Linkage between community diversity of sulfate-reducing microorganisms and methylmercury concentration in paddy soil

Yu-Rong Liu · Yuan-Ming Zheng · Li-Mei Zhang · Ji-Zheng He

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Abstract Sulfate-reducing microorganisms (SRM) have been thought to play a key role in mercury (Hg) methylation in anoxic environments. The current study examined the linkage between SRM abundance and diversity and contents of methylmercury (MeHg) in paddy soils collected from a historical Hg mining area in China. Soil profile samples were collected from four sites over a distance gradient downstream the Hg mining operation. Results showed that MeHg content in the soil of each site significantly decreased with the extending distance away from Hg mine. Soil MeHg content was correlated positively with abundance of SRM and the contents of organic matter (OM), NH_4^+ , SO_4^{2-} , and Hg. The abundances of SRM based on dissimilatory (bi) sulfite reductase (*dsrAB*) gene at 0–40 cm depths were higher than those at 40–80 cm depth at all sites. The SRM community composition varied in the soils of different sampling sites following terminal restriction fragment length polymorphism (T-RFLP) and phylogenetic analyses, which appeared to be correlated with contents of MeHg, OM, NH_4^+ , and SO_4^{2-} through canonical correspondence analysis. The dominant groups of SRM in the soils examined belonged to *Deltaproteobacteria* and some unknown SRM clusters that could have potential for Hg methylation. These results advance our understanding of the relationship between SRM and methylmercury concentration in paddy soil.

Keywords Rice field · Methylmercury · Sulfate-reducing microorganisms (SRM) · Microbial community · Phylogenetic analysis

Introduction

Inorganic mercury (Hg^{2+}) can be transformed into highly neurotoxic methylmercury (MeHg) via microbial processes that involve sulfate or iron reduction in anaerobic environments (Pak and Bartha 1998; Schaefer et al. 2011; Yu et al. 2012; Hsu-Kim et al. 2013). Most Hg in soil is detected as ionic Hg^{2+} species and shows relatively low toxicity, however, it still poses some environmental risk due to potential for Hg methylation followed by bioamplification in soil-plant systems (Meng et al. 2010; Liu et al. 2012). Earlier studies have examined microbial methylation of Hg in estuarine, wetland, and freshwater sediments (Gilmour et al. 1992; Heyes et al. 2006; Duran et al. 2008; Todorova et al. 2009), but little attention to date has been paid to the linkage between specific types of microorganism and Hg methylation in soil environments. Since some studies reported high MeHg contents in both of soil and rice in the Wanshan Hg mine area of Guizhou province in southwest China (Feng et al. 2008; Zhang et al. 2010), concerns have been raised about the fate of Hg and potential production of MeHg in the local soil-rice system. However, the mechanism of Hg methylation by microorganism in environment remains poorly understood (Park et al. 2013).

Sulfate-reducing microorganisms (SRM) is a phylogenetically diverse microbial group that contributes important function in soil including sulfate reduction, terminal oxidation of organic carbon, Hg methylation, and biodegradation of organic pollutants in anaerobic environments (Coates et al. 1996; Dhillon et al. 2003; Fitzgerald et al. 2007; He et al. 2010). Since the study on SRM pure-culture strains *Desulfovibrio desulfuricans* first demonstrating its capacity for Hg methylation (Choi et al. 1994), many studies linking Hg methylation to SRM species were conducted in natural habitats (Compeau and Bartha 1985; King et al. 2000; Benoit

