Available online at www.sciencedirect.com
ScienceDirect
Systematic and Applied Microbiology 32 (2009) 342–350
SYSTEMATIC AND APPLIED MICROBIOLOGY
www.elsevier.de/syapm

Diversity analysis of magnetotactic bacteria in Lake Miyun, northern China, by restriction fragment length polymorphism ☆

Wei Lin[a,b], Jinhua Li[a,b], Dirk Schüler[c], Christian Jogler[c], Yongxin Pan[a,*]

[a]*Biogeomagnetism Group, Paleomagnetism and Geochronology Laboratory, State Key Laboratory of Lithospheric Evolution, Institute of Geology and Geophysics, Chinese Academy of Sciences, Beijing 100029, China*
[b]*Graduate University of Chinese Academy of Sciences, Beijing 100039, China*
[c]*Department Biologie I Bereich Mikrobiologie, Ludwig-Maximillians-Universität München, Maria-Ward-Street 1a, 80638 München, Germany*

Received 31 July 2007

Abstract

Magnetotactic bacteria (MTB) synthesize intracellular nano-scale crystals of magnetite or greigite within magnetosomes. MTB are ubiquitous in limnic and marine environments. In order to understand the diversity of MTB better, sediment samples were examined from Lake Miyun near Beijing by restriction fragment length polymorphism (RFLP). First, *in silico* analysis was used to evaluate the effectiveness of 12 sets of restriction endonucleases for distinguishing MTB sequences retrieved from the GenBank database. It was found that the tested restriction endonucleases had different power in the ability to differentiate the operational taxonomic units (OTUs) of MTB. Specifically, of the 12 sets of enzymes, *Msp*I plus *Rsa*I was found to be the most effective for correctly differentiating the OTUs of selected MTB sequences and it could detect 16 OTUs with appropriate OTUmin and OTUmax values (96.7% and 97.7%, respectively). The *Msp*I plus *Rsa*I RFLP analysis was then utilized to investigate the diversity of MTB in Lake Miyun sediment and it identified 8 OTUs (74.5% of the whole library) as MTB. Among these, 5 were affiliated to *Alphaproteobacteria*, while the rest belonged to the *Nitrospira* phylum. Interestingly, OTUs C, D and I displayed 91.8–98.4% similarity to "*Magnetobacterium bavaricum*". Together, these results demonstrated that the *Msp*I plus *Rsa*I RFLP analysis was useful for studying the diversity and change in community composition of uncultivated MTB from environmental samples.

Keywords: Magnetotactic bacteria; Restriction fragment length polymorphism; Phylogenetic analysis; 16S rRNA; Transmission electron microscopy

Introduction

Magnetotactic bacteria (MTB) are a group of Gram-negative prokaryotes that possess intracellular, membrane-bound crystals of magnetic iron oxide (magnetite) or iron sulphide (greigite) minerals called magnetosomes, which are responsible for their magnetic orientation [2]. These bacteria exhibit diverse cell morphologies, such as coccoid, rod-shape, vibrioid, spirilloid and multicellular

Abbreviation: MTB, magnetotactic bacteria; RFLP, restriction fragment length polymorphism; OTUs, operational taxonomic units; TEM, transmission electron microscopy.

☆*Note*: Nucleotide sequence data of OTUs A, B, C, D, F, G, H and I are available in the GenBank database under the EU780674–EU780681.

*Corresponding author. Tel.: +86 10 6200 7913/8299 8406; fax: +86 10 6201 0846.
E-mail address: yxpan@mail.iggcas.ac.cn (Y. Pan).

0723-2020/$ - see front matter © 2008 Elsevier GmbH. All rights reserved.
doi:10.1016/j.syapm.2008.10.005

[5,18,20,21,42]. So far, most of the identified MTB affiliate with the *Alphaproteobacteria* [1], whereas many-celled magnetotactic prokaryotes and *Desulfovibrio magneticus* strain RS-1 belong to the *Deltaproteobacteria* [9,33]. In addition, "*Magnetobacterium bavaricum*" clusters close to the *Nitrospira* phylum [42]. Recently, at least some indications suggest the presence of rod-shaped MTB producing greigite crystals in the *Gammaproteobacteria* [37].

Various magnetotactic bacteria are found in diverse aquatic environments, e.g., marine habitats, lakes, rivers, estuary or deep-sea sediments and even in wet soil [3]. Despite the ubiquitous occurrence of MTB and their high abundance, so far, only a few MTB species have been isolated from the environment and grown in pure culture. However, MTB can be enriched from environmental samples by taking advantage of their magnetotactic behaviour. Consequently, the diversity of magnetically enriched MTB could be analyzed relatively easily without the need for cultivation [12,17,22,28,34,35,40,42,43,45].

Restriction fragment length polymorphism (RFLP) analysis is one broadly accepted method used to survey the phylogenetic diversity of bacteria [27]. Restriction endonucleases are used in this approach to produce different RFLP patterns, which can be defined as operational taxonomic units (OTUs) that can be inferred as single populations within a community [23]. Like other 16S rRNA gene-based methods, RFLP analysis lacks the limitations of culture-dependent techniques and has been used to identify bacterial diversity in the deep sea, hydrothermal vent systems [23,24], water columns [13,26], sediments [31,38,46,47] and soil [10,48,49]. Recently, RFLP analysis was applied to screen the 16S rRNA gene library of enriched MTB from environmental samples for investigating their community structures [12,28].

