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Distribution and diversity of magnetotactic bacteria in sediments of the Yellow Sea continental shelf

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Abstract

Purpose Magnetotactic bacteria (MTB) are cosmopolitan and ubiquitous in sediments of freshwater and marine habitats. Most studies of marine MTB have concerned intertidal zones, and there have been few reports of MTB from seafloor sediments. The objective of this study was to investigate the diversity and spatial distribution of MTB on the continent shelf of the Yellow Sea.

Materials and methods Sediments from the continental shelf were collected from the Yellow Sea in August 2015 (summer) and January 2016 (winter). Magnetotactic bacteria were enriched by a permanent magnet. The number of MTB was counted. The diversity of MTB was identified based on high-throughput sequencing and Silva database and nt/nr database. Environmental factors including bottom temperature and salinity, total carbon (TC), total nitrogen (TN), total sulfur (TS), total organic carbon (TOC), concentration of iron, and the sediment grain size were measured.

Results and discussion Magnetotactic bacteria were distributed widely in the Yellow Sea, at abundances of 5–2400 ind./dm^3 in summer and 5–820 ind./dm^3 in winter. The dominant MTB at most sampling stations were cocci, although rod-shaped MTB dominated at one station (H17). High-throughput sequencing distinguished 2669 reads for putative MTB in summer and 3271 in winter, and these were classified into 72 operational taxonomic units (OTUs). In both summer and winter, most reads (> 74%) and OTUs (> 50%) of putative MTB belonged to the *Nitrospirae*. The MTB abundance was significantly correlated with sediment grain size. The MTB abundance was highest at station H17, where the sediment grain size was larger than at other stations.

Conclusions We present a systematic assessment of the spatial distribution and diversity of MTB in sediments of the continental shelf of the Yellow Sea. Our data suggest that diverse MTB are widely distributed in the seafloor sediment of the Yellow Sea, with *Nitrospirae* likely being the dominating group, and that the abundance of MTB are influenced by sediment grain size.

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1 Introduction

Prokaryotes that exhibit magnetotaxis, collectively known as magnetotactic bacteria (MTB), have directional motility that is influenced by the Earth's geomagnetic field and by externally applied magnetic fields (Bazylinski et al. 2013). The term MTB has no taxonomic meaning (Bazylinski 1999), and they are a morphologically, phylogenetically, and metabolically diverse group of microorganisms. They form variously shaped intracellular biomineral magnetic crystals, either magnetite (Fe_3O_4) or greigite (Fe_3S_4), termed magnetosomes, which cause the MTB to passively align along magnetic field lines as they swim (Bazylinski et al. 2013). On the basis of 16S rRNA

gene sequences, MTB have been classified into the phyla *Proteobacteria*, *Nitrospirae*, *Candidatus* Omnitrophica (previously known as OP3), and probably the candidate phylum *Latescibacteria* (Kolinko et al. 2012; Bazylinski et al. 2013; Lagkouvardos et al. 2014; Lin and Pan 2015) and *Alphaproteobacteria* are often the dominant group in freshwater and marine environments (Mann and Sparks 1990; Spring et al. 1995; Amann et al. 2007).

Magnetotactic bacteria are a group of ubiquitous aquatic microorganisms found worldwide in chemically stratified water columns and sedimentary environments. Their distribution is influenced by several environment factors, but in general MTB occur predominantly at the oxic–anoxic transition zone (OATZ) (Simmons et al. 2004; Flies et al. 2005a; Abreu et al. 2007; Pan et al. 2008; Lin et al. 2009; Lefèvre and Bazylinski 2013). The temporal and spatial distribution of some species of MTB has been the subject of several reports. Lin et al. (2010) investigated the temporal variation of MTB communities in freshwater sediments using the 16S rRNA gene and UNIFRAC analyses, and found a strong correlation between nitrate concentration and the main genetic variability of MTB communities. Postec et al. (2012) found that the occurrence of populations of magnetotactic cocci on the French Mediterranean coast was correlated with the redox state of sulfur compounds. The annual variation in abundance of multicellular magnetotactic prokaryotes, a peculiar group of MTB, was studied in the Araruama Lagoon (Brazil). The abundance peaked in summer, and variations in abundance were possibly related to environmental factors including organic matter, salinity, and iron (Martins et al. 2009; Sobrinho et al. 2011; Martins et al. 2012). Comparison of MTB communities at several locations in China and the USA has indicated that both environmental heterogeneity and geographic distance play significant roles in shaping dominant populations and community composition of MTB, and that their biogeography was relatively more influenced by environmental factors (Lin et al. 2013).

Marine MTB have mostly been reported from coastal sediments, including sediments of intertidal zones (Pan et al. 2008; Zhu et al. 2010; Postec et al. 2012; Zhang et al. 2013b; Chen et al. 2015, 2016), estuaries (Bazylinski 1996; Bazylinski et al. 1988; Lin et al. 2012a), lagoons (Martins et al. 2009; Du et al. 2015; Fuduche et al. 2015), and mangrove swamps (Lin et al. 2012a); however, MTB cells or DNA sequences and magnetosomes have also been found in seafloor sediments. Magnetotactic bacteria were found at three sampling sites in the Santa Barbara Basin (598 m; west coast USA), with vibrio and rod-shaped cells present but coccoid cells being the most common morphology (Stolz et al. 1986). Several morphotypes of MTB and magnetosomes have been reported from the Pacific and Atlantic oceans (Vali et al. 1987; Vali et al. 1989; Petermann and Bleil 1993). Partial 16S rRNA gene sequences (500 bp) were obtained from marine sediments (200–500 m) near the Nansha Islands (South China Sea) (Dai et al. 2002). Magnetosomes and 402 reads detected by high throughput sequencing for six OTUs likely belonging to MTB genera were detected at nine study sites (approximately 5000 m) in the east Pacific Manganese Nodule Province (Dong et al. 2016). However, there have been no systematic reports concerning MTB in continental shelf areas of China Seas. In this study, we investigated the distribution and diversity of MTB in sediments of the Yellow Sea in summer and winter, and infer that the abundance of MTB is influenced by heterogeneous environmental conditions, with sediment grain size being the dominant factor.

2 Materials and methods

2.1 Sample collection

Sediments from the continental shelf were collected from the Yellow Sea in August 2015 (summer) and January 2016 (winter) aboard the research vessel Dongfanghong Erhao. The samples were collected at 26 stations during the summer field trip and 23 stations during the winter (Fig. 1), using a box corner. Aboard the vessel, subsampling of each surface sediment (0–3 cm) was carried out immediately. This involved transfer of approximately 10 cm^3 of the sediment into a sterilized 10-ml centrifuge tube for DNA extraction and environmental factors measurement (stored at − 20 °C), and transfer of approximately 300 cm^3 of sediment into a bottle together for MTB observed, with seawater (1:1 ratio) that covered the collected sediment in the box corner.

