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3 **A novel MAs(III)-selective ArsR transcriptional repressor**

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9 **Supplemental Materials**

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Table S1: Oligonucleotide primers used in this study

Primer	Sequence	
pBAD- <i>SparsR</i> :		
SparsRF:	5'-CATG <u>CCATGGT</u> GAAACATAGCCGATATGAAT-3'	(<i>Nco</i> I site underlined)
SparsRR:	5'-GCGT <u>CGACCT</u> GGCCACTACAACATTGATCC-3'	(<i>Sal</i> I site underlined)
pACYC184- <i>ParsP-gfp</i> :		
ParsPF:	5'-CG <u>GGATCC</u> ATTGGCCTCATCTTATTAAG-3'	(<i>Bam</i> HI site underlined)
ParsPR:	5'-GCGT <u>CGACT</u> TTTTACCTCTGTGGTATTTATA-3'	(<i>Sal</i> I site underlined)
pBAD- <i>SpArsR_{AC}</i> :		
SparsRF:	5'-CATG <u>CCATGGT</u> GAAACATAGCCGATATGAAT-3'	(<i>Nco</i> I site underlined)
HSpArsR ₁₀₂ R:	5'-CCCA <u>AAGCTT</u> ACAACATTGATCCTGTAAAAAAT-3'	(<i>Hind</i> III site underlined)
HAfArsR ₉₇ F:	5'-CCCA <u>AAGCTT</u> ACCCGGGACTGTGCCCTATCCG-3,	(<i>Hind</i> III site underlined)
HAfArsR ₉₇ R:	5'-ATGATGATGATGATGATGGT <u>CGACCT</u> GGT-3'	(<i>Sal</i> I site underlined)
Cysteine residue mutagenesis		
C83S forward	5'-AAGGAAGGGTGTGTAC <u>AGCGT</u> GCCTGATTATGAG-3'	
C83S reverse	5'-CTCATAATCAGGCAC <u>GCT</u> GTACAACACCCTTCCTT-3'	
C101S forward	5'-GCATTTTTTACAGGATCAA <u>AGTT</u> GTAGTGGCCAGCATCA-3'	
C101S reverse	5'-TGATGCTGGCCACTACA <u>ACT</u> TTGATCCTGTAAAAAATGC-3'	
C102S forward	5'-CATTTTTTACAGGATCAATGT <u>AGT</u> AGTGGCCAGCATCATCATC-3'	
C102S reverse	5'-GATGATGATGCTGGCCACT <u>ACT</u> TACATTGATCCTGTAAAAAATG-3'	
Reverse transcription polymerase chain reaction (RT-PCR)		
ArsP forward	5'-GTGGGCATTCTACAAGCCTTTATCA-3'	579bp
ArsP reverse	5'-TAATGAACCGAGTAAAACACCCACA-3'	
ArsR forward	5'-AATGTAGAGAATGCAGCTAAGGTAT-3'	266bp
ArsR reverse	5'-TGATCCTGTAAAAAATGCACTAACC-3'	
ArsPR forward	5'-GATAAAGGCTTGTAGAATGCCCACT-3'	644bp
ArsPR reverse	5'-TGATCCTGTAAAAAATGCACTAACC-3'	

11 **Legends to the Supplemental Figures:**

12

13 **Supplemental Fig. S1. Multiple alignment of SpArsR orthologs.** Representative ArsR
14 orthologs (accession numbers in parentheses) are from: *A. ferrooxidans* (ACK80311),
15 *Ralstonia solanacearum* (NP_522690), *Caulobacter crescentus* (NP_420316),
16 *Mesorhizobium loti* (NP_103579), *Sinorhizobium meliloti* 1021 (NP_385183),
17 *Agrobacterium fabrum* (NP_354498), *Rhodopseudomonas palustris* (NP_947601),
18 *Desulfospira joergensenii* (WP_027362847), *Serratia liquefaciens* FK01 (GAK27386),
19 *Endozoicomonas elysicola* (WP_020582082), *Enterovibrio calviensis* (WP_017007765),
20 *Psychromonas ossibalaenae* (WP_026339319), *Glaciecola agarilytica* NO2
21 (GAC07582.1), *Photobacterium* sp. AK15 (ELR66560), *Moritella marina*
22 (WP_019439555) and *S. putrefaciens* (ADV53698). The multiple alignment was
23 calculated with CLUSTAL W.

