Comparison of four Cupriavidus metallidurans strains using Phenotype MicroArray™ analysis

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Overview

- Introduction: *Cupriavidus metallidurans*

- Comparative Genome Hybridization (CGH) of 14 *C. metallidurans* strains

- Results and discussion of Phenotype MicroArray™ analysis of 4 *C. metallidurans* strains

- Conclusions
Cupriavidus metallidurans

- Often isolated from industrial sites
  - mining-, metallurgical-, and chemical industries

- And other
  - space-related environments
  - patients with cystic fibrosis
  - causative agent of an invasive human infection

- Type strain: CH34

- Full genome sequence is available

Janssen *et al.*, 2010; Langevin *et al.*, 2011; Mijnendonckx *et al.*, 2013
Cupriavidus and Ralstonia genera

- Class: β-Proteobacteria; Order: Burkholderiales; Family: Burkholderiaceae

- Degradation of xenobiotics
  - Volcanic ashes
  - Industrial biotopes
  - Nitrogen fixation
  - Hydrogenotrophy

- Industrial biotopes
  - Opportunistic pathogens
  - Plant pathogens

- Cupriavidus metallidurans

- Cupriavidus pinatubonensis JMP 134
  - Cupriavidus bahamensis
  - Cupriavidus basilevskyi
  - Cupriavidus bilatensis

- Cupriavidus campinensis
  - Ralstonia insidiosa
  - Ralstonia pickettii

- Ralstonia mannitolilytica
  - Ralstonia solanacearum
  - Ralstonia syzygii

- Pandoraea apista
Numerous contamination events of ISS water systems by C. metallidurans

Potable water on ISS:
- Water used for crew consumption, including food rehydration

Two types of water coexist on ISS
- Russian standards
  - Silver (1.9 µM ≤ [Ag] ≤ 4.6 µM)
  - Not removed before consumption
- US standards
  - Iodine ([Total iodine] = 6.0 mg/L, 1.0 mg/L ≤ [residual iodine] ≤ 4.0 mg/L)
  - Removed before consumption

Russian and US water are not mixed

Van Houdt et al., 2012; Mijnendonckx et al., 2013
Isolated from different sources:
  Industrial – potable water – human

Associated risks \( \rightarrow \) especially potable water ISS

Astronauts \( \rightarrow \) decreased immune system

Pathogenic potential ?

What are differences between different strains ?
  Data from comparative genome hybridization
Cupriavidus metallidurans strains

- Comparative whole genome hybridization
- A set of 14 different strains
- To gain insights in:
  - Conservation of genes (and traits)
  - Horizontal transfer/acquisition of genes
  - Evolutionary forces shaping the species
<table>
<thead>
<tr>
<th>Strain</th>
<th>Isolation site</th>
<th>Isolation place</th>
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<tbody>
<tr>
<td>CH34&lt;sup&gt;T&lt;/sup&gt;</td>
<td>Decantation tank, zinc factory</td>
<td>Liège, Belgium</td>
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<tr>
<td>CH42</td>
<td>Polluted sediments, zinc factory</td>
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<td>CH79</td>
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<td>31A</td>
<td>Galvanisation tank, metal factory</td>
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<td>Mine tailings</td>
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<tr>
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<td>NE12</td>
<td>Assembly facility Kennedy Space Center</td>
<td>Florida, USA</td>
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<td>NA1</td>
<td>SVO-ZV with Russian ground-supplied water</td>
<td>International Space Station</td>
</tr>
<tr>
<td>NA2</td>
<td>American contingency water container</td>
<td>International Space Station</td>
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<tr>
<td>NA4</td>
<td>filter of the Russian SRV-K module</td>
<td>International Space Station</td>
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CGH: general comparison

| Van Houdt et al., 2012 |
### CGH: genomic islands on CHR1

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<td>putative fimbrial operon</td>
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Van Houdt *et al.*, 2012
Cupriavidus metallidurans

- Metal resistance determinants are highly conserved among C. metallidurans strains independent from their isolation place
  - acquired these functions long ago? (biased towards anthropogenic environments?)

- GI/MGEs more specific for metal polluted environments
  - Acquired by HGT, interaction with different populations? More environmental pressure?

