Comparison of four Cupriavidus metallidurans strains using Phenotype MicroArray™ analysis

A. Van Assche[1], P. Monsieurs[2], C. Gaudreau[3], B. Lievens[1], K. Willems[1], N. Leys[2], & R. Van Houdt[2]

[1] Laboratory for Process Microbial Ecology and Bioinspirational Management, Departement of Microbial and Molecular Systems, KU Leuven association, Thomas More, campus De Nayer, Scientia Terrae Research Institute, Sint Katelijne Waver, Belgium
[2] Unit of Microbiology, Belgian Nuclear Research Centre, SCK•CEN, Mol, Belgium
[3] Microbiologie Médicale et Infectiologie, Centre Hospitalier de l'Université de Montréal-Hôpital Saint-Luc, Montréal, Québec, Canada

SCK•CEN
STUDIECENTRUM VOOR KERNENERGIE
CENTRE D'ETUDE DE L'ENERGIE NUCLEAIRE

Université de Montréal

KU LEUVEN THOMAS MORE

Scientia Terrae
RESEARCH INSTITUTE

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

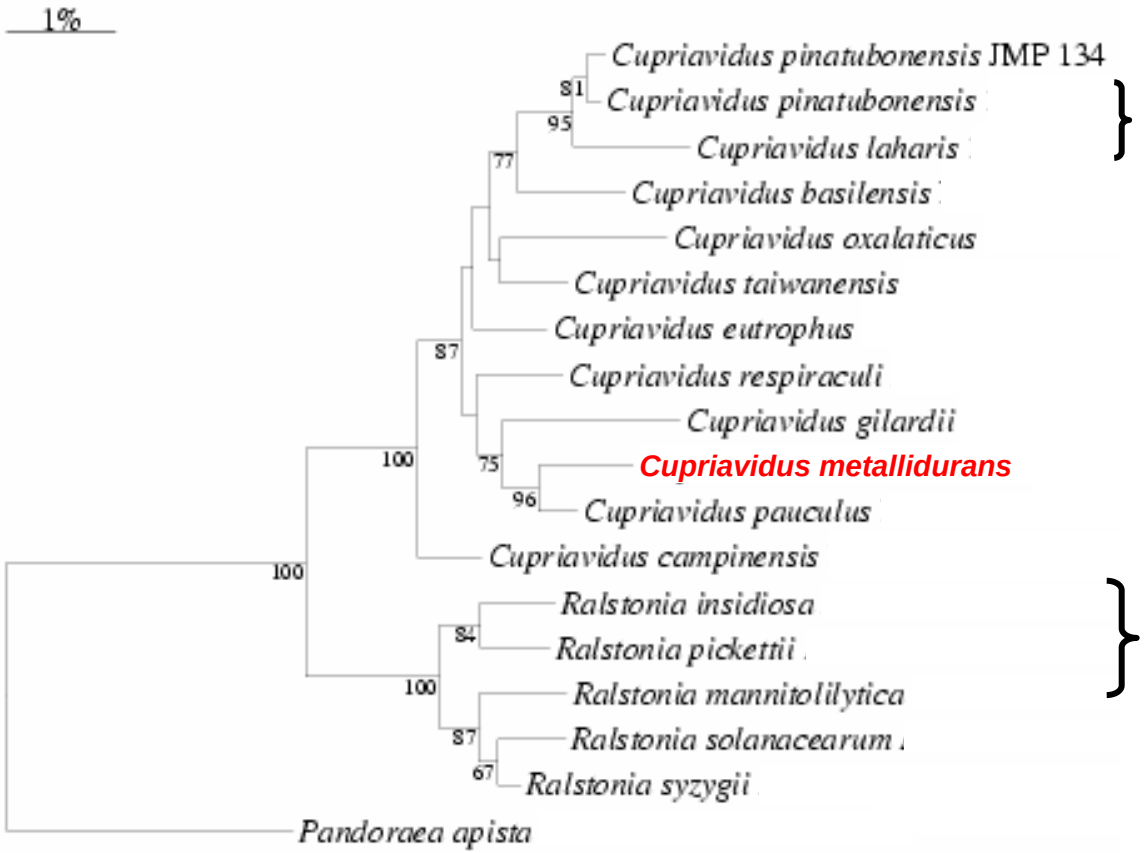
Cupriavidus metallidurans

- 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*



Cupriavidus and International Space Station

- 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

Cupriavidus metallidurans

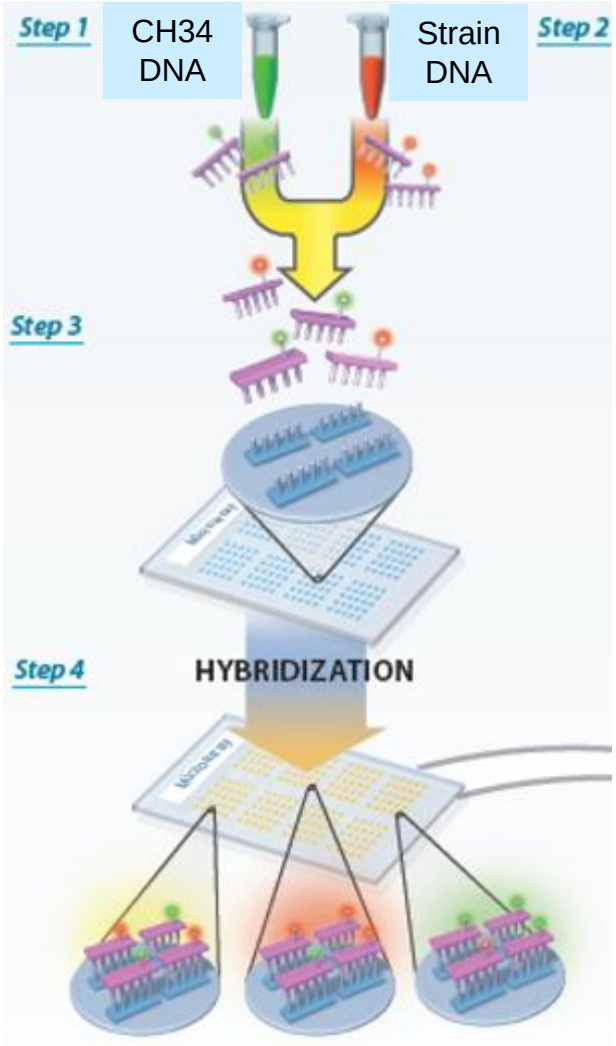
- Isolated from different sources:

  Industrial – potable water – human

- Associated risks ? → especially potable water ISS
- Astronauts → 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

