Composition, Reactivity, and Regulation of Extracellular Metal-Reducing Structures (Nanowires) Produced by Dissimilatory Metal Reducing Bacteria

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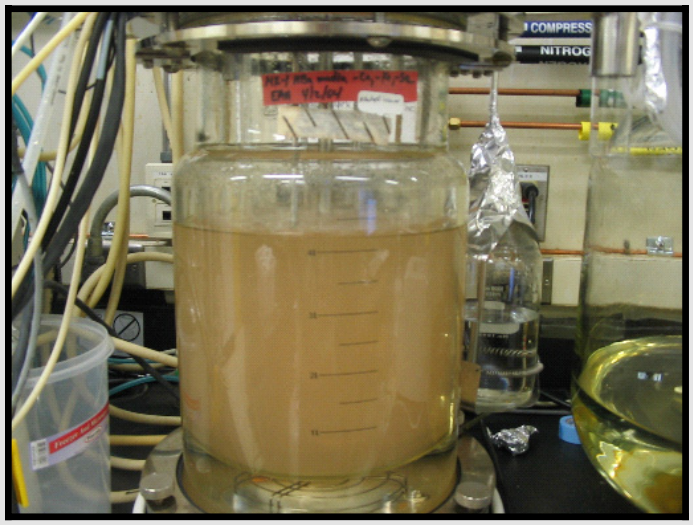
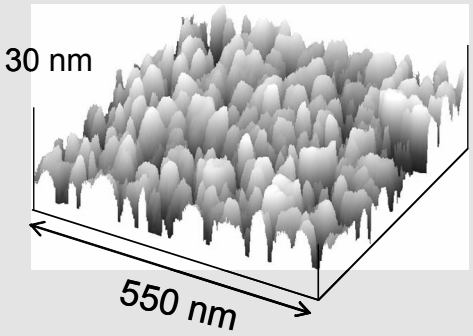
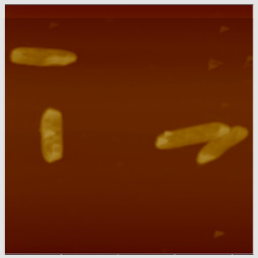
Shewanella oneidensis strain MR-1 cultivated with high agitation

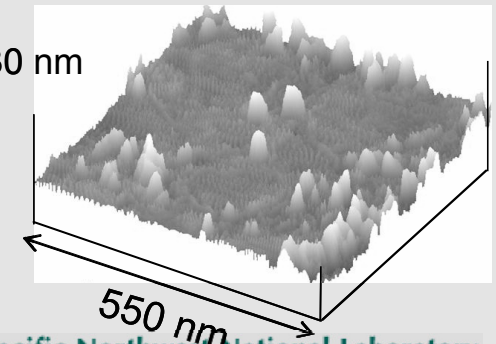


Electron Acceptor Limited (EAL)

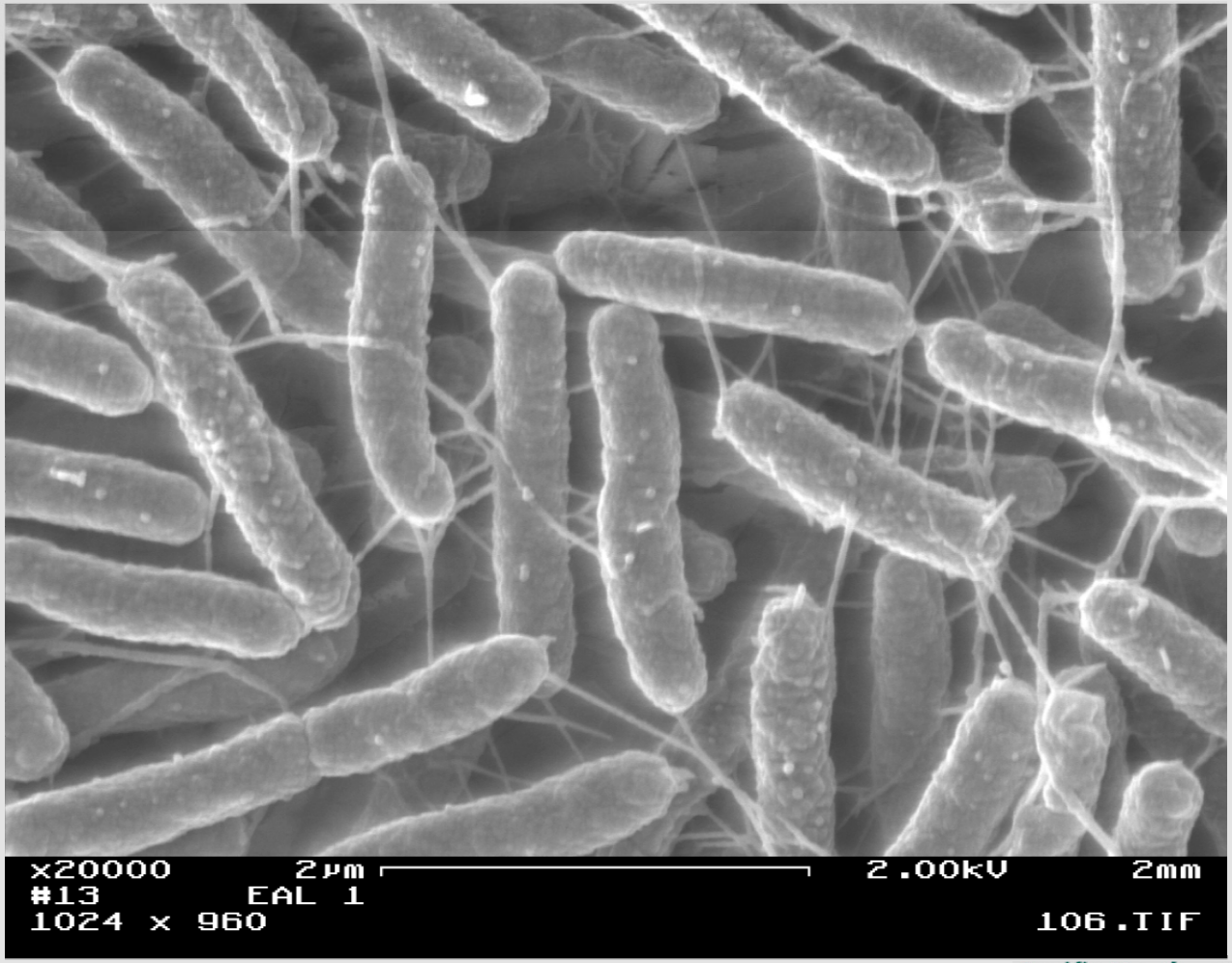
Electron Acceptor Excess (EAX)







SEM image of MR-1 cultivated with O_2-limitation and low agitation (50 rpm)



