Supporting Information

Arsenic uptake by rice is influenced by microbe-mediated As redox changes in the rhizosphere

Yan Jia,[†,‡] Hai Huang,[†] Zheng Chen,[†] Yong-Guan Zhu[†,§,*]

[†] State Key Lab of Urban and Regional Ecology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China.

[‡] Institute of Process Engineering, Chinese Academy of Sciences, Beijing 100190, China.

[§] Key Lab of Urban Environment and Health, Institute of Urban Environment, Chinese Academy of Sciences, Xiamen 361021, China.

[*]Corresponding author phone: 86-592-6190997; fax: 86-592-6190997; e-mail: ygzhu@rcees.ac.cn

MATERIALS AND METHODS

Soil DNA extraction. Total microbial DNA was isolated from roots, rhizosphere soil and bulk soil samples from the pot experiment, using a FastDNA SPIN Kit for Soil (MP Biomedicals, USA) and the FastPrep Instrument (MP Biomedicals, USA), following the manufacturer's instructions. DNA concentration was measured with a Nanodrop ND-1000 UV-Vis spectrophotometer (NanoDrop Co., USA).

Quantification of 16S rDNA, *aroA*-like, *arrA* and *arsC* gene copy numbers. Soil DNA (10 fold dilution) was conducted in real-time quantitative PCR (iQ^{TM}5 Thermocycler, BioRad, USA) to quantify 16S rDNA, *arrA* gene and *arsC* gene abundance in each sample. For the quantification of 16S rDNA, each reaction performed in a total volume of 25 μL, containing 12.5 μL Premix Ex taq^{TM} (TaKaRa, Japan), 1 μL DNA template, 1 μL of each 10 μM primer bacteria-(1369F/1492R), 0.5 μL 10 μM probe (TM1389F CTTGTACACACCGCCCGTC) and 9μL PCR degrade water. The PCR mixtures were firstly incubated at 95ºC for 10 s, followed by 35 cycles at 95ºC for 15 s and at 56ºC for 1 min. The bacterial standard template DNA was prepared by PCR amplified 16S rDNA using the bacteria-(1369F/1492R) specific primer pair. The amplicons from the ordinary PCR were ligated to the pGEM-T Easy vector (Promega, USA) and transformed into *Escherichia coli* JM109 cells. These clones were sequenced and the most abundant one was used as an internal standard. Plasmid DNA was extracted and the concentration was measured by Nanodrop ND-1000 UV-vis spectrophotometry (NanoDrop Co., USA).

For quantification of the *aroA*-like gene, primers of *AroA*deg1F/*AroA*deg1R and *AroA*deg2F/*AroA*deg2R were used. The PCR was performed in 25 μL volume containing 12.5 μL SYBR Premix Ex taq^{TM} (TaKaRa, Japan), 1 μL DNA template, 1 μL of each 10 μM primer pair

and 9.5 μL PCR grade water. The PCR thermal cycling parameters were as follows: 94ºC denatured for 5 min followed by 40 cycles at 94 ºC for 40 s, 60ºC for 45 s, 72ºC for 1 min; after the cycles the reaction mixtures were further incubated at 72ºC for 10 min. For quantification of the *arsC* gene, the primer pair amlt-42-f/amlt-376-r and smrc-42-f/smrc-376-r was used following the thermal cycling program: 95ºC for 5 min; 40 cycles at 95ºC for 30 s, 56ºC for 40 s and 72ºC for 1 min; after the cycles, the mixtures were further incubated at 72ºC for 5 min. For quantification of the *arrA* gene, the primer pair HAArrA-D1F/HAArrA-G2R was used following the thermal cycling program: 95ºC for 5 min; 40 cycles at 95ºC for 30 s, 53.5ºC for 30 s and 72ºC for 30 s; after the cycles, the mixtures were further incubated at 72ºC for 5 min. The preparation of standard curves and the quantification of *aroA*-like, *arsC* and *arrA* DNA were the same as 16S rDNA except for the primers and PCR amplification program. The efficiencies and the standard curves of these real time PCR assays are given in Table S1.