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Y.-R. Liu · Y.-M. Zheng · L.-M. Zhang · J.-Z. He (✉)
State Key Laboratory of Urban and Regional Ecology,
Research Center for Eco-Environmental Sciences,
Chinese Academy of Sciences, Beijing 100085, China
e-mail: jzhe@rcees.ac.cn

et al. 2001; Ekstrom et al. 2003). Recently, it was suggested that only SRM belonging to the *Deltaproteobacteria* group can methylate Hg (Ranchou-Peyruse et al. 2009). Therefore, it was very essential to link the diversity of SRM to Hg methylation in environments because long-term historical evolution or related functional gene transfer may result in different potential for Hg methylation among various SRM. As a consequence, taxonomic diversity of SRM associating to Hg methylation in environment has been characterized by recent studies (Yu et al. 2010, 2012), which has enhanced our understanding on the contribution of SRM to MeHg production. Recently, activity and diversity of SRM in rice field have been widely reported (Scheid and Stubner 2001; Liu et al. 2009; Lin et al. 2010). However, no study is available regarding on the relationship of SRM community diversity with MeHg concentration in paddy soil.

Paddy soil usually produces an anaerobic environment attributing to the depletion of oxygen in the rice bulk after flooding, which can result in prevalence of anaerobic functional microbes (Liu et al. 2009). Wanshan area is one of important rice producing regions in southwest China, where mean (and range) of MeHg content in paddy soil was 2.7 (0.27–12) ng g⁻¹ according to previous reports (Feng et al. 2008; Zhang et al. 2010), a level that poses a serious threat to soil ecosystems and safety for human consumption of rice from this area. Therefore, it is essential to understand the methylation of Hg by the microorganisms in the paddy soil. The objective of this study was to link ecological characteristics of SRM to the MeHg concentration in the paddy soil, and the obtained results might be helpful to understand microbial methylation of Hg in rice field. The abundance and diversity of SRM were characterized based on the dissimilatory (bi) sulfite reductase (*dsrAB*) gene, which has been widely used as a biomarker for determining the SRM abundance and diversity in various environments (Geets et al. 2006; Liu et al. 2009; Lin et al. 2010).

Materials and methods

Study area and sampling

All soil samples examined here were collected from rice fields in the Wanshan Hg mining area, which is located in the eastern part of Guizhou province, southwest China (Fig. 1). The Wanshan area is a typical hilly and karstic terrain that has a subtropical humid climate. The Hg mining site is rated the largest Hg deposit in China, with mining activities first initiated in the Qin Dynasty (circa 221 B.C.). Large-scale production, however, ceased officially in 2001. A long-term history of Hg mining in the area has led to serious soil Hg contamination attributing to discharge of waste from the Hg mine. Four sites (referred to as S1, S2, S3, and S4, respectively)

were selected according to their distance away from the Hg mining site. S1, S2, and S3 are located in Wanshan district while S4 is located in Tongren district, in close proximity to Wanshan district. S4 was used as the control site in the current study, as it was at the greatest distance from the mine site and is considered to have little Hg contamination. Totally 48 soil samples (three replicates for each one) were taken at different depths (0–20, 20–40, 40–60, and 60–80 cm) at the 4 sites. Soil samples were passed through a 2.0-mm sieve and air-dried for general chemical analyses. For Hg, MeHg and molecular analyses, some of sieved soils were stored at -20 °C. Some basic soil chemical characteristics from the four sites are listed in Table 1.

Chemical analysis

Soil pH was determined using a soil to distilled water ratio of 1:2.5, and soil organic matter (OM) was determined using the K₂CrO₇ oxidation titration method (Walkley and Black 1934). Soil sulfate was extracted with deionized water in a ratio 1:5 and measured via ion chromatography using an AS14 column and an ECD50 conductivity detector (Dionex, USA). Ammonium in soil samples was extracted with 2 M KCl and determined using a Continuous Flow Analyser (SAN++, Skalar, Holland). For Hg analysis, soil samples were first digested with HNO₃ + HCl (10 ml, 1:1 v/v) in a teflon tube at 100 °C for 2 h. Total Hg concentration was then determined using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Soil samples were prepared for MeHg analysis using CuSO₄-methanol/solvent extraction according to the method outlined by (Liang et al. 2004), following which MeHg contents were determined using HPLC-ICP-MS.

