

Supplementary Material

Identification of resistance genes and response to arsenic in *Rhodococcus aetherivorans* BCP1

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Table S1. Primer sets used in this study.

Target gene	Primer name	Sequence (5' to 3')
<i>arsA</i>	arsA-RT-For	AAGAAGGTGCTGCTGGTCTC
	arsA-RT-Rev	GACGATGCGTTCACGGTAGG
<i>arsD</i>	arsD-RT-For	ATGAGCACCATCGCAGTCTT
	arsD-RT-Rev	CCCTGATCCTTCAGCCACTG
<i>arsC1</i>	arsC1-RT-For	CTCAACGCCCTCTCGGTG
	arsC1-RT-Rev	GTGTCCCAGTTCTCCACGTA
<i>arsR</i>	arsR-RT-For	GACCATCTCGCACCATCTGA
	arsR-RT-Rev	GATCCCGGATTCGACGGAC
<i>arsC2</i>	arsC1'-RT-For	CCGAACTCGGACTCGATCTC
	arsC1'-RT-Rev	ACCACCTCGAGGGATTGTCC
<i>arsC3</i>	arsC1_1-RT-For	GACATCTCCACCCAATCCCC
	arsC1_1-RT-Rev	CCAGTCGCGGTAGCTCTTG
<i>acr3</i>	acr3-RT-For	CGCCATGGTCATCATCTGGA
	acr3-RT-Rev	GTGGTCTGTTTCGAGTCCGAG
<i>duf2703</i>	duf2703-For	CGAATCCAACCGCATCTGG
	duf2703-Rev	TTGACGATCAGTCGTTTCGGG
<i>arsO</i>	arsO-RT-For	GTGACATCGTGATGGTCCCG
	arsO-RT-Rev	CGGTGTCGTCCCAGAAGATG
<i>pstB</i>	pstB-RT-For	CAATCCGTTCCCCACCATGT
	pstB-RT-Rev	CCTTGACCTCGTTCCACAGG
<i>pstC</i>	pstC-RT-For	GAGGCCACGATTACCACTCC
	pstC-RT-Rev	ATCACCGAGATGAGAACGGC
<i>mshD</i>	mshD-RT-For	GAACTGCTGCGGGTCAATG
	mshD-RT-Rev	ACTTTCGTCCAGTGGAAGCC

Table S2. Functional profile of Actinobacteria *ars* genes associated to *ars* gene clusters^{a,b}

