New Insights into the Mechanism of Bacterial Metal Respiration

Thomas J. DiChristina

School of Biology
Georgia Tech

Fe: Charles Moore, Carolyn Haller, David Bates
Mn: Brian Burnes, Karen Sankovich
U: Roy Wade, Jason Dale
Se: Ellen Taratus, Sean Eubanks
Tc: Amanda Payne

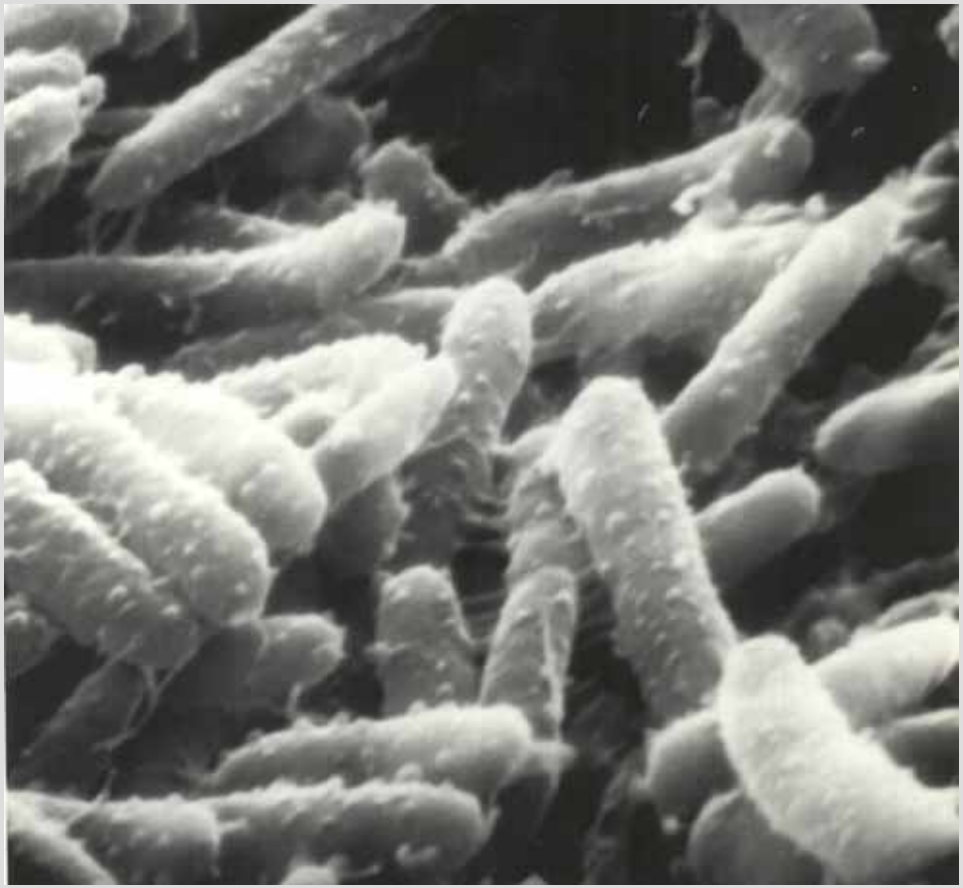
PROJECT GOAL

Identify genes and gene products required for microbial metal reduction

- Reductive dissolution of **iron**
- Reductive dissolution of **manganese**
- Reductive precipitation of **selenium**
- Reductive precipitation of **uranium**
- Reductive precipitation of **technetium**

Model Metal Reducing Bacteria:

Shewanella putrefaciens strain 200
Shewanella oneidensis strain MR-1



Shewanella Respiratory Capability

Electron Donors:

Organic acids
Amino acids
Sugars
Hydrogen

Electron Acceptors:

Oxygen [O_2]
Nitrogen compounds [NO_3^-, NO_2^-, NO]
Manganese oxides [Mn^{4+}, Mn^{3+}]
Ferric iron [Fe^{3+}]
Sulfur compounds [SO_3^{2-}, $S_2O_3^{2-}$, S(0), DMSO]
Uranium [U^{6+}]
Technetium [Tc^{7+}, Tc^{4+}]
Selenium [Se^{4+}]
Trimethylamine-*N*-oxide [TMAO]
Fumarate
AQDS (electron shuttle)
Others: Arsenate, Chromate, Vanadate, Neptunium(V), Deaminated histidine, Phenazines

EXPERIMENTAL STRATEGY

Genetic complementation analysis of metal reduction-specific mutants

1. Mutagenize WT via chemical or transposon mutagenesis
2. Identify metal reduction-specific mutants
3. Mobilize WT gene clone bank into mutants
4. Identify transconjugates with restored metal reduction capability
5. Subclone, analyze nucleotide sequence of complementing gene

Random Mutagenesis

1. Metal reduction-specific
2. High-throughput (may need to screen 20,000)

Clarke-Carbon equation:

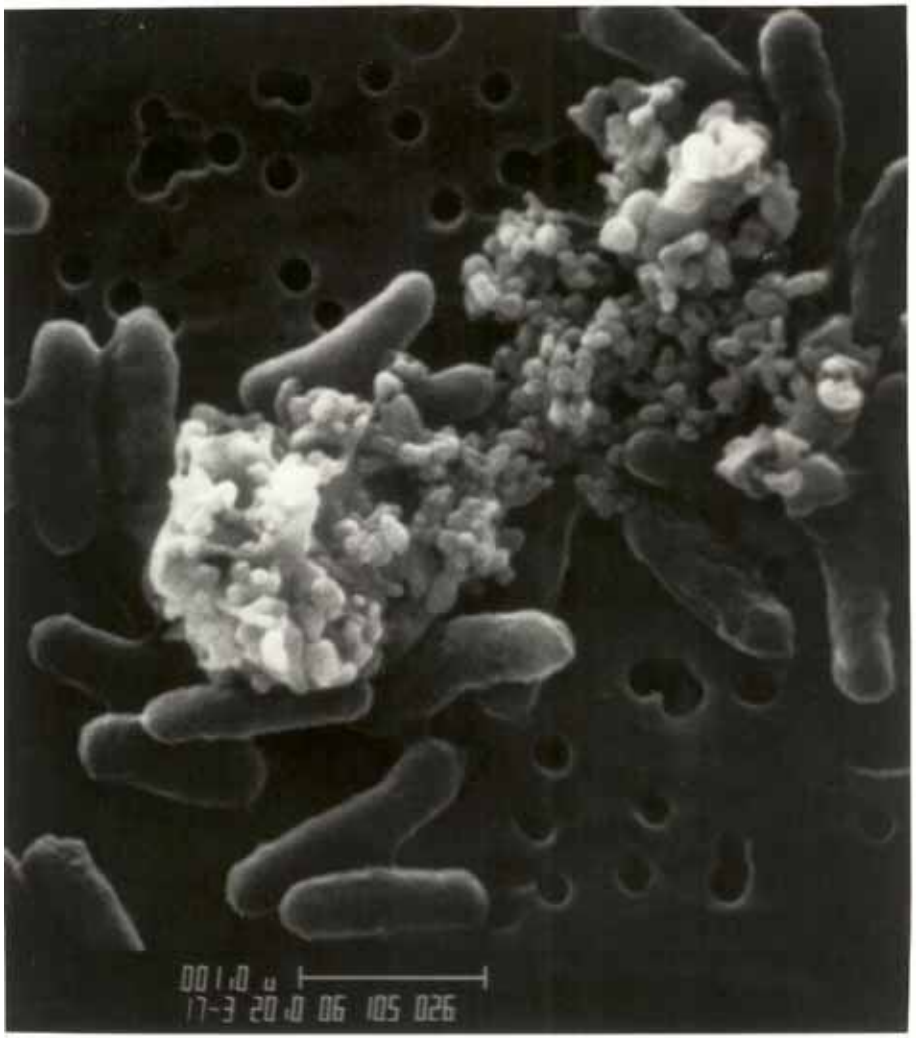
$$P = 1-(1-f)^N$$

P = probability of identifying mutant (99%)
f = absolute frequency (1/4000 genes)
N = number of random mutants that must be screened to ensure a 99% probability of identifying mutant in 4000 gene chromosome if phenotype encoded by one gene: N = 20,000

- red ping pong ball analogy

Microbial Fe(III) respiration

Physiological problem associated with Fe(III) respiration



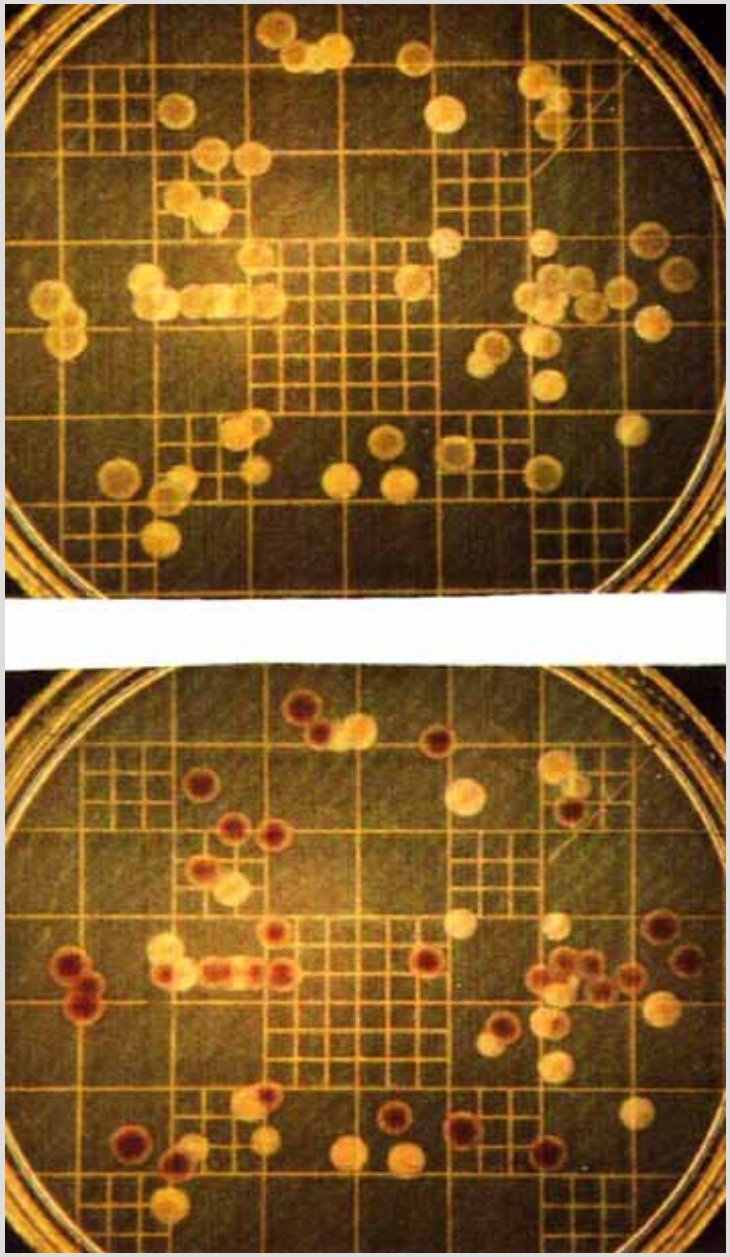
Problem:

Anaerobic respiration on insoluble electron acceptor

Potential solutions:

1. Transport Fe(III) in
2. Transport Fe(III) reductase out
3. Exogenous electron shuttles
4. Endogenous electron shuttles

