

# Mechanisms of U(VI) Reduction and Sediment Growth in *Desulfovibrio*

Lee Krumholz

**Department of Botany and Microbiology**

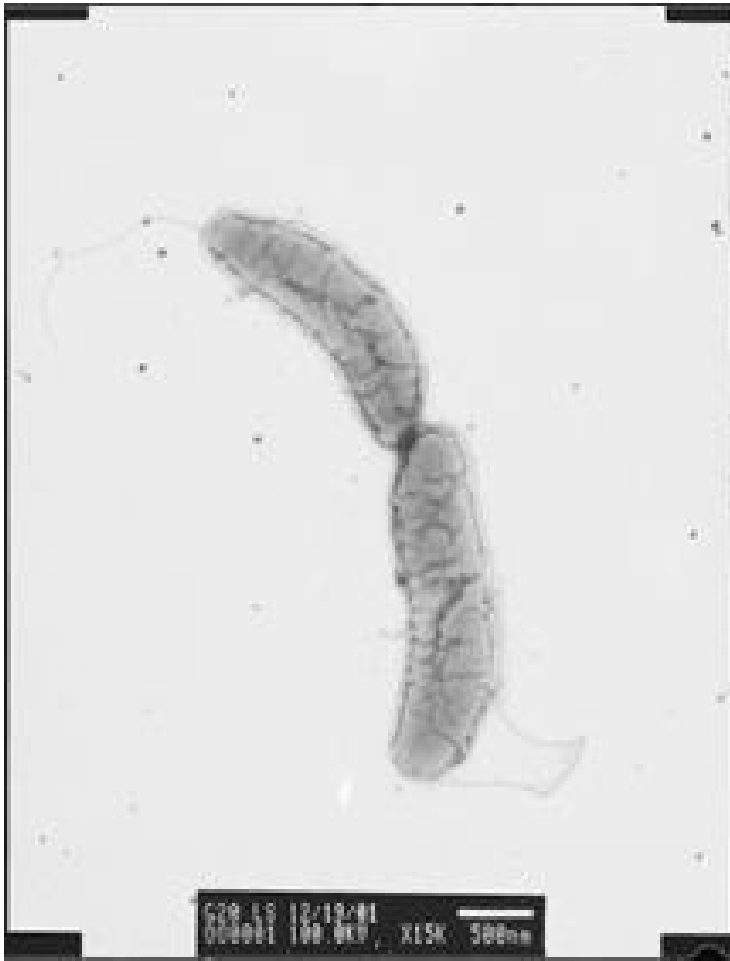


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# Goals

- Identify mechanisms of survival of sediment bacteria in U(VI) contaminated sediments.
  - This was further refined to
    1. Identification of mechanisms of dealing with U(VI)
    2. Identification of mechanisms of survival in Anoxic sediments.

