Impacts of human activities on distribution of sulfate-reducing prokaryotes and antibiotic resistance genes in marine coastal sediments of Hong Kong

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ABSTRACT

Sulfate-reducing prokaryotes (SRPs) and antibiotic resistance genes (ARGs) in sediments could be biomarkers for evaluating the environmental impacts of human activities, although factors governing their distribution are not clear yet. By using metagenomic approach, this study investigated the distributions of SRPs and ARGs in marine sediments collected from 12 different coastal locations of Hong Kong, which exhibited different pollution levels and were classified into two groups based on sediment parameters. Our results showed that relative abundances of major SRP genera to total prokaryotes were consistently lower in the more seriously polluted sediments (P-value < 0.05 in 13 of 20 genera), indicating that the relative abundance of SRPs is a negatively correlated biomarker for evaluating human impacts. Moreover, a unimodel distribution pattern for SRPs along with the pollution gradient was observed. Although total ARGs were enriched in sediments from the polluted sites, distribution of single major ARG types could be explained neither by individual sediment parameters nor by corresponding concentration of antibiotics. It supports the hypothesis that the persistence of ARGs in sediments may not need the selection of antibiotics. In summary, our study provided important hints of the niche differentiation of SRPs and behavior of ARGs in marine coastal sediment.

INTRODUCTION

Marine sediment covers most of the surface of our planet. It is commonly regarded as a long-term reservoir of both terrigenous and aquatic pollutants. For the offshore zone, diversity and abundances of macrobenthic invertebrates living on and inside the sediment have been commonly adopted as bioindicators of health status of the benthic ecosystem for decades (Phillips 1977).

More recently, researchers have realized that microorganisms, especially for bacteria living inside the sediment, can also serve as important bioindicators in relation to the benthology and biogeochemical process of the benthic ecosystem as they involve in decomposition of organic matter and recycling of materials such as carbon, sulfur and nitrogen (Sun et al. 2012; Dang et al. 2013). Thus, these tiny creatures are not only the dwellers, but also the system service providers (Prokopenko et al. 2013).

In the last two decades, with the advancement of molecular techniques, it has become feasible to use environmental microbes and their genetic materials as indicators for pollution evaluation (Rasmussen and Sørensen 1998; Gillings et al. 2015). Like the macroinfauna, the diversity of the poorly movable microbes is reliable to reflect sediment quality, since the microbial diversity often correlates well with concentrations of pollutants such as trace metals and persistent organic pollutants (POPs) in the sediment (Xu et al. 2014). At the same time, the microbes in marine sediments are food resources and maintain- ers of the benthic ecosystem (Alongi 1988). For example, anaerobic sulfate-reducing prokaryotes (SRPs) biochemically interact with the niches and the macroinfauna. They are responsible for the major anaerobic carbon cycling in the marine sediment (Muyzer and Stams 2008). Moreover, SRPs may change the sedimentary niche by (i) producing H2S, which is toxic to benthic an- imals (Wang and Chapman 1999); (ii) undergoing mineralization of ionic metals by forming sulfides, which could immobilize and detoxify the metals (Gadd and Griffiths 1977; Wang and Chap- man 1999); and (iii) facilitating methylation of mercury (Muyzer and Stams 2008; Mosher et al. 2012). All these processes link the SRPs with macroinfauna and the other microbiota in sediments. Besides of the traditional pollutants such as nitrogen, phosphate and trace metals, antibiotic resistance genes (ARGs) har- bored by living or dead microorganisms are currently considered as an emerging pollutant (Czekalski, Dı´ez and Bu¨

rgmann 2014). Unlike the chemical pollutants, the ARGs are self-replicating and horizontally transferable among microorganisms (Pruden et al. 2006). Most importantly, these genes cause high risk to hu- man health by compromising the antibiotic therapies. Although ARGs naturally exist, intensified human activities such as dis- charges of untreated and partially treated wastewater effluents, and contaminated surface runoff could dramatically increase their abundance and transferability in the marine environment (Zhang and Zhang 2011; Li et al. 2015). Profiling the distribution of ARGs in marine sediments could provide a perspective on understanding of the extent of human impacts on the benthic ecosystem.

Although there were many reports focusing on the diversity and abundance of SRPs and ARGs in sediments (Sua´rez-Sua´rez et al. 2011; Chen et al. 2013), a few studies focused on their distributions among sites within a small range but with different levels of human impacts. In this study, we performed a metagenomic survey on 12 sediments samples collected along a pollution gradient in the coastal marine environment of Hong Kong (Fig. S1, Supporting Information). Two major topics are ad- dressed by integrating the metagenomic data and the sediment parameters: (i) whether the distribution of SRPs and ARGs are related to the impact of human activities; (ii) which sediment factors were implicated and associated with the distribution of the relative abundance of SRPs and ARGs. Our results showed that relative abundances of most SRPs were generally negatively affected by the pollution level in the sediment, although a non- linear distribution pattern for SRPs along with the pollution gra- dient was observed. Moreover, the dominant ARG types showed no correlation with the abundances of corresponding antibiotics in the sediment. These drive curiosity on further examination on the niche differentiation of SRPs and the fate of ARGs in coastal marine sediments.

