Using Copper-Resistant Bacteria to Reduce Copper Toxicity in *Shewanella oneidensis* Cr(VI) Reduction Studies

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Introduction

- The most common oxidized states of chromium are hexavalent and trivalent states
  - Cr(VI) is soluble, highly mobile in the environment, and carcinogenic
  - Cr(III) is less mobile and less toxic
- *Shewanella oneidensis* reduces Cr(VI) to Cr(III) under various conditions:
  - aerobic and anaerobic environments
  - in high and low nutrient levels
  - in the presence of other bacteria, sorbing agents, cationic metals

- *Shewanella* is inhibited by Cu^{2+} at >10 ppm
- Cu^{2+} is often present in Cr(VI)-contaminated wastewaters
- *Pseudomonas* is resistant to copper
  - Can sequester copper in extracellular polymeric substances (EPS); bind to proteins in the periplasm
- The use of *Pseudomonas* to remove copper toxicity for *Shewanella* in a bioremediation system was investigated

Experimentation

- Cr(VI) reduction by pure and mixed cultures of DSP10 and 4 *Pseudomonas* strains
- Growth dynamics of DSP10 and 0788-7 in low nutrients
- Resistance of *Pseudomonas* to Cu^{2+}

⌘Cr(VI) reduction by column bioreactor

Reaction column with circulation line through a peristaltic pump

Materials and Methods

LB broth was spiked with concentrations of K_2CrO_4

↓

Samples removed at various time intervals

↓

Cr(VI) was measured using Standard Method 3500-Cr Colorimetric Method

Chromate Assay:

0.5 ml supernatant from centrifuged sample

0.5 ml of 0.2N H_2SO_4

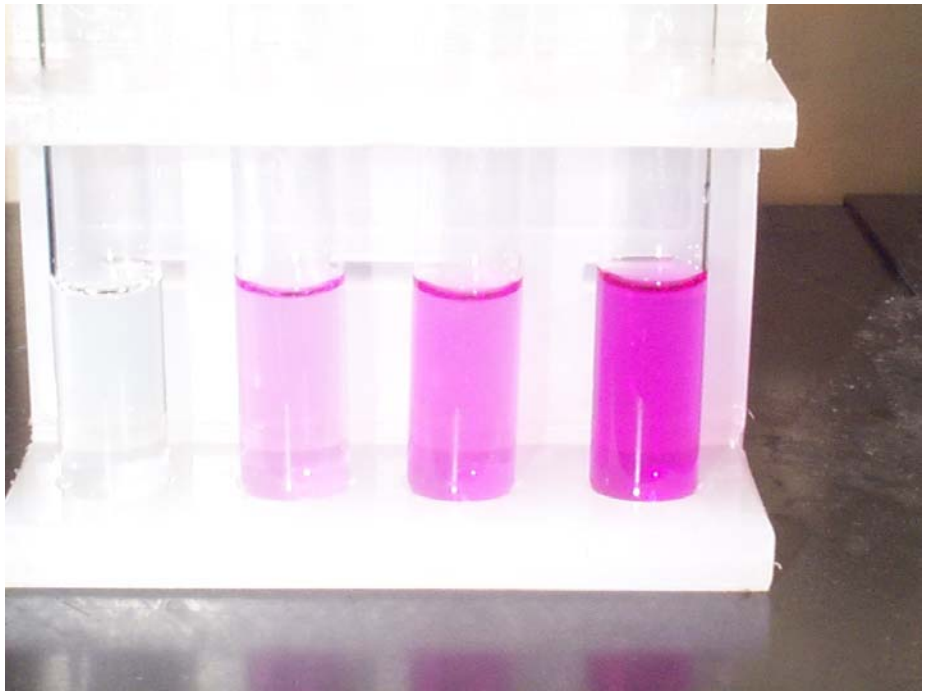
0.1 ml diphenylcarbazide solution

Mix and let stand 5-10 minutes for full color development

Transfer to a 1-cm absorption cell and measure absorbance at 540 nm

Use distilled water as reference (blank)