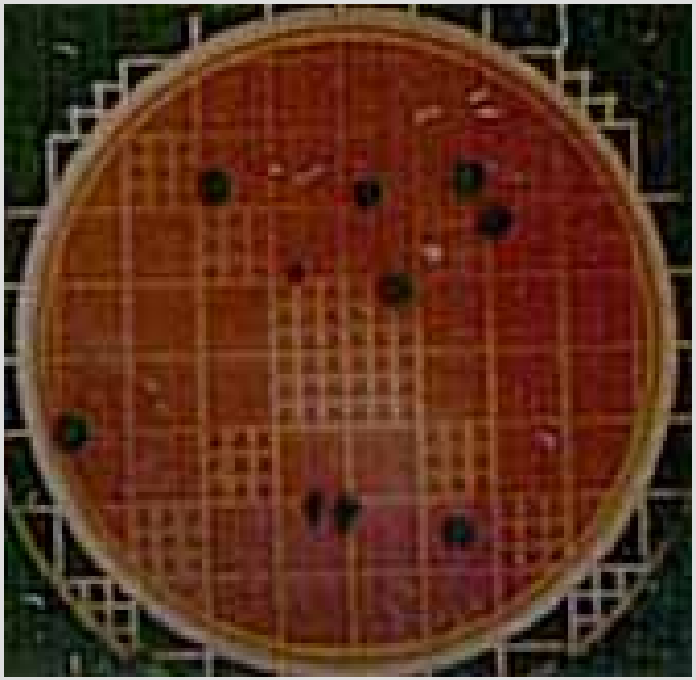
Ferrozine spray

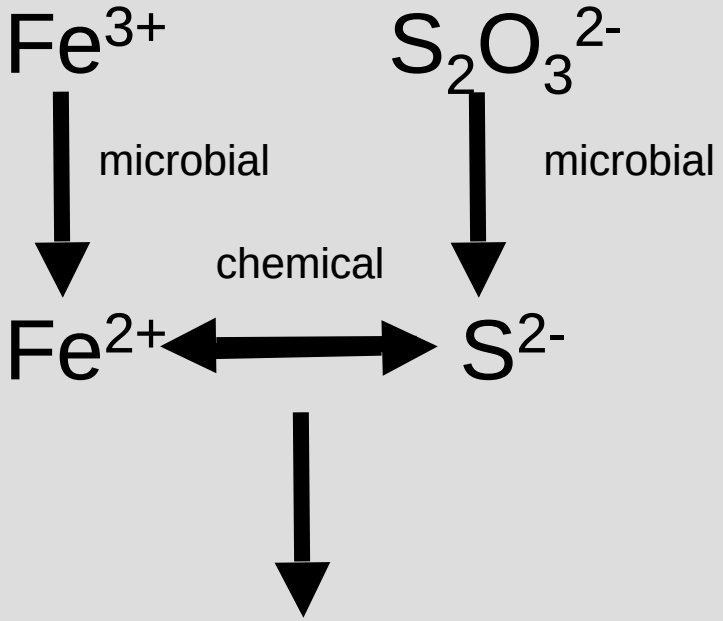


Before spray

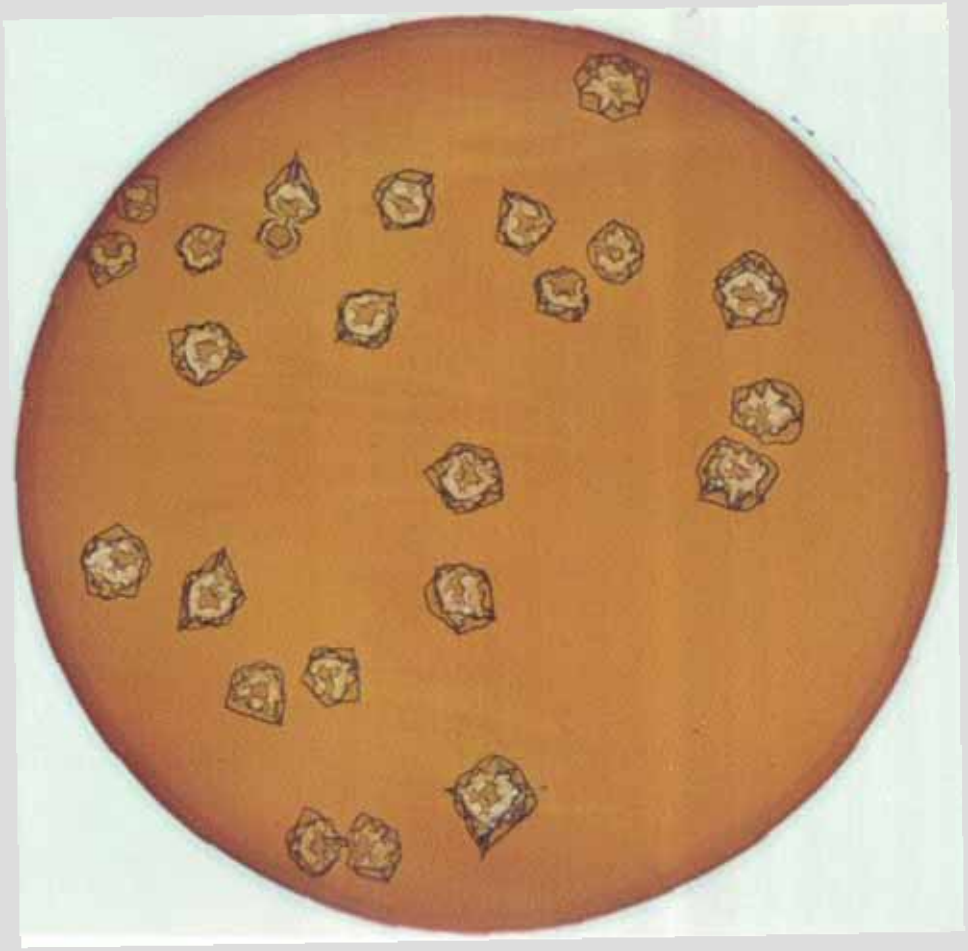
After spray

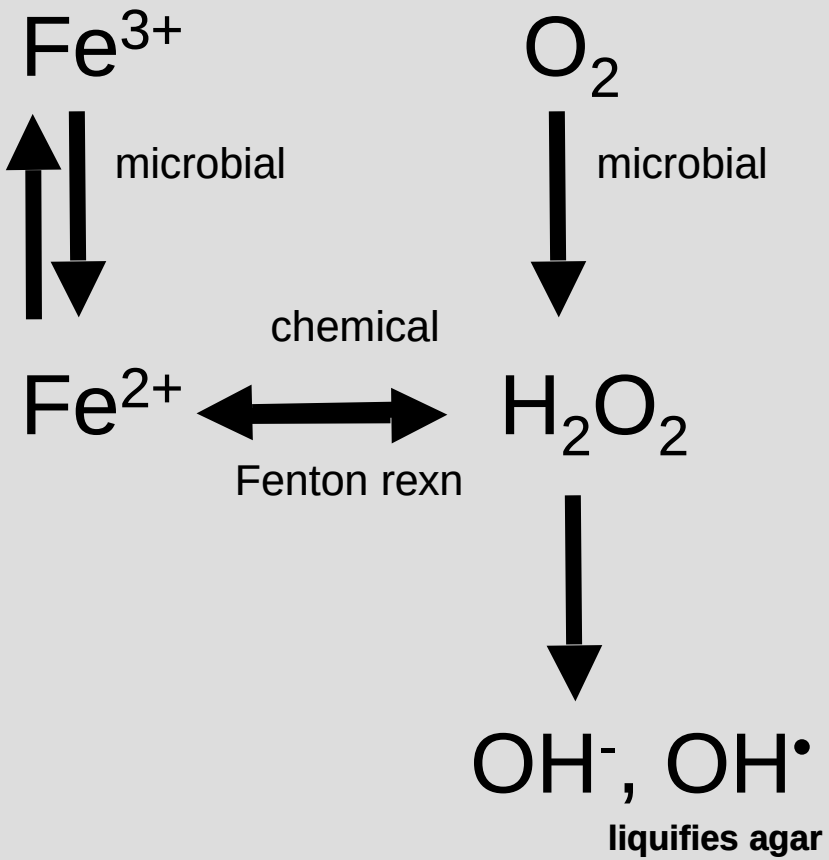
TSI Agar Phenotype



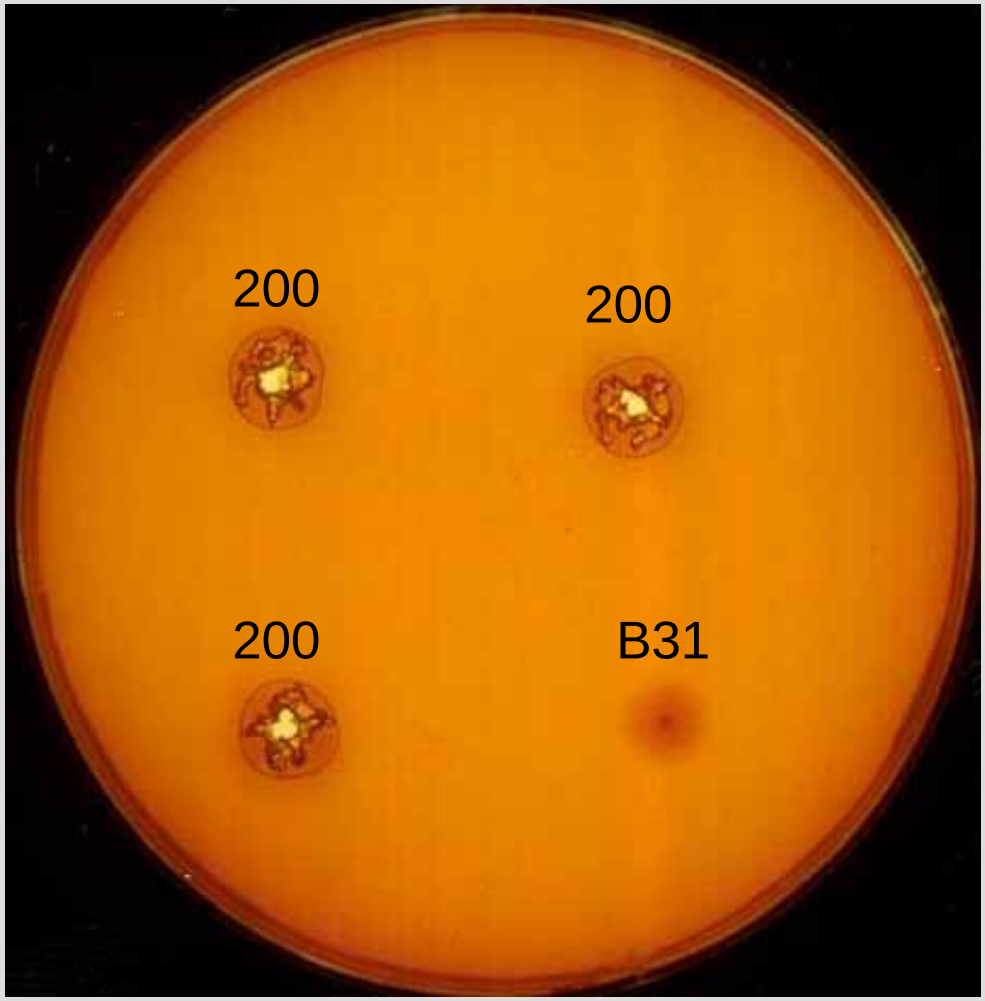


AGR phenotype

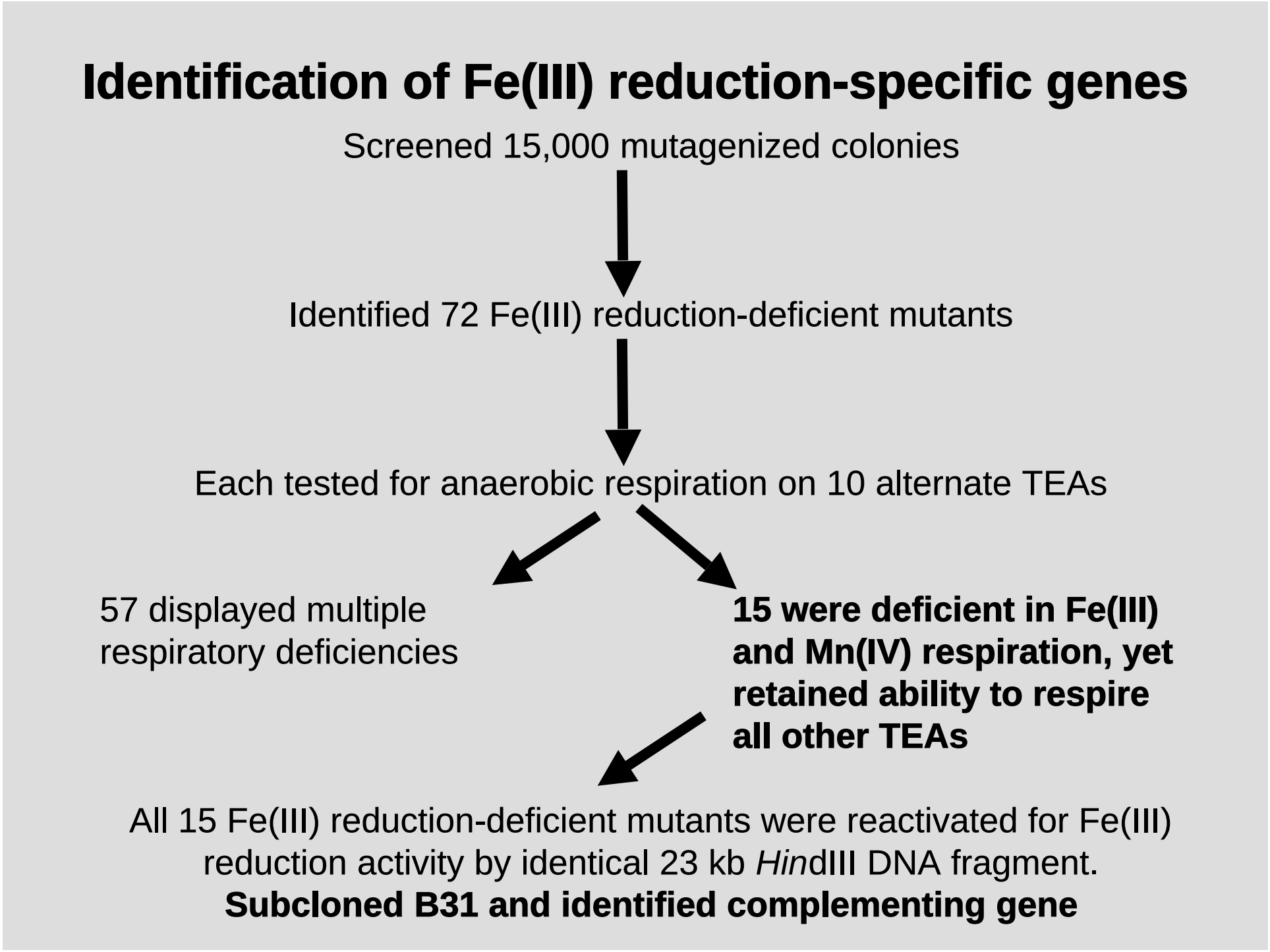




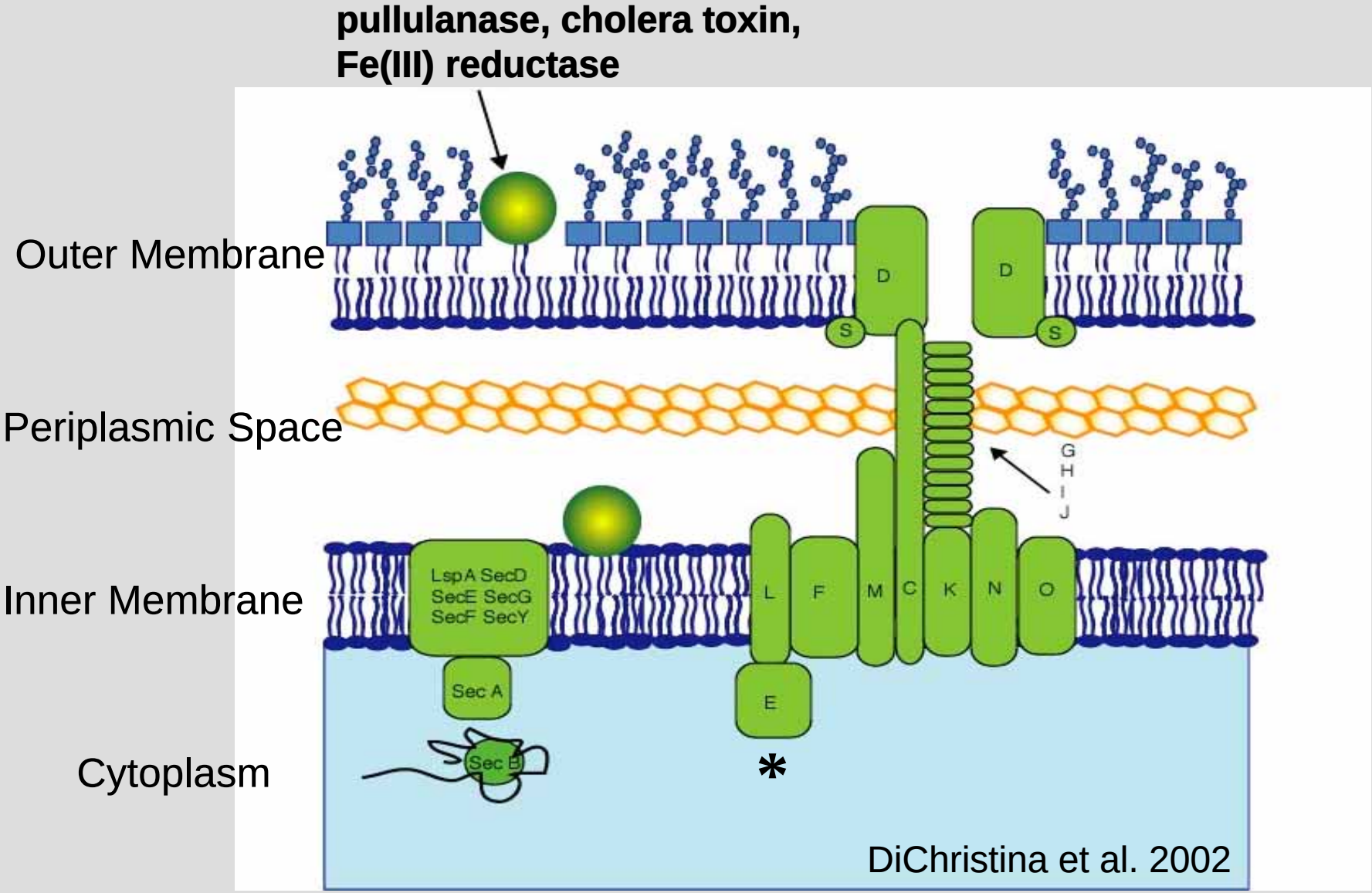
AGR mutant phenotype



Identification of Fe(III) reduction-specific genes

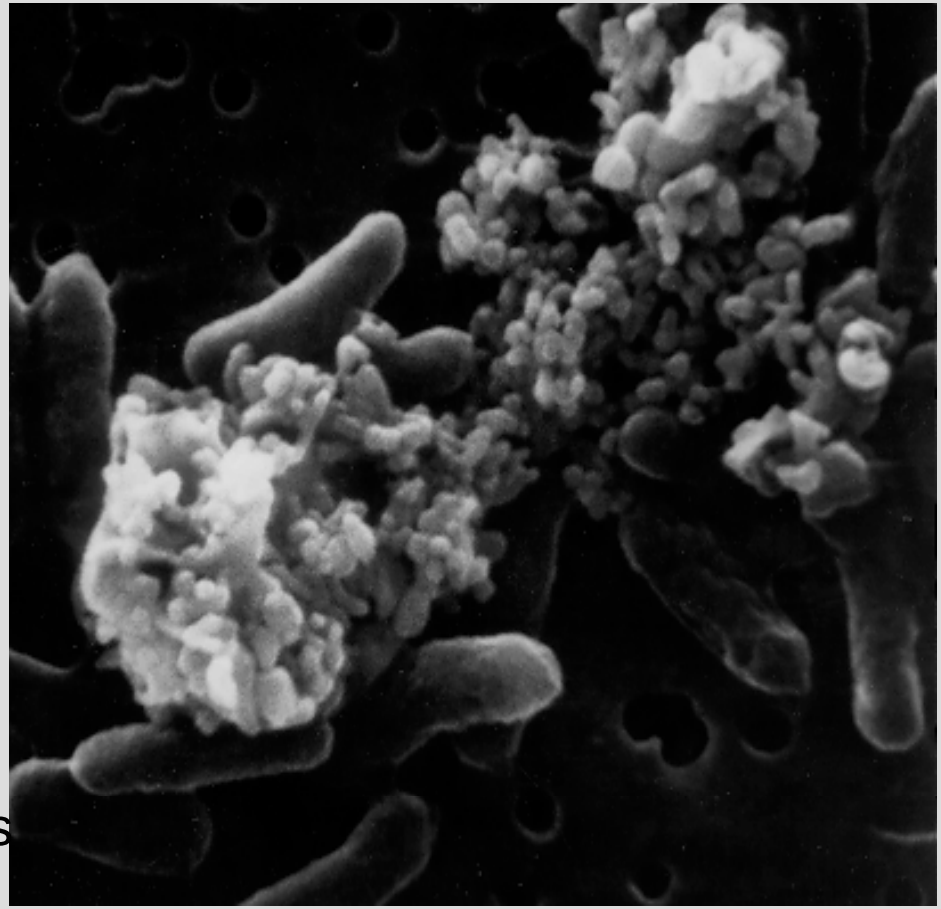


Type II Protein Secretion



WORKING HYPOTHESIS:

Fe(III)-reducing *Shewanella* employ a Type II protein secretion system to target the Fe(III) terminal reductase to the outside face of the outer membrane where it transfers electrons to insoluble Fe(III) substrates



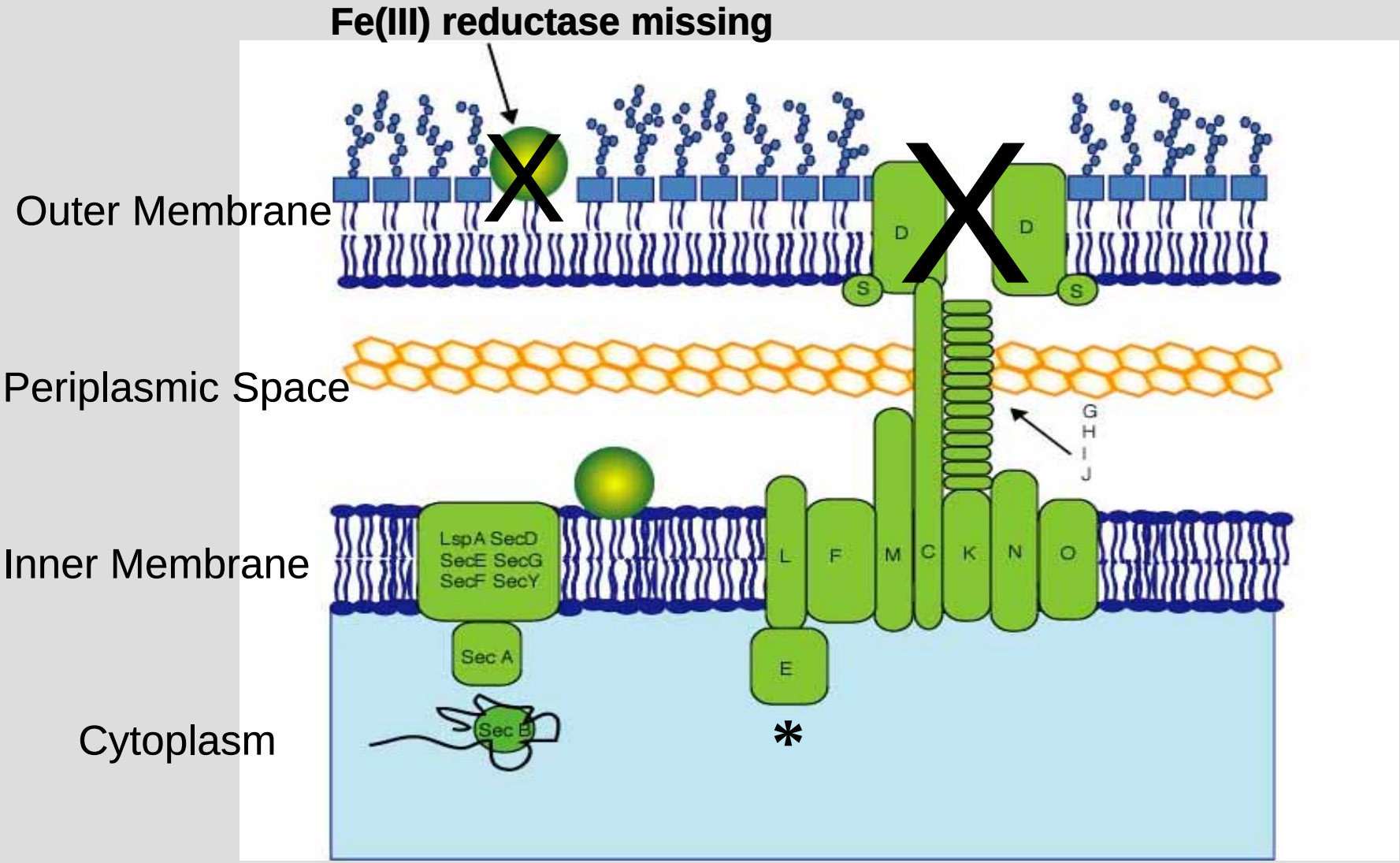
- Delete *gspD* in *S. oneidensis* MR-1
- 0.5 M KCl wash to detach peripheral proteins from outside face of outer membrane of wild-type and Δ*gspD.*
- Compare profiles of Fe(III) reductases attached to periphery of wild-type and Δ*gspD* mutant.
- Isolate and ID Fe(III) reductase from wild-type strain (missing in Δ*gspD*)

Type II Secretion Genes in *S. oneidensis* MR-1 genome

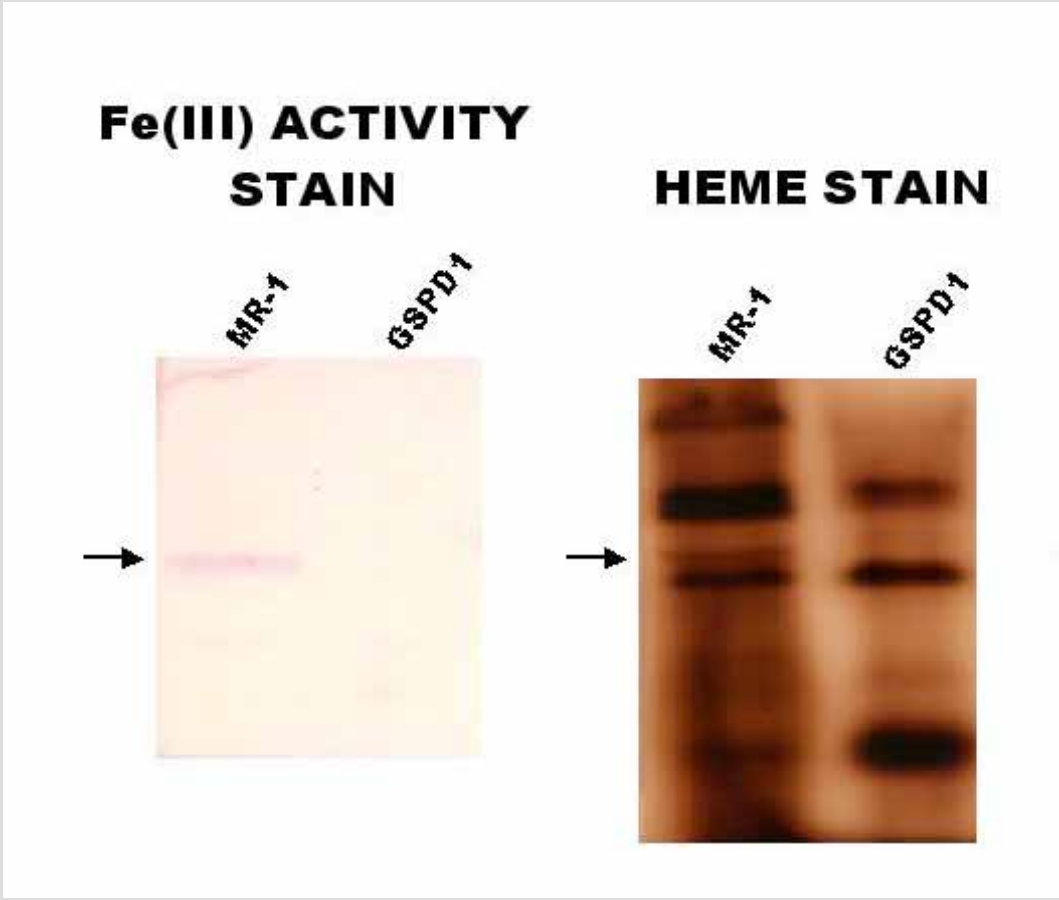
BLAST Results of predicted *S. putrefaciens* strain MR-1 Type II genes

	pulC	39%	55%	1 X 10^{-51}	*A. salmonicida exeC*
*	*pulD*	53%	70%	0.0	*V. cholera epsD*
	pulE	69%	81%	0.0	*V. cholera epsE*
	pulF	63%	77%	1 X 10^{-132}	*A. hydrophila exeF*
	pulG	77%	88%	1 X 10^{-59}	*A. hydrophila exeG*
	pulH	32%	53%	2 X 10^{-21}	*A. hydrophila exeH*
	pulI	33%	54%	2 X 10^{-15}	*A. hydrophila exeI*
	pulJ	39%	57%	1 X 10^{-34}	*A. hydrophila exeJ*
	pulK	40%	58%	2 X 10^{-55}	*A. hydrophila exeK*
	pulL	42%	58%	1 X 10^{-75}	*A. hydrophila exeL*
	pulM	41%	66%	1 X 10^{-31}	*A. hydrophila exeM*
	pulN	27%	44%	3 X 10^{-23}	*A. hydrophila exeN*

Type II Protein Secretion



Fe(III) reductase missing from peripheral proteins detached from outside face of *S. oneidensis* MR-1 outer membrane



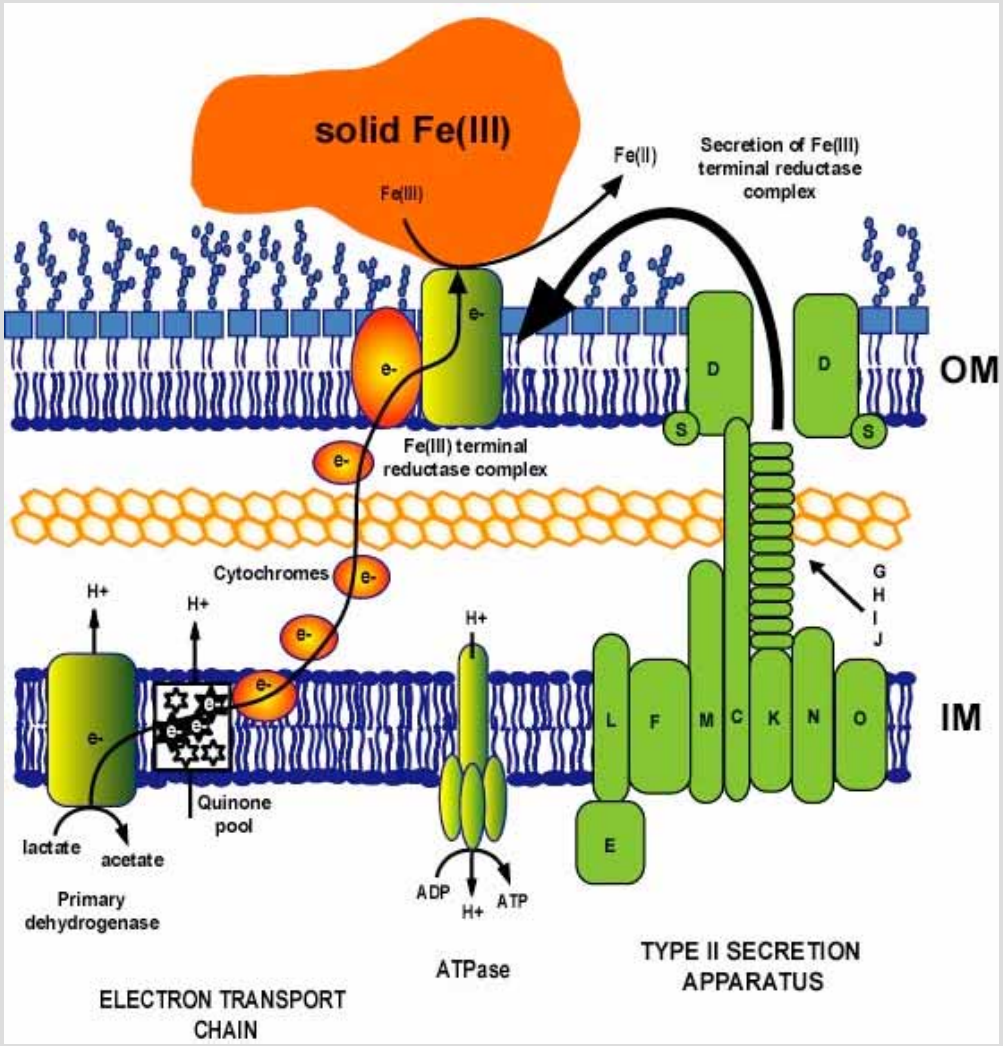
MALDI-TOF MS analysis of polypeptides in Fe(III) reductase complex of *S. oneidensis* MR-1

- Five polypeptides
- First three polypeptides have been identified:

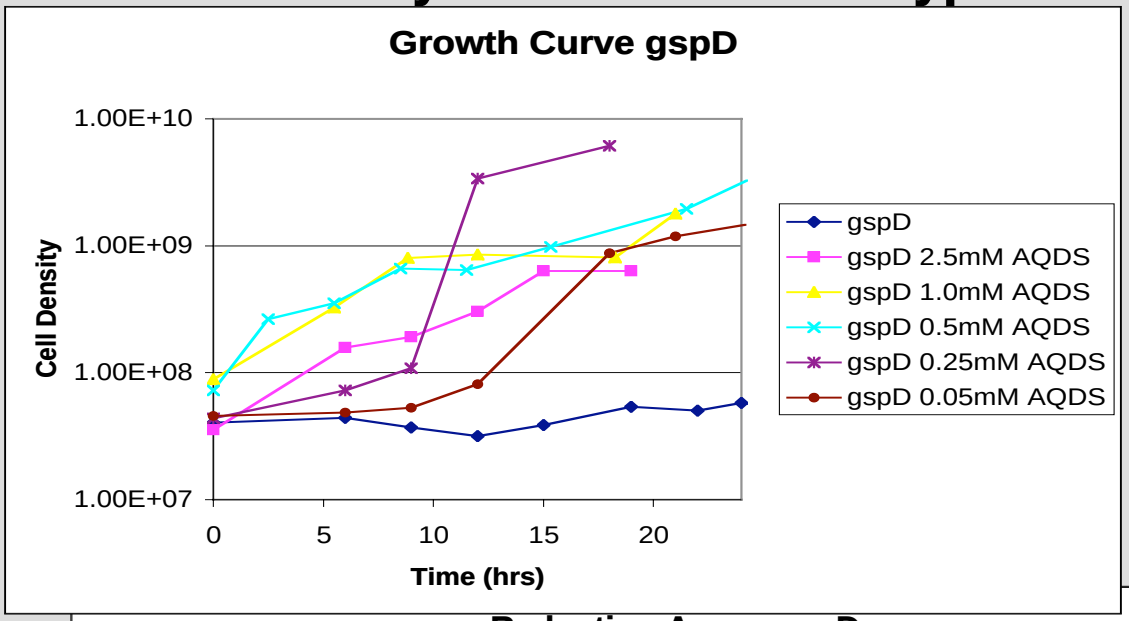
  1. Protease
  2. Omc B - Decaheme *c*-type cytochrome
  3. SoxB - Component of sulfur oxidation pathway (77% similar to *Vibrio parahaemolyticus* SoxB homolog)

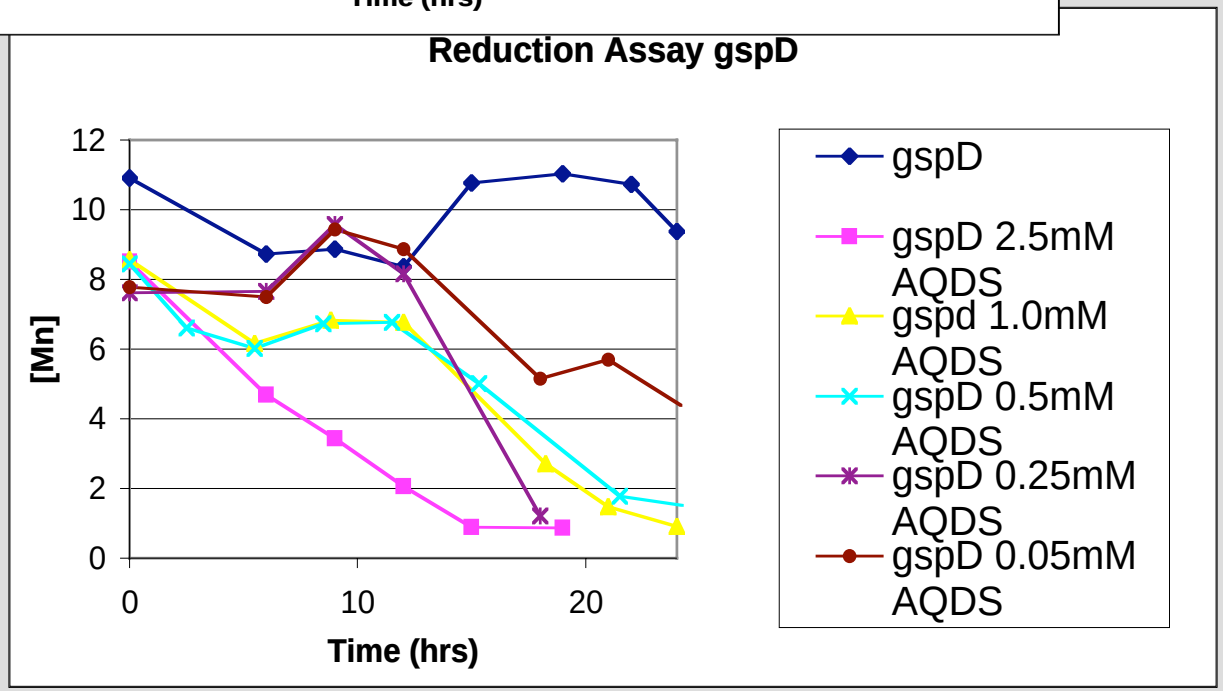
- *S. oneidensis* MR-1 genome contains a full complement of *Vibrio*-like *sox* homologs (*A - H*) (but not known to oxidize S).
- Possible evolutionary link between microbial Fe(III) reduction and S oxidation?