MATERIALS AND METHODS

Sampling site and sediment parameters

Twelve marine sediments (0–10 cm depth and all the seawater salinities are over 30 g L^{-1}) were collected along a pollution gradient in the marine environmental of Hong Kong during 5– 8 June 2012 (Table S1 and Fig. S1, Supporting Information). The representative environmental pollutant parameters (i.e. chemical oxygen demand (COD), ammonia nitrogen (NH_3-N), copper (Cu), zinc (Zn), total phosphate (TP) and benzoapyrene) in the sediments were measured by the Environmental Protection Department (EPD) of the Hong Kong Special Administrative Region Government and such data can be readily downloaded from the EPD website (http://www.epd.gov.hk/epd/). Homogenized sediments were kept on ice during transportation and lyophilized immediately in the laboratory before a long-term storage at −20°C.

Antibiotic analysis

For each sediment sample, triplicate subsamples were randomly taken for quantifying the composition and concentration of antibiotics. To extract antibiotics from the sediment, ultrasonic approach was applied according to the method recommended by Xu et al. (2007) via the optimized solid phase extraction process. Antibiotics in sediment samples were pre-concentrated, and then twenty antibiotics were examined using the ultraperformance liquid chromatography–tandem mass spectrometer (UPLC-MS-MS; Acquity, Waters, USA), following the procedure from Li et al. (2009).

DNA extraction and metagenomic sequencing

Around 0.5-g lyophilized sediment samples were weighed and used in DNA extraction. The DNA was extracted by using the FastDNA SPIN® kit for Soil (Qbiogene, Inc., US), which lyses mi- crobial cells by mechanical and chemical methods. For each sample, 2–4 replications were extracted to yield over 3-μg DNA. The DNA yield, purity and fragmentation were evaluated by a spectrophotometer (NanoDrop, ND-1000, Thermo Scientific, USA) and electrophoresis in agarose gel. A ratio of OD_{260}/OD_{280} over 1.6 was ensured for the metagenomic library construction. The 12 DNA samples were sent to Beijing Genome Institute for library construction (800 bp insertions) and shotgun sequencing via the Illumina Hiseq2000 platform (PE-100 strategy). The 12 li- braries were sequenced in two lanes (six samples per lane) in a single sequencing run. The raw sequence data have been up- loaded into Sequence Read Archive of NCBI (accession number from *SRR2134631* to *SRR2134634, SRR2134636 to SRR2134637* and *SRR2134639 to SRR2134644*).

Microbial community and SRPs analysis

The metagenomic reads were filtered by requiring the average Q-value over 20 (99% accuracy). The reads were performed by BLASTn (Blast+ version 2.2.27) against the SILVA SSU database (release 119; Quast *et al.* 2012) and outputs were set at e-value of 10^{-20}, alignment length over 90 bp and 50 top hits. Family- level community structure was determined by using the MEGAN 4 annotation under the default setting (Huson *et al.* 2007).

The short read length of 16S rRNA gene is not sufficient for SRP identification into a finer taxonomic rank, such as the genus level. Thus, the SRP taxa were investigated by referring to the dissimilatory sulfite reductase subunit A and B (*dsr*AB). A sub-database annotation pipeline was applied to detect and classify the *dsr*AB gene (Yu and Zhang 2013). Briefly, all reads were performed BLASTx (Blast+ version 2.2.27) against a customized collection of *dsr*AB genes from a reference (Zverlov *et al.* 2005). Hit reads satisfying over 50% similarity and over 25 amino acid (AA) length were extracted. The obtained candidate *dsr*AB sequences were further conducted BLASTx against the full NR database. By MEGAN annotation (Huson *et al.* 2007), only those reads with the best hit against a known SRP genus (by manually checking the hit genera), and similarity over 70% for over 25 AA were deter- mined as a *dsr*AB sequence from the corresponding SRP taxa. These operations substantially shortened the searching time and precluded a high proportion of false-positive hits that were actually reverse type dissimilatory sulfite reductase from sulfur- oxidizing organisms.