Cr(VI) Detection by the Diphenylcarbazide Method 3500-Cr



Left Tube: Control (reagent blank; no Cr(VI) added)

2nd Tube: 5 ppm Cr(VI)

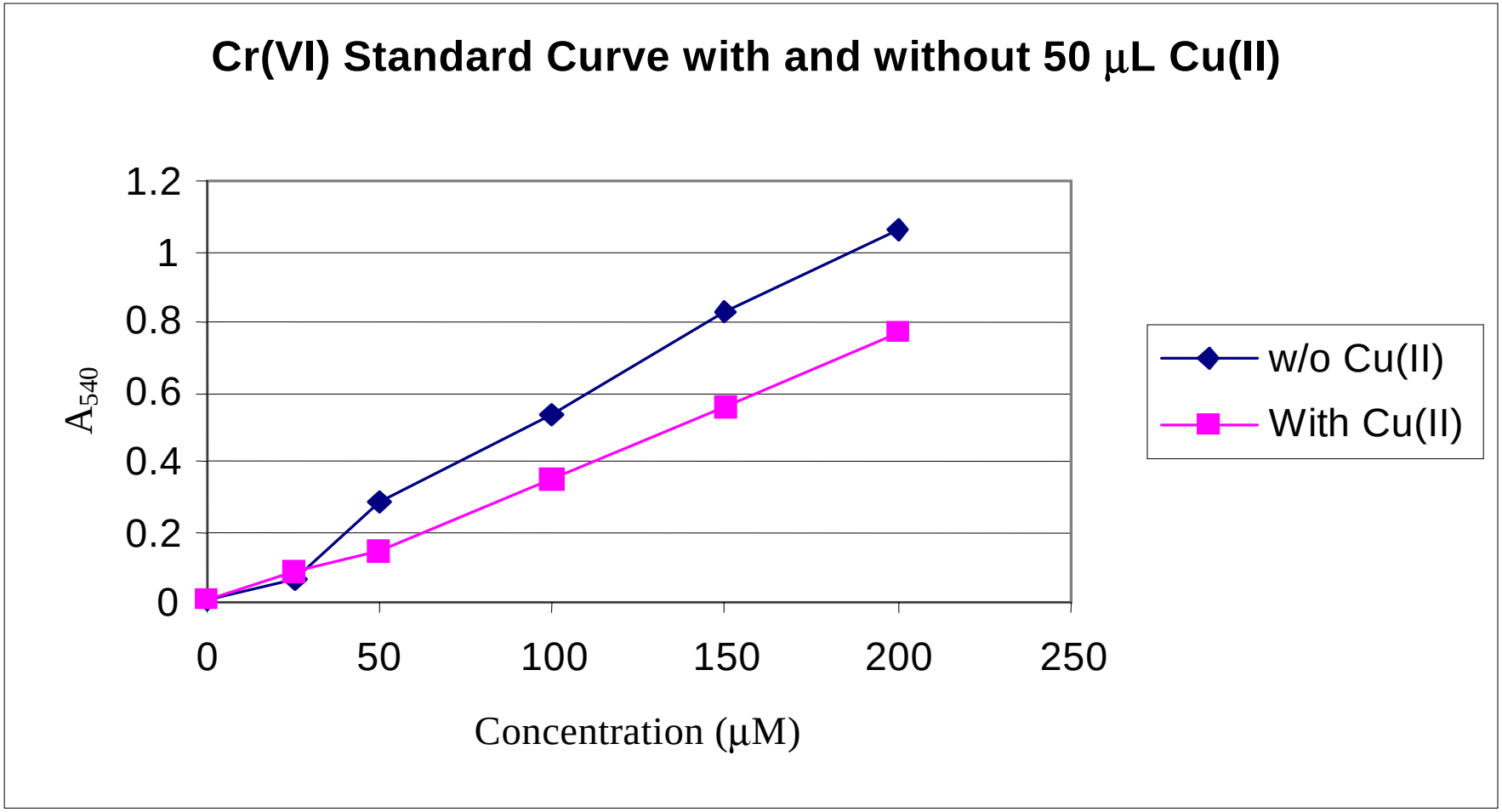
3rd Tube: 10 ppm Cr(VI)