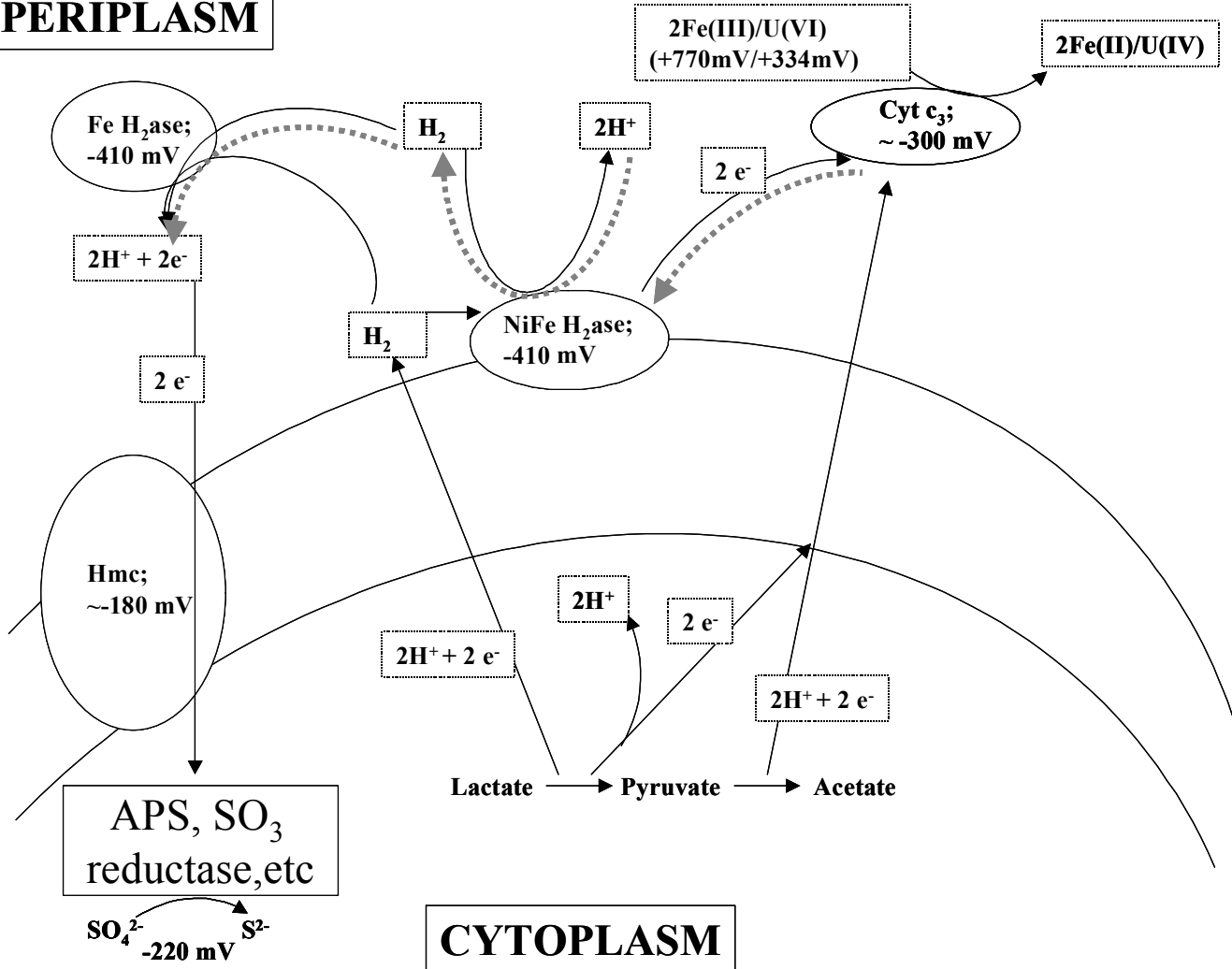
# *Desulfovibrio* G20



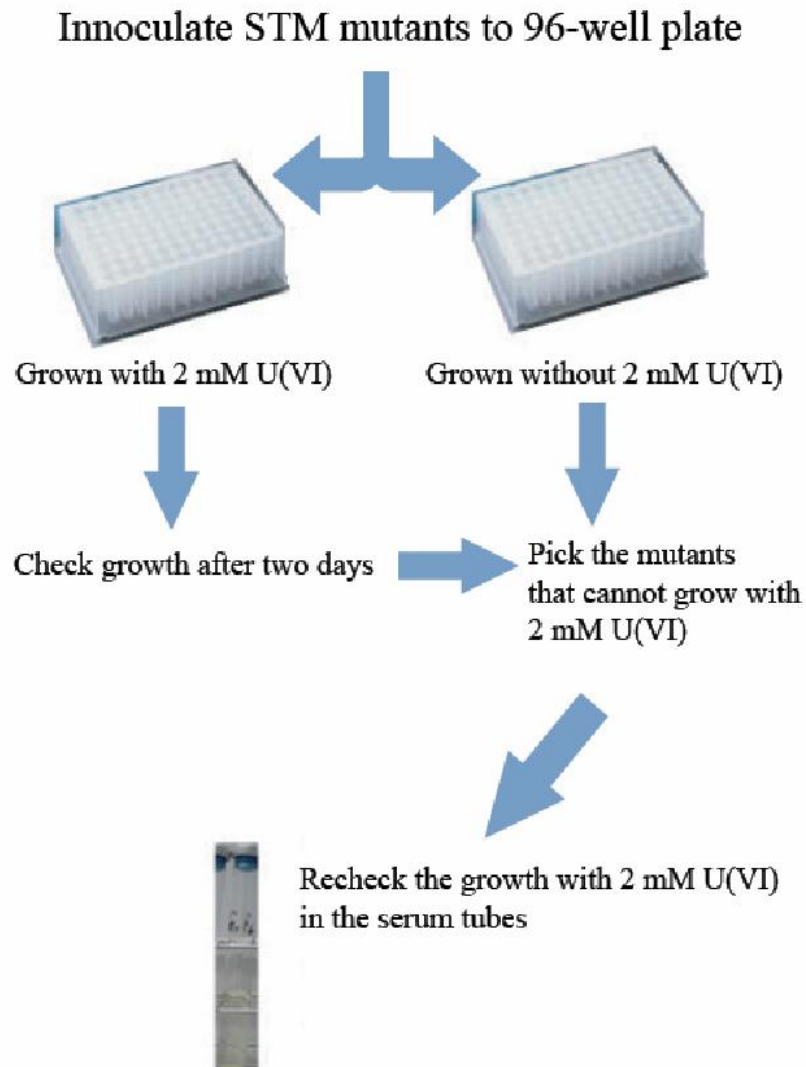
- Isolated from an oil producing reservoir.
- 4.1 Mb, 3598 candidate genes
- Doubling time is ~4.6 hours