Soil DNA Extraction and Quantification of *dsrAB* gene

DNA was extracted from 0.5 g of soil samples using Ultra-cleanTM soil DNA Isolation Kits (MoBio Laboratory, USA) according to the manufacturer's protocol. Real-time PCR assay is considered a highly sensitive method to quantify microbial abundance in environmental samples (Smith et al. 2006; Cao et al. 2012). Abundance of SRM was determined here using a quantitative real-time PCR method using an iCycler iQ5 thermocycler (Bio-Rad, USA), based on *dsrAB* gene sequences. *DsrAB* gene abundance was quantified in a 25-μl reaction mixture containing SYBR[®] Premix Ex Taq[™] (Takara Biotechnology), using the primer set as described by previous study (Liu et al. 2009).

To prepare standard curves, *dsrAB* gene sequences were amplified from extracted DNA using the primers DSRM2060F/DSR4R primers (Geets et al. 2006). PCR products were cloned into the pGEM-T Easy Vector (Promega). Plasmids used as standards for quantitative analyses were extracted from the corrected clones of

Fig. 1 Map of the study area and sampling locations in Wanshan Hg mining area (S1, S2, S3, and S4 represent site 1, site 2, site 3, and site 4, respectively)

target gene. Copy numbers of target gene was calculated directly from the concentrations of extracted plasmid DNA. Tenfold serial dilutions of a known copy number of the plasmid DNA were run in real-time PCR assays in triplicate to generate an external standard curve.

Terminal restriction fragment length polymorphism (T-RFLP) analysis

The method of T-RFLP analysis is one of most common molecular techniques for profiling microbial community

Table 1 Chemical properties of the paddy soil samples in the Wanshan Hg mining area^a

Sample (cm)	pH (H ₂ O)	OM (%)	SO ₄ ²⁻ (mg kg ⁻¹)	NH ₄ ⁺ (mg kg ⁻¹)
S1 (0–20)	7.43 ±0.04Ab ^b	4.13 ±0.70Aa	179.48 ±7.04Ab	11.35±0.70 Aa
S1 (20–40)	7.59±0.03 Aa	3.41±0.40 Aab	209.75±7.34 Aa	4.69 ±0.42Ab
S1 (40–60)	7.45 ±0.05ABb	2.37 ±0.14Ac	170.73 ±12.2Ab	5.32 ±1.80Ab
S1 (60–80)	7.46 ±0.05Ab	2.64 ±0.22Abc	171.08 ±18.48Ab	4.22 ±0.32Ab
S2 (0–20)	7.52±0.03 Ba	4.87 ±0.06ABa	361.28±29.24 Bb	14.61 ±1.33Ba
S2 (20–40)	7.43±0.03 Bb	4.12 ±0.55Ab	293.78±24.32 Bc	5.75 ±0.53Ab
S2 (40–60)	7.36 ±0.05Ac	3.94 ±0.40Bb	385.45 ±12.23Bb	5.65 ±0.66Ab
S2 (60–80)	7.16 ±0.04Bd	4.08±0.24 Bb	436.13 ±25.14Ba	5.09 ±0.68Ab
S3 (0–20)	7.40 ±0.04Ab	6.66±0.24 Ba	324.18 ±1.31Ca	23.29±1.40 Ca
S3 (20–40)	7.47 ±0.02Bb	6.39±0.09 Bab	247.95±3.40 Cb	8.08 ±1.37Bb
S3 (40–60)	7.52 ±0.09Ba	5.57±1.08 Cb	160.00±18.65Ac	6.94±0.50 Bb
S3 (60–80)	7.50 ±0.04Ab	5.14±0.17 Cb	158.03 ±16.01Ac	8.18±1.53 Bb
S4 (0–20)	7.01±0.08 Cc	5.22±0.60 Aa	168.83±19 Aa	8.97±0.59 Da
S4 (20–40)	7.46 ±0.05Bb	3.62 ±0.35Ab	62.87 ±14.73Db	3.48±3.32 Cb
S4 (40–60)	7.77±0.06 Ba	2.44±0.15 Ac	40.17 ±6.57Cb	3.58±0.46 Cb
S4 (60–80)	7.64 ±0.16Cb	1.57±0.19 Dd	52.75 ±4.95Cb	3.03±0.78 Cb