4th Tube: 15 ppm Cr(VI)

Absorbance measured at 540 nm in Milton Roy Spec20

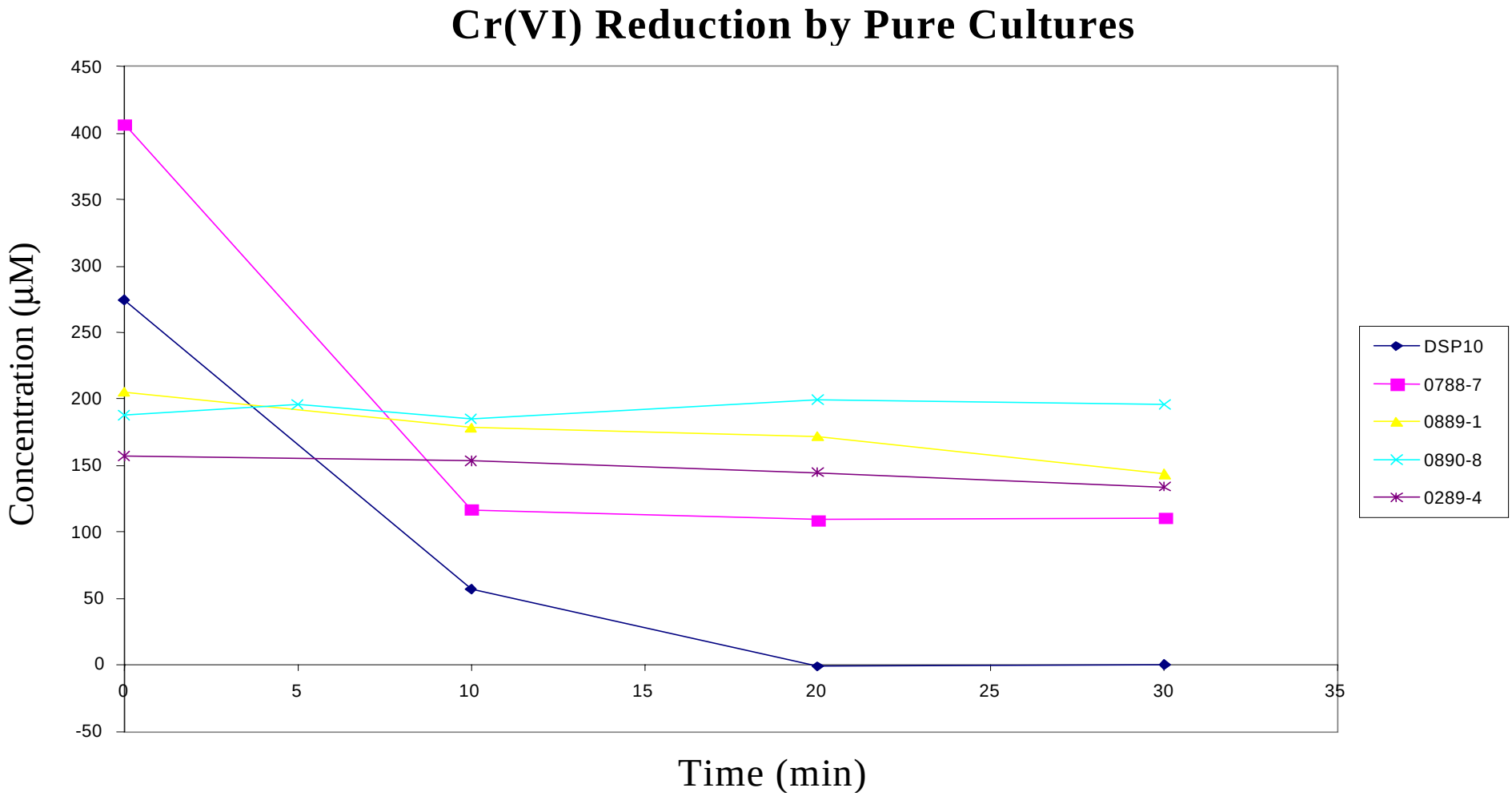
Micrograms Cr(VI) determined by reference to the standard calibration curve

