Horizontal Gene Transfer(!!) in the Contaminated Subsurface

Patricia Sobecky
School of Biology
Georgia Institute of Technology
Atlanta, Georgia

Prokaryotic Prevalence & Distribution

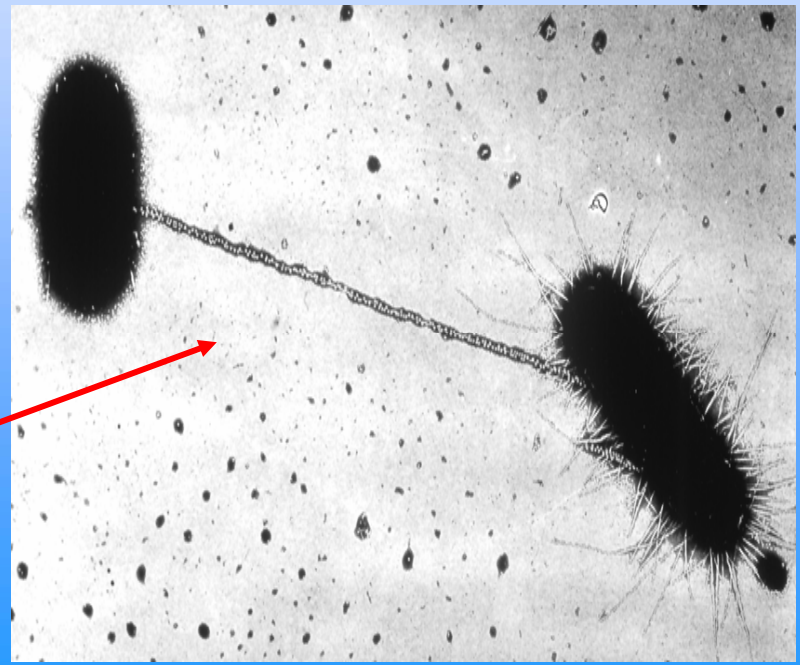
Habitat	Population Size
Marine heterotrophs	
Above 200m	3.6×10^{28}
Below 200m	8.2×10^{28}
Marine autotrophs	2.9×10^{27}
Soil	2.6×10^{29}
Oceanic subsurface	3.6×10^{30}
Terrestrial subsurface	1.4×10^{30}
Domestic mammals	4.3×10^{24}

adapted from Whitman *et al.*, *PNAS* 95:6578-6583

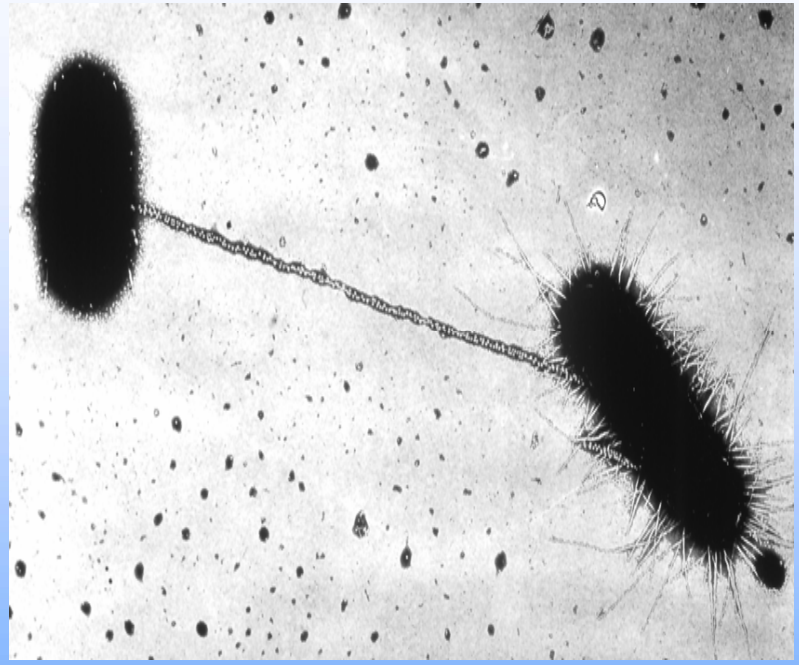
Horizontal Gene Pool

….comprised of mobile genetic elements (MGEs) including plasmids, bacteriophages, IS elements, transposons, integrons, other mobile genetic elements and their genes…...

Broad-Host-range Transfers: Plasmid-mediated conjugation (and phage-mediated transduction) occurs between related and (*highly*) unrelated microbes!



Lack knowledge on…



- Extent of the evolution, diversity & persistence of plasmid populations in aquatic (e.g., marine, freshwater) and terrestrial (e.g., subsurface, deep subsurface) habitats

- Extent of host ranges of phages and plasmids (especially those promoting broad-host-range)

- Nature of genetic information present in the HGP

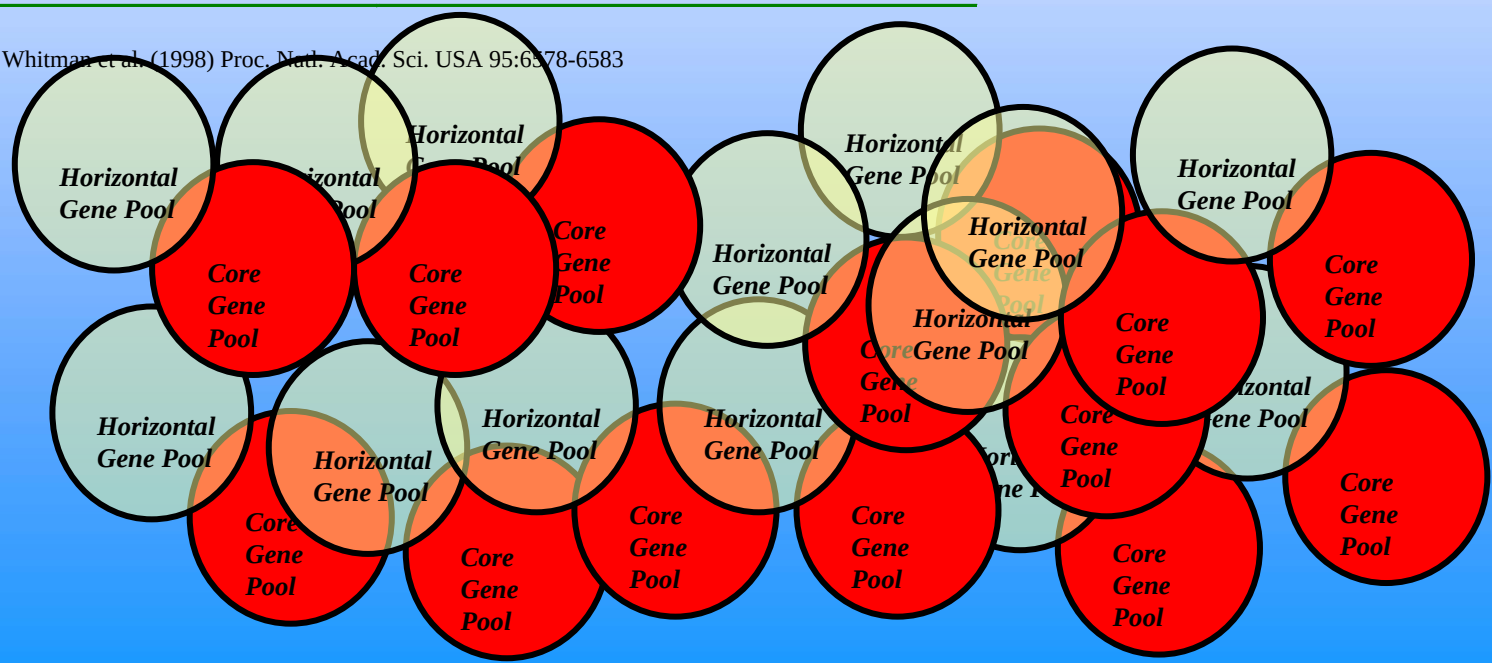
- Nature and movement (flow) of traits within & between environmental niches (temporal, spatial) and ecosystems