Actinobacterial Genus	# genomes	<i>glpF</i>	<i>arsM</i>	<i>ars</i> <i>H</i>	<i>ars</i> <i>P</i>	<i>ars</i> <i>J</i>	<i>arsC</i> ^c	<i>ars</i> <i>B</i>	<i>arcR</i> <i>3</i>	<i>ars</i> <i>A</i>	<i>ars</i> <i>D</i>	<i>ars</i> <i>O</i>	<i>ars</i> <i>T</i>	<i>ars</i> <i>X</i>	<i>ars</i> <i>R</i> ^d	<i>arsI</i>
<i>Acidimicrobium</i>	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Acidipropionibacterium</i>	1	-	-	-	-	-	1	-	-	1	1	-	-	-	1	-
<i>Acidothermus</i>	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Actinoallotheicus</i>	1	-	-	-	-	-	1	-	1	-	-	-	-	-	1	1
<i>Actinobacteria</i>	4	4	-	-	-	-	4	-	-	-	-	-	-	-	-	-
<i>Actinoplanes</i>	4	3	-	-	-	-	4	-	-	-	-	-	2	-	4	-
<i>Actinotignum</i>	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Adlercreutzia</i>	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Amycolaptosys</i>	7	1	5	-	-	-	6	-	6	-	-	1	-	-	6	2
<i>Arcanobacterium</i>	1	-	-	-	1	-	1	-	-	-	-	-	-	1	1	-
<i>Arthrobacter</i>	2	2	-	-	-	-	2	-	2	1	-	2	2	-	2	-
<i>Atopobium</i>	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Beutenbergia</i>	1	-	-	-	-	-	1	-	-	-	-	-	-	-	1	-
<i>Bifidobacterium</i>	38	-	-	-	1	-	1	-	1	-	-	-	-	-	1	-
<i>Blastococcus</i>	1	-	-	-	-	-	1	-	1	-	-	-	-	-	1	1
<i>Brachibacterium</i>	1	-	-	-	-	-	1	-	1	-	-	1	-	-	1	-
<i>Brevibacterium</i>	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Catenurispora</i>	1	1	-	-	-	-	1	-	-	-	-	-	-	-	1	-
<i>Cellulomonas</i>	3	1	3	-	3	-	3	-	3	-	-	-	3	-	3	-
<i>Clavibacter</i>	3	-	-	-	-	-	3	-	-	-	-	-	-	-	3	-
<i>Conexybacter</i>	1	-	1	-	-	-	1	-	1	-	-	-	-	-	1	-
<i>Coriobacterium</i>	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Corynebacterium</i>	63	-	-	-	3	-	63	-	63	1	1	-	-	-	63	-
<i>Cutibacterium</i>	12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Criptobacterium</i>	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Cutibacterium</i>	12	-	-	-	-	-	-	-	-	1	1	-	-	-	10	-
<i>Dermacococcus</i>	1	-	-	-	-	-	1	1	1	-	-	-	-	-	1	-
<i>Eggerthella</i>	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Frankia</i>	5	5	-	-	-	-	5	-	5	-	-	-	-	-	5	5
<i>Gardnerella</i>	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Geodermatophilus</i>	1	1	-	-	-	-	1	-	1	-	-	-	-	-	1	-
<i>Glutamibacter</i>	1	-	-	-	-	-	1	-	1	-	-	-	-	-	1	-
<i>Gordonia</i>	3	-	-	-	-	-	3	-	3	-	-	-	-	-	3	2
<i>Gordonibacter</i>	1	-	-	-	-	-	1	-	1	-	-	-	-	-	1	-
<i>Hoyosella</i>	1	-	-	-	-	-	1	-	1	-	-	-	-	-	1	1
<i>Illumatobacter</i>	1	-	-	-	-	1	1	-	1	-	-	-	-	-	1	-
<i>Intrasporangium</i>	1	-	-	-	-	-	1	-	1	1	1	-	-	-	1	-
<i>Isoptericola</i>	1	-	-	-	-	-	1	-	1	-	-	-	-	-	1	-
<i>Jonesia</i>	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Kinecoccus</i>	1	-	-	-	-	-	1	-	1	-	-	-	-	-	1	-
<i>Kitasatospora</i>	1	-	-	-	-	-	1	-	1	-	-	-	-	-	1	-
<i>Kytococcus</i>	1	-	-	-	-	-	1	-	1	-	-	-	-	-	1	1