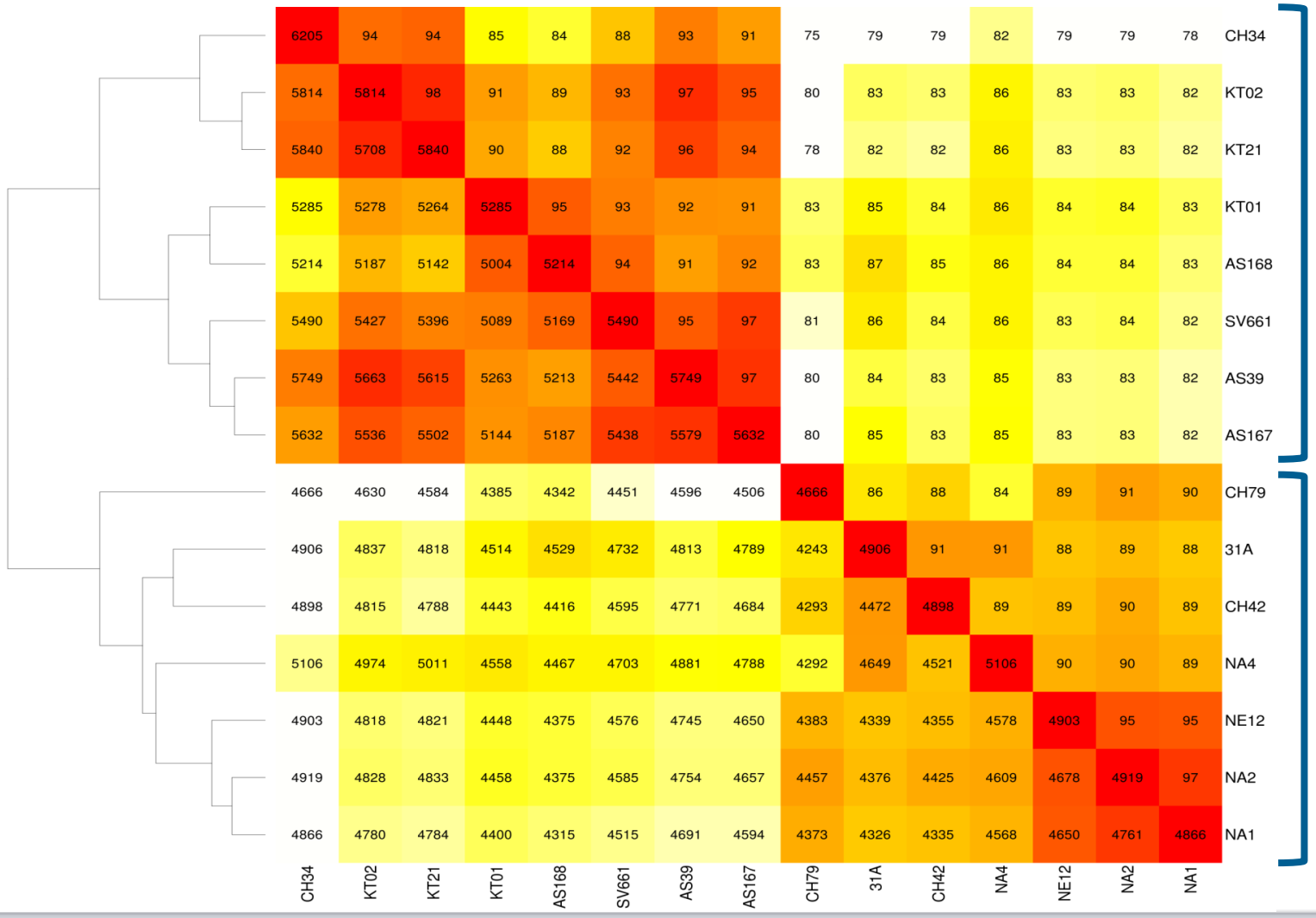


Van Houdt *et al.*, 2012

Cupriavidus metallidurans strains

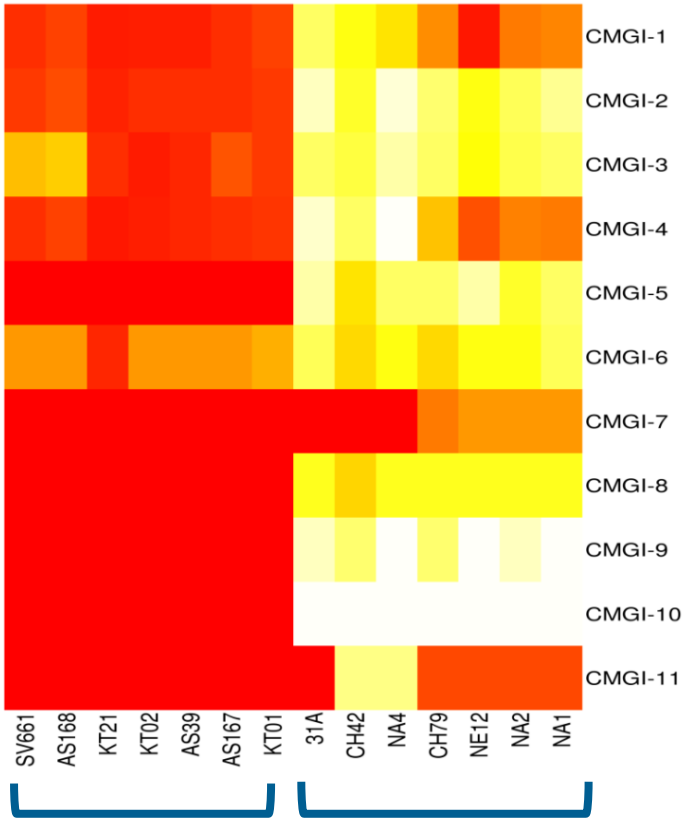
Strain	Isolation site	Isolation place
CH34^T	Decantation tank, zinc factory	Liège, Belgium
CH42	Polluted sediments, zinc factory	Liège, Belgium
CH79	Polluted sediments, zinc factory	Liège, Belgium
31A	Galvanisation tank, metal factory	Holzminden, Germany
AS39	Mine tailings	Likasi-Sud, Congo
AS167	Mine tailings	Likasi-Sud, Congo
AS168	Mine tailings	Likasi-Sud, Congo
KT01	Wastewater treatment plant	Göttingen, Germany
KT02	Wastewater treatment plant	Göttingen, Germany
KT21	Wastewater treatment plant	Göttingen, Germany
SV661	Non-ferrous industry	Beerse, Belgium
NE12	Assembly facility Kennedy Space Center	Florida, USA
NA1	SVO-ZV with Russian ground-supplied water	International Space Station
NA2	American contingency water container	International Space Station
NA4	filter of the Russian SRV-K module	International Space Station

CGH: general comparison



Van Houdt *et al.*, 2012

CGH: genomic islands on CHR1



Size	Family	Features
109 kb	PAGI-2	island from *P. aeruginosa* from cystic fibrosis patient
101 kb	Tn*4371*	hydrogenotrophy, metabolism aromatic compounds
97 kb	Tn*4371*	CO_2 fixation, hydrogenotrophy
56 kb	Tn*4371*	
25 kb		
17 kb		
15 kb		arsenic resistance
12 kb		
20 kb		
20 kb		
10 kb		putative fimbrial operon