- (Molecular) triggers promoting conjugative plasmid transfer (e.g., pheromones/pAD1, opines/Ti); expression of plasmid-encoded virulence genes

Habitat	Population Size
Marine heterotrophs	
Above 200m	3.6×10^{28}
Below 200m	8.2×10^{28}
Marine autotrophs	2.9×10^{27}
Soil	2.6×10^{29}
Oceanic subsurface	3.6×10^{30}
Terrestrial subsurface	1.4×10^{30}
Domestic mammals	4.3×10^{24}

Table adapted from Whitman et al. (1998) Proc. Natl. Acad. Sci. USA 95:6578-6583

Vast potential for genetic innovation and plasmid diversity



What ‘biome’ factors are critical (or minimally required) for HGT?

- Presence/Abundance of Mobile elements
- Diversity and Distribution of “Mobilome” (and hosts)
- Metabolic activity (individual hosts, community)
- Selection/adaptation
- Extreme conditions?

So are we searching for HGT in all the right places? All the right ways? All the right scales? Retrospective methods *vs* real-time??




Gene transfer in an acidic contaminated subsurface site

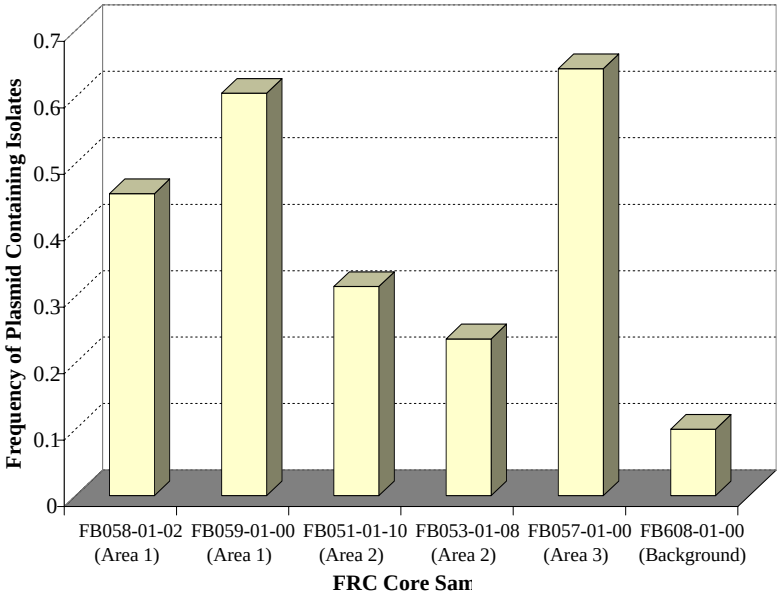
Former waste ponds at Oak Ridge, TN represent an *extreme environment*; radionuclide & heavy metal contamination, low pH (3-4), high $N0_3^{2-}$ (> 100 mM)

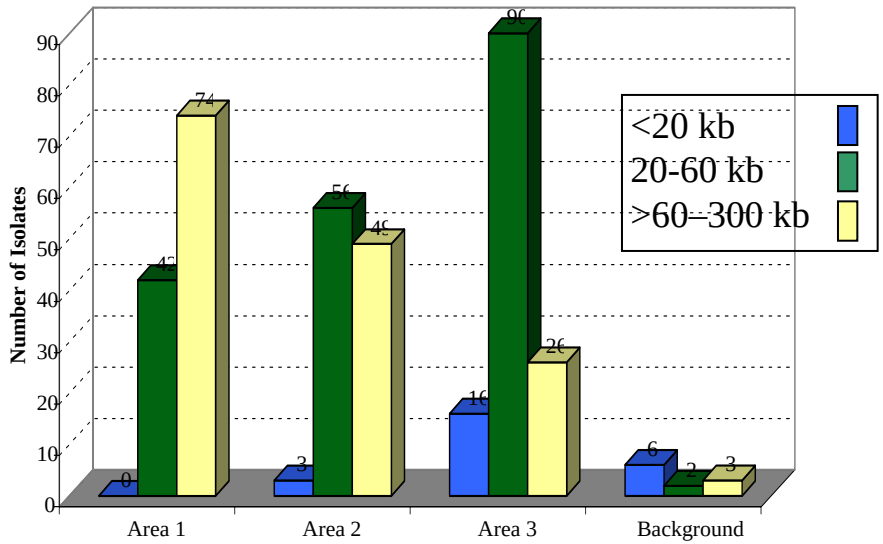
% Gram positive subsurface isolates resistant to metals

Metal	Oak Ridge FRC	Hanford[a]	Savannah River Site[a]
Cd(II)	20	nd[b]	nd
Cr(VI)	17	74	64
Hg(II)	17	1	3
Pb(II)	56	57	39

[a] data derived from Benyehuda et al. (2003)

