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BIOGRAPHY

- Jonathan Caguiat is an Associate Professor in the Department of Biological Sciences and the Center for Applied Chemical Biology at Youngstown State University. He received his Bachelor's degree in Biology with a concentration in Molecular Biology from the University of Michigan and his Ph.D. in Microbiology from Michigan State University. His research focuses on metal resistant bacteria. He has reviewed manuscripts for Bioremediation Journal, Current Microbiology and Applied Microbiology and Biotechnology.

Research Interests

- Mechanisms of metal resistances in bacteria
- Particularly, selenium homeostasis
  - Selenium is an important trace element for bacterial growth
  - In the presence of toxic levels of selenite, how do resistant bacteria process selenite without poisoning themselves?

Recent Publications

- **CAGUIAT, J.** (2014). Generation of *Enterobacter* sp. YSU Auxotrophs Using Transposon Mutagenesis, *Journal of Visualized Experiments* (*JoVE*), in press.
- Jasenec A., N. Barasa, S. Kulkarni, N. Shaik, S. Moparthi, V. Konda and **J. CAGUIAT** (2009). Proteomic profiling of cysteine induced selenite resistance in *Enterobacter* sp. YSU. *Proteome Science* 7:30.
- Holmes A., A Vinayak, C. Benton, A. Esbenshade, C. Heinselman, D. Frankland, S. Kulkarni, A. Kurtanich and **J. CAGUIAT** (2009). Comparison of two multimetal resistant bacterial strains: *Enterobacter* sp. YSU and *Stenotrophomonas maltophilia* ORO2. *Current Microbiology* 59:526. DOI 10.1007/s00284-009-9471-2
- Summers, A. O. and **J. CAGUIAT** (2004). Metal binding proteins, recombinant host cells and methods. Patent Number 6,750,042.
- Song, L., **J. CAGUIAT**, Z. Li, J. Shokes, R. A. Scott, L. Olliff and A. O. Summers (2004). Engineered single chain, antiparallel, coiled coil mimics MerR metal binding site. *J. Bacteriol.* 186:1861-1868.
- **CAGUIAT, J. J.**, A. L. Watson and A. O. Summers (1999). Cd(II)-responsive and constitutive mutants implicate a novel domain in MerR. *J. Bacteriol.* 181:3462-3471.

Poplar Creek

- Y-12 plant used to produce materials for nuclear weapons
- Early 40's – uranium enrichment
- 1950's and 60's lithium enrichment using mercury
- Mercury and other metal wastes contaminated East Fork Poplar Creek in Oak Ridge, TN

Widner et al (1996). Health Phys. 71:457-459
Revis et al (1988). Patent # 4,728,427

Poplar Creek Strains

- *Stenotrophomonas maltophilia* OR02 (*S. maltophilia* 02)
- *Enterobacter* sp. YSU
- MIC experiments in liquid cultures
  - Growth detected by turbidity

MICs for *E. coli* strain HB101, *Enterobacter* sp. YSU and *S. maltophilia* OR02

	Minimal Inhibitory Concentration (mM)		
Metal Salt	*E. coli* HB101	*Enterobacter* sp. YSU	*S. maltophilia* OR02
$HgCl_2$	0.02	0.07	0.24
$CdCl_2$	0.14	0.24	0.33
$ZnCl_2$	0.5	0.8	5
$CuSO_4$	3	3	5
$HAuCl_4 \bullet H_2O$	0.02	0.05	0.25
K_2CrO_4	0.4	0.4	7
$AgNO_3$	0.03	0.08	0.03
$NaAsO_2$	7	14	14
Na_2SeO_3	70	40	40
$Pb(NO_3)_2$	2	2	2

