

Support Information

Microbial Arsenic Methylation in soil and Rice Rhizosphere

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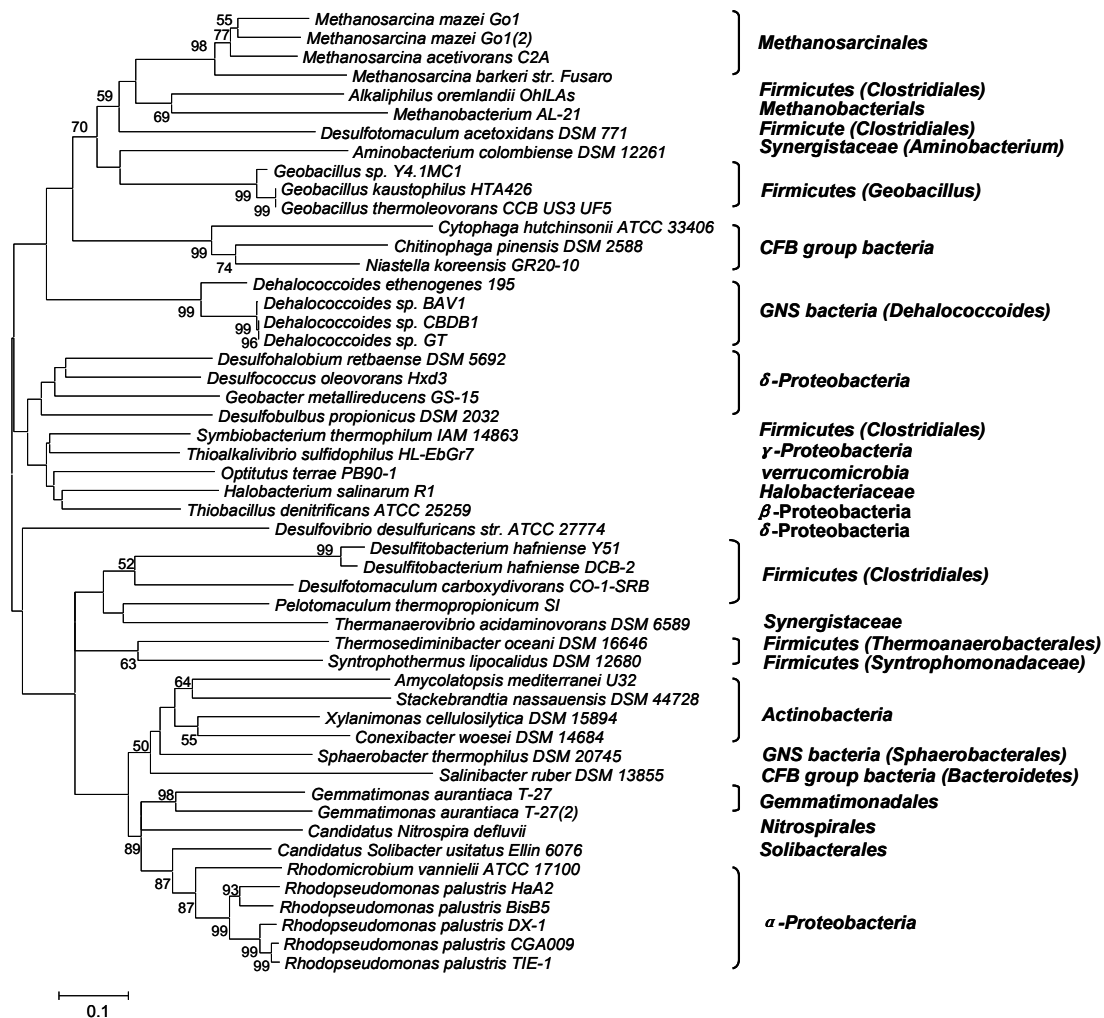


Figure S1. Neighbor-joining phylogenetic tree of prokaryotic full length *arsM* sequences from the NCBI database. The bars correspond to 10 mutations per 100 residues.



Figure S3. PCR products of the partial *arsM* gene sequences using the designed primer pairs. This shows the PCR products from rhizosphere soil grown with Yangdao without rice straw addition into the soil. From the left to the right are: (a) marker (DL2000, Takara Bio Inc., Japan); products using the primer pair (b) *arsMF1/arsMR1*, (c) *arsMF1/arsMR2*, (d) *arsMF1/arsMR3*, (e) *arsMF2/arsMR1*, (f) *arsMF2/arsMR2*, (g) *arsMF2/arsMR3*, (h) *arsMF3/arsMR1*, (i) *arsMF3/arsMR2*, (j) *arsMF3/arsMR3*, respectively.