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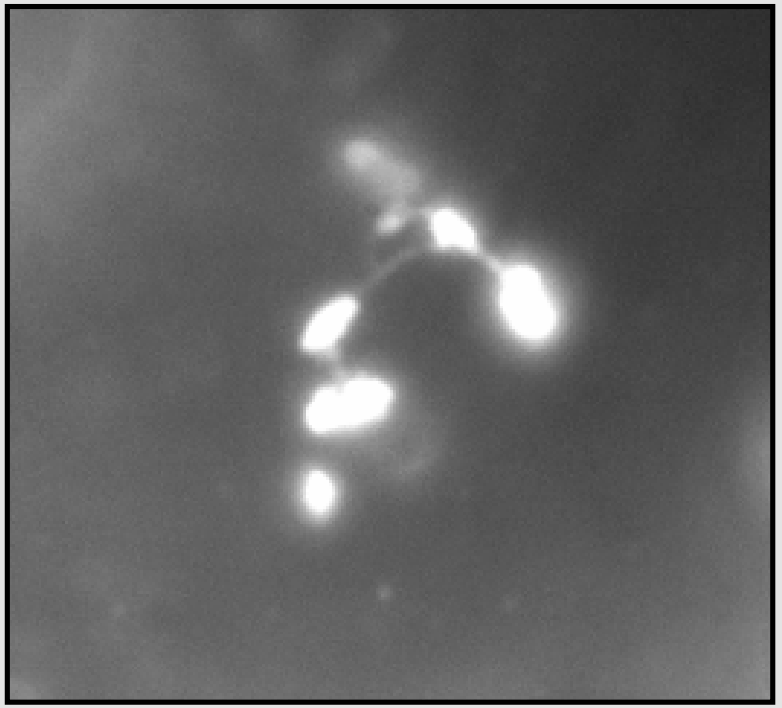
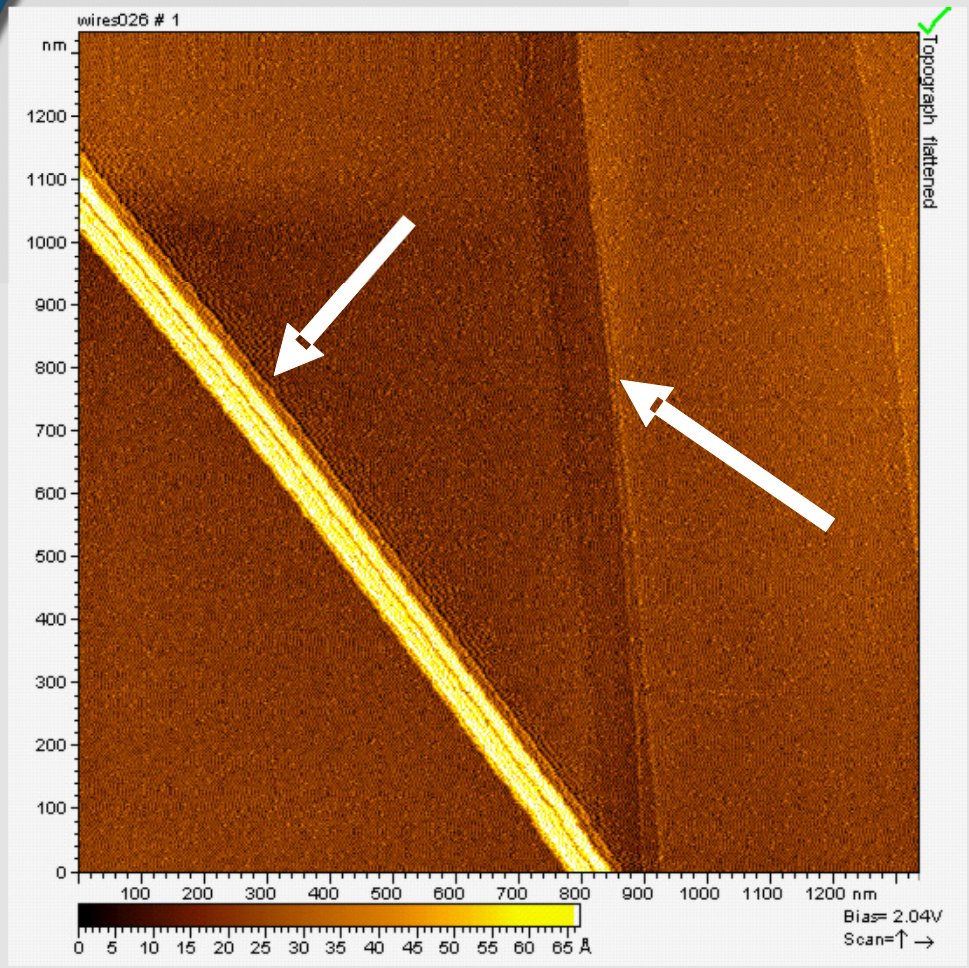
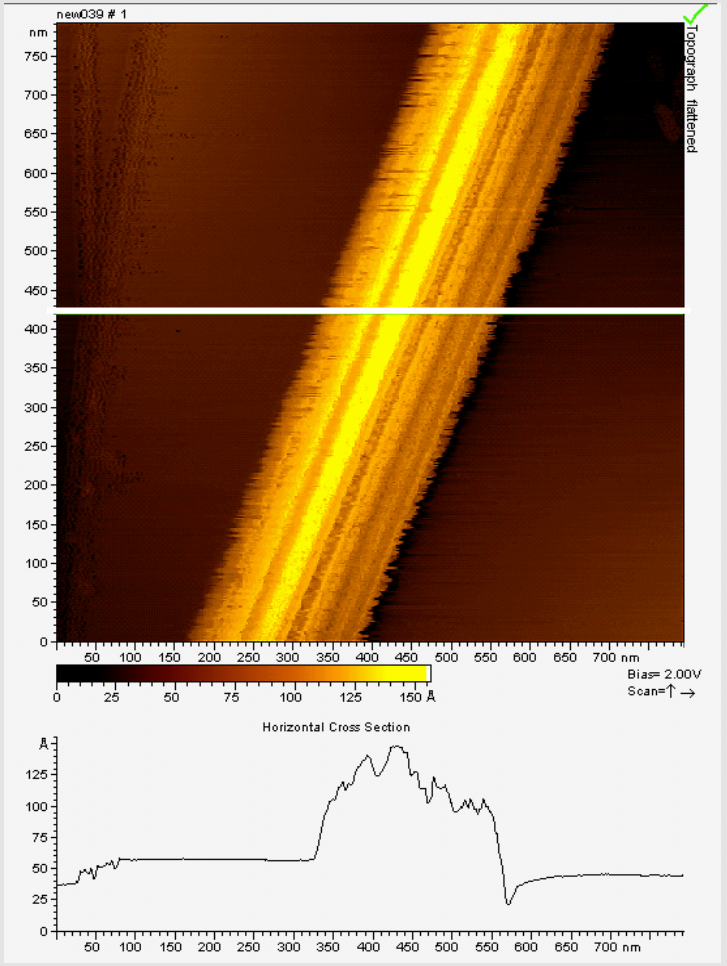