Standard Curve



Shift of absorbance values due to presence of copper in solution

Pure Culture Cr(VI) Reduction



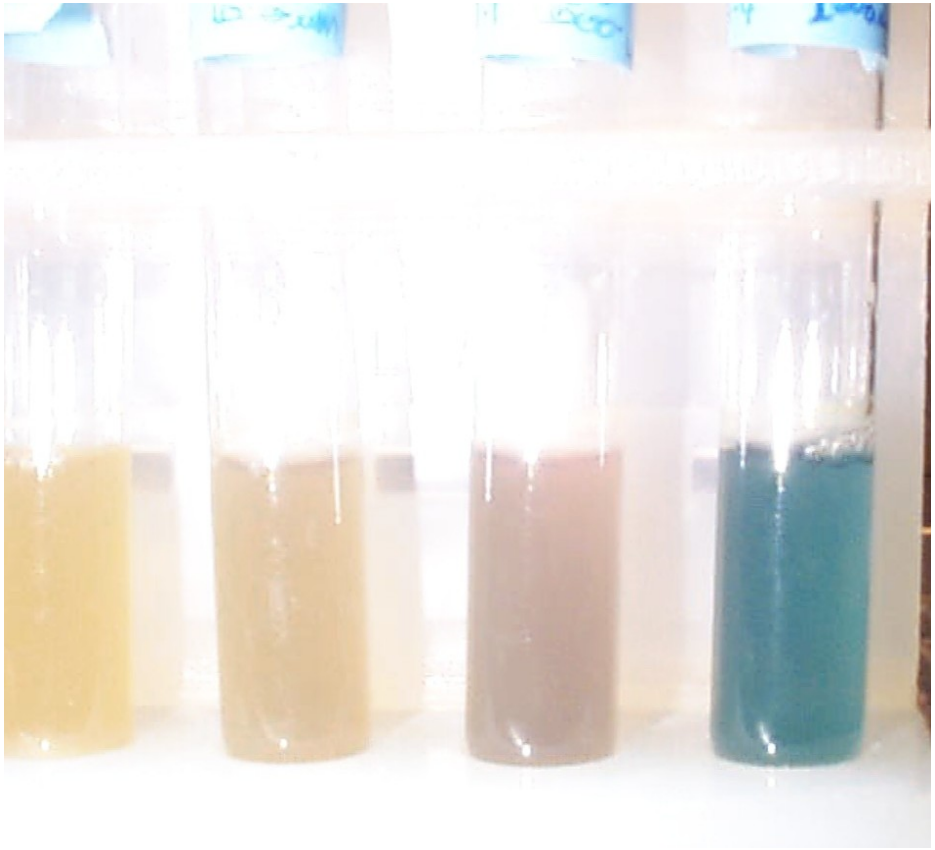
Superior Cr(VI) reduction by DSP10 than 4 pseudomonads