OM means organic matter

^a S1, S2, S3, and S4 represent site 1, site 2, site 3, and site 4, respectively

^b Concentrations do not share the same letter are significantly different ($P<0.05$)

Fig. 2 Hg and methylmercury (MeHg) concentrations in the different depth soil from the four sites. Capital letters indicate the significant differences among sites at the same soil depth ($P < 0.05$). Lowercase letters indicate the significant differences among soil depths at the same site ($P < 0.05$). Small bars show standard errors

(Li et al. 2013). For the T-RFLP analysis of *dsrAB* gene, the 5' end of DSRAB_F forward primer was labeled with 5-carboxyfluorescein (FAM) for PCR amplification (Schmalenberger et al. 2007). A 50- μ l reaction mixture contained 4 μ l of template DNA, 5 μ l 10 $\times$ *Ex Taq* buffer (Mg^{2+} plus), 1 μ l bovine serum albumin (BSA, 25 mM), 4 μ l dNTPs (2.5 mM) and 1 μ l of each primer of *dsrAB* gene

(10 μ M), and 0.5 μ l *Ex Taq HS* (5U μ l⁻¹; Takara, Japan). The thermal profile for amplification was as follows: 3 min at 94 $^{\circ}$ C; 35 cycles of 1 min at 94 $^{\circ}$ C, 1 min at 60 $^{\circ}$ C, and 3 min at 72 $^{\circ}$ C; and a final extension at 72 $^{\circ}$ C for 10 min. FAM-labeled PCR products were purified using the Wizard[®] SV Gel and PCR Clean-Up System (Promega, USA). Aliquots (5 μ l) were digested with 5 U *Hha I* (Takara, Japan) in a final volume of 20 μ l at 37 $^{\circ}$ C for 1.5 h. Digested amplicons were precipitated with 2 μ l of 3 M NaOAc and 40 μ l of absolute alcohol at -20 $^{\circ}$ C for 30 min. DNA pellets were washed in 70 % ethanol and then dissolved in distilled water. Fluorescently labeled fragments were separated and detected by capillary electrophoresis in an ABI PRISM 3700 DNA Analyzer (Applied Biosystems, USA) under GeneScan mode. T-RFLP profiles were analyzed with GeneMapper software (Applied Biosystems, USA). The relative abundance of the individual terminal restriction fragment (T-RF) was estimated by reference to the internal size standard GeneScan-1000 LIZ (Applied Biosystems, USA). Proportional abundances of individual T-RFs were determined as the peak area of the respective T-RF divided by the total peak area of all T-RFs detected within a fragment size that ranged between 50 and 1,000 bp. The relative abundance of a T-RF was calculated by dividing the peak height of the T-RF by the total peak height of all T-RFs in the profile. Peaks with heights $\leq 1\%$ of the total peak height were not included in further analyses.

Cloning, sequencing and phylogenetic analysis

In order to characterize *dsrAB* gene diversity based on the T-RFLP analysis, two clone libraries were constructed from top soil (0–20 cm depth) samples at S1 and S2, the sites that possessed the most diverse SRM communities. The *dsrAB* gene was amplified using the same primers as described above without FAM labeling. Purified PCR products (800 bp) were ligated into the pGEM-T Easy Vector (Promega, USA) and then transformed into *Escherichia coli* JM109 competent cells (Takara, Japan) following the manufacturer's instructions. In total, 200 *dsrAB* gene clones were sequenced. Sequences sharing >97% *dsrAB* gene nucleotide homology were grouped into the same

Table 2 Correlations between abundance of sulfate-reducing microorganisms (SRM), pH, and concentrations of SO_4^{2-} , OM, NH_4^+ , Hg, and MeHg in the soil samples

