Supplementary Material

Taxonomically-linked growth phenotypes during arsenic stress among arsenic resistant bacteria isolated from soils overlying the Centralia coal seam fire

Taylor K Dunivin,[1,2] Justine Miller,[3] Ashley Shade[1,4*]

* Correspondence: Ashley Shade: shadeash@msu.edu

1 Supplementary Tables and Figures

1.1 Supplementary Tables

Supplementary Table 1. Phenotypes of arsenic resistant isolates.

Air temperature (°C)	Soil temperature (°C)	Organic matter (360°C)	Organic matter (500°C)	NO_3^- (ppm)	NO_4^+ (ppm)	pH	S (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)	Fe (ppm)	As (ppm)
13.3	57.4	3.1	7.1	4.6	1.7	8	28	37	2545	114	67.1	2.58

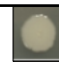
Supplementary Table 2. Degenerate primers used for end point PCR.

Gene	Primer Sequence (5'-3')	Name	Source
16S	AGAGTTTGATCCTGGCTCAG	Uni-27F	Weisburg *et al*., 1991
16S	GGTTACCTTGTTACGACTT	Uni-1492R	Weisburg *et al*., 1991
16S	GTGCCAGCMGCCGCGGTAA	U515F	Baker *et al*., 2003
arsB	GGTGTGGAACATCGTCTGGAAYGCNAC	darsB1F	Achour *et al*., 2007
arsB	CAGGCCGTACACCACCAGRTACATNCC	darsB1R	Achour *et al*., 2007
ACR3(1)	GCCATCGGCCTGATCGTNATGATGTAYCC	dacr1F	Achour *et al*., 2007
ACR3(1)	CGGCG ATGGCCAGCTCYAAYTTYTT	dacr1R	Achour *et al*., 2007
ACR3(2)	TGA TCTGGGTCATGATCTTCCCVATGMTGVT	dacr5F	Achour *et al*., 2007
ACR3(2)	CGGCCACG GCCAGYTCRAARAARTT	dacr4R	Achour *et al*., 2007
arsC	TCGCGTAATACGCTGGAGAT	amlt-42-f	Sun *et al*., 2004
arsC	ACTTTCTCGCCGTCTTCCTT	amlt-376-r	Sun *et al*., 2004
arsC	TCACGCAATACCCTTGAAATGATC	smrc-42-f	Sun *et al*., 2004
arsC	ACCTTTTCACCGTCCTCTTTCGT	smrc-376-r	Sun *et al*., 2004
arsC	AGCCAAATGGCAGAAGC	P52F	Cavalca, *et al*., 2010
arsC	GCTGGRTCRTCAAATCCCCA	P323R	Cavalca, *et al*., 2010
arrA	CGAAGTTCGTCCCGATHACNTGG	AS1F	Song *et al*., 2009
arrA	GGGGTGCGGTCYTTNARYTC	AS1R	Song *et al*., 2009
arrA	GTCCCNATBASNTGGGANRARGCNMT	AS2F	Song *et al*., 2009
arrA	ATANGCCCARTGNCCYTGNG	AS2R	Song *et al*., 2009
aioA	CCACTTCTGCATCGTGGGNTGYGGNTA	aoxBM1-2F	Quemeneur *et al*., 2008
aioA	TGTCGTTGCCCCAGATGADNCCYTTYTC	aoxBM3-2R	Quemeneur *et al*., 2008
arsM	TCYCTCGGCTGCGGCAAYCCVAC	arsMF1	Jia *et al*., 2013
arsM	GTGCTCGAYCTSGGCWCCGGC	arsMF2	Jia *et al*., 2013
arsM	GGCATCGACGTGCTKCTBTCSGC	arsMF3	Jia *et al*., 2013
arsM	AGGTTGATGACRCAGTTWGAGAT	arsMR1	Jia *et al*., 2013
arsM	CGWCCGCCWGGCTTWAGYACCCG	arsMR2	Jia *et al*., 2013
arsM	GCGCCGGCRAWGCAGCCWACCCA	arsMR3	Jia *et al*., 2013

Supplementary Table 3. Isolates with short *arsC* sequences (<200 bp).