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

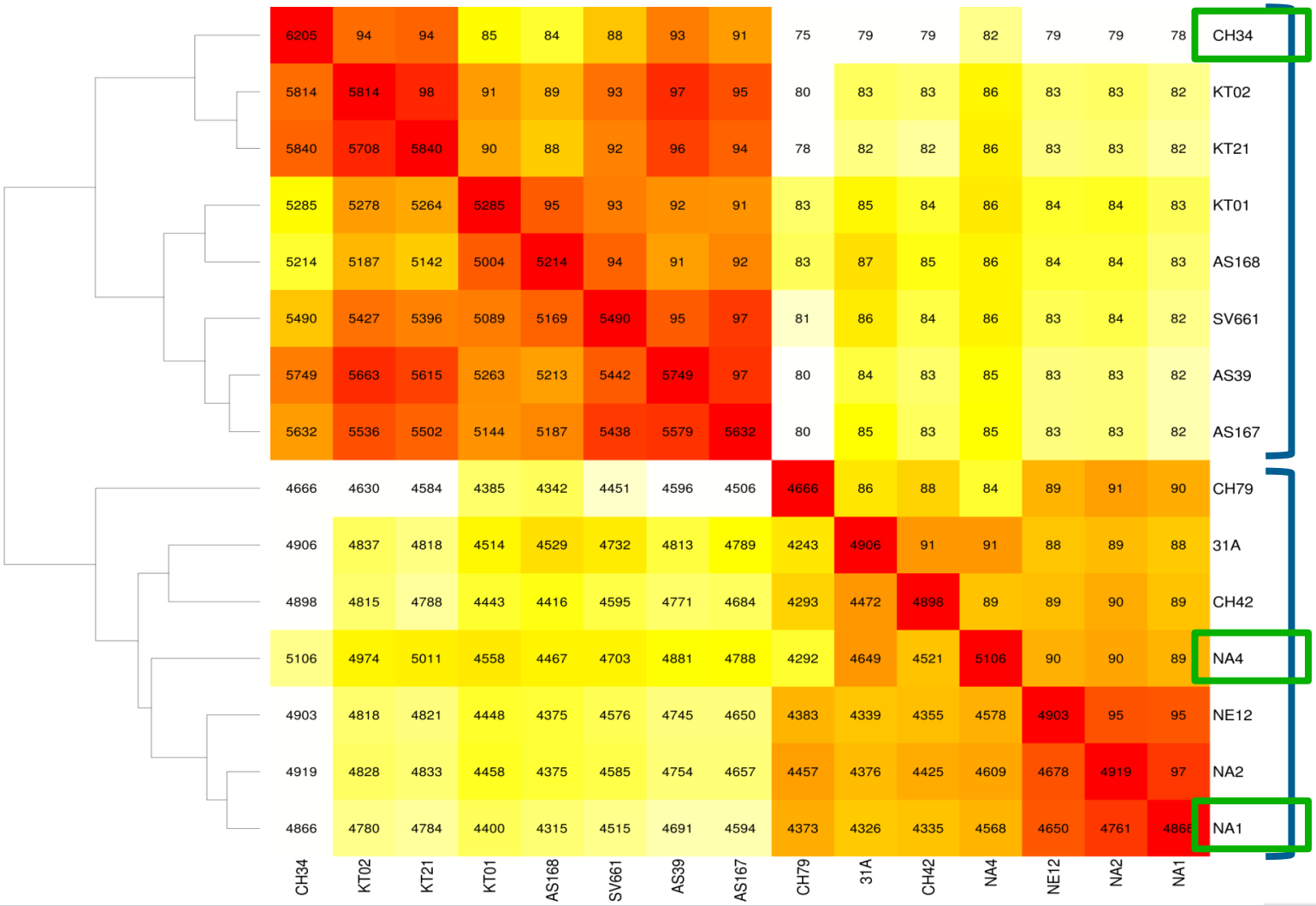
Cupriavidus metallidurans

Biolog shows us the way…

… It's the Phenotype MicroArray™

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

Selected strains



+ H1130

Van Houdt *et al.*, 2012