	Kendall's correlation coefficient					
	Abundance of SRM	SO ₄ ²⁻	OM	pH	NH ₄ ⁺	Hg
SO ₄ ²⁻	0.182					
OM	0.606**	0.344**				
pH	-0.145	0.335**	0.210*			
NH ₄ ⁺	0.413**	0.333**	0.566**	0.210*		
Hg	0.11	0.559**	0.196	-0.134	0.304**	
MeHg	0.374*	0.320*	0.306*	-0.02	0.335**	0.480**

* $P < 0.05$; ** $P < 0.01$, values in bold are statistically significant

Fig. 3 Sulfate-reducing microorganisms (SRM) abundance based on dissimilatory (bi) sulfite reductase (*dsrAB*) gene sequences. Capital letters indicate the significant differences among sites at the same soil depth ($P < 0.05$). Lowercase letters indicate the significant differences among soil depths at the same site ($P < 0.05$). Small bars show standard errors

Operational Taxonomy Units (OTUs). Representative sequences of each OTU were aligned and analyzed with Clustal X 1.81 (Thompson et al. 1994). A phylogenetic tree was constructed using MEGA version 5.0 employing a neighbor-joining method applying a Bootstrap value of 1,000. Sequences from different OTUs were deposited in GenBank nucleotide sequence database under the following accession numbers: JX976939–JX976996.

Statistical analysis

Copy numbers of *dsrAB* gene sequences were log-transformed to normalize their distributions for analysis and then subjected to Canonical Correspondence Analysis (CCA; Canoco 4.5 for

Windows) to explore the relationship between the various species detected (characterized by T-RFs with interspecies distances) and environmental factors (soil pH and the contents of OM, NH_4^+ , SO_4^{2-} , Hg, and MeHg, respectively). Variables to be included in the model were chosen by forward selection at the 0.05 baseline. Significance of the constrained ordination process was tested using a Monte Carlo permutation test. Two-way analysis of variance (ANOVA) was used to assess differences of all the obtained variables of soils among the depths and sites, and all the results were represented as means with associated standard errors. Statistical significance was assessed using SPSS 13.0 software. Bivariate correlations were conducted to link different parameters. $P < 0.05$ was considered to be significant.

Results and discussion

Hg and MeHg contents in the experimental soils

There was significant difference ($P < 0.05$) of Hg content among the top soils from the four sites (Fig. 2). Average Hg contents in the soils declined with geographical distance away from the historic Hg mine site, which could be attributed to the different levels input of Hg by atmospheric dispersing or surface water runoff. For example, the S1 is closest to Hg mine site, where the Hg contents were both highest in the top soil of S1 compared to the other sites. Furthermore, the lowest Hg contents in the top soil of S4 was comparable to background levels reported elsewhere across China (CNEMS 1990), suggesting that Hg contamination from mining had not reached S4 and also could be used as

Fig. 4 Terminal restriction fragment length polymorphism (T-RFLP) patterns for the *dsrAB* gene in different soil profiles

Fig. 5 Neighbour-joining tree of *dsrAB* gene sequences retrieved from top soils in the Wanshan Hg mining area. Designation of the clones includes the following information: site (S1, S2, S3, and S4), clone code, nucleotide sequence accession number in the GenBank, followed by the length of terminal restriction fragments and the number of clones with

similarities above 97 % to the listed sequence in the clone library in the parentheses. Clones in **bold** indicated the dominant T-RFs detected by T-RFLP analysis. Bootstrap values (>50 %) were indicated at branch points. The scale bar represented 5 % estimated sequence divergence

Fig. 5 (continued)

an adequate control site in the current study. Lower ($P < 0.05$) Hg contents in bottom soil (60–80 cm) of the four sites suggested this region originally probably had low natural Hg background. However, Hg content in top soil of S1 was approximately 20 times higher than mean Hg background levels. Since top soil is generally considered to be the arable layer, high Hg level in this layer poses a serious health risk to local rice safety due to its possible transformation and accumulation by rice roots.