- What are the differences in phenotype?

Van Houdt et al., 2012; Mijnendonckx et al., 2013
Cupriavidus metallidurans

Biolog shows us the way...

... It’s the Phenotype MicroArray™

(Slightly modified from the lyrics of “No hidden path” by Neil Young)

Van Houdt et al., 2012; Mijnendonckx et al., 2013
<table>
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**Selected strains**
Set up of PM analysis:

According to Biolog’s instruction with minor modifications

- **Inoculum:**
  - Grown on LB agar for 48 hours at 30 °C
  - Suspended in IF-0, OD$_{600}$ = 0.2
  - 1:50 dilution in IF-0 with dye mix A

- **Carbon source used for PM 3, 6, 7, and 8**
  - 2 mM of sodium succinate and 2 µM of ferric citrate

- **Incubation at 30 °C for 6 days**

- **Data analysis with OmniLog® PM kinetic analysis software (version 1.6) and OPM an R package**
  - Based on max height
  - Heat maps and radial plots
Phenotype MicroArray™ analysis
PM 1 MicroPlate™: Carbon sources

- Heat map of PM 1: carbon sources

Mostly carboxylic acid and some amino acids
Almost all carbohydrates

NA4
CH34
H1130
NA1
Phenotype MicroArray™ analysis
PM 1 MicroPlate™: Carbon sources

- Heat map of PM 1: carbon sources

D-aspartic acid was not used by strain CH34
Sodium formate was not used by strain H1130
D-alanine was not used by strain NA1
Heat map of PM 2A: carbon sources

- All carbohydrates, all polymers, all alcohols, most amine, amide and esters
- Carboxylic acids and amino acids
Radial plot of sorbic acid, \( \gamma \)-amino-N-butyric acid, L-valine, L-Leucine, and glycine
Phenotype MicroArray™ analysis
PM 3 MicroPlate™: Nitrogen sources

- Heat map of PM 3B: nitrogen sources

- Very weak signal or no signal when inorganic nitrogen is the N-source
- Amino acids
  - No signal when L-arginine or L-methionine is the N-source
  - Strong signals for all strains when L-serine, L-aspartic acid, L-threonine, L-asparagine, L-glutamic acid, L-histidine, L-glutamine, L-proline, or L-phenylalanine is the N-source
Radial plot of L-leucine, L-alanine, D-asparagine, L-valine, and L-tryptophan

- Amino acids
  - Type strain CH34 is not able to use L-tryptophan
    - Reduced signals on PM6, 7 and 8 when Trp is part of a dipeptide
• Importance of L-tryptophan for type strain CH34

N-source: L-tryptophan or a Trp dipeptide

• No or reduced signal for type strain CH34

• Type strain CH34 has a stop codon in the kynB gene

Kurnasov et al., 2006
Phenotype MicroArray™ analysis
PM 9 & 10 MicroPlate™: Osmolytes and pH

- **PM9: Osmolytes**
  - All strains are sensitive to sodium chloride concentrations (> 1 %)
  - Other dose ranges of osmolytes give similar metabolic activity curves

- **PM10: pH**
  - ≥ 5
  - Type strains CH34 shows better growth in acidic pHs when certain amino acids are present
Chemical sensitivity plates
Strain CH34 (black), strain NA1 (blue), strain NA4 (yellow), and strains H1130 (purple)

- Amoxicillin: NA4 is more resistant
- Nafcillin: CH34 is more resistant
- Harmane: H1130 is more resistant
- Cytosine arabinoside: NA1 is more resistant
- No clear difference in heavy metal resistance
Conclusions

- Preferred carbon sources are carboxylic acids or amino acids, other carbon sources are less or not at all used.

- Strains show clear differences in amino acid preference as their nitrogen source.

- Differences in preferred carbon and nitrogen sources will be exploited for conjugation/mating assays between different C. metallidurans strains.

- Differences are observed in the chemical sensitivity plates:
  - No clear difference is observed on heavy metal resistance which is in agreement with previous studies.
  - Antibiotic resistance is not markedly higher/more diverse for H1130.

- Observed phenotypic differences will be further validated and studied by full genome sequencing.
Acknowledgements

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