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ORIGINAL ARTICLE

Survey of sulfur-oxidizing bacterial community in the Pearl River water using *soxB*, *sqr*, and *dsrA* as molecular biomarkers

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Abstract

In this study, we surveyed the abundance and diversity of three sulfur oxidation genes (*sqr*, *soxB,* and *dsrA*) using quantitative assays and Miseq high-throughput sequencing. The quantitative assays revealed that *soxB* is more abundant than *sqr* and *dsrA* and is the main contributor to sulfur oxidation. In the diversity analysis, the SOB community mainly comprised the classes *Nitrospira*, *Alphaproteobacteria*, *Betaproteobacteria*, and *Gammaproteobacteria*. The genera *Gallionella*, *Hydrogenophaga*, *Limnohabitans*, *Methylomonas*, *Nitrospira*, *Rhodoferax,* and *Sulfuritalea* were abundant in the communities for *sqr*; *Dechloromonas*, *Limnohabitans*, *Paracoccus*, *Sulfuritalea*, *Sulfitobacter,* and *Thiobacillus* were abundant in communities for *soxB*; *Sulfuritalea*, *Sulfurisoma*, and *Thiobacillus* were abundant in communities for *dsrA*. This study presented a high diversity of SOB species and functional sulfur-oxidizing genes in Pearl River via high-throughput sequencing, suggesting that the aquatic ecosystem has great potential to scavenge the sulfur pollutants by itself.

Keywords Sulfur-oxidizing bacteria · Pearl River · *soxB* · *sqr* · *dsrA* · High-throughput sequencing

Introduction

With the reform and opening, China's economy has grown quickly and is maintaining a high growth rate, producing nearly 11.6% of the total global GDP and consuming about 46.3% of the world's total steel in 2012 (Zhao et al. 2017). The Pearl River Delta Region is one of the most developed and industrialized zones in China. It contributed to 9.1% of the national GDP in 2014. However, rapid population growth, industrial development, and agricultural production have led to increasing environmental pollution in this region. The Pearl River is the 2nd largest river in China and the 13th largest river in the world. Anthropogenic activity and economic development have caused an unforeseen damage to the ecosystem of the Pearl River due to the presence of large amount of pollutants, such as the sulfur compounds that are being illegally discharged from the cloth printing and dyeing factories, tanneries, paper mills, petrochemical refineries, etc. Thus, a good understanding of sulfur biogeochemistry in the Pearl River ecosystem is helpful to know how the ecosystem itself scavenges sulfur pollutants.

Biological oxidation of inorganic sulfur compounds is an important process in the global sulfur cycle. In this process, the energy generated from sulfur oxidation is used by aerobic chemoautotrophs for carbon dioxide fixation, while the electrons derived from the reduced sulfur compounds are used by anoxygenic photoautotrophs for carbon dioxide reduction (Friedrich et al. 2005). Because sulfur occurs in a broad range of oxidation states (− 2 to + 6), a wide variety of redox enzymes produced by different microorganisms have been detected in artificial or natural ecosystems. These enzymes include the sulfur-oxidizing (Sox) enzyme system, which is encoded by the *soxTRSVWXYZABCDEFGH* gene cluster (Rother et al. 2001; Friedrich et al. 2005; Ghosh and Dam 2009); the sulfide:quinone oxidoreductase (SQR), which is encoded by *sqr* (Ghosh and Dam 2009; Chan et al. 2009); and the reverse dissimilatory sulfite reductase (Dsr)

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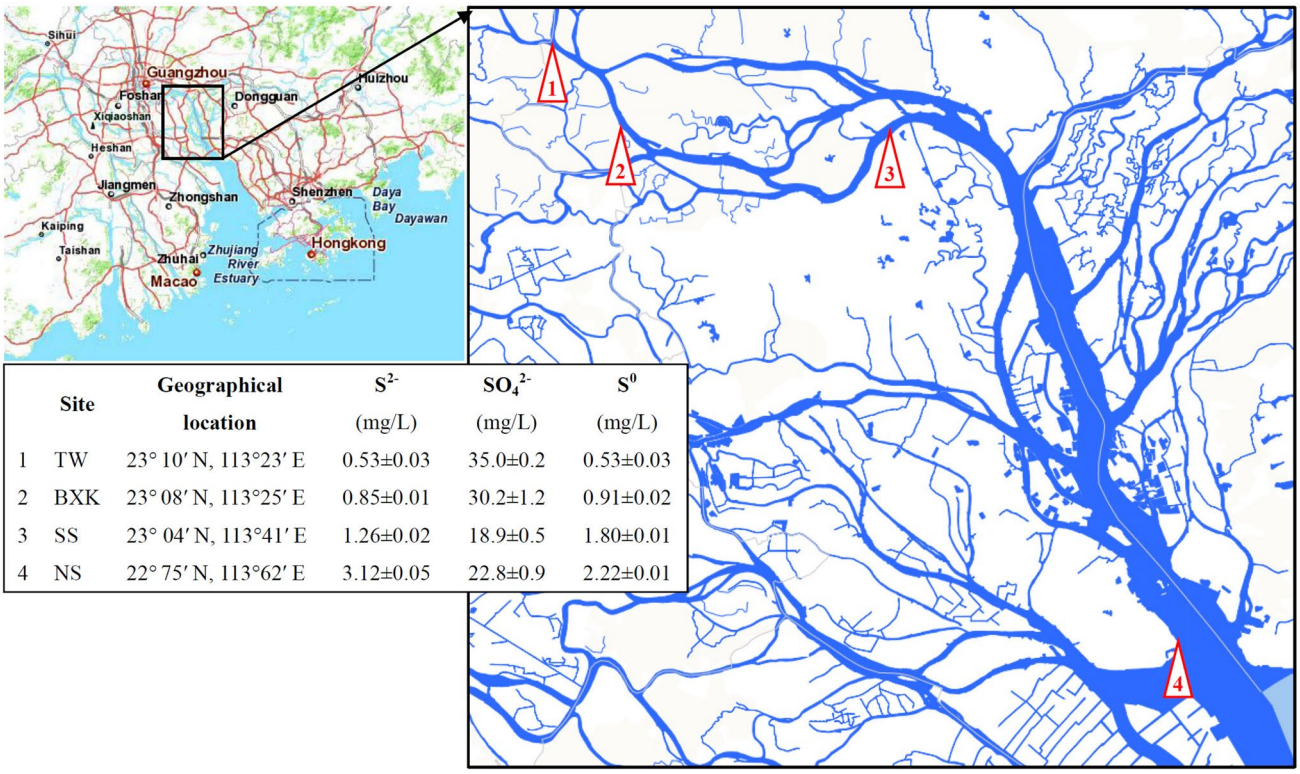
system, which is encoded by the *dsrABEFHCMKLJOPNRS* gene cluster (Dahl et al. 2005).