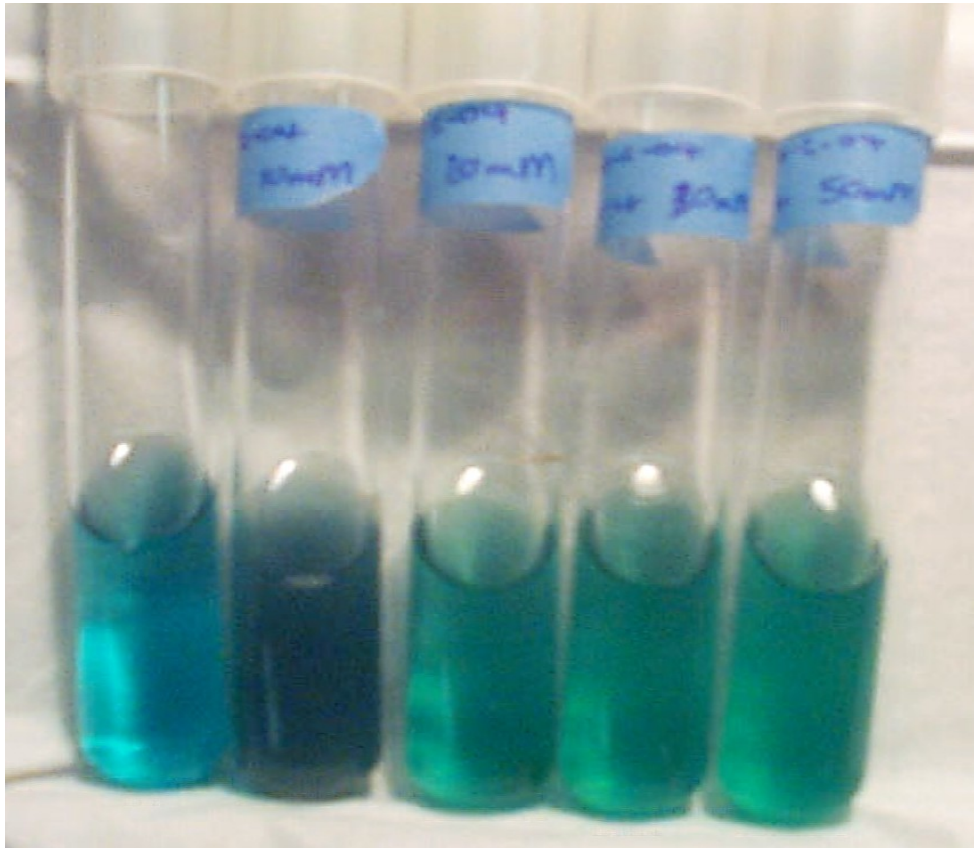
Low Nutrient Growth of DSP10 + 0788-7

Bacterial Strain	Day 1 (10^{-6})	Day 3 (10^{-6})	Day 6 (10^{-5})
DSP10 colonies	365	320	662
0788-7 colonies	5	21	180
Ratio	73	15.3	3.7

- Ratio of DSP10 to 0788-7 decreased over time
- Growth was balanced in comparison to previous tests