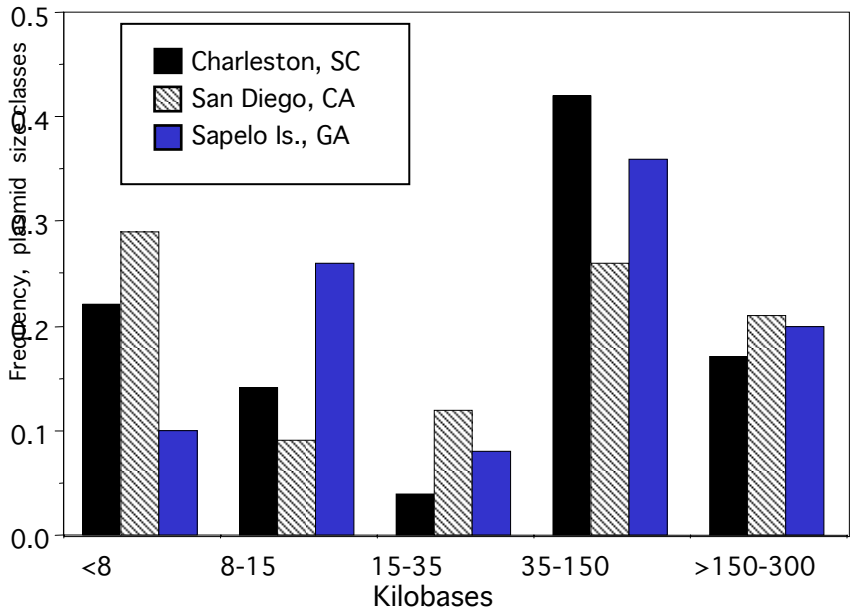
[b] not determined





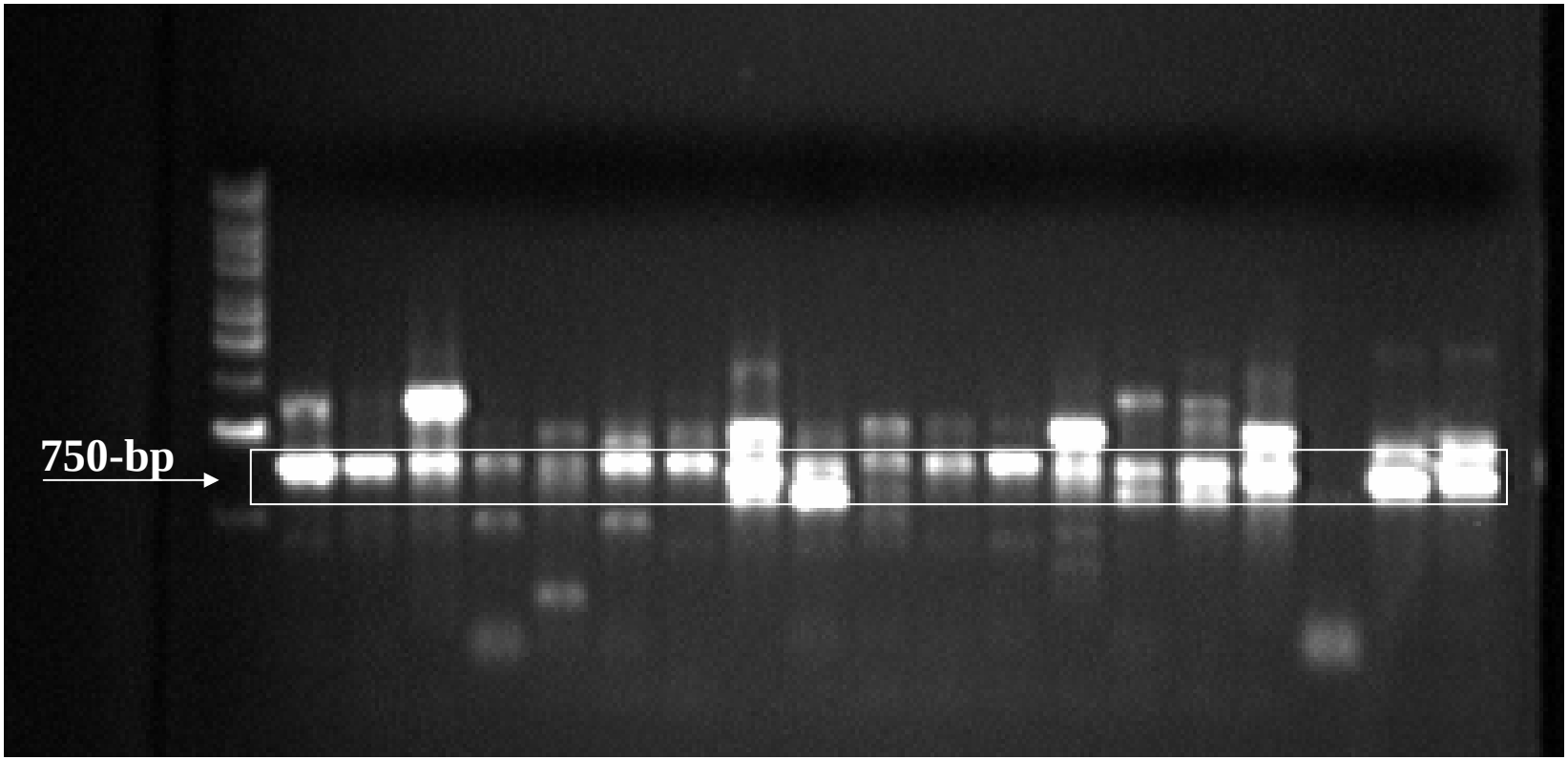
25-60% of FRC isolates contained plasmids; (single replicons)

FRC size classes > 60kb; (lacked bi-modality typical of marine plasmid populations)