24

Fig. S1

Acidithiobacillus	1	-----MEPLQDPAQIVARLEALASPVRLIEIFRLLVVEQEPTGLV
Ralstonia	1	-----MEETDAIRSLAALAHSLRLRVFRMLVVGAPAGLT
Caulobacter	1	-----MLSALSHEGRLAIFRLLVQAGPAGVA
Mesorhizobium	1	-----MDKATALQALAALGQETRLVFRLLVRAGAKGVP
Sinorhizobium	1	-----MDTVDIIAFAALAQPTRLDAFRFLVKHEPDGLP
Agrobacterium	1	-----MDNIGAIAAISALAQTTTLETFRLLVQHEPEGIP
Rhodopseudomonas	1	-----MDNDSAIASIGALAQGTTRLDVFRLLVRHEPDGLA
Desulfospira	1	-----MENETAAKKLAELGHTTTRLSIFRYLVKVGQCAS
Serratia	1	-----MELNEVANALKELGHPTRLSIYKQLVRAGHGGLP
Endozoicomonas	1	-----MNIDDYAKTLKELGHPVRLSIYKRLVKAGRSVIP
Enterovibrio	1	-----MELDAVAKALKELGHPTRLTIFRRLVRAGHNGIA
Psychromonas	1	-----MELEVIAKALKELGHPTRLAIFKRLVKSGFQOGIA
Glaciecola	1	-----MDIENTAKALKELGHPTRLNIYRSVVRAGYQOGIA
Photobacterium	1	-----MDIEIVAKALKELGHPTRLAIFKSVVRAGYQOGIA
Moritella	1	-----MDIDIIAKALKELGHPTRLTIFKSIIRAGYQOGIA
Shewanella	1	-----MNIADMNVADMVVENAAKVLKELGHPTRLALFRLLVKGGYTQVA

95/95

Acidithiobacillus	39	SGDIAEHLGQPHNGISFHLKNLQHAGLVTVQREGRYQRYRAAMPVVRALVAYLTENCCHG
Ralstonia	35	PCATAEQLDVPGATLSFHLKELMNAALVTQERDGRHLIYRAAFDHMNGLLGFLTENCQCG
Caulobacter	27	AGETARALNVLPNLSLSANLNVLSHSGLIASRRDGRSIIYTYADYDAMTGLLGFLMEDCCAG
Mesorhizobium	35	AGETATRLSTVQNTMSAHLKILGQAGLVHAERDGRSIRYADMTGFRDLLAYLMEDCCNG
Sinorhizobium	35	AGETARLINVPHNTMSTHTAQLQRAGLVTTTRRQSRSSIIYRADLDGLRHVVSFILKDCAG
Agrobacterium	35	AGETARLTHVPQNTMSAHLATLARAGLVKSERQSRSSIIYRADLEGLRALTLFLLKDCGG
Rhodopseudomonas	35	AGETARQLDVPQNTMSAHLGILARAGIVRSEHSRSIIYRADLDGLRALTLFLVKDCAG
Desulfospira	35	VGGIQQEKLGIPIGSLTSHHRRRLVAAGLTKQVRESRVLYCIPQFDVLDRLLEFLKSECCTE
Serratia	35	VGGIQQEKLGIPIGSLTSHHIAALLSVGLVHQQREGRTLFCPLQYDVLNGIVAFLEIECCAE
Endozoicomonas	35	VGGIQQEKLGIPIGSLTSHHLSALVSVGLVQQIRDGRLLYCIPQFQVFDQLLDFLRDQCCVD
Enterovibrio	35	VGGIQQEKLGIPIGSLTSHHLSLMSAGLITQRREGRVLYCVPQFEQLVAVLFLQDECCVD
Psychromonas	35	VGGIQQEKLGIPIGSLTSHHISGLVSAAGLVTQRREGRVLYCVAEYKKLESVITFLQDECCAD
Glaciecola	35	VGGIQQEKLGIPIGSLTSHHISGLLSAGLITQRRESRTLFCVAEYDKLQSVIDFLQDECCAD
Photobacterium	35	VGGIQQEKLGIPIGSLTSHHISGLLSAGLITQRREGRVLYCVAEYDKLQAVIGFLQDECCLD
Moritella	35	VGGIQQEKLGIPIGSLTSHHISGLLSAGLITQRREGRVLYCVAEYDKLKAVIGFLQDECCID
Shewanella	45	VGGIQQEKLGIPIGSLTSHHISALMSAGIISQRREGRVLYCVPDYELQGLVHFLQDQCCSG