<i>Kocuria</i>	1	-	-	-	-	-	1	-	1	-	-	-	-	-	-	-
<i>Kutzneria</i>	1	-	-	-	-	-	1	-	1	-	-	-	-	-	1	1
<i>Kribella</i>	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Leifsonia</i>	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Microbacterium</i>	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Micrococcus</i>	1	-	-	-	-	-	1	-	1	-	1	-	-	-	1	-
<i>Microlunatus</i>	1	1	1	-	-	-	1	-	1	-	-	-	1	-	1	-
<i>Micromonospora</i>	2	-	-	-	-	-	1	-	1	-	-	-	-	-	1	-
<i>Mobiluncus</i>	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
<i>Modestobacter</i>	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
<i>Mycobacterium</i>	54	-	-	-	-	-	42	-	42	-	-	-	-	-	42	52
<i>Mycolicibacter</i>	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
<i>Mycolicibacterium</i>	8	1	-	-	2	-	8	-	8	2	2	-	-	-	8	6
<i>Nakamurella</i>	1	-	-	-	1	-	1	-	-	-	-	-	-	-	1	-
<i>Nocardia</i>	4	-	-	-	-	-	3	-	3	-	-	-	-	-	3	3
<i>Nocardioides</i>	1	-	-	-	-	-	1	-	1	-	-	-	-	-	1	1
<i>Nocardiosis</i>	2	-	-	-	-	-	2	-	2	-	-	-	-	-	2	2
<i>Olsenella</i>	1	-	-	-	-	-	1	-	1	-	-	-	-	-	1	-
<i>Paenaarthrobacter</i>	1	1	-	-	-	-	1	-	1	-	-	-	1	-	1	-
<i>Propionibacterium</i>	1	-	-	-	-	-	1	-	-	1	1	-	-	-	1	-
<i>Pseudoarthrobacter</i>	2	2	-	-	-	-	2	-	-	-	-	-	1	1	1	-
<i>Pseudonocardia</i>	1	-	-	-	-	-	1	-	1	-	-	-	-	-	1	1
<i>Pseudopropionibacterium</i>	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Renibacterium</i>	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1
<i>Rhodococcus</i>	9	-	-	-	-	-	9	-	9	4	4	9	2	-	9	5
<i>Rhodoluna</i>	1	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-
<i>Rothia</i>	2	-	-	-	-	-	-	-	-	2	2	-	-	-	2	-
<i>Rubrobacter</i>	3	-	2	2	-	-	3	-	3	-	-	-	-	-	3	-
<i>Saccharomonospora</i>	1	-	-	-	-	-	1	-	1	-	-	-	-	-	1	1
<i>Saccharopolispora</i>	1	-	-	-	-	-	1	-	1	-	-	-	-	-	1	1
<i>Saccharotrix</i>	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
<i>Salinispora</i>	2	1	-	-	-	-	2	-	2	-	-	-	-	-	1	1
<i>Sanguibacter</i>	1	-	-	-	-	-	1	-	1	-	-	-	1	-	1	-
<i>Segniliparus</i>	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
<i>Slackia</i>	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
<i>Stackebrandtia</i>	1	-	-	-	-	-	1	-	1	-	-	-	-	-	1	-
<i>Streptomyces</i>	25	3	5	-	-	-	19	-	19	-	-	-	2	-	19	15
<i>Streptosporangium</i>	1	-	-	-	-	-	1	-	1	-	-	-	-	-	1	-
<i>Thermobifida</i>	1	-	-	-	-	-	1	-	1	-	-	-	-	-	1	1
<i>Thermobispora</i>	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1
<i>Thermomonospora</i>	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1
<i>Tropheryma</i>	2	-	-	-	-	-	1	-	1	-	-	-	-	-	-	-
<i>Trueperella</i>	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Tsukamurella</i>	1	-	-	-	-	-	1	-	1	-	-	-	-	-	1	1
<i>Verrucosipora</i>	1	-	-	-	-	-	1	-	1	-	-	1	-	-	1	-

^a The numbers reported in the table correspond to the number of actinobacterial strains possessing each gene organized in cluster with other genes involved in arsenic resistance

^b The actinobacterial genomes and plasmids present in the JGI database with complete sequence have been included in the analysis

^c These numbers include both thioredoxin dependent arsenate reductases (ArsC2/3) and mycothiol arsenate transferases (ArsC1)

^d These numbers include both *arsR* and *arsR** predicted to regulate mechanisms of resistance to inorganic and organic arsenic compounds, respectively.

Table S4. Fold change of the *mshD*, *pstB* and *pstC* genes (see Supplementary Figure S6) in *R. aetherivorans* BCP1 cells exposed to 6 and 33 mM of As(V)^{1,2}

Target genes	Gene Product	6 mM As(V)	33 mM As(V)
<i>pstB</i>	Phosphate transport system protein	2.01 ± 0.01	0.26 ± 0.05
<i>pstC</i>	Phosphate transport system permease protein	1.08 ± 0.01	0.25 ± 0.03
<i>mshD</i>	Mycothiol synthase	0.58 ± 0.03	0.78 ± 0.01

¹ Data reported are the fold of expression compared to the glucose-growth conditions without As(V) supplied to the medium, using 16S rRNA as reference gene.