ATPase (*zntA*/*cadA*/*pbrA* loci)
Nested PCR

50 Pb^r isolates tested; 26 ATPase loci obtained



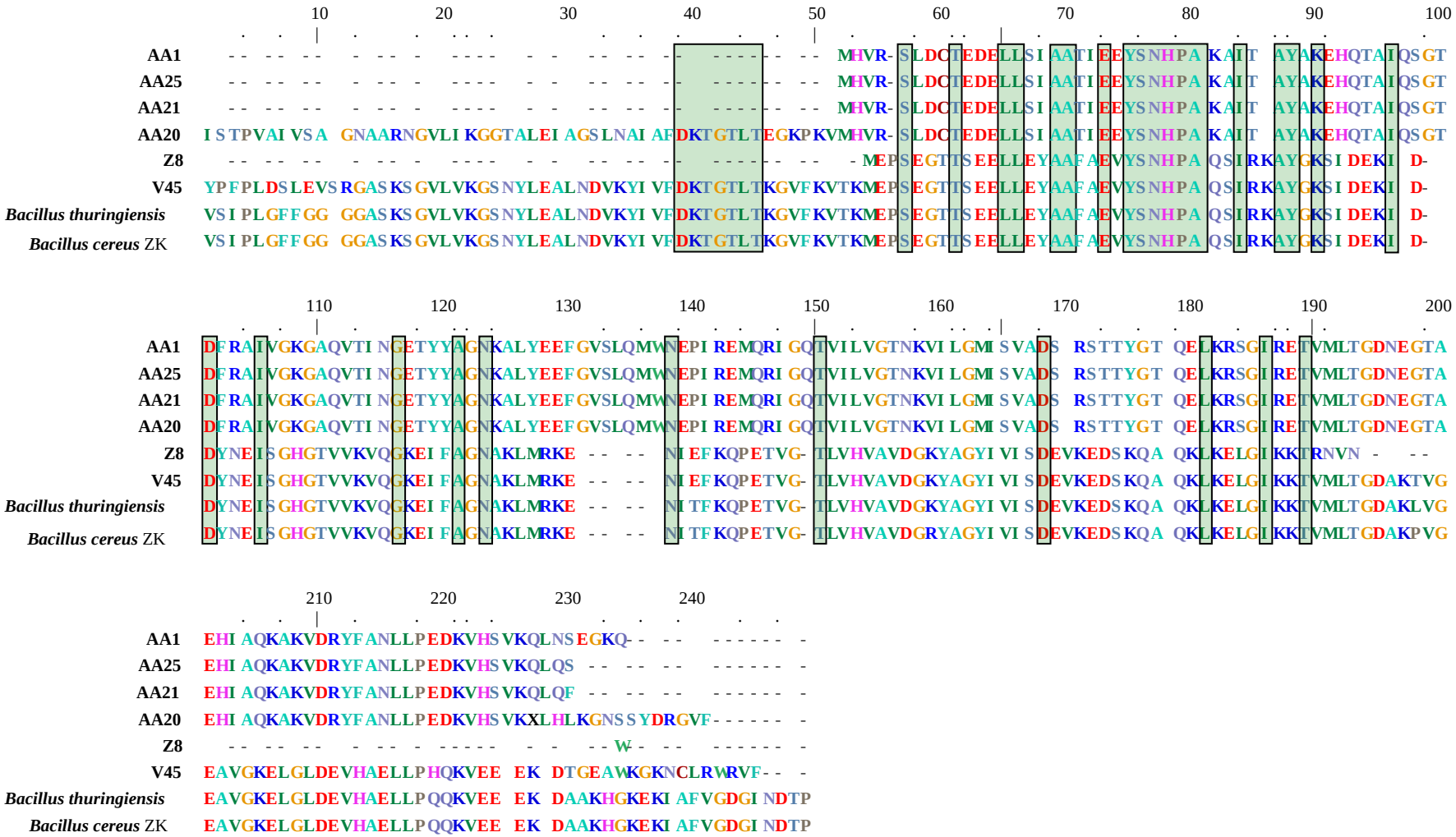
P-type ATPases: **metal homeostasis “stressor genes”; confer resistance to heavy metals by enzymatic detoxification and efflux pumping**

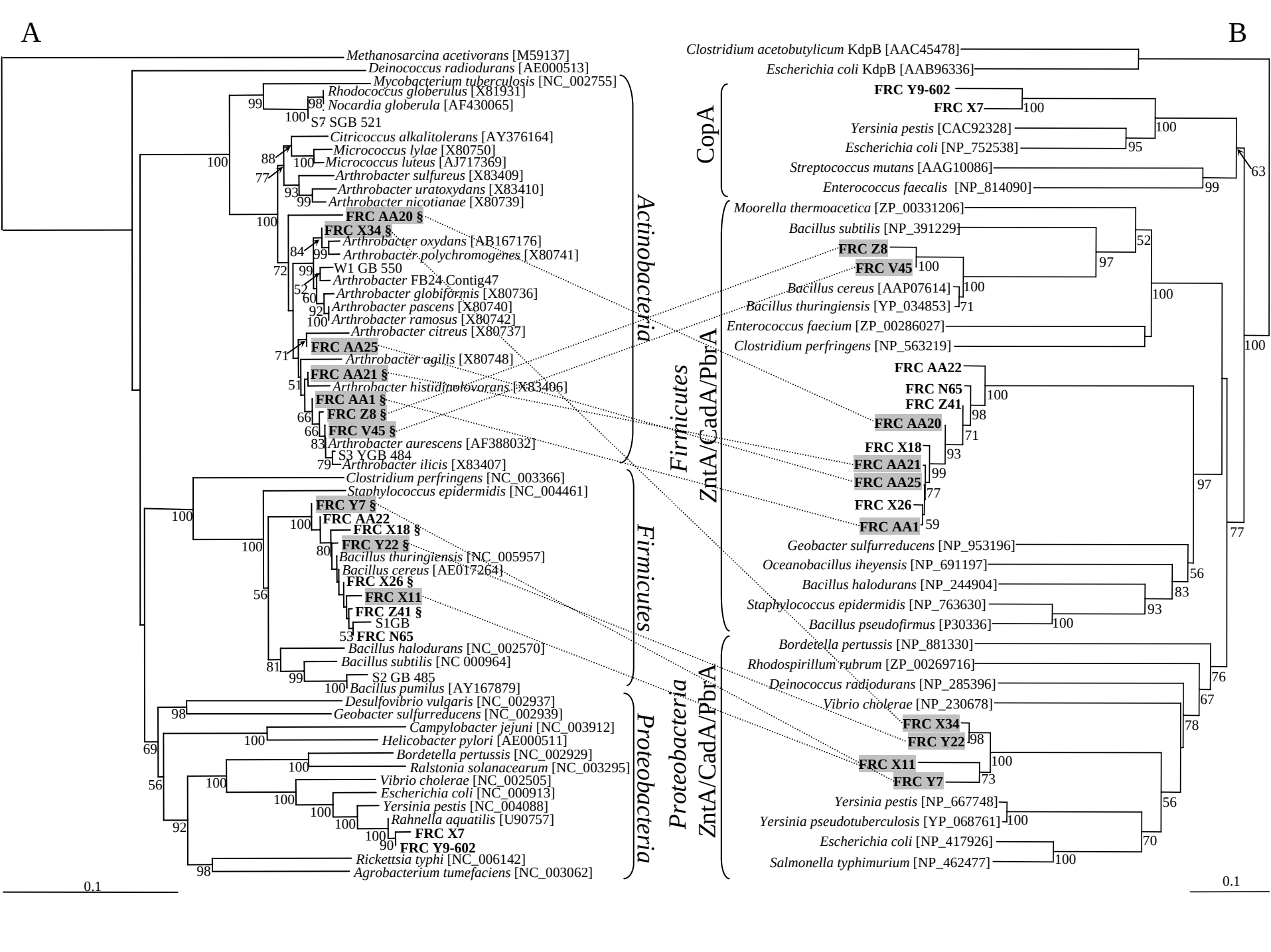
Oligonucleotide primers used during nested PCR reactions to obtain P_{IB}-type ATPase and number of isolates which yielded amplicons