Quantification of ARGs types and subtypes

ARG determination followed the pipeline of our previous report (Yang *et al.* 2013). The database is the Structured Non-redundant Clean Antibiotic Resistance Genes Database (SNC-ARDB) containing 2998 non-redundant sequences belonging to 618 sub- types and 25 types. The reads were aligned against the SNC- ARDB using BLASTx. A read was classified as a certain ARG only if its best hit sequence had over 90% similarity with a reference for over 25 AA hit length. Because abundances of most single ARG subtypes were low, the subsequent analysis was conducted only at

155 the type-level.

156

157 **Data analysis**

158 Calculation of diversity indexes, principal components analysis (PCA), non-metric
159 multidimensional scaling (NMDS) were per- formed using the PAST 3 software
160 (Hammer, Harper and Ryan 2001). Redundancy analysis (RDA) for linear-model
161 ARG distribution was realized in the CANOCO 4.5 software (Lepš and Šmilauer
162 2003). The abundances of *dsrAB* and ARG reads were normalized by the number of
163 16S rRNA gene reads. The comparison of relative abundances of SRPs and bacterial
164 families be- tween sediment sample groups was conducted using the STAMP
165 software (Parks and Beiko 2010). Since multiple *t*-tests comparisons were involved,
166 Benjamini–Hochberg FDR correction was applied as suggested by the STAMP
167 program (Benjamini and Hochberg 1995). Correlation analysis between the
168 abundances of ARGs and concentrations of antibiotics was performed using IBM
169 SPSS version 19.0 software.

170

171 **RESULTS**

172 **Different human activities indicated by sediments parameters**

173 According to the PCA results for the environmental parameters (Table S1, Supporting
174 Information; Fig. 1), the sediment samples could be cataloged into two groups (Group I and Group
175 II), which were determined by their COD, heavy metals and benzoapyrene concentrations. Group
176 I includes the seven less polluted sites (low COD, etc.), including HKSD-10, 34, 45, 68, 75, 90

and 118. Group II consists of sites HKSD-3, 53, 54, 104 and 107, which are suggested to suffer from more serious impacts from human ac- tivities and higher pollution degree. For example, the HKSD-53 and 54 sites located along a water channel that is surrounded by a high density of inhabitants. The HKSD-3, which suffers from discharges of both harbor and municipal pollutants, shows the highest total phosphorus and NH_3-N concentrations, and thus, this site is different from the other four sites in Group II. The average values of all parameters are higher in Group II than in Group I. Therein, COD and the concentrations of the two heavy metals show significant difference ($P < 0.05$; Fig. 1).

Data profile for the metagenomic sequencing

The data sizes of the 12 metagenomic datasets ranged from 2.80 to 5.99 Gb, as listed in Table 1. Therein, 7178–20 812 reads were classified as the small subunit of ribosomal RNA (SSU rRNA) sequences. In most metagenomes, the percentage of prokaryotic 16S rRNA to total SSU rRNA sequences was above 85%, which could serve as the potential evidence that the most dominant biomass within the samples is from Bacteria and Archaea. The 16S rRNA gene sequences from Bacteria are 10.7–60.3 times higher than those from Archaea. However, about 45% SSU rRNA reads in HKSD-53 were from eukaryotic cells. It is suggested that this sample contains a high abundance of eukaryotic DNA. Additionally, we have conducted the sequence assembly (CLC Ge- nomics Workbench 6.0) to get longer sequences (contigs) with higher taxonomic precision; however, only very few short contigs were revealed (N_{50} < 500 and less than 5% reads recovery). This result could indirectly reflect the extremely high microbial diversity in the sediment samples. Therefore, only read-

based annotation and quantification were performed in the sub- sequent analysis.

Major bacterial communities and SRP groups in sediments with different pollution levels

For the bacterial SSU reads, only a minor proportion of them (35.2%–46.3%) could be assigned into a defined family with MEGAN annotation. This might be derived from the compromised taxonomic precision for the short reads, as well as the existence of many family-level novel organisms without formal nomenclature currently. Major families that were over 0.5% at least in one sample (35 families in total) accounted for 21.5%– 31.6% of total bacteria. As shown in Fig. 2, the top five families are Desulfobacteraceae (1.7%–7.6%), Planctomycetaceae (1.4%–4.5%), Bacillaceae (0.07%–15.1%), Desulfobulbaceae (0.5%–3.4%) and Nitrospiraceae (0.8% – 2.9%). Two of the top ten families, i.e. Bacillaceae and Peptostreptococcaceae, showed a highly discrepant distribution. Bacillaceae was only enriched in HKSD-104 and HKSD-107 samples, and Peptostreptococcaceae was sparse in three samples (HKSD-34, HKSD-68 and HKSD-107). In general, other families showed an even distribution, suggesting that these sediments have similar bacterial community at least at the taxonomic rank over family level.