Many studies using one or more of these functional sulfur oxidation genes as molecular biomarkers have been conducted to examine communities of sulfur-oxidizing bacteria (SOB) and their roles in various environments. *Thiobacillus*-like *Betaproteobacteria* has been found to be the dominant SOB group in soil after sulfur application using *soxB* clone library sequencing (Tourna et al. 2014); *Sulfurimonas, Thiobacillus, Thioclava, Thiohalomonas*, and *Dechloromonas* have been detected as the most frequent SOB in water-flooded petroleum reservoirs (Tian et al. 2017); *Thioalkalivibrio, Halothiobacillus, Marinobacter*, and *Halochromatium* have been detected in hypersaline and soda lakes using *soxB* as molecular marker (Tourova et al. 2013); *soxB* clone library analyses suggested that *Chromatiales* and *Thiotrichales* were dominant SOB members in vegetated salt marsh sediments (Thomas et al. 2014). In a previous work, we had designed three primer pairs specific for *soxB*, *sqr,* and *dsrA*, which are known parts of sulfur oxidation genes, to reveal the main SOB that participated in the removal of sulfide from sulfide-rich wastewater in a treatment plant. The results as well as some other reports based on our designed primers validated the suitability of these primers for studying the SOB community (Luo et al. 2011; Kojima et al. 2014). Until now, the SOB communities distributed in various environments were studied using functional genes-based clone library, but no study has been reported via high-throughput sequencing on Illumina (Miseq and Hiseq) or Roche (454 pyrosequencing) platforms. In this study, we applied the Miseq high-throughput sequencing method to survey the SOB community in the Pearl River water using three kinds of sulfur oxidation genes as molecular biomarkers. Diverse SOB species were detected along the Pearl River and the result provides useful information regarding the potential bacterial groups for scavenging sulfur pollutants in the Pearl River.

Materials and methods

Water sampling

Four water samples were collected from the Guangzhou section of the Pearl River in September 2014; distribution of the sampling sites represents the upper, middle, and lower reaches of the Pearl River that flows through Guangzhou City; the geographical location and chemical characteristics of each site are shown in Fig. 1. For each site, five water samples were randomly collected from the top 0.5 m, mixed



	Site	Geographical location	S^{2-} (mg/L)	SO_4^{2-} (mg/L)	S^0 (mg/L)
1	TW	23° 10′ N, 113°23′ E	0.53±0.03	35.0±0.2	0.53±0.03
2	BXK	23° 08′ N, 113°25′ E	0.85±0.01	30.2±1.2	0.91±0.02
3	SS	23° 04′ N, 113°41′ E	1.26±0.02	18.9±0.5	1.80±0.01
4	NS	22° 75′ N, 113°62′ E	3.12±0.05	22.8±0.9	2.22±0.01

Fig. 1 Geographical locations and sulfur compound concentrations of water sampling sites. *TW* Tanwei dock, *BXK* Baixianke dock; *SS* Suishi dock; *NS* Nansha dock

together, and considered as one water sample. The samples were pre-filtered through a filter paper to remove macroparticles and then filtered through a 0.22 µm membrane (Millipore, USA) to obtain a microbial pellet. The pellets were stored at − 80 °C until extraction of total genomic DNA. The filtered water was used to determine the concentrations of sulfide, sulfur, and sulfate.

Determination of sulfur compounds

The concentration of sulfate was measured by ion chromatography (ICS 900, Dionex, USA) using a precolumn Dionex IonPac® AS14, with 10 mM carbonate buffer solution as the mobile phase. The sulfide concentration was measured by the methylene blue method (Fogo and Popowsky 1949). Elemental sulfur was extracted with chloroform, and the air-dried extract was dissolved in ethanol and oxidized by alkaline sodium hypobromite solution, as described by Tabatabai and Bremner (Tabatabai and Bremner 1970); the resultant sulfate was measured by ion chromatography as described above.

Genomic DNA extraction

Genomic DNA from water samples was extracted using the BioFast Soil Genomic DNA extraction kit (Biofux, Hangzhou, China), according to the manufacturer's instructions. The extracted DNA was dissolved in Tris–EDTA (TE) buffer (50 mmol L^{-1}; pH 8.0) and its purity was determined using a UV spectrophotometer (NanoDrop 2000, Thermo Scientific, USA) and by agarose gel electrophoresis. The DNA samples were stored at – 80 °C for further analysis such as PCR amplification and quantitative PCR.

High-throughput sequencing of functional genes

The three functional genes, *sqr*, *soxB,* and *dsrA*, were PCR-amplified using previously designed primer pairs *sqr-473F/982R*, *soxB-704F/1199R,* and *dsrA-625F/877R,* respectively (Luo et al. 2011). A unique 8 bp barcode was added at the 5′-end of each forward primer. PCRs were performed in a 25 µl reaction mixture containing 1 µl of DNA template, 12.5 µl of PCR mix (TIANGEN, China), and 1 µl of forward and reverse primers (10 µM). The PCR products were purified using the TaKaRa Agarose Gel DNA Purification Kit ver. 4.0 (TaKaRa, Dalian, China) and quantified using a spectrophotometer (NanoDrop 2000, Thermo Scientific, USA). Sequencing was performed on a MiSeq 300 sequencer (Illumina, San Diego, USA) by IGE Biotechnology (Guangzhou, China).

Clean data were obtained after filtering reads containing ambiguities and mismatches with specific primers or those with average quality values less than 20. Data were processed using Mothur (Schloss et al. 2009) and QIIME (Caporaso et al. 2010) and OTUs were clustered at the 90 and 95% nucleotide sequence identity levels. The taxonomic classifications of *sqr, soxB,* and *dsrA* were determined using the BLAST× program in BLAST+ by searching against the NR database.