# Mechanisms of U(VI) reduction

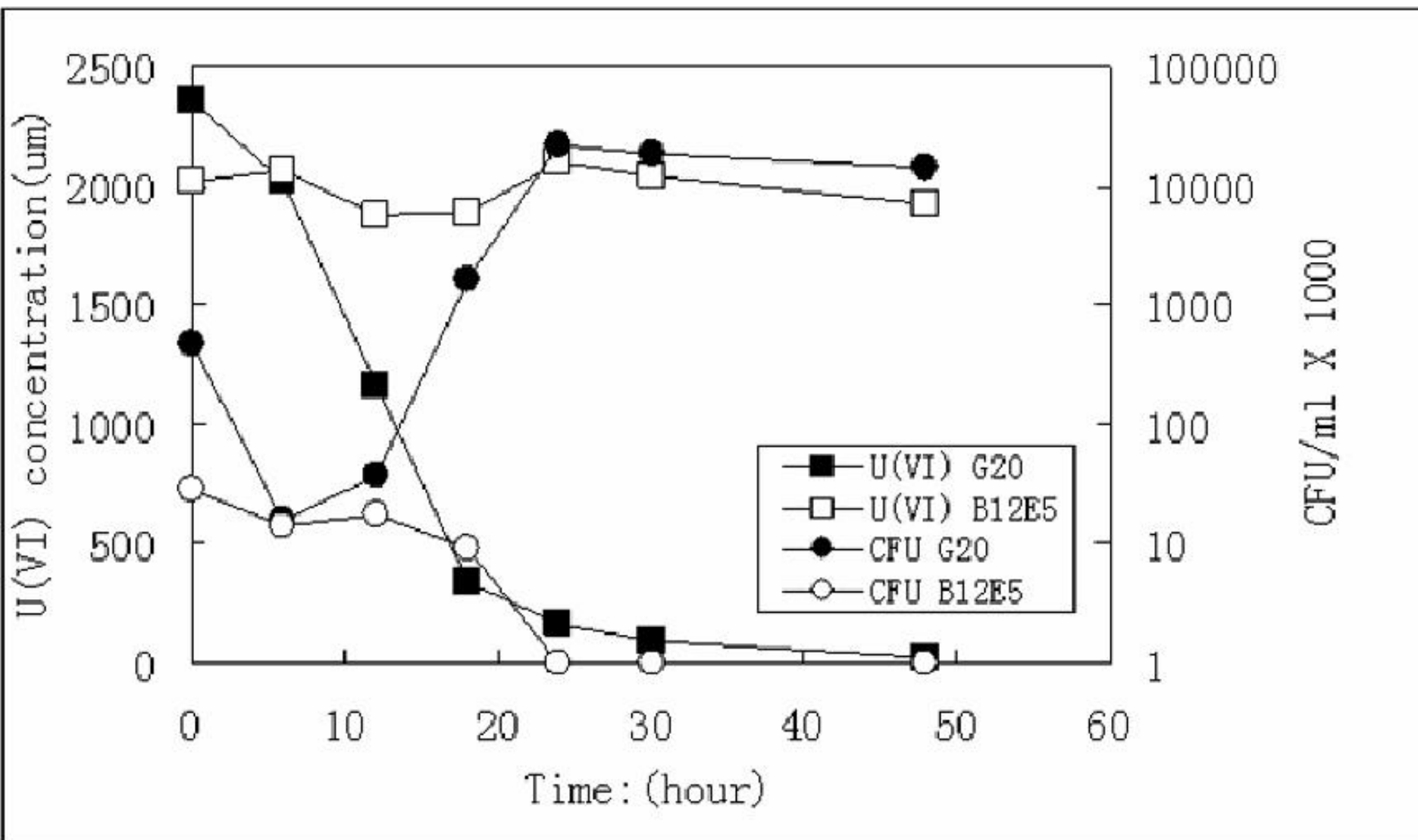
**PERIPLASM**



# Identification of Genes involved in U(VI) Response



# 10 mutants

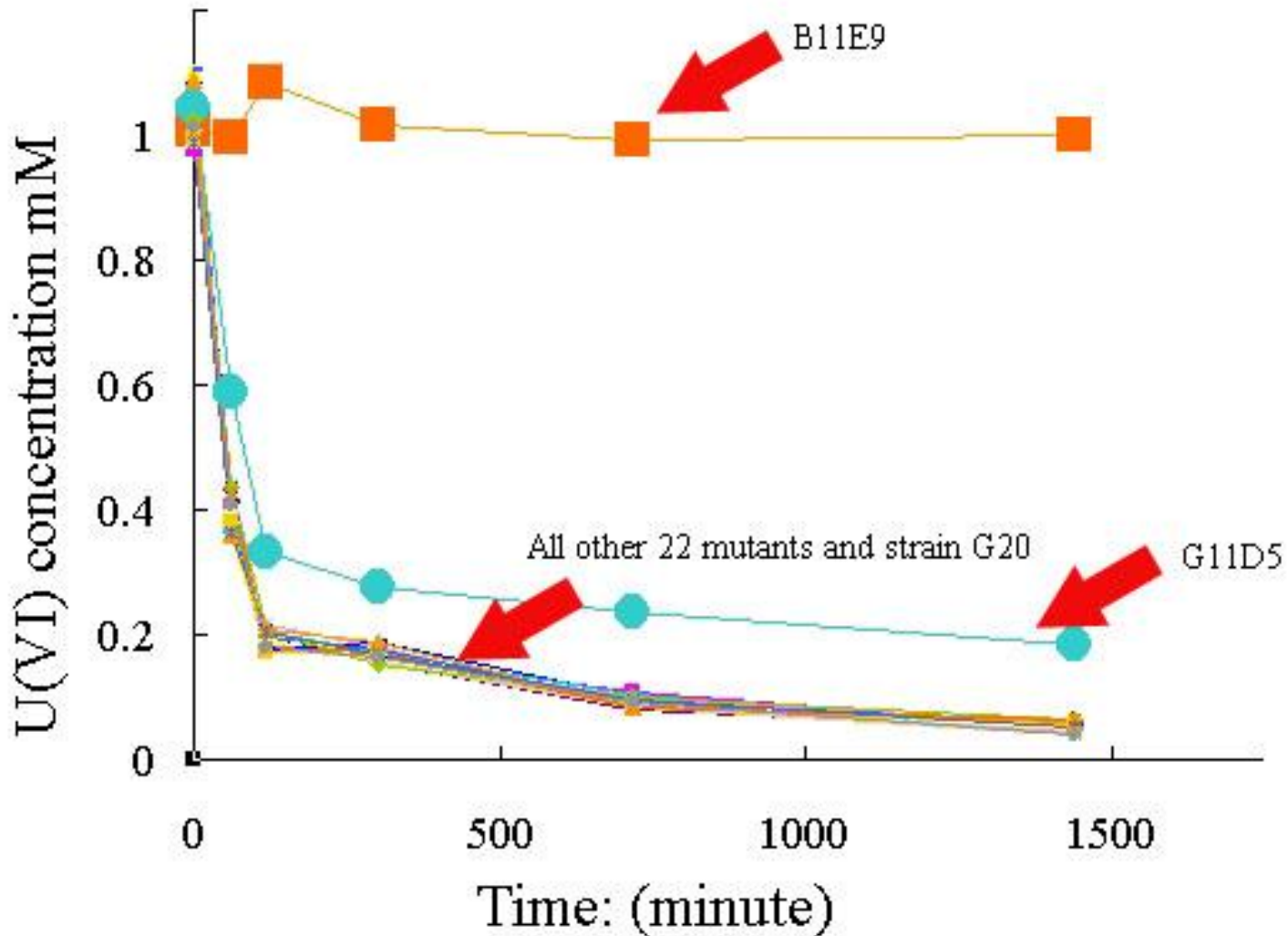


# Potential Functions of mutated genes in U(VI) Sensitive Mutants

- DNA repair
- rRNA methylation
- Protein Renaturation