Isolate	***arsC* sequence**
A2707	cgatgctgatttagtctgttacgcttttgtggccatgcggatgctgtttgtccgtctactccgccgcatgtgaatcgagttcactggggatttgacgacccagcaa
A2723	gctggatttagtctgtnacnctttgtggtcacgcagatgctgtctgtccnncaacacctccgcacgtgaancgagttcactggggatttgacgacccagcaa
A2733	caatgaanatcnggatggtttgattccgttatgatcgatgtctacttcgtttcattgctttaattgcnttcggattcactccgtgtgcctcnatacccgcagaaantac
A2735	cgatgctgatttagtctgttacgctttgtggtcacgcagatgctgtctgtccnncaacacctcctcacgtgaancgagttcactggggatttgacgacccagcaaa

Supplementary Table 4. Phenotypes of arsenic resistant isolates.

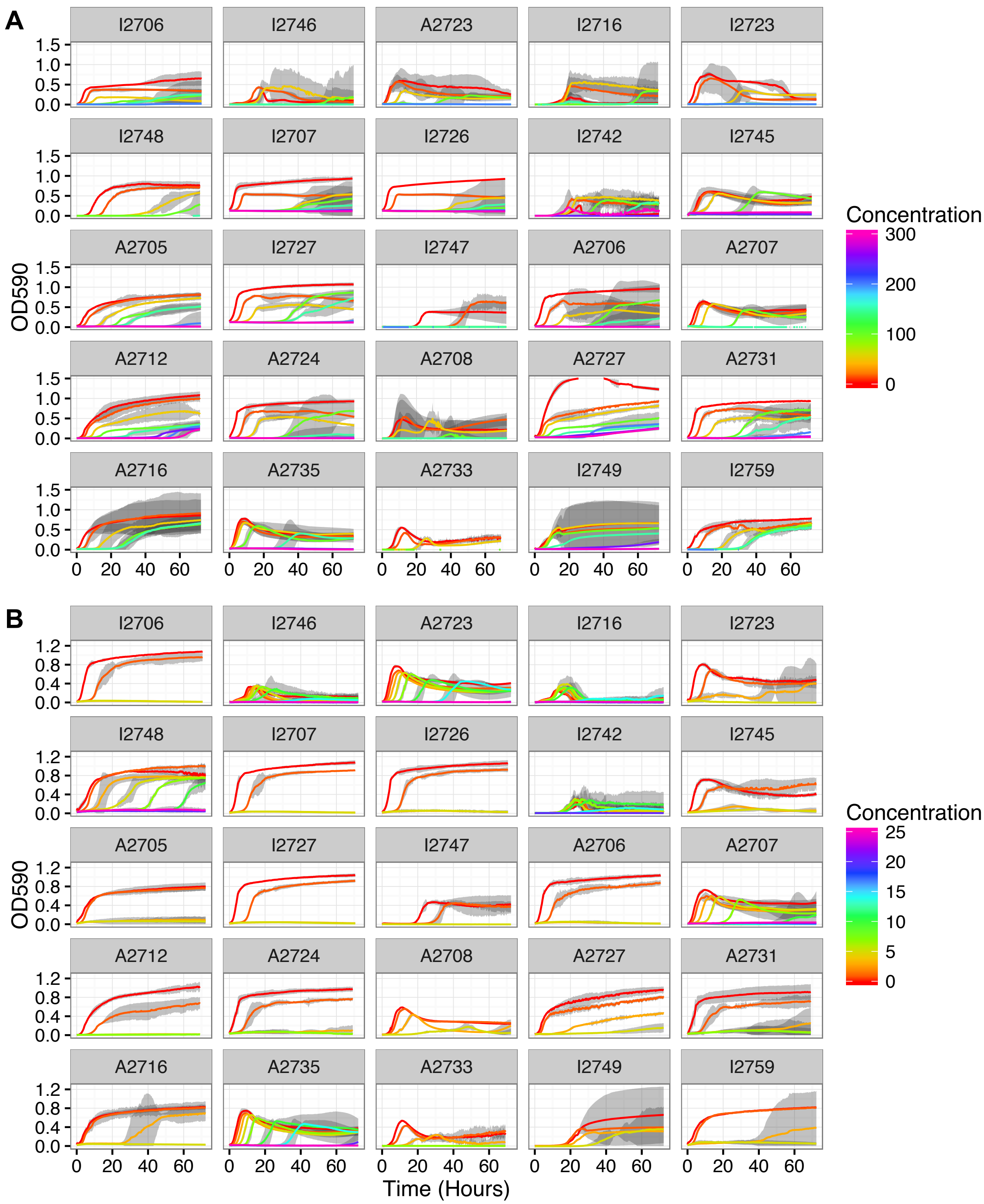
Isolate	Closest 16S rRNA gene sequence described (% similarity)	Colony Morphology	Temperature Maximum (°C)	Length (μm)	Width (μm)
I2706	*Enterobacter absuriae* JM 6051 (99.43%)		44.3	1.43	1.21
I2707	*Enterobacter absuriae* JM 6051 (99.35%)		44.3	1.34	1.16
I2716	*Bacillus nealsonii* DSM 150-7577 (99.49%)		44.3	4.63	1.16
I2723	*Bacillus anthrasis* ATCC 14578 (100%)		44.3	4.46	1.36
I2726	*Enterobacter absuriae* JM 6051 (99.5%)		44.3	2.37	1.45
I2727	*Enterobacter absuriae* JM 6051 (99.56%)		44.3	2.89	1.05
I2742	*Bacillus nealsonii* DSM 150-7577 (99.49%)		44.3	2.60	0.85
I2745	*Bacillus anthrasis* ATCC 14578 (99.86%)		44.3	4.11	0.90
I2746	*Paenibacillus xylanilytius* XIL14 (98.63%)		44.3	1.68	1.46
I2747	*Paenibacillus xylanilytius* XIL14 (98.58%)		39.7	3.96	1.12