However, it has been found that the utilization of different restriction endonucleases resulted in a different resolution of the RFLP data for the distinction of *Mycoplasma*, *Pseudomonas* (sensu stricto) and mycobacteria [8,30,44]. Thus, it is necessary to evaluate the potential and power of RFLP for identifying the actual population of MTB. In the present study, firstly, the resolving power of 12 sets of commonly used restriction endonucleases was assessed on 23 selected 16S rRNA gene sequences of diverse MTB by *in silico* experiments. In addition, the diversity of magnetically enriched MTB from Lake Miyun near Beijing was investigated by using the evaluated RFLP analysis.

Materials and methods

Selection of MTB sequences

MTB are widespread in environmental sediments and *Alphaproteobacteria* are normally the dominant phylum [12,17,28,40,41,43,45]. In addition to the *Alphaproteobacteria* phylum, only a few 16S rRNA gene sequences are listed in the NCBI GenBank database. While alphaproteobacterial sequences show high-similarity values, 16S rRNA gene sequences of MTB from other phyla are much more diverse. Thus, in the present study, *Alphaproteobacteria* was mainly focused on. Of 68, 16S rRNA gene sequences obtained from MTB within the NCBI GenBank database, 23 were selected for subsequent *in silico* analysis based on two criteria: (i) the length of sequences should be >1300 bp and (ii) that these sequences spanned different types of MTB, including cocci, spirilla, vibrioid and rod-shaped bacteria (Supplementary Table 1). All species belonged to *Alphaproteobacteria*, except *D. magneticus* strain RS-1 that branched within the *Deltaproteobacteria*. Multiple sequence alignments were performed by using Vector NTI Advance 10.1.1 (Invitrogen) with default parameters. Then, the exterior ends of these sequences were trimmed manually to a length of approximately 1350 bp. As the identity between two well-studied model organisms, *Magnetospirillum magnetotacticum* MS-1 and *M. magnetotacticum* AMB-1, is 97.7% we arbitrarily defined identities between 2 MTB of $>98\%$ as one OTU [32]. According to this criterion, the 23 MTB selected for this study were affiliated in 18 OTUs (Supplementary Table 1).

Computer generation of RFLPs

Twelve commonly used sets of restriction endonucleases were evaluated in the present study: *Rsa*I; *Msp*I; *Alu*I plus *Rsa*I; *Msp*I plus *Hae*III; *Msp*I plus *Rsa*I; *Hha*I plus *Hae*III; *Hin*P1I plus *Msp*I; *Msp*I plus *Hha*I; *Rsa*I plus *Hind*III; *Hha*I, *Rsa*I plus *Hae*III; *Hha*I, *Rsa*I plus *Bst*UI and *Alu*I, *Dde*I plus *Msp*I. The restriction site determination, the size of RFLP fragments and the expected results of the analysis via gel electrophoresis were simulated *in silico* by using Vector NTI Advance 10.1.1 (Invitrogen). Restriction fragments shorter than 99 bp were not considered and fragments that differed by about 7 bp or less in size were treated as identical in this study [44,46]. Two parameters were defined: OTUmin, representing the minimal identity of nucleic acid sequences between two strains in the same OTU, and, OTUmax, reflecting the maximal identity of nucleic acid sequences between two strains in different OTUs. Both parameters were utilized to evaluate the resolution of the selected restriction endonucleases. The ideal value of OTUmin and OTUmax should be identical to the similarity threshold for different MTB species (i.e., 98% in this study).

Sampling and magnetic collection of MTB from lake sediment

The sampling of MTB-rich sediments was described elsewhere [17]. Briefly, sediment from the first 5–10 cm

was collected from Lake Miyun, located in the Yanshan Mountains, approximately 80 km northeast of Beijing city, at a water depth of 1–2 m. To set up microcosms, sediment samples were transferred to 600 ml plastic flasks covered with approximately 100 ml of water from the sampling sites and stored at room temperature in dim light. After several weeks of incubation, blooming events of MTB near the water/sediment interface were observed. MTB were first collected from sediments in microcosms by using magnets, as described previously [34]. Collected MTB were then purified from non-magnetic contaminations by using a double-ended open magnetic separation apparatus (Jogler et al., personal communication). MTB were collected under a homogenous magnetic field, whose strength was 5 times greater than the Earth's magnetic field.

Transmission electron microscopy (TEM) analysis

A drop of magnetically enriched MTB was deposited onto a copper grid (230 meshes) covered by a carbon-coated formvar film. The cells were allowed to settle on the grid for 1 h. Excess liquid was removed with a filter paper. All grids were rinsed at least twice with distilled water before TEM observation. In case of negative staining, wet grids were treated with a drop of negative staining solution (1.5% uranyl acetate) for 1 min and an FEI Tecnai 20, with an accelerating voltage of 120 kV, was used for TEM and negative staining analyses.

PCR–RFLP analysis

Two universal bacterial primers, 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) [6], were used to amplify 16S rRNA genes directly from magnetically enriched MTB cells, as described previously [17]. Amplification products were cloned using a commercially available pMD19-T vector cloning kit (TaKaRa). Ligation and transformation were carried out according to the manufacturer's instructions. The transformed cells were then plated onto Luria–Bertani agar plates with ampicillin. A total of 49 colonies were randomly picked and screened for 16S rRNA gene inserts, which were amplified (at a 30 μl scale) with the primers specific for the pMD19-T vector. Ten microliters of PCR products were digested with 0.1 U of *Msp*I plus 0.1 U of *Rsa*I (MBI Fermentas) overnight at 37 °C. The resulting fragments were separated by gel electrophoresis in 4% (wt/vol) agarose in 1 × TAE buffer. RFLP patterns were compared, and OTUs were defined, by using the Quantity One v4.6.2 (Bio-Rad Laboratories).