Holmes et al (2009).
Current Microbiology 59:526

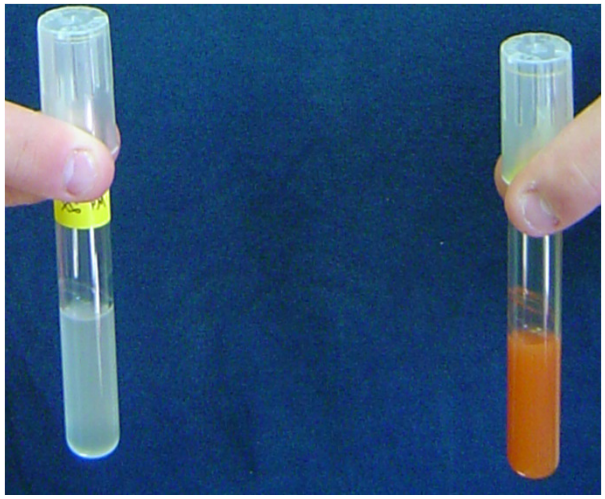
MIC Results

- *S. maltophilia* 02 is more resistant to most of the tested metal salts than *Enterobacter* sp. YSU
  - $AgNO_3$ is an exception
- *Enterobacter* sp. YSU is more resistant to the tested metal salts than *E. coli* strain HB101
  - $CuSO_4$ and K_2CrO_4 are exceptions

Holmes et al (2009). *Current Microbiology* 59:526

MIC Results

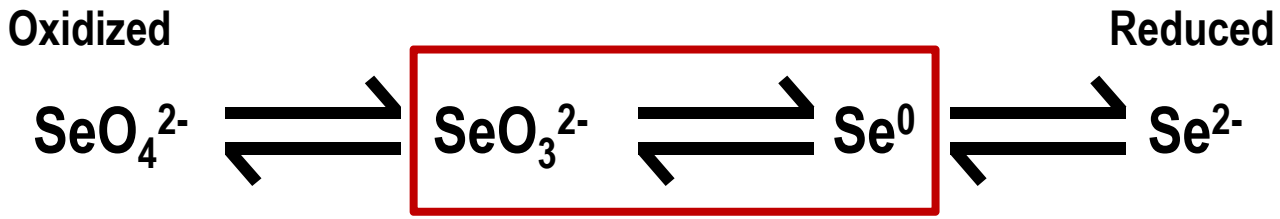
- $Pb(NO_3)_2$ MIC difficult to determine because it is precipitates in most growth media
- Na_2SeO_3 MIC difficult to determine because red color produced by cell reduction of selenite (SeO_3^{2-}) to elemental selenium interfered with turbidity readings



No selenite Selenite

Selenium Characteristics

Compound	Formula	Oxidation State
Selenate	SeO_4^{2-}	+6
Selenite	SeO_3^{2-}	+4
Elemental Selenium	Se^0	0
Selenide	Se^{2-}	-2



Reduction of selenite to elemental selenium

Selenite Resistance Study

- Selenite transported into *E. coli* using two pathways
- Sulfate transport – CysA, CysW and CysT
  - The non-specific pathway
- Undetermined selenite-specific pathway
  - The specific pathway
- *Enterobacter* sp. YSU requires cysteine for selenite resistance in M-9 minimal salts medium