I2748	*Mirobaterium paraoxydans* F36 (99.85%)		47.7	1.19	1.14
I2749	*Olivibater oleidegrans* TBF2/20.2 (99.42%)		44.3	1.58	1.23
I2759	*Acinetobacter baumanii* ATCC 19606 (99.78%)		44.3	1.20	1.16
A2705	*Acinetobacter baumanii* ATCC 19606 (99.64%)		44.3	1.25	1.19
A2706	*Enterobacter absuriae* JM 6051 (99.50%)		44.3	2.07	1.10
A2707	*Bacillus anthrasis* ATCC 14578 (100%)		44.3	3.83	0.91
A2708	*Bacillus subtilis subsp. inoquosorum* KT 13429 (99.93%)		52.0	3.26	0.90
A2712	*Pseudomonas hibisicola* ATCC 19867 (99.36%)		39.7	2.01	1.01
A2716	*Acinetobacter baumanii* ATCC 19606 (99.78%)		44.3	1.12	0.96
A2723	*Bacillus anthrasis* ATCC 14578 (99.73%)		44.3	3.20	1.64
A2724	*Enterobacter absuriae* JM 6051 (99.57%)		44.3	1.19	1.09
A2727	*Pseudomonas geniculata* ATCC 19374 (99.78%)		39.7	1.23	0.79
A2731	*Enterobacter absuriae* JM 6051 (99.49%)		44.3	1.18	1.15
A2733	*Bacillus subtilis subsp. inoquosorum* KT 13429 (99.93%)		52.0	3.61	0.91
A2735	*Bacillus anthrasis* ATCC 14578 (99.85%)		44.3	4.30	2.04

Supplementary Table 5. Comparison of As resistance gene sequences with NCBI references. Top BLAST hit is recorded for each As resistance gene contig. %GC of the contig is also listed alongside average %GC of reference genomes (with standard deviation shown) of the same taxonomy. Sequences were accessed from NCBI on January 12, 2017.