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Acidithiobacillus	99	-TRDCALSGET----RSPSVQEGNQ-----
Ralstonia	95	EA--CPEFSAA----GCKC-----
Caulobacter	87	APQICAPLSAI----VSGGMACGAKA-----
Mesorhizobium	95	APELCQPILQA----VTCKC-----
Sinorhizobium	95	HPDVCAPLVAD----LTPCCSPMDRPARSHRSEGGMDGEAADRKN
Agrobacterium	95	ATELCAPLIAE----LTPCCQAEAL-----
Rhodopseudomonas	95	APELCAPLLAE----LTPCC-----
Desulfospira	95	AKCKM-----
Serratia	95	QQPHC-----
Endozoicomonas	95	SGVSSF-----
Enterovibrio	95	ESSAS-----
Psychromonas	95	MEDIG-----
Glaciecola	95	EKT-----
Photobacterium	95	EQASDDM-----
Moritella	95	EVEFSATASNK-----
Shewanella	105	Q-----

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27 **Supplemental Fig. S2. Response of the bacterial biosensor with SpArsR to As(III)**

28 **and MAs(III). A)** Diagrammatic representation of the construction of the bacterial

29 biosensor with SpArsR. In pBAD-*SparsR*, *SparsR* is under control of the *ara* promoter. In

30 pACYC184-*ParsP-gfp*, the *gfp* reporter is under control of the *arsP* promoter. **B)**

31 Conditions for constitutive, repressed or derepressed *gfp* expression. In cells of *E. coli*

32 AW3110 with both plasmids, *SparsR* is not expressed in the absence of arabinose, and

33 *gfp* expression is constitutive, producing cellular fluorescence. In the presence of

34 arabinose, *SparsR* is expressed, and *gfp* is repressed, so the cells are not fluorescent.

35 In the presence of both arabinose and arsenical inducer, *gfp* expression is derepressed,

36 and the cells are fluorescent. **C)** The bacterial biosensor with *SparsR* responds only to

37 MAs(III). Expression of the *gfp* reporter gene was assayed as described under

38 Experimental Procedures. Cells of *E. coli* strain AW3110(DE3) bearing wild type *SparsR*

39 were grown without arabinose, 0.2% arabinose, or 0.2% arabinose and arsenicals at the

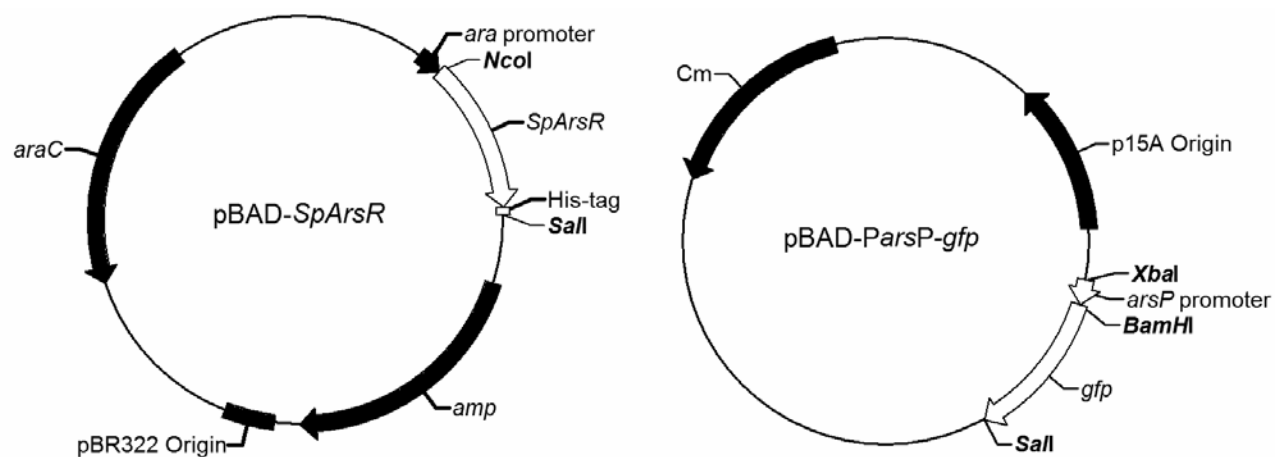
40 indicated concentrations. Fluorescence intensities were quantified by spectrofluorometry.

41 The data are the mean \pm SE (n = 3).

42

Fig. S2

A



B

Condition	arabinose	As	<i>arsR</i>	<i>gfp</i>	fluorescence
Constitutive	-	-	repressed	expressed	yes
Repression	+	-	expressed	repressed	no
Derepression	+	+	induced	expressed	yes

C