Alpha diversity indices including the Chaol, Shannon, and Simpson were calculated and the SOB communities from all the sites were compared using the Mothur program. A neighbor-joining tree was created using Mega 5.0 software (Tamura et al. 2011) after computing the evolutionary distances via the Poisson correction method. A heat map analysis was conducted to compare the communities in different samples using the vegan package in R ver. 3.2.3 (https://www.r-project.org/). Correlations between the communities and environmental variables were tested by canonical correlation analysis (CCA), followed by a manual deselection of collinear environmental variables, and significance tests of Monte Carlo permutations were performed to construct optimal models (ter Braak and Smilauer 2002).

Quantitative PCR

PCR products of *sqr*, *soxB,* and *dsrA* from the genomic DNA isolated from the river water were cloned using the Mighty TA-cloning kit (TaKaRa, Dalian, China). Plasmids containing *sqr*, *soxB,* or *dsrA* were extracted using the TaKaRa MiniBEST Plasmid Purification kit (TaKaRa, Dalian, China) and digested using *Eco*RV (TaKaRa, Dalian, China). After separation on an agarose gel, the *sqr*, *soxB,* and *dsrA* fragments were purified using the TaKaRa Agarose Gel DNA Purification kit (TaKaRa, Dalian, China) and used to construct the respective standard curves. Concentration of DNA was determined using a spectrophotometer (NanoDrop 2000, Thermo Scientific, USA). Standard curves were prepared using 6 serial tenfold dilutions ranging from 10^3 to 10^8 gene copies/mL. The DNA was quantified by determining the copy number as well as the concentration and base pair composition of related functional genes. Quantitative PCR was performed in triplicate on an ABI 7500 Fast real-time PCR system (Applied Biosystems) using SYBR Premix Ex *Taq*™ II (TaKaRa, Dalian, China). The cycling protocol was as follows: initial denaturation for 30 s at 95 °C, followed by 40 cycles of denaturation for 10 s at 95 °C, annealing for 30 s at 52 °C for *sqr*, 55 °C for *soxB*, and 60 °C for *dsrA*, and elongation for 45 s at 72 °C. The correlation coefficients (r^2) of the standard curves were > 0.999. The amplification efficiencies (*E*) of *sqr*, *soxB,* and *dsrA* were 87.6, 90.3, and 83.6%, respectively.

Nucleotide sequence accession number

Sequence data are available in the NCBI Sequence Read Archive under accession number SRR3623345 for *sqr*, SRR3624939 for *soxB*, and SRR3624940 for *dsrA*.

Results

Chemical characteristics and functional gene content of the water

The concentrations of sulfide, sulfate, and sulfur in the water ranged from 0.53 to 3.12, 18.9 to 35.0, and 0.53 to 2.22 mg L^{-1}, respectively; the content of sulfide and sulfur in the four sampling sites increased in the downstream direction, while that of sulfate decreased (Fig. 1). Abundance of *sqr*, *soxB*, and *dsrA* in the samples ranged from 1.2 to 8.1×10^4 copies mL^{-1}, 1.2 to 3.0×10^5 copies mL^{-1}, and 3.7 to 8.1×10^3 copies mL^{-1}, respectively; in the sampling sites, *sqr* was most abundant in BXK; *soxB*, in NS; and *dsrA*, in TW (Fig. 2).

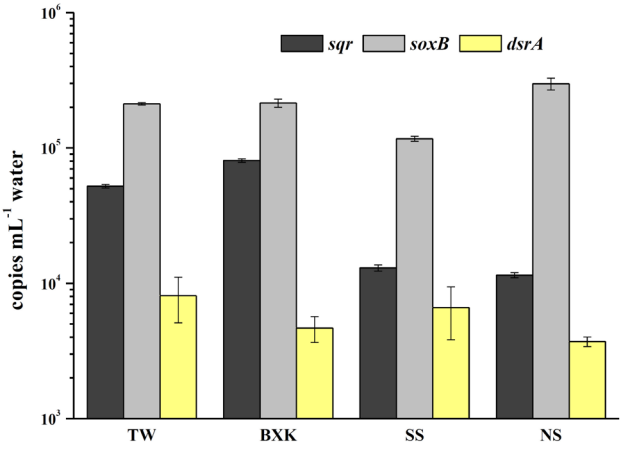


Fig. 2 Abundances of *sqr*, *soxB* and *dsrA* genes in TW, BXK, SS, and NS

Diversity of sulfur oxidation genes in the Pearl River water

High-throughput sequencing generated an average of 42,937, 8364, and 47,045 filtered reads for *sqr*, *soxB*, and *dsrA*, respectively. The Shannon–Wiener estimator indicated that the three genes were enriched in the Pearl River water, with *sqr*, *soxB*, and *dsrA* abundant in NS, SS, and TW, respectively (Table 1). In the BLASTx analysis, 12,988 reads, accounting for 7.05–8.39% of the total *sqr* reads had no hits; 2157 reads, accounting for 0.77%–2.42% of the total *soxB* reads, had no hits; and 3040 reads, accounting for 0.70%–2.28% of the total *dsrA* reads, had no hits (data not shown).

The remaining sequences were assigned to taxonomic classifications and are shown in Fig. 3. Approximately, 46.92–59.96% of the total *sqr* reads, 50.79–74.77% of the total *soxB* reads, and 61.10–69.17% of the total *dsrA* reads were assigned to the uncultured or unclassified bacteria. The remaining *sqr* sequences were assigned to four classes, *Nitrospira* (2.34–4.58%), *Alphaproteobacteria* (3.06–7.35%), *Betaproteobacteria* (29.58–39.49%), and *Gammaproteobacteria* (2.76–8.02%). They covered 11