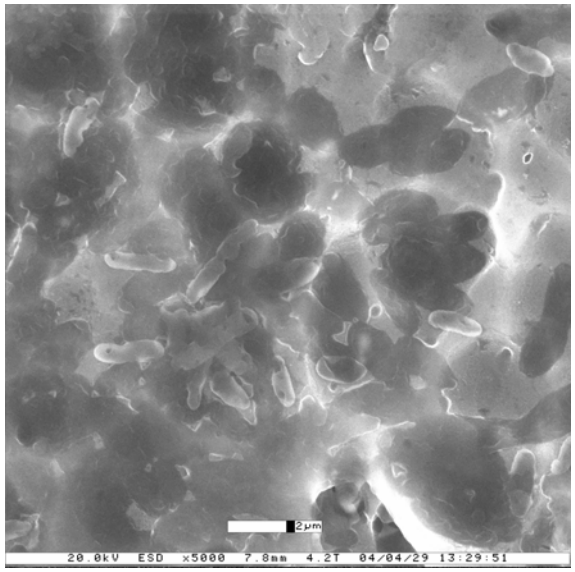
Resistance of *Pseudomonas* to Cu^{2+}



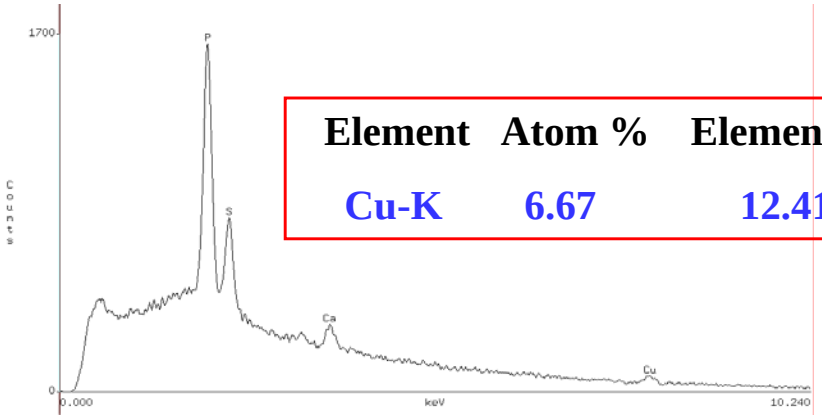


1 mM 2 mM 5 mM 10 mM Control 10mM 20mM 30mM 50mM

Pseudomonas 0889-1 *Pseudomonas* 0289-4

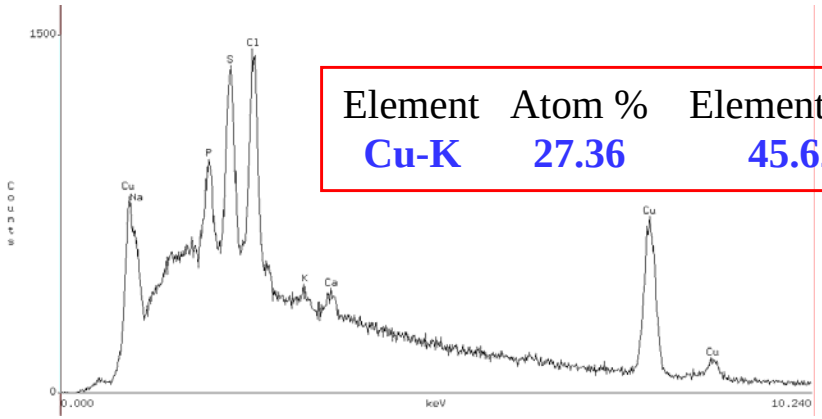


Environmental scanning electron micrograph (ESEM) of *Pseudomonas* sp. 0788-7 cells grown in LB + **1 mM copper sulfate** for 24 h at 25°C with shaking at 125 rpm. **Image shows copious amounts of extracellular polysaccharides (EPS)**



Element	Atom %	Element Wt %
Cu-K	6.67	12.41

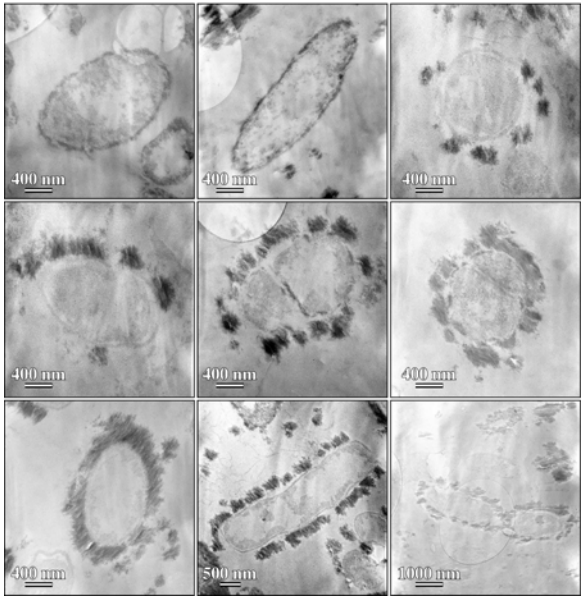
Energy dispersive spectroscopy (EDS) analysis of the extracellular polymeric material from the **1 mM copper sulfate** 24 h culture (sample rinsed 3X in distilled water before ESEM and EDS analysis)



Element	Atom %	Element Wt %
Cu-K	27.36	45.62

EDS analysis from strain 0788-7 cells grown in LB with **4 mM copper** sulfate. Note: EDS data showed similar results for the other 3 copper-resistant pseudomonads used in this study