Neither geographic nor pollution-dependent pattern could be detected in the clustering, based on the Euclidean distances of the 35-family communities (Fig. 2). Only several closely located samples showed a high similarity among their community structure, such as HKSD-3 and HKSD-10. Merely two of the 35 families, i.e. Syntrophobacteraceae and Phycisphaeraceae distributed differently (P = 0.033 and 0.015, respectively, two-tail t-test) between Group I and Group II sites.

However, the Benjamini–Hochberg FDR correction (in STAMP software) further increased the *P*-values to no difference (corrected $P = 0.402$ and 0.296 for Syntrophobacteraceae and Phycisphaeraceae, respectively). This result suggested that the general bacterial family- level composition could not differentiate the pollution of the sediments in our study. The NMDS plot (Bacillaceae was excluded due to its high abundance only in HKSD104 and 107) also suggested that the family-level community could not be simply differentiated by the chemical contamination in the sediment samples (Fig. 3A).

Totally, over 50 SRP genera were detected referring to MEGAN annotation of the *dsr*AB. They were phylogenetically affiliated with five phyla, i.e. Proteobacteria, Firmicutes, Nitrospira, Ther- modesulfobacteria and Euryarchaeota. Normalized by the number of 16S rRNA gene reads, the abundance of total SRPs ranged from 0.084 to 0.33 per 16S rRNA sequence, which indicated a high proportion of SRPs in the total microbial communities. Nonetheless, the value should be influenced by the factors of longer length of *dsr*AB than 16S rRNA gene, and the variation of the copy number of 16S rRNA gene. Since the top 20 SRP genera accounted for >87% of detected SRPs in all samples, only their abundances were shown in Fig. 4.

Different from the bacterial family-level community, 16 out of 20 detected SRP genera showed a significantly different relative abundance ($P < 0.05$, two-tail *t*-test) between the two groups. It was without exception that average abundances of all SRP genera are more abundant in Group I than in Group II. Thirteen genera still held a significant difference ($P < 0.05$) between the two groups after the Benjamini–Hochberg FDR correlation (Fig. 4). The top three hit SRP genera (in their average

246 abundance among all samples) were *Desulfotomaculum* (corrected P = 0.024),
247 *Desulfococcus* (corrected P = 0.061) and *Desulfobacter* (corrected P = 0.042). Because
248 we compared the SRP abundance by using the data normalized by the total 16S
249 rRNA reads in the dataset, it is worth pointing out that no difference (P = 0.255, two-
250 tail *t*-test) of 16S rRNA abundance (16S rRNA reads per M reads) was detected
251 between the two groups. Therefore, the different relative abundances of SRPs in
252 Group I and Group II should not be a false signal, derived from the background
253 density of 16S rRNA gene.
254 Although most SRPs were less abundant in polluted sediments, the structure of SRPs was still
255 not a good indicator for sediment pollution level. The results of NMDS showed that the SRP
256 communities of two groups did not separate from each other clearly (Fig. 3B). The exceptions are
257 HKSD-75 in Group I and HKSD-107 in Group II (Fig. 4). Interestingly, the two samples had the
258 lowest COD and Cu concentrations in Group I and II, respectively. This potentially implied a
259 non-linear variation pattern that the relative abundances of SRPs may decrease if COD
260 are very low (HKSD-75) or very high (samples in Group II except for HKSD-107),
261 and they will be more competitive in moderate COD concentration (samples in Group I
262 except for HKSD-75 and HKSD-107). This suggested a non-linear unimodel
263 distribution of SRPs along the increased COD concentration gradient.
264 To further test the results of SRP distribution, we also per- formed a parallel analysis
265 on the relative abundance of sulfate- reducing bacteria (SRB) in total bacteria based
266 on the high- throughput sequencing of the V4 region of 16S rRNA gene (See Fig.
267 S2, Supporting Information). For each sample, two DNA- level replications were
268 involved. Although the signal decreased to some extent, the SRB distribution between

Group I and Group II still exhibit significant difference ($P < 0.05$) if requiring >90% similarity cut-off or using all hits that were the closest to a SRB reference. The difference could be more significant if the HKSD- 75 is excluded from Group I ($P < 0.01$). However, it should be noted that the boundary of rRNA sequences between SRPs and non-SRPs is unknowable and variable. It might partially con- tribute to the lowering difference between the two groups.