Similarly, average MeHg contents in the soils decreased with geographical distance away from the historic Hg mine site. Higher MeHg concentrations of top soil over those of deep soil were found at S1 and S2, while no significant difference of them was observed in the S3 and S4. These could be explained partially by that the MeHg concentration in the soils was significantly correlated with Hg content (Table 2). Generally, the soil having higher Hg content would probably provide more

bioavailable Hg. Therefore, total Hg content in soil could be one of important factors potentially influencing MeHg concentration. Besides, MeHg concentration in the soils was also covaried by several other environmental factors including the abundance of SRM and contents of OM, SO_4^{2-} , and NH_4^+ . SRMs are considered as main producers of MeHg⁺ in anaerobic environments (Compeau and Bartha 1985; Compeau et al. 1992), so the abundance of SRM can potentially impact the MeHg content in the paddy soils. The correlation here between MeHg content and SRM abundance might further supported this point. In addition, our results are in accordance with previous studies suggesting that SO_4^{2-} concentration was also an important factor that governs Hg methylation capacity in the environment (Branfireun et al. 1999; Acha et al. 2005; Sitte et al. 2010). High SO_4^{2-} content in the soils examined might be helpful to the prevalence of some SRM that produced MeHg (Han et al. 2010), and this could explain partially why the SO_4^{2-}

concentration are significantly correlated to the MeHg content. It was also suggested that OM can form strong complexes with MeHg^+ (Khwaja et al. 2010), which would be absorbed and fixed on soil. The correlation between the contents of MeHg and OM in the paddy soils might further support the previous observation. Therefore, avoidance of exogenous OM input into rice field during aerobic cultivation of rice plants could be an effective way to reduce the accumulation of MeHg in soil. Intriguingly, the content of NH_4^+ covariates on the MeHg concentration in the soils according to the Table 2, which could attribute to that the OM degradation in soils induced to the accumulation of NH_4^+ under anaerobic conditions.

Abundance of SRM in soils

The abundance of SRM in tested soil samples was positively correlated with the contents of OM, NH_4^+ and MeHg (Table 2, Fig. 3). Average abundance of *dsrAB* gene in these soils was 6.04×10^8 , which was comparable to the previous studies based on the sites without Hg or other pollution (Scheid and Stubner 2001; Liu et al. 2009). These could indicate that the SRM abundance was not influenced by the level of pollution. No significant relationship was observed between the abundance of SRM and the Hg contents in the soils at the four sites, suggesting that long-term Hg contamination did not changed abundance of SRM in the present study. This result was different from the previous studies based on short-term incubation experiments showing a negative influence of Hg pollution on the abundance of micro-organism (Liu et al. 2010; Zhou et al. 2012). These different effects could be attributed to their different polluted duration by Hg, and some microbial groups in soil might exhibit some resistance or adaptation to long-term Hg stress (Barkay et al. 2003). For each site, total abundances of the *dsrAB* gene in 0–40 cm soil were significantly higher than those in the 40–80 cm soil ($P < 0.05$). Those could be partially explained by that OM contents in the layers were higher compared to those in 40–80 cm depth soils. Abundance of SRM was correlated significantly with OM contents based on results of the correlation analysis, suggesting that relatively high levels of OM may provide favorable conditions for SRM growth in the soils. Additionally, more OM content in soil might induce more ammonification resulting in accumulation of NH_4^+ in anaerobic conditions, and this may be why NH_4^+ content was significantly correlated with SRM abundance.

Diversity of SRM and phylogenetic analysis

The present study used T-RFLP method to analysis the community structure of SRM in the paddy soils with different Hg contents. A total of 29 T-RFs were detected at the four soil sites where 59, 96, 113, and 158 bp T-RFs dominated (>50 %) for SRM (Fig. 4). Phylogenetic analysis of *dsrAB* gene

revealed 9 distinct clusters, and their affiliations to distinct T-RFs are evident in Fig. 5. Less than 30 % of sequences obtained were grouped into a known SRM cluster. The 113 bp T-RF were mainly grouped into *Desulfobacteraceae* in the both of clone libraries, while most 96 bp T-RFs were affiliated with the *Syntrophobacteraceae* group. Overall, 40 % of detected sequences was grouped into unknown cluster 2, which mainly consisted of most 59 and 158 T-RFs.