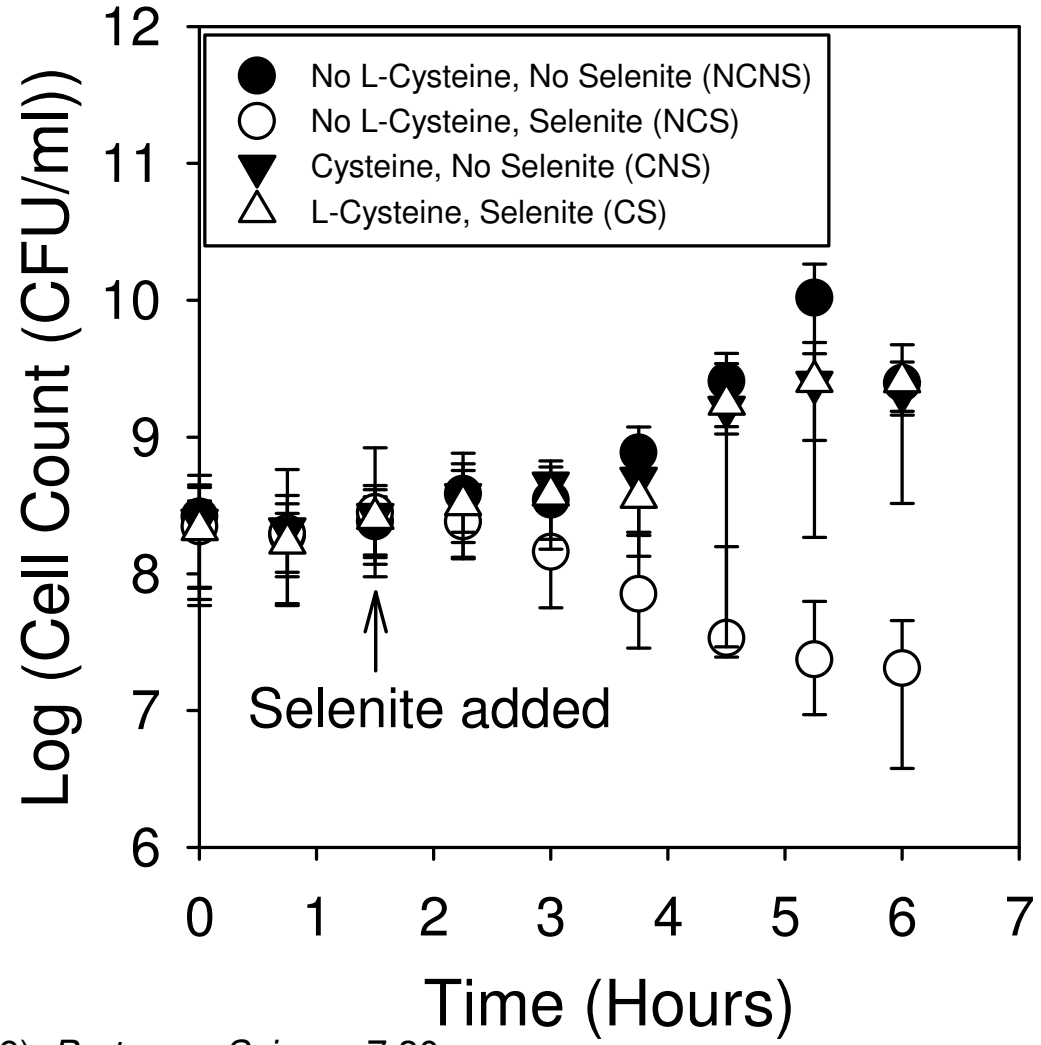
Sabine Müller · Johann Heider · August Böck The path of unspecific incorporation of selenium in Escherichia coli Arch Microbiol (1997) 168 : 421–427c

METHODS - GROWTH

- Grow cells in the presence and absence of cysteine to early-log phase
- Split the cultures – original set
  - No cysteine, no selenite (NCNS) and
  - Cysteine, no selenite (CNS)
- Add 40 mM selenite to the second set
  - No cysteine, selenite (NCS) and
  - Cysteine, selenite (CS)
- Follow growth by viable cell count

Jasenec et al. (2009). *Proteome Science* 7:30

Cysteine Requirement for Selenite Resistance



Jasenec et al. (2009). *Proteome Science* 7:30

HYPOTHESIS

- Cysteine confers selenite resistance by blocking transport through the non-specific (sulfate permease) pathway