Table 1 Alpha diversity of high-throughput sequencing

Sample	No of reads	Observed OTUs		Chaol	Shannon	Ace	Simpson	Good's coverage
		90%	95%					
sqr								
TW	22,272	1064	1735	2626	771	2843	0.98	096
BXK	33,911	1314	2180	3141	729	3491	0.97	097
SS	23,892	1419	2096	3119	696	3494	0.94	095
NS	91,675	2518	4362	4684	776	4974	0.95	099
soxB								
TW	8261	907	1327	1843	791	2067	0.97	090
BXK	9615	1023	1570	2163	854	2449	0.98	090
SS	5824	1074	1138	1723	909	2036	0.99	080
NS	9759	961	1195	1599	718	1761	0.95	094
dsrA								
TW	49,696	768	1764	3268	844	3416	0.99	099
BXK	25,201	754	1627	2471	705	2610	0.96	097
SS	76,055	1037	2809	2609	684	2651	0.97	098
NS	37,228	961	2483	2936	813	3059	0.97	098

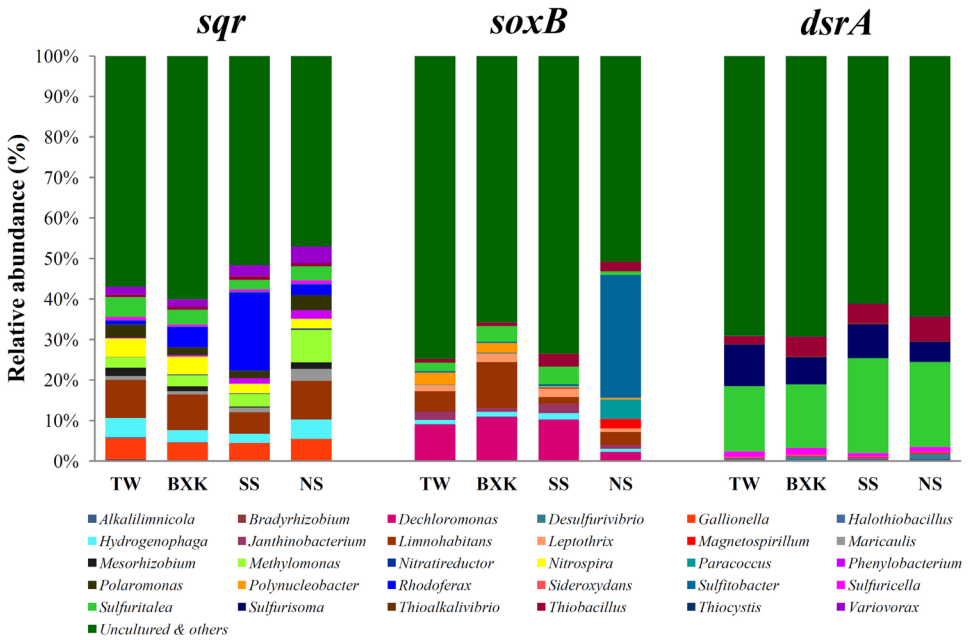


Fig. 3 Relative abundance as a percentage of the *sqr*, *soxB,* and *dsrA* bacterial community at the genus level in TW, BXK, SS, and NS

orders, 13 families, and 18 genera. The dominant genera were *Gallionella* (4.33–5.37%), *Hydrogenophaga* (2.22–4.76%), *Limnohabitans* (5.32–9.51%), *Methylomonas* (2.52–7.99%), *Nitrospira* (2.34–4.58%), *Rhodoferax* (0.91–19.26%), and *Sulfuritalea* (2.30–4.90%). The remaining *soxB* sequences were assigned to three classes, *Alphaproteobacteria* (0.51–37.54%), *Betaproteobacteria* (11.54–33.50%), and *Gammaproteobacteria* (0.07–0.12%). They covered 6 orders, 8 families, and 12 genera. The dominant genera were *Dechloromonas* (2.22–10.90%), *Limnohabitans* (1.73–11.41%), *Paracoccus* (0.10–4.79%), *Sulfuritalea* (0.88–4.36%), *Sulfitobacter* (0.31–30.39%), and *Thiobacillus* (0.84–3.14%). The remaining *dsrA* sequences were assigned to four classes, including Alphaproteobacteria (0.13–0.27%), Betaproteobacteria (29.31–37.66%), Gammaproteobacteria (0.31–0.91%), and Deltaproteobacteria (0.48–1.22%). They covered seven orders, eight families, and ten genera. The dominant genera were *Sulfuritalea* (15.70–23.41%), *Sulfurisoma* (4.99–10.32%), and *Thiobacillus* (1.97–6.02%).

Dominant SOB members in the Pearl River water

Among the *sqr* sequences, 27 OTUs showed a relative abundance of > 1%. Heat map analysis of these key OTUs revealed that OTU5726 represented sequences that were most abundant in TW (6.64%) and BXK (6.77%), and OTU15178 and OTU15918 represented sequences that were most abundant in SS (18.58%) and NS (3.73%), respectively (Fig. 4a). Phylogenetic analysis revealed that OTU5726 was closely related to uncultured bacteria; OTU15178, to the genus *Rhodoferax*; and OTU15918, to the genus *Variovorax* (Fig. 4a). Among the *soxB* sequences, 17 OTUs showed a relative abundance of > 1%. Heat map analysis of these key OTUs revealed that OTU6751, OTU9791, OTU11231, and OTU509 represented sequences that were most abundant in TW (10.23%), BXK (6.45%), SS (2.85%), and NS (16.26%), respectively (Fig. 4b). Phylogenetic analysis revealed that OTU6751 and OTU11231 were closely related to uncultured bacteria; OTU9791, to the genus *Limnohabitans*; and OTU509, to the genus *Sulfitobacter* (Fig. 4b). Among the *dsrA* sequences, 38 OTUs showed a relative abundance of > 1%. Heat map analysis of these key OTUs revealed that OTU5122, OTU1059, OTU3027, and OTU4024 represented sequences that were most abundant in TW (12.71%), BXK (13.80%), SS (4.99%), and NS (14.48%), respectively (Fig. 4c). Phylogenetic analysis revealed that these OTUs were closely related to uncultured bacteria (Fig. 4c).Techni ques for measuring body composition

Correlation of SOB communities with environmental factors

CCA suggested a high species–environmental correlation for the first two axes (53.1 and 25.9% for *sqr*; 60.5 and 36.7% for *soxB*; 47.6 and 30.2% for *dsrA*) (Fig. 5). The SS sample was divergent from the other samples along the CCA axis 2. TW and BXK showed similar values for *soxB* and *dsrA*, indicating that their bacterial communities were similar. The abundance of *sqr*, *soxB*, and *dsrA* sequences in NS correlated positively with the sulfide and sulfur content and negatively with sulfate content; this is contrary to the result in BXK and TW. In the SS sample, the abundance of most *sqr* and *soxB* sequences correlated