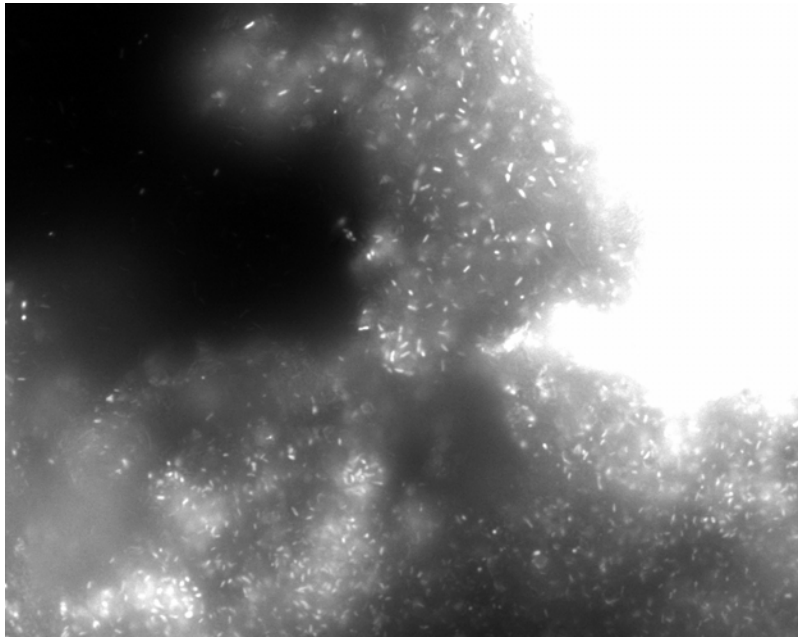
Pseudomonas sp. 0788-7



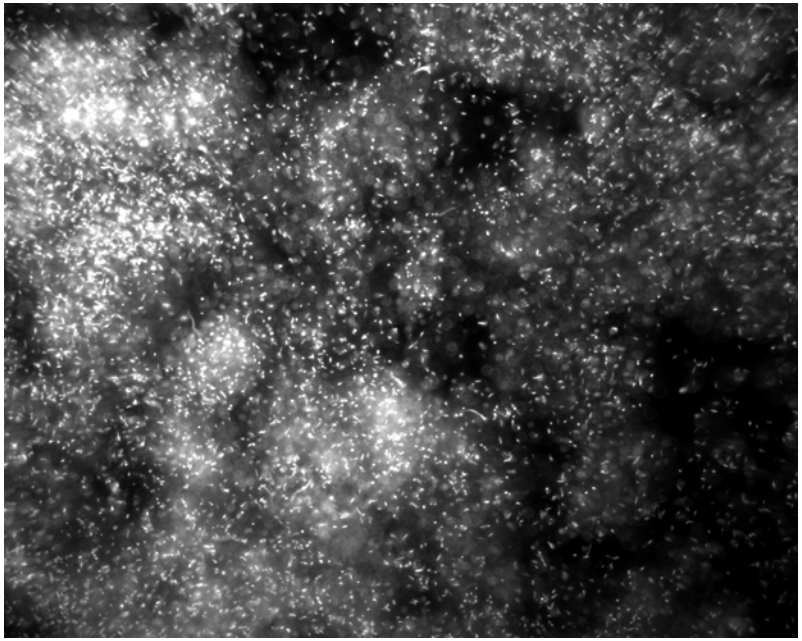
EC-TEM images of **copper precipitates in the EPS surrounding cells** of a copper-resistant pseudomonad

Provided by Richard Ray, Dr. Brenda Little and Dr. Tyrone Daulton (NRL/Codes 73330 and 7430, Stennis Space Center, MS 39529

Degeneration of Cell Mats in Column by Copper



Day 23: Intact DSP10 biomass before addition of copper – Live cell stain



Day 30: DSP10 mat after repeated addition of copper – Many dead cells

Conclusions

- *Pseudomonas* is extremely resistant to Cu^{2+}
- A 12.5% LB environment helps balance growth between DSP10 and 0788-7
- Column bioreactors are effective as bioremediation systems
  - Bacteria should become established before repeated addition of copper
  - Cell biomats protect DSP10 from Cu^{2+} to some extent
  - A strategy for long-term survival of DSP10 against copper must be developed

Further Research

- Investigate Cr(VI) reduction by mixed cultures in flasks with copper
- Low nutrient growth of mixed cultures using DSP10 and other pseudomonads and with the addition of metals (chromium and copper)
- Column studies
  - Addition of other copper-resistant bacteria
  - Addition of extracellular polysaccharide (EPS) material from pseudomonads
  - Addition of heat killed *Pseudomonas* cells

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