2.2 Measurement of environmental factors

The sediment grain size was measured over the range 0–500 μm using a particle size analyzer (Cilas 940L, France). Total carbon (TC), total nitrogen (TN), total sulfur (TS), and total organic carbon (TOC) were measured using an elemental analyzer (Vario Macro CNS, Elementar, Germany), and the concentration of iron was measured using inductively coupled plasma-optical emission spectroscopy (ICP-OES; Thermo Fisher Scientific, USA). The bottom temperature and salinity were measured using a conductivity–temperature–pressure (CTD) sensor (Sea-Bird 911, USA). The redox potential was measured every 2 cm from the sediment surface using a redox tester (JENCO 6173, Shanghai, China).
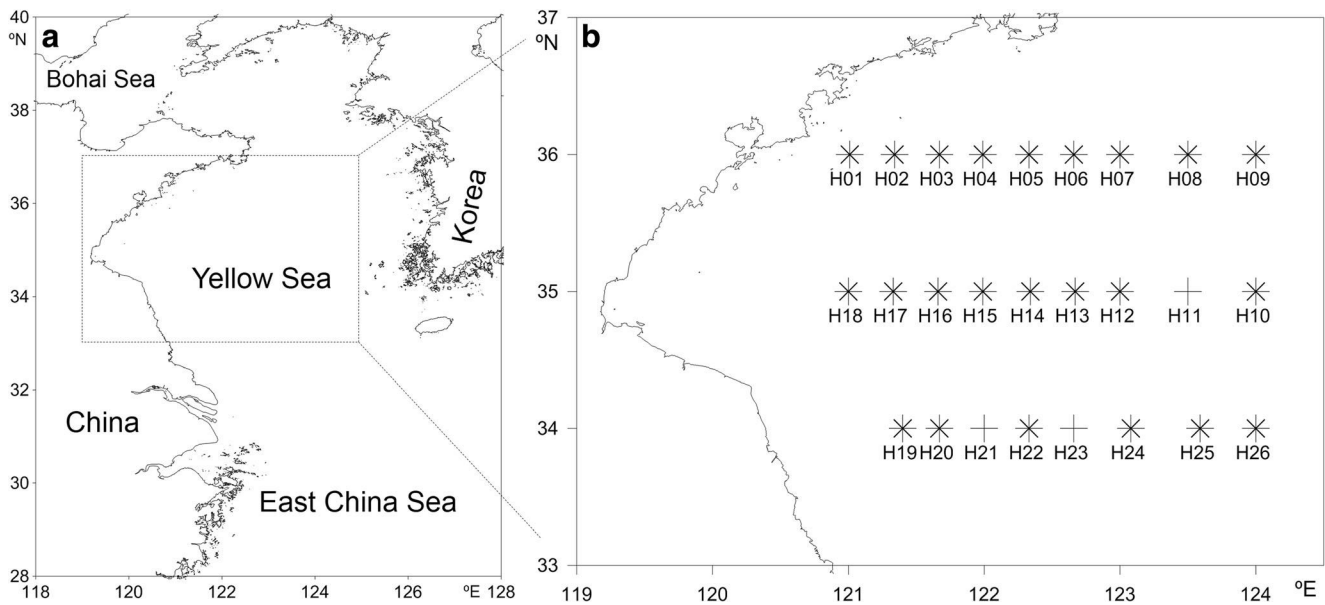
Fig. 1 Location of the sediment sampling stations in the Yellow Sea. The study area (**a**) and an expansion of the included panel (**b**), showing the locations of the sampling stations. Sediment was collected in both summer and winter at the stations designated ✳ in (**b**); stations where sediment was only collected in summer are designated by +

2.3 Optical and electron microscopy

Magnetotactic bacteria in each sediment sample were enriched by shaking the sample bottle to mix the water and sediment, and placing a permanent magnet (0.37 T each) at the water/sediment interface outside the bottle. After approximately 30 min, MTB cells accumulated to form a dark pellet at the water/sediment interface adjacent to the magnet. The pellets were removed using a Pasteur pipette and transferred into a 1.5-ml centrifuge tube. Because of the low abundance of MTB, the centrifuge tube was stood for approximately 5 min, during which time the MTB accumulated in the bottom of the centrifuge tube. A 30-μl volume was then removed from the bottom of the centrifuge tube and used to prepare a hanging drop. This was observed under an applied magnetic field using an Olympus CX31 microscope, and the number (N) of MTB was counted. The volume (V) of sediment in bottle was measured. The abundance (A) of MTB was calculated as $A = N/V$.

For transmission electron microscopy (TEM) a 3-μl volume of the magnet-enriched sample was removed from the bottom of the centrifuge tube and deposited on a Formvar-coated copper grid, which was washed with distilled water and air dried. The morphology of the MTB was examined using a TEM (Hitachi H8100, Japan) operating at 75 kV. Chemical composition and crystal structure analyses were performed using high-resolution transmission electron microscopy (HRTEM; JEM 2100F, JEOL, Japan; operating at 200 kV) equipped with energy dispersive X-ray spectroscopy (EDXS).

2.4 DNA extraction and 16S rRNA gene sequencing and analysis

Genomic DNA was extracted from 0.3 g wet weight of sediment from each of 14 stations in summer and 13 stations in winter where MTB were observed and station H24, using the FastDNA Spin Kit for Soil (MP Biomedicals, LLC, USA) and the Fast PrepTM FP120 cell disrupter (Thermo Electron Corp, USA). Following a quality check of the extracted DNA using a Nanodrop ND-1000 spectrophotometer (LabTech, Washington, DC, USA), the hypervariable region V3–V5 of bacterial 16S rRNA genes was amplified by PCR using the bar-coded universal primers 338F (5′-ACTCCTACGGGAGGCAGCA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′). Library construction and sequencing were performed by Majorbio Technology (Shanghai, China) using an Illumina MiSeq platform, following the manufacturer's protocol.