Model No. 1. Direct contact

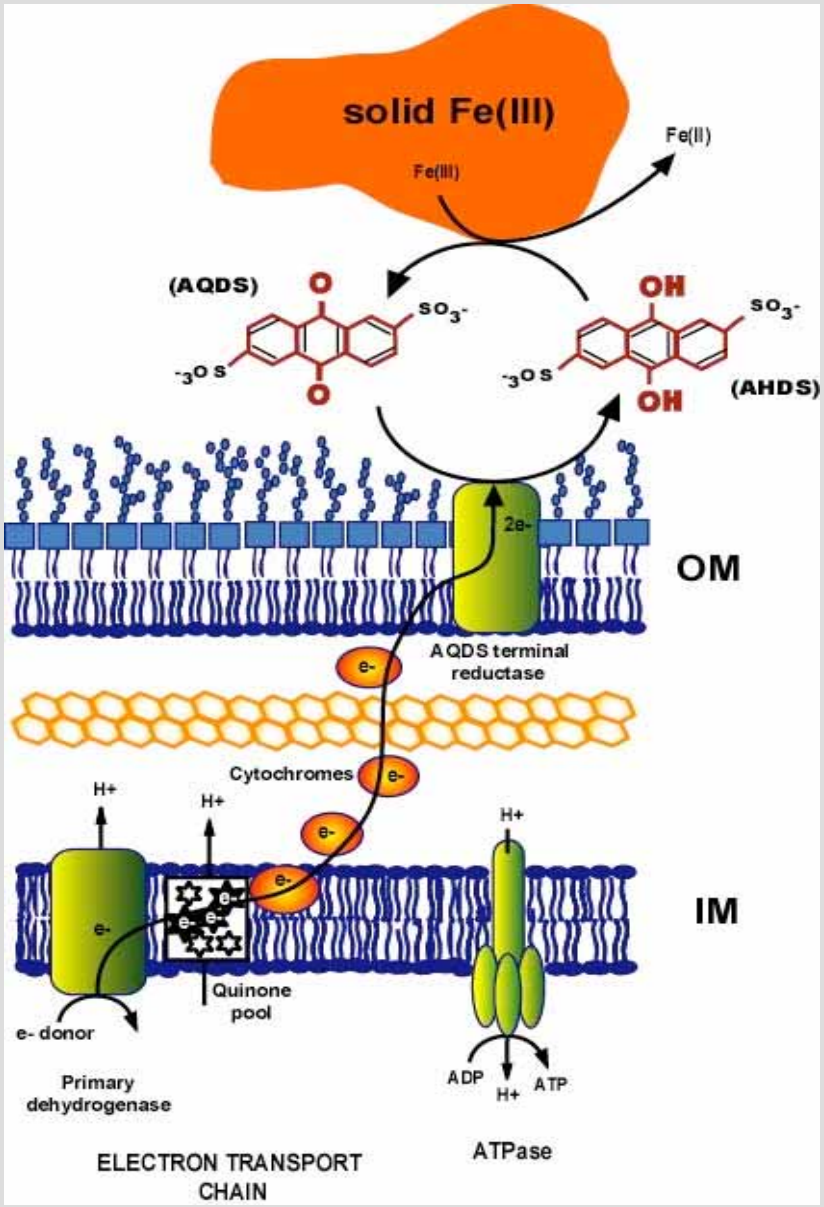


Addition of exogenous electron shuttle AQDS overcomes Fe(III) and Mn(IV) reduction deficiency of *S. oneidensis* Type II secretion mutants





Model 4. Exogenous electron shuttle



- Note Type II secretion system and OM-targeted Fe(III) reductase are missing in *gspD* mutant.

- Facilitates identification of AQDS reductase in *gspD* mutant background [I.e., Fe(III) reduction activity solely due to AQDS reduction].

Identification of AQDS shuttle genes

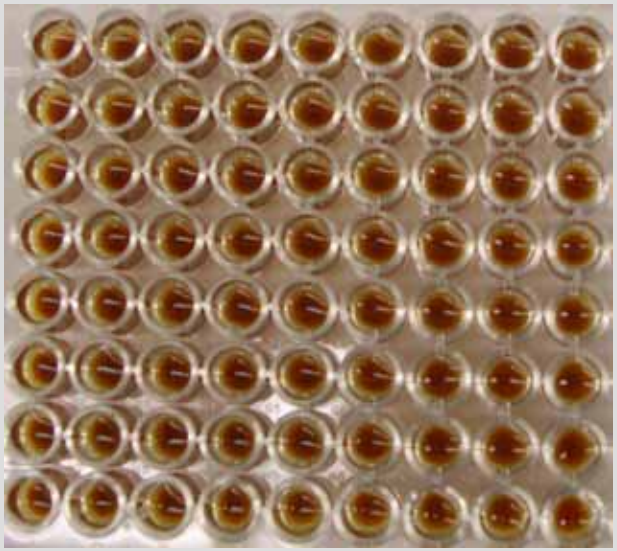
0 hr

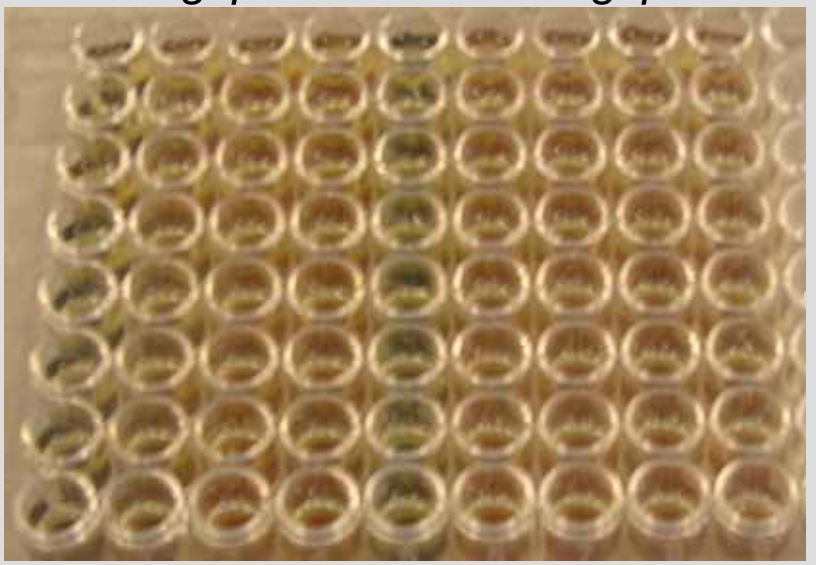
200 *gspE* MR HK *gspD*

24 hr

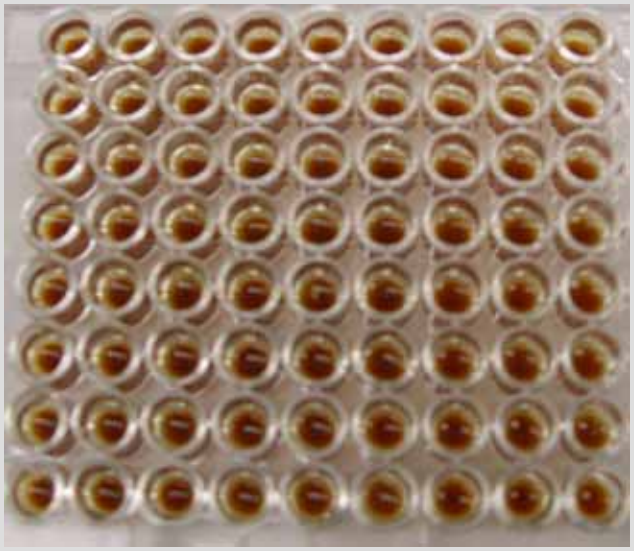
200 *gspE* MR HK *gspD*

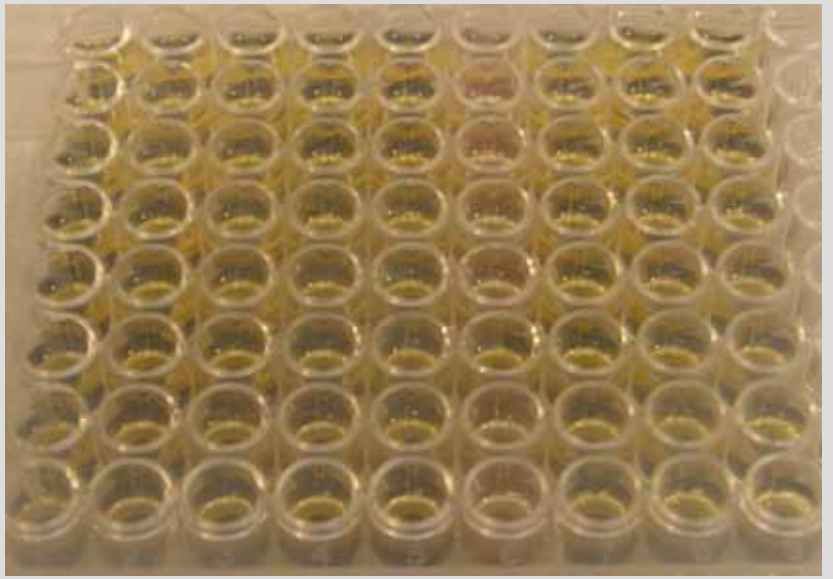
HFO





HFO + AQDS

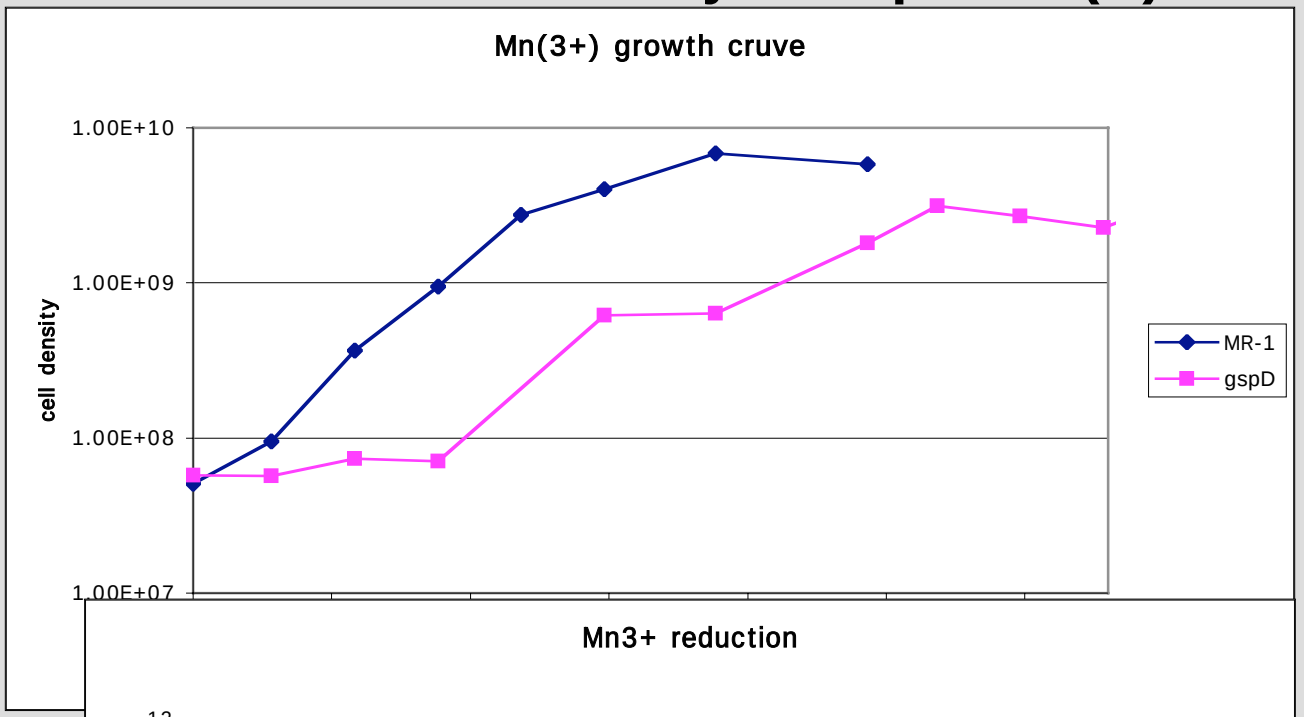




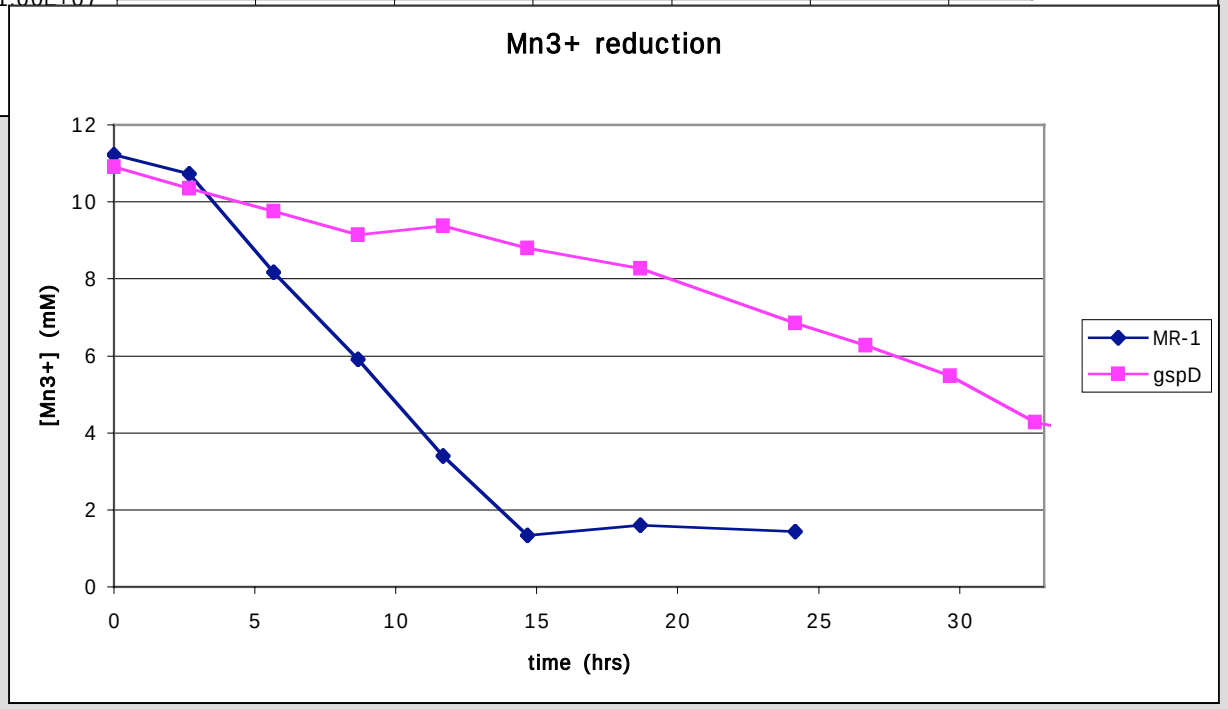
Microbial Mn(IV) Respiration

Mn(IV) reduction-deficient Type II secretion mutant *gsp*D of *S. oneidensis* retains ability to respire Mn(III)

Cell growth



Mn(III) depletion



WORKING HYPOTHESIS:

Mn(IV) reduction proceeds step-wise via two successive one-electron transfer reactions catalyzed by separate Mn(IV) and Mn(III) reductases

Mn(IV) Reduction:

$Mn^{4+} \longrightarrow Mn^{3+} \longrightarrow Mn^{2+}$

Mn(II) Oxidation:

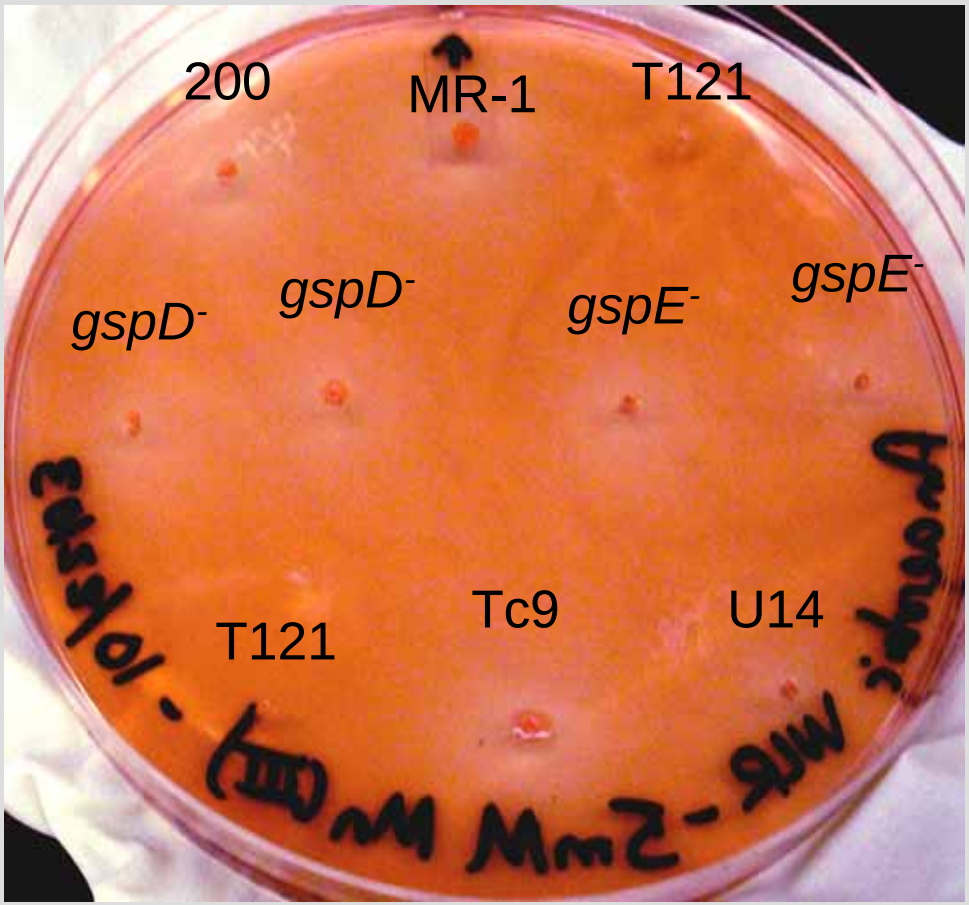
$Mn^{2+} \longrightarrow Mn^{3+} \longrightarrow Mn^{4+}$

(DFO trap; Tebo)

Mn(III) Reduction

Mn^{3+} ⟶ Mn^{2+}

soluble, pinkish → soluble, clear



- Mn(III) reduction-deficient mutant screening technique (WT = clearing zone)
- EMS and *Tn*5 mutagenesis
- Genetic complementation analysis with MR-1 clone bank
- Identify genes required for Mn(III) reduction
- Do Mn(III) reduction-deficient mutants grow anaerobically on Mn(IV)?