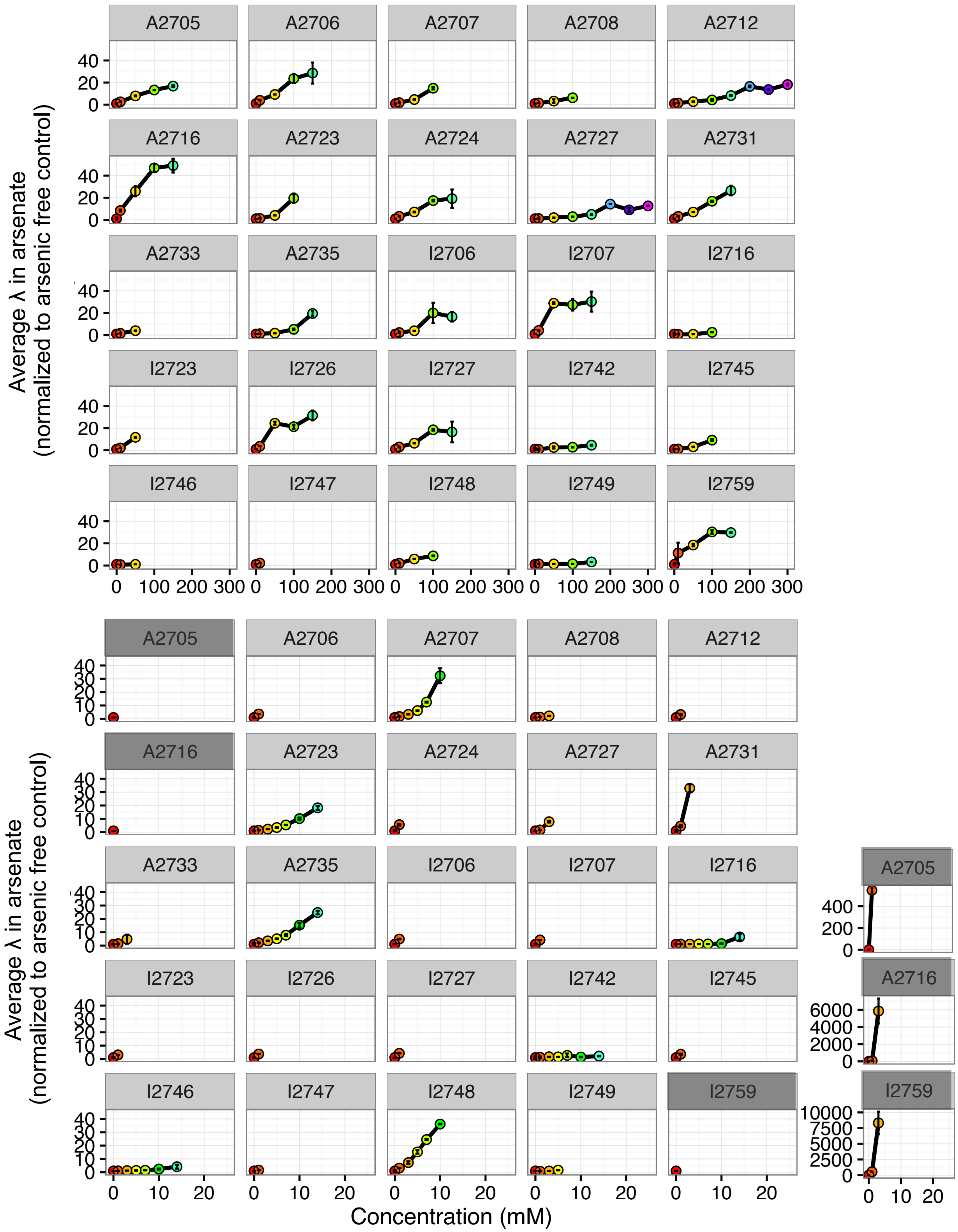
Isolate	**Genus**	**Gene**	**Closest NCBI match (% similarity)**	**%GC gene**	**%GC reference genomes**
I2706	*Enterobacter*	*arsB*	*Enterobacter cloacae* isolate MBRL1077 (97%)	59.62	55 ± 0.37
I2707	*Enterobacter*	*arsB*	*Enterobacter cloacae* isolate MBRL1077 (97%)	59.42	55 ± 0.37
I2726	*Enterobacter*	*arsB*	*Enterobacter cloacae* isolate MBRL1077 (97%)	59.51	55 ± 0.37
I2727	*Enterobacter*	*arsB*	*Enterobacter cloacae* isolate MBRL1077 (97%)	59.83	55 ± 0.37
I2759	*Acinetobacter*	*arsB*	*Enterobacter cloacae* isolate MBRL1077 (97%)	59.69	39.03 ± 0.11
A2706	*Enterobacter*	*arsB*	*Enterobacter cloacae* isolate MBRL1077 (97%)	59.14	55 ± 0.37
A2724	*Enterobacter*	*arsB*	*Enterobacter cloacae* isolate MBRL1077 (97%)	59.78	55 ± 0.37
A2731	*Enterobacter*	*arsB*	*Enterobacter cloacae* isolate MBRL1077 (97%)	60	55 ± 0.37
A2712	*Pseudomonas*	*ACR3(2)*	*Stenotrophomonas maltophilia* strain ISMMS2 (84%)	62.96	66.54 ± 0.25
A2727	*Pseudomonas*	*ACR3(2)*	*Stenotrophomonas maltophilia* strain ISMMS2 (95%)	64.69	66.54 ± 0.25