Distribution of ARGs in different sediments

ARGs abundances in the sediment samples were from 2.6×10^{-3} to 3.8×10^{-2} read per 16S rRNA read (Table S2, Supporting Information), which was similar with previous reports on fish pond sediment samples (4×10^{-3}–3.1×10^{-2}; Li *et al.* 2015). The abundant ARG types were genes encoding resistance to multidrug (3.2×10^{-3} in average), bacitracin (1.2×10^{-3} in average) and sulfonamide (1.4×10^{-3} in average). The result of *t*-test for the relative abundance of total ARGs indicated a nearly significant difference between two groups ($P = 0.050$). However, the result of RDA indicated that Zn ($P = 0.015$) and COD concentration ($P = 0.019$) was confident explainable factors among the six sediment parameters. Distributions of the most single major ARGs types could not be well explained by the levels of sediment pollutants (Fig. 5). Moreover, the two factors could only robustly interpret the distribution of total ARG and the miscellaneous type resistance genes. The other specific ARG types seemed not to be influenced by any of the sediment parameters analyzed.

Ten out of the 20 surveyed antibiotics were detected in at least one sediment sample (Table S3, Supporting Information). Therein, Roxithromycin (0.02–775.53 $\mu g\ kg^{-1}$),

Sulfad azine (1.49–157.16 μg kg^{-1}) and Sulfamethoxazole (4.07–249.52 μg kg^{-1}) were ubiquitously detected and corresponding to two ARG types, i.e. the resistance genes for Macrolide-lincosamide-streptogramin and Sulfonamides. Correlation analyses (both Spearman correlation and Kendall correlation were tested since the data of antibiotic concentrations were not normally distributed) were performed to examine the potential implications between antibiotics and ARGs. However, the statistics showed no significant correlation ($P > 0.1$ in all cases, bootstrap $N = 1000$). This result suggested that the occurrence of ARGs could not be explained by the local distribution of the two major antibiotics classes. This is reasonable to consider that the absolute antibiotic concentration in sediment seems too low to selectively enrich the ARG-containing microbes.

DISCUSSION

Marine sediments contain extremely diverse microbes because of the complex physicochemical gradients therein (Sogin et al. 2006). Metagenomics have been applied to study the microbial distribution and functions in marine sediments and given chance to discover new phenomena and solve problems in microbial ecology (Biddle et al. 2008). It is powerful to examine the ecological concerns of functional microbiota and genes. In this study, the distributions of SRPs and ARGs in marine sediments under different impacts of human activities were investigated with the metagenomic analysis. Without the potential PCR biases as suggested previously (Zverlov et al. 2005; Li et al. 2015), the current methodology based on the next-generation sequencing gives more comprehensive SRPs and ARGs profiles with high fidelity.

315 The community of bacteria and SRPs in several marine sediments of Hong Kong has
316 been studied previously with the clone library methods (Zhang, Ki and Qian 2008;
317 Zhang *et al.* 2008). With more samples and less biased metagenomic profiling, we
318 have found that pollutants such as COD, Cu and benzoapyrene may govern the
319 distribution pattern of SRPs in the sediment. Moreover, the results also suggest that
320 these pollutants may in- fluence the relative abundance of SRPs to total microbes
321 under a unimodel paradigm. In the present study, either low or high concentrations
322 of pollutants will decrease relative abundance of SRPs in total microbes. It should
323 be pointed out that the absolute abundance of SRPs (e.g., gene copies or cell num-
324 bers per gram sediment) in the sediment was not determined in this study because we
325 failed to get confident quantitative PCR results due to the unknown inhibitors in the
326 DNA extractions. Thus, to the great extent, our results implied a niche occupation of
327 SRPs or competition between SRPs and other microbes in the marine sediments.
328 Although a study has shown that some SRPs are slow-growing microbes even in
329 laboratory reactors, typically with doubling time of weeks (Girguis, Cozen and
330 DeLong 2005), the *in situ* growth rates of different SRPs in the marine sediment are
331 unknown. However, it is reasonable that other bacterial taxa with higher growth rate
332 will outcompete SRPs if they can adapt to the more seriously polluted sediments or
333 more 'natural' sediments.
334 According to our finding, the relative abundance of SRPs could serve as a good
335 indicator for the impact of human activity, in particular chemical pollution in coastal
336 marine sediments, due to the consistent responses from diverse taxa. However, the
337 distribution pattern of SRPs in different marine sediments should be further

investigated to test the proposed unimodel model and their niche differentiation in detail. It is interesting to further investigate if the absolute abundance of SRPs can be affected by the pollution level in the sediment.