Jasenec et al. (2009). *Proteome Science* 7:30

METHODS - PROTEOMICS

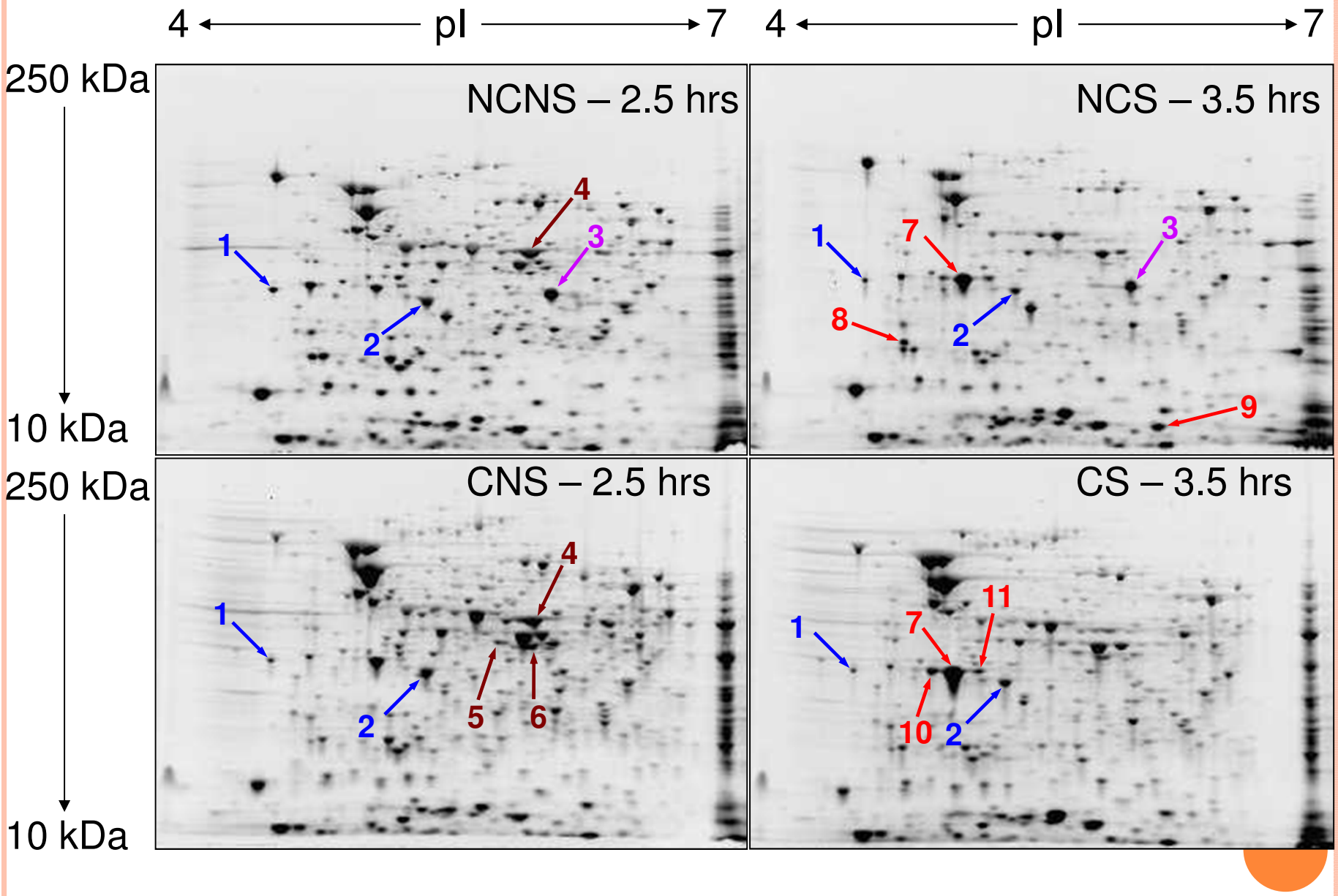
- Grow cells in the presence and absence of cysteine to early-log phase
- Remove no selenite samples
  - No cysteine, no selenite (NCNS) and
  - Cysteine, no selenite (CNS)
- Add 40 mM selenite
- Remove selenite samples one hour after exposure
  - No cysteine, selenite (NCS) and
  - Cysteine, selenite (CS)

Jasenec et al. (2009). *Proteome Science* 7:30

METHODS - PROTEOMICS

- Extract proteins from each sample and separate equal amounts by charge (isoelectric focusing) and size (SDS-polyacrylamide gel electrophoresis)

Jasenec et al. (2009). *Proteome Science* 7:30



Jasenec et al. (2009). *Proteome Science* 7:30

FINDINGS

- Controls – all four conditions
  - Spot 1 - OmpF porin
  - Spot 2 - protein chain elongation factor EF-Ts
- Unique spot from samples lacking cysteine (NC)
  - Spot 3 - conserved hypothetical lipobinding protein
- Spots from samples lacking selenite (NS)
  - Spots 4, 5 and 6 - translation elongation factor EF-Tu
- Spots from samples treated with selenite (S)
  - Spot 8 - putative tellurium resistance protein C
  - Spot 9 - small heat shock protein