A2733	*Bacillus cereus*	*ACR3(2)*	*Stenotrophomonas maltophilia* D457 (83%)	64.83	43.89 ± 0.71
I2716	*Bacillus nealsonii*	*arsC*	*Bacillus cereus* ATCC 14579 (95%)	39.5	35.1
I2723	*Bacillus cereus*	*arsC*	*Bacillus cereus* ATCC 10987 (96%)	41.2	35.26 ± 0.18
I2726	*Enterobacter*	*arsC*	*Bacillus cereus* F837/76 (99%)	40.97	55 ± 0.37
I2727	*Enterobacter*	*arsC*	*Bacillus cereus* ATCC 10987 (96%)	40.89	55 ± 0.37
I2745	*Bacillus cereus*	*arsC*	*Bacillus thuringiensis* strain KNU-07 (98%)	37.67	35.26 ± 0.18
I2746	*Paenibacillus*	*arsC*	*Bacillus cereus* strain A1 (98%)	37.8	50.9 ± 0.14
I2747	*Paenibacillus*	*arsC*	*Bacillus* sp. ABP14 (95%)	39.29	50.9 ± 0.14
A2707	*Bacillus cereus*	*arsC*	*Bacillus cereus* D17 (92%)	43.28	35.26 ± 0.18
A2708	*Bacillus subtilis*	*arsC*	*Bacillus anthracis* strain Tyrol 4675 (95%)	40.38	43.89 ± 0.71
A2723	*Bacillus cereus*	*arsC*	*Bacillus* sp. CH19 (86%)	42.79	35.26 ± 0.18
A2733	*Bacillus subtilis*	*arsC*	*Bacillus cereus* strain FORC_024 (88%)	40.1	43.89 ± 0.71
A2735	*Bacillus cereus*	*arsC*	*Bacillus* sp. CH19 (88%)	42.29	35.26 ± 0.18