Sequencing and phylogenetic analysis

Representative clones of all OTUs were chosen for sequencing. All sequences were checked for chimera formation with the CHECK_CHIMERA software of the Ribosomal Database Project. The resulting sequences were compared against the NCBI GenBank database by using the BLAST algorithm [4] to identify sequences with high similarity. Vector NTI Advance 10.1.1 (Invitrogen) was used for subsequent alignment. Phylogenetic analyses were conducted by using MEGA version 3.1 [16] based on the neighbour-joining analysis of selected sequences. Bootstrap analysis of 100 replicates was performed to validate the reproducibility of the branching pattern of the tree. The sequences retrieved in this study were deposited in the GenBank database under EU780674–EU780681.

Results

In silico evaluation of restriction endonucleases for RFLP analysis of MTB

All 23 selected MTB in this study affiliated to 18 OTUs (Supplementary Table 1). For all of them, the theoretical restriction patterns of 12 sets of restriction endonucleases were calculated and performed *in silico*. Initial observation revealed that the restriction endonucleases used had different power in the ability to differentiate the OTUs of MTB (Table 1). *Msp*I plus *Hae*III, *Alu*I, *Dde*I plus *Msp*I and *Msp*I plus *Rsa*I were able to detect a large number of different OTUs (18, 17 and 16 OTUs, respectively), while *Rsa*I, *Rsa*I plus *Hind*III and *Hha*I, *Rsa*I plus *Bst*UI could only distinguish 12 OTUs. The average number of restriction fragments per strain for each set of restriction endonucleases was estimated (Table 1). The results showed a range of 4–6 fragments per strain. Additionally, the percentages of successfully identified OTUs divided by the real number of OTUs (18 OTUs) were presented (Table 1). Of 12 sets of restriction endonucleases, *Alu*I, *Dde*I plus *Msp*I, *Msp*I plus *Rsa*I and *Msp*I plus *Hae*III were most efficient for correct identification of OTUs (83.3% and 77.8%). Furthermore, the identities of nucleic acid sequences were compared between the same and different OTU(s) acquired by the three sets of restriction endonucleases mentioned above (Table 2). The higher OTUmax of *Msp*I plus *Hae*III and *Alu*I, *Dde*I plus *Msp*I, 99.6% and 99.8%, respectively, meant that using these two sets of restriction endonucleases might overestimate the number of OTUs, i.e., the retrieved number of OTUs by these enzymes was more than the real number of OTUs for some MTB. Correspondingly, the lower OTUmin of *Msp*I plus

Table 1. Summary of *in silico* RFLP analysis for 23 selected 16S rRNA genes of MTB.

Restriction endonuclease	Number of detected OTUs	Mean number of restriction fragments per strain	Successful affiliations[a] (%)	OTUs which cannot be differentiated by this set of enzymes[b]
MspI plus *HaeIII*	18	5	77.8	OTU II, XI and XII
AluI plus *DdeI* plus *MspI*	17	5	83.3	OTU II and XII; OTU V and VI
MspI plus *RsaI*	16	5	77.8	OTU II and XII; OTU V and VI
AluI plus *RsaI*	15	6	66.7	OTU II and XII; OTU VII, IX and X; OTU V and VI
RsaI, *HhaI* plus *HaeIII*	15	5	66.7	OTU VII and IX; OTU V and X; OTU XV and XVIII
MspI	15	4	66.7	OTU II and XII; OTU V and VI; OTU X and XI
Hinp1I plus *MspI*	14	4	66.7	OTU II and XII; OTU V, VI and VIII
MspI plus *HhaI*	14	4	66.7	OTU II and XII; OTU V, VI and VIII
HaeIII plus *HhaI*	13	6	55.6	OTU I and III; OTU V, VII, IX and X; OTU XV and XVIII
RsaI	12	4	50.0	OTU II, V, VI, X and XII; OTU VII and IX
RsaI Plus *HindIII*	12	4	50.0	OTU II, V, VI, X and XII; OTU VII and IX; OTU XV and XVI
HhaI, RsaI plus *BstUI*	12	5	38.9	OTU II and XII; OTU VII and IX; OTU V, VI and X; OTU VIII and XI; OTU XV and XVI

[a]Percentage of successfully identified OTUs divided by the real number of OTUs (18 OTUs, Supplementary Table 1).
[b]Detailed information for the OTUs is shown in Supplementary Table 1.

Table 2. Comparison of OTUmin and OTUmax for three sets of restriction endonucleases.

Restriction endonucleases	OTUmin (%)[a]	OTUmax (%)[b]
*Msp*I plus *Hae*III	89.40	99.60
*Alu*I, *Dde*I plus *Msp*I	96.90	99.80
*Msp*I plus *Rsa*I	96.70	97.70

[a]Minimal identity of nucleic acid sequences between two strains in the same OTU.
[b]Maximal identity of nucleic acid sequences between two strains in different OTUs.