# Washed Cell U(VI) reduction test by 24 mutants

## Growth Experiment with B11E9





# The Region surrounding the mutation in B11E9

G20



*D. vulgaris*

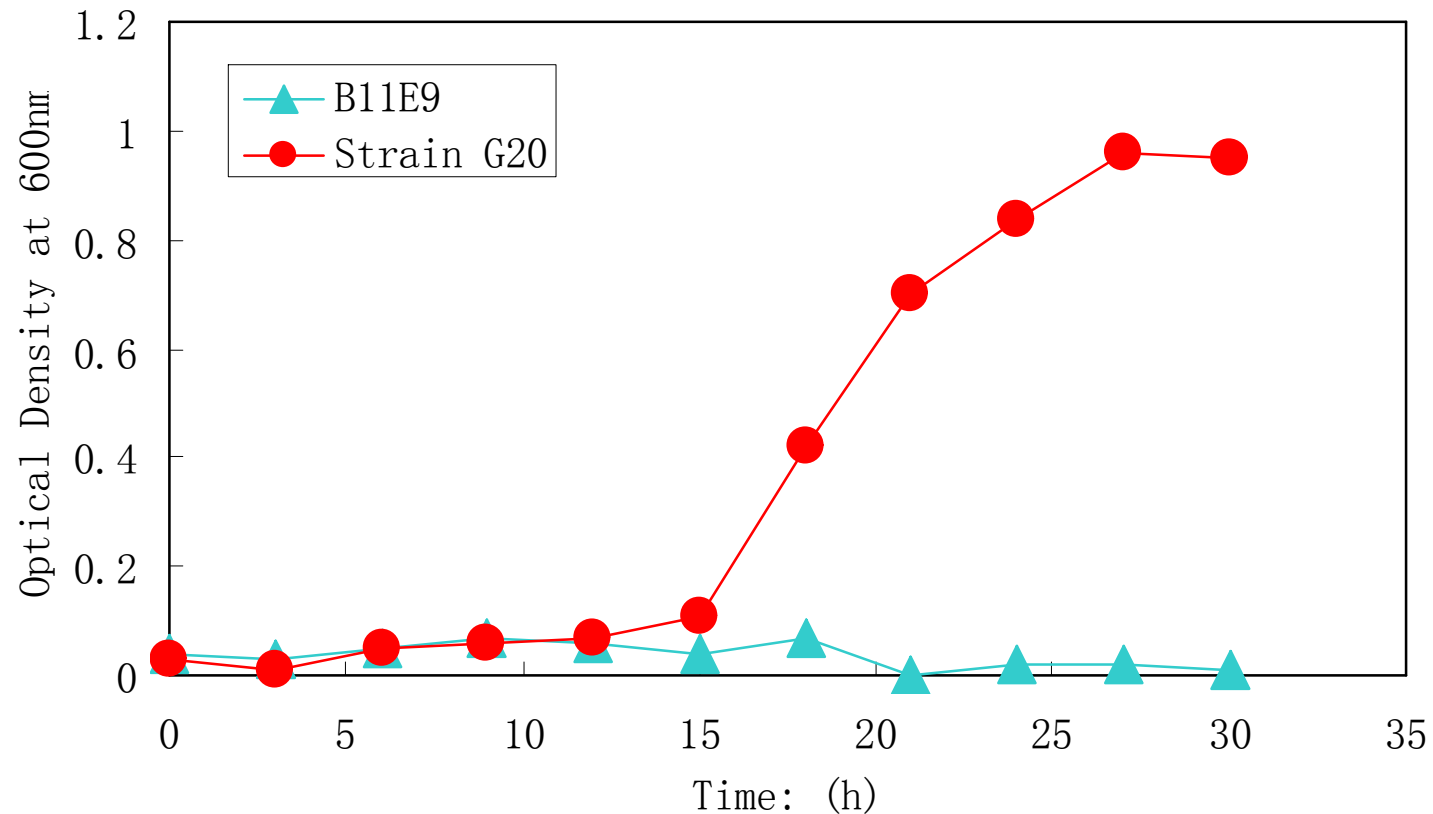


# Mechanism of As(VI) Reduction

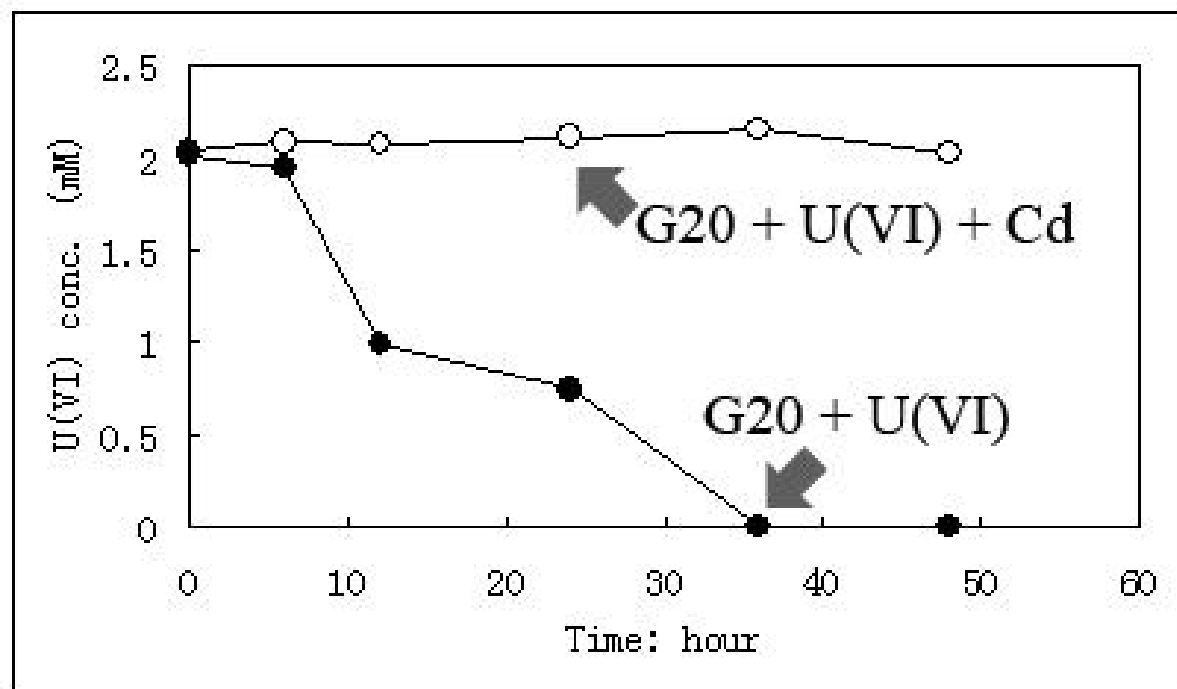
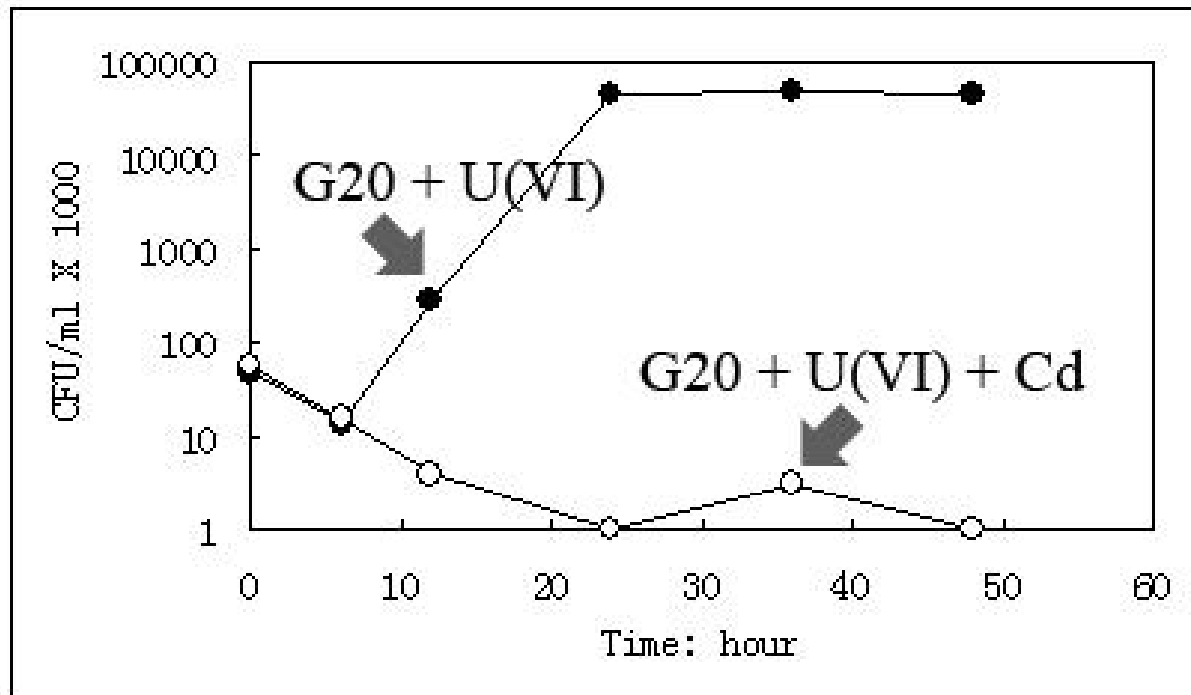
QuickTime™ and a  
None decompressor  
are needed to see this picture.