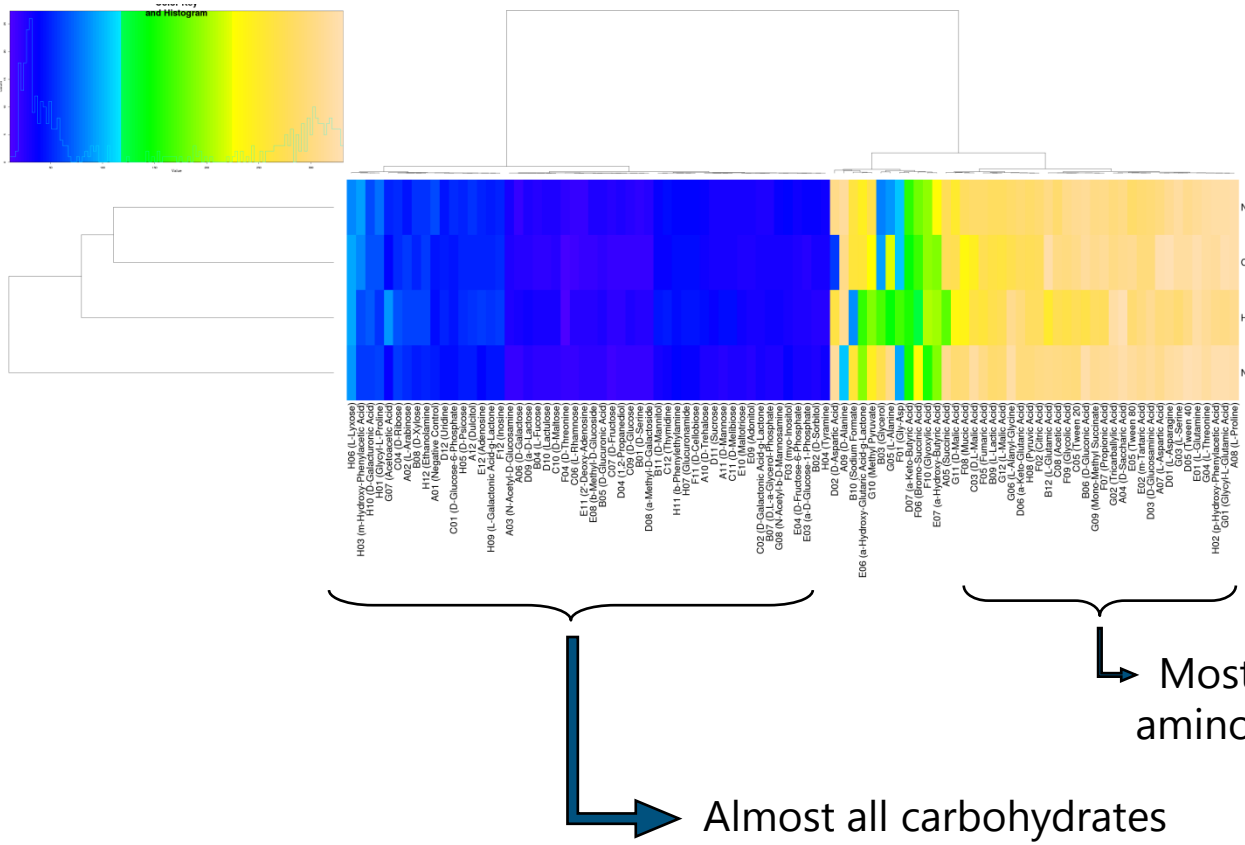
Phenotype MicroArray™ analysis
Set up

- 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



NA4
CH34
H1130
NA1

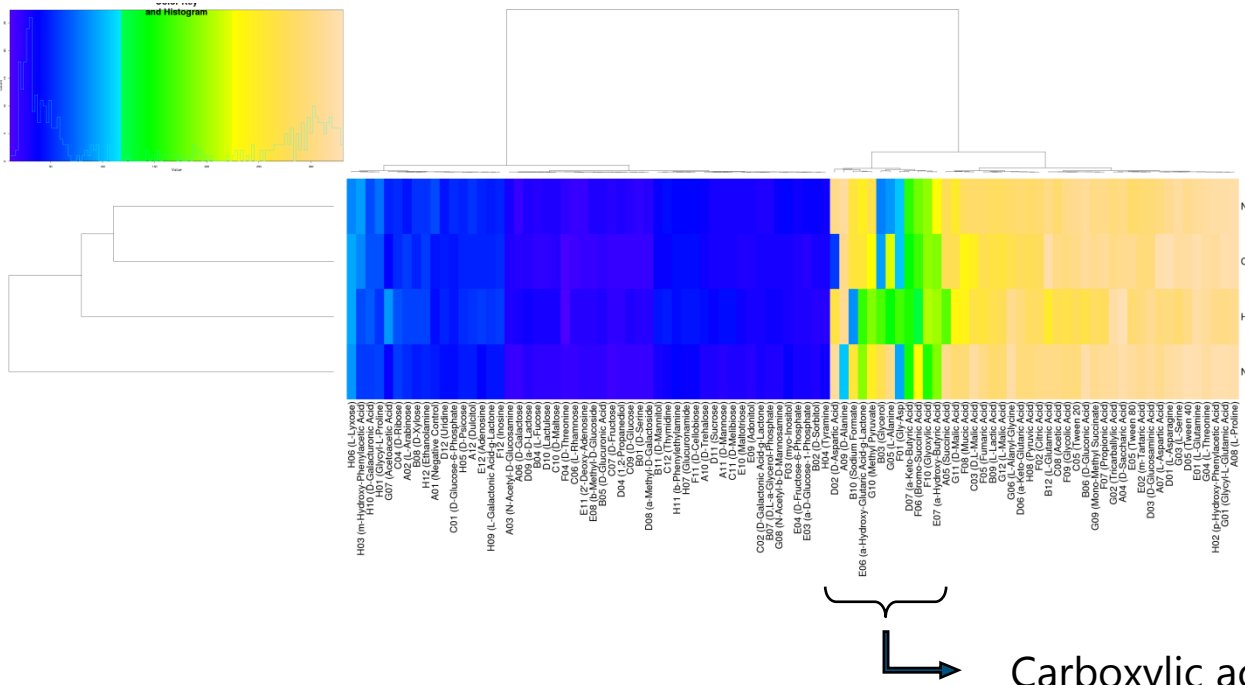
Mostly carboxylic acid and some amino acids

Almost all carbohydrates