² Data is presented as the mean ± SD (standard deviation) of three replicates

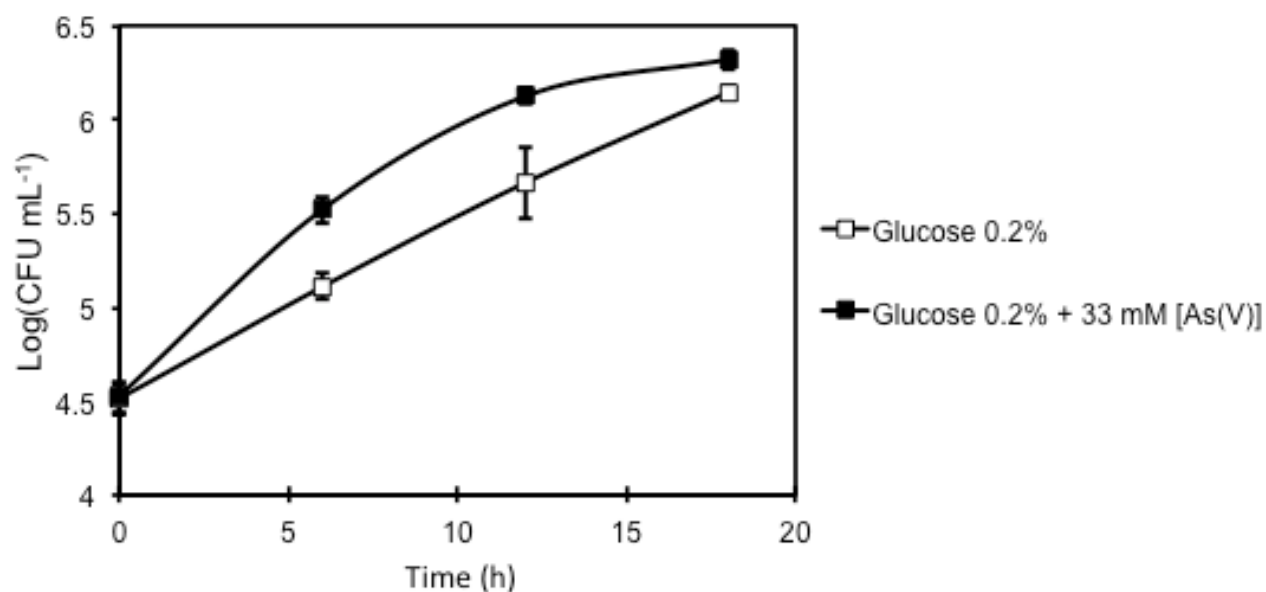


Figure S1 Growth curves of *R. aetherivorans* BCP1 without (white squares) and in the presence of 33 mM As(V) (black squares). Data are presented as the mean $\pm$ SD of five replicates.

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BCP1_ArsI      --MSRVQLALNVDDLQEAVTFYSKLFGTGPAKVPGYANFAIAEPPLKLVLIENA-GKG- 56
DSM43183_ArsI --MSRVQLALRVDPLEASIGFYSKLFGTGPVKVRPGYANFAIAEPPLKLVLIEGA-GEDA 57
MD1_ArsI      --MKYAHVGLNVTNLEKSIEFYSKLFGAEPVKVKPDYAKFLLESPGLNFTLNLRDEV-NG 57
PCC7120_ArsI  MSVMKTHVALNVTNIEKSVTFYRAMFGLPEVKYKTDYAKFDIPNPALNLTNLNTNNVQIG 60
               :  .:.*. * :: : *  : **  *. * : . ** : *  * : . *
               :  .:.*. * :: : *  : **  *. * : . ** : *  * : . *

BCP1_ArsI      GSIHHLGVEVESSEKVVHSEIARLTDEGL-FTDEEIGTTCCFATQDKVWWTGPAGEKWEVY 115
DSM43183_ArsI  TRLDHLGVEVEDSAQVGHAARRLKESGL-ATVEENDTACCYAVQDKVWWTGPGGEPWEVY 116
MD1_ArsI      NQVGHFGIQVESTEEVVAHKNRLAENGILSQYDEINTTCCYALQDKFWIHDPDGNEWEFF 117
PCC7120_ArsI  GALSHLGVQVESTQEVQSAIERFNEAGL-DLFTEDNTDCCYALQDKVWWTDPDGNRWEVF 119
               :. * : : : * : : * : : * : * : * : * : * : * : * : * : * :
               :. * : : : * : : * : : * : * : * : * : * : * : * : * : * :