# Loss of As(V) Tolerance.

- B11E9 also lost As(V) resistance when grown in lactate sulfate media with 20mM As(V)



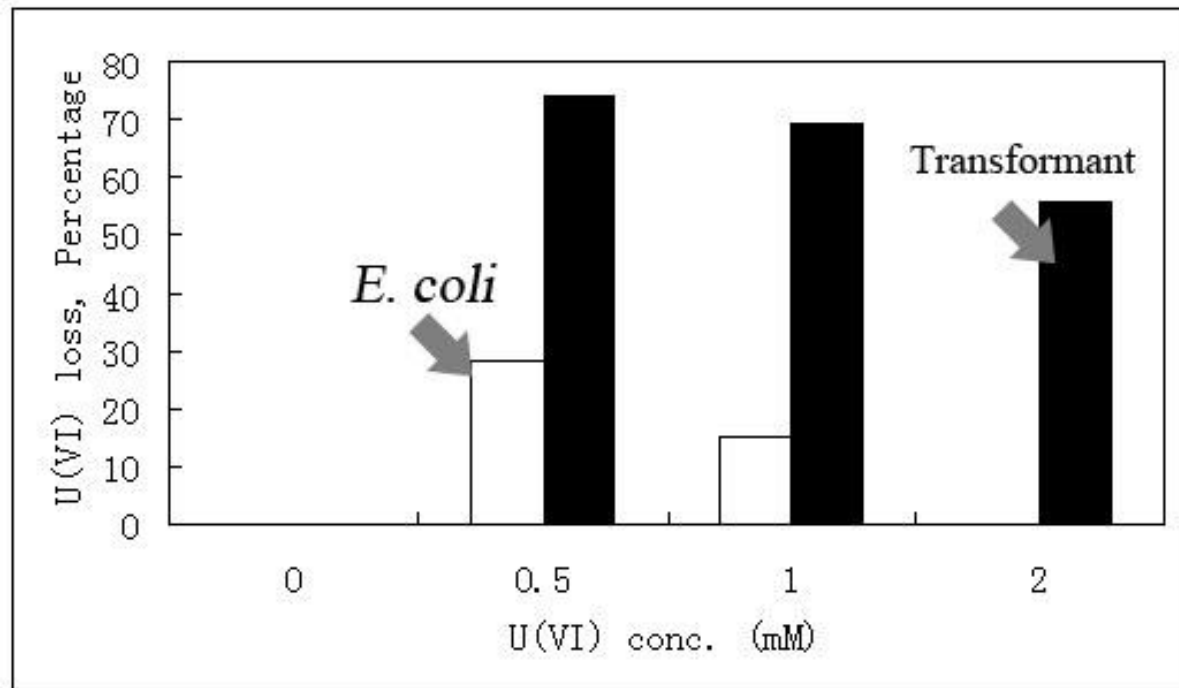
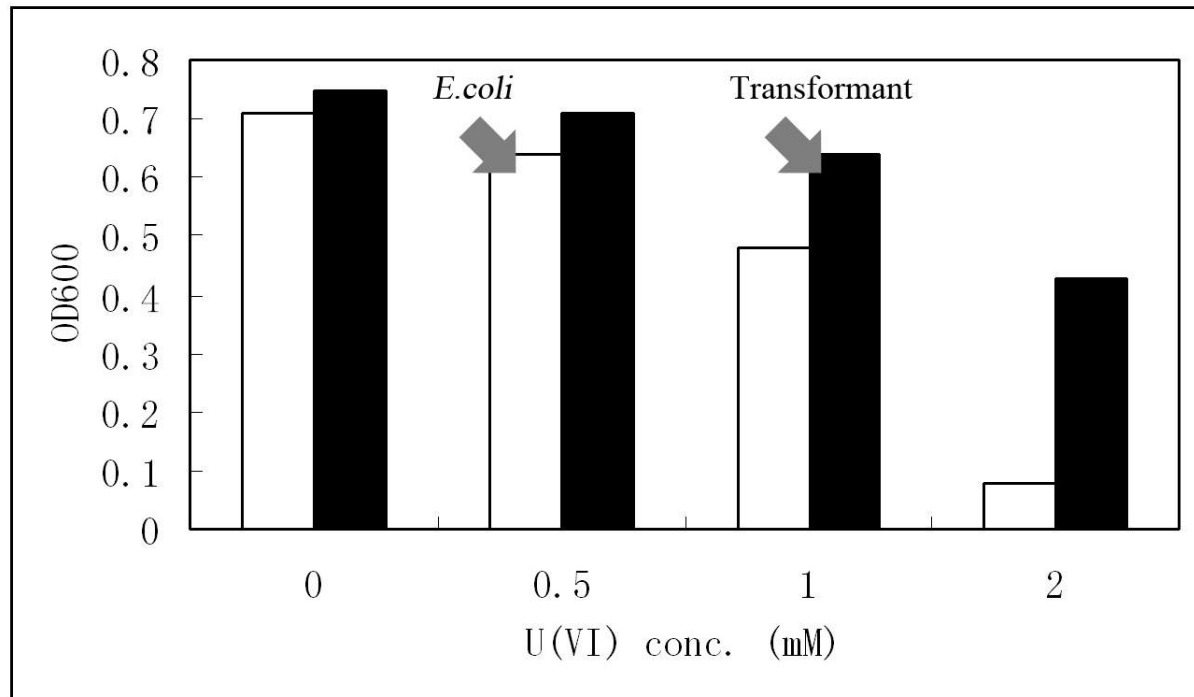
# Growth and U(VI) reduction of G20 with Cd