Construction of the *aroA*-like, *arrA* and *arsC* clone library. DNA from the rhizosphere soil and rice roots (Yangdao growth without rice straw addition, each with 4 replicates) in the pot experiment were used for the construction of *aroA*-like, *arrA* and *arsC* clone libraries. The 50-μL PCR reaction mixture contained 5.0 μL 10×PCR buffer, 4 μL dNTPs (2.5 mM each), 2 μL each 10 μM primer pair, 1.0 μL 5 U/μL *Taq* HS polymerase (Takara Bio Inc., Japan), 2 μL DNA template (1-10 ng) and 36 μL PCR grade water. Purified PCR products were ligated into pGEM-T Easy Vector and then transformed into *E. coli* JM109 according to the manufacturer's protocols. Positive clones (about 50 per library) were selected randomly from these clone libraries and sequenced. Sequences displaying more than 98% identity with each other were grouped into the same operational taxonomic units (OTUs). The representative sequences were then compared with entries in the NCBI database using the Basic Local Alignment Search Tool

(BLAST). Phylogenetic analysis was performed using MEGA, and the neighbor joining trees were constructed using Kimura two-parameter distance with 1000 replicates to produce bootstrap values. The cloned sequences were submitted to the NCBI database from JX489044-JX489133 (*arrA* gene from JX489044-JX489050; *aroA*-like gene from JX489051-JX489101; *arsC* gene from JX489102-JX489133).

Terminal restriction fragment length polymorphism (T-RFLP) analysis of *aroA*-like gene. DNA extracts from the rhizosphere soil and bulk soil were used for T-RFLP analysis. PCR amplification of *aroA*-like gene was conducted with each forward primer labeled by 6-carboxyfluorescein (FAM). The labeled PCR products were gel-purified with the Wizard SV Gel and PCR Clean-Up System and then digested by the restriction enzyme *Taq* I (Takara, Japan) at 37°C for 3 h. Digestion products were determined with 3130XL Genetic Analyzer (Applied Biosystems, USA). Relative abundances of each individual terminal restriction fragments (T-RFs) were calculated based on peak areas in relation to total peak area. Peaks that occurred in at least three replicates and with a percentage > 2% were listed.