After removal of lower quality raw reads, the high-quality reads were clustered (USEARCH version 7.1; http://qiime.org/) into operational taxonomic units (OTUs) using a 97% identity threshold. Taxonomic classification was carried out using RDP Classifier (Wang et al. 2007) via the Silva database (Quast et al. 2013) (Silva release 123; http://www.arb-silva.de), based on a confidence threshold of 70%. OTU sequence blast to nt/nr database was done for MTB identification. The 16S rRNA gene sequences were aligned using the CLUSTAL W multiple alignment software. The phylogenetic tree was constructed via the neighbor-joining method using MEGA VERSION 6.0, and boot-strap values were calculated from 1000 replicates.

3 Results

3.1 Physical and chemical characteristics of bottom waters and sediments

The temperature average (11.58 °C) and range (7.03–22.80 °C) of bottom waters in summer was higher than in winter (9.53 °C and 8.05–11.02 °C, respectively). The salinity in summer ranged from 30.66 to 33.26 (average 32.11), while in winter ranged from 31.74 to 33.02 (average 32.04). The median sediment particle size varied from 5.28 to 149.44 μm in summer and 6.58 to 159.07 μm in winter, and peaked at station H17 in both seasons. The average nutrient percentages (summer and winter) were 1.14 and 1.07% for TC, 0.09 and 0.09% for TN, 0.12 and 0.15% for TS, and 0.55 and 0.48% for TOC. The concentration of Fe averaged 28.11 mg/g in summer and 28.89 mg/g in winter. There were correlations between the median sediment size and the nutrient percentage for TC (summer: $r = -0.665$, $P < 0.01$; winter: $r = -0.717$, $P < 0.01$), TN (summer: $r = -0.726$, $P < 0.01$; winter: $r = -0.651$, $P < 0.05$), TS (summer: $r = -0.613$, $P < 0.05$; winter: $r = -0.644$, $P < 0.05$), and TOC (summer: $r = -0.625$, $P < 0.05$; winter: $r = -0.662$, $P < 0.05$), and the concentration of Fe (summer: $r = -0.742$, $P < 0.01$; winter: $r = -0.721$, $P < 0.01$). The percentages of TC, TN, TS, and TOC, and the concentration of Fe were lower at station H17 than at other or most other stations (Table 1).

3.2 Distribution and morphology of MTB in sediments of the Yellow Sea

Microscopy observations for the presence of MTB were made immediately after field-collected sediment was brought onboard, to ensure that the abundance and morphology of MTB reflected that in situ. In summer, MTB were found at 12 of the 26 stations (38.46%) at an average abundance of 290 ind./dm³ (5–2400 ind./dm³), while in winter they were observed at 7 of the 23 stations (30.43%) at an average abundance of 130 ind./dm³ (5–820 ind./dm³). These results suggest that sediments from a higher percentage of stations had a higher abundance of MTB in summer than in winter. In both summer and winter, the stations where MTB were observed were distributed throughout the study area, from near-shore to far offshore (Fig. 2a, b). The number of MTB was highest at station H17 in both seasons (2400 ind./dm³ in summer, and 820 ind./dm³ in winter). At most stations except H17, the dominant morphology of MTB was coccoid. At H17, rod-shaped MTB (Fig. 3) that varied in size (average $14.36 \pm 2.72 \times 3.38 \pm 0.59$ μm; $n = 44$) dominated in both summer and winter, and intracellular interface and magnetosome chain-like structures were observed within MTB cells.

Diverse morphologies of MTB from station H17 were observed using TEM. Most of the rod-shaped MTB had a single magnetosome chain (Fig. 4a–g), but one cell had magnetosomes in a bundle (Fig. 4h). The magnetosome chains could be continuous (Fig. 4a–d, f, g) or broken (Fig. 4e), and the chain(s) were lengthwise paralleled (Fig. 4b–g) or widthwise paralleled (Fig. 4a). The coccoid MTB contained one or two magnetosome chain(s) (Fig. 4i–m), and the chain was broken in one cell (Fig. 4m). The magnetosome crystals included all the previously described morphologies including prismatic (Fig. 4a–c, i–m), tooth-shaped (Fig. 4d), and bullet-shaped (Fig. 4e–h). EDXS and HRTEM analysis showed that these magnetosomes were composed of magnetite (Fig. 5).

3.3 Correlation between MTB abundance and environmental factors

The abundance of MTB at each station where MTB were observed was significantly positive correlated with the median sediment grain size (0–500 μm) in both summer and winter (summer: $r = 0.710$, $P < 0.01$; winter: $r = 0.820$, $P < 0.01$). This suggests that the spatial distribution of MTB was regulated by the size of sediment particles. In the range of grain sizes examined (0–500 μm diameter), the abundance of MTB increased with increasing sediment coarseness. The abundance of MTB at station H17 in summer and winter was at least ten times of that at other stations, and the sediments at H17 were significantly coarser than at any other station in both summer and winter (Table 1). There was also a correlation between sediment particle size and MTB cell morphology. Cocci MTB dominated at the stations with larger particles while rod-shaped MTB dominated at stations with smaller particles. In addition, a trend of increasing MTB abundance with decreasing percentages of TC, TN, TS, and TOC, and a decreasing concentration of Fe, was also observed.

3.4 Phylogenetic diversity of MTB in sediments of the Yellow Sea

The number of optimized bacterial 16S rRNA gene reads obtained for each station ranged from 23,930 to 37,981, with an estimated coverage > 95% (95.08–97.43%). The reads were classified into 2738–3974 OTUs at each station, based on 97% sequence similarity. Based on the nt/nr database, 72 OTUs were likely related to MTB (Fig. 6). All 26 *Alphaproteobacteria* OTUs, 7 *Deltaproteobacteria* OTUs, and 38 *Nitrospirae* OTUs were present in both summer and winter, while one *Nitrospirae* OTU occurred only in winter. There were 19–41 OTUs of putative MTB at each station in summer (the maximum number occurred at station H05, and the minimum at station H21), and 26–46 OTUs occurred at

Table 1 Physical and chemical properties at the sampling stations from which live MTB were observed, or the DNA of MTB was obtained and sequenced