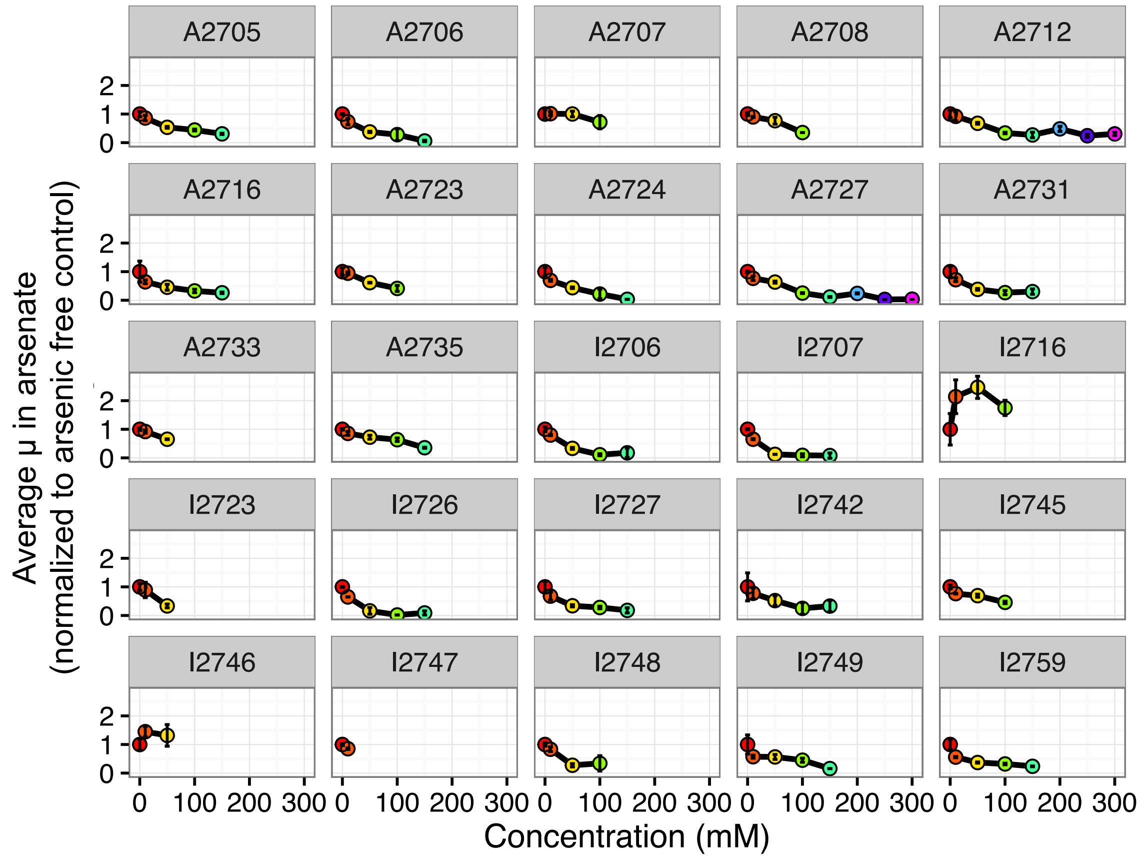
1.1 Supplementary Figures

Supplementary Figure 1. Average OD_{590} over 72 h in TSB50 with in increasing concentrations of arsenate **(A)** or arsenite **(B)**. Grey ribbon represents 95% confidence intervals from three replicates. Note the difference in color scales for A and B.



Supplementary Figure 2. Growth phenotypes in TSB50 with increasing concentrations of arsenate and arsenite normalized to growth in TSB50 without arsenic. Points are averages from three technical replicates, and error bars show standard deviation. Note the different scale for λ in arsenite for isolatesA2705, A2716, and I2759.