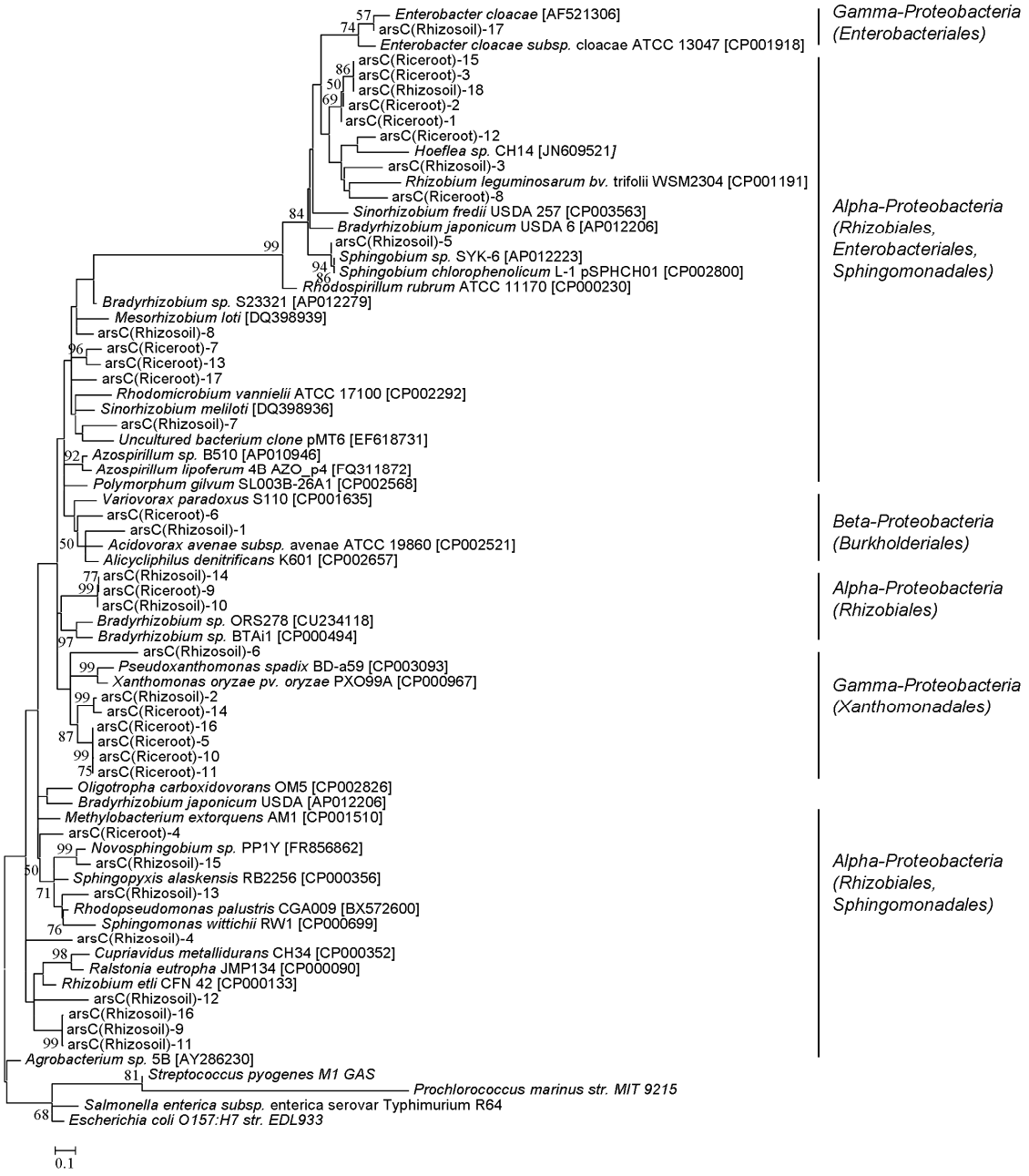

Figure S1. Neighbor-join phylogenetic tree of representative *arsC* sequences retrieved from the rice roots and rhizosphere soil.