BCP1_ArsI      TVLADSDTFGTSPKLL---DQGENSEGVCCGTAAEQEPATGTEKAPAATTCC-      164
DSM43183_ArsI  VVKGDADTLAKAD-----DSACCTPRD-----SGSAGAAVGADCC-      151
MD1_ArsI      YTKTTVEENSTHPPT-CCVNEPNVEKAEECSPTASSNK-----DTSNCCS      161
PCC7120_ArsI  VVKVADTAPEKNLATVSSSGEIQAVKKSCCA-----              150
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Figure S2 Multi-sequence alignment of the *R. aetherivorans* BCP1 As-C lyase ArsI against ArsI orthologs from *Bacillus* sp. MD1 (MD1_ArsI, AIA09488), *Thermomonospora curvata* DSM 43183 (DSM43183_ArsI, WP_012854466.1), and *Nostoc* sp. PCC 7120 (PCC 7120_ArsI, WP_010995277.1). Black triangle indicate the cysteine pair probably involved in MAs(III) binding. Sequence alignment was performed with Clustal Omega (<https://www.ebi.ac.uk/Tools/msa/clustalo/>)

SP200_ArsR	VGQLQEALQIPGSTLSHHISALMSAGIISQRREGRVLYCVPDYELLQGLVHFLQDQCCSG	104	
BCP1_ArsR *	TCDLASAVGLTESTVSHHLGQLRKAGMVQSTRRGMMVHHRACNEALQALRLVLDPNCC--	118	
PD630_ArsR *	TCDLATGVGLSESTVSHHLGQLKKAGMVQPVRRGMNVFY SARADALDALRVVLDPNCC--	118	
RHA1_ArsR *	TCDLATGVGLSESTVSHHLGQLKKAGMVQSVRRGMNVFY SARADALDALRVVLDPNCC--	118	
B4_ArsR *	TCDLATGVGLSESTVSHHLGQLKKAGMVQSVRRGMNVFY GARPDALDALRVVLDPHCC--	118	organic As
SB3094_ArsR1*	TCDLATGVDLAESTVSHHLGQLRKAGMVESERRGMNVFYRARAESLEALRAVLDPNCC--	118	
SB3094_ArsR2*	TCDLAAGVDLAESTVSHHLGQLRKAGMVESERRGMNVYHARAESLEALRVVLDPNCC--	118	
103S_ArsR*	TCDLAVGVGLAESTVSHHLGQLRKAGMVESTRRGMNVYRVRGESLDALRLVLDPNCC--	118	
SB3094_ArsR2	VCDISGSFDLSQPTISHHLRLVLEAALLECERRGTWYYYWVPAALRQLSDVLGIEPTEV	101	
B4_ArsR1	VCDISATIDLSQPTISHHLKVLRAAGLLDCERRGTWYYYWVIPSALQQLSAVLLTESGPV	117	
PD630_ArsR1	VCDISESFDLSQPTISHHLKVLRAAGLLDCERRGTWYYYWVIPSALQQLSAVLLTESGPV	117	
B4_ArsR2	VCDISASFDLSQPTISHHLKVLRAAGLLDCERRGTWYYYWVIPSALQQLSAVLLTEGGPA	117	
RHA1_ArsR	VCDISESFDLSQPTISHHLKVLRAAGLLDCERRGTWYYYWVIPSALQQLSAVLLTESGTA	117	inorganic As
BCP1_ArsR	VCDISPSFDLSQPTISHHLKVLREAGLLDCERRGTWVHYRVVPSALAQLSAVLSVESGIV	117	
SB3094_ArsR1	VCDISPAFDLSQPTISHHLKVLREAGLLDCERRGTWYYRVIPSALTQLSAVLSPERGVV	86	
103S_ArsR	VCDISPAFDLSQPTISHHLKVLREAGLLDSERRGTWYYRVIPSALQLSTVLSAAWGGV	115	
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Figure S3 Multi-sequence alignment of the MAs(III)-responsive ArsR* transcriptional repressor of *Shewanella putrefacens* 200 (originally named as Sp200_ArsR) against putative MAs(III)-selective ArsR* (together indicated as “organic As”) and As(III)-responsive ArsR (together indicated as “inorganic As”) proteins. Black arrows highlights the cysteine pair conserved in the MAs(III) binding site of the ArsR*. For simplicity only the C-terminal region is shown. The locus tags in GenBank and JGI are: BCP1_ArsR* (JGI: Ga0035244_05158, NCBI: N505_RS21280), BCP1_ArsR (JGI: Ga0035244_05182, NCBI: N505_RS21405); *R. equi* 103S 103S_ArsR (JGI/NCBI: REQ_04560), 103S_ArsR* (JGI/NCBI: REQ_04620); *R. opacus* B4: B4_ArsR1 (JGI/NCBI: ROP_00150), B4_ArsR* (JGI/NCBI: ROP_40650), B4_ArsR2 (JGI/NCBI: ROP_29950); *R. pyridinivorans* SB3094: ArsR1 (JGI: Rpyr3094_04607; NCBI: Y013_RS15395), ArsR1* (JGI: Rpyr3094_04612; NCBI: Y013_RS15420), ArsR2* (JGI: Rpyr3094_04678, NCBI: Y013_RS25175), ArsR2 (JGI: Rpyr3094_04684, NCBI: Y013_RS25210); *R. jostii* RHA1 (JGI: RHA1_ro04133-ro04132; NCBI: RHA1_RS20100-RS20095).