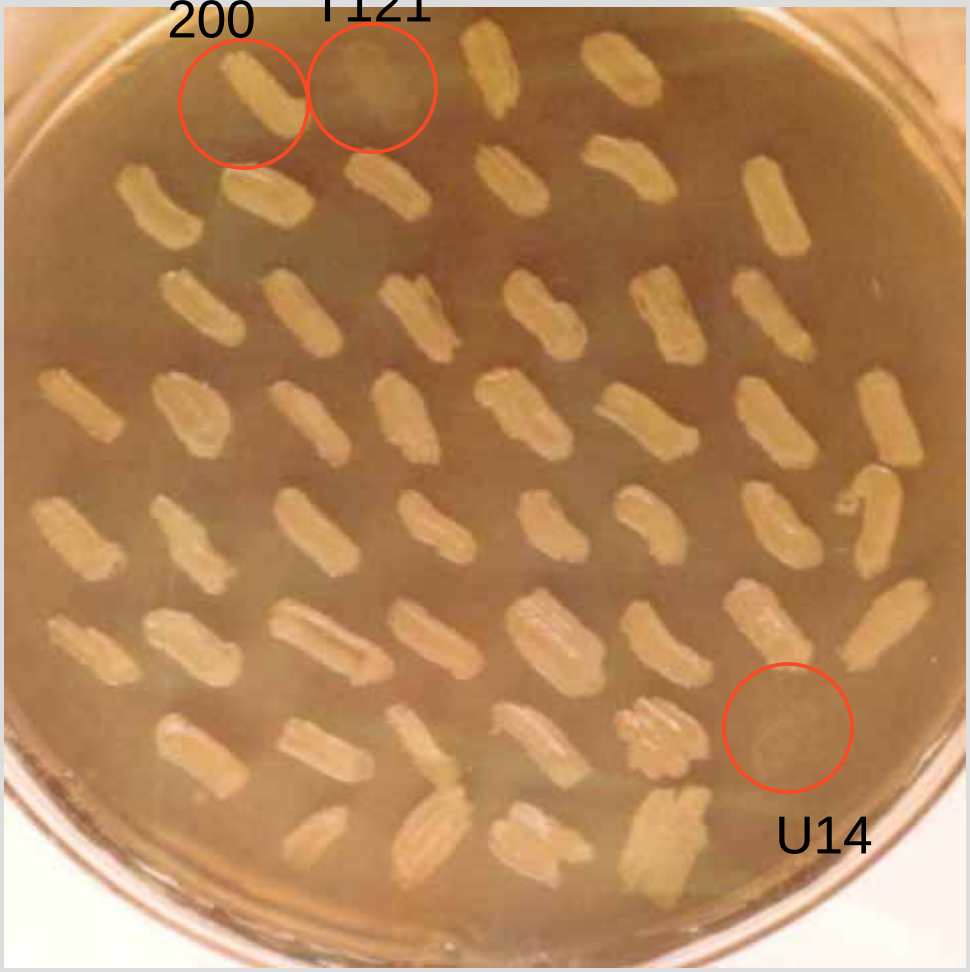
Microbial U(VI) Respiration

Uranium reduction

U(VI) ⟶ U(IV)

Soluble, Clear

Insoluble, Brown Precipitate



Anaerobic Respiratory Capability of Uranium Reduction-deficient Mutants of *S. putrefaciens* 200

	U(VI)	Fe(III)	MnO_2	TMAO	Fumarate	NO_3^-	NO_2^-	$S_2O_3^{2-}$	SO_3^{2-}
200R	+	+	+	+	+	+	+	+	+
U14	-	+	+	+	+	+	-	+	+
U97	-	-	-	-	+	+	-	+	-
U106	-	-	-	-	-	+	-	+	-
U110	-	-	-	-	-	-	-	-	-

+ growth; - no growth

WORKING HYPOTHESIS:

The U(VI) and NO_2^- reduction systems of *Shewanella* share common respiratory chain components, possibly including the terminal NO_2^- reductase itself

Three types of energy-transducing bacterial NO_2^- reductases

Denitrification:

1. Cu-containing NirK $NO_2^- \rightarrow NO$
2. cytochrome *cd*$_1$ NirS $NO_2^- \rightarrow NO$

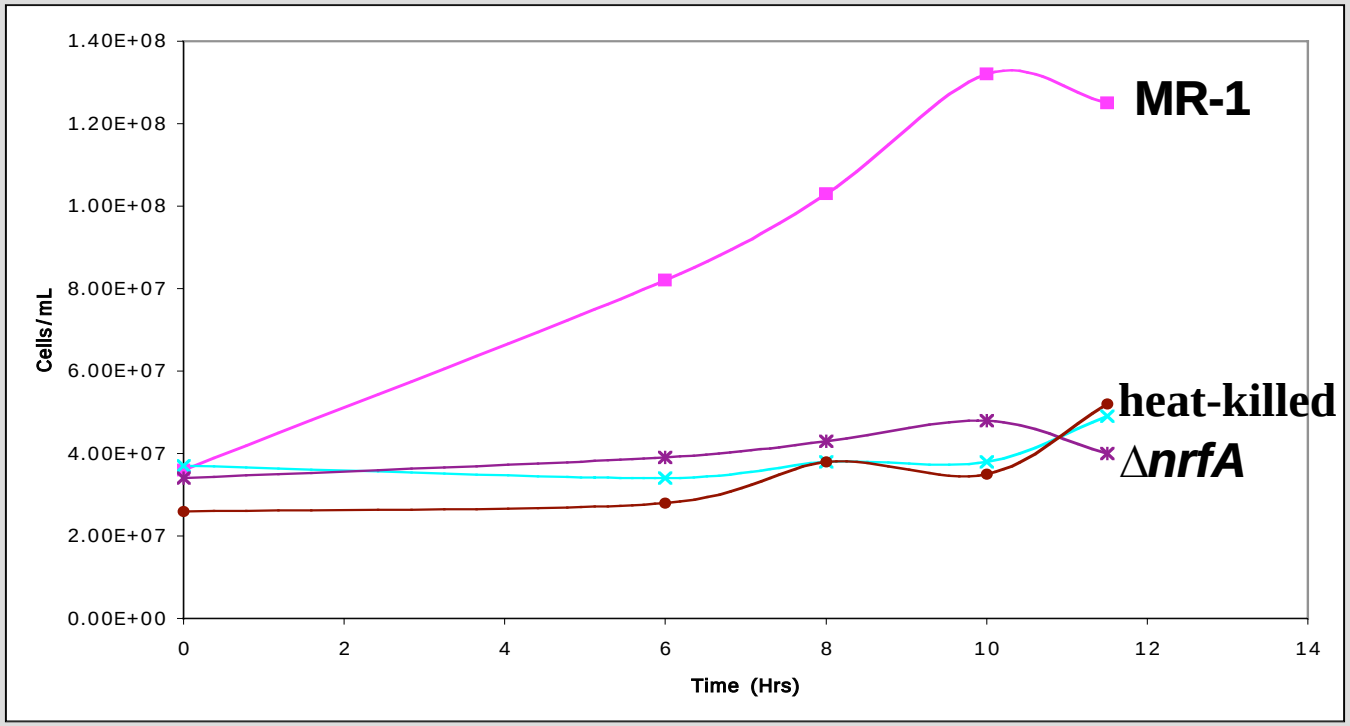
Dissimilatory nitrate ammonification:

3. NrfA: cytochrome c_{552} $NO_2^- \rightarrow NH_3$

$SO_3^{2-} \rightarrow S^{2-}$

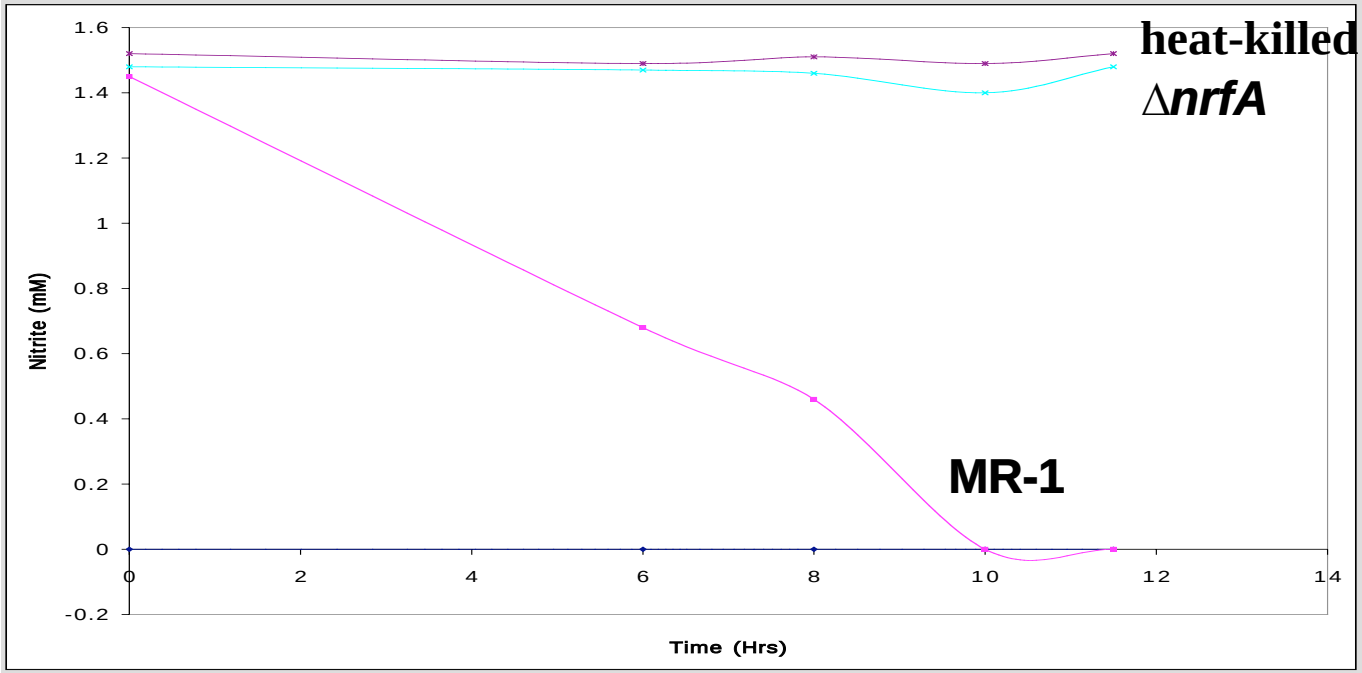
***S. oneidensis* genome scan:**

- ***E. coli*-like NrfA homolog was detected (79% similar)**
- **Generate ∆*nrfA* deletion mutant, test for U(VI) reduction**

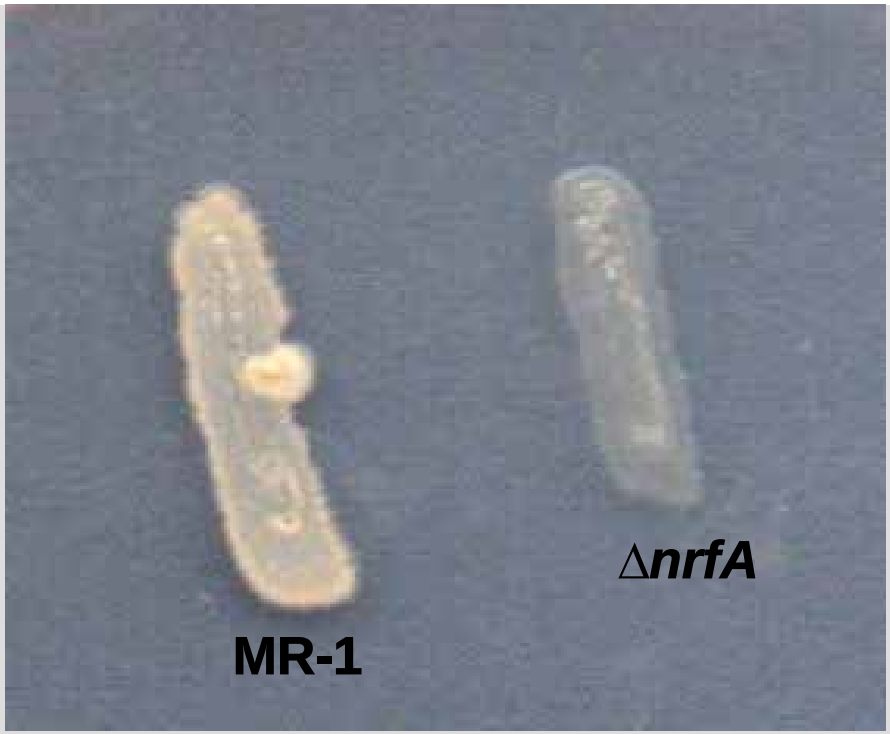


***S. oneidnesis* ΔnrfA deletion mutant is unable to respire NO_2^-**

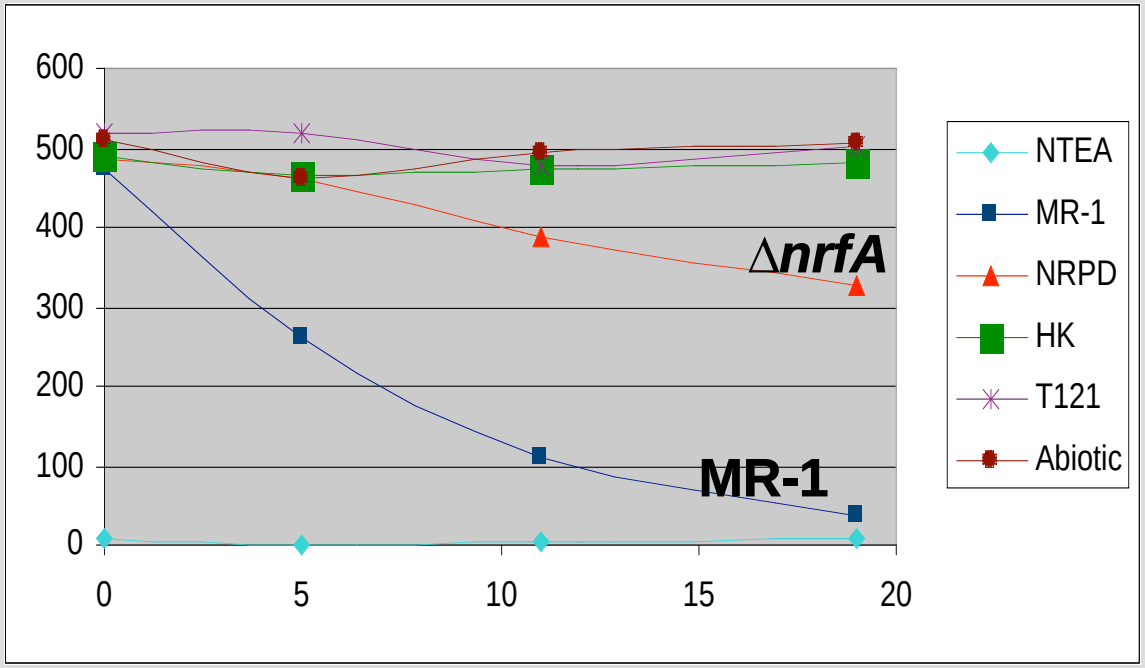
Anaerobic growth on NO_2^-



NO_2^- depletion



S. oneidensis Δ*nrfA* deletion mutant displays a U(VI) reduction-deficient phenotype on U(VI) reduction plate screen



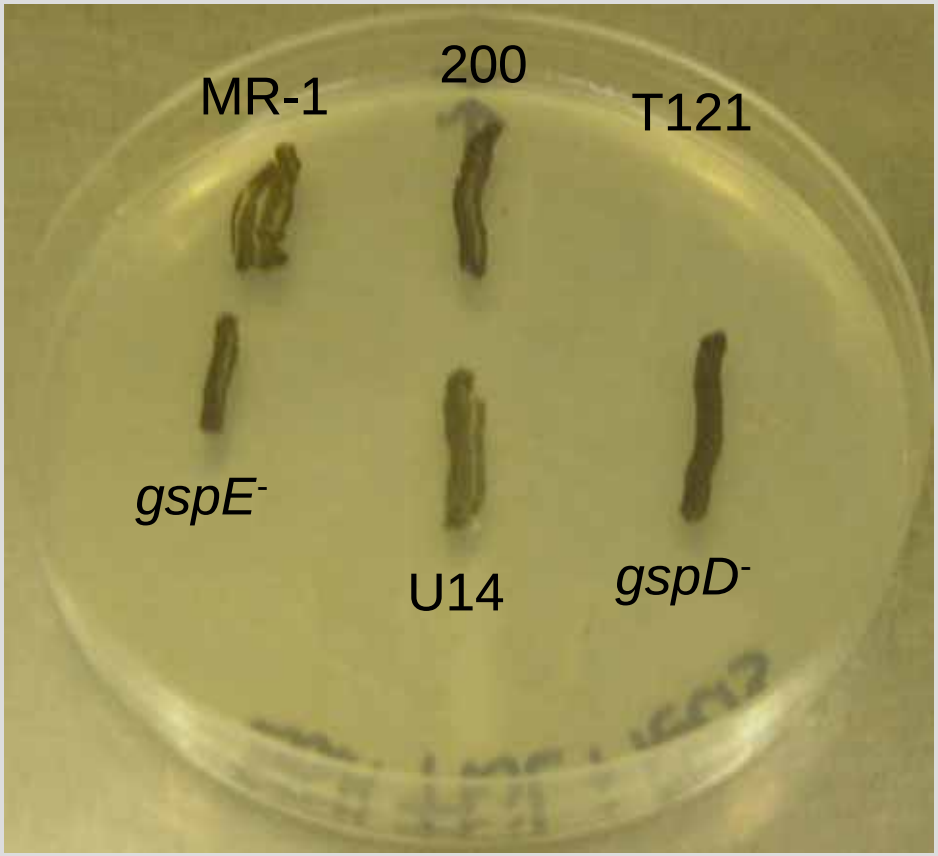
S. oneidensis Δ*nrfA* deletion mutant is severely impaired in U(VI) reduction activity in anaerobic liquid culture

Microbial Tc(VII) Reduction

Technetium reduction

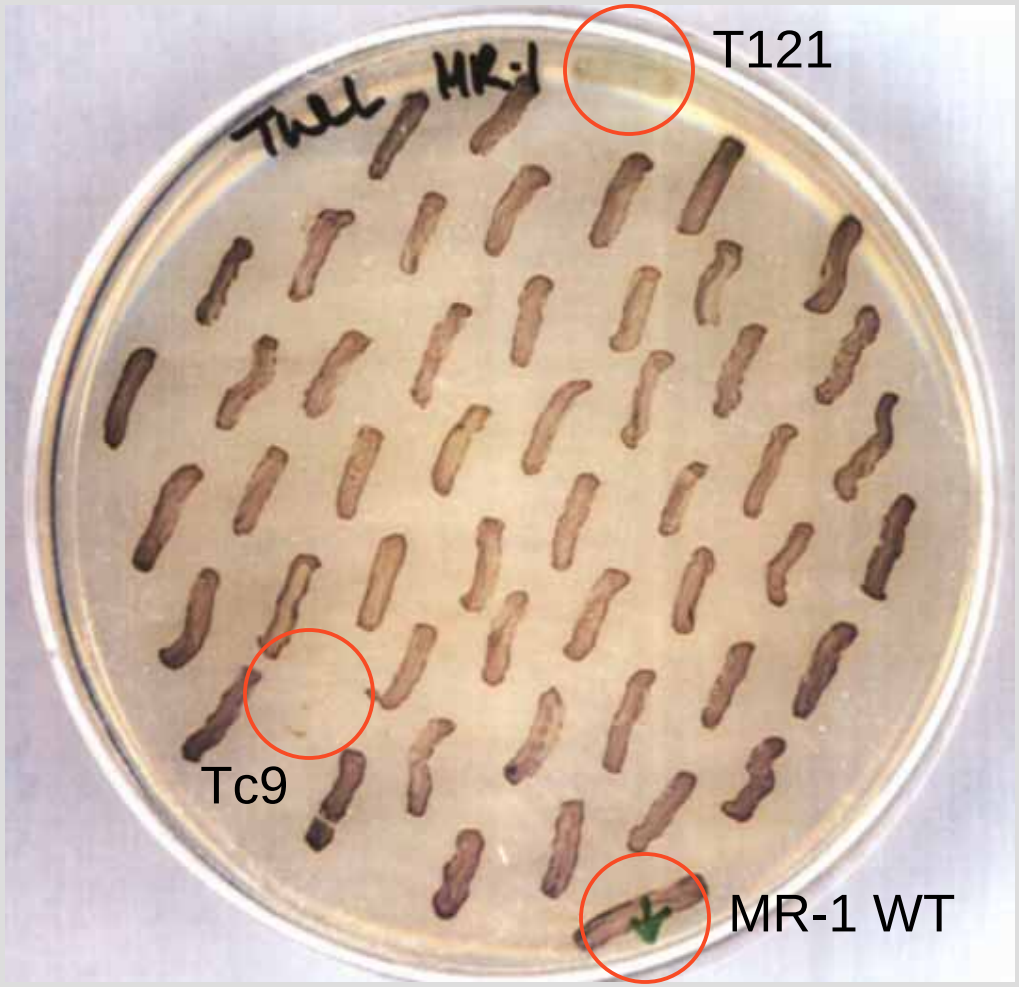
Tc^{7+} → Tc^{4+}

clear, soluble → black precipitate

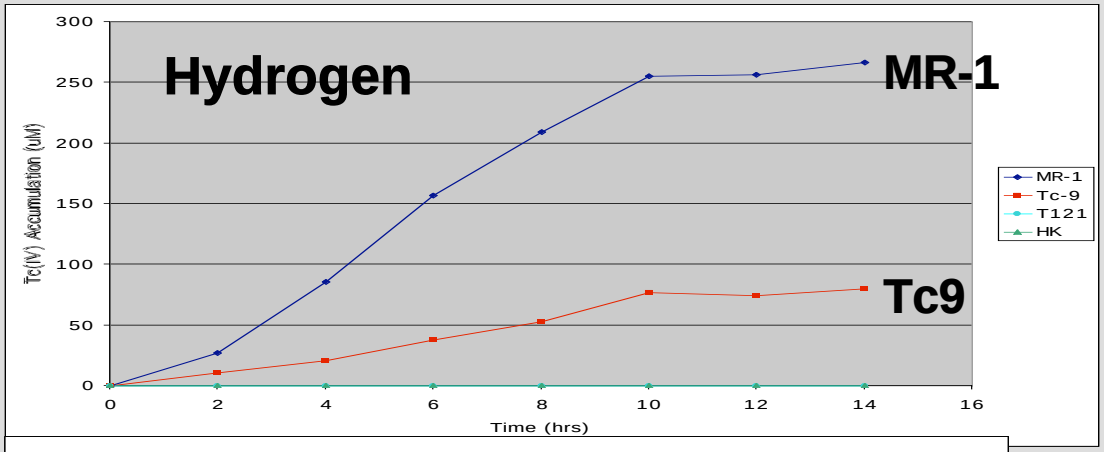


- Jon Lloyd (1997): Tc^{7+} reduced by *E.coli* HycE (H_2-evolving Ni/Fe H_2ase of formate hydrogen lyase complex)
- De Luca (2001): Tc^{7+} reduced by Ni/Fe H_2ase of SRB *Desulfovibrio fructosovorans*
- *S. oneidensis* MR-1 genome contains two *Wolinella*- and *Thermotoga*-like hydrogenases that do not display homology to above hydrogenases.

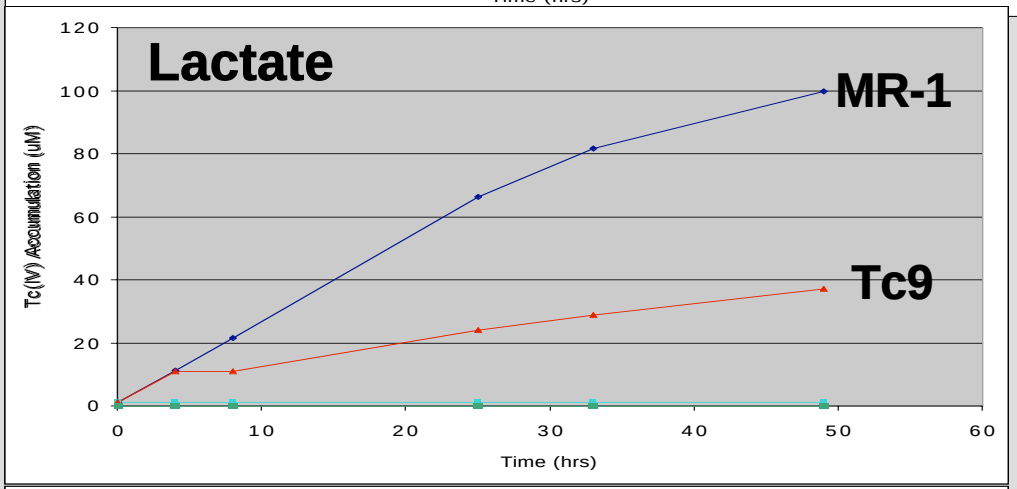
Identification of Tc(VII) reduction-deficient mutants



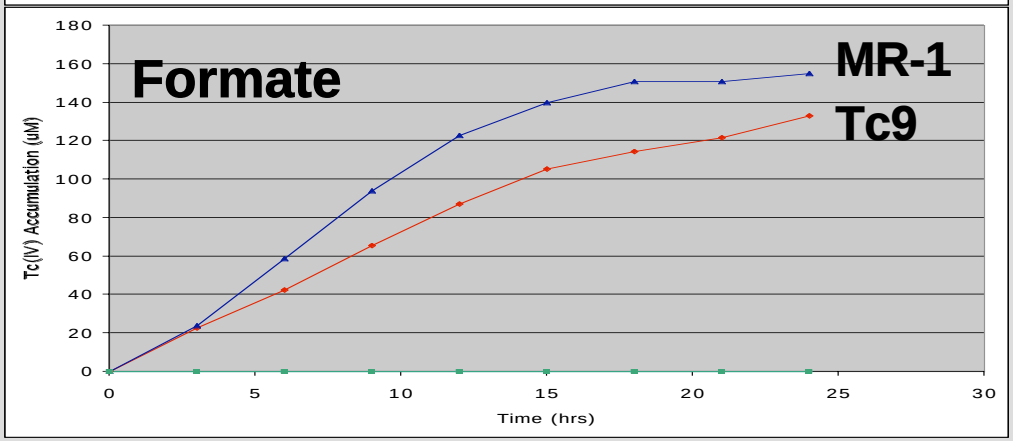
….and Tc9 retains ability to respire all other TEAs



Tc(VII) reduction-deficient mutant Tc9 is unable to reduce Tc(VII) with either hydrogen…….