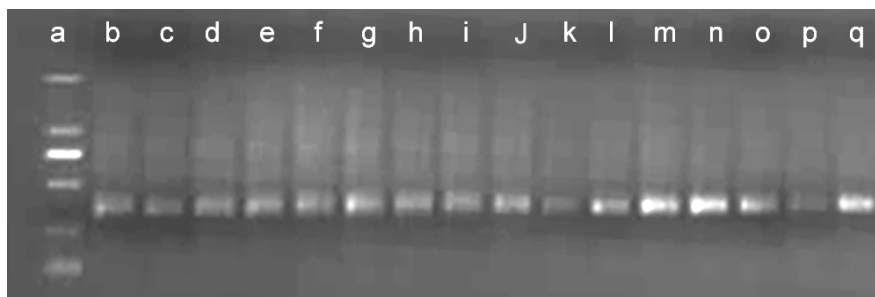


Figure S4. PCR products of the partial *arsM* sequences using the primer arsMF1/arsMR2 from microbial DNA of different soils and roots. The PCR products from left to right were: (a) marker (DL2000, Takara Bio Inc., Japan); (b) CZ1, As impacted paddy soil from Chenzhou 1, Hunan province, China; (c) CZ2, As impacted paddy soil from Chenzhou 2, Hunan province, China; (d) QY1, As impacted paddy soil from Qiyang 1, Hunan province, China; (e) QY2, As impacted paddy soil from Qiyang 2, Hunan province, China; (f) FQ, arid land soil from Fengqiu, Henan province, China; (g) GL, paddy soil from Guilin, Guangxi province, China; (h) CP, wheat land soil from Beijing, China; (i) TP, park soil (Taoranting) from Beijing, China; (j) DP, park soil (Daguanyuan) from Beijing, China; (k) Park soil (Longtan Lake) from Beijing, China; (l) OP, park soil (Olympic park) from Beijing, China; (m) WP, park soil (World park) from Beijing, China; (n) EI, grass rhizosphere soil (Research center for Eco-Environmental Sciences) from Beijing, China; (o) HLJ, Organic soil from Heilongjiang province, China; (p) R1, rice root of Yangdao from the pot experiment; (q) R2, rice root of Nongken from the pot experiment.

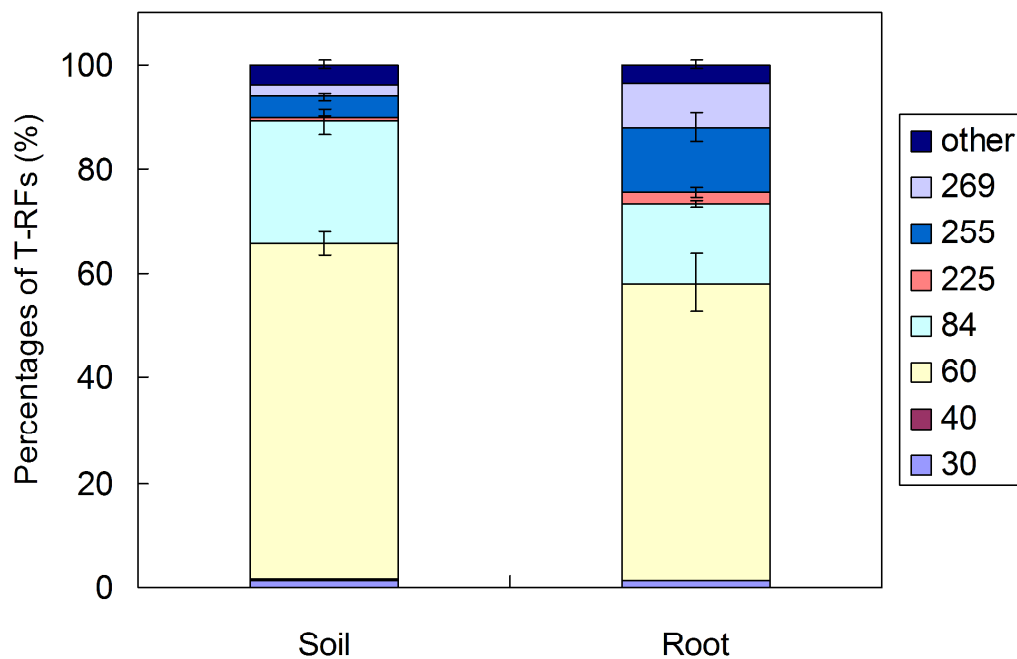


Figure S5. Community structure of *As* methylating prokaryotes in rice roots and rhizosphere soil (Yangdao grown without rice straw addition) reflected by the terminal restriction fragment length polymorphism (T-RFLP) analysis. Terminal restriction fragments (T-RFs) were digested by *Taq* I enzyme of PCR products of *arsM* genes from rhizosphere soil and rice roots. Bars represent standard errors ($n = 4$).