Scanning Tunneling Microscopic images of nanowires from wild type MR-1





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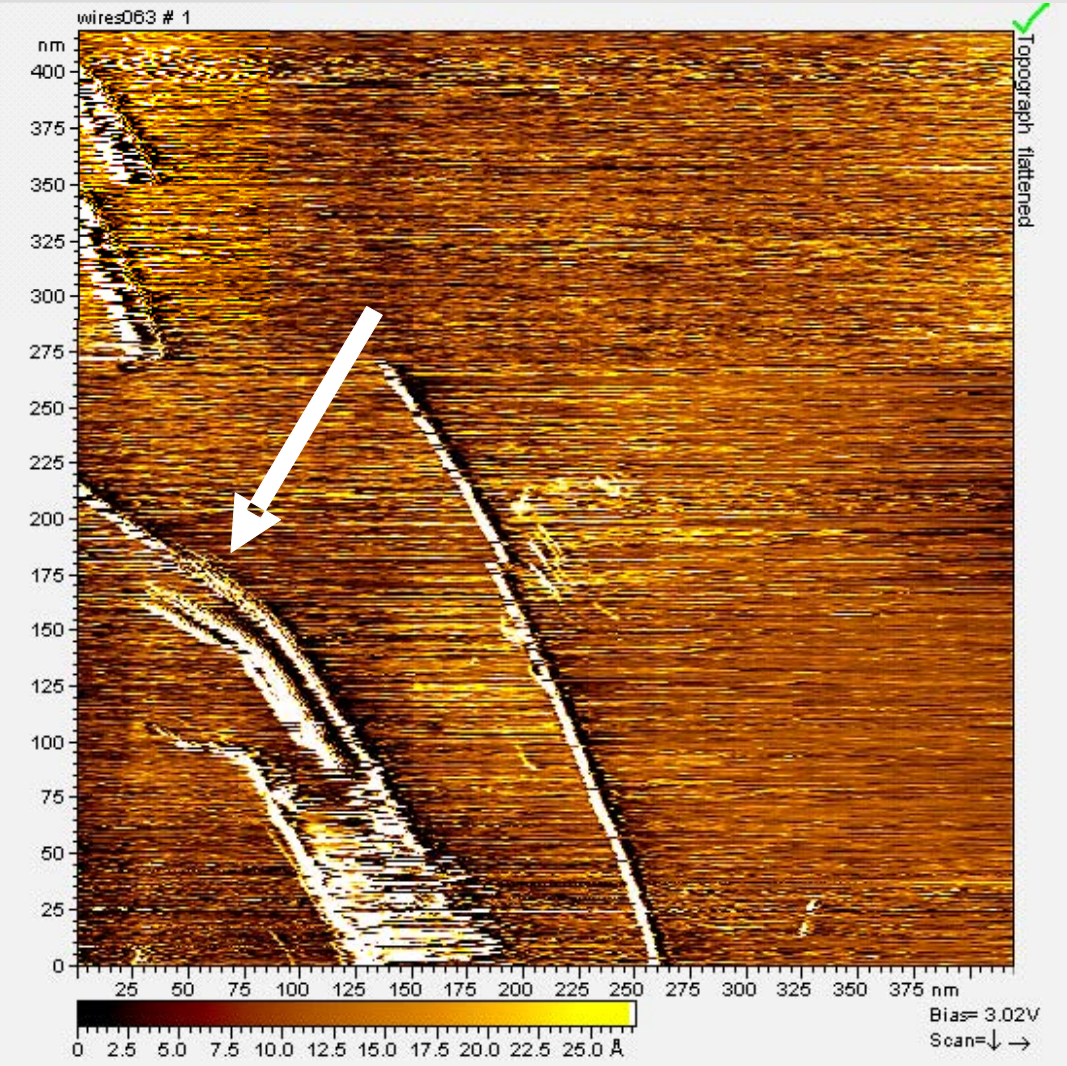
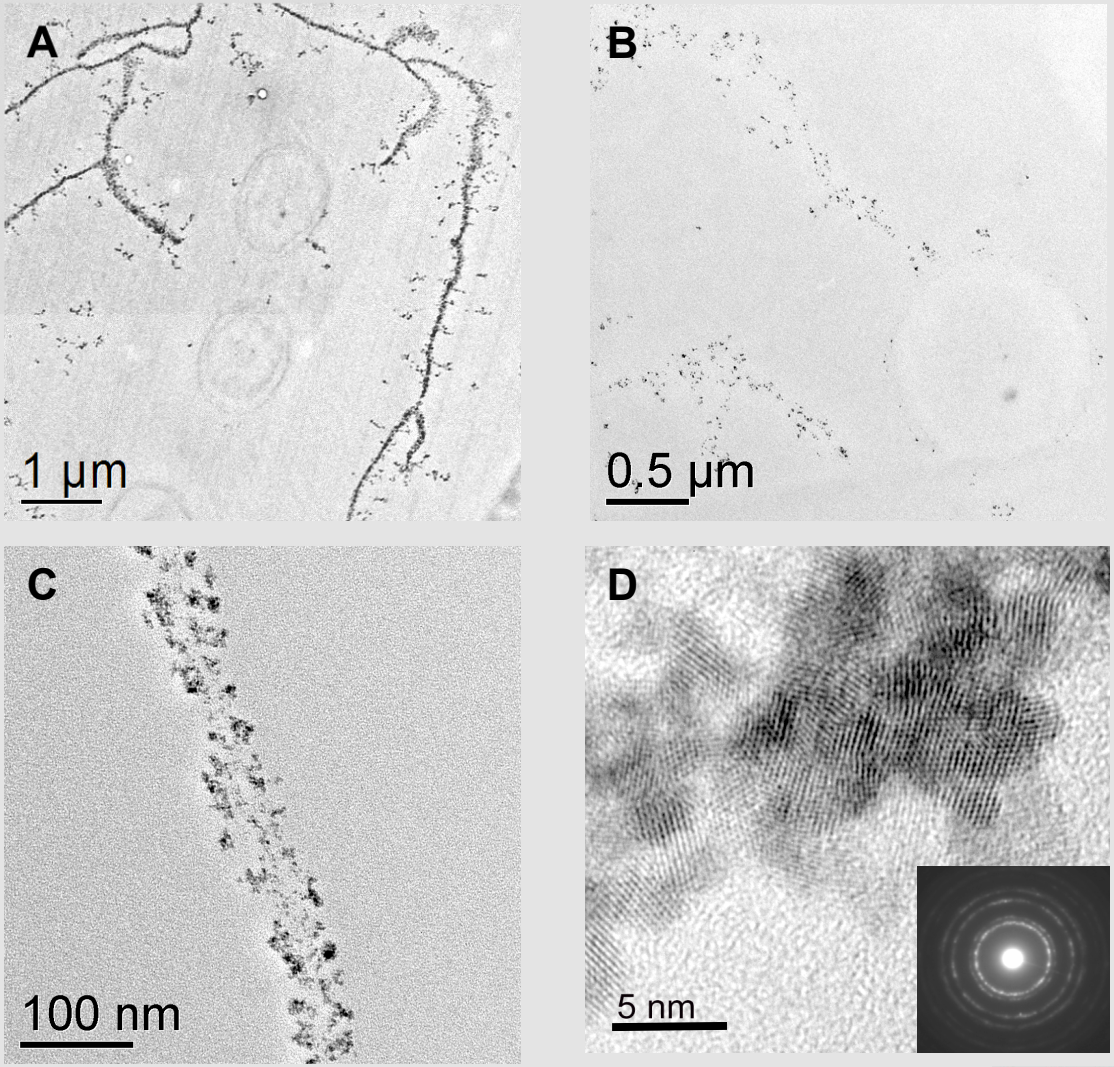