Station	Longitude (°E)	Latitude (°E)	Depth (m)	Temperature (°C)		Salinity (psu)		Sediment median size (μm)		TC%		TN%		TS%		TOC%		Fe (mg kg^{-1})	
				S.	W.	S.	W.	S.	W.	S.	W.	S.	W.	S.	W.	S.	W.	S.	W.
H01	121.01	36	20	14.79	8.36	31.48	31.84	15.39	11.43	1.89	2.01	0.08	0.05	0.01	0.09	0.27	0.34	36.81	46.86
H02	121.34	36	36	12.73	8.90	31.54	31.89	41.68	32.43	0.65	0.78	0.04	0.06	0.09	0.13	0.34	0.29	20.80	20.50
H05	122.33	36	50	8.56	8.99	32.33	31.92	6.66	6.85	1.36	1.43	0.13	0.15	0.22	0.22	0.65	0.75	35.51	35.92
H06	122.66	36	65	8.30	9.65	32.41	32.09	5.28	5.64	1.42	1.46	0.16	0.19	0.19	0.22	1.36	0.91	37.86	38.15
H08	123.50	36	72	8.43	ND	32.81	ND	6.69	6.40	1.37	1.44	0.18	0.19	0.17	0.27	1.31	0.89	36.40	36.43
H09	124.00	36	74	7.03	9.20	32.68	32.31	6.30	8.78	1.89	1.44	0.18	0.19	0.22	0.26	1.32	0.97	35.55	34.94
H10	124.00	35	78	8.70	9.64	32.96	33.02	7.58	9.92	0.92	0.98	0.10	0.12	0.13	0.16	0.70	0.58	29.11	26.78
H15	121.99	35	56	9.13	10.48	32.24	31.98	15.34	20.10	0.78	0.78	0.07	0.07	0.15	0.13	0.38	0.41	21.70	22.36
H16	121.66	35	45	9.62	10.45	31.86	31.96	80.58	97.22	0.58	0.38	0.03	0.03	0.08	0.07	0.19	0.14	16.82	15.64
H17[a]	**121.33**	**35**	**32**	**15.32**	**9.49**	**31.51**	**31.83**	**149.44**	**159.07**	**0.27**	**0.33**	**0.03**	**0.02**	**0.07**	**0.07**	**0.17**	**0.14**	**18.36**	**16.41**
H18	121.00	35	36	13.54	9.54	31.58	31.74	40.44	35.85	1.81	0.65	0.05	0.03	0.08	0.08	0.27	0.16	27.80	28.32
H21	122.00	34	20	22.80	8.05	30.66	31.77	104.29	ND.	0.80	ND.	0.02	ND.	0.04	ND.	0.08	ND.	23.09	ND.
H22	122.33	34	37	13.13	10.17	32.24	31.87	51.81	64.18	0.99	1.14	0.03	0.03	0.08	0.07	0.19	0.17	22.54	22.23
H24	123.08	34	67	10.10	11.02	33.26	32.30	9.04	10.16	1.18	1.14	0.10	0.09	0.19	0.15	0.47	0.43	31.12	31.04

S summer, *W* winter, *ND* not done

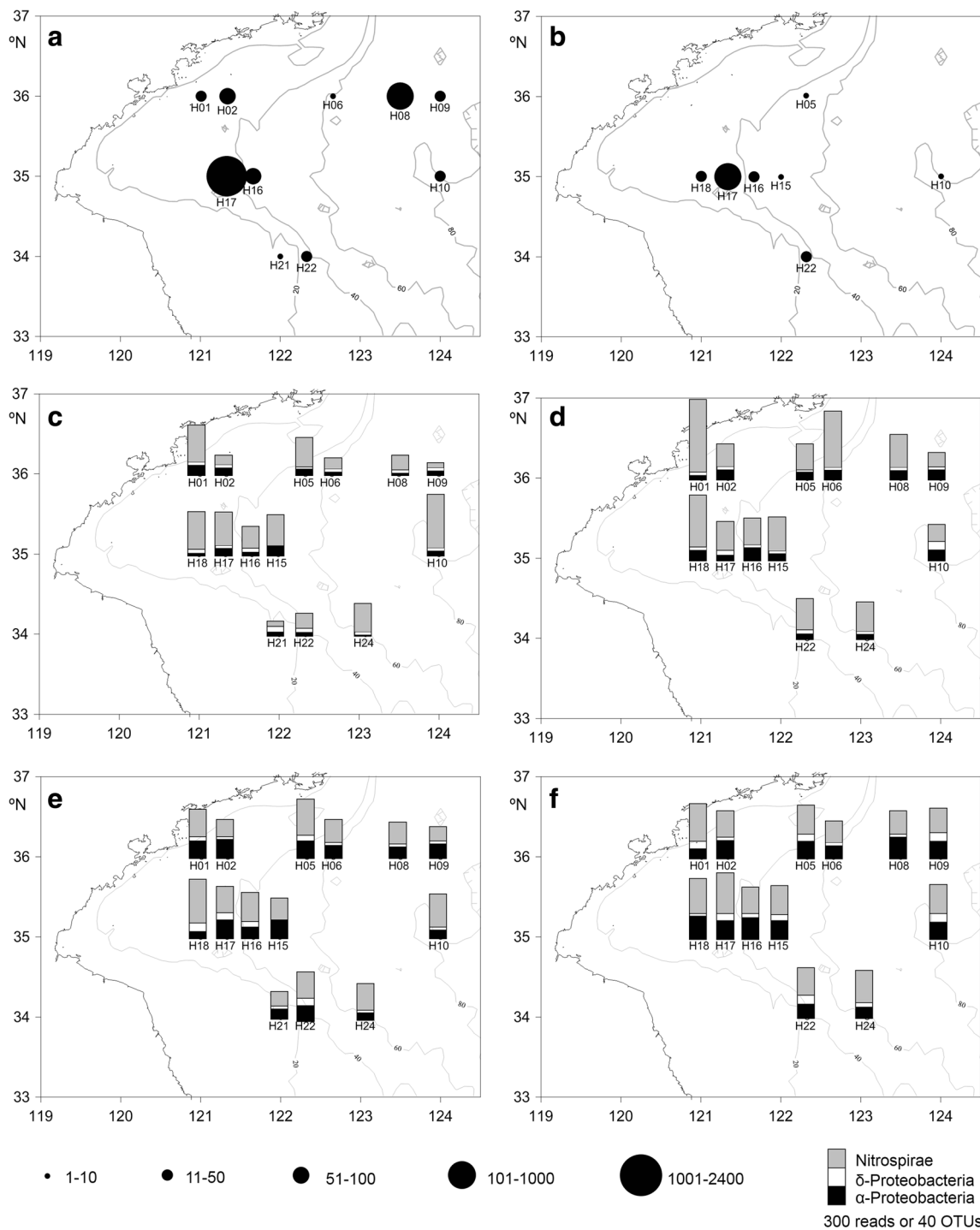[a] The environment factors of H17 are shown in bold

Fig. 2 Distribution of MTB abundance, and MTB reads and OTUs. The abundance (ind./dm³) of MTB in summer (**a**) and winter (**b**). The number of MTB reads in summer (**c**) and winter (**d**). The number of MTB OTUs in summer (**e**) and winter (**f**)

each station in winter (maximum at station H17, minimum at station H06). A greater number of putative MTB reads was found in winter (total 3271 reads, *Alphaproteobacteria* 657, *Deltaproteobacteria* 174, *Nitrospirae* 2440) than in summer (total 2447 reads, *Alphaproteobacteria* 448, *Deltaproteobacteria* 150, *Nitrospirae* 1849). In summer, 71–333 MTB reads were obtained at each station; the maximum

number of MTB reads was found at station H10 and the minimum was at station H09. In winter, 151–439 MTB reads were obtained at each station; the maximum was at station H01 and the minimum was at station H09 (Fig. 2c–f).