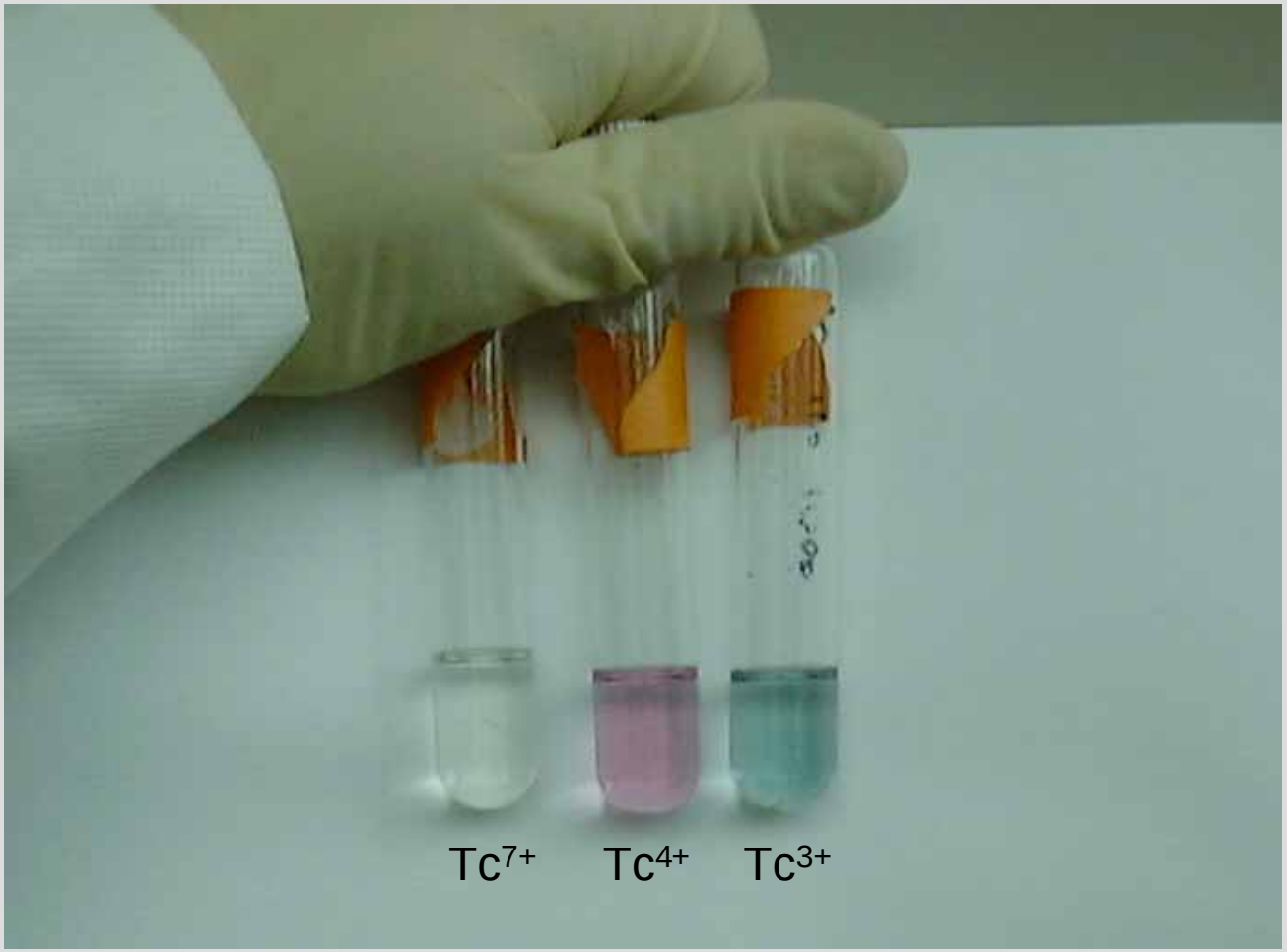


or lactate as electron donor, yet…..



retains near-wild type Tc(VII) reduction activity with formate as electron donor.

Tc(VII) Reduction in 50 mM Bicarbonate Buffer



WORKING HYPOTHESIS:

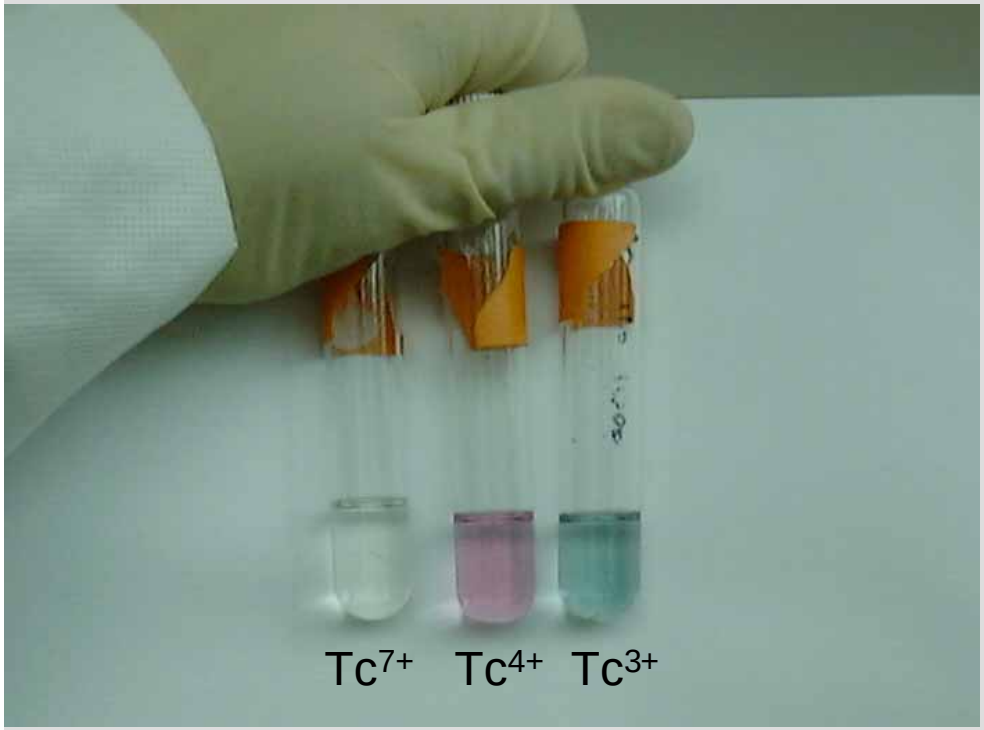
Tc(VII) reduction proceeds step-wise via two successive electron transfer reactions catalyzed by separate Tc(VII) and Tc(IV) reductases

Tc(VII) Reduction:

$Tc^{7+} \longrightarrow Tc^{4+} \longrightarrow Tc^{3+}$

Tc(III) Oxidation:

$Tc^{3+} \longrightarrow Tc^{4+} \longrightarrow Tc^{7+}$



Rapid screen for identification of Tc(IV) reduction-deficient mutants:

Mutagenize and identify mutants which remain pink-colored

Take Home Messages

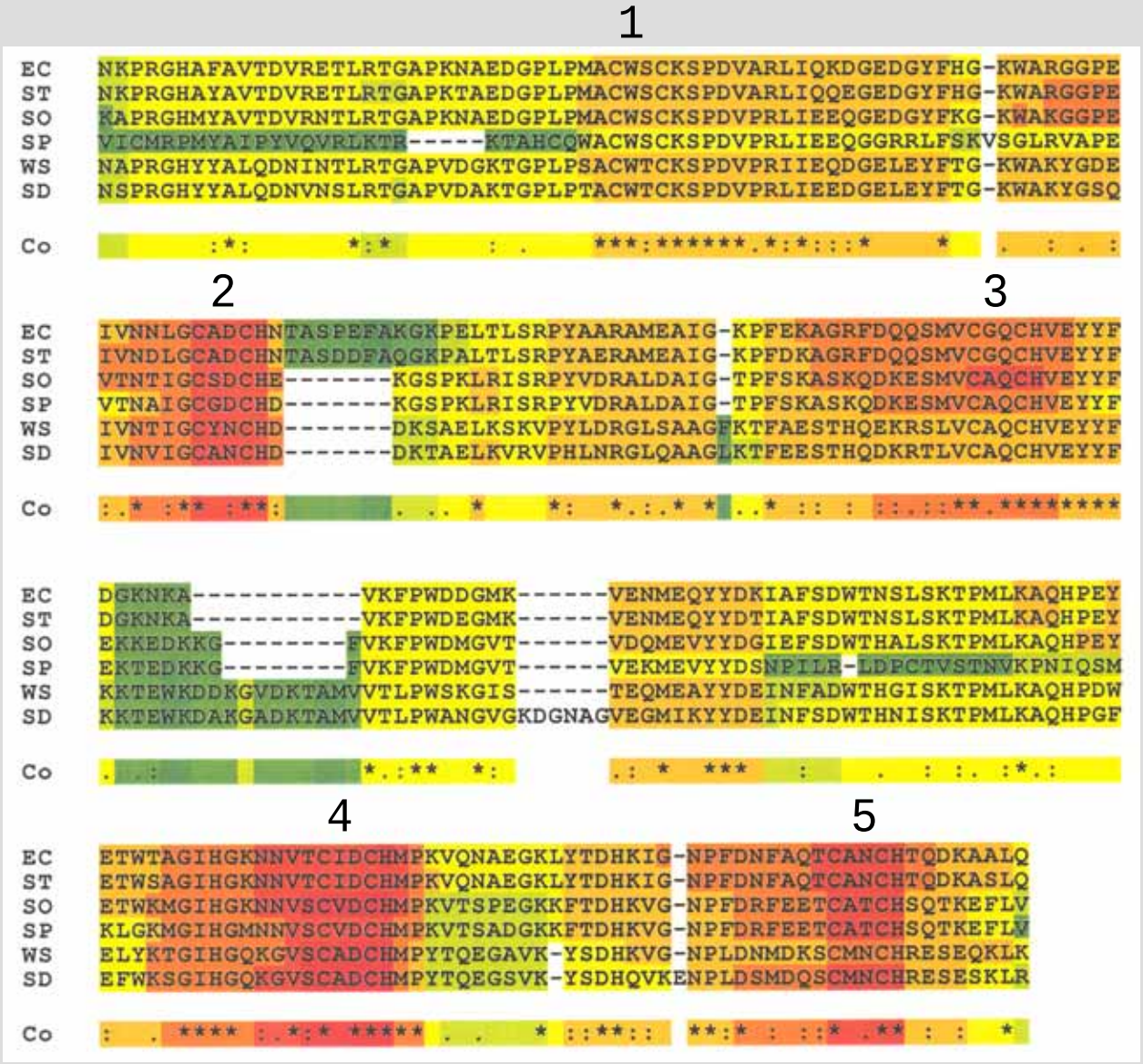
1. **Fe(III) reduction**
   - Fe(III) reductase targeted to cell periphery via Type II protein secretion system in all *Shewanella*
   - Fe(III) reductase complex may include protease, *c*-type cytochrome and component of S oxidation
   - reduction of exogenous electron shuttle AQDS

2. **Mn(IV) reduction**
   - Also requires Type II protein secretion system
   - Two successive one electron transfer steps via Mn(III) reductase

3. **U(VI) reduction**
   - Nitrite reductase NrfA is involved in U(VI) reduction

4. **Tc(VII) reduction**
   - Electron donor-dependent
   - Two successive electron transfer steps via Tc(IV) reductase

NrfA is highly conserved between species



Nucleotide sequence analysis of DNA fragment complementing B31

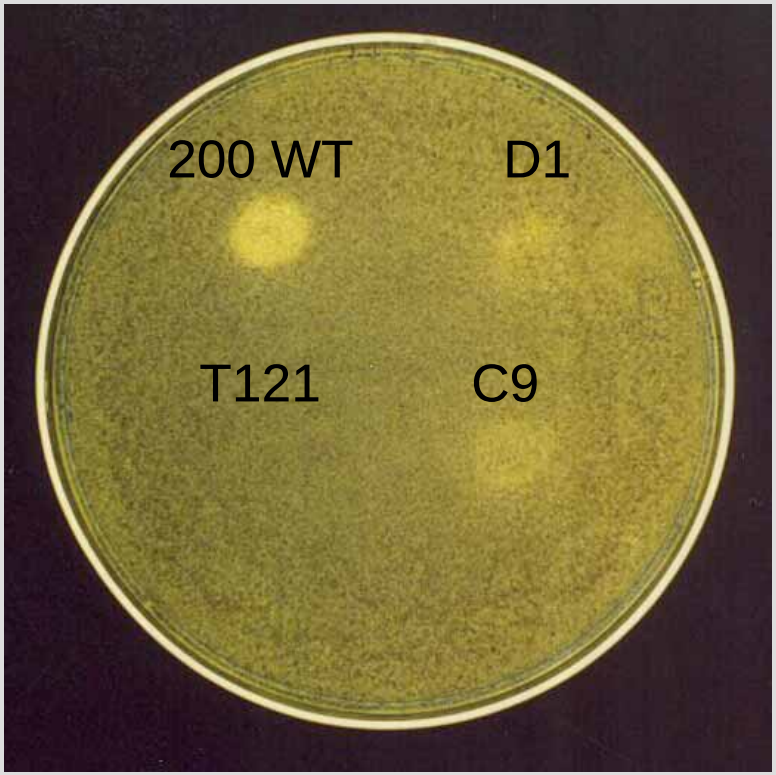
	Gene	Identity	Positive	Expect	Translated product (best hit)
	ferD'	57%	70%	8×10^{-70}	*V. cholera epsD*
*	*ferE*	75%	87%	0.0	*A. hydrophila exeE*
	ferF	56%	69%	1×10^{-114}	*A. hydrophila exeF*
	ferG	41%	55%	4×10^{-13}	*E. carotovora outG*

B4-2S

Manganese Reduction

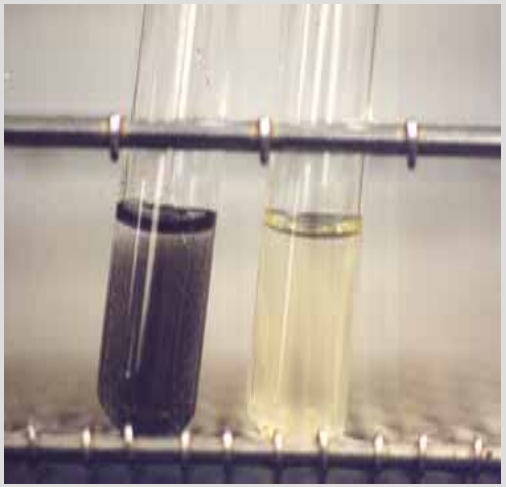
MnO_2 ⟶ Mn^{2+}

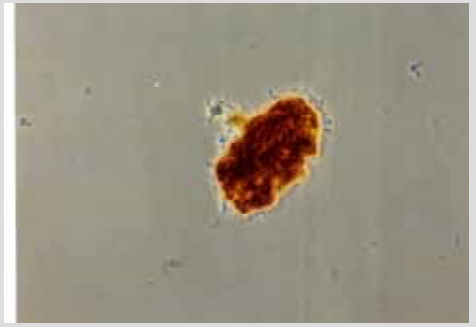
Insoluble, black precipitate soluble, toxic

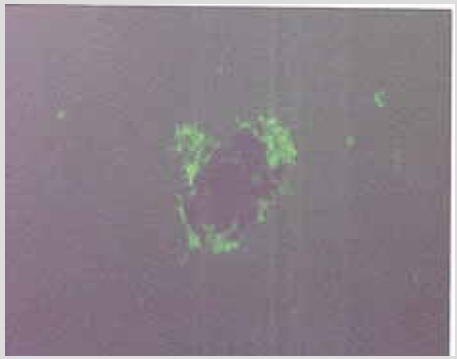


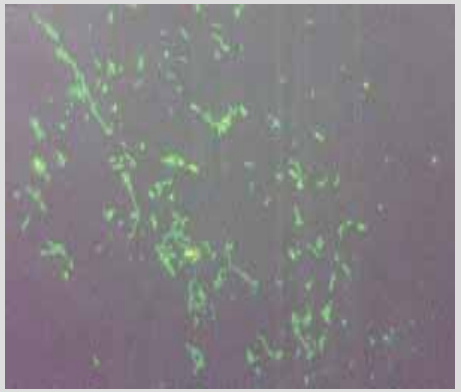
- Screened 10,000 *Tn*5-mutagenized colonies for Mn(IV) reduction activity
- Identified 34 putative Mnr mutants
- 32 of the 34 Mnr mutants displayed multiple respiratory deficiencies.
- Two Mnr mutants (D1 and C9) were deficient in Mn(IV) respiration, yet retained the ability to respire all other terminal electron acceptors, including Fe(III).

Anaerobic growth on Mn(IV)









Genetic analysis of Mnr mutant D1

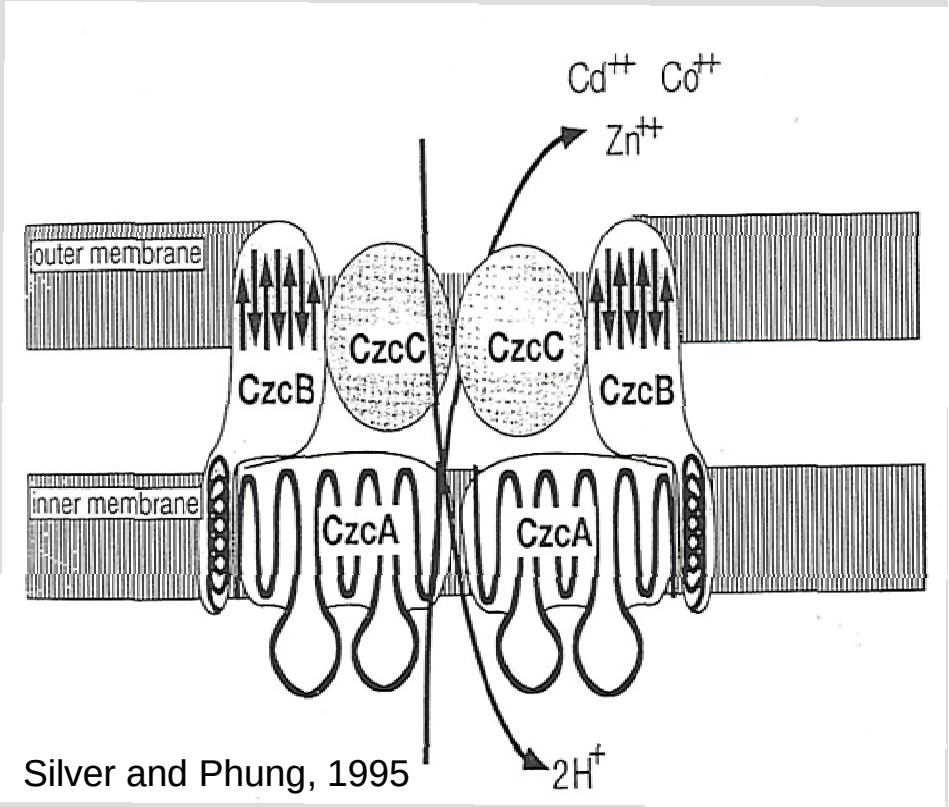
- *Tn*5 inserted in an ORF displaying 46% similarity to the *Alcaligenes eutrophus* cation efflux protein CzcD.

- *A. eutrophus* CzcD is involved in regulation of the proton/divalent cation antiporter CzcCBA that mediates resistance to Co^{2+}, Zn^{2+} and Cd^{2+}.

- *S. oneidensis* contains a full complement of *czc* genes (*CBA*, *DRS, IN)* with 42 - 58% similarity to the *A. eutrophus* homologs.

- **WORKING HYPOTHESIS:**

  ***S. putrefaciens* employs a Czc-like proton/divalent cation antiporter to efflux Mn^{2+} that otherwise accumulates intracellularly to toxic levels during anaerobic Mn(IV) respiration**



Silver and Phung, 1995

Divalent metal cations Co^{2+}, Zn^{2+}, Cd^{2+} and Mn^{2+} inhibit growth and accumulate in *czcD* mutant of *S. putrefaciens* 200

Experimental design:

- Grow WT and *czcD* mutant in the presence of increasing concentrations of divalent cations.
- Compare minimum inhibitory concentration of divalent cations in WT to *czcD* mutant.
- Measure divalent cation accumulation in cytoplasm of WT and *czcD* mutant via atomic absorption spectroscopy.