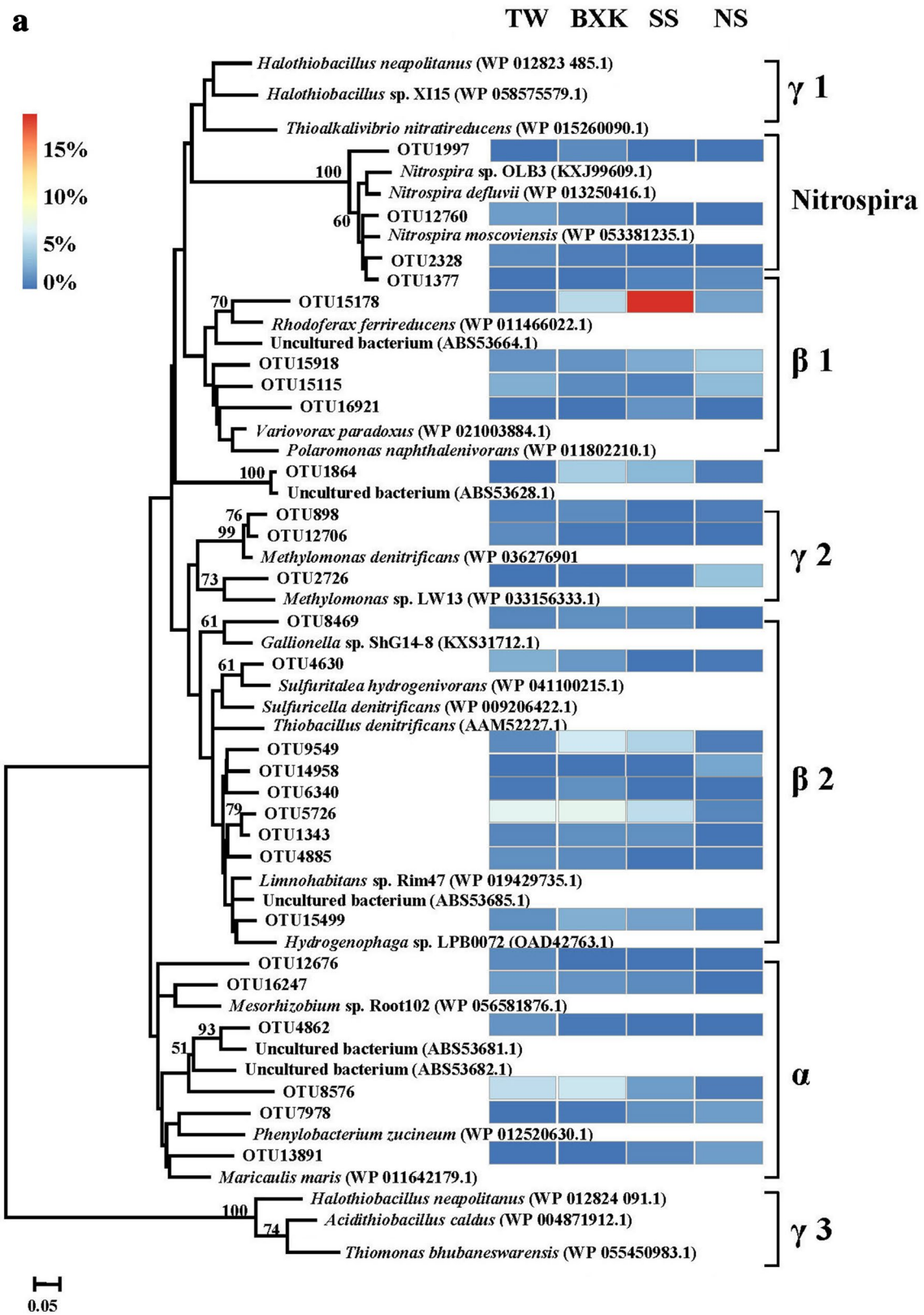


Fig. 4 Phylogenetic tree and heat maps of key OTUs (abundance > 1%) of *sqr* **a**, *soxB* **b**, and *dsrA* **c** in high-throughput sequencing. Neighbor-joining tree was created after computing the evolutionary distances via Poisson correction method. Bootstrap values over 50% based on 1000 replicates are shown