A2705
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I2727
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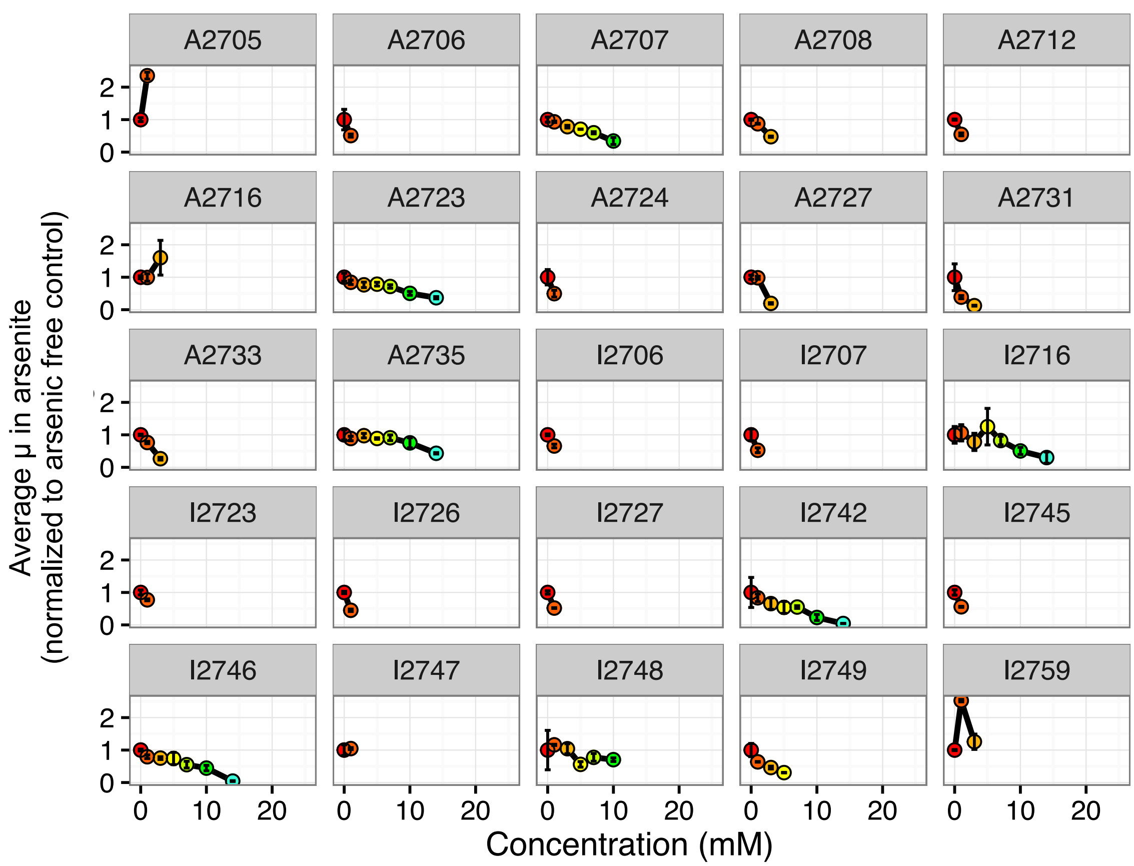
I2745
I2746
I2747
I2748
I2749
I2759
Average μ in arsenate
(normalized to arsenic free control)
Concentration (mM)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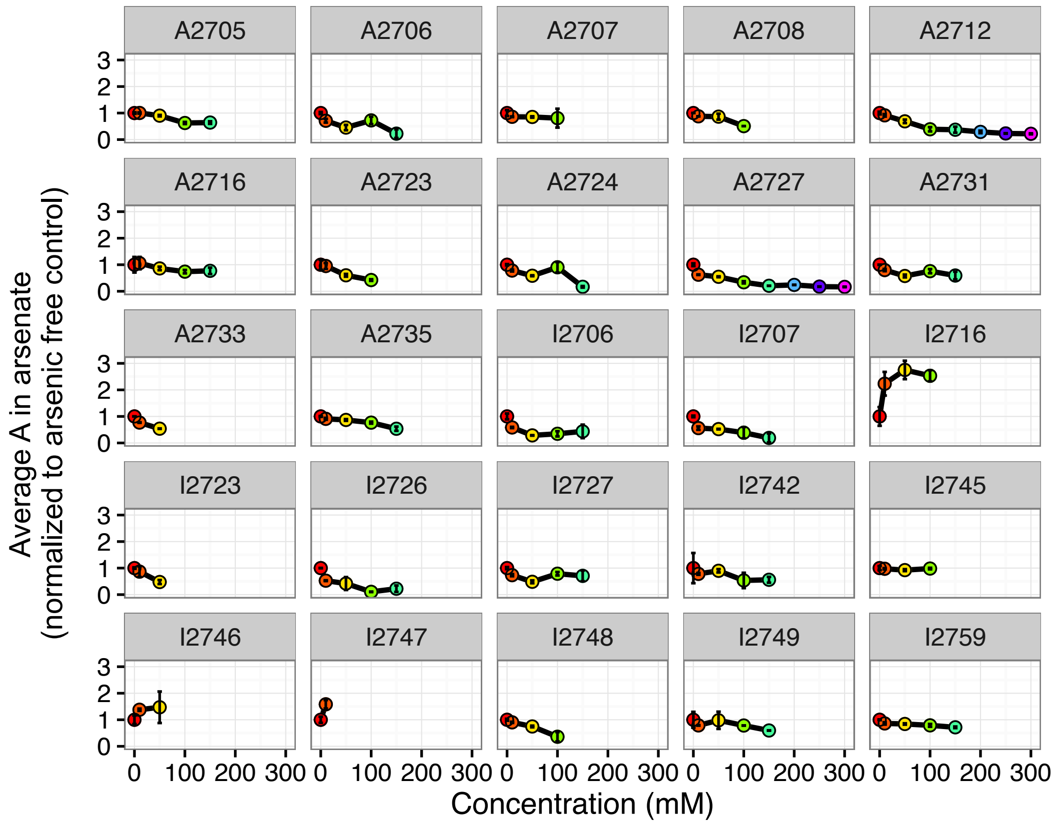
I2749
I2759