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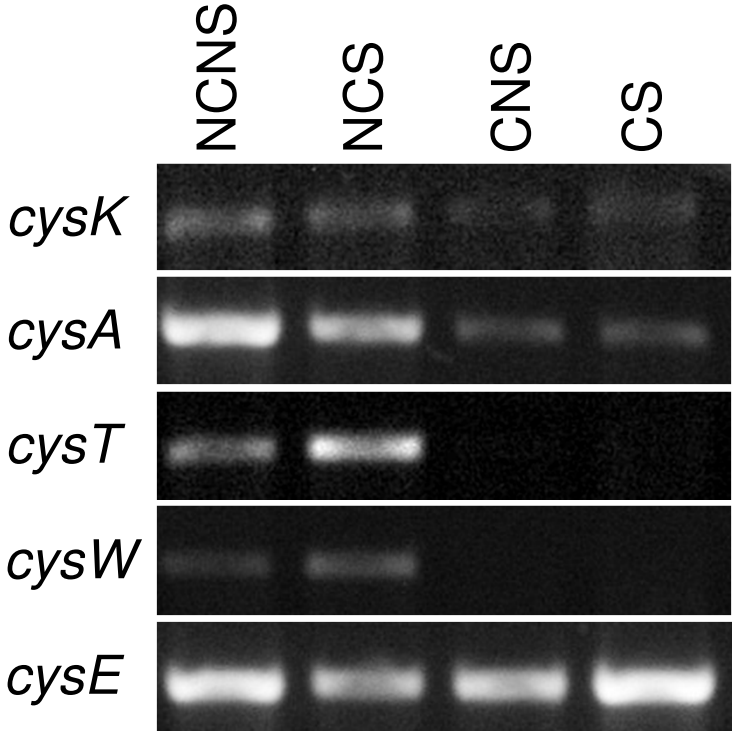
FINDINGS

- Spots from samples treated with selenite (S)
  - Spots 10 and 11 (CS) – proteins for outer membrane protein + hydrogenase component
  - Spot 7 (NCS) contains CysK
  - Spot 7 (CS) lacked CysK
- Does the addition of cysteine also decrease the expression levels of other non-specific sulfate transport genes?

Jasenec et al. (2009). *Proteome Science* 7:30

Reverse Transcriptase – PCR (RT-PCR)

- Grow cells – Mid-log phase
- Take NS samples
- Add Se
- Take samples after 1 hr (S samples)
- Purify RNA
- Make cDNA
- PCR with primers for sulfate transport genes



Jasenec et al. (2009). *Proteome Science* 7:30

CONCLUSIONS

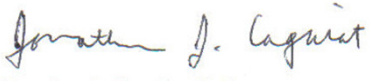
- *Enterobacter* sp. YSU requires Cysteine for resistance to 40 mM selenite
- Proteomic and RT-PCR data support the hypothesis that cysteine confers resistance by blocking selenite transport through the sulfate permease pathway
- Red color of the cultures containing cysteine and selenite suggest that the selenite still enters through a selenite specific pathway and is reduced to elemental selenium

Jasenec et al. (2009). *Proteome Science* 7:30

Other Work

- Identify other metal resistances gene from *Enterobacter* sp.YSU and *S. maltophilia* OR02 using a transposon mutagenesis system
- 2,500 colonies isolated from Poplar creek in Oak Ridge.
  - Screen for resistances to Hg(II) and other metals
  - Identify metal resistance genes
  - Compare them to the metal resistance genes in *Enterobacter* sp. YSU and *S. maltophilia* OR02

Digital Signature



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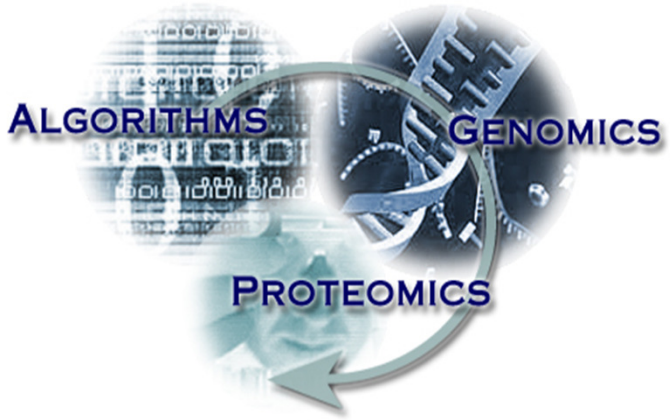
- Journal of Pharmacogenomics & Pharmacoproteomics
- Journal of Data Mining in Genomics & Proteomics



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