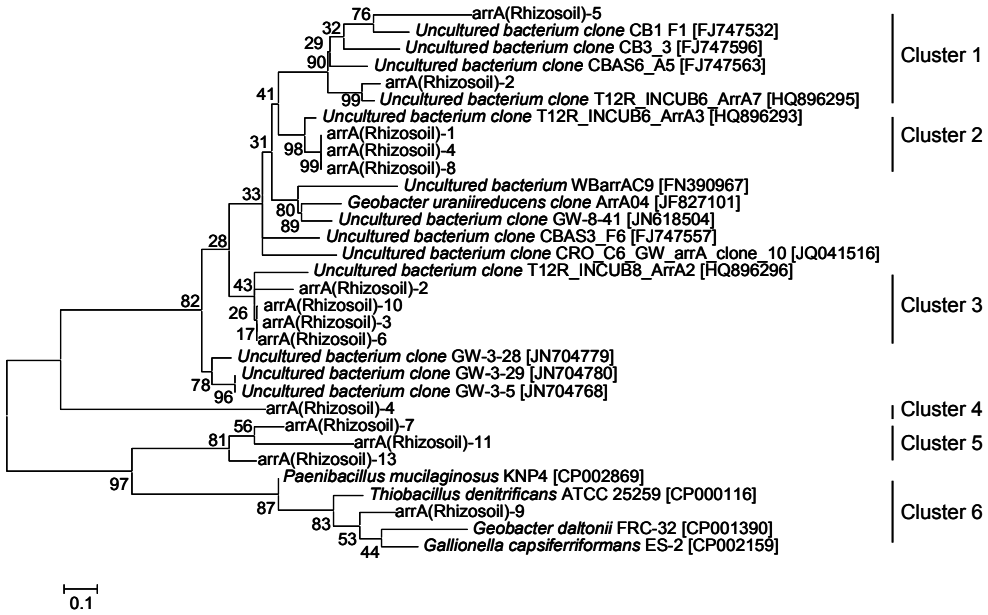

Figure S2. Neighbor-join phylogenetic tree of representative *arrA* sequences retrieved from the rice roots and rhizosphere soil. These sequences with the sequence number of "FJ" were amplified from the anaerobic aquifer sediments, estuarine sediment; these sequences "HQ" were from Meuse river basin; these sequences "FN" were from aquifer sediments; these sequences "JF" were from water treatment reactors.

Figure S3. Neighbor-join phylogenetic tree of representative *aroA*-like sequences retrieved from the rice roots and rhizosphere soil; the *in-silico* T-RFs are given in the trees.

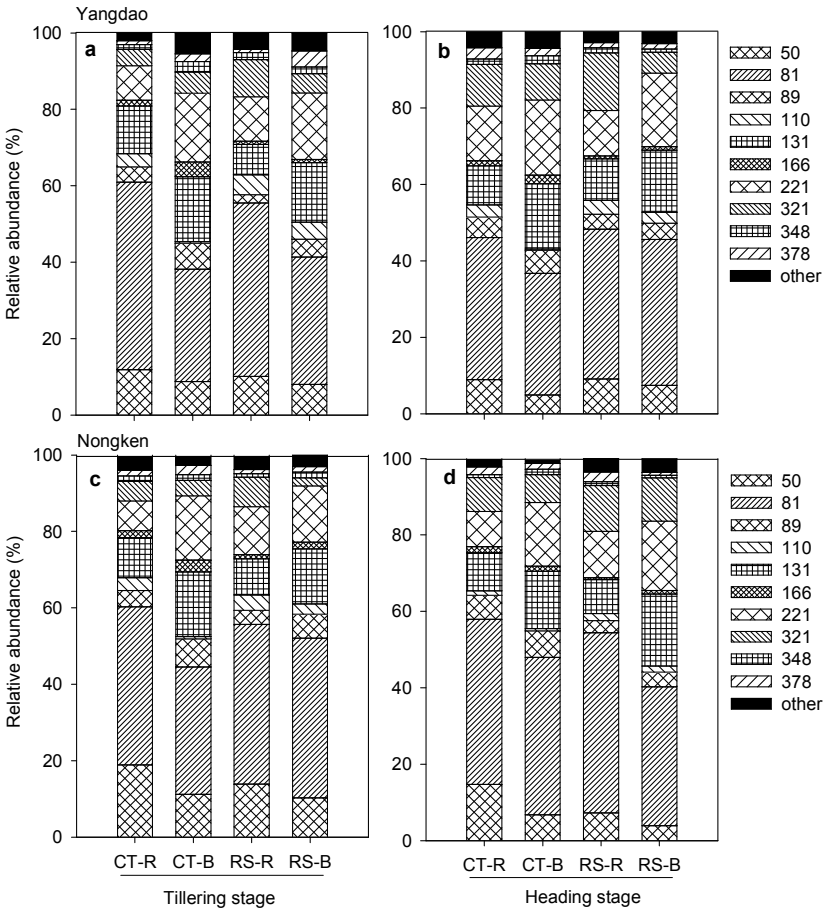

Figure S4. Community structure of *aroA*-like gene in the rhizosphere and bulk soils of Yangdao (a, b) and Nongken (c, d) grown with or without rice straw application at tillering and heading stages. CT-R, rhizosphere soil without rice straw application; CT-B, bulk soil without rice straw application; RS-R, rhizosphere soil with rice straw application; RS-B, bulk soil with rice straw application. The relative abundances of terminal restriction fragments (T-RFs) are digested by *Taq* I enzyme from PCR products of *aroA*-like genes.