B

PR4_ArsMyc_Trans	-MSKPSVL FVCVKNNGGKSQMAAGLMRKAAGDQVDVYSAGTKPGDAVNLSAETLLEVGVD	59
BCP1_ArsC1	-VRTPSVL FVCVKNNGGKSQMAAALMRQAAGGRIAVHSAGTAPGPALNLSVQALEEVGAP	59
Cg_ArsC2	---MKSVL FVCVGNNGGKSQMAAALQKAYSADSVETHSAGTKPAQGLNQLSVESAIEVGAD	57
Cg_ArsC1	MINNPQSVL FVCVGNNGGKSQMAAALAKKHAGDALKVYSAGTKPGTKLNQSLDSTAIEVGAD	60
	***** *****.* :: *. . ::**** *. :* * :: **.	
	▼	
PR4_ArsMyc_Trans	ISGETPTLIDPQLVRNVDLVLTGSEAKVDPV--AGTDFENMDTDEPSERGIIDGIERMRL	117
BCP1_ArsC1	VDGVEPKPIDPQLLREVLDVLTGDRARDA----AYVENMDTDEPSERGIIDGIERMRL	114
Cg_ArsC2	MSQGIKPIDPELRLTVDRVVLGDGAQVDMPESAQGALERWSIEEPAQ---GMERMRI	114
Cg_ArsC1	MSQGFPGIKIDQELLKRVDRVVLGAEAQLMPEIDANGILQRWVTDEPSERGIEGEMRMRL	120
	:: * . ** :*: ** ** ** :*::: .::* **: ** : *::****:	
PR4_ArsMyc_Trans	VRDDIAARVDALAGRLTT----- 135	
BCP1_ArsC1	VRDDIAARVDALAA RLDSASPTR 138	
Cg_ArsC2	VRDQIDNRVQALLAG----- 129	
Cg_ArsC1	VRDDIDARVQNLVAELTQNA---- 140	
	***.* **.* **.* **.* **.*	

Figure S4 Multi-sequence alignment of *R. aetherivorans* BCP1 arsenate reductases BCP1_ArsC2/3 (A) and BCP1_ArsC1 (B) with the corresponding homologs from *Corynebacterium glutamicum*, *R. erythropolis* PR4 and *Streptomyces coelicolor* A3. Sequence alignment was performed with Clustal Omega (<https://www.ebi.ac.uk/Tools/msa/clustalo/>)