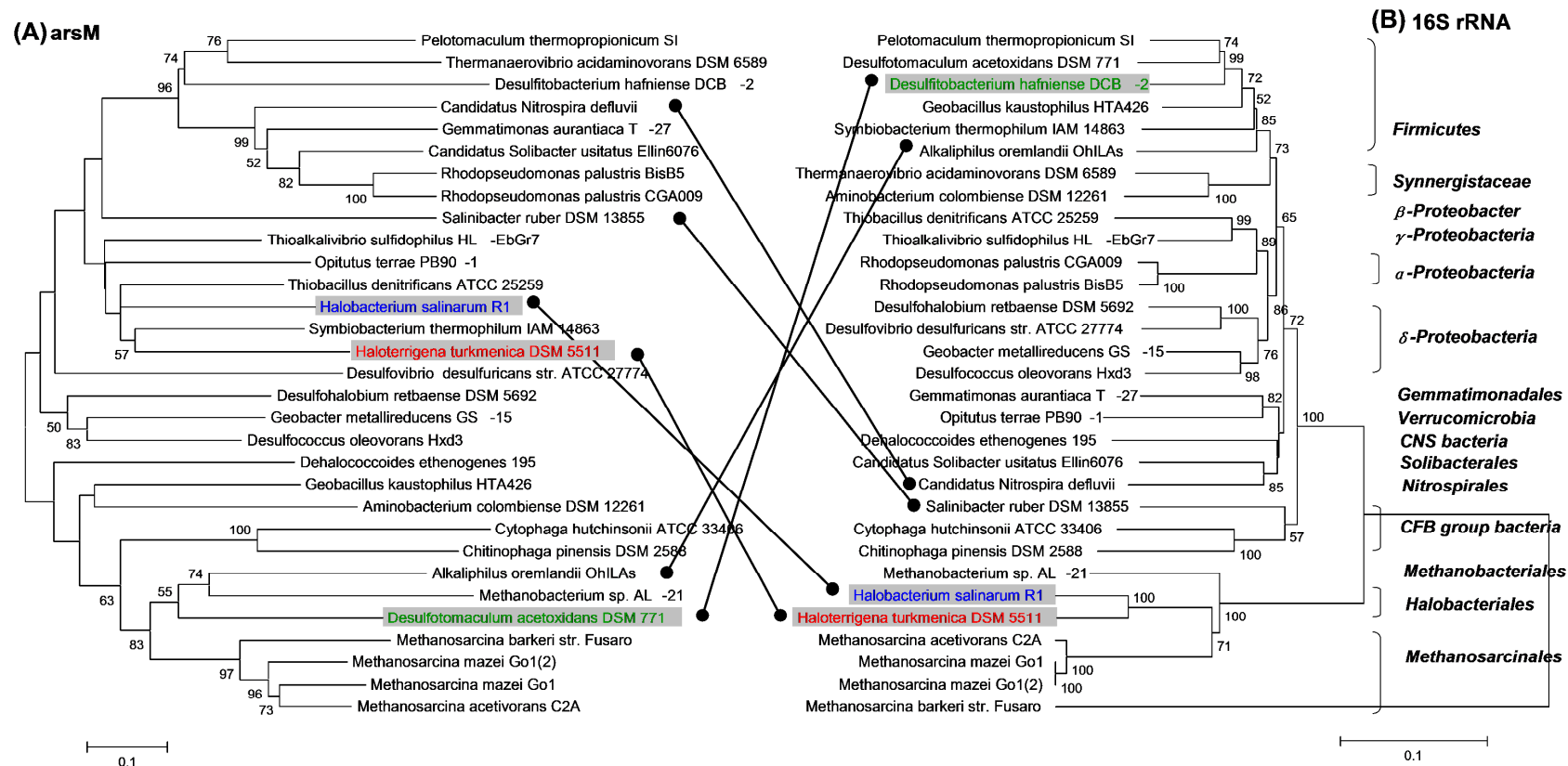


Figure S6. Phylogenetic congruence of microorganisms on the basis of full length *arsM* gene (A) and 16S rRNA gene (B). The sequences were retrieved from microbial genomes in the NCBI database, of which, *arsM* from *Halobacterium salinarum* R1 was plasmid-born. The lines show the inconsistency in the phylogenetic trees of *arsM* and 16S rRNA gene sequences, providing evidence for the potential horizontal gene transfer (HGT). The shadowed ones show the potential HGT between bacterial and archaea. The bars correspond to 10 mutations per 100 residues.