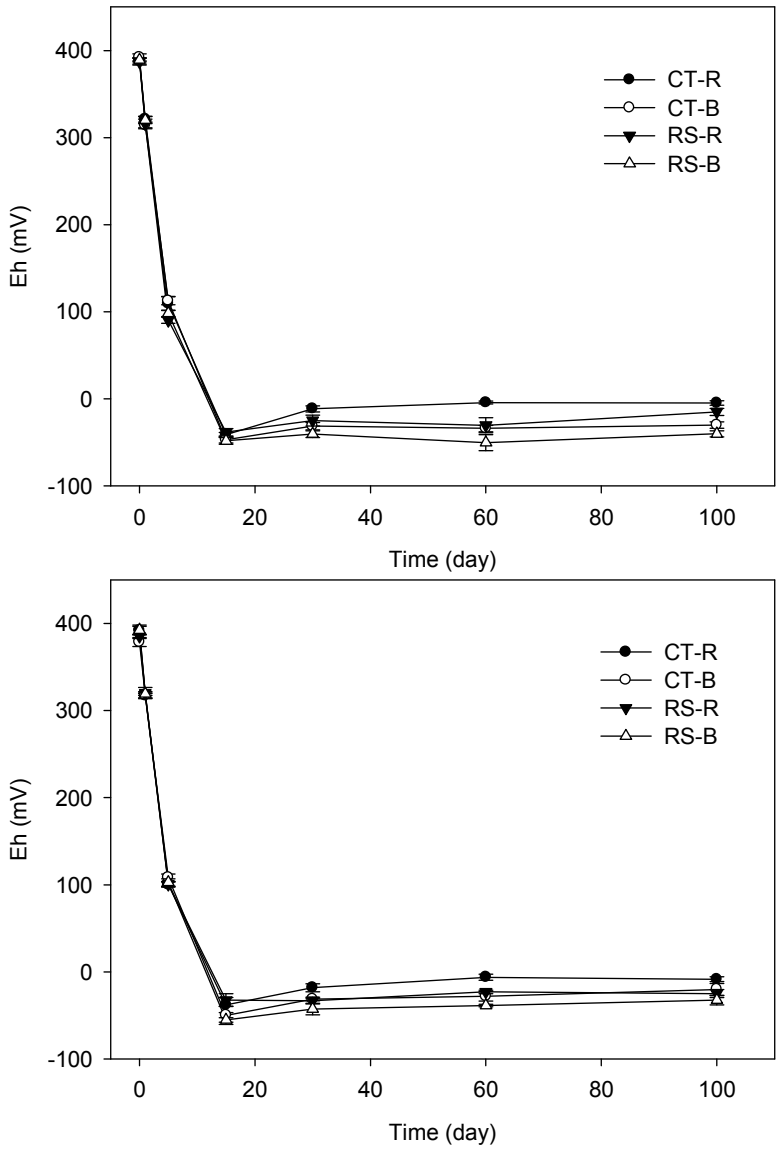

Figure S5. Redox potential (Eh) of the rhizosphere and bulk soils of Yangdao (a) and Nongken (b) during the experiment with or without rice straw application in paddy soil, in tillering and heading stage, respectively. CT-R, rhizosphere soil without rice straw application; CT-B, bulk soil without rice straw application; RS-R, rhizosphere soil with rice straw application; RS-B, bulk soil with rice straw application. Bars represent standard errors (n = 4).