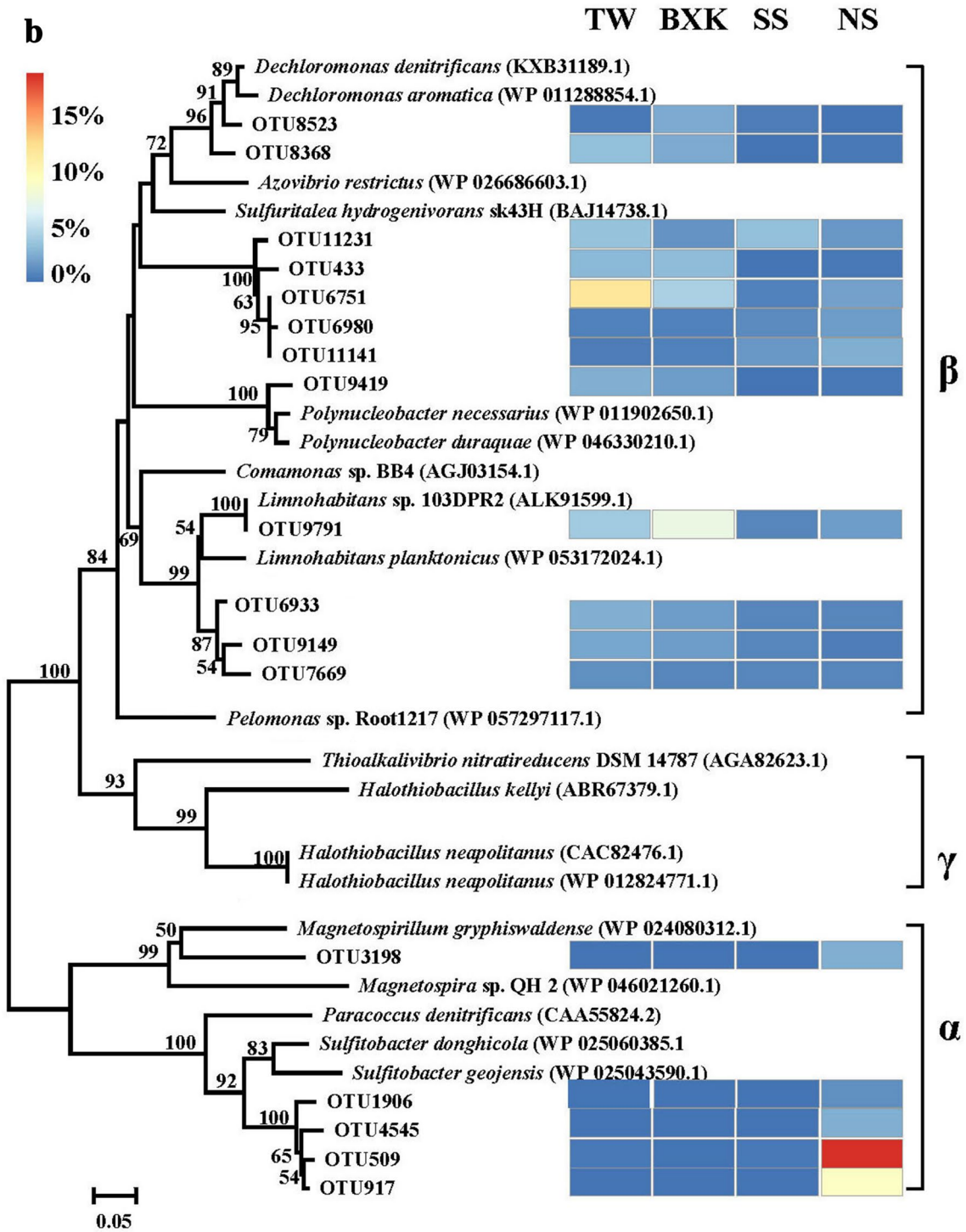


Fig. 4 (continued)

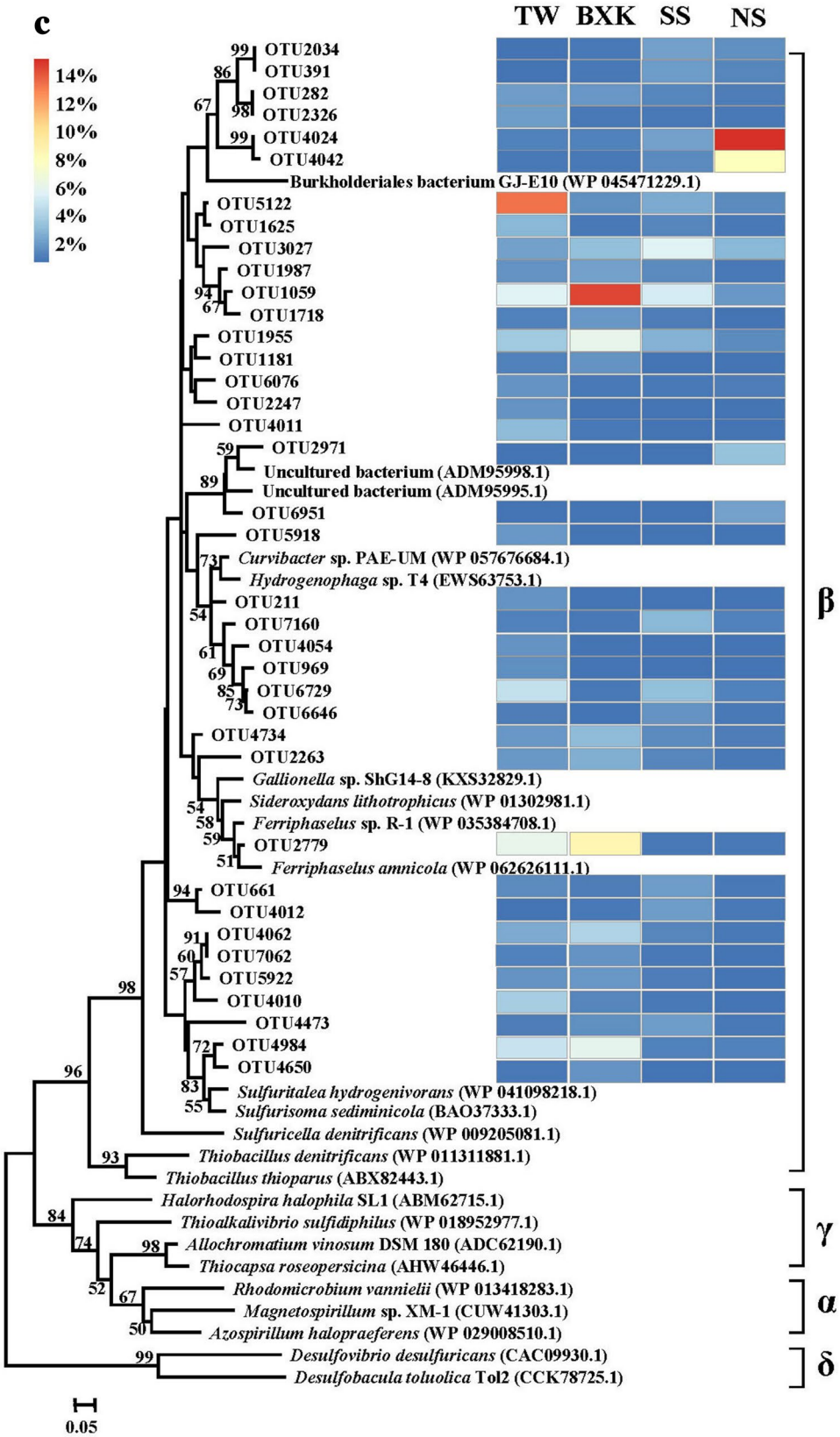


Fig. 4 (continued)