*Hae*III (89.4%) showed that different strains of MTB could share the same RFLP patterns, which led to an underestimate of community diversity. However, *Msp*I plus *Rsa*I gave appropriate values of OTUmin and OTUmax (96.7% and 97.7%, respectively, Table 2), which were close to the ideal value of 98%. Thus, of the three sets of restriction endonucleases, *Msp*I plus *Rsa*I outperformed the others and could reflect the diversity of selected MTB sequences better.

Morphological diversity of magnetically enriched MTB from Lake Miyun

Approximately, 151 of Lake Miyun sediments were divided into more than 50 microcosms, which were then analysed with respect to the occurrence and community structure of MTB. At the beginning of the first year, most samples were dominated by magnotactic cocci and their sequences were related to previously identified uncultivated magnetotactic cocci in the *Alphaproteobacteria* [17]. However, after 1 year's incubation, the population of MTB in most samples obviously changed, as revealed by light microscopy and TEM analysis. In this study, the MTB were magnetically enriched from the 2-year-aged samples, which were composed of various morphotypes of MTB, such as cocci, rod-shaped bacteria and occasionally spirilla (Fig. 1A–F). Interestingly, a large magnetotactic rod-shaped bacterium was found to be dominant in some microcosms, and it was morphologically identical (length: 5–10 μm; width: 1.5 μm; up to 600 bullet-shaped magnetosomes) to the previously described "*M. bavaricum*" found in the mesotrophic Lake Chiemsee (Fig. 1I) [42]. Furthermore, bullet-shaped magnetosomes were also found in a distinct magnetotactic rod and a peculiar magnetotactic coccus (Fig. 1G and H).

RFLP and sequencing analyses of magnetically enriched MTB from Lake Miyun

MTB enriched from more than 50 2-year-aged microcosms were pooled and a clone library of 16S rRNA genes was subsequently constructed. A total of 49 clones were randomly picked and screened and 47 of these clones contained insets of the proper size.

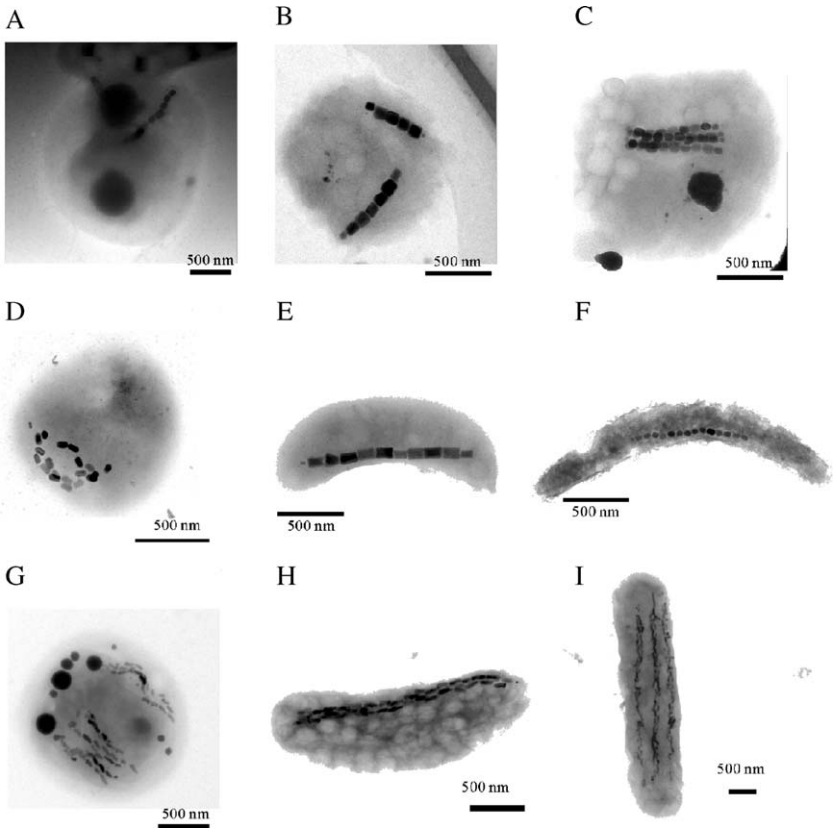

Fig. 1. Representative electron micrographs of different MTB collected from Lake Miyun. These included cocci (A–D and G), vibroid to rod-shaped bacteria (E, H and I) and spirilla (F). Three different morphotypes of bacteria were found with several chains of bullet-shaped magnetosomes (G–I).

The community of the recovered clones was examined by the RFLP analysis with the aid of *Msp*I plus *Rsa*I, and 17 OTUs were identified (Fig. 2). Representative clones from each OTU were selected for sequencing analysis, and the nearly full length 16S rRNA gene sequences were determined. By using the CHECK_CHIMERA program, two clones (representing OTUs P and Q) were found to be chimeras and they were thus eliminated from further analysis (Fig. 2). The resulting sequences were compared against the NCBI nucleotide database using the BLAST algorithm. It was shown that 8 out of 15 OTUs, accounting for 74.5% of all the 16S rRNA genes cloned, were composed of MTB. Among these OTUs, five groups were most similar to magnetotactic cocci and thus affiliated into *Alphaproteobacteria*, which accounted for 48.9% of the examined 16S rRNA gene clones (Fig. 3). Additionally, there were three OTUs, accounting for 28.9% of the entire clone library, that were most similar to magnetotactic rod MHB-1 and "*M. bavaricum*" which could be affiliated with the *Nitrospira* phylum [12,42] (Fig. 3). OTU I had a maximum identity of 97.8% with magnetotactic bacterium *M. bavaricum*. OTU C was found to be most similar to magnetotactic rod MHB-1 (98.8% identity). OTU D was 94.0% and 95.3%