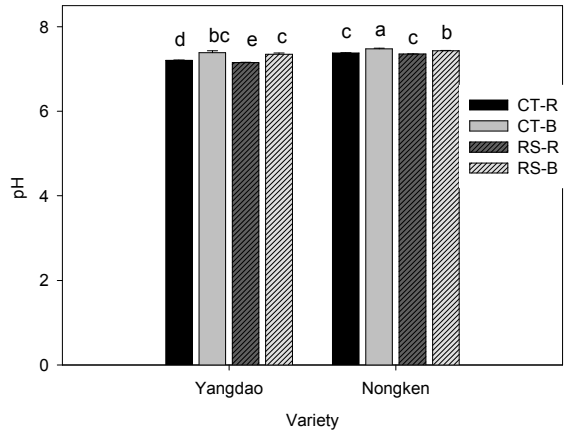

Figure S6. pH of the rhizosphere and bulk soils after the harvest of Yangdao and Nongken rice cultivars, with or without rice straw application in the paddy soil, respectively. pH of the soil after the rice cultivation was measured in a ratio of 1: 2.5 of soil and 1 mol L^{-1} KCl solution after the soil was air dried. CT-R, rhizosphere soil without rice straw application; CT-B, bulk soil without rice straw application; RS-R, rhizosphere soil with rice straw application; RS-B, bulk soil with rice straw application. Bars represent standard errors (n = 4). Different letters means significant differences between the treatments.

Table S1. Efficiency of the Q-PCR test for 16S rRNA, *aroA*-like, *arsC* and *arrA* gene.

	PCR efficiency (%)	R^2	Slope	Y-intercept
16S rRNA	97.2	0.998	-3.391	9.41
aroA-like	90.2	0.995	-3.524	2.40
arsC	90.9	0.992	-3.426	6.23
arrA	91.3	0.998	-3.521	6.02

Table S2. ANOVA test for copy number of 16s rRNA, *aroA*-like, *arsC* and *arrA* gene.

Factor	16s rRNA		*aroA*-like		*arsC*		*arrA*	
	F value	*P*	F value	*P*	F value	*P*	F value	*P*
Soil	61.56	0.00	15.85	0.00	30.04	0.00	4.85	0.03
Straw	151.58	0.00	96.79	0.00	62.49	0.00	11.19	0.00
Cultivar	13.49	0.00	19.76	0.00	10.76	0.00	0.54	0.47
Stage	337.75	0.00	40.67	0.00	33.67	0.00	0.91	0.35
Soil × Straw	25.98	0.00	4.91	0.03	5.28	0.03	0.02	0.90
Soil × Cultivar	0.00	0.99	0.07	0.80	1.49	0.23	0.09	0.76
Soil × Stage	43.01	0.00	4.22	0.05	4.81	0.03	1.15	0.29
Straw × Cultivar	4.06	0.05	18.63	0.00	35.93	0.00	1.50	0.23
Straw × Stage	82.79	0.00	30.82	0.00	6.04	0.02	0.49	0.49
Cultivar × Stage	6.91	0.01	0.10	0.75	14.68	0.00	5.31	0.03
Soil × Straw × Cultivar	0.05	0.82	0.01	0.93	1.28	0.26	0.90	0.35
Soil × Straw × Stage	24.28	0.00	4.89	0.03	0.67	0.42	0.25	0.62
Soil × Cultivar × Stage	0.00	0.95	1.05	0.31	6.81	0.01	2.14	0.15
Straw × Cultivar × Stage	1.26	0.27	0.50	0.48	35.01	0.00	5.69	0.02
Soil × Straw × Cultivar × Stage	0.15	0.70	0.63	0.43	6.53	0.01	0.97	0.33

Soil, rhizosphere soil or bulk soil; Straw, with or without rice straw addition; Cultivar, Yangdao or Nongken rice plants; Stage, stage of tillering and the beginning of the heading. $P < 0.05$ was taken as the significant differences.

Table S3. Plant biomass of Yangdao and Nongken grown with or without rice straw application into paddy soil.

Rice	Rice straw	Shoot (g)	Root (g)	Sum (g)
Yangdao	No	24.5 ± 2.1	6.3 ± 0.4	30.8 ± 1.2
	Yes	28.4 ± 2.2	7.2 ± 0.3 *	35.6 ± 2.0 *
Nongken	No	22.3 ± 1.8	6.0 ± 0.3	28.3 ± 1.7
	Yes	22.8 ± 0.9	5.8 ± 0.6	28.6 ± 0.9

Data are mean ± SE (n = 4). "*" means significant differences of plant biomass between the treatments with or without rice straw application in shoot, root biomass and total biomass of Yangdao and Nongken, respectively ($P < 0.05$).