Reduction and precipitation of uranium by MR-1

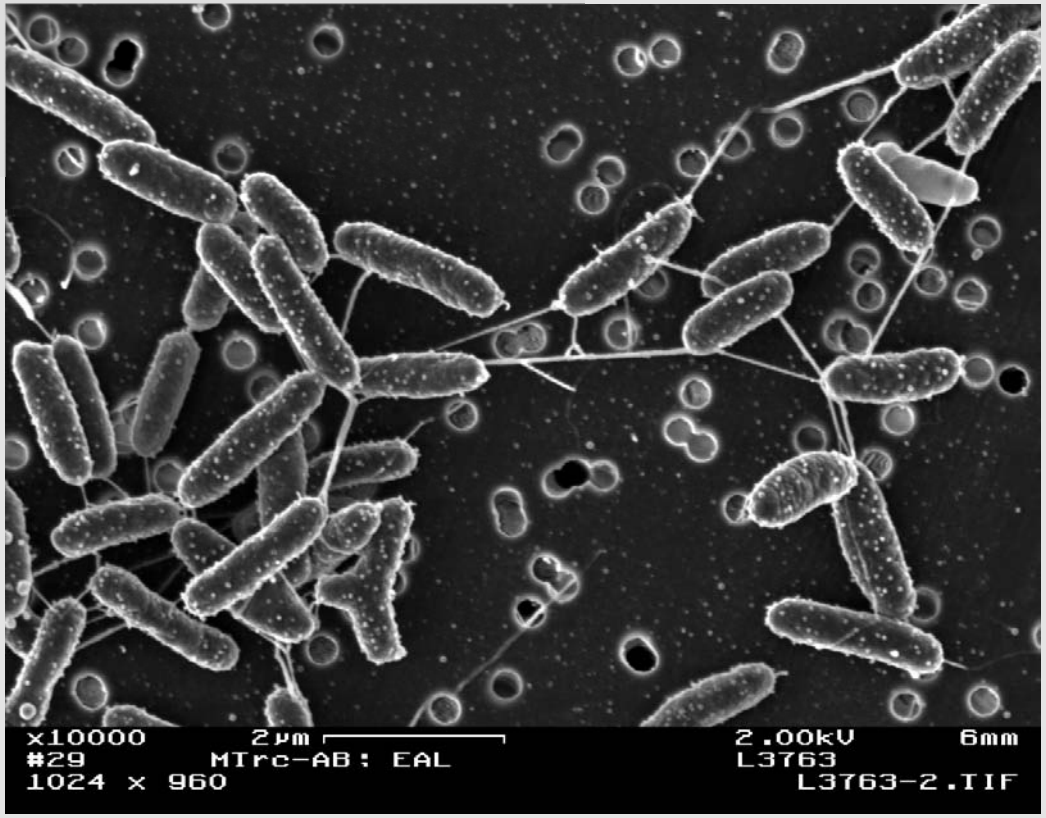


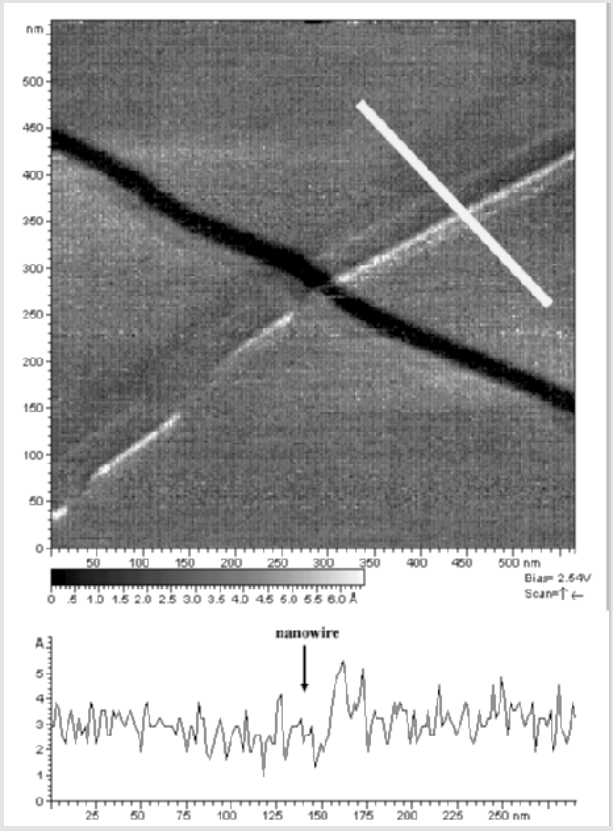
Fractionation protocol

Proteins detected in extracellular fraction

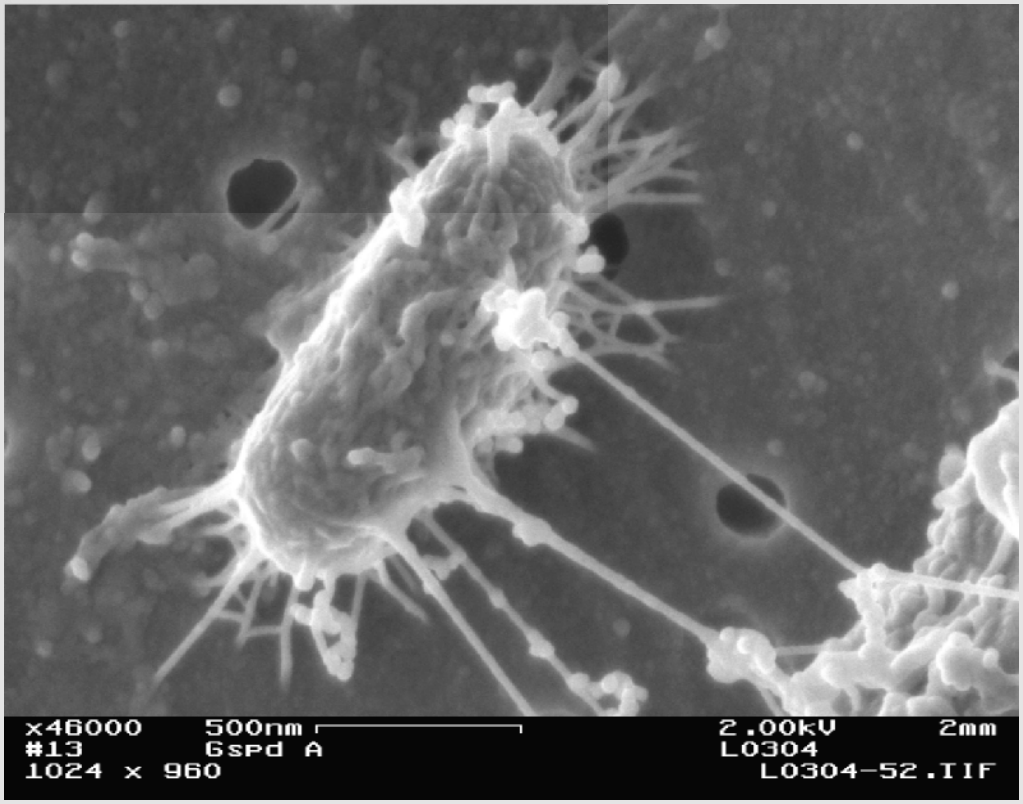
- Outer membrane cytochromes
  - MtrC, OmcA, and MtrB
- Type II secretion protein
  - GspG pseudopilin
- Cell shape determining protein
  - MreB (prokaryotic actin)
- Methyl accepting chemotaxis proteins
- ATP synthase

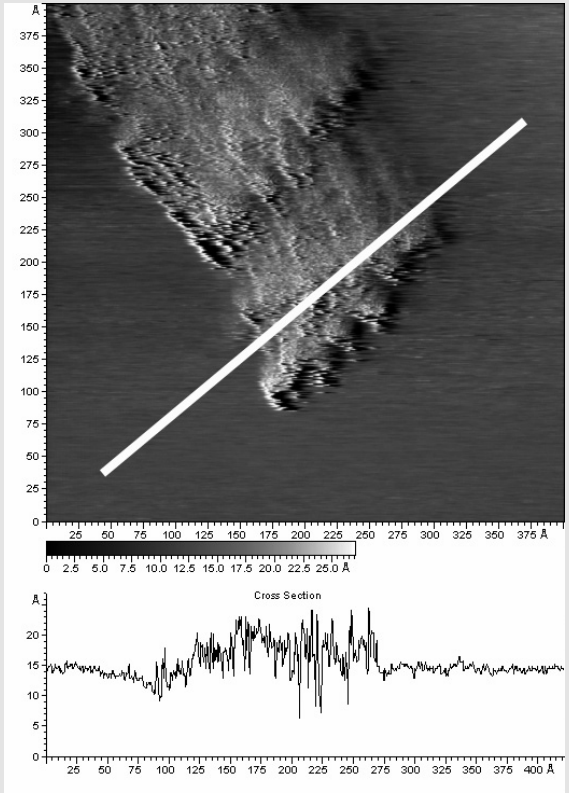
Non-conductive nanowires produced by Δ *mtrC*



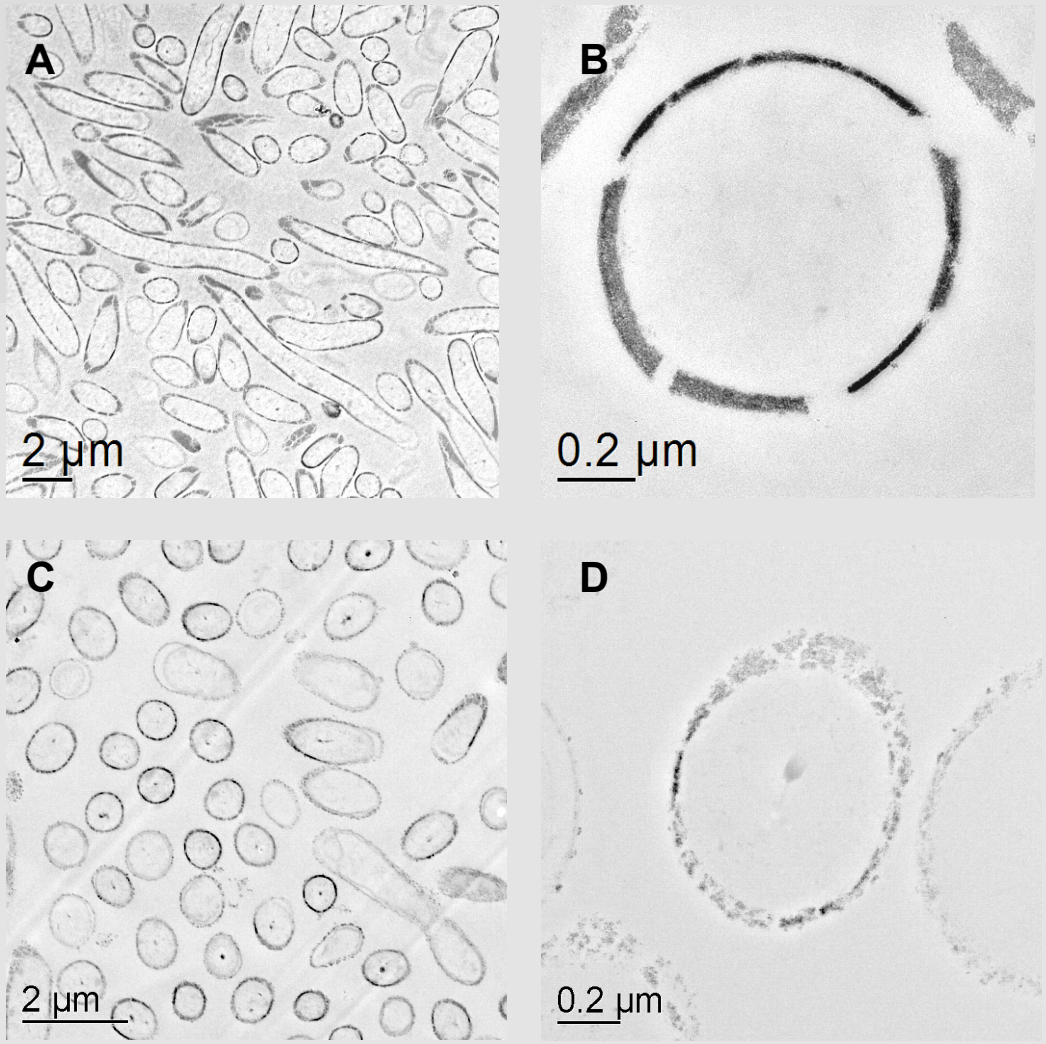


Non-conductive nanowires produced by GspD- mutant





Uranium reduction and precipitation by mutants producing non-functional nanowires