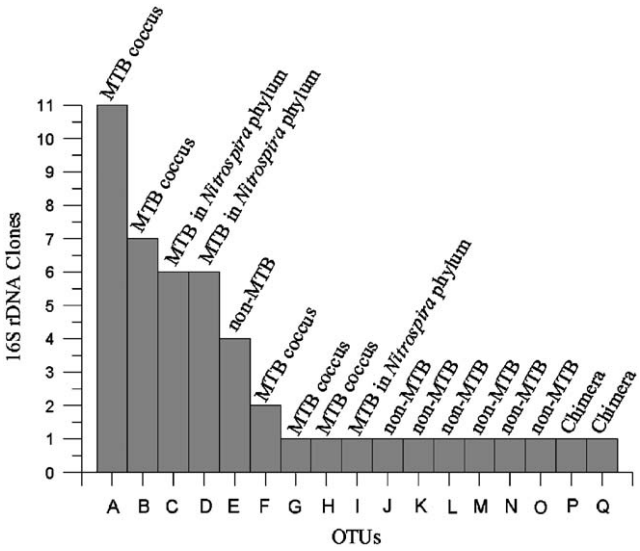

Fig. 2. Distribution of 16S rRNA gene clones from magnetically enriched samples among different OTUs. The OTUs were numbered from A to Q.

identical to "*M. bavaricum*" and *Magnetococcus* CF3, respectively. In addition, 22.2% of the clones in the library probably originated from non-magnetic

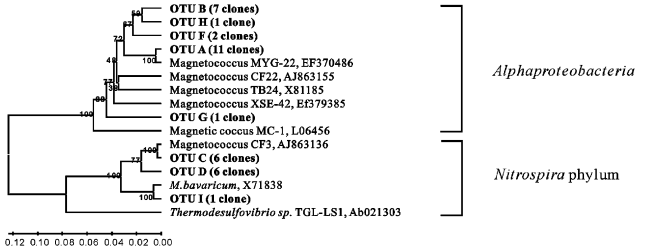

Fig. 3. Preliminary phylogenetic tree showing the relationships between the 8 MTB OTUs in *Alphaproteobacteria* and the *Nitrospira* phylum. The tree was generated using neighbour-joining analysis. The sequences determined in this study are written in bold. The numbers at nodes represent bootstrap values for the nodes (100 times resampling analysis).

contamination, as indicated by the lack of similarity to known MTB sequences.

Discussion

Diverse MTB with a high abundance might play a significant role in iron and sulphur cycling within aquatic ecosystems [36]. Investigation of MTB diversity is of great interest since it could not only improve the understanding of the correlation between the MTB population and environmental factors, but also extend our knowledge of their origin and evolution. Like other uncultivated environmental bacteria, a reliable approach for studying MTB diversity is a challenging task.

RFLP analysis enables a rapid investigation of community diversity. However, the restriction endonucleases used play a critical role in the analysis of the results. Moyer et al. [25] first systematically evaluated the rationale for selecting restriction endonucleases and found that the choice of numbers and types of restriction enzymes could enhance the information gained from the same bacterial community. Similar results were also reported by Engebretson et al. and Stakenborg et al. [11,44]. In this study, we focused on the evaluation of appropriate restriction endonucleases, which are efficient at detecting different MTB. Our results demonstrated that simply using more restriction endonucleases did not increase the resolution of MTB differentiation. For instance, *Msp*I was able to resolve more OTUs than several sets of two or three restriction endonucleases (Table 1). Although a set of *Msp*I plus *Hae*III and a set of *Alu*I, *Dde*I plus *Msp*I detected more OTUs in this study, some of them were false-OTUs because their OTUmax was very high (99.60% and 99.80%, Table 2). Furthermore, an OTUmin of 89.4% with a set of *Msp*I plus *Hae*III suggested that this set of enzymes can not effectively differentiate all selected sequences. Our findings demonstrated that exclusive use of the RFLP method alone to analyze the community structure of MTB should be treated with care, since this method might bias the diversity of communities by using inappropriate sets of enzymes. *Rsa*I plus *Hind*III and *Msp*I were previously used to investigate the diversity of MTB in microcosms and marine samples [12,28]. However, both of them could only correctly detect 50% and 66.7% of the OTUs (Table 1), which suggests that these two sets of restriction endonucleases were not appropriate to unravel the real community structure of uncultivated MTB. According to our *in silico* analysis, the *Msp*I plus *Rsa*I RFLP analysis should have a better resolution power for screening the diversity of MTB. It should be noted that given the faster advances and cheaper price of DNA sequencing technology, MTB clone libraries might be screened by direct sequencing in the future rather than using traditional fingerprint methods, such as RFLP. However, RFLP could be directly applied to the PCR products retrieved from MTB communities by 16S rRNA gene primers in order to characterize compositional changes over time and space, and further link these changes to environmental factors. This may facilitate future studies on the ecology and distribution of MTB.