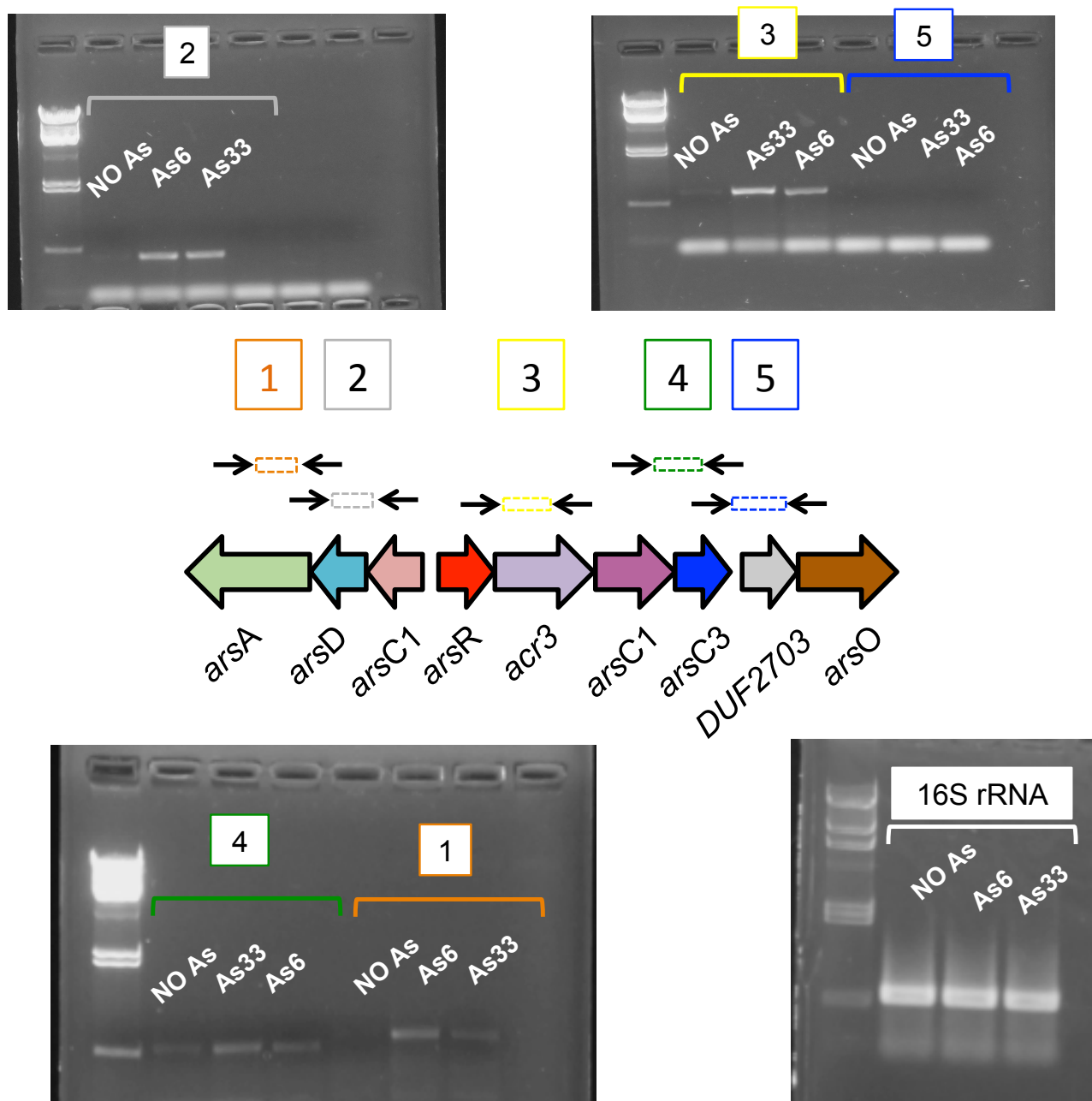


Figure S5 Analysis of *ars* genes co-transcription in *R. aetherivorans* BCP1. cDNA was retro-transcribed from mRNA extracted from BCP1 cells grown in the presence of 6mM and 33 mM As(V) (As6 or As33) or in the absence of the oxyanion (NO As). Arrows above the *ars* genes represent the position of the primers used in RT-PCR (see Supplementary Table S1) to evaluate the co-transcription of adjacent *ars* genes. The RT-PCR of 16S rRNA is also shown as reference gene.

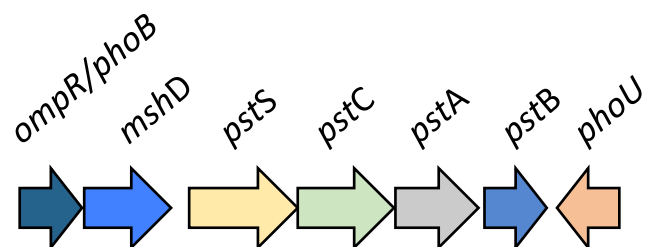


Figure S6 Organization of the *pst* genes and *mshD* gene in *R. aetherivorans* BCP1 genome. The genes displayed have JGI locus tag from Ga0035244_01310 to Ga0035244_01316, while the gene products have GenBank ID from KDE14905 to KDE14911. The predicted gene products are (in order from the left): OmpR/PhoB-type DNA-binding domain protein, mycothiol synthase MshD, phosphate transport system substrate-binding protein PstS, phosphate transport system permease protein PstC, phosphate ABC transporter PstA, phosphate transport system ATP-binding protein PstB, phosphate transport system protein PhoU.