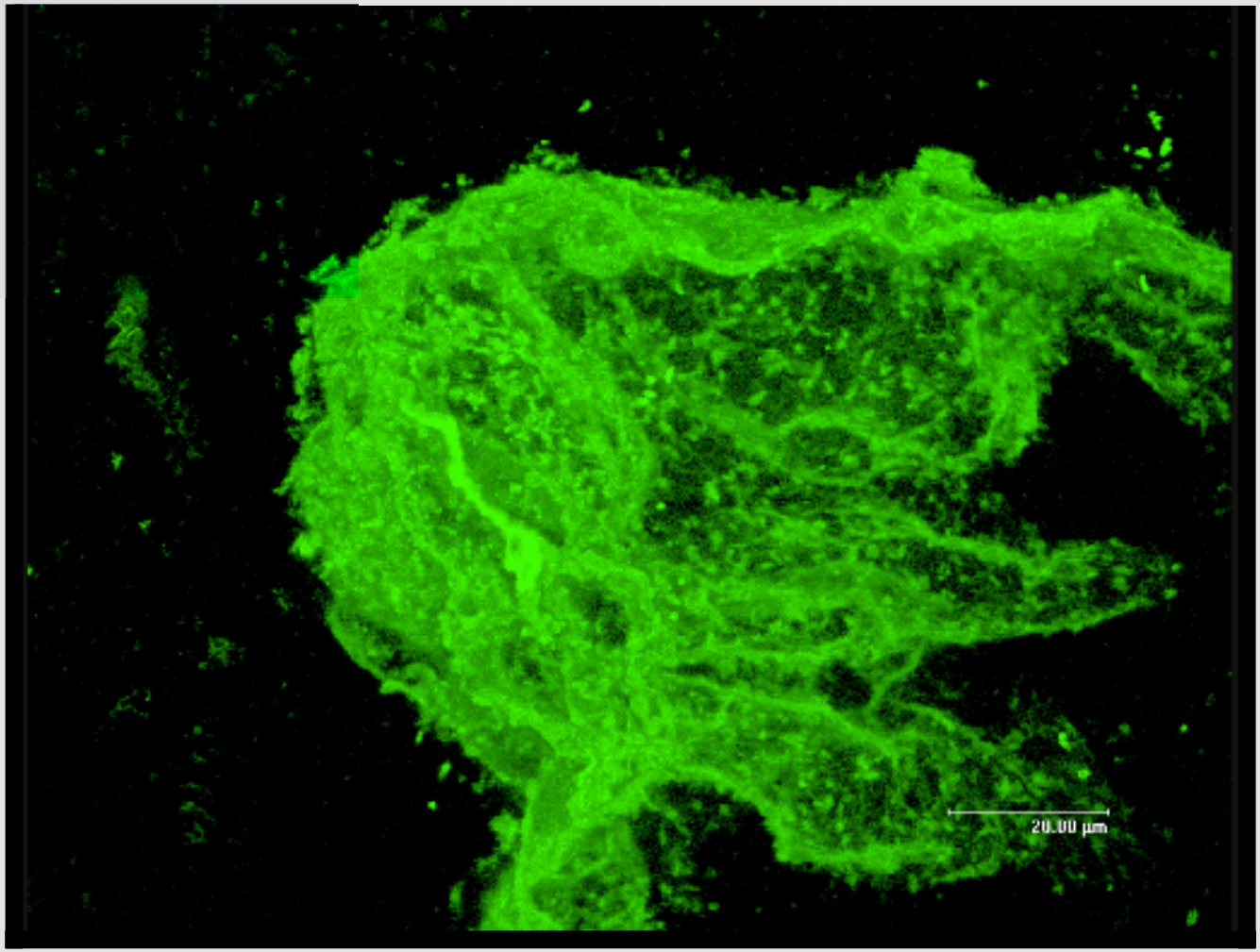


Iron oxide reduction and electricity generation by wild type and mutants of MR-1

Strain	Ferrihydrite reduction extractable Fe(II), mM	Electrochemical activity pA
MR-1	10.14±0.01	68.0±7.8
ΔmtrC	3.11±0.03	4.6±0.2
GSPD	0.8±0.02	ND

Effect of Agitation on Microbial Metabolism and Physiology under Electron Acceptor Limitation

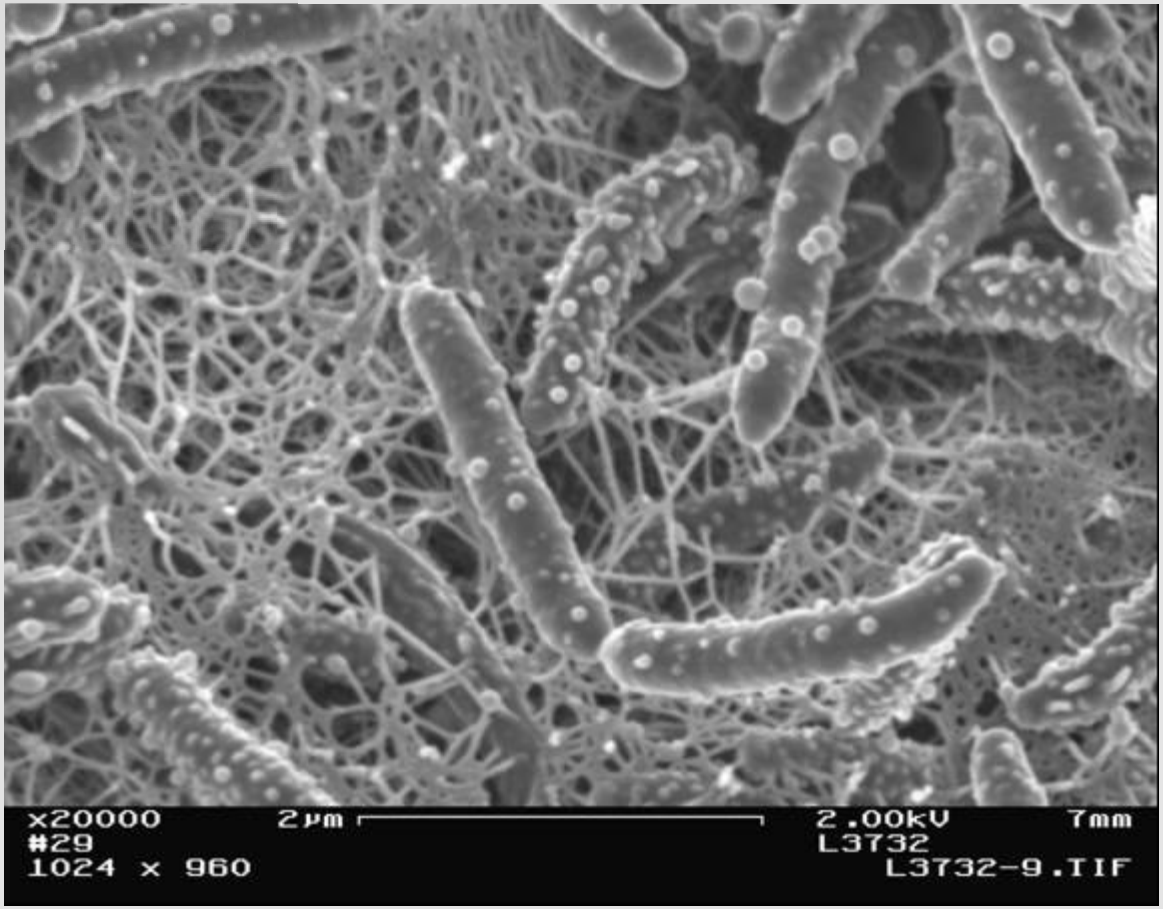
Surface Biofilm of MR-1 Grown in Static Batch Culture



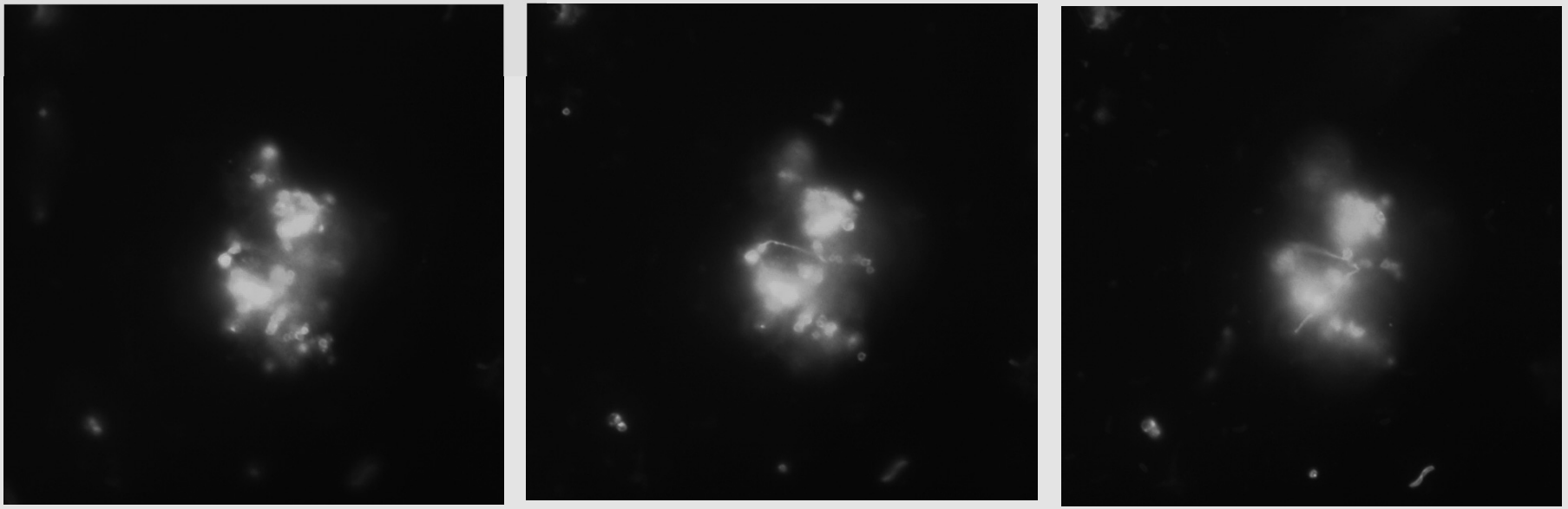
Iron oxide reduction, electricity generation, and biofilm (pellicle) formation by wild type and mutants of MR-1