PCR Reaction	Primer set[c]	Primer sequence	Annealing temperature (C)	No. Pb^r isolates yielded PCR product
1[a]	79JC	5' TGACTGGCGAATCGGTBCCBG 3'	59	8
	84JC	5' GGAGCATCGTTAATDCCRTCDCC 3'		
	132JC	5' CTAACTGGCGAATCAGTCCC 3'	55	25
	84JC	5' GGAGCATCGTTAATDCCRTCDCC 3'		
2[b]	81JC	5' GGATGTCCTTGTGCTYTART 3'	49	5
	84JC	5' GGAGCATCGTTAATDCCRTCDCC 3'		
	133JC	5' CCCTCACCTTGTGCYCTGG 3'	49	23
	84JC	5' GGAGCATCGTTAATDCCRTCDCC 3'		

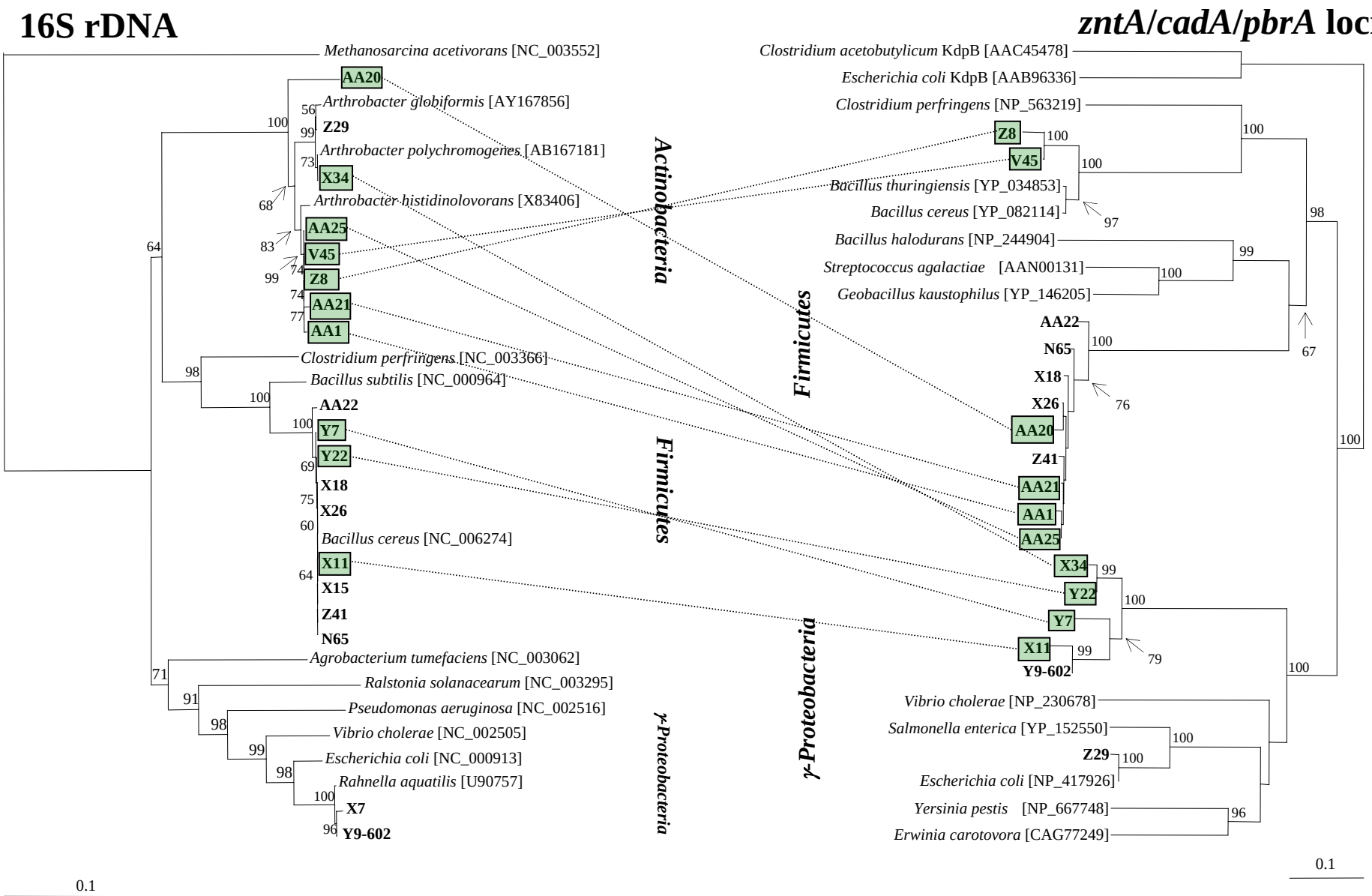
[a] expected product size, approximately 1.2 kb
[b] expected product size, approximately 0.75 kb
[c] See Coombs and Barkay (8) for specific thermocycling parameters