However, since 22 of the 23 selected MTB sequences in this study belonged to *Alphaproteobacteria*, the *Msp*I plus *Rsa*I set might only be effective for detecting MTB within this class. So far, only a few known 16S rRNA MTB gene sequences affiliate outside the *Alphaproteobacteria* and the similarities between them are not very high, although it was found in this study that *Msp*I plus *Rsa*I could still correctly distinguish them. However, it is believed that more sequences of MTB affiliating within or outside the *Alphaproteobacteria* will be detected in the future, and then *Msp*I plus *Rsa*I might be inappropriate for describing the community composition of MTB for any new sequences spanning several phyla.

MTB are ubiquitous in aquatic sediment samples, no matter whether they are freshwater or marine. Coccoid-shaped cells are the most abundant MTP morphotype occurring in nature. So far, diverse morphology and phylogeny of magnetotactic cocci have been analyzed by culture-independent approaches and nearly all of them are affiliated within the *Alphaproteobacteria* [7,12,17,19,22,28,39,40,43,45]. Five magnetotactic cocci OTUs were retrieved in the present study and all of them had a phylogenetic affiliation with the *Alphaproteobacteria* as well (Fig. 3). Based on their sequence identities (91.5–96.9%, Supplementary Table 2), these OTUs may represent five different species. Four further OTUs (OTU A, OTU B, OTU F and OTU H), together with the previously cloned 16S rRNA gene MYG-22 [17], comprised a monophyletic group and were 93–97% similar to the published magnetotactic cocci (Fig. 3). OTU G was most closely related to the uncultured magnetotactic coccus clone CF 22 [12]; however, the

similarity of 92% was relatively low. In addition, OTU G was 91–92% similar to the other four OTUs (Supplementary Table 2). Consequently, OTU G is probably a new species, which would be distinct from known magnetotactic cocci at the genus level.

In our study, another three OTUs (OTU C, OTU D and OTU I) were found to affiliate within the *Magnetobacterium* group in the *Nitrospira* phylum. OTU I shared high similarity with "*M. bavaricum*" (97.8%), which was detected from the calcareous sediments of the mesotrophic Lake Chiemsee (near Munich, Germany). It contained more than 600 bullet-shaped magnetosomes arranged in several chains [14,29]. On the other hand, OTU C from the present study was 98.8% identical to the 16S rRNA gene sequence of MHB-1, which was found in a microcosm obtained from the eutrophic Waller Lake (Bremen, Germany). MHB-1 exhibits the same morphology of magnetosomes as those from "*M. bavaricum*" and they are aligned in multiple chains as well [12]. OTU D is also related to the *Magnetobacterium* group and it is most similar to MHB-1 (similarity of 95%). Thus, OTU D probably represents a new species of MTB within the *Nitrospira* phylum, in addition to *M. bavaricum* and MHB-1. Recently, besides Lake Chiemsee and Waller Lake [42], "*M. bavaricum*"-like bacteria were also found in ponds near Munich (Jogler and Lin, unpublished observation) and in the River Seine upstream from Paris, France [15]. Our result of MTB affiliated within the *Nitrospira* phylum is so far the first report of the occurrence of these bacteria in China, which in addition suggests a micro-diversity of "*M. bavaricum*"-related bacteria in natural habitats, and that these bacteria are more ubiquitous in environmental samples than previously imagined. This finding is of great interest, given the fact that "*M. bavaricum*" was shown to represent a significant proportion of the microbial biomass (30%) within its micro-habitat and its supposed dominant ecological role in this sediment layer [42]. Further *in situ* studies of diverse lakes in different locations could provide deeper insights into the distribution and abundance of "*M. bavaricum*"-like bacteria. In addition, more 16S rRNA gene sequences from distinct habitats of these bacteria are desirable in order to address the question of whether "*M. bavaricum*"-like bacteria belong to the *Nitrospira* phylum, or form a new phylum.

Acknowledgements

We thank Aihua Deng at the Institute of Microbiology, CAS, for her suggestions and excellent technical assistance during the experiments. We are grateful to Rudolf Amann and two anonymous reviewers for valuable comments, which significantly improved the manuscript. This work was supported by CAS project KZCX-3-SW-150 and NSFC grants 40821091 and 40325011. W. Lin is grateful to the Max Planck/CAS doctoral promotion program (DPP) for supporting his stay in the LMU Munich.

Appendix A. Supplementary materials

The online version of this article contains additional supplementary data. Please visit doi:10.1016/j.syapm.2008.10.005.

