mediated by a single important gene in a single strain that is robust to environmental perturbations

= Dehalococcoides sp.

= reductive dehalogenase genes
Reductive dehalogenation of chloroethenes as a microbial community process
Reductive dehalogenation of chloroethenes as a microbial community process

Dehalobacter sp.
Desulfitobacterium sp.
Dehalospirillum sp.
Geobacter sp.
Dehalococcoides sp.

Dehalococcoides sp.
Reductive dehalogenation of chloroethenes as a microbial community process

Dehalobacter sp.
Desulfitobacterium sp.
Dehalospirillum sp.
Geobacter sp.
Dehalococcoides sp.

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Müller et al. AEM 2004
16S rRNA gene-based phylogeny

Dehalococcoides sp. GT
Dehalococcoides sp. BAV1
Dehalococcoides sp. CBDB1
Dehalococcoides ethenogenes 195
Dehalococcoides sp. VS
Dehalogenimonas lykanthroporepellens BL-DC-9

hupL-based phylogeny

Dehalococcoides sp. GT
Dehalococcoides sp. CBDB1
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Evolution of reductive dehalogenation traits in *Dehalococcoides sp.*

Reductive dehalogenase genes move as mobile elements in *Chloroflexi* populations

The ‘old’ paradigm

The new paradigm

= Dehalococcoides sp.

= reductive dehalogenase genes
Reductive dehalogenation of chloroethenenes as a microbial community process

Dehalobacter sp.
Desulfitobacterium sp.
Dehalospirillum sp.
Geobacter sp.
Dehalococcoides sp.

Dehalococcoides sp.
Reductive dehalogenation of chloroethenenes as a microbial community process

Dehalobacter sp.
Desulfitobacterium sp.
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Geobacter sp.
Dehalococcoides sp.

Dehalococcoides sp.

$0.5 - 2 \text{ nM } H_2$

PCE $\rightarrow$ TCE $\rightarrow$ cisDCE $\rightarrow$ VC $\rightarrow$ Ethene

$\text{pceA}$ $\rightarrow$ $\text{tceA}$ $\rightarrow$ $\text{vcrA}$

Acetate
CH$_4$
H$_2$S
Fe$^{2+}$

$\text{vcrA}$
$'bvcA'$

primary fermenters secondary fermenters

$\text{H}_2$ thresholds
30-300 nM $H_2$
$\sim 10$ nM $H_2$
1-10 nM $H_2$
How to assess gene dynamics and H$_2$ flux in heterogeneous environments?
How to assess gene dynamics and H$_2$ flux in heterogeneous environments?

Design of a tiled functional gene array

Hydrogenase gene array (H$_2$ase Chip) 

~2300 diverse hydrogenase genes

Reductive dehalogenase gene array (Rdh Chip) 

~280 diverse rdh genes
H₂ flux in complex environments

5L Lactate/PCE Chemostat

Analysis by H₂ase gene chip

Hydrogenase chip can predict H₂ flux in complex communities

How do different (indirect) H₂ donor effect Dhc populations?

Dynamic changes in *Dehalococcoides* populations upon change in H$_2$ source

The new paradigm

Bioremediation is largely due to activity of mobile genes in heterogeneous populations that exhibit fluctuations upon environmental perturbations.

Electron donor: formate, lactate, propionate

= Dehalococcoides sp.

= reductive dehalogenase genes
How do competing *electron acceptors* effect Dhc populations?
How do competing electron acceptors effect Dhc populations?

**SO₄²⁻ addition**

**SO₄²⁻ addition**

Dynamic change in Dehalococcoides population upon alternate acceptor addition
PM5L chemostat batch cultures (sulfide vs. sulfate addition experiments)

Sulfide addition is sufficient to explain *Dehalococcoides* population shift
Useful molecular tools reveal a shift in paradigm for reductive dehalogenation in complex environments.

The new paradigm

Chloroethene bioremediation is largely due to dynamic, evolving, and heterogeneous populations of dehalogenating *Chloroflexi* that respond differentially to environmental perturbations.
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