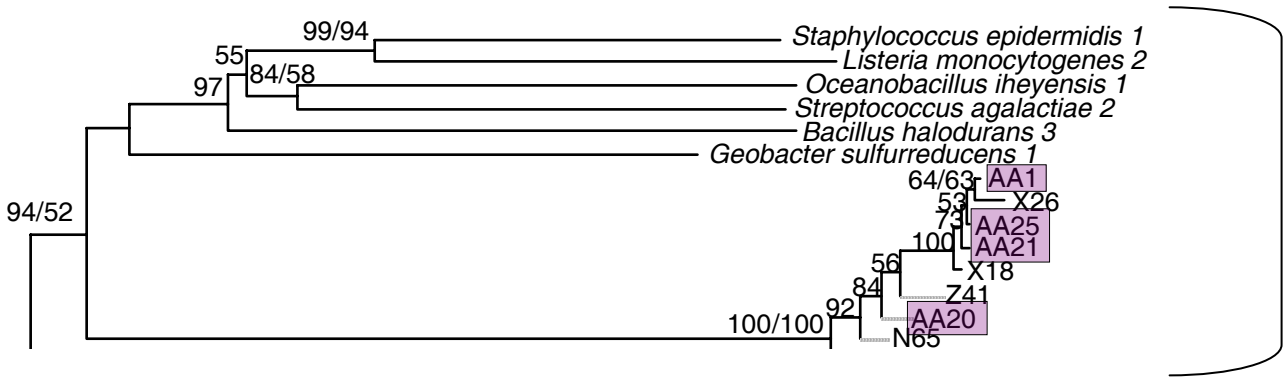
Arthrobacter sp. P_{IB}-type ATPase alignment




A
B
Methanosarcina acetivorans [M59137]
Deinococcus radiodurans [AE000513]
Mycobacterium tuberculosis [NC_002755]
Rhodococcus globerulus [X81931]
Nocardia globerula [AF430065]
S7 SGB 521
Citricoccus alkalitolerans [AY376164]
Micrococcus lylae [X80750]
Micrococcus luteus [AJ717369]
Arthrobacter sulfureus [X83409]
Arthrobacter uratoxydans [X83410]
Arthrobacter nicotianae [X80739]
FRC AA20 §
FRC X34 §
Arthrobacter oxydans [AB167176]
Arthrobacter polychromogenes [X80741]
W1 GB 550
Arthrobacter FB24 Contig47
Arthrobacter globiformis [X80736]
Arthrobacter pascens [X80740]
Arthrobacter ramosus [X80742]
Arthrobacter citreus [X80737]
FRC AA25
Arthrobacter agilis [X80748]
FRC AA21 §
Arthrobacter histidinolovorans [X83406]
FRC AA1 §
FRC Z8 §
FRC V45 §
Arthrobacter aurescens [AF388032]
S3 YGB 484
Arthrobacter ilicis [X83407]
Actinobacteria
Clostridium perfringens [NC_003366]
Staphylococcus epidermidis [NC_004461]
FRC Y7 §
FRC AA22
FRC X18 §
FRC Y22 §
Bacillus thuringiensis [NC_005957]
Bacillus cereus [AE017264]
FRC X26 §
FRC X11
FRC Z41 §
S1GB
FRC N65
Bacillus halodurans [NC_002570]
Bacillus subtilis [NC 000964]
S2 GB 485
Bacillus pumilus [AY167879]
Firmicutes
Desulfovibrio vulgaris [NC_002937]
Geobacter sulfurreducens [NC_002939]
Campylobacter jejuni [NC_003912]
Helicobacter pylori [AE000511]
Bordetella pertussis [NC_002929]
Ralstonia solanacearum [NC_003295]
Vibrio cholerae [NC_002505]
Escherichia coli [NC_000913]
Yersinia pestis [NC_004088]
Rahnella aquatilis [U90757]
FRC X7
FRC Y9-602
Rickettsia typhi [NC_006142]
Agrobacterium tumefaciens [NC_003062]
Proteobacteria
0.1
Clostridium acetobutylicum KdpB [AAC45478]
Escherichia coli KdpB [AAB96336]
CopA
FRC Y9-602
FRC X7
Yersinia pestis [CAC92328]
Escherichia coli [NP_752538]
Streptococcus mutans [AAG10086]
Enterococcus faecalis [NP_814090]
Moorella thermoacetica [ZP_00331206]
Bacillus subtilis [NP_391229]
FRC Z8
FRC V45
Bacillus cereus [AAP07614]
Bacillus thuringiensis [YP_034853]
Enterococcus faecium [ZP_00286027]
Clostridium perfringens [NP_563219]
ZntA/CadA/PbrA
Firmicutes
FRC AA22
FRC N65
FRC Z41
FRC AA20
FRC X18
FRC AA21
FRC AA25
FRC X26
FRC AA1
Geobacter sulfurreducens [NP_953196]
Oceanobacillus iheyensis [NP_691197]
Bacillus halodurans [NP_244904]
Staphylococcus epidermidis [NP_763630]
Bacillus pseudofirmus [P30336]
Bordetella pertussis [NP_881330]
Rhodospirillum rubrum [ZP_00269716]
Deinococcus radiodurans [NP_285396]
Vibrio cholerae [NP_230678]
FRC X34
FRC Y22
FRC X11
FRC Y7
Yersinia pestis [NP_667748]
Yersinia pseudotuberculosis [YP_068761]
Escherichia coli [NP_417926]
Salmonella typhimurium [NP_462477]
ZntA/CadA/PbrA
Proteobacteria
0.1

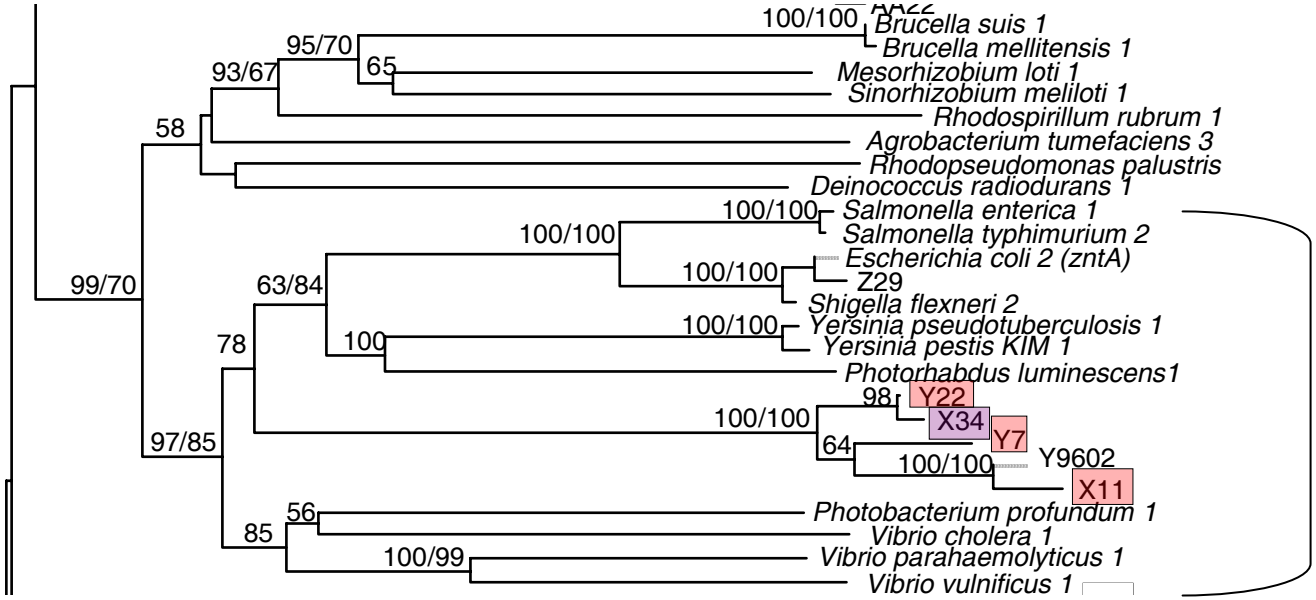
Subsurface Bacteria 16S rDNA and ATPase Phylogeny