Strain	Ferrihydrite reduction extractable Fe(II), mM	Electrochemical activity pA	Biofilm formation
MR-1	10.14±0.01	68.0±7.8	+++
ΔmtrC	3.11±0.03	4.6±0.2	-+
GSPD	0.8±0.02	ND	-

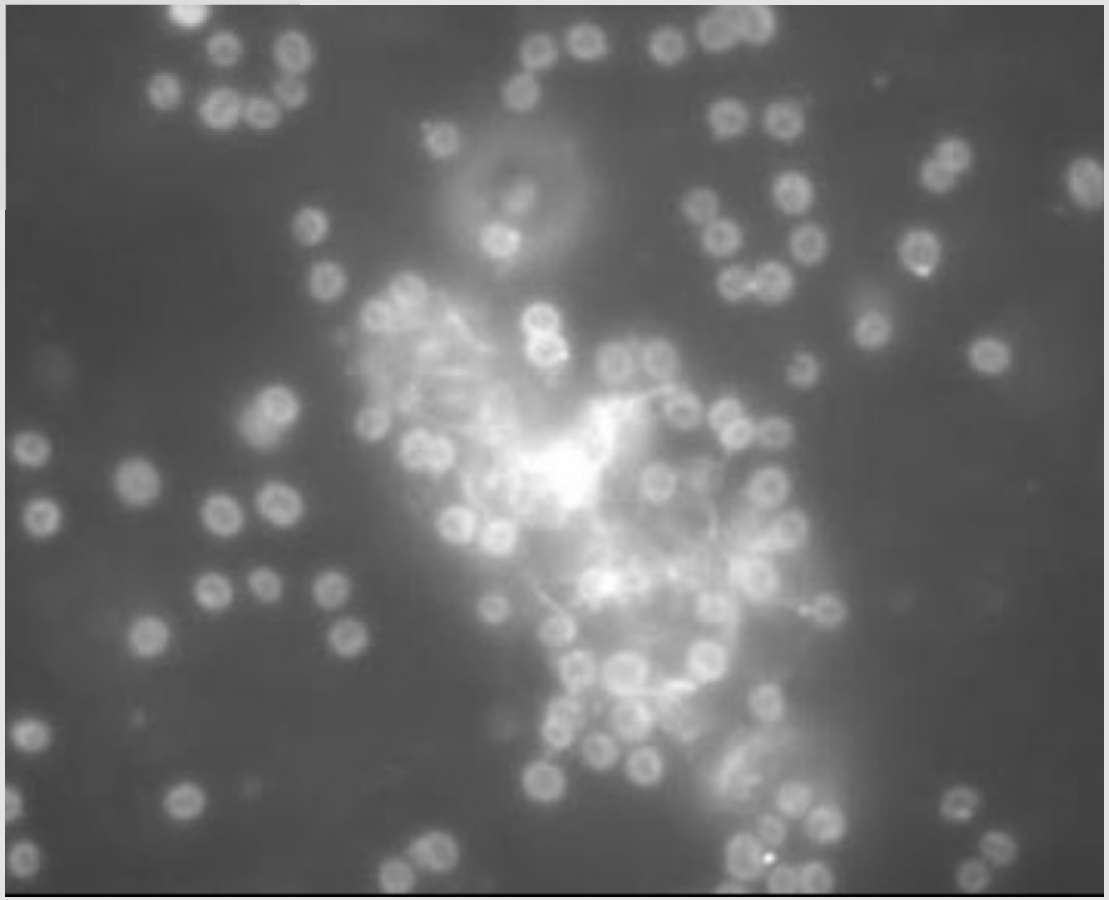
Nanowire produced by *Geobacter sulfureducens* cultivated in a fumarate-limited chemostat



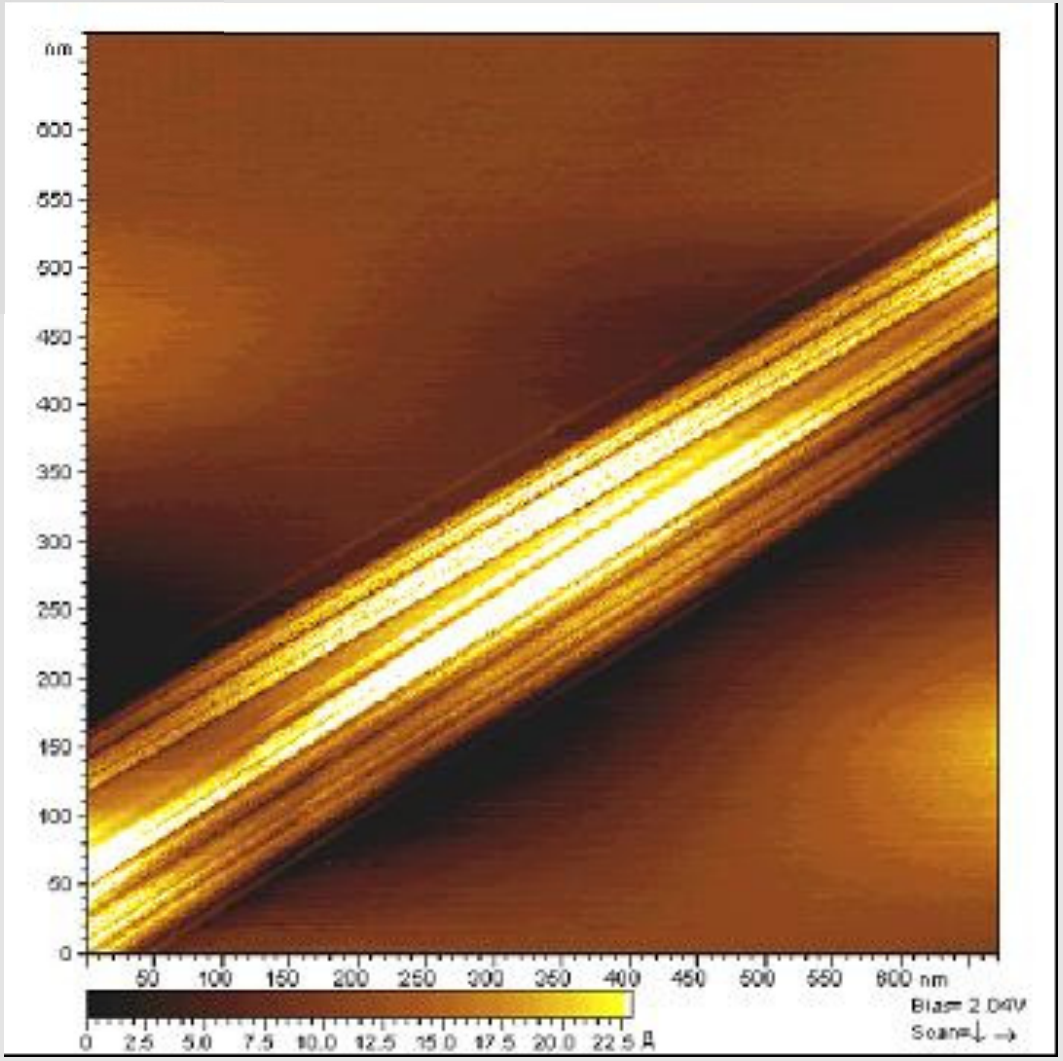
Confocal image of a methanogenic/sulfate reducing co-culture (Dave Stahl)