References

[1] R. Amann, J. Peplies, D. Schüler, Diversity and Taxonomy of Magnetotactic Bacteria, Springer, Berlin, 2006.
[2] D.L. Balkwill, D. Maratea, R.P. Blakemore, Ultrastructure of a magnetic spirillum, J. Bacteriol. 141 (1980) 1399–1408.
[3] D. Bazylinski, R. Frankel, Magnetosome formation in prokaryotes, Nat. Rev. Microbiol. 2 (2004) 217–230.
[4] D.A. Benson, I. Karsch-Mizrachi, D.J. Lipman, J. Ostell, D.L. Wheeler, Genbank, Nucleic Acids Res. 33 (2005) D34–D38.
[5] R.P. Blakemore, D. Maratea, R.S. Wolfe, Isolation and pure culture of a freshwater magnetic spirillum in chemically defined medium, J. Bacteriol. 140 (1979) 720–729.
[6] M.T. Brandl, B. Quinones, S.E. Lindow, Heterogeneous transcription of an indoleacetic acid biosynthetic gene in *Erwinia herbicola* on plant surfaces, Proc. Natl. Acad. Sci. USA 98 (2001) 3454–3459.
[7] B.L. Cox, R. Popa, D.A. Bazylinski, B. Lanoil, S. Douglas, A. Belz, D.L. Engler, K.H. Nealson, Organization and elemental analysis of P-, S-, and Fe-rich inclusions in a population of freshwater magnetococci, Geomicrobiol. J. 19 (2002) 387–406.
[8] T. De Baere, R. de Mendonca, G. Claeys, G. Verschraegen, W. Mijs, R. Verhelst, S. Rottiers, L. Van Simaey, C. De Ganck, M. Vaneechoutte, Evaluation of amplified rDNA restriction analysis (ARDRA) for the identification of cultured mycobacteria in a diagnostic laboratory, BMC Microbiol. 2 (2002) 4.
[9] E.F. Delong, R.B. Frankel, D.A. Bazylinski, Multiple evolutionary origins of magnetotaxis in bacteria, Science 259 (1993) 803–806.
[10] J. Dunbar, S.M. Barns, L.O. Ticknor, C.R. Kuske, Empirical and theoretical bacterial diversity in four Arizona soils, Appl. Environ. Microbiol. 68 (2002) 3035–3045.
[11] J.J. Engebretson, C.L. Moyer, Fidelity of select restriction endonucleases in determining microbial diversity by terminal-restriction fragment length polymorphism, Appl. Environ. Microbiol. 69 (2003) 4823–4829.
[12] C.B. Flies, J. Peplies, D. Schüler, Combined approach for characterization of uncultivated magnetotactic bacteria

from various aquatic environments, Appl. Environ. Microbiol. 71 (2005) 2723–2731.
[13] J.A. Fuhrman, A.A. Davis, Widespread archaea and novel bacteria from the deep sea as shown by 16S rRNA gene sequences, Mar. Ecol.-Prog. Ser. 150 (1997) 275–285.
[14] M. Hanzlik, M. Winklhofer, N. Petersen, Pulsed-field-remanence measurements on individual magnetotactic bacteria, J. Magn. Magn. Mater. 248 (2002) 258–267.
[15] A. Isambert, N. Menguy, E. Larquet, F. Guyot, J.P. Valet, Transmission electron microscopy study of magnetites in a freshwater population of magnetotactic bacteria, Am. Miner. 92 (2007) 621–630.
[16] S. Kumar, K. Tamura, M. Nei, MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment, Brief. Bioinform. 5 (2004) 150–163.
[17] W. Lin, L. Tian, J. Li, Y. Pan, Does capillary racetrack-based enrichment reflect the diversity of uncultivated magnetotactic cocci in environmental samples?, FEMS Microbiol. Lett. 279 (2008) 202–206.
[18] U. Lins, M. Farina, Organization of cells in magnetotactic multicellular aggregates, Microbiol. Res. 154 (1999) 9–13.
[19] U. Lins, F. Freitas, C.N. Keim, M. Farina, Electron spectroscopic imaging of magnetotactic bacteria: magnetosome morphology and diversity, Microsc. Microanal. 6 (2000) 463–470.
[20] F. Meldrum, S. Mann, B.R. Heywood, R.B. Frankel, D.A. Bazylinski, Electron microscopy study of magnetosomes in a cultured coccoid magnetotactic bacterium, Proc. R. Soc. Lond. B 251 (1993) 231–236.
[21] F.C. Meldrum, S. Mann, B.R. Heywood, R.B. Frankel, D.A. Bazylinski, Electron microscopy study of magnetosomes in two cultured vibrioid magnetotactic bacteria, Proc. R. Soc. Lond. B 251 (1993) 237–242.
[22] T.T. Moench, W.A. Konetzka, A novel method for the isolation and study of a magnetotactic bacterium, Arch. Microbiol. 119 (1978) 203–212.
[23] C.L. Moyer, F.C. Dobbs, D.M. Karl, Estimation of diversity and community structure through restriction-fragment-length-polymorphism distribution analysis of bacterial 16s ribosomal-RNA genes from a microbial mat at an active, hydrothermal vent system, Loihi Seamount, Hawaii, Appl. Environ. Microbiol. 60 (1994) 871–879.
[24] C.L. Moyer, F.C. Dobbs, D.M. Karl, Phylogenetic diversity of the bacterial community from a microbial mat at an active, hydrothermal vent system, Loihi Seamount, Hawaii, Appl. Environ. Microbiol. 61 (1995) 1555–1562.
[25] C.L. Moyer, J.M. Tiedje, F.C. Dobbs, D.M. Karl, A computer-simulated restriction fragment length polymorphism analysis of bacterial small-subunit rRNA genes: efficacy of selected tetrameric restriction enzymes for studies of microbial diversity in nature?, Appl. Environ. Microbiol. 62 (1996) 2501–2507.
[26] T.D. Mullins, T.B. Britschgi, R.L. Krest, S.J. Giovannoni, Genetic comparisons reveal the same unknown bacterial lineages in Atlantic and Pacific bacterioplankton communities, Limnol. Oceanogr. 40 (1995) 148–158.
[27] A. Nocker, M. Burr, A.K. Camper, Genotypic microbial community profiling: a critical technical review, Microb. Ecol. 54 (2007) 276–289.
[28] H.M. Pan, K.L. Zhu, T. Song, K. Yu-Zhang, C. Lefevre, S. Xing, M. Liu, S.J. Zhao, T. Xiao, L.F. Wu, Characterization of a homogeneous taxonomic group of marine magnetotactic cocci within a low tide zone in the China Sea, Environ. Microbiol. 10 (2008) 1158–1164.
[29] Y. Pan, N. Petersen, M. Winklhofer, A. Davila, Q. Liu, T. Frederichs, M. Hanzlik, R. Zhu, Rock magnetic properties of uncultured magnetotactic bacteria, Earth Planet. Sci. Lett. 237 (2005) 311–325.
[30] L.A. Porteous, F. Widmer, R.J. Seidler, Multiple enzyme restriction fragment length polymorphism analysis for high resolution distinction of *Pseudomonas* (sensu stricto) 16S rRNA genes, J. Microbiol. Methods 51 (2002) 337–348.
[31] K. Ravenschlag, K. Sahm, J. Pernthaler, R. Amann, High bacterial diversity in permanently cold marine sediments, Appl. Environ. Microbiol. 65 (1999) 3982–3989.
[32] M. Sait, P. Hugenholtz, P.H. Janssen, Cultivation of globally distributed soil bacteria from phylogenetic lineages previously only detected in cultivation-independent surveys, Environ. Microbiol. 4 (2002) 654–666.
[33] T. Sakaguchi, A. Arakaki, T. Matsunaga, *Desulfovibrio magneticus* sp. nov., a novel sulfate-reducing bacterium that produces intracellular single-domain-sized magnetite particles, Int. J. Syst. Evol. Microbiol. 52 (2002) 215–221.
[34] D. Schüler, S. Spring, D.A. Bazylinski, Improved technique for the isolation of magnetotactic spirilla from a freshwater sediment and their phylogenetic characterization, Syst. Appl. Microbiol. 22 (1999) 466–471.
[35] S.L. Simmons, D.A. Bazylinski, K.J. Edwards, Population dynamics of marine magnetotactic bacteria in a meromictic salt pond described with qPCR, Environ. Microbiol. 9 (2007) 2162–2174.
[36] S.L. Simmons, K.J. Edwards, Geobiology of Magnetotactic Bacteria, Springer, Berlin, 2006.
[37] S.L. Simmons, S.M. Sievert, R.B. Frankel, D.A. Bazylinski, K.J. Edwards, Spatiotemporal distribution of marine magnetotactic bacteria in a seasonally stratified coastal salt pond, Appl. Environ. Microbiol. 70 (2004) 6230–6239.
[38] S. Sjoling, D.A. Cowan, High 16S rDNA bacterial diversity in glacial meltwater lake sediment, Bratina Island, Antarctica, Extremophiles 7 (2003) 275–282.
[39] S. Spring, R. Amann, W. Ludwig, K.-H. Schleifer, D. Schüler, K. Poralla, N. Petersen, Phylogenetic analysis of uncultured magnetotactic bacteria from the alpha-subclass of *Proteobacteria*, Syst. Appl. Microbiol. 17 (1994) 501–508.
[40] S. Spring, R. Amann, W. Ludwig, K.H. Schleifer, N. Petersen, Phylogenetic diversity and Identification of nonculturable magnetotactic bacteria, Syst. Appl. Microbiol. 15 (1992) 116–122.
[41] S. Spring, R. Amann, W. Ludwig, K.H. Schleifer, D. Schüler, K. Poralla, N. Petersen, Phylogenetic analysis of uncultured magnetotactic bacteria from the alpha-subclass of *Proteobacteria*, Syst. Appl. Microbiol. 17 (1995) 501–508.
[42] S. Spring, R. Amann, W. Ludwig, K.H. Schleifer, H. van Gemerden, N. Petersen, Dominating role of an unusual magnetotactic bacterium in the microaerobic zone of a freshwater sediment, Appl. Environ. Microbiol. 59 (1993) 2397–2403.