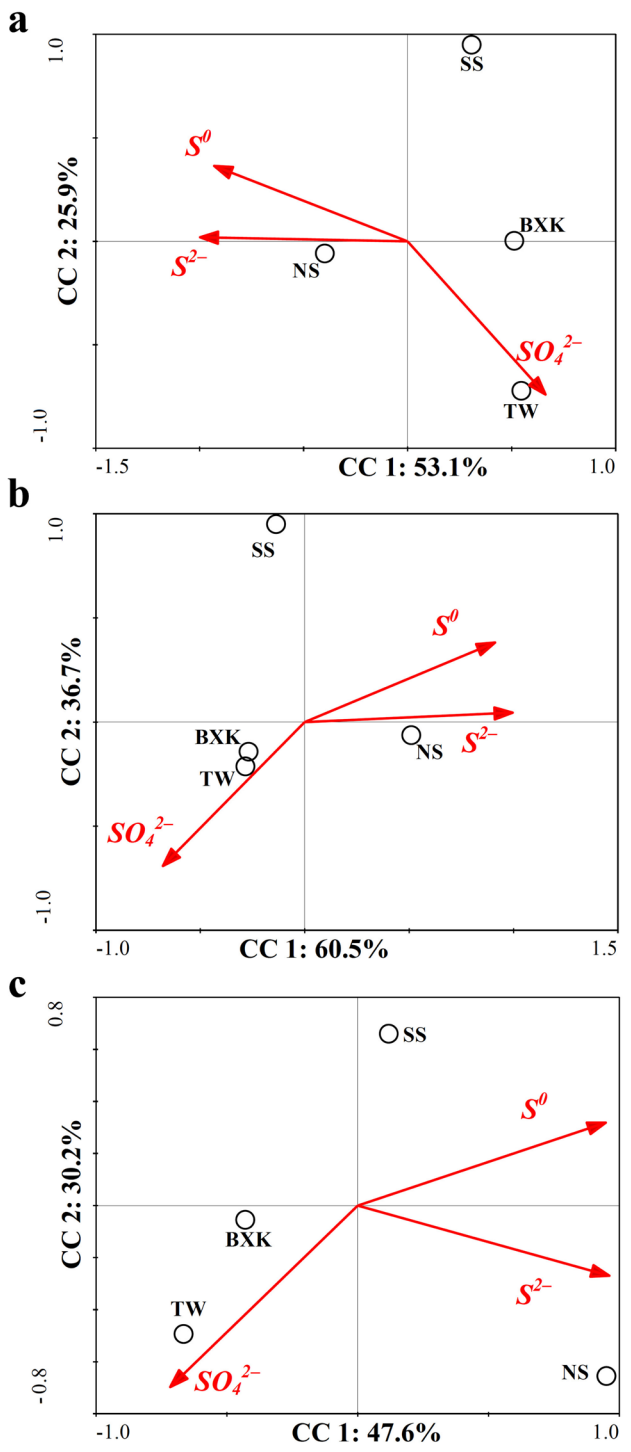


Fig. 5 CCA of the *sqr* **a**, *soxB* **b**, and *dsrA* **c** bacterial community with the environmental variables elemental sulfur, sulfide, and sulfate. CCA models were produced through the manual deselection of collinear variables and automatic forward selection via Monte Carlo permutation significance tests

negatively with sulfide and sulfate content and was independent of sulfur content; the abundance of most *dsrA* sequences correlated positively with sulfide content and negatively with sulfur content.

Discussion

Key enzyme system responsible for sulfur oxidation

The quantitative survey indicated that *soxB* was more abundant than *sqr* and *dsrA* in the Pearl River water, which suggested that Sox was probably the key enzyme that catalyzed sulfur oxidation in this environment. The Sox enzyme complex not only converts thiosulfate to sulfate without the formation of any free intermediate, but also oxidizes sulfide and sulfur by feeding HS^- and S^0 into the Sox pathway as appropriate intermediates via enzymatic or non-enzymatic conjugation to a carrier protein SoxY (Ghosh and Dam 2009). However, the Sqr and reverse Dsr mostly convert hydrogen sulfide to sulfur and intracellular sulfur to sulfate, respectively (Dahl et al. 2005; Ghosh and Dam 2009; Chan et al. 2009). The Sox system commonly controls sulfur oxidation in aerobic chemotrophic and anaerobic phototrophic *Alphaproteobacteria* and a shortened *sox* gene cluster was identified in the genomes of other chemotrophic or phototrophic bacteria (including colorless sulfur bacteria, green sulfur bacteria, purple sulfur bacteria, purple non-sulfur bacteria); this has given rise to the hypothesis about emergence of a common mechanism in SOB (Friedrich et al. 2001, 2005; Ghosh and Dam 2009). From the above analysis, we infer that the sulfur oxidation in the Pearl River water was mainly catalyzed by the Sox enzyme system.

SOB members that contribute to sulfur oxidation

High-throughput sequencing revealed that most of the SOB belonged to the classes *Nitrospira*, *Alphaproteobacteria*, *Betaproteobacteria*, and *Gammaproteobacteria*. *sqr* was predominantly expressed in the genera *Gallionella*, *Hydrogenophaga*, *Limnohabitans*, *Methylomonas*, *Nitrospira*, *Rhodoferax*, and *Sulfuritalea*; *soxB*, in *Dechloromonas*, *Limnohabitans*, *Paracoccus*, *Sulfuritalea*, *Sulfitobacter*, and *Thiobacillus*; and *dsrA*, in *Sulfuritalea*, *Sulfurisoma*, and *Thiobacillus*. Of these, *Sulfuritalea* and *Thiobacillus* were the predominant genera in the communities positive for all three functional genes; *Limnohabitans* and *Hydrogenophaga*, in communities positive for *sqr* and *soxB*; *Gallionella*, *Methylomonas*, *Nitrospira*, and *Rhodoferax* in communities positive for *sqr*; *Dechloromonas*, *Paracoccus*, and *Sulfitobacter*, in communities

positive for *soxB* community; and *Sulfurisoma*, in communities positive for *dsrA*.