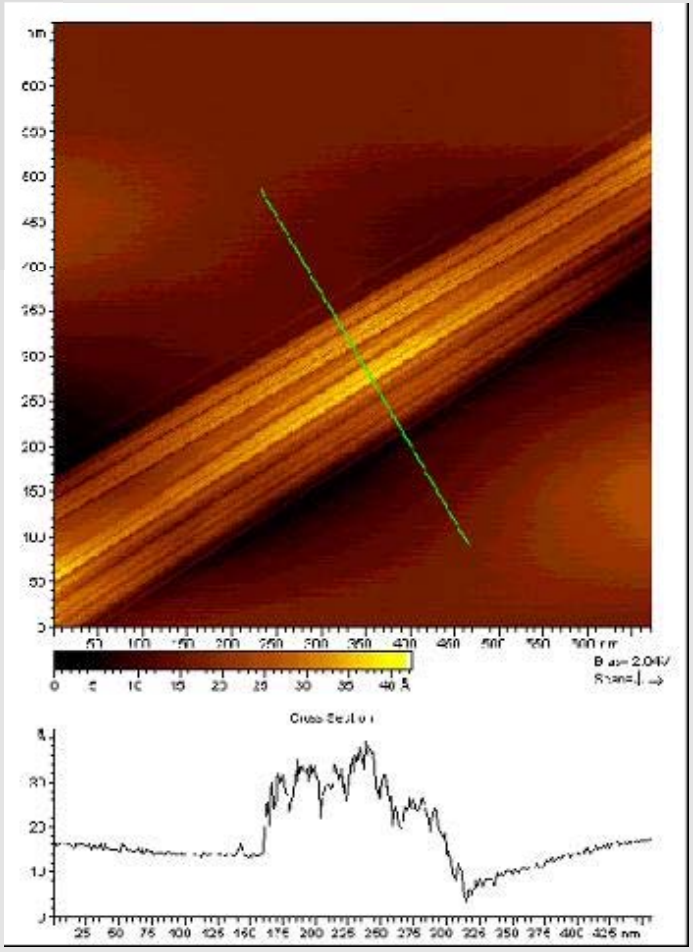
Nanowires produced by *Synechocystis* strain 6803 cultivated in a CO_2-limitation



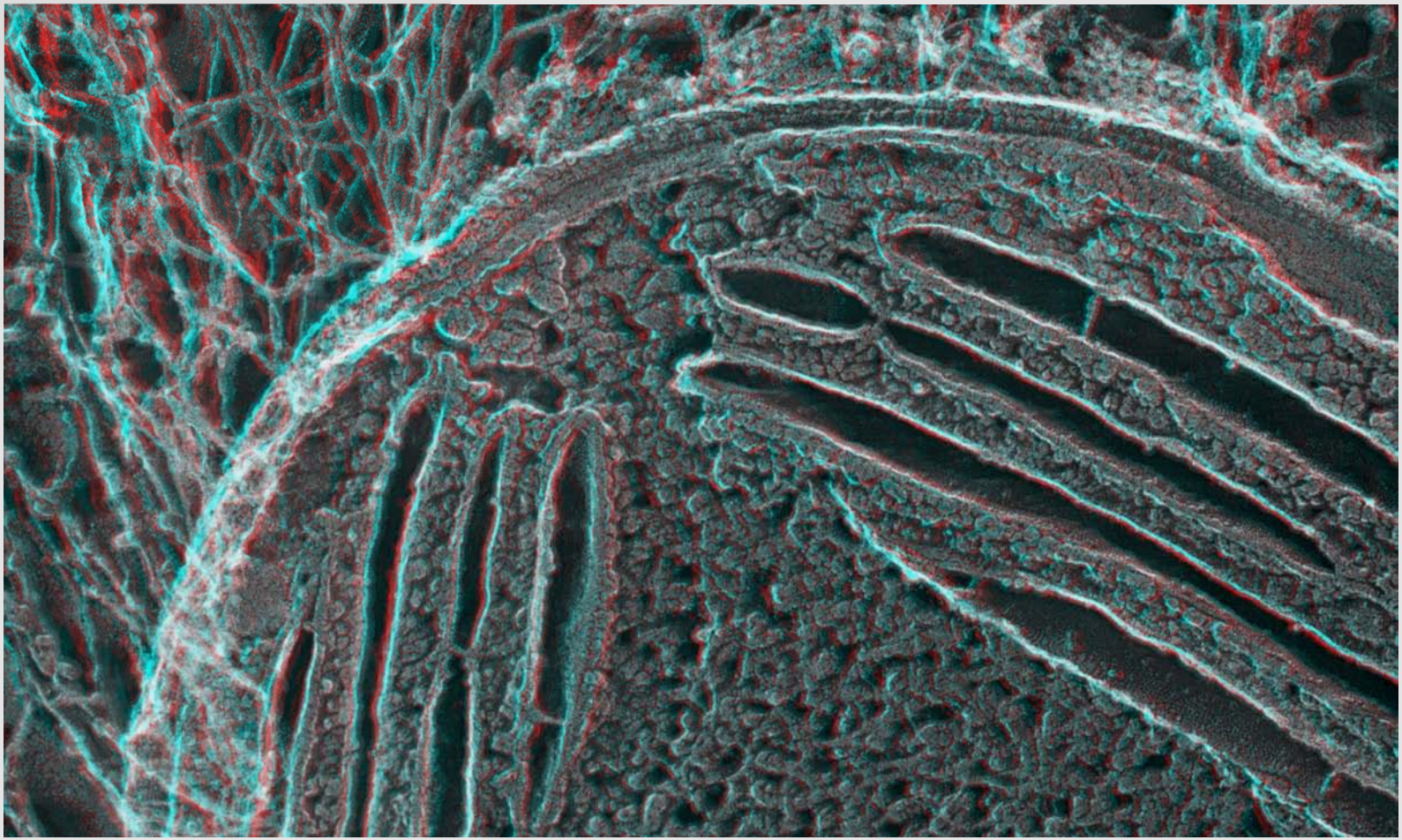
STM image of a nanowire from *Synechocystis* strain 6803



STM image of a nanowire from *Synechocystis* strain 6803



Deep-etch SEM image of *Synechocystis* 6803
(Himadri Pakrasi)



Deep-etch SEM image of *Synechocystis* 6803
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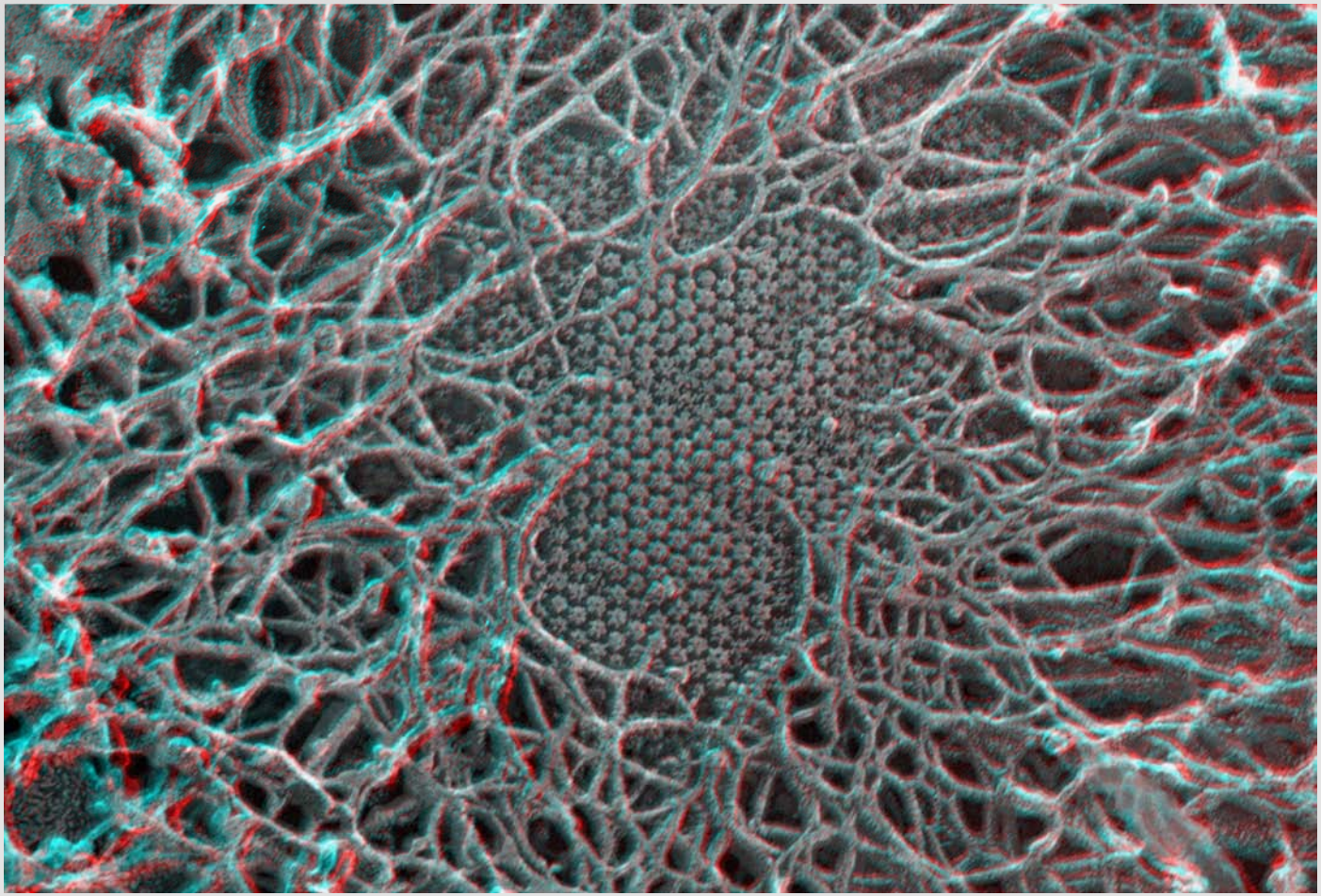

Summary

- Nanowires are produced in response to electron acceptor limitation
  - Low concentration of dissolved electron accetors (O_2, fumarate)
  - Poor accessibility to reactive sites on metal oxide surfaces
- Nanowire are electrically conductive and redox reactive
- Nanowires are complex assemblages
  - Cytochromes (conductivity and redox reactivity
  - Pilins (structural role)
  - Polysaccharides?
- Loss of functionality impacts
  - reduction of solid phase electron acceptors
  - Location of biogenic UO_2 nanocrystals
  - Biofilm formation
  - Ability to produce electricity in a biological fuel cell

Summary (cont.)