Average μ in arsenite
(normalized to arsenic free control)
Concentration (mM)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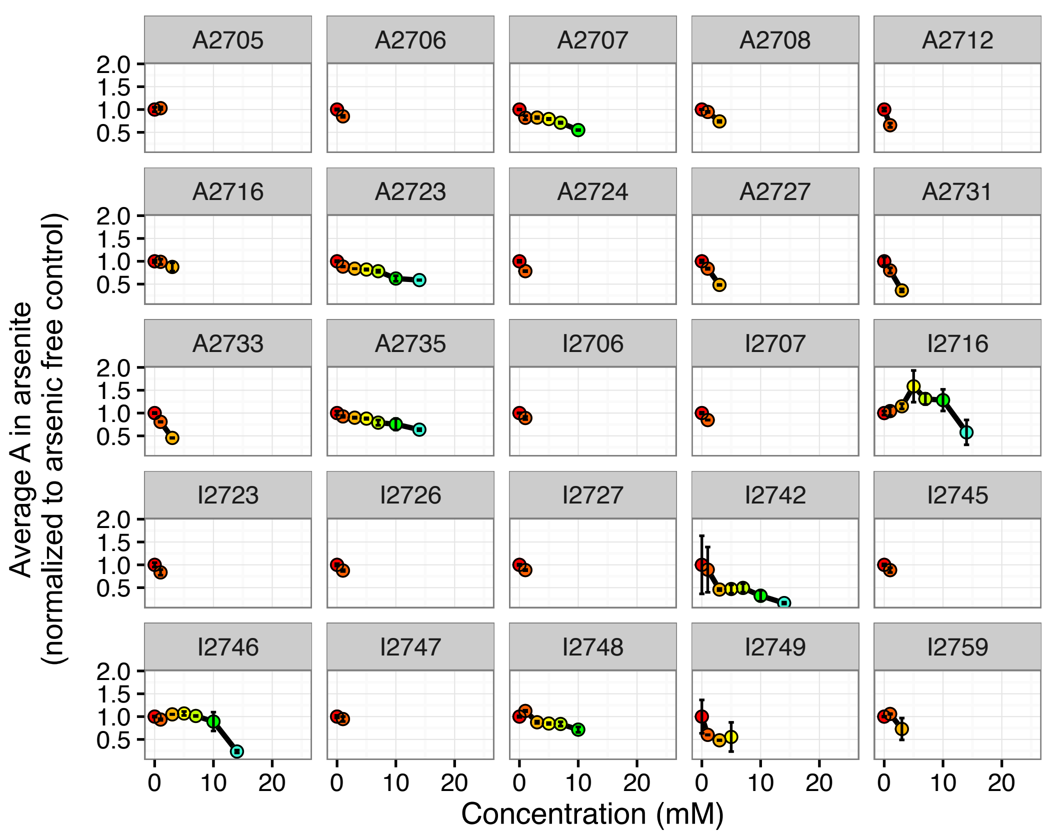
I2759
Average A in arsenate
(normalized to arsenic free control)
Concentration (mM)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Average A in arsenite
(normalized to arsenic free control)
Concentration (mM)