# Mechanism of U(VI) Reduction

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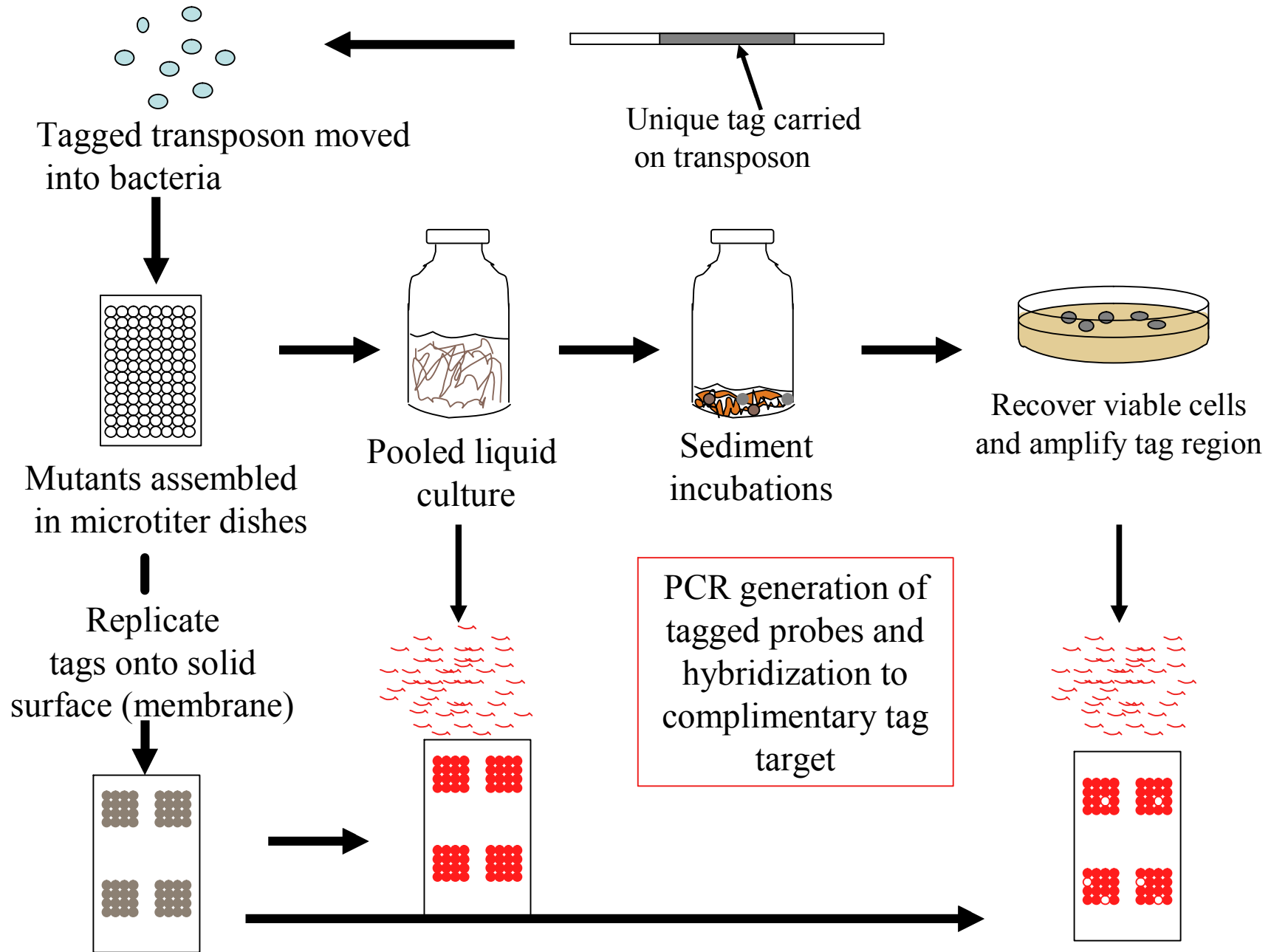
# U(VI) Reduction by *E. coli* transformant



# Signature Tagged Mutagenesis

- First developed to identify virulence genes in pathogens.
- Use the technique to identify functions involved in sediment growth. Different from functions needed for lab growth.

# Overview of signature tagged mutagenesis (STM)







Screened ~5000 mutants for each bacterium by STM and identified 100 mutants in G20 and 46 mutants in MR-1

## **Recurring Themes**

DNA repair

Transcriptional regulators

Phage-related proteins

Transport proteins (multidrug efflux)

Conserved and hypothetical proteins

# DNA repair

<i>In situ</i> CI	<i>In vitro</i> CI	Gene	Product
0.11±0.002	0.571	recG	ATP-dependent DNA helicase RecG
0.39±0.05	ND		helicase (SO0368)
0.4±0.42	ND		conserved hypothetical (SO1652)
0.85±0.38	ND		helicase (SO2744)

- sediment conditions may be mutagenic
- these genes may correct DNA defects resulting from sediment mutagens (e.g., organic acid fermentation products)

# Response to mutagens


- One mutant B12(pF11) belongs to **UmuC** family.
- It has 53.83% similarity to *D. vulgaris* umuC protein.

# Protection from a Severe Mutagenic Event

## The SOS response

QuickTime™ and a  
TIFF (LZW) decompressor  
are needed to see this picture.

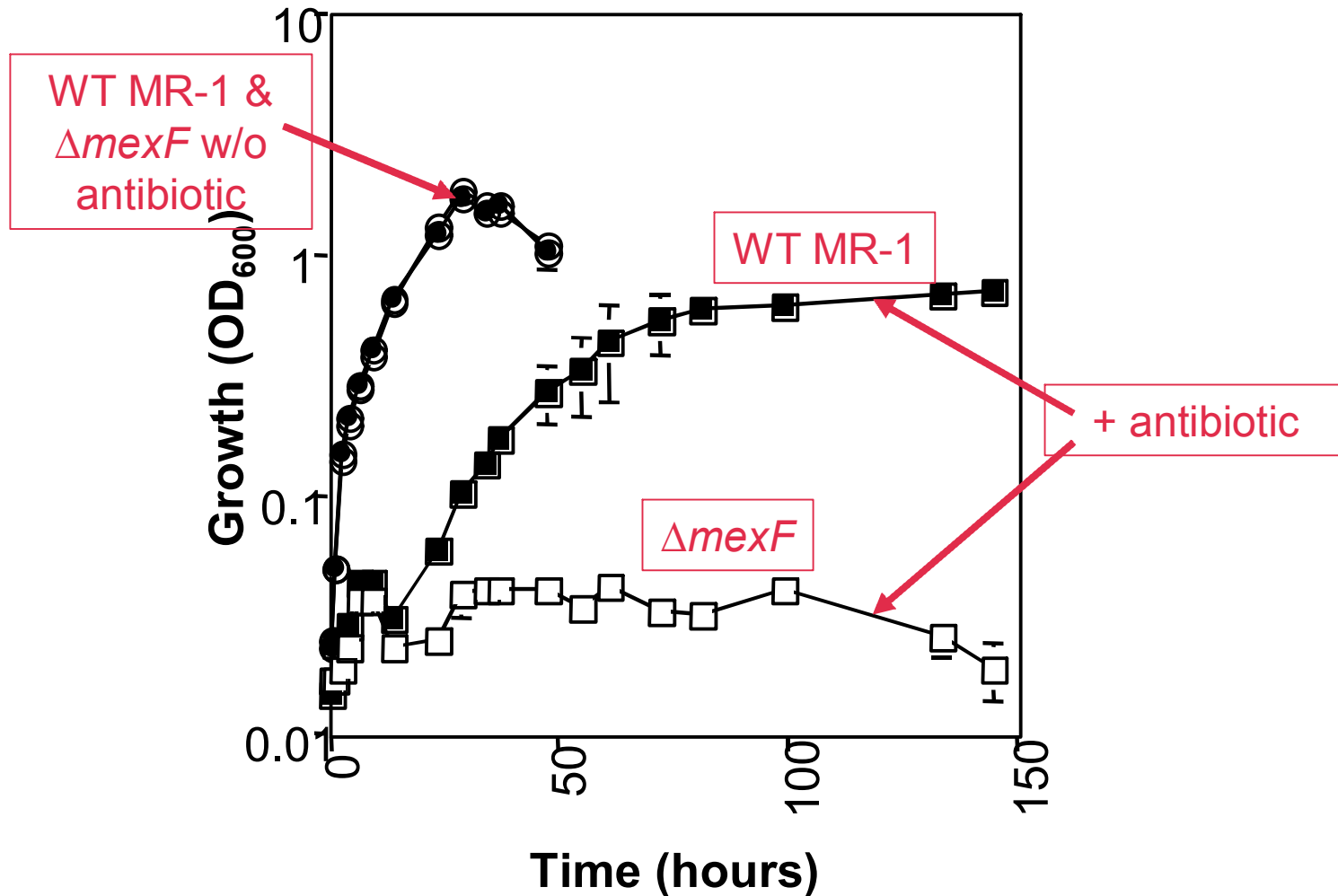
QuickTime™ and a  
TIFF (LZW) decompressor  
are needed to see this picture.