Last but not least, our results indicated that the distribution of ARGs was not determined by the local antibiotic pollution in the coastal sediments. Although the total ARGs abundance seemed to be positively influenced by human impact, the distribution of a few major ARG types only could be well explained by the general factors, such as COD and Zn, instead of the corresponding antibiotics. It is contradictory with the common belief that the enrichment of ARGs is usually related to abuse of antibi- otics in hospital and animal farming (Spellberg *et al.* 2008; Martinez 2009), where the ARG concentrations are much higher than natural systems. In addition, it has been pointed that there were some silent ARGs that might not be involved in the resistance of certain hosts (but indeed confer the resistance to some other hosts), which could also induce the poor correlation between antibiotics and ARGs (Dantas and Sommer 2012). It should be also noticed that heavy metals, such as Cu and Zn, were higher in Group II sediments than in Group I. Thus, another potential mechanism of maintaining certain ARGs in sediment is by the co-selections of heavy metals (Baker-Austin *et al.* 2006). Our results support the idea that the occurrence and persistence of ARGs in the marine sediments may not be directly associated with the *in situ* stress of the antibiotic residues in sediment. They could be derived from the direct continuous input of biomass or non-selective effect. For example, microbes containing the ARGs may prefer the more polluted sediments. For the slowly growing and relatively stable microbial communities in marine sediments,

the loss of ARGs along with the diminishing of the ARG- containing microbes could be a long-term process. In fact, this increases the risk of the resuscitation and transmission of ARGs. Long-term monitoring and laboratory-scale experiments may provide further evidences for understanding the persistence of ARGs and their environmental risk for coastal benthic ecosystems.

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SUPPLEMENTARY DATA

https://academic.oup.com/femsec/article/92/9/fiw128/2197721

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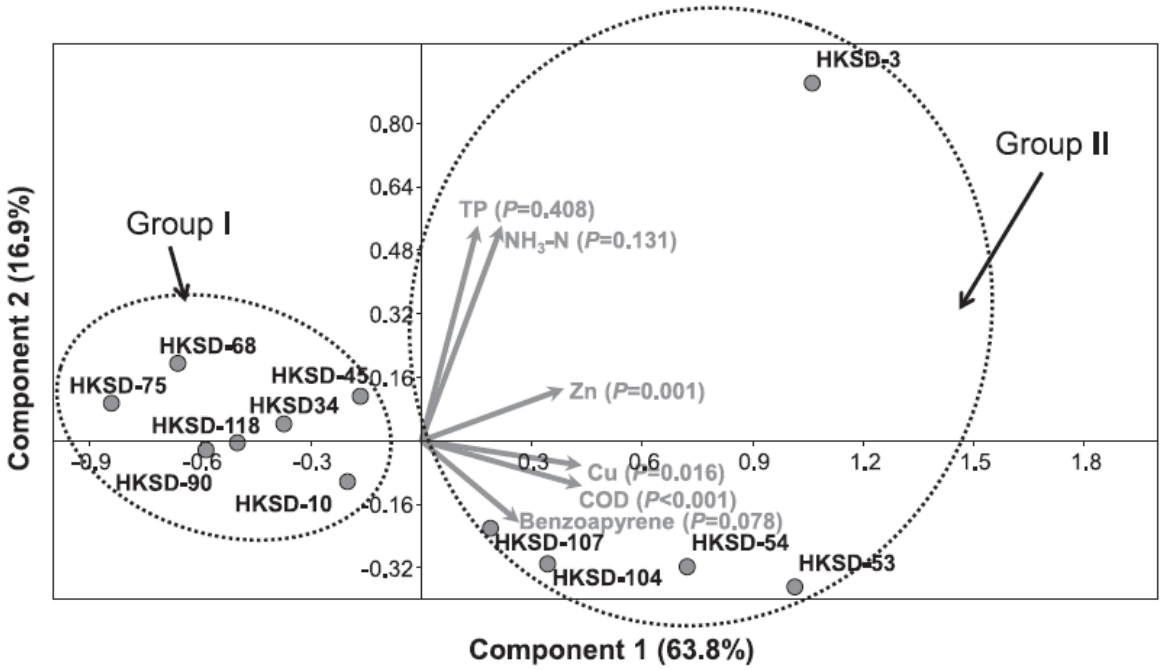
LIST OF FIGURES AND TABLES



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382 **Figure 1.** Principal component analyses for the 12 sediment sites of the marine
383 environment of Hong Kong based on the concentrations of selected parameters in the
384 sediment. The different environmental parameters were normalized using the
385 minimum—maximum normalization procedure. Vector matrices of the key pollutant
386 parameters are shown by the individual gray lines and each with an arrow; the longer
387 the line the more important this parameter is in relation to the separation among
388 different sites. The *P*-value of each parameter is based on the student's *t*-test between
389 the two groups. One includes HKSD-10, 34, 45, 68, 75, 90 and 118 and the other
390 includes HKSD-3, 53, 54, 104 and 107.