Phenotype MicroArray™ analysis

PM 1 MicroPlate™: Carbon sources

- Heat map of PM 1: carbon sources



NA4

CH34

H1130

NA1

Carboxylic acids and amino acids

D-aspartic acid was not used by strain CH34

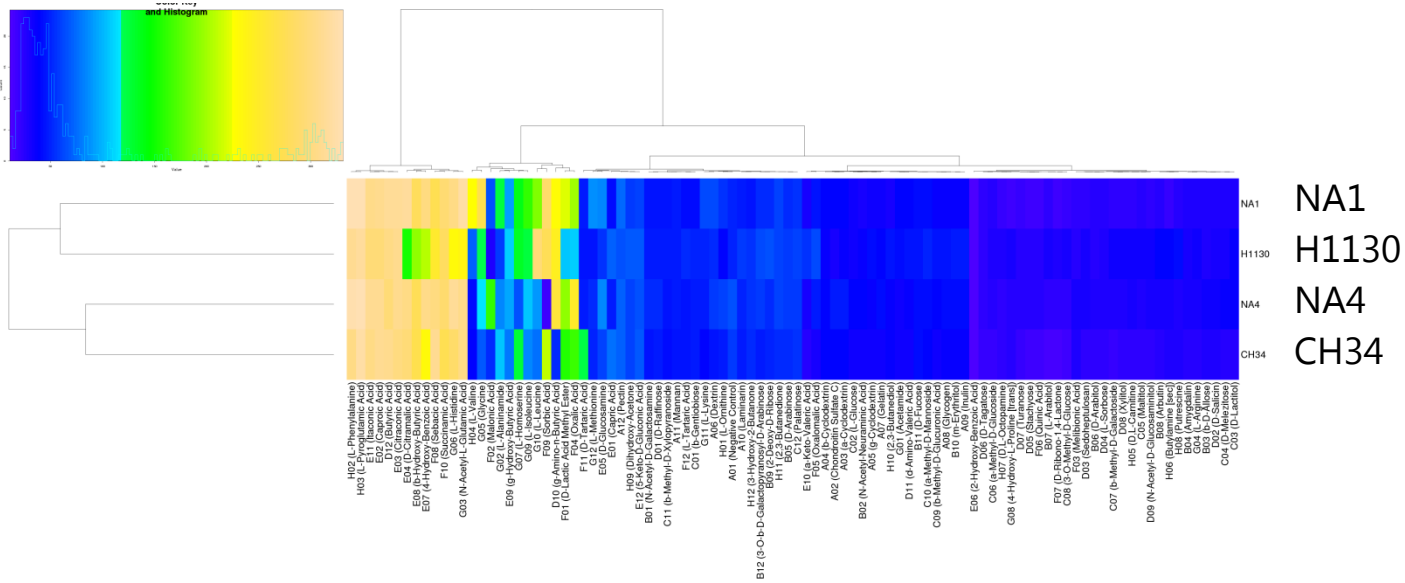
Sodium formate was not used by strain H1130