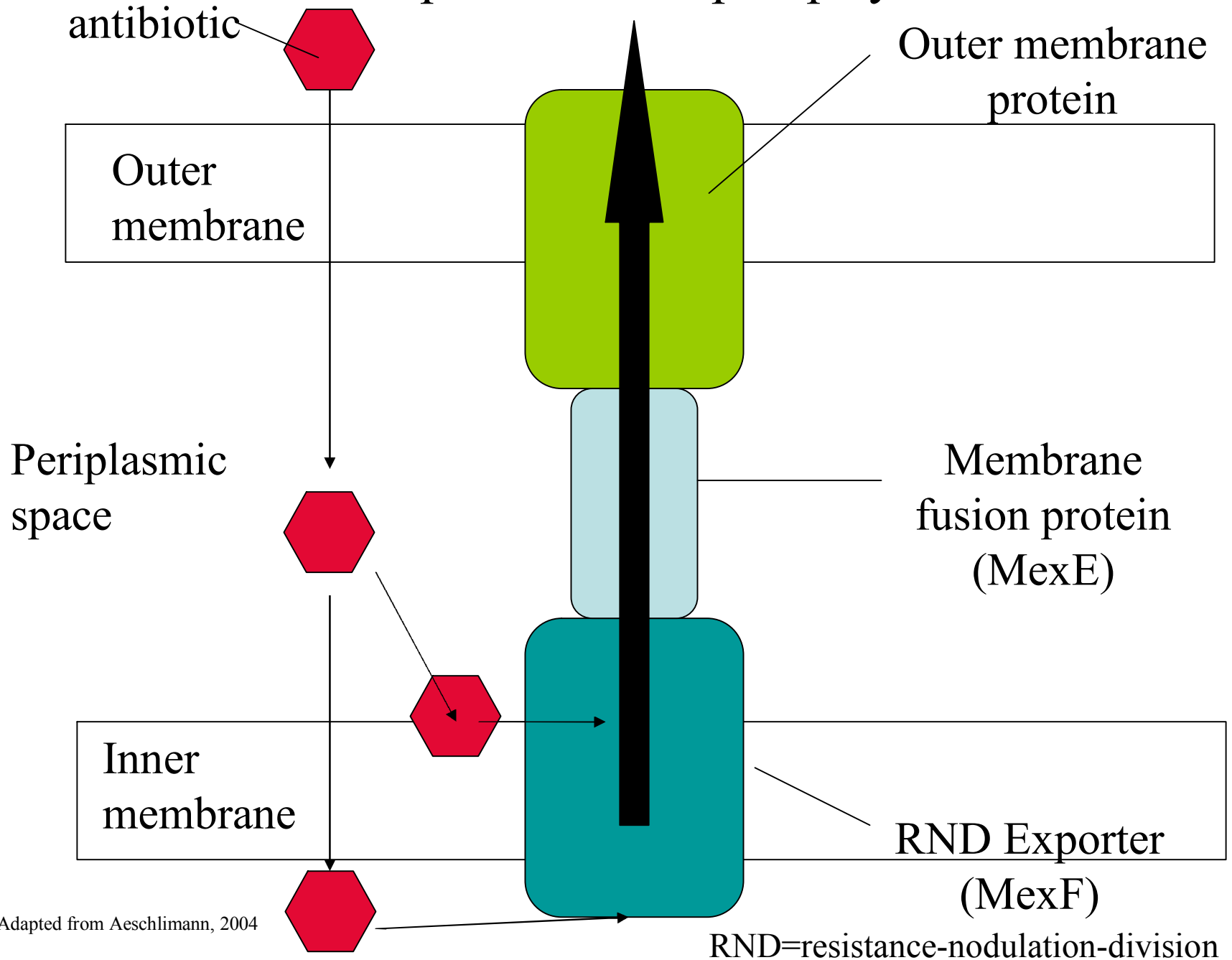
## STM mutants in MR-1 with potential role in drug efflux

<i>In situ</i> CI	<i>In vitro</i> CI	Gene	Product	COG
0.28±0.06	1.3±0.26	mexF	RND multidrug efflux transporter MexF	<b>Cation/Multidrug efflux pump</b>
0.59±0.07	1.3±0.54		HlyD family secretion protein	<b>Multidrug resistance efflux pump</b>
0.43±0.14	ND		toxin secretion, membrane fusion protein	<b>Multidrug resistance efflux pump</b>
0.3	1.3±0.78		transcriptional regulator, TetR family	<b>Transcriptional regulator</b>

# *mexF* deletion cannot grow in the presence of 5 $\mu\text{g/ml}$ chloramphenicol



# Tripartite efflux pump system

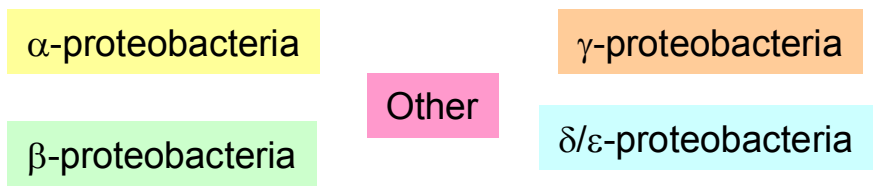




Other environmental bacteria proteins similar to MR-1 MexF from NCBI pblast; Gaps range from 0-3%