No significant difference of diversity (Shannon index) was observed for among the soils from different sites or depths based on T-RFLP analysis of *dsrAB* (data not shown). However the percentage of specific sequences in each soil at the four sites was different (Fig. 4), which could exert different functions of Hg methylation because of taxonomic diversity (Yu et al. 2010). High proportion of 214 T-RF was found in the soil of S1 where the MeHg contents were highest, while no or small level of the 214 T-RF could be observed at the other sites. This might suggest that this uncultured group could have adapted and exhibited some resistance to the Hg stress. Intriguingly, the CK site (S4) had the lowest proportion of 96 bp T-RF (dominated by *Syntrophobacteraceae* group) compared to that at the other higher MeHg content sites. However, it is difficult to clarify its associations with the MeHg contents in the soils examined according to this study, which need further evidence to confirm this point. In addition, relatively high abundance of 604 bp T-RF belonging to *Peptococcaceae* was found in S4, which seemed to be sensitive to Hg stress and just grow well in a low Hg environment.

Fig. 6 Canonical correspondence analysis (CCA) between the SRM community and environmental variables of pH value, the contents of organic matter (OM), total Hg, methylmercury (MeHg), NH_4^+ , and SO_4^{2-} in the soils. The meaning of symbols: circle represents S1, box represents S2, right triangle represents S3, and asterisk represents S4

All of known SRM in the present study belonged to *Deltaproteobacteria* that had been suggested the only group can participate in Hg methylation (Ranchou-Peyruse et al. 2009). Some of unknown SRM could also play crucial role in Hg methylation, however up to date no evidence can prove it. Nearly half of the known *dsrAB* sequences detected here belonged to the *Syntrophobacteraceae*. This may result from high sulfate concentrations in the soils since *Syntrophobacteraceae* have been suggested to be more common in presence of high sulfate content (Leloup et al. 2007). The majority of uncultured *dsrAB* sequences detected here were related to environmental sequences retrieved from mudflat (Leloup et al. 2006), everglades (Castro et al. 2002), marine sediments (Jiang et al. 2009), hot springs (Fishbain et al. 2003), and paddy soil (He et al. 2010).

Relationship of SRM communities and soil environmental factors

Results of the CCA analysis based on the T-RFLP data suggested that SRM community structure in the soils studied was influenced or covariated substantially by soil environmental variables, and the effect of individual environmental factors was different in each of the four sites (Fig. 6). The four soil horizon samples taken at each site in general, clustered together in the CCA profile, indicating that distance from the Hg mining site influenced SRM community distribution.

It appears that soil SRM community structure at S1 could be influenced by MeHg content according to the CCA profiles, which might be associated with the higher MeHg content posing a relatively higher toxicity to soil microorganisms. Soil OM and NH_4^+ contents appeared to have a strong effect on the SRM community diversity at S3. Previous studies have suggested that the OM levels play important role on influencing microbial communities (He et al. 2007; Leloup et al. 2009), and results of our study add partial support for this view. As a substrate for sulfate reduction in soils, sulfate concentration may also exert a crucial role in influencing SRM community composition according to the CCA analysis. To get a more comprehensive insight into the effects of environmental factors on the SRM community in paddy soils, further studies using samples from different soil types and at a larger spatial scale are therefore needed.

Conclusions

This study showed a linkage between the SRM diversity and MeHg levels, indicated by the analysis of correlation between the abundance of SRM and concentrations of MeHg in the soils. The association implied the importance of SRM for MeHg concentration in anaerobic rice fields. However, the content of OM, SO_4^{2-} , NH_4^+ , and Hg could also impact the MeHg concentration in paddy soil. Soil SRM community

composition varied among the different sites, which could be determined by several environmental variables, such as OM content. Some new unknown SRM groups were reported in this study, which is of importance to recognize the relationship between SRM diversity and Hg methylation in paddy soil.

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