In both summer and winter, the percentage of putative MTB reads and the number of OTUs affiliated to *Nitrospirae* was highest (reads: approximately 75%; OTUs:
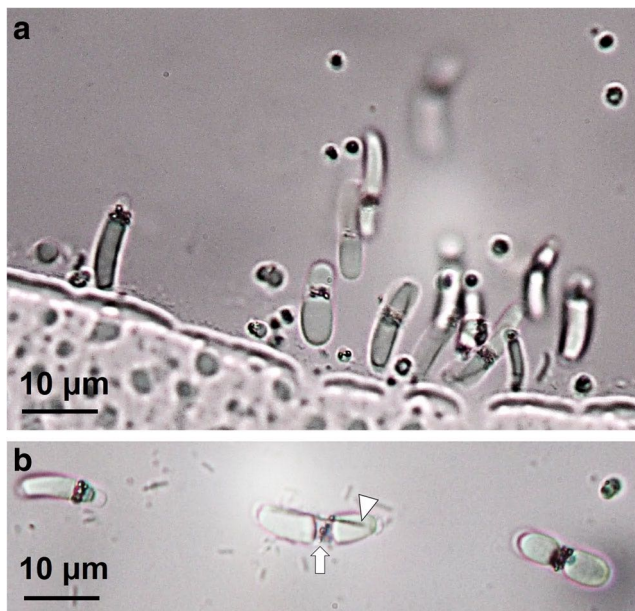
Fig. 3 Morphology of MTB from station H17, as viewed by optical microscopy. **a** Large rod-shaped MTB of various sizes were the dominant morphotype. **b** Magnetotactic bacteria having intracellular interfaces (indicated by the arrows) and the chain-like magnetosome structures (indicated by the triangles)

Fig. 4 Morphology of MTB from station H17, based on TEM. **a–h** Rod-shaped MTB. **i–m** Coccoid MTB. The magnetosomes in **h** were in a bunch. Cells in **a–g**, **j–k**, and **m** had single magnetosome chains. Cells in **i** and **l** had two magnetosome chains. The chains in **e** and **m** were broken. The magnetosomes in **a** were widthwise paralleled
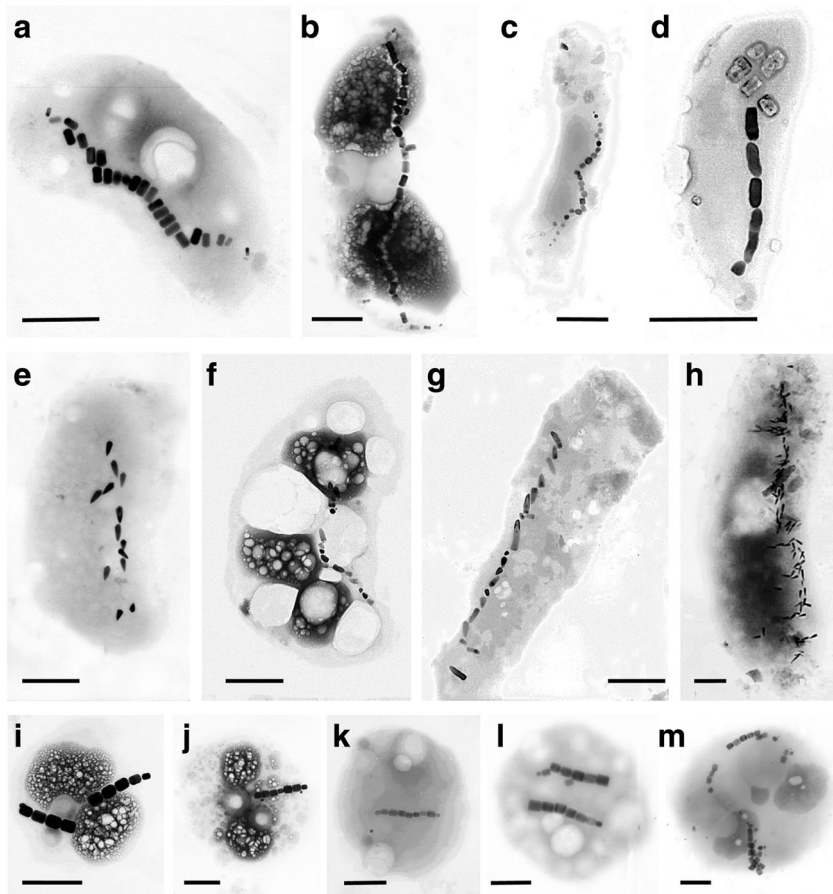
Bar=0.5 μm

> 50%); at 11 stations in summer and 12 in winter, the percentage of *Nitrospirae* reads was > 50% of the total. Blast analysis revealed that the putative *Nitrospirae* OTUs included 23 that were closest to uncultured *Magnetobacterium* sp. clone P1B_23 (FN687033.1); 8 that were closest to uncultured *Magnetobacterium* sp. clone PM5_6.4-14 (JQ177589.1); 7 that were closest to *Candidatus* Magnetobacterium bavaricum (FP929063.1); and 1 (OTU224) that was closest to *Candidatus* Magnetoovum mohavensis strain LO-1 (GU979422.1).