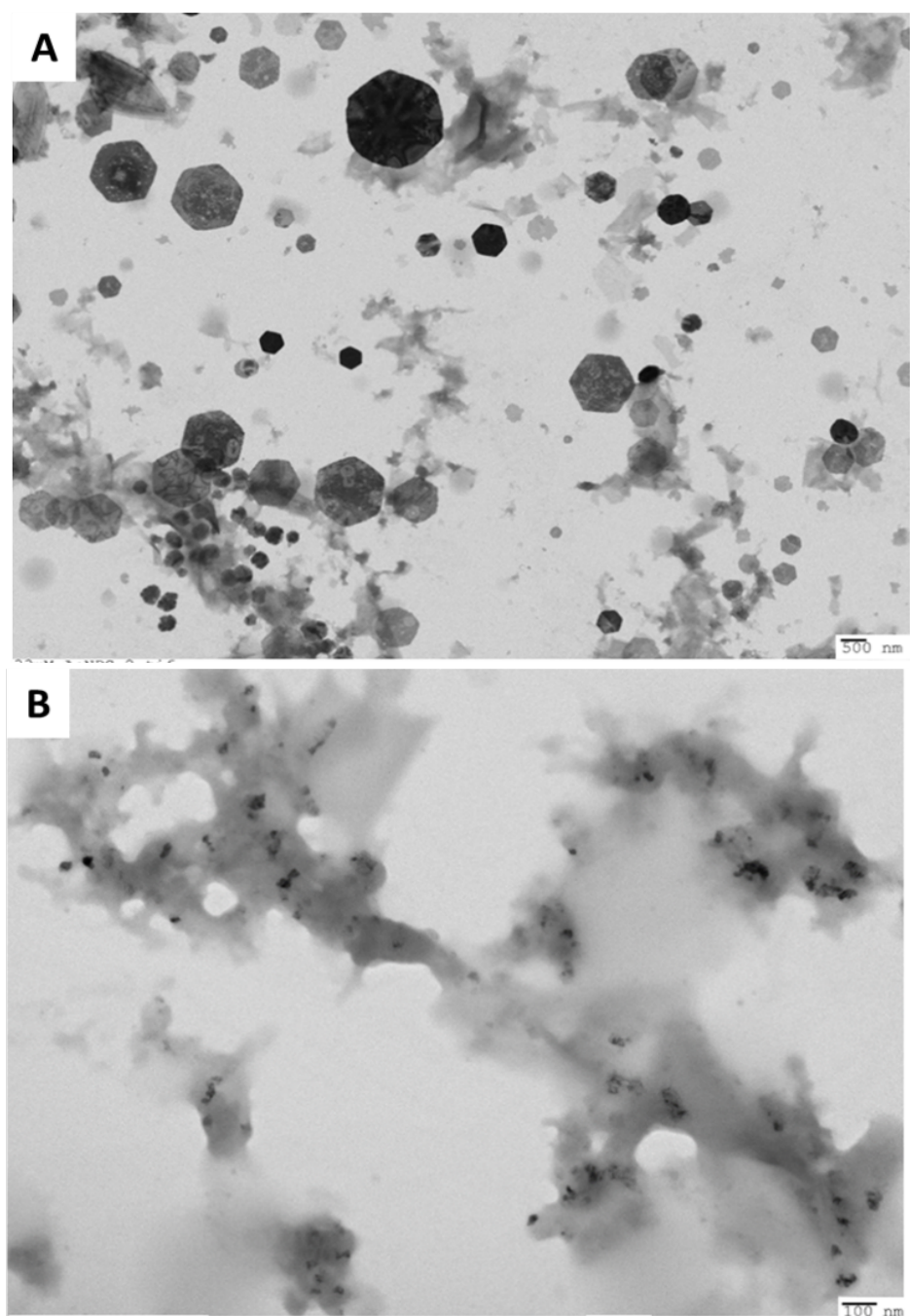


Figure S7 Transmission Electron Microscopy (TEM) images of cell extracts obtained through sonication of BCP1 cells grown for 96 hours on M9 medium and glucose 0.2% w/v as only carbon and energy source in the presence of 33 mM As(V) (Panel A) or 6 mM As(V) (Panel B). Electron dense nanoparticles are visible in the cellular extract of BCP1 cells incubated with arsenate.