organism	size (aa)	identity	similarity
<i>B. japonicum</i> USDA 110	1062	600/1035 (57%)	745/1035 (71%)
<i>Magnetococcus</i> MC-1	1041	362/1058 (34%)	555/1058 (52%)
<i>M. magnetotacticum</i> MS-1	1059	461/1037 (44%)	642/1037 (61%)
<i>Mesorhizobium loti</i> MAFF303099	1067	475/1039 (45%)	637/1039 (61%)
	1061	415/1044 (39%)	608/1044 (58%)
	1055	380/1040 (36%)	584/1040 (56%)
<i>Rhodopseudomonas palustris</i> CGA009	1049	370/1035 (35%)	563/1035 (54%)
	1050	366/1036 (35%)	581/1036 (56%)
	1046	381/1048 (36%)	597/1048 (56%)
	1050	358/1044 (34%)	553/1044 (52%)
	1043	365/1045 (34%)	564/1045 (53%)
<i>Rhodospirillum rubrum</i>	1055	354/1041 (34%)	551/1041 (52%)
	1052	611/1041 (58%)	752/1041 (72%)
	1052	364/1051 (34%)	557/1051 (52%)
	1059	375/1044 (35%)	567/1044 (54%)
<i>Silicibacter pomeroyi</i> DSS-3	1050	376/1054 (35%)	576/1054 (54%)
	1042	411/1050 (39%)	620/1050 (59%)
<i>Sinorhizobium meliloti</i> 1021	1042	411/1050 (39%)	620/1050 (59%)
	1058	474/1042 (45%)	654/1042 (62%)
<i>Azoarcus</i> sp. EbN1	1058	402/1033 (38%)	611/1033 (59%)
	1048	382/1048 (36%)	570/1048 (54%)
	1077	399/1036 (38%)	592/1036 (57%)
	1053	379/1053 (35%)	585/1053 (55%)
<i>Dechloromonas aromatica</i> RCB	1062	592/1037 (57%)	734/1037 (70%)
	1040	406/1048 (38%)	601/1048 (57%)
<i>Methylobacillus flagellatus</i> KT	1062	589/1042 (56%)	729/1042 (69%)
	1037	409/1039 (39%)	598/1039 (57%)
	1037	358/1037 (34%)	547/1037 (52%)
<i>Nitrosomonas europaea</i> ATCC 19718	1048	492/1037 (47%)	665/1037 (64%)
	1064	400/1050 (38%)	597/1050 (56%)
<i>Polaromonas</i> JS666	1065	612/1025 (59%)	740/1025 (72%)
<i>Ralstonia eutropha</i> JMP134	1057	390/1046 (37%)	589/1046 (56%)
	1051	375/1045 (35%)	560/1045 (53%)
	1050	363/1053 (34%)	560/1053 (53%)
<i>Ralstonia metallidurans</i> CH34	1063	637/1049 (60%)	767/1049 (73%)
	1063	388/1046 (37%)	590/1046 (56%)
	1050	383/1045 (36%)	569/1045 (54%)
	1048	375/1054 (35%)	564/1054 (53%)
<i>R. solanacearum</i> GMI1000	1069	621/1042 (59%)	750/1042 (71%)
<i>Rubrivivax gelatinosus</i> PM1	1080	623/1036 (60%)	754/1036 (72%)
<i>T. denitrificans</i> ATCC 25259	1073	573/1054 (54%)	737/1054 (69%)
	1054	380/1049 (36%)	579/1049 (55%)

organism	size (aa)	identity	similarity
<i>Acinetobacter</i> ADP1	1059	348/1048 (33%)	559/1048 (53%)
<i>Idiomarina loihiensis</i> L2TR	1033	383/1035 (37%)	583/1035 (56%)
<i>M. capsulatus</i> str.Bath	1055	545/1042 (52%)	715/1042 (68%)
<i>Photobacterium profundum</i> SS9	1051	401/1055 (38%)	606/1055 (57%)
	1044	580/1040 (55%)	755/1040 (72%)
<i>Pseudomonas fluorescens</i> PfO-1	1032	379/1046 (36%)	579/1046 (55%)
	1043	370/1049 (35%)	564/1049 (53%)
	1054	367/1057 (34%)	558/1057 (52%)
<i>Psychrobacter</i> 273-4	1075	358/1039 (34%)	568/1039 (54%)
<i>S. oneidensis</i> MR-1	1044	375/1048 (35%)	562/1048 (53%)
<i>V. fischeri</i>	1037	398/1038 (38%)	597/1038 (57%)
<i>Xylella fastidiosa</i> 9a5c	1055	363/1042 (34%)	557/1042 (53%)
<i>Xylella fastidiosa</i> Ann-1	1055	361/1042 (34%)	562/1042 (53%)
<i>X. fastidiosa</i> Temecula1	1055	362/1042 (34%)	558/1042 (53%)
<i>D. psychrophila</i> LSv54	1047	370/1058 (34%)	566/1058 (53%)
<i>Desulfovibrio</i> strain G20	1055	386/1053 (36%)	583/1053 (55%)
<i>D. vulgaris</i> Hildenborough	1046	426/1045 (40%)	616/1045 (58%)
	1047	412/1037 (39%)	605/1037 (58%)
<i>Geobacter metallireducens</i> GS-15	1070	371/1044 (35%)	563/1044 (53%)
	1044	392/1054 (37%)	606/1054 (57%)
	1053	366/1057 (34%)	566/1057 (53%)
<i>G. sulfurreducens</i> PCA	1052	371/1056 (35%)	571/1056 (54%)
<i>Wolinella succinogenes</i> DSM 1740	1030	412/1031 (39%)	593/1031 (57%)
	1044	362/1034 (35%)	67/1034 (54%)
uncultured <i>Acidobacteria</i>	1040	385/1035 (37%)	572/1035 (55%)
<i>Chlorobium tepidum</i> TLS	1063	354/1032 (34%)	574/1032 (55%)
<i>Bacteroides thetaiotaomicron</i> VPI-5482	1048	406/1042 (38%)	590/1042 (56%)
	1034	383/1042 (36%)	578/1042 (55%)
<i>Rhodopirellula baltica</i> SH 1	1072	458/1039 (44%)	662/1039 (63%)
	1037	431/1035 (41%)	612/1035 (59%)
<i>Anabaena variabilis</i>	1057	410/1040 (39%)	603/1040 (57%)
<i>C. watsonii</i> WH8501	1041	363/1032 (35%)	578/1032 (56%)
<i>G. violaceus</i> PCC 7421	1050	402/1038 (38%)	576/1038 (55%)



# What have we learned from STM

- Identified a number of genes and cell functions that are required for life in the real world but not needed for growth in culture.
- Demonstrated for the first time the importance of specific genes as cells are growing in natural environments.
- Raised many questions regarding function of specific genes. Regulatory genes. Protection from toxins. DNA repair, etc.

# Acknowledgments

- Funding from DOE ERSP program.
- Qingwei Luo, Jennifer Groh, Xiangkai Li and Nydia Castaneda.
- EMSL