Subsurface (host) strain	Nearest Relative (16S rRNA)	% Identity	ATPase G+C Content
Arthrobacter sp. AA1	*Arthrobacter* sp. RG-56	99	38%
Arthrobacter sp. AA20	*Arthrobacter* sp. AD-1	91	38%
Arthrobacter sp. AA21	*Arthrobacter* sp. RG-56	99	39%
Arthrobacter sp. AA25	*Arthrobacter* sp. RG-56	100	38%

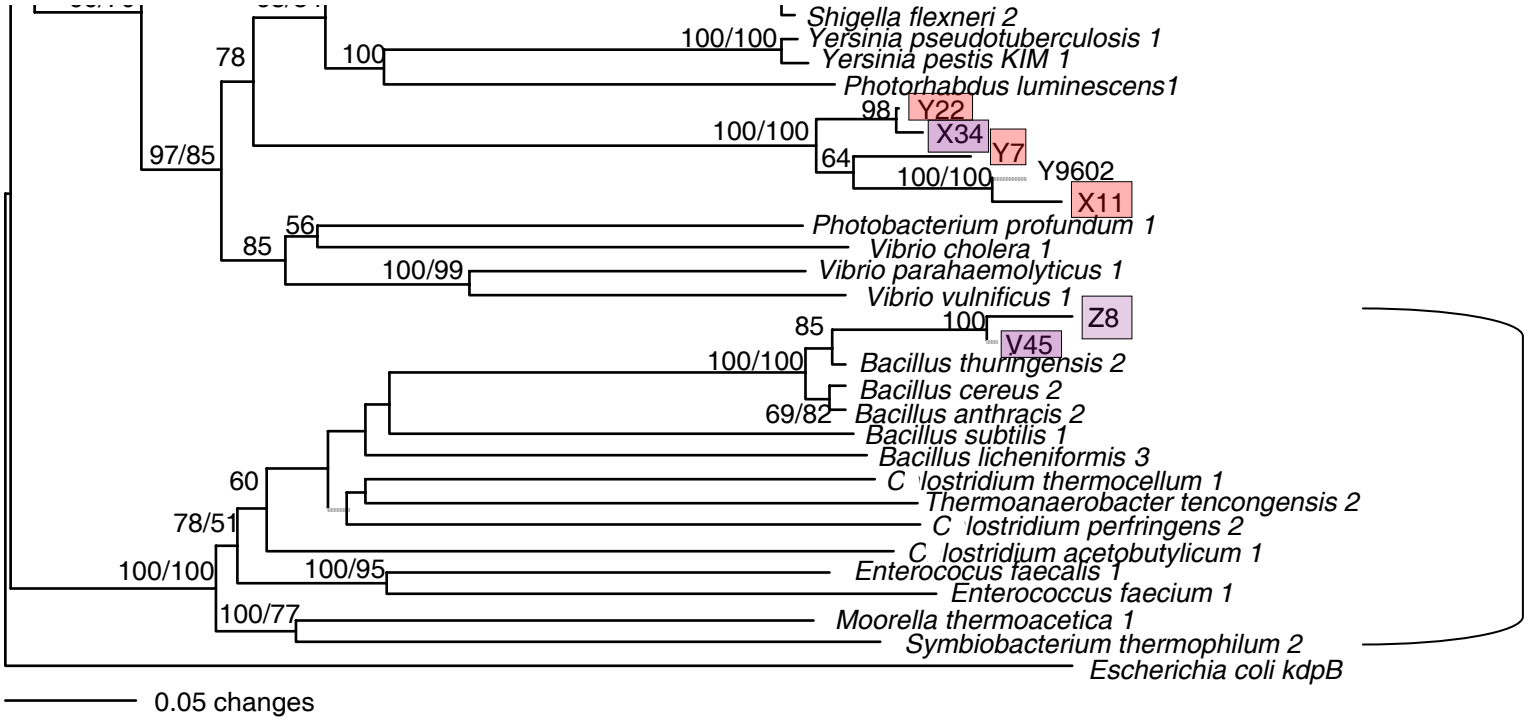
Example 1: HGT of P_{IB}-type ATPase loci among *Actinobacteria* hosts (high G+C) and *Firmicutes* (low G+C)



γ-*Proteobacteria*

Subsurface (host) strain	Nearest Relative (16S rRNA)	% Identity	ATPase G+C Content
Arthrobacter sp. X34	*Arthrobacter* sp. M4	100	41.5%
Bacillus sp. Y7	*Bacillus* sp. 12	99	59.2%
Bacillus sp. Y22	*Bacillus* sp. 12	97	58.3%
Bacillus sp. X11	*Bacillus* sp. 12	99	58.6%

Example 2: HGT of P_{IB}-type ATPase loci among *Actinobacteria* and *Firmicutes* hosts and γ-*Proteobacteria*



Subsurface (host) strain	Nearest Relative (16S rRNA)	% Identity	ATPase G+C Content
Arthrobacter sp. V45	*Arthrobacter* sp. RG-56	100	35.6%
Arthrobacter sp. Z8	*Arthrobacter* sp. RG-56	99	35.4%

Example 3: HGT of P_{IB}-type ATPase loci among *Actinobacteria* hosts and *Firmicutes*

Support for acquisition of P_{IB}-type ATPases by HGT in subsurface isolates from radionuclide and metal contaminated soils