4 Discussion

The dominant group of MTB at each study station was affiliated to *Nitrospirae* (average 53%), which is quite different from most of other MTB communities, in which *Alphaproteobacteria* MTB have usually been found to be dominant (Spring et al. 1995). In sediments from 200 to 500 m depth near Nansha Islands (South China Sea) (Dai et al. 2002), one sequence belonging to the *Alphaproteobacteria* was obtained, while four OTUs
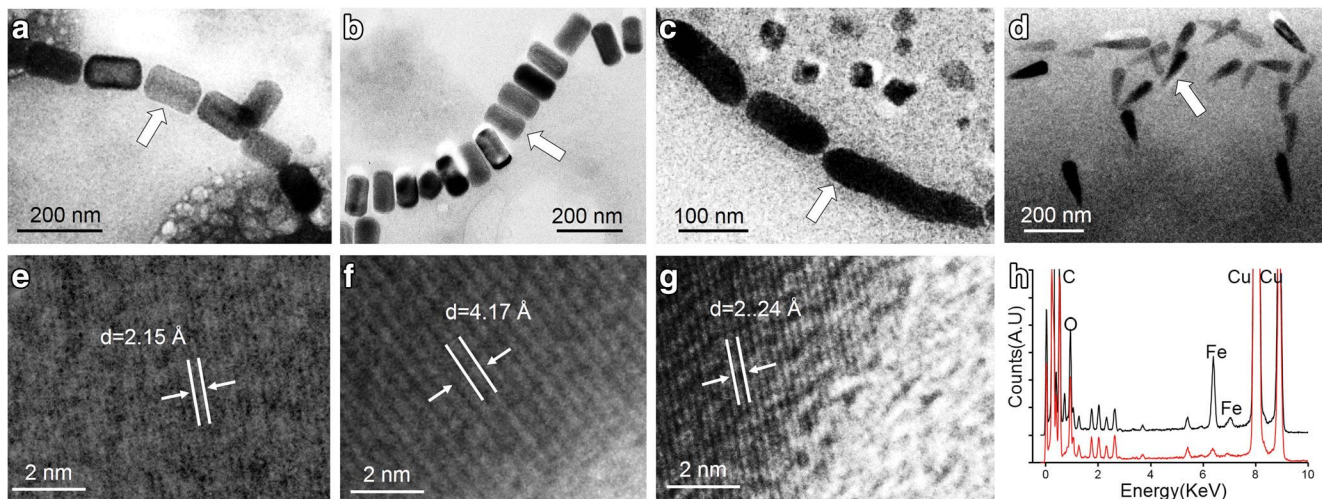
Fig. 5 HRTEM and EDXS analysis of magnetosomes. Prismatic-shaped magnetosomes (**a**, **b**). Tooth-shaped magnetosomes (**c**). Bullet-shaped magnetosomes (**d**). **e**–**g** are HRTEM images of the magnetosomes arrowed in **a**–**c**, respectively. **h** shows an EDXS analysis of the magnetosome arrowed in (**d**). HRTEM and EDXS analysis showed that these magnetosomes were magnetite

affiliated to the *Alphaproteobacteria* and one to the *Deltaproteobacteria* were obtained from sediments (approximately 5000 m depth) in the east Pacific Ocean (Dong et al. 2016). It is notable that the dominant group of MTB from near Nansha Islands and in the east Pacific Ocean, which comprise the only two reports involving taxonomic information on MTB from seafloor sediments, were members of the *Alphaproteobacteria*. Furthermore, the dominant group of MTB in our study comprised members of the *Nitrospirae*; this contrasts with previous reports of MTB in ocean habitats, which have found no evidence of the presence of this bacterial phylum. The closest sequence matches to the putative *Nitrospirae* MTB in the Yellow Sea were from three freshwater habitats (Petersen et al. 1989; Lefèvre et al. 2011; Rotaru et al. 2012) and a saline lake (Cangemi et al. 2010). This indicates that *Nitrospirae* MTB could occur in both freshwater and marine sediments and have a wider distribution than previously considered.