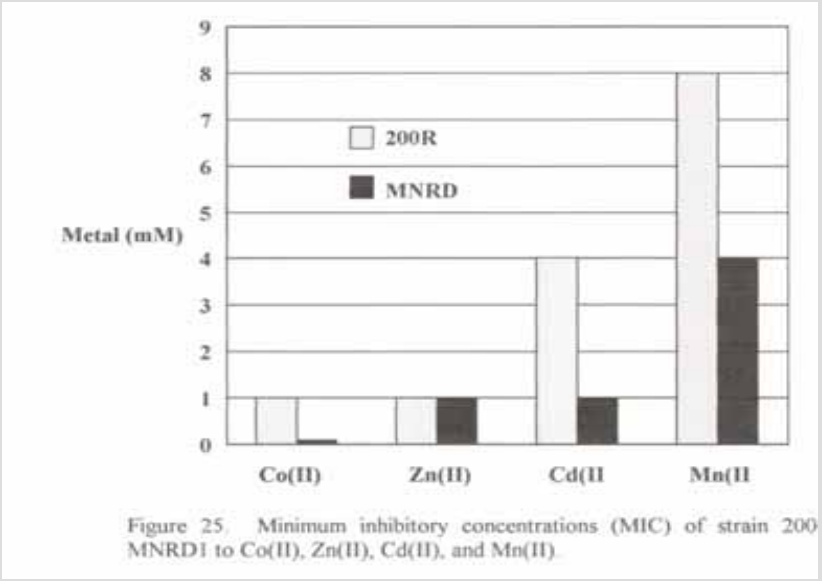
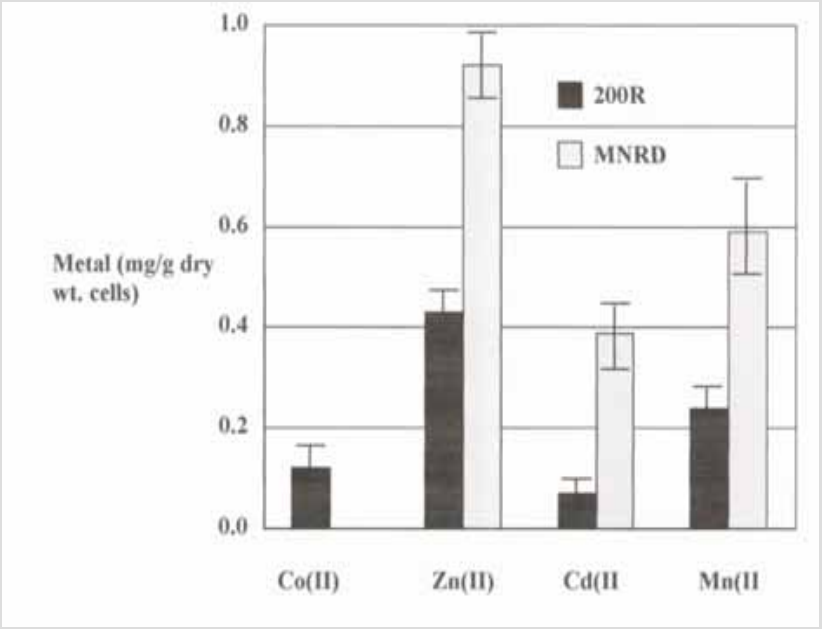
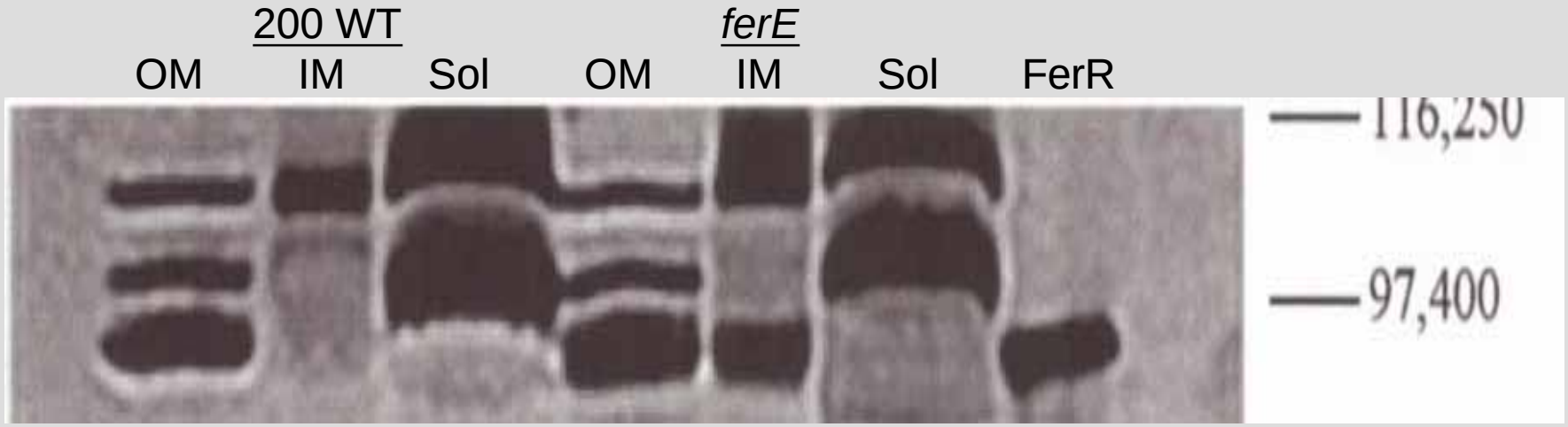


Figure 25. Minimum inhibitory concentrations (MIC) of strain 200 MNRD1 to Co(II), Zn(II), Cd(II), and Mn(II).



Fe(III) reductase accumulates in the periplasmic space of Type II protein secretion mutants of *S. putrefaciens* 200

Heme stain of outer membrane (OM), inner membrane(IM) and soluble (Sol) fractions of *S. putrefaciens* 200 WT and *ferE* mutant B31



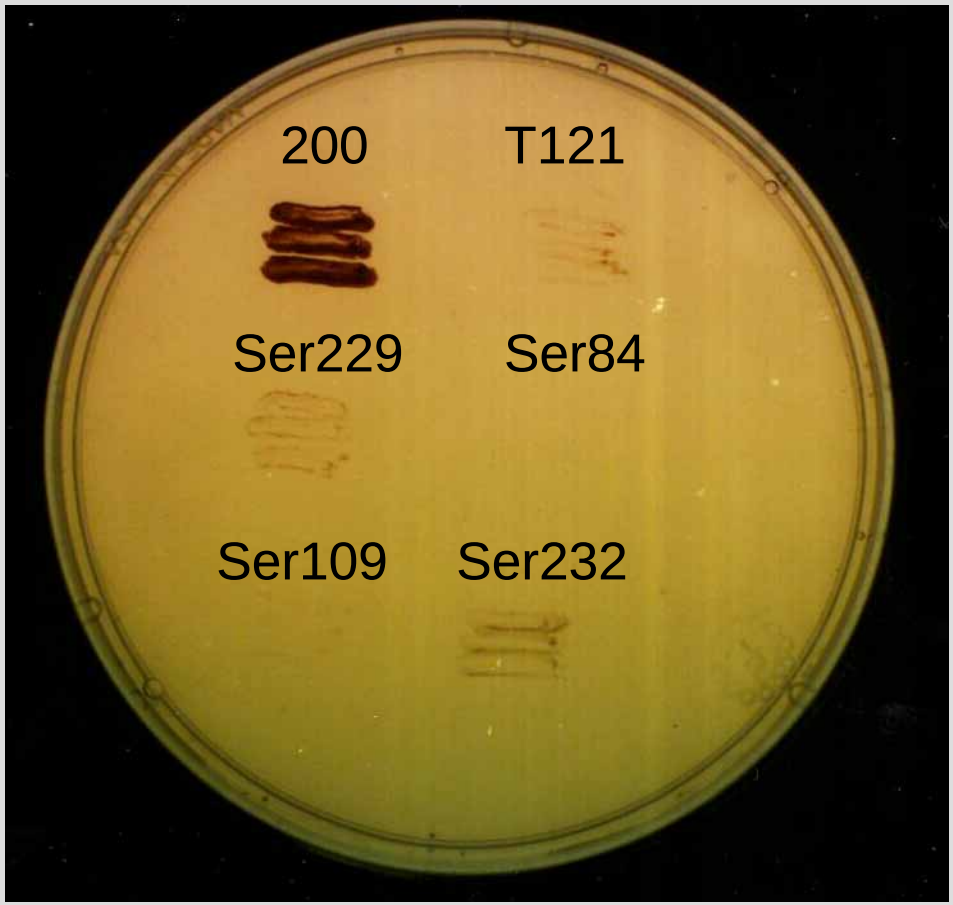
S. oneidensis Nrf homologies

Nrf	Function	MR-1 Hits	E Value	% Identity
A	c_{552} Nitrite Reductase	1	e^{-172}	63 (79% similar)
B	*c*-type pentaheme cytochrome	4	e^{-12} - e^{-14}	29-34
C	Fe-S Protein	6	e^{-17} - e^{-61}	30-56
D	NADH quinone	3	e^{-34} - e^{-54}	29-38
E	cytochrome assembly	2	e^{-40} - e^{-54}	30-34
F	cytochrome assembly	2	e^{-12} - e^{-21}	38-52
G	cytochrome synthesis / assembly	2	e^{-14} - e^{-26}	33-39
H	transmembrane *c*-type (NapC/NirT family) cytochrome (reduces NrfA of *Wolinella*)	0	n/a	n/a
I	heme delivery in periplasm	0	n/a	n/a
J	assembly/synthesis (ccsA and ResA-like)	0	n/a	n/a

Selenite reduction

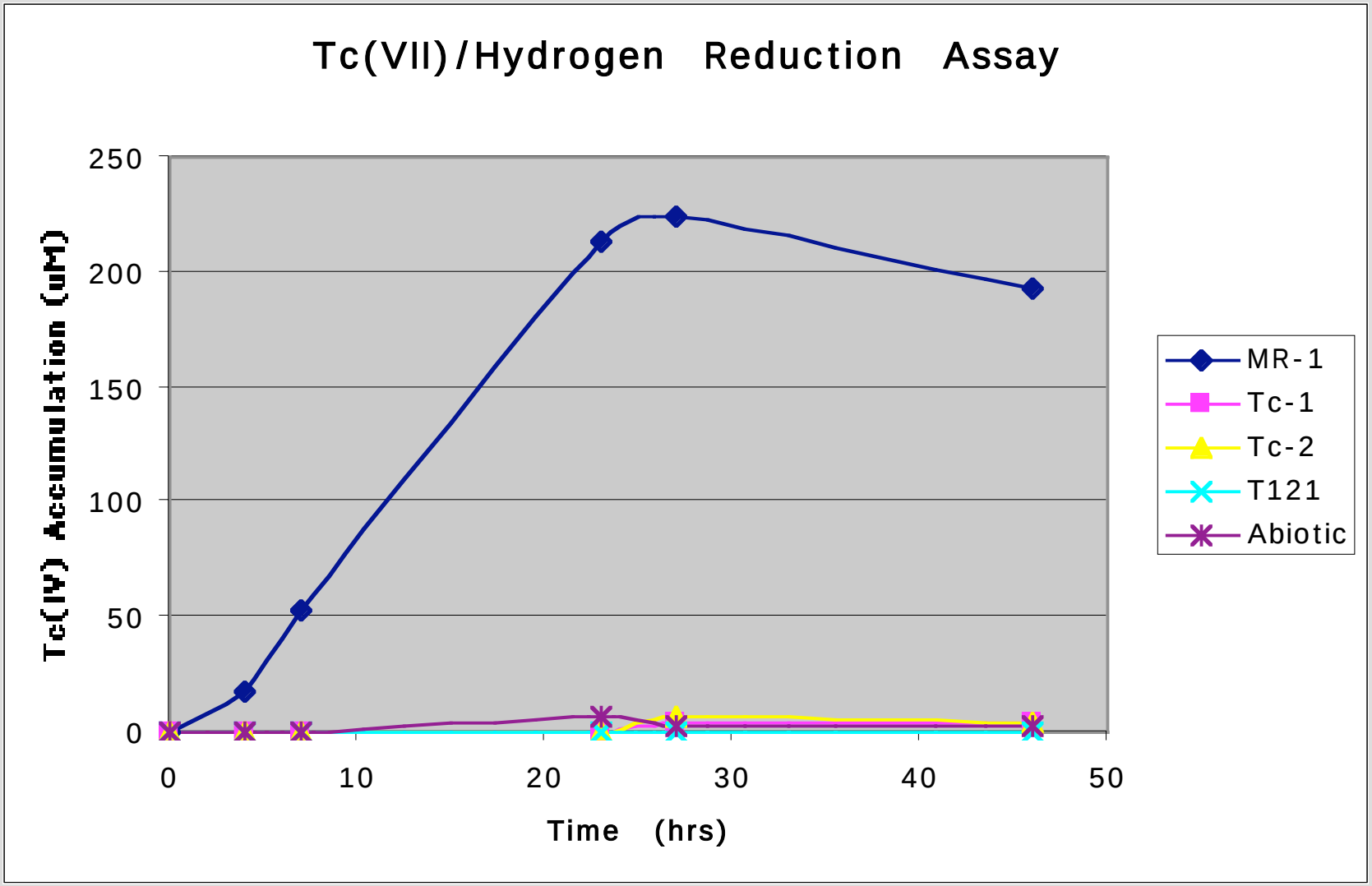
Se(IV) ⟶ Se(0)

Soluble, clear Insoluble, red precipitate

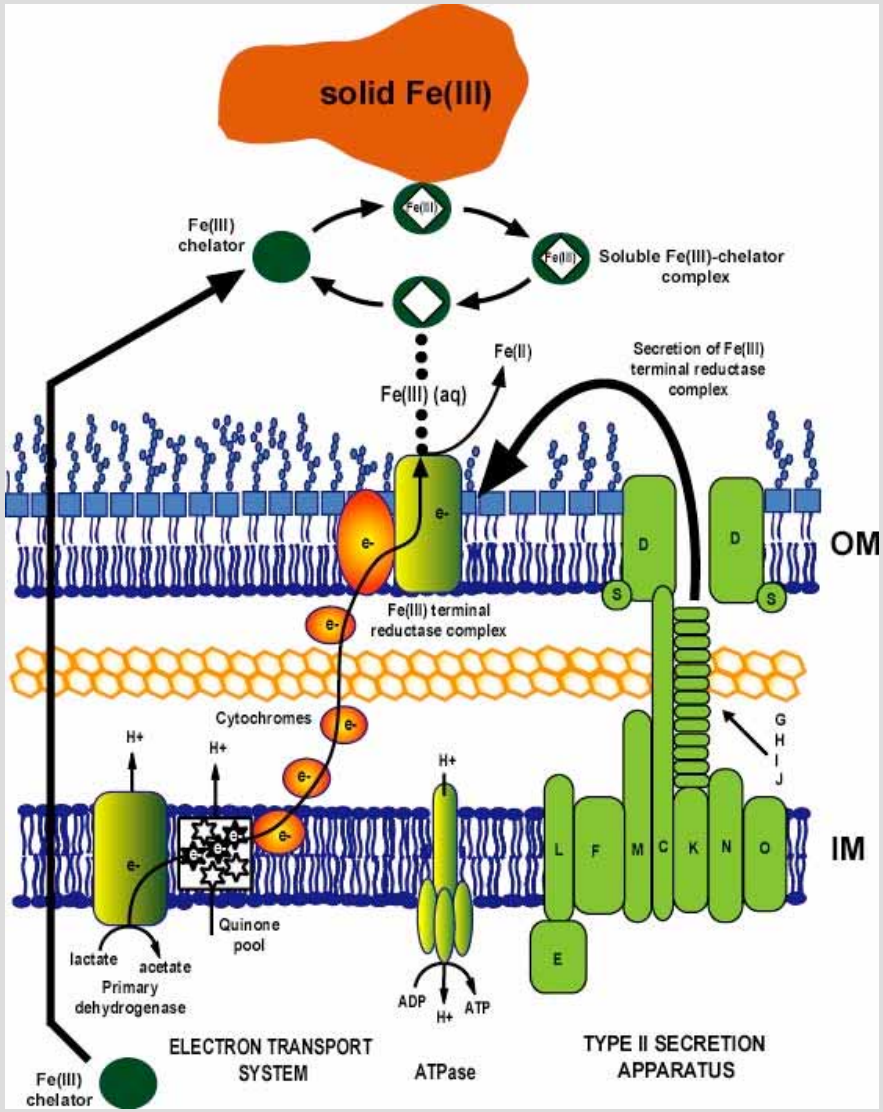


Respiratory capability of Ser mutants

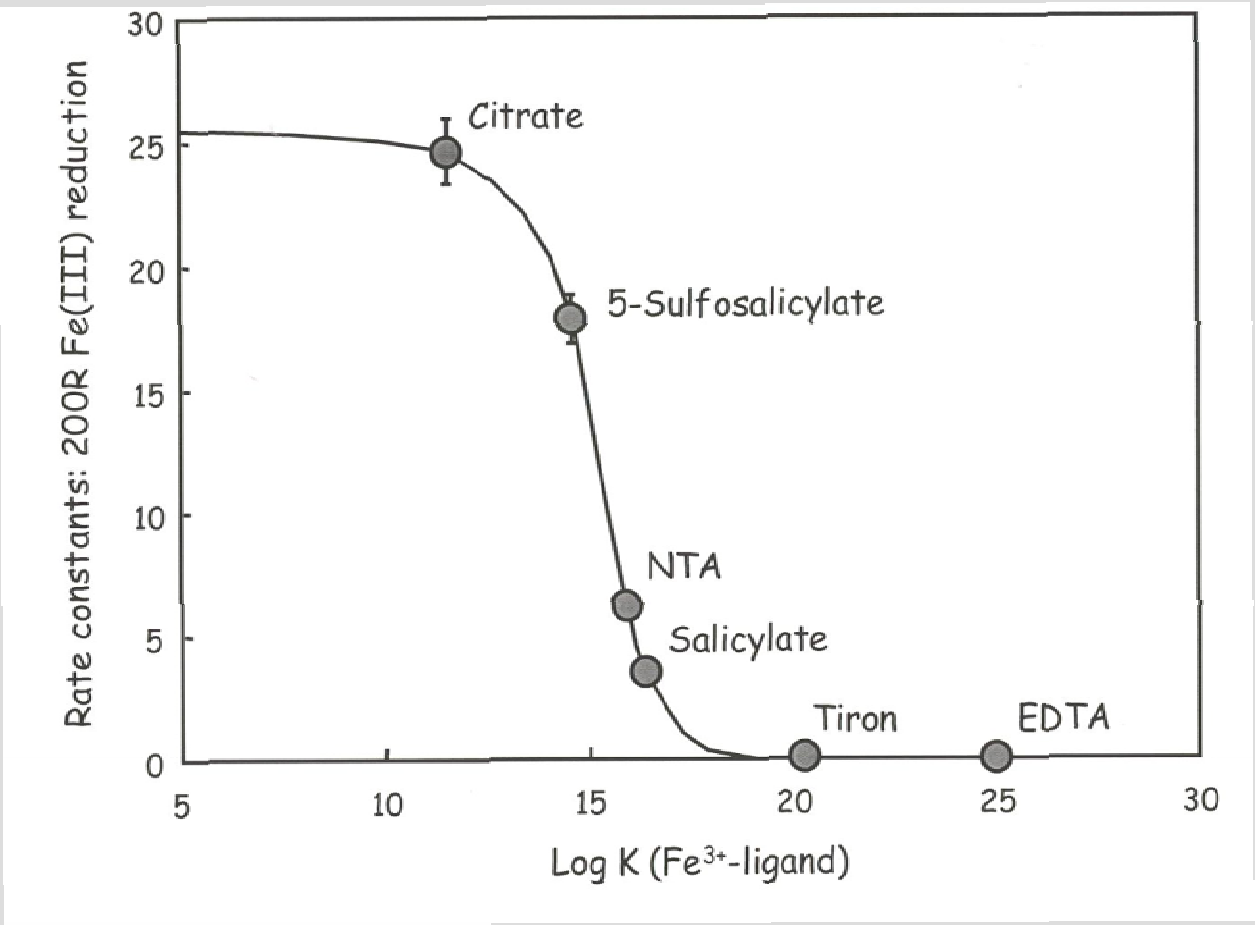
Strain	O_2	Se(IV)	Fe^{3+}	TMAO	Fum	NO_2^-	NO_3^-	SO_3^{2-}	$S_2O_3^{2}$	Mn^{4+}
WT	+	+	+	+	+	+	+	+	+	+
T121	+	-	-	-	-	-	-	-	-	-
Ser84	+	-	-	-	-	-	-	-	-	-
Ser109	+	-	-	-	-	-	-	-	-	-
Ser229	+	-	+	+	-	-	-	+	+	+
Ser232	+	-	+	+	-	-	-	+	+	+


Tc(VII)/Hydrogen Reduction Assay
Tc(IV) Accumulation (uM)
250
200
150
100
50
0
0
10
20
30
40
50
Time (hrs)
MR-1
Tc-1
Tc-2
T121
Abiotic

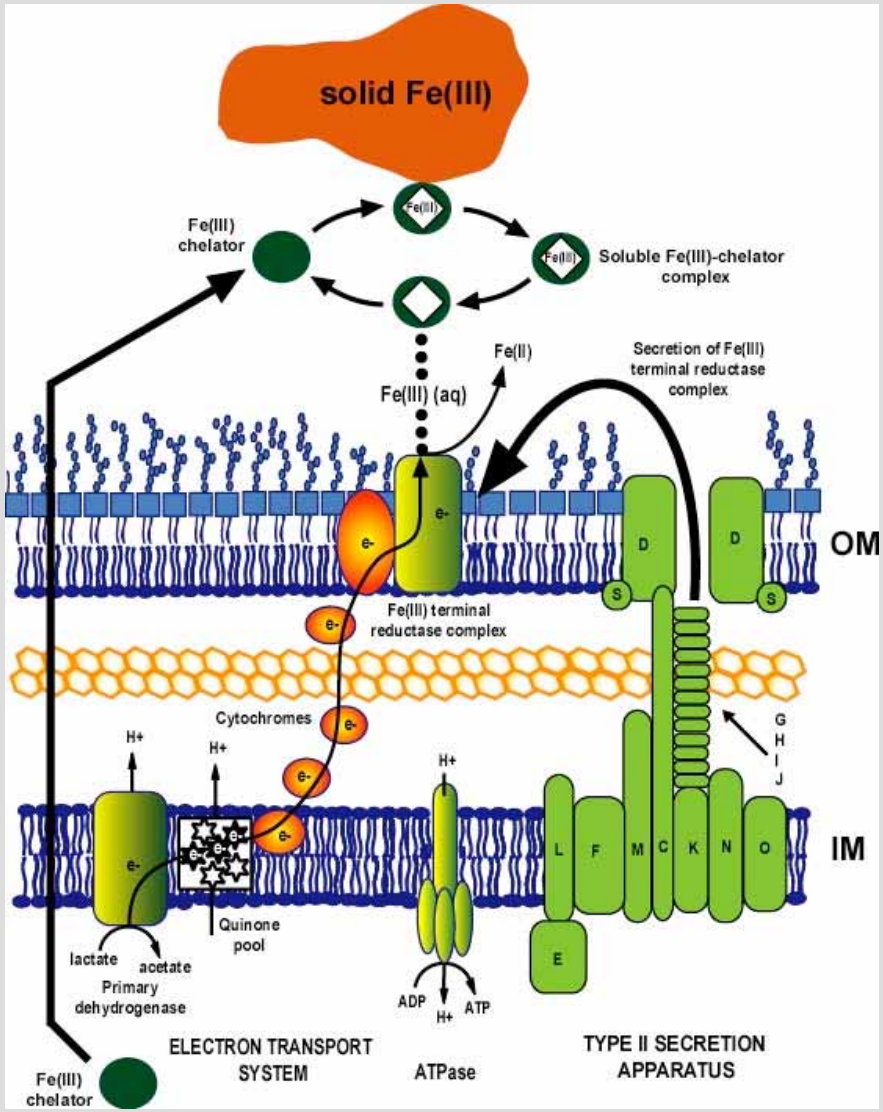
Model 2. Endogenous Fe(III) Chelator



Fe(III) reduction is inhibited by chelating compounds with strong Fe(III) binding capability