D-alanine was not used by strain NA1

Phenotype MicroArray™ analysis

PM 2 MicroPlate™: Carbon sources

- Heat map of PM 2A: carbon sources



NA1
H1130
NA4
CH34

All carbohydrates, all polymers, all alcohols, most amine, amide and esters

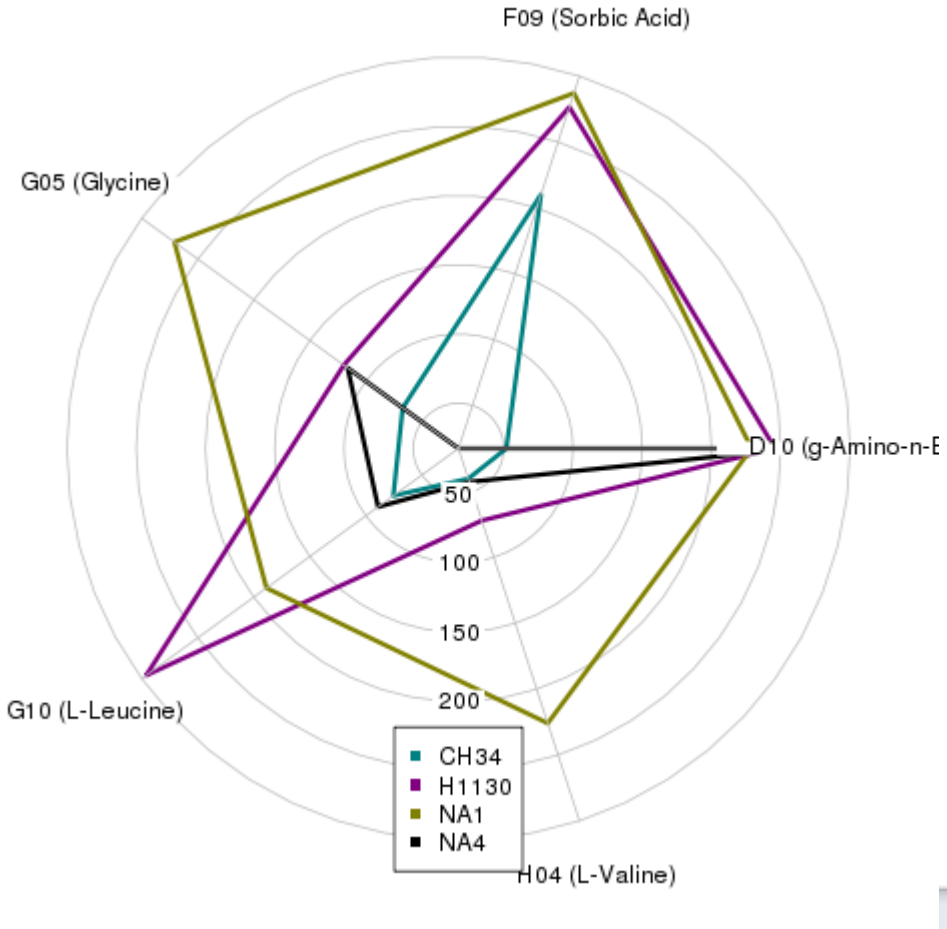
Carboxylic acids and amino acids

Carboxylic acids and amino acids

Phenotype MicroArray™ analysis

PM 2 MicroPlate™: Carbon sources

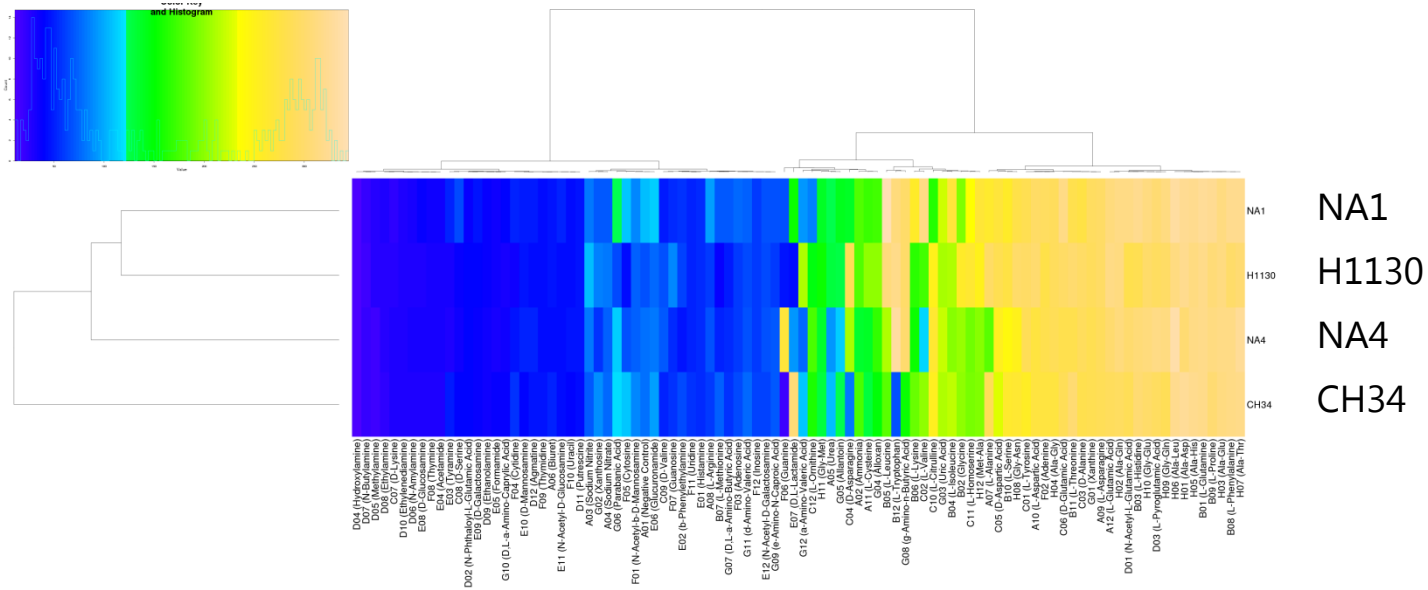
- Radial plot of sorbic acid, γ-amino-N-butyric acid, L-valine, L-Leucine, and glycine