The abundance of MTB in sediments of the continental shelf of the Yellow Sea in summer and winter did not exceed 2400 ind./dm^3, which is orders of magnitude lower than the levels generally reported for environments where these bacteria have been found (Blakemore 1982), and is also lower than the abundance of MTB in the intertidal zone of the Yellow Sea, including Lake Yuehu (10^3–10^4 ind./cm^3) (Du et al. 2015). The only report of the abundance of MTB in seafloor sediment comes from the South Atlanta Ocean (depth = 600 m), where the max number found was 6×10^5 ind./cm^3 (Petermann and Bleil 1993); this is also higher than abundance of MTB in the Yellow Sea. The Yellow Sea is located between mainland China and the Korean Peninsula. It has a wide and shallow shelf that receives inputs of significant amount of terrigenous materials (Liu et al. 2015). The relatively low abundance of MTB in the sediment of the Yellow Sea continental shelf suggests that this environment is relatively unsuitable for MTB compared with the intertidal zone and the deep sea. Additionally, the number of reads from MiSeq sequencing of sediment genomic DNA can also somehow reflect the abundance of MTB, but the relative abundance based on the number of reads was not consistent with the abundance determined by microscopy. The number of reads is approximately quantitative within species, but between-species comparisons might be biased (Amend et al. 2010). Phylogenetic studies based on 16S rRNA gene sequence analysis have revealed that MTB are scattered within various taxa, including the classes *Alphaproteobacteria*, *Deltaproteobacteria*, *Gammaproteobacteria*, and the phylum *Nitrospirae* (Bazylinski et al. 2013), the phylum *Omnitrophica* (Kolinko et al. 2012; Lagkouvardos et al. 2014), and probably the candidate phylum *Latescibacteria* (Lin and Pan 2015). The use of 16S rRNA gene classification for MTB is limited by the limited number of MTB sequences in the available databases, making it difficult to identify novel MTB by comparison with known MTB sequences. Another factor that may contribute to the discrepancy is that the DNA of some weakly motile MTB was obtained from cells that were not observed by microscopy. And there are some MTB species that shared more than 98% of similarity on the 16S rRNA gene with nonmagnetotactic bacteria, species that probably lost the ability of synthetize magnetosomes. Hence, some nonmagnetotactic bacteria may be regarded as MTB. Different sampling methods for microscopy and sequencing can also cause discrepancies. In our study, the sample for microscopy was from the upper 3 cm of the sediment, and approximately 300 cm^3 of sediment was mixed prior to the extraction of MTB. Therefore, what was observed by microscopy was those MTB inhabiting the range to 3 cm,
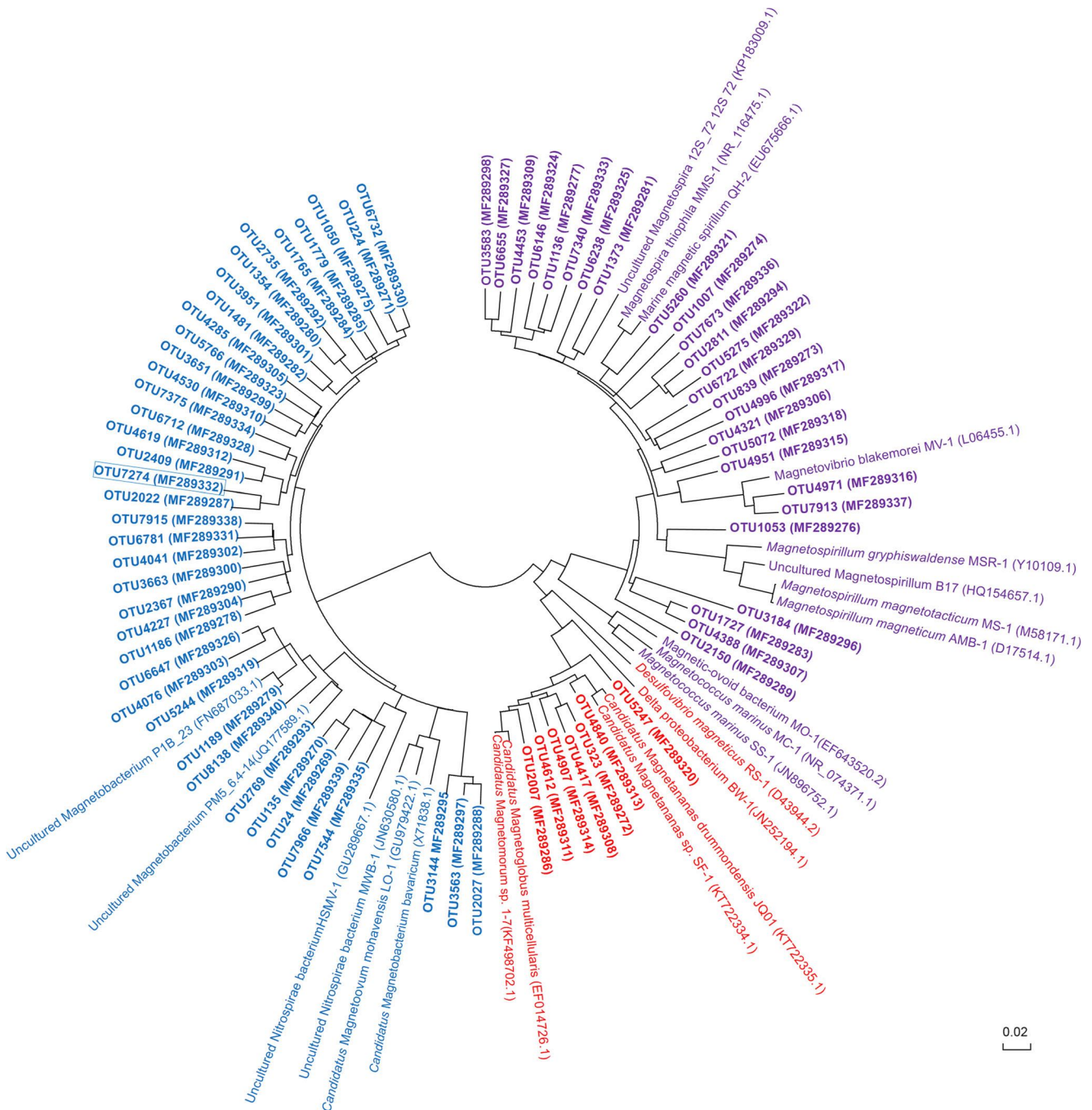
Fig. 6 Phylogenetic tree showing the relationships of the 72 putative MTB OTUs to the closest database matches, based on sequence similarity. The tree was generated using MEGA 6.0 and the neighbor-joining method, based on 425 bp sequence alignments for the 16S rRNA gene. The sequences obtained in this study are indicated in bold. The GenBank accession numbers for the sequences used are indicated in parentheses. Scale bar, 0.02 substitutions per nucleotide position. The genus classifications were based on the Silva database, release 123. All OTUs occurred in both summer and winter, except for OTU7274 (in box), which was only detected in winter

while the sample for sequencing was 300 mg of sediment randomly collected from 0 to 3 cm. Based on previous reports (Zhang et al. 2013a) and the vertical distribution of the MTB we studied (a core sample was obtained from station H17, was cut into 2-cm sections from the surface, and the abundance of MTB in each section was determined; see Electronic Supplementary Material), the abundance of MTB varies in the upper 3 cm of sediment.

Previous studies have shown that environmental factors, including temperature (Lin et al. 2012b), salinity (Martins et al. 2009; Lin et al. 2012a), nitrate (Lin and Pan 2010), and sulfur compounds (Postec et al. 2012), can explain the

distribution of MTB at local scales (Lin et al. 2013). The abundance of MTB at a sampling site in summer is higher than that in winter (Du et al. 2015), and vertically, it is related to the concentration of some chemical factors (Flies et al. 2005b). Little is known about the horizontal distribution of MTB across large spatial scales, other than for one previous study based on analysis of communities of MTB and their environments at several sampling sites in China and the USA (Lin et al. 2013), which showed that the main environmental influence was salinity. In this study, we found that MTB were more abundant in summer than in winter, which is a similar finding to those of previous reports. The sediments we collected from each station were from the top 1–3 cm, where most MTB at station H17 (and in a previous study (Zhang et al. 2013a)) were shown to occur.

The correlation between sediment grain size and the abundance of MTB raises questions about why and how grain size so strongly affects their distribution. Our present hypothesis is that sediment grain size is the most important factor influencing the movement of MTB. Magnetotactic bacteria are known to be chemolithoautotrophic and their source of electrons is in the deeper layer while terminal acceptor of electrons is in the upper layer. (Bazylinski and Frankel 2004; Williams et al. 2006; Lefèvre et al. 2012). By magnetotaxis, MTB move recurrently downwards to uptake sources of electrons and upwards to oxide them to obtain energy. (Bazylinski et al. 2013). Therefore, movement is indispensable for the metabolism and survival of MTB; however, motility may be restricted by sediments having very small grain sizes. The percentage of sediment particles > 190 μm in diameter at station H17 was at least twice that of the other stations, which may have been a critical factor affecting their occurrence (Fig. 7a, b). A simple model relating particles and space shed some light on the mechanism involved (Fig. 7c, d). For five particles forming a space, the area of the narrowest cross section is defined by $S = R^2 \times (\sqrt{3} - -\pi/2)$, and this is positively correlated with the size of the particles. Based on microscopy observations of the turning motion of the MTB, and that the area needed to turn was approximately a circle having a radius equivalent to the MTB cell length, we hypothesize that to swim freely the MTB need spaces twice the cell length (l). Therefore, to maximize the potential for movement, the diameter (D) of an inscribed circle at the narrowest point in the sediment should be twice the MTB cell length. Based on the geometric