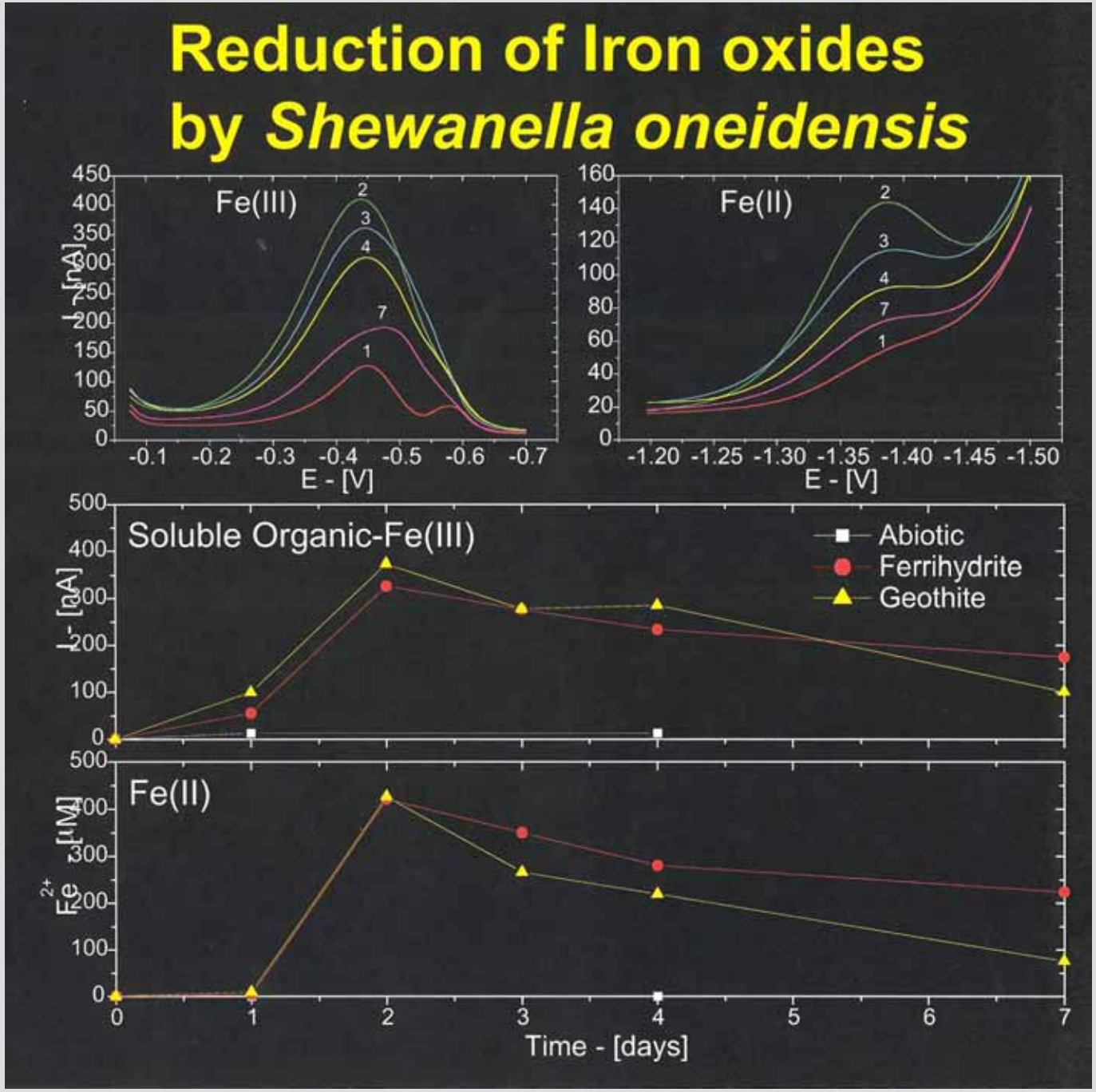


Model 3. Exogenous Fe(III) Chelator

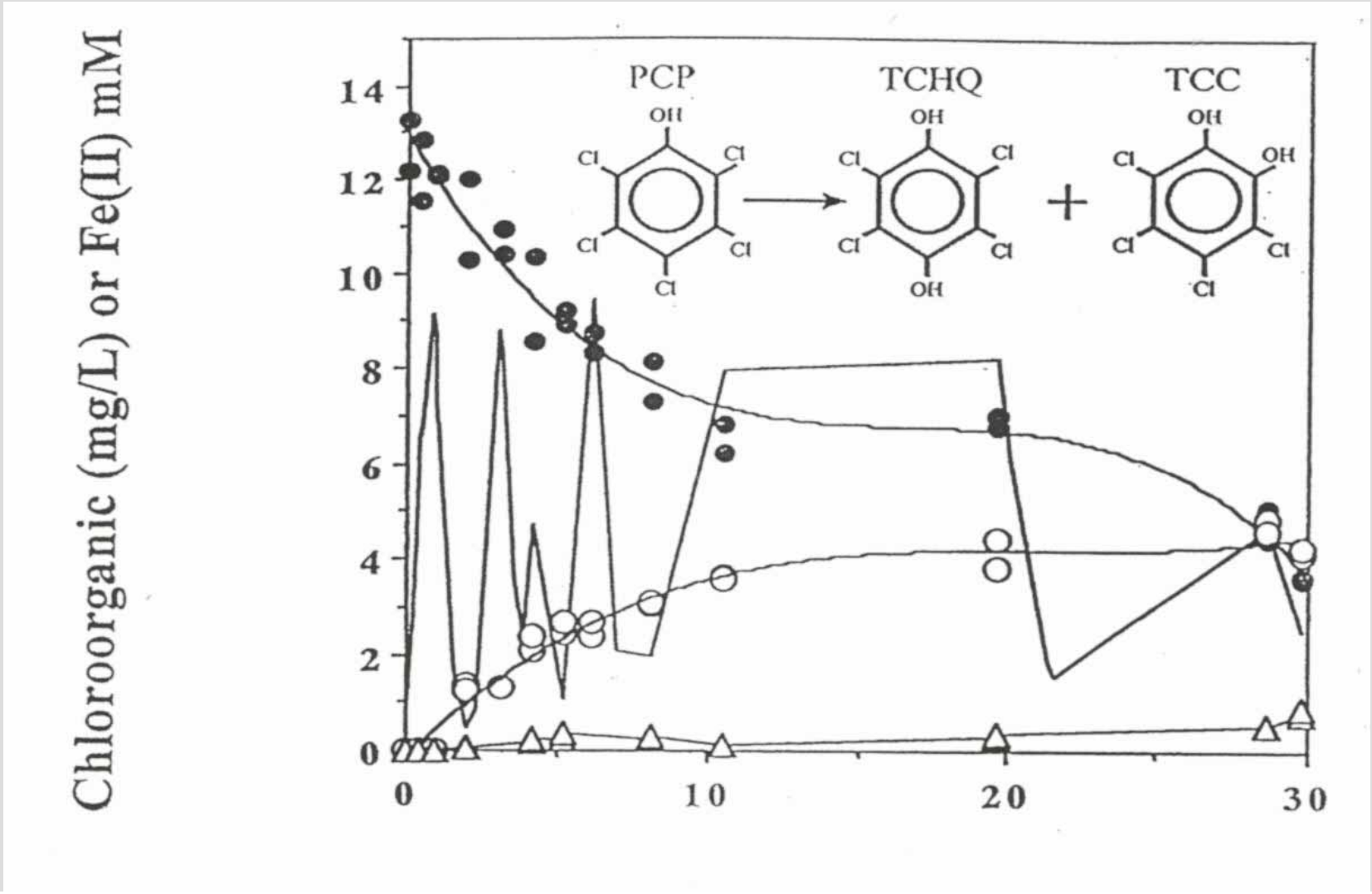


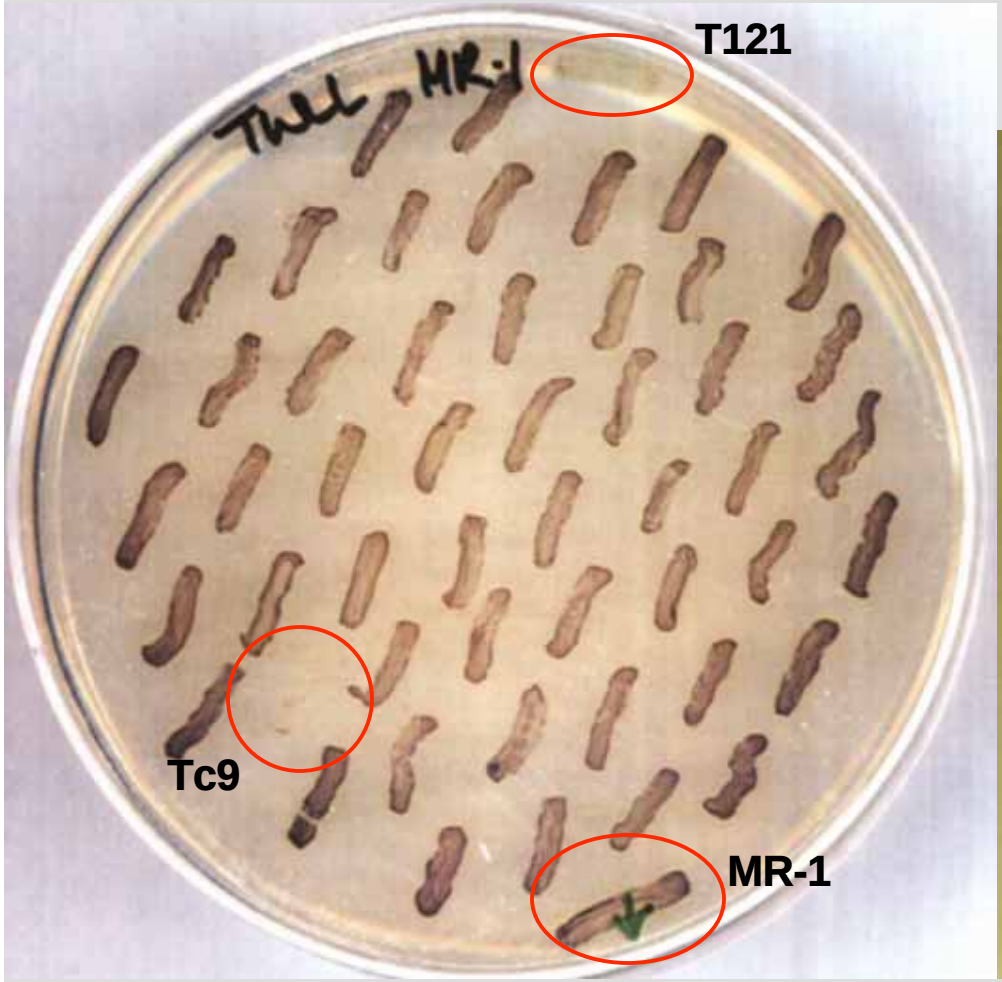
Model No. 4 Endogenous electron shuttle

not detected

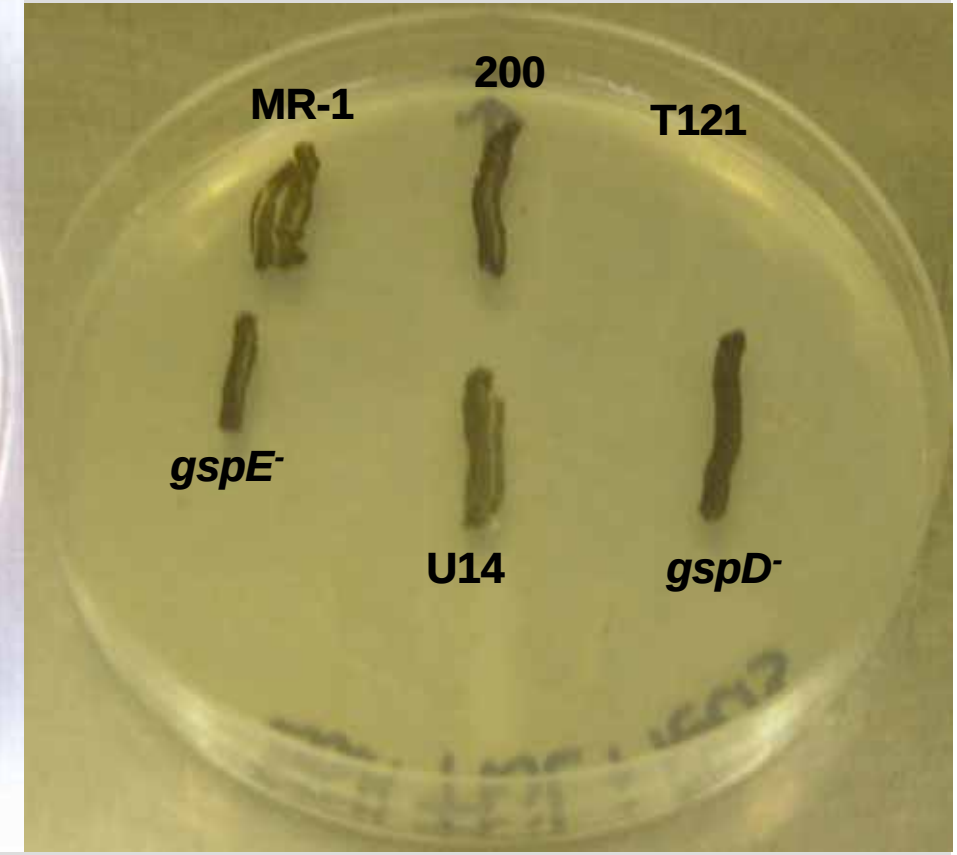

Reduction of Iron oxides
by Shewanella oneidensis
Fe(III)
Fe(II)
i - [nA]
E - [V]
Soluble Organic-Fe(III)
Abiotic
Ferrihydrite
Geothite
i - [nA]
Fe2+ [µM]
Time - [days]

Microbially-driven Fenton reaction for pentachlorophenol degradation

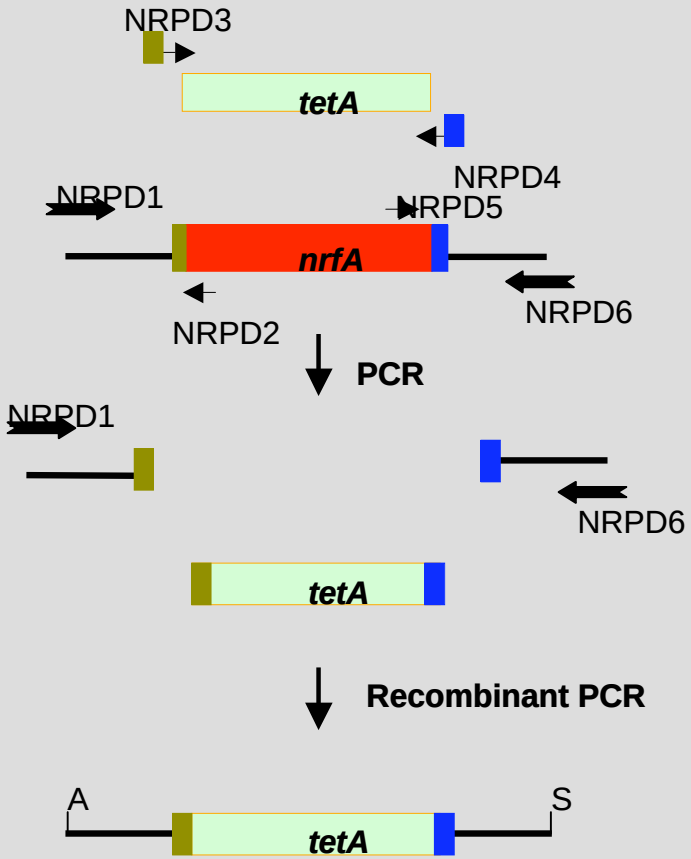




Color Image 1. Actual Tcr screening plate used to identify Tc(VII) reduction-deficient mutant Tc9 [row 6, column 2]. Colonies resulting from EMS-treated cells were grown under microaerobic conditions for 2 days at 30°C on agar growth medium supplemented with 125 μM Tc(VII). Note black Tc(IV) precipitate on colony surface of all strains except Tc9 and negative control strain T121 [row 1, column 3]. Positive control strain [wild-type *S. oneidensis* MR-1] is located at row 9, column 1.

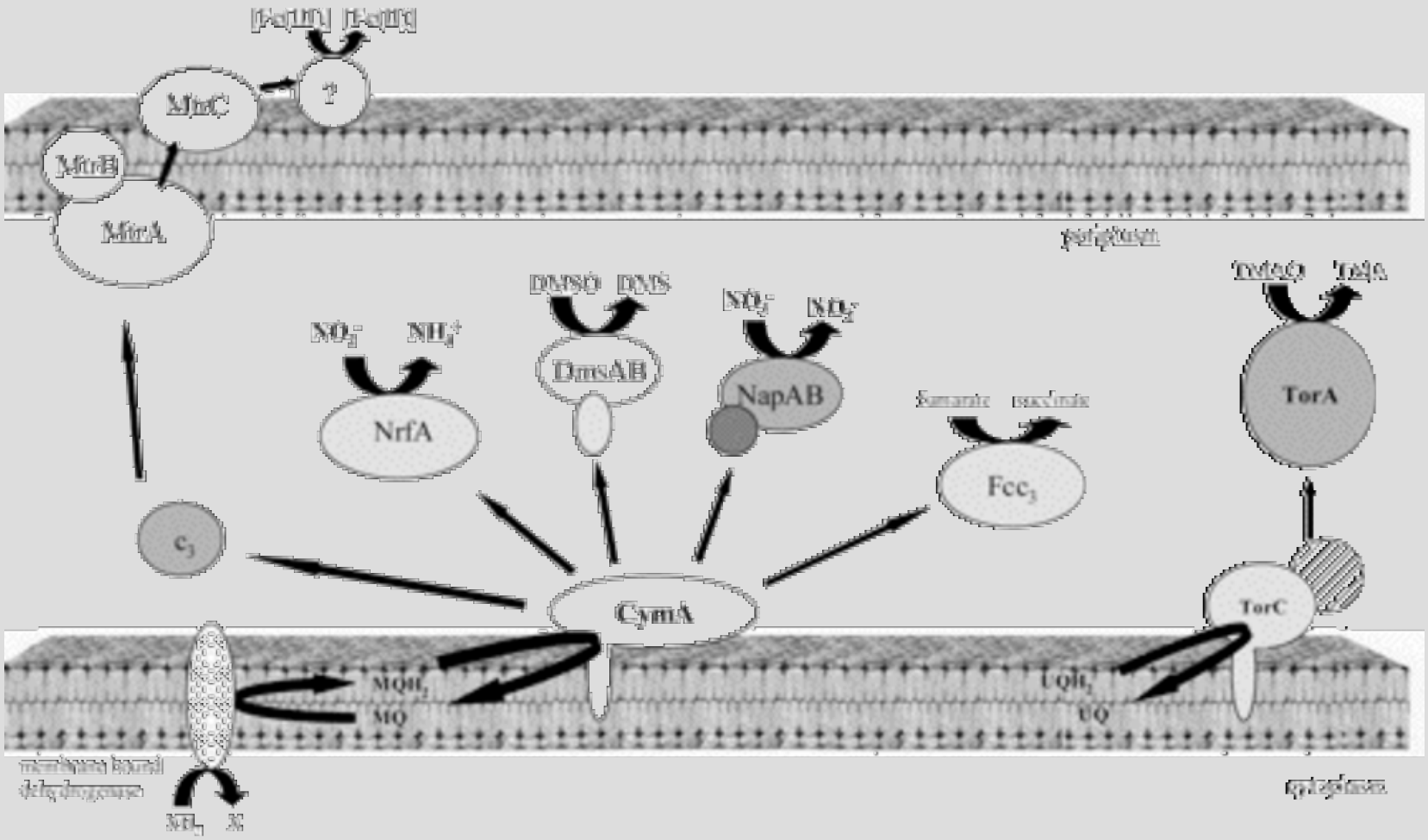


Color Image 3. Tc(VII) reduction phenotypes of *S. oneidensis* strains MR-1 [wild-type] and *gspD*$^{-}$ [Fe(III) reduction-deficient type II secretion mutant] and *S. putrefaciens* strains 200 [wild-type], T121 [anaerobic respiratory mutant], *gspE*$^{-}$ [Fe(III) reduction-deficient type II secretion mutant] and U14 [U(VI) reduction-deficient mutant]. Strains were grown under microaerobic conditions for 2 days at 30°C on agar growth medium supplemented with 125 μM Tc(VII). Note black Tc(IV) precipitate on colony surface of all strains except T121 [previously found to lack the ability to respire anaerobically on any electron acceptor].

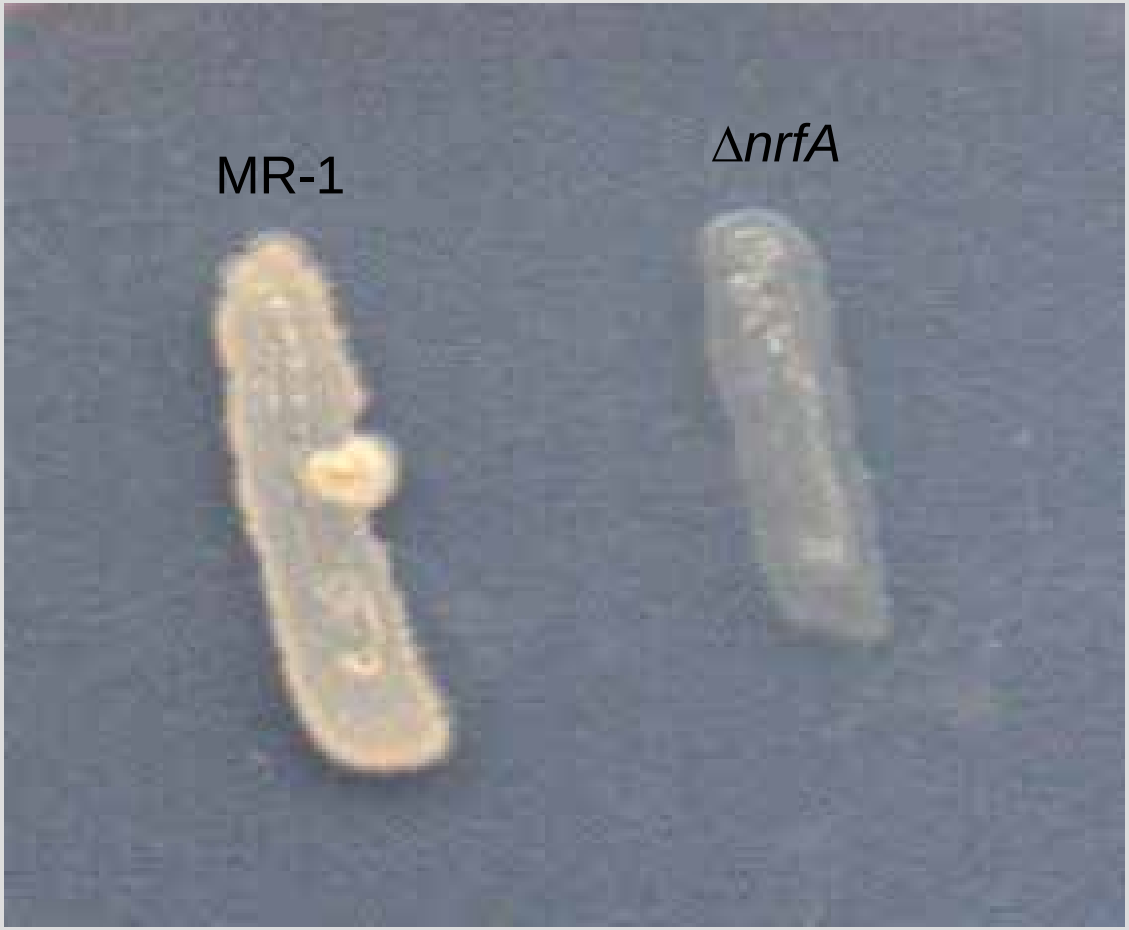


Color Image 8. Recombinant PCR strategy for deletion mutagenesis. Top - Three DNA fragments were amplified independently: a tetracycline-resistance cassette *tetA*, and the upstream and downstream sequences of *nrfA*. Primers NRPD3 and NRPD4 were designed so that their products hybridized with either end of the upstream and downstream sequences of *nrfA*. THe three sequences were combined as templates for recombinant PCR to generate a linear fragment of 2.7 kb. The final product was cloned into suicide vector pNTPS139 for gene replacement. Bottom - Primers used for amplification.