Sulfuritalea, an important genus of communities positive for *sqr*, *soxB*, and *dsrA*, is usually isolated from oxic or anoxic habitats and capable of facultative autotrophic growth by oxidation of thiosulfate, elemental sulfur, and hydrogen (Kojima and Fukui 2011, 2014). Kojima et al. (2014) have also identified *Sulfuritalea* as a major planktonic sulfur oxidizer in a stratified freshwater lake using *aprA*, *dsrA*, *soxB*, *sqr*, and 16S rRNA gene clone libraries. Furthermore, sulfur oxidation genes, such as *sqr*, *sox* operons *soxXYZABEF*, and *dsrABEFHCMKLJOPNR,* have been identified in the genome of *Sulfuritalea* (Watanabe et al. 2014). *Thiobacillus*, including the species *Thiobacillus denitrificans*, *Thiobacillus aquaesulis*, *Thiobacillus thioparus*, and *Thiobacillus thiophilus*, are typical SOB that are commonly found to be predominant in various environments (Koenig et al. 2005; Maestre et al. 2009; Luo et al. 2011). More than 50 genes (*sqr*, *soxWXYZABEF*, *dsrABEFHCMKLJOPNR*, and genes encoding sulfite dehydrogenase, APS reductase, ATP sulfurylase, rhodanese, etc.) associated with sulfur oxidation have been identified in the genome of *Thiobacillus denitrificans* (Beller et al. 2006). Bacteria of the genus *Limnohabitans* may have a prominent role in the freshwater ecosystem because of their high rates of substrate uptake and diverse metabolic pathways (Newton et al. 2011; Kasalický et al. 2013). A large number of *Limnohabitans* were found in diverse freshwater bodies and are predicted to be involved in the oxidation of reduced sulfur and nitrogen compounds (Kasalický et al. 2013; Herrmann et al. 2015). Although there is no direct evidence for their sulfur-oxidizing ability, genes associated with sulfur oxidation, including *sqr* (annotated as pyridine nucleotide-disulfide oxidoreductase) and the *soxBXAZYDCR* gene cluster, have been identified in the genome of *Limnohabitans* strains (Zeng et al. 2012). Bacteria from the genus *Hydrogenophaga* are facultative heterotrophs and are frequently detected in wastewater treatment systems (Cytryn et al. 2005; Chung et al. 2007). Several *Hydrogenophaga* species have been identified as being capable of oxidizing thiosulfate (Graff and Stubner 2003; Chung et al. 2007; Yoon et al. 2008).

Members of *Gallionella* belong to the group of organisms called "iron bacteria" owing to their ability to grow chemolithotrophically by oxidizing iron (Lütters-Czekalla 1990; Dworkin et al. 2006). Although *Gallionella* is commonly found in salt waters, marine bays, and acid mine waters (Dworkin et al. 2006), the diversity of *Gallionella* is evinced by their isolation from neutrophilic habitats, such as freshwaters (Wang et al. 2011; Emerson et al. 2013) and soils (Wang et al. 2009). The ability to oxidize sulfide and thiosulfate was observed in *Gallionella ferruginea* strain BD (Lütters-Czekalla 1990) and the sulfur oxidation genes, including *sqr*, *tetH*, and the *dsrABEFHCMKLJOPN* cluster, were found in an assembled genome of a *Gallionella* species (Bertin et al. 2011); however, neither growth on reduced sulfur compounds nor sulfur oxidation genes were observed in *Gallionella capsiferriformans* ES-2 (Emerson et al. 2013). In the communities positive for *soxB*, *Dechloromonas* species were predominant in samples from TW, BXK, and SS, while *Sulfitobacter* species predominated in samples from NS. The *soxFRCDYZAXB* gene cluster has been identified in the genome of *Dechloromonas* (Salinero et al. 2009); this cluster probably encodes a Sox system that couples sulfur oxidation with perchlorate reduction (Sahu et al. 2009). Most *Sulfitobacter* strains are able to oxidize sulfite (Sorokin 1995; Pukall et al. 1999; Park et al. 2007). *Sulfurisoma*, comprising only one taxonomically assigned species, *Sulfurisoma sediminicola*, was the second largest genus in the communities positive for *dsrA*. *Sulfurisoma sediminicola* is a facultative autotroph capable of oxidizing thiosulfate, elemental sulfur, and hydrogen; moreover, *sqr, soxB*, and *dsrA* genes have been detected in this strain (Kojima and Fukui 2014).

Remarkably, *Nitrospira* and *Rhodoferax* species were found to be predominant in the communities positive for *sqr*. Although *Nitrospira* are among the most diverse and widespread nitrifiers in natural ecosystems and biological wastewater treatment systems, they have hardly been studied and are mostly uncultured (Lücker et al. 2010). Although *sqr* have been identified in the genomes of *Nitrospira defluvii*, *Nitrospira moscoviensis*, and some other *Nitrospira* sp., no sulfur-oxidizing potential has been reported (Lücker et al. 2010; Koch et al. 2015; Speth et al. 2016). Similarly, no sulfur-oxidizing potential has been reported for the genus *Rhodoferax*; moreover, *Rhodoferax ferrireducens*, too, is unable to utilize sulfide or thiosulfate (Finneran et al. 2003). These discrepancies could be attributed to lateral gene transfer (LGT). In fact, many studies have concluded that LGT plays an important role in oxidative sulfur metabolism by facilitating the acquisition of *sqr*, *sox*, and *dsr* (Meyer et al. 2007; Gregersen et al. 2011; Kleiner et al. 2012). Theissen et al. (2003) have reported that Sqr is not a well-conserved protein and that its gene has undergone a few lateral transfers during evolution, making it difficult to discern its precise lineage by phylogenetic methods.

Conclusion

Quantitative assays revealed that *soxB* is more abundant than *sqr* and *dsrA*, indicating that the Sox enzyme system has more important effect on scavenging sulfur pollutants in the Pearl River than the SQR and reverse Dsr systems. High-throughput sequencing analyses suggested that SOB groups closely related to classes *Nitrospira*, *Alphaproteobacteria*,

Betaproteobacteria, and *Gammaproteobacteria* were dominants in the Pearl River. This study presented high diversity of SOB and abundance of functional sulfur oxidation genes in the Pearl River, which enable the ecosystem to scavenge the inorganic sulfur compounds by itself. This survey was conducted at the level of functional genes; further studies using meta-omics and identification of unknown groups are necessary to obtain insights into the role of SOB in sulfur oxidation in this ecosystem.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest in the publication.

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