Genus	Strain designation	G+C content (%)	Phylogenetic incongruency	Support for HGT	P_{IB}-type ATPase most closely related to:
Arthrobacter	FRC-AA1	38	+	+	*Firmicutes*
	FRC-AA20	38	+	+	*Firmicutes*
	FRC-AA21	38	+	+	*Firmicutes*
	FRC-AA25	38	+	+	*Firmicutes*
	FRC-V45	36	+	+	*Firmicutes*
	FRC-X34	59	+	**Maybe**	***γ-Proteobacteria***
	FRC-Z8	35	+	+	*Firmicutes*
Bacillus	FRC-AA22	38	-	-	*Firmicutes*
	FRC-N65	38	-	-	*Firmicutes*
	FRC-X11	59	+	+	*γ-Proteobacteria*
	FRC-X18	38	-	-	*Firmicutes*
	FRC-X26	38	-	-	*Firmicutes*
	FRC-Y7	58	+	+	*γ-Proteobacteria*
	FRC-Y22	58	+	+	*γ-Proteobacteria*
	FRC-Z41	38	-	-	*Firmicutes*
Rahnella	FRC-X7	59	-	-	*γ-Proteobacteria*
	FRC-Y9602	59	-	-	*γ-Proteobacteria*

Viable cell counts as determined after washing, 1 h incubation pH 4 either with or without 200 µM uranyl acetate.
Note: At pH 4 U(VI) occurs primarily as uranyl ion (UO_2^{2+}).

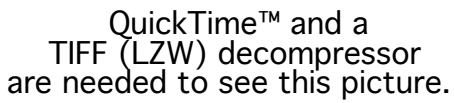
Genus	Strain Designation	CFU [a] (washed)	CFU [b] (without 200 µM U)	CFU (with 200 µM U)
Arthrobacter	**FRC-AA1**	**$1.79 \cdot 10^8 \pm 0.24$ [c]**	**$1.59 \cdot 10^8 \pm 0.25$**	**$1.45 \cdot 10^8 \pm 0.24$**
	FRC-AA21	**$1.53 \cdot 10^8 \pm 0.18$**	**$7.78 \cdot 10^7$**	**$6.45 \cdot 10^7 \pm 0.19$**
	FRC-AA25	**$2.31 \cdot 10^8$**	**$1.39 \cdot 10^8$**	**$9.20 \cdot 10^7 \pm 0.24$**
	FRC-V45	**$1.67 \cdot 10^8 \pm 0.20$**	**$9.13 \cdot 10^7$**	**$6.85 \cdot 10^7$**
	FRC-X34	**$1.83 \cdot 10^8$**	**$1.71 \cdot 10^8$**	**$1.07 \cdot 10^8$**
	S3	$5.10 \cdot 10^9$	$7.25 \cdot 10^9$	$6.45 \cdot 10^7$
	W1	$1.98 \cdot 10^{10}$	$1.98 \cdot 10^{10} \pm 0.24$	$2.17 \cdot 10^{10}$
	A. histidinolovorans ATCC 11442	$1.57 \cdot 10^8$	$6.10 \cdot 10^7 \pm 0.25$	$4.35 \cdot 10^7 \pm 0.51$
Rhodococcus	S7	$1.92 \cdot 10^9 \pm 0.24$ [d]	$2.28 \cdot 10^9 \pm 0.34$	$1.43 \cdot 10^9$
Bacillus	**FRC-N65**	**$2.99 \cdot 10^8$**	**$1.12 \cdot 10^8 \pm 0.19$**	**$<1 \cdot 10^4$**
	FRC-X18	**$1.87 \cdot 10^8 \pm 0.25$**	**$3.51 \cdot 10^7 \pm 0.57$**	**$4.03 \cdot 10^5$**
	FRC-Y9-2	**$2.55 \cdot 10^7 \pm 0.32$**	**$3.12 \cdot 10^6 \pm 0.53$**	**$<1 \cdot 10^4$**
	S1	$4.56 \cdot 10^8$	$<3 \cdot 10^6$	$<3 \cdot 10^6$
	S2	$1.54 \cdot 10^8 \pm 0.25$	$<3 \cdot 10^6$	$<3 \cdot 10^6$
	B. cereus ATCC 14579	$9.70 \cdot 10^7 \pm 0.26$	$<1 \cdot 10^4$	$<1 \cdot 10^4$
Escherichia	*E. coli* JM109	$8.00 \cdot 10^7 \pm 0.19$	$4.65 \cdot 10^7 \pm 0.15$	$<1 \cdot 10^4$
Rahnella	**FRC-Y9-602**	**$1.10 \cdot 10^8$**	**$5.40 \cdot 10^7 \pm 0.20$**	**$1.54 \cdot 10^6 \pm 0.46$**

[a] CFU-colony forming unit. All strains were plated immediately following a 2x 0.1M NaCl wash at pH 4
[b] strains were incubated 1 h at pH 4
[c] standard error is indicate only for those FRC isolates greater than 10%
[d] standard deviation is indicated for isolates greater than 10% as previously indicated by Suzuki and Banfield (56)

Some hypotheses on HGT "drivers" at FRC

Stresses endemic to the FRC (e.g., heavy metals, radionuclides, nitrates) "require" microbial innovation and adaptation and HGT provides a rapid and effective way to survive and adapt to these stresses

It's the Guar Gum!!



Guar gum is a polysacharide composed of galactose and mannose. Present in Area 2 (site we happen to detect the most HGT of ATPase genes….).

So over the years the FRC treatments and activities that promote microbial stimulation(s) are providing the large-scale HGT experiments….wonder what HGT events the Criddle, Istok and Schiebe experiments are promoting???

***misc factoids*: Guar gum retards ice crystal growth non-specifically by slowing mass transfer across solid/liquid interface.**

What ‘biome’ factors are critical (or minimally required) for HGT?

- Presence/Abundance of Mobile elements
- Diversity and Distribution of “Mobilome” (and hosts)
- Metabolic activity (individual hosts, community)
- Selection/adaptation
- Extreme conditions

So are we searching for HGT in all the right places? All the right ways? All the right scales? Retrospective methods *vs* real-time??




Acknowledgments

Rob Martinez

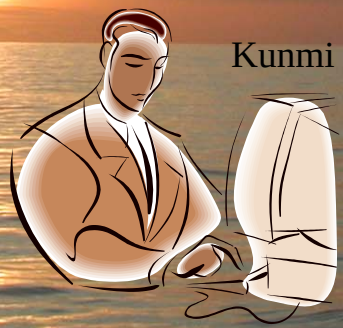
Stacy Shinneman

Mike Humphrys

Melanie Raimondo

Tracy Hazen

Yanling Wang



Kunmi Ayanbule

and Danielle Kennedy

Collaborators: Tamar Barkay, Jonna Coombs & special thanks to Dave Watson

Funding: Department of Energy NABIR