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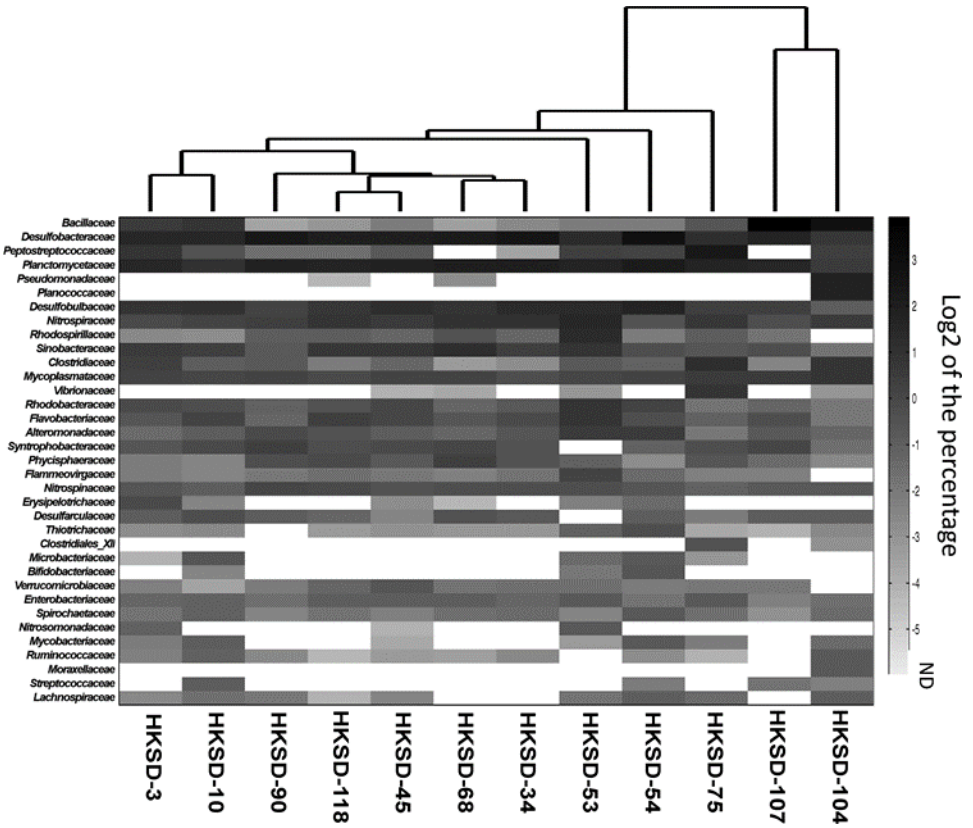

Figure 2. Distribution of the top 35 bacterial families in the 12 sediment samples and the clustering analysis based on the Euclidean distances between samples. The listed families are over 0.5% of relative abundance in at least one sample

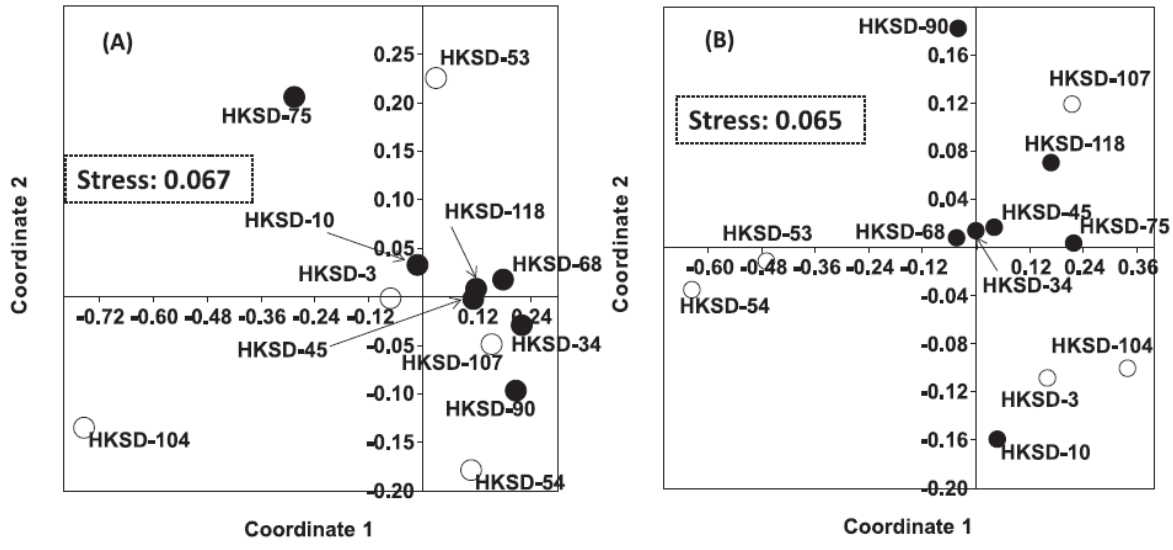

Figure 3. Non-metric multidimensional scaling for the sediment samples based on the

400 Euclidean distances of top 34 bacterial families (Bacillaceae excluded) (A) and top
401 20 hit SRP genera (B). Black circles are samples in Group I and open circles represent
402 sediments in Group II.