- Nanowires are produced diverse metabolic groups
  - DMRB
  - SRB (conductivity measurements pending)
  - Cyanobacteria
  - Iron and manganese oxidizing bacteria (preliminary)
  - Biofilm organisms…..

Future Research

- Immunogold labeling
  - Peptide specific antibodies (Marshall and Beliaev)
  - Determine location, orientation
- Characterize mechanism of electron transfer
  - Microscopic/spectroscopic techniques
  - Heme to heme transfer?
  - Topic for Biogeochemical Grand Challenge (EMSL)
- Identify components in other metabolic groups
  - Functionally similar but compositionally distinct?
  - Origin of components in mixed communities

Collaborators

Alex Beliaev
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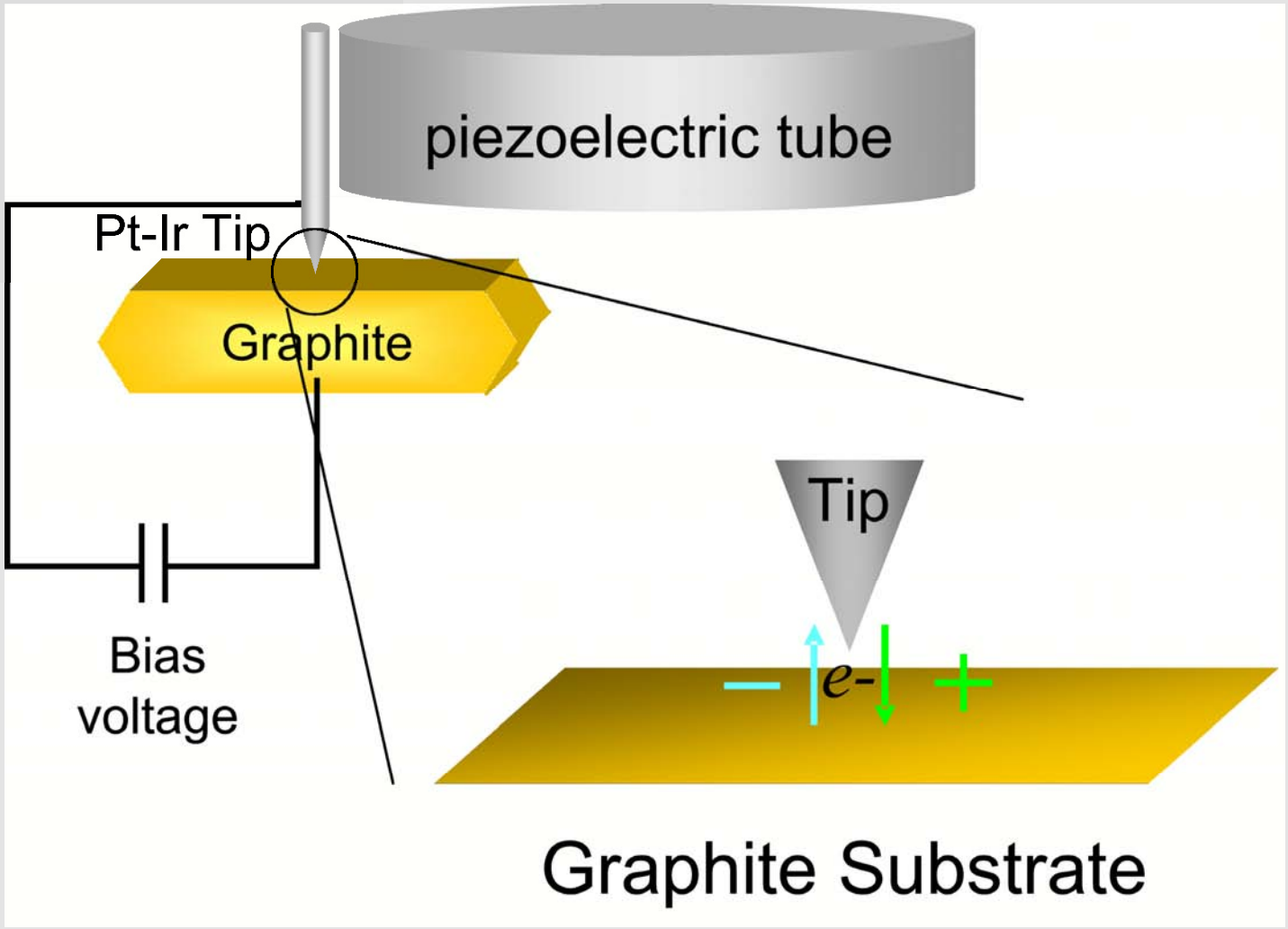
Dwayne Elias
Jim Fredrickson
Tom Gihring
Carol Giometti
Eric Hill
David Kennedy
Peter Lu

Matt Marshall
Jeff McLean
Ken Nealson
Duohai Pan
Grigoriy Pinchuk
Bree Reed
Margie Romine
Kevin Rosso
Daad Saffarini
Svetlana Yanina
John Zachara

Scanning Tunneling Microscopy



Type II General Secretion Pathway

Type II Secretion Pathway

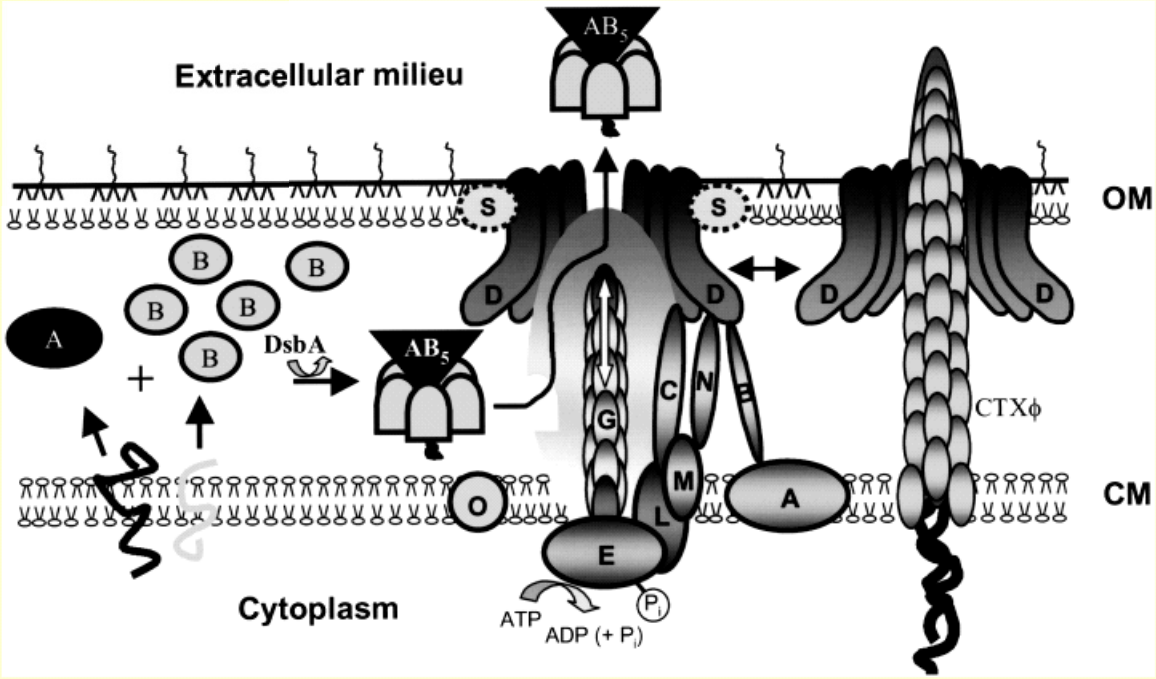


Figure above from Sandkvist, M., Biology of Type II Secretion . Mol. Microbiol. 2001. 40(2):271-283.
In this figure DsbA and DsbB are the proteins secreted. We do not yet know which proteins are secreted by the *Shewanella* type II pathway, but we do know that this pathway is required for respiration of Fe and DMSO.

Operon Structure (created at MGBD site)

TEM image of thin sectionS of strain MR-1 cultured in agarose medium with electron acceptor limitation