Table S1. Site identification and description of soil samples analyzed using degenerated primers to amplification of *arsM* genes.

Site ID ^a	Sample description	Location	pH ^b	Organic matter ^c (g kg ⁻¹)	As (mg kg ⁻¹)	Copy numbers of <i>arsM</i> gene ^d (10 ⁷ g ⁻¹ dry soil)	Copy numbers of 16S rRNA gene ^e (10 ¹⁰ g ⁻¹ dry soil)
QY1	Paddy soil	Qiyang, Hunan province, China	7.36	55.0	56.0	3.04	1.91
QY2	Paddy soil	Qiyang, Hunan province, China	7.48	38.3	73.0	3.16	1.83
CZ1	Paddy soil	Qiyang, Hunan province, China	7.25	41.3	77.2	4.20	2.00
CZ2	Paddy soil	Chenzhou, Hunan province, China	6.96	45.2	69.6	4.45	2.10
FQ	Arid land	Fengqiu, Henan province, China	7.91	9.5	7.8	2.23	1.54
GL	Paddy soil	Guilin, Guangxi province, China	7.61	28.6	10.6	9.30	4.53
CP	Wheat field	Changping, Beijing, China	7.82	16.4	15.3	3.54	1.32
TP	Park soil	Taoranting park, Beijing, China	8.14	56.5	12.2	3.07	3.10
DP	Park soil	Daguanyuan park, Beijing, China	8.57	30.6	7.8	2.51	3.18
LP	Park soil	Longtanhu park, Beijing, China	8.56	26.6	9.6	2.92	1.36
OP	Park soil	Olympic park, Beijing, China	8.57	17.6	7.4	6.81	2.44
WP	Park soil	World park, Beijing, China	8.75	16.7	8.6	1.18	1.84
EI	Grass rhizosphere	RCEES, Beijing, China	8.58	32.3	10.3	2.66	1.87
HLJ	Wetland soil	Sanjiang wetland, Heilongjiang, China	5.88	57.9	7.9	3.07	1.05

^a Site identification corresponded to abbreviation in Figure S2;

^b pH were determined in a soil: water ratio of 1: 2.5;

^c Contents of organic matter were measured by method of hydrated heat potassium dichromate oxidation-colorimetry;

^d Copy numbers of *arsM* in the microbial DNA of different fresh soils were assessed by the quantitative PCR using the primer pair *arsMF1/arsMR2*.

^e Copy numbers of 16S rRNA gene in the microbial DNA of different fresh soil were assessed targeted for amplification using the primers Bact 1369F and Prok 1492R and the *TaqMan* probe TM 1389F.

Table S2. ANOVA test for copy numbers of *arsM* gene from the rhizosphere and bulk soils grown with rice plants of Yangdao and Nongken, with or without rice straw addition, respectively.

Factor	F value	Significance (<i>p</i> value)
Soil	11.17	0.002*
Straw	90.60	0.000*
Variety	13.31	0.001*
Stage	11.65	0.001*
Soil × Straw	0.41	0.524
Soil × Variety	0.32	0.569
Soil × Stage	0.21	0.645
Straw × Variety	12.77	0.001*
Straw × Stage	41.37	0.000*
Variety × Stage	30.72	0.000*
Soil × Straw × Variety	0.15	0.699
Soil × Straw × Stage	0.86	0.356
Soil × Variety × Stage	2.12	0.151
Straw × Variety × Stage	27.59	0.000*
Soil × Straw × Variety × Stage	0.01	0.921

Soil, rhizosphere soil or bulk soil; Straw, with or without rice straw addition; Variety, Yangdao or Nongken rice plants; Stage, stage of tillering and the beginning of the heading. “*” means the significant difference at the level of $p < 0.05$.

Table S3. Percentages of the theoretical terminal restriction fragments (T-RFs) of in situ digestion of sequences from clone library (using the primer pair arsMF1/arsMR2) of *arsM* genes from rhizosphere soil and rice root. Clones were supposed to be digested by *Taq* I enzyme.

T-RF	Clone library ^a	
	Root	Soil
30		3.9
40		2.0
60	46.3	33.3
84	14.8	43.1
138	3.7	
201	1.9	
204	1.9	
211	0.0	2.0
225	14.8	9.8
235	0.0	2.0
255	7.4	0.0
269	9.3	3.9
Other		

^a The percentage of each theoretical T-RFs accounts for the total clones in the clones library from root and rhizosphere soil, respectively.