Phenotype MicroArray™ analysis

PM 3 MicroPlate™: Nitrogen sources

- Heat map of PM 3B: nitrogen sources



NA1

H1130

NA4

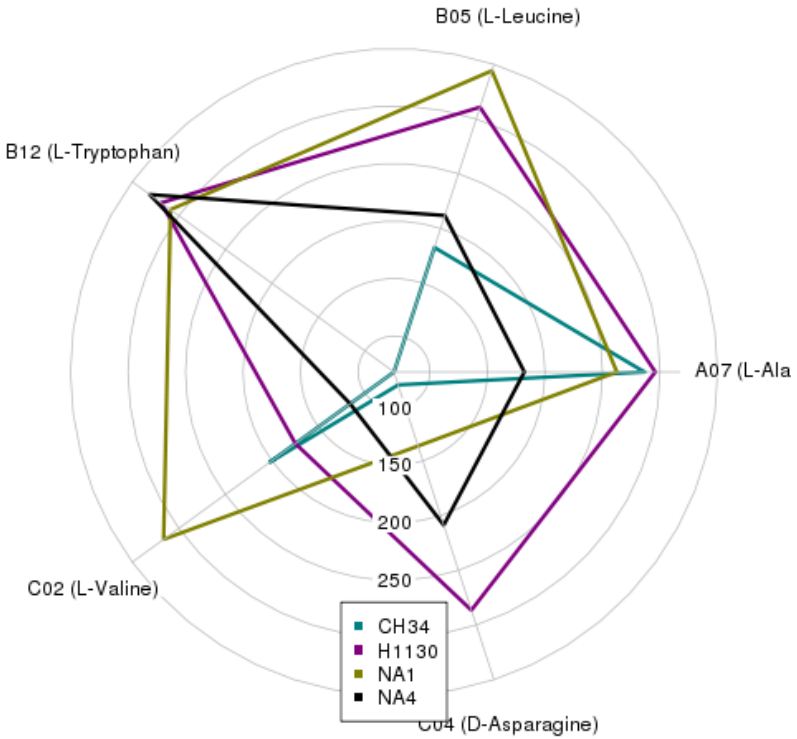
CH34

- 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-aspargine, L-glutamic acid, L-histidine, L-glutamine, L-proline, or L-phenylalanine is the N-source

Phenotype MicroArray™ analysis

PM 3 MicroPlate™: Nitrogen sources

- Radial plot of L-leucine, L-alanine, D-aspargine, L-valine, and L-tryptophan

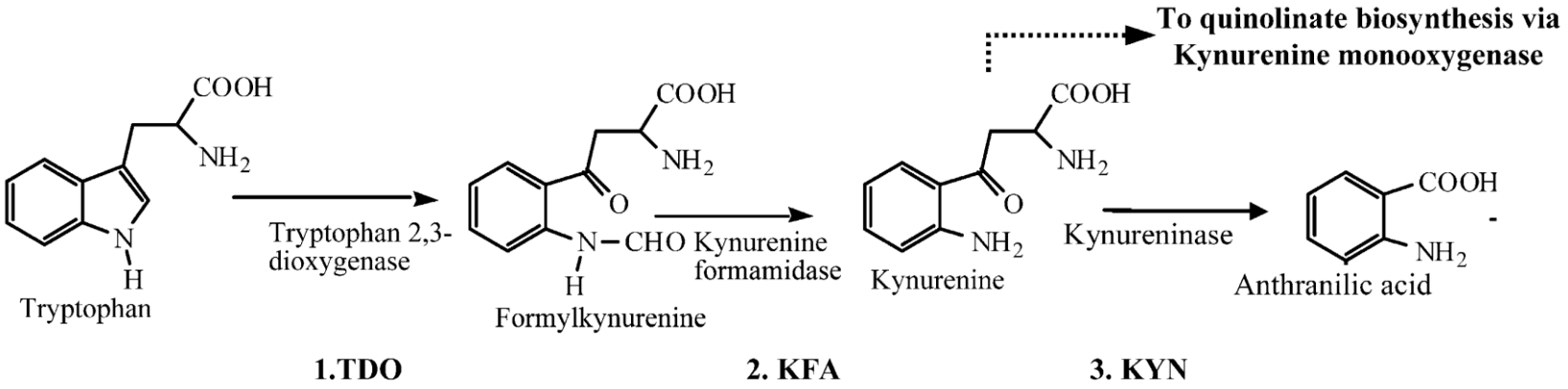


- Amino acids
  - Type strain CH34 is not able to use L-tryptophan
    - Reduced signals on PM6, 7 and 8 when Trp is part of a dipeptide