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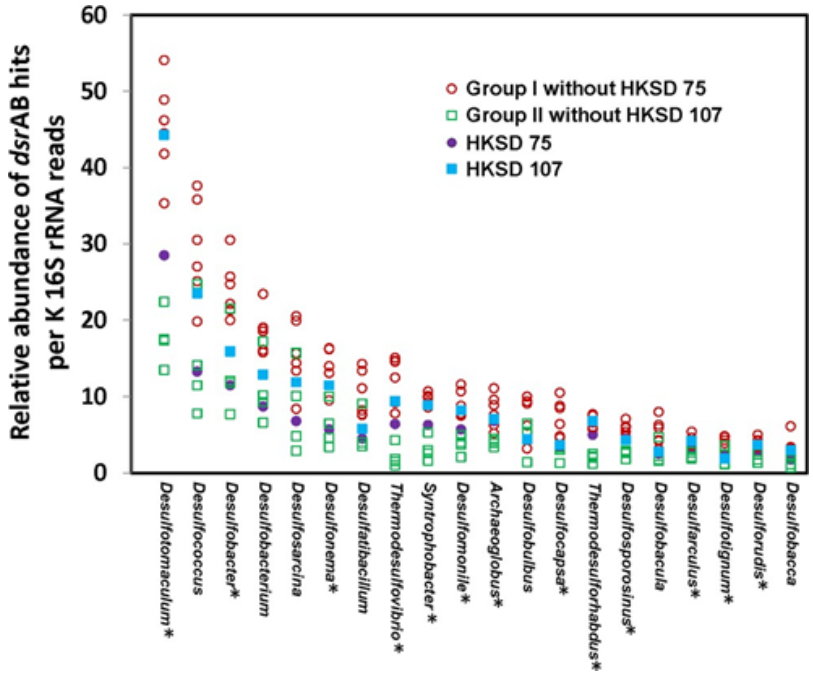

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405 Figure 4. Distribution of the top 20 SRB genera in the sediments. The abundances of
406 SRPs were normalized by 1000 16S rRNA sequences. Two-tailed equal variance t
407 test and the Benjamini–Hochberg multiple test correlation were performed in the
408 STAMP program (Parks and Beiko 2010). The genus name was marked with asterisk
409 if the difference between the two groups was significant (corrected P value <0.05).
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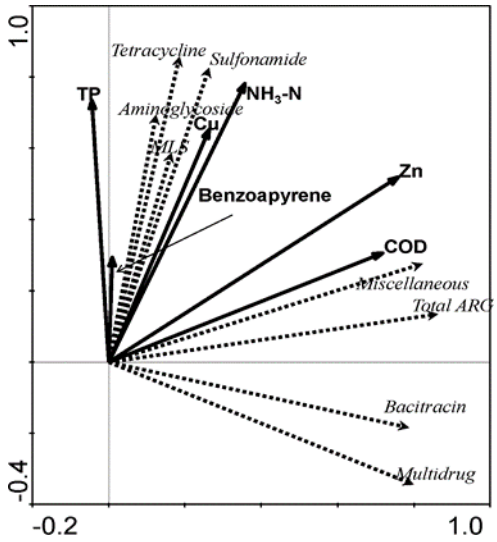

Figure 5. RDAfor the distribution of major antibiotics resistance genes and sediment parameters. Solid lines are environmental variables and dash lines represent the ARGs. The P-values of Monte Carlo permutation tests (n = 999) for the parameters are as follows: 0.015 for Zn, 0.019 for COD, 0.079 for NH3-N, 0.365 for Cu, 0.432 for TP and 0.866 for benzoapyrene.

Table 1. Data profile and the ribosomal RNA compositions for the metagenomes.

Sample	Data size (million reads)	No. of SSU rRNA reads	No. of bacterial SSU rRNA reads	Percentage of prokaryotic SSU to total SSU reads (%)
HKSD-3	56.2	16 681	15 967	98.27
HKSD-10	28.0	10 125	8145	87.93
HKSD-34	32.8	8702	7867	93.85
HKSD-45	28.8	8811	7945	92.21
HKSD-53	43.3	13 356	7002	55.57[a]
HKSD-54	59.9	20 812	17 713	86.52
HKSD-68	27.0	7178	6879	99.07
HKSD-75	38.1	15 325	13 329	91.89
HKSD-90	37.2	11 627	9998	88.24
HKSD-104	39.1	20 735	17 812	91.60
HKSD-107	31.6	11 772	11 161	96.64
HKSD-118	30.4	9498	8712	96.64

[a]The sample of HKSD-53 has a high percentage of eukaryotic DNA in the metagenomic data.

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