Name	Sequence (5' ® 3')	Use
NRPD1	GCCTACAGGGCCCGAACCCTTCTGGCAGCAT	PCR amplification of sequence upstream of *nrfA*, includes 5' *Apa*I recognition sequence.
NRPD2	GGCGCGAAGCGACTTACAAGTAATC	
NRPD3	GATTACTTGTAAGTCGCTTCGCGCCTTATCCGGTAACTATCGT	PCR amplification of the *tetA* gene from pACY184
NRPD4	GCATTAAGTGCATTGGTTGCCGGAGTGGTGAATCCGTTAGC	
NRPD5	CCGGCAACCAATGCACTTAATGC	PCR amplification of the sequence downstream of *nrfA*, includes 3' *Spe*I recognition sequence.
NRPD6	GCCTACAACTAGTGCGTGTTTTCAAACTCGGTG	


MtrC
MtrB
MtrA
?
periplasm
DMSO DMS
NO_3^- NH_4^+
NO_2^- NO_2^-
TMAO TMA
NrfA
DmsAB
NapAB
TorA
Fcc$_3$
c$_3$
CymA
TorC
MQH_2
MQ
UQH_2
UQ
cytoplasm

S. oneidensis Δ*nrfA* deletion mutant displays a U(VI) reduction-deficient phenotype



- Derek Lovley (1993): U(VI) and Cr(VI) reduced in vitro by cytochrome c_3 of *Desulfovibrio vulgaris*
- Judy Wall (2002): U(VI) reduced in vivo by cytochrome c_3 of *Desulfovibrio desulfuricans*
- *S. oneidensis* MR-1 genome contains SRB-like cytochrome c_3, but Δc_3 deletion mutant retains U(VI) reduction activity

Identification of Fe(III) reduction-specific genes

Screened 15,000 mutagenized colonies via
ferrozine spray, TSI and AGR assays

Identified 72 Fer mutants

Tested each for anaerobic respiration on suite of 10 alternate TEAs

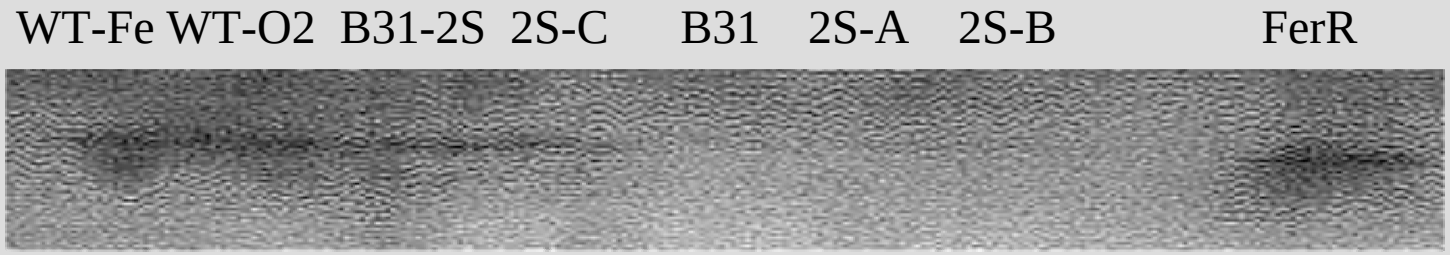
57 displayed multiple respiratory deficiencies

15 were deficient in Fe(III) and Mn(IV) respiration, yet retained ability to respire all other TEAs

All 15 Fer mutants were reactivated for Fe(III) reduction by
an identical 23 kb *Hin*dIII DNA fragment)

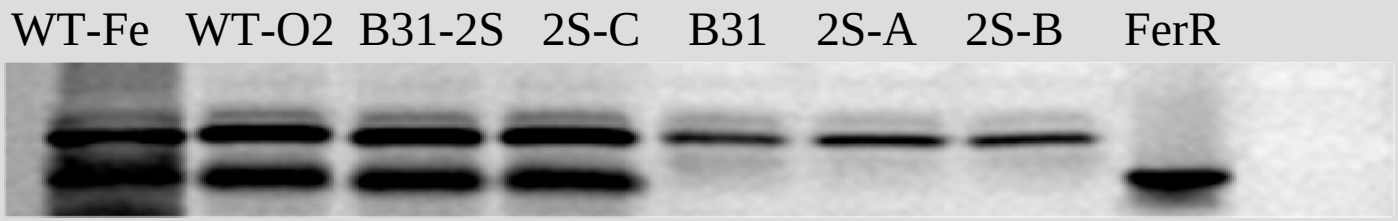
Subcloned B31 and identified complementing gene

KCl wash to detach peripheral proteins from cell surface of WT and *gspE*



Native-PAGE
Ferrozine stain

- Fe(III) reductase is missing from periphery of *gspE* mutant B31



SDS-PAGE
heme stain

- Fe(III) reductase contains heme (cytochrome *c*?)

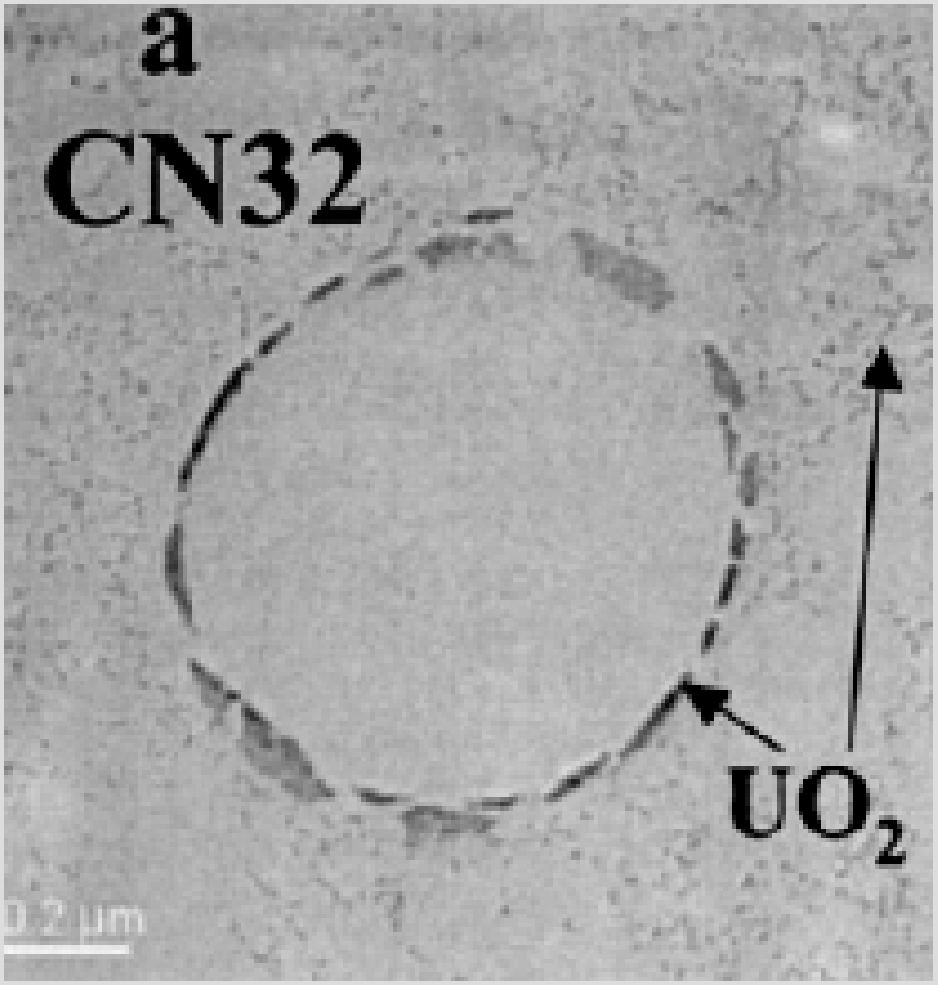
- **18 amino acid N-terminal sequence of heme-positive Fe(III) reductase displayed no significant homology to any predicted protein-encoding ORF in database (including conserved hypotheticals).**

Reduction kinetics of Fe(III), Co(III), U(VI) Cr(VI) and Tc(VII) in cultures of dissimilatory metal-reducing bacteria

Liu CX, Gorby YA, Zachara JM, Fredrickson JK, Brown CF

BIOTECHNOLOGY AND BIOENGINEERING

80 (6): 637-649 DEC 20 2002



- **NrfA is located in periplasmic space of *E. coli*, *W. succinogenes* and *Sulfurospirillum deleyianum***

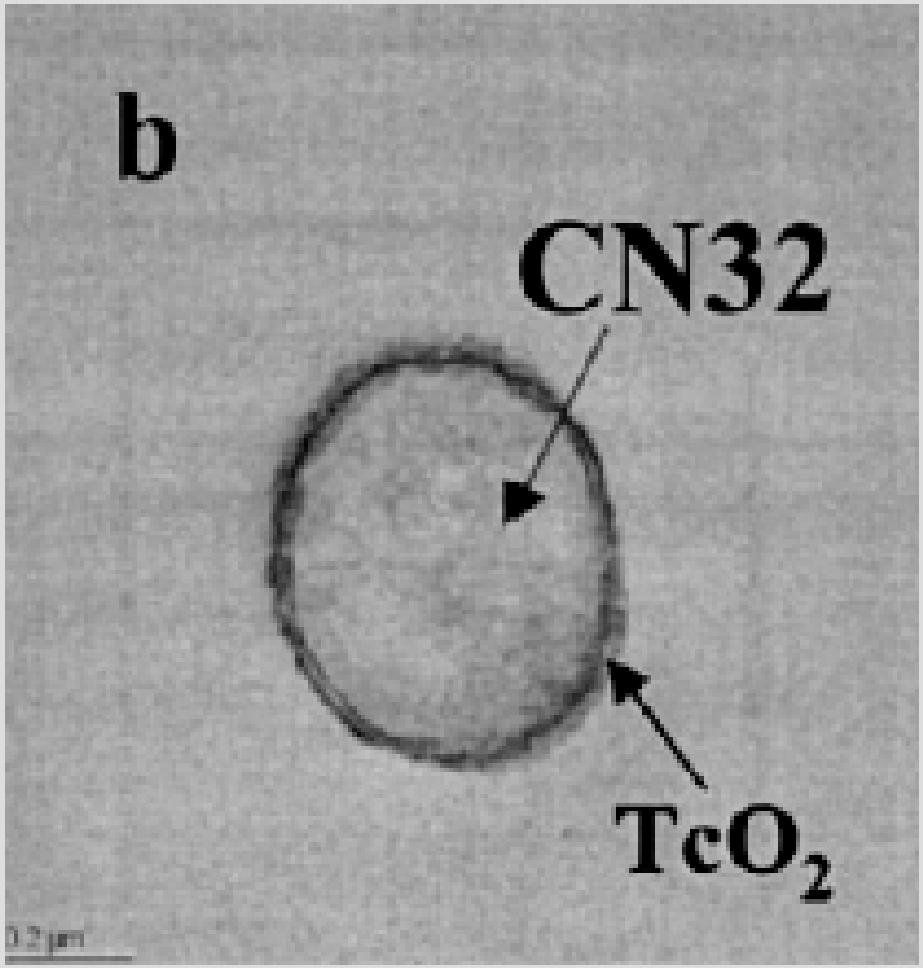
- **Accumulation of U(IV) in periplasmic space of *S. putrefaciens* CN32**

Reduction kinetics of Fe(III), Co(III), U(VI) Cr(VI) and Tc(VII) in cultures of dissimilatory metal-reducing bacteria

Liu CX, Gorby YA, Zachara JM, Fredrickson JK, Brown CF

BIOTECHNOLOGY AND BIOENGINEERING

80 (6): 637-649 DEC 20 2002

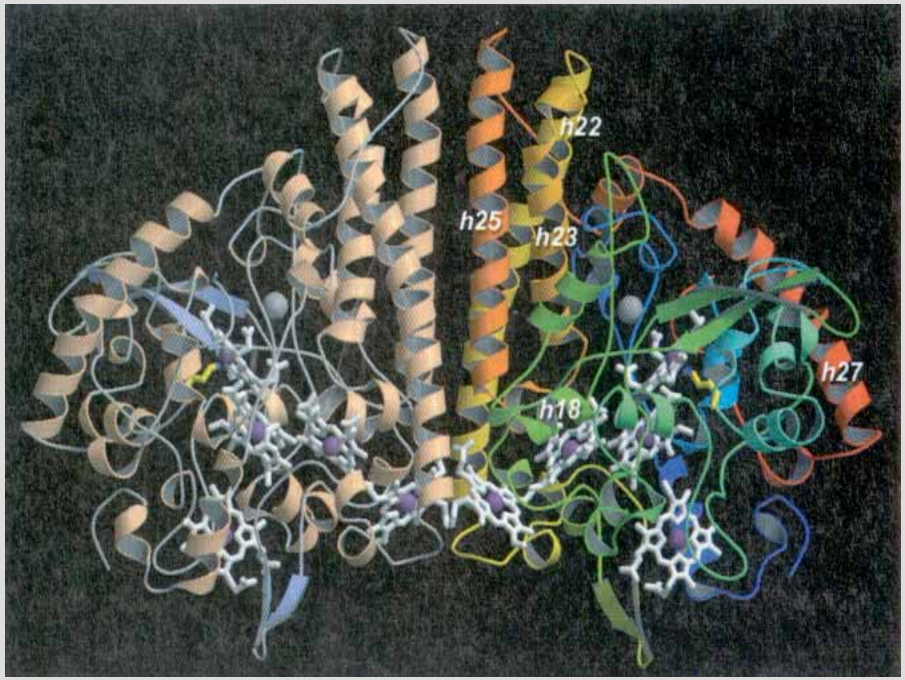


- **Tc(IV) accumulates in periplasmic space of *S. putrefaciens* CN32**
- **Tc(VII) reductase located in periplasmic space of *S. oneidensis* MR-1?**

Shewanella oneidensis MR-1

- **isolated from Oneida Lake by Ken Nealson's group in 1988**
- **anaerobic respiratory capability identical to *S. putrefaciens* 200**
- **MR-1 genome sequenced by DOE-JGI in 2002**
- **4,758 ORFs (46% NOT assigned biological function, 22% conserved hypotheticals, 24% unique); 32% most similar to *Vibrio cholera* genes**
- **39 *c*-type cytochromes, including 8 that contain 10 hemes each**
- **3 of the 8 decahemes are located in OM**

Crystal structure of *Sulfurospirillum deleyianum* NrfA

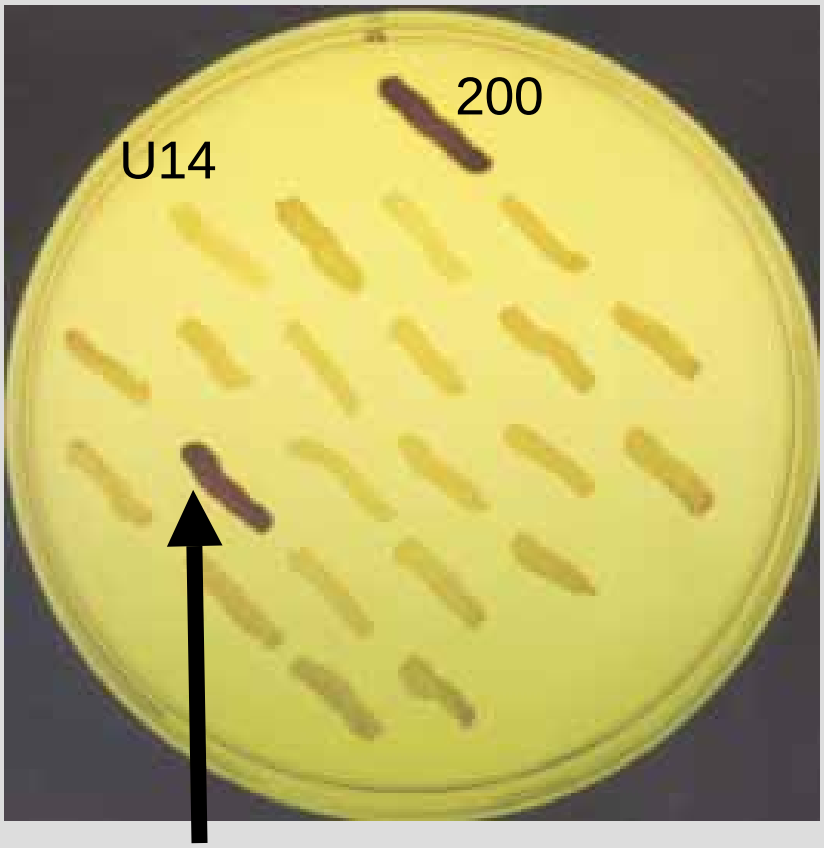


(Einsle et al., 1999)

- homodimer with 5 close-packed hemes per monomer
- orientation of 5 heme groups is nearly identical to hydroxylamine oxidoreductase of *Nitrosomonas europaea* (NH_2OH oxidized to NO_2^-)
- active site heme of NrfA is lysine-coordinated as opposed to histidine-coordinated HAO in *N. europaea* (electron sink as opposed to electron donor)
- electropositive entrance channel for NO_2^- and electronegative exit channel for NH_4^+

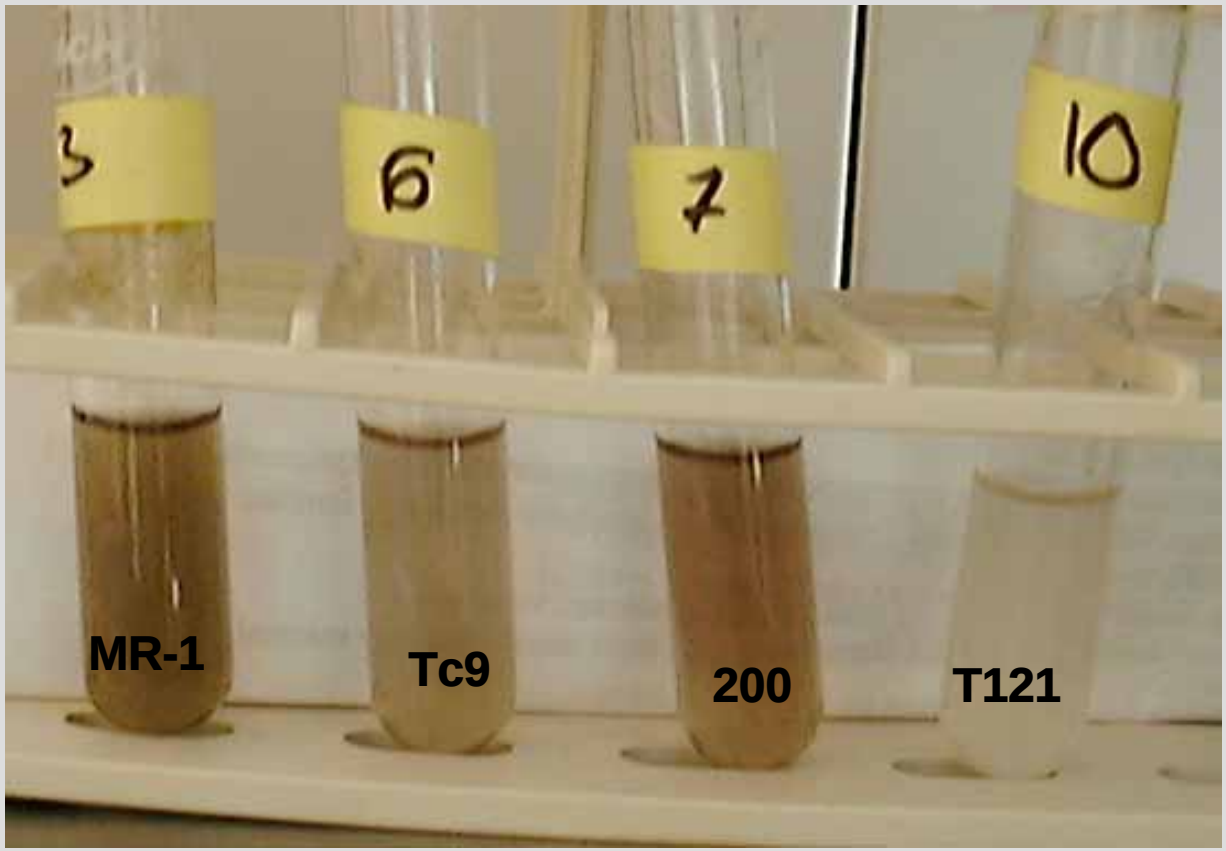
- *nrfA* deleted from *S. oneidensis* chromosome

Restoration of U(VI) reduction capability to Urr mutant U14



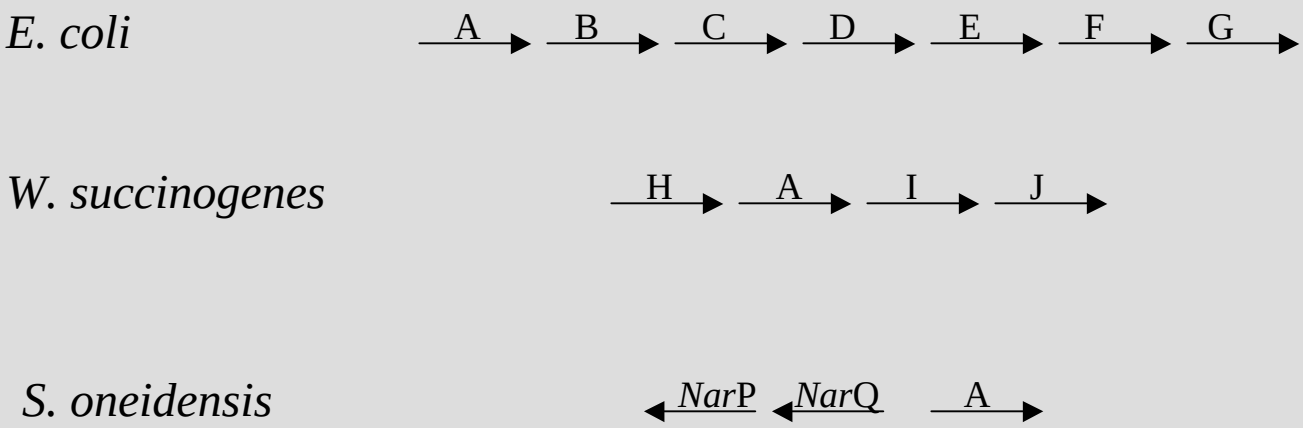
Transconjugant U14-D14 with restored U(VI) reduction capability

- Currently subcloning complementing DNA fragment



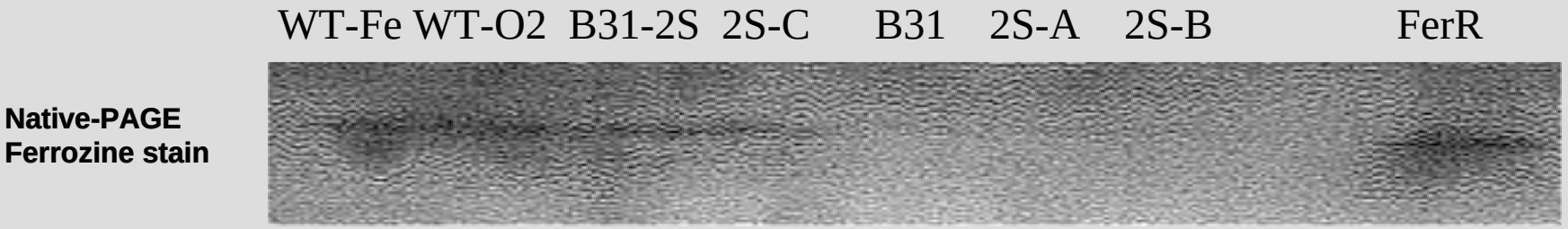
Tc9 retains ability to respire all other TEAs

S. oneidensis genome scan

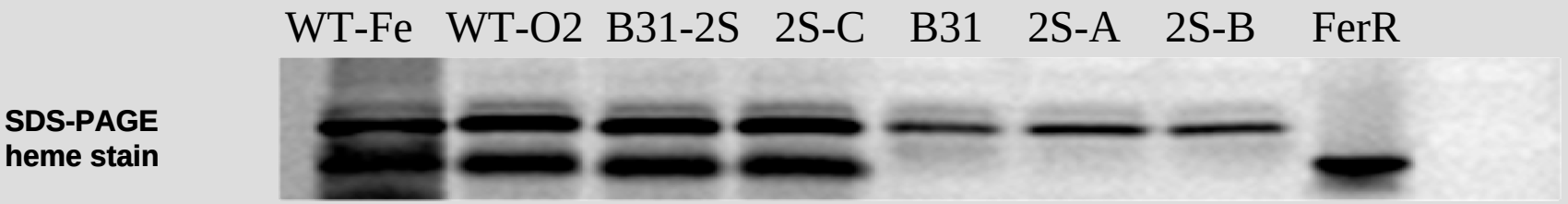


- **Cu-containing and cytochrome *cd*$_1$ (NirK/S) homologs were not detected**
- ***W. succinogenes*-like NrfH - J homologs were not detected**
- ***E. coli*-like NrfA - G homologs were detected but scattered throughout genome (79% similar)**

KCl wash to detach peripheral proteins from cell surface of WT and *gspE*



- Fe(III) reductase is missing from periphery of *gspE* mutant B31



- Fe(III) reductase contains heme (cytochrome *c*?)

2003 - Repeat in *Shewanella oneidensis* MR-1