Phenotype MicroArray™ analysis

PM 3 MicroPlate™: Nitrogen sources

- Importance of L-tryptophan for type strain CH34

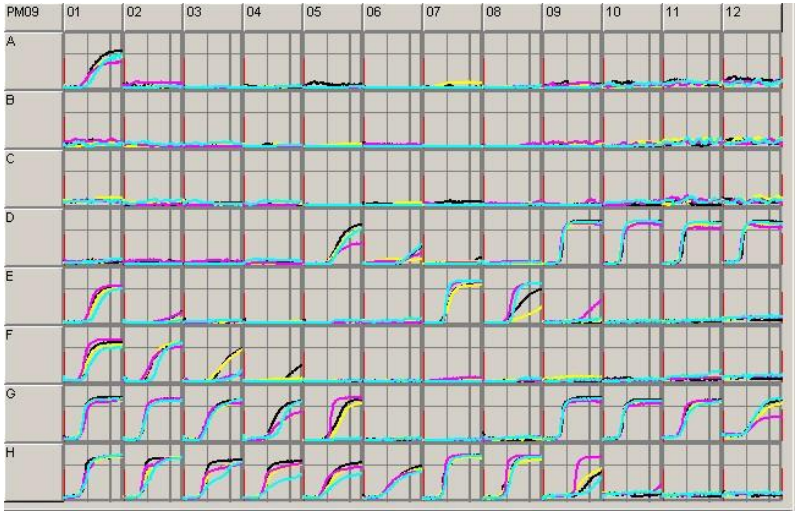


- N-source: L-tryptophan or a Trp dipeptide
  - No or reduced signal for type strain CH34
    - Type strain CH34 has a stop codon in the *kynB* gene

Kurnasov *et al.*, 2006

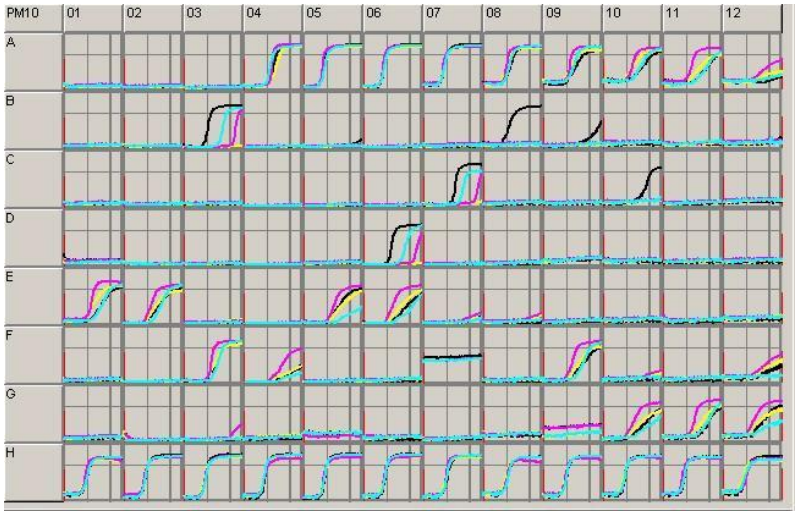
Phenotype MicroArray™ analysis

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



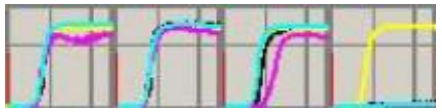
Phenotype MicroArray™ analysis

PM 11 - 20 MicroPlate™: Chemical sensitivity

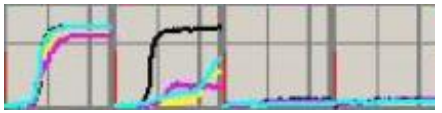
- Chemical sensitivity plates

Strain CH34 (black), strain NA1 (blue),strain NA4 (yellow), and strains H1130 (purple)

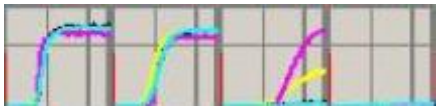
- Amoxicillin: NA4 is more resistant



- 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

- Corresponding author: Rob Van Houdt (rob.van.houdt@sckcen.be)
- SCK•CEN: Ann Provoost, Kristel Mijnendonckx, and Max Mergeay
- Biolog: Andre Chouankam and Barry Bochner

- Funding: European Space Agency and Belgian Science Policy (COMICS project; C90356).

Thank you for your attention

SCK•CEN
Studiecentrum voor Kernenergie
Centre d'Etude de l'Energie Nucléaire
Belgian Nuclear Research Centre

Stichting van Openbaar Nut
Fondation d'Utilité Publique
Foundation of Public Utility

Registered Office: Avenue Herrmann-Debrouxlaan 40 – BE-1160 BRUSSELS
Operational Office: Boeretang 200 – BE-2400 MOL