Fig. 7 Grain size distribution spectra for the sampling stations, and a model representing the space among particles. The grain size distribution spectrum in summer (a) and winter (b). The spectra for station H17 are shown in bold lines. The spectra for station H17 was clearly different from that of the other stations, and the percentage of particles > 190 μm diameter at H17 was at least twice that at the other stations. The simple model for the space (arrow) among particles (c and d). The narrowest place in the space is shown in green in panel c, and is shown in panel d. The area of the void (yellow) was calculated as $S = R^2 \times (3^{0.5} - \pi/2)$, and was positively correlated with the size of particles
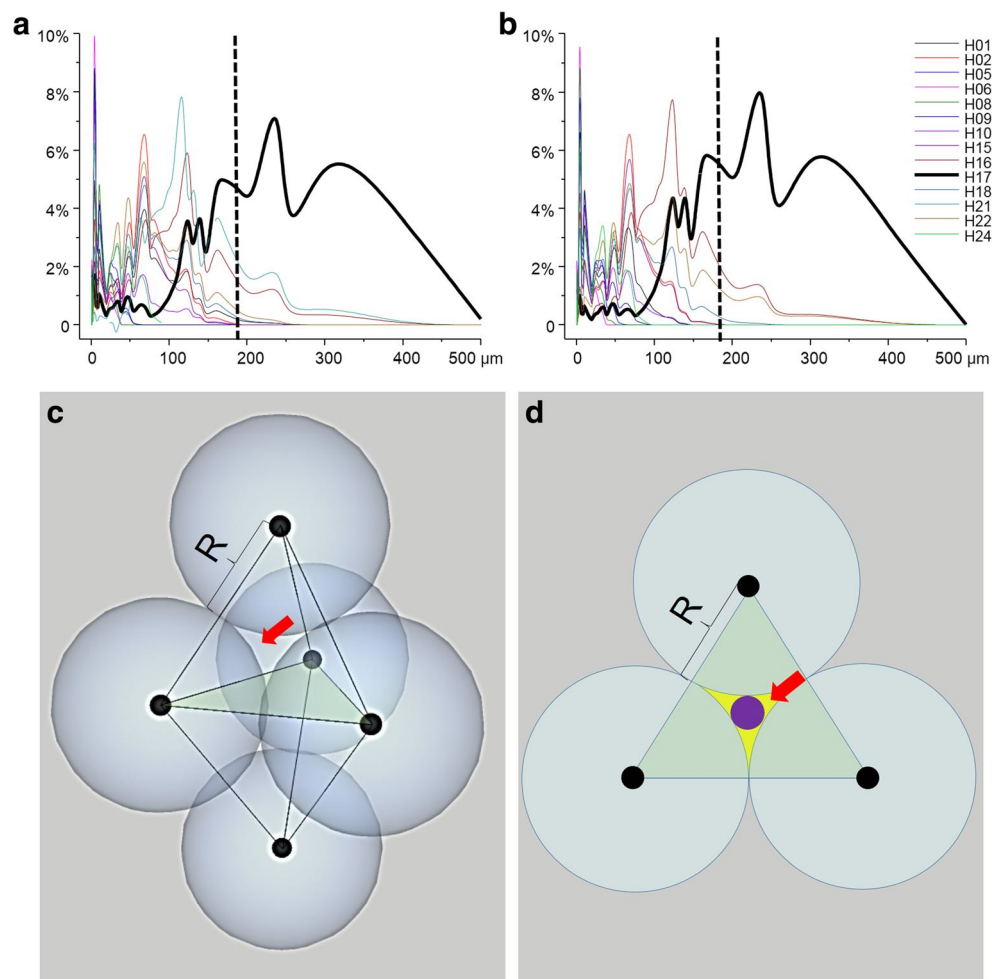
relationship $l = \frac{1}{2}D \cdot \frac{2\sqrt{3}-3}{3}$, the diameter of sediment particles should be no less than 190 μm and the percentage of particles > 190 μm was much higher (> 30%) at station H17 than at other stations (average < 1%). The above suggests that a minimum percentage of larger particles is essential to enable MTB swimming in sediments. Several studies have shown that sediment grain size is important for other benthic organisms including Foraminifera (Alve 1999), although whether they prefer fine (Debenay et al. 2001) or coarse (Diz et al. 2004) particles remains contentious (Armynot du Chatelet et al. 2009). However, the sediment grain size has been reported to be a limiting factor influencing the distribution of benthic Foraminifera (Armynot du Chatelet et al. 2009). This study is the first to report grain size as a critical factor influencing the abundance of MTB. More sophisticated modeling will be necessary to fully explain this phenomenon.

Biogeochemical parameters co-varied with particle size, decreasing with increasing particle size, which is a finding consistent with one previous study (Zhao and Yu 1983). In addition to the parameters we measured, the concentrations of Ti, P, Cu, Co, Ni, Zn, Cr, Li, and V have also been reported to decrease as particle size increases (Zhao and Yu 1983). In general, relative low concentrations of nutrients appear more favorable for the isolation of MTB compared with richer media (Bazylinski et al. 2013), and it may be that this applies in natural environments. In summer and winter the lowest percentages of TC, TN, TS, TOC, and concentration of Fe predominately occurred at station H17. Furthermore, the concentrations of Ti, P, Cu, Co, Ni, Zn, Cr, Li, and V at station H17 and in adjacent areas are evidently lower than at other locations in the Yellow Sea (Zhao and Yu 1983). It has been reported that Cu, Co, and Zn inhibit the motility of MTB, making them swim slowly and in some cases they became non-motile (Bahaj et al. 1994). Thus, the low concentrations of nutrients and heavy metals may have contributed to the high abundance of MTB at station H17. However, the abundance of MTB is probably influenced by multiple factors; the interactions between these and the grain-size dependent distribution of MTB need to be further studied.

5 Conclusions

This work demonstrates the diversity and distribution of MTB in seafloor sediment of the Yellow Sea. *Nitrospirae* are likely to be the dominant group and the abundance of MTB are strongly influenced by sediment grain size. In the range of grain sizes examined (0–500 μm diameter), the abundance of MTB increased with increasing sediment coarseness. Large particles can provide MTB with enough space and lower concentration of some chemical elements, which are suitable for MTB's survival. This is a systematic investigation concerning MTB in sediments of a continental shelf of the Yellow Sea. Knowledge of the distribution of MTB on the continental shelf of the Yellow Sea may serve as a general guideline to investigate the locations of MTB in seafloor habitats.

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