[43] S. Spring, U. Lins, R. Amann, K. Schleifer, L. Ferreira, D. Esquivel, M. Farina, Phylogenetic affiliation and ultrastructure of uncultured magnetic bacteria with unusually large magnetosomes, Arch. Microbiol. 169 (1998) 136–147.

[44] T. Stakenborg, J. Vicca, P. Butaye, D. Maes, T. De Baere, R. Verhelst, J. Peeters, A. de Kruif, F. Haesebrouck, M. Vaneechoutte, Evaluation of amplified rDNA restriction analysis (ARDRA) for the identification of *Mycoplasma* species, BMC Infect. Dis. 5 (2005).

[45] R.H. Thornhill, J.G. Burgess, T. Matsunaga, PCR for direct detection of indigenous uncultured magnetic cocci in sediment and phylogenetic analysis of amplified 16S ribosomal DNA, Appl. Environ. Microbiol. 61 (1995) 495–500.

[46] H. Urakawa, K. Kita-Tsukamoto, K. Ohwada, Microbial diversity in marine sediments from Sagami Bay and Tokyo Bay, Japan, as determined by 16S rRNA gene analysis, Microbiology-UK 145 (1999) 3305–3315.

[47] M.X. Xu, P. Wang, F.P. Wang, X. Xiao, Microbial diversity at a deep-sea station of the Pacific nodule province, Biodivers. Conserv. 14 (2005) 3363–3380.

[48] J.Z. Zhou, M.E. Davey, J.B. Figueras, E. Rivkina, D. Gilichinsky, J.M. Tiedje, Phylogenetic diversity of a bacterial community determined from Siberian tundra soil DNA, Microbiology-UK 143 (1997) 3913–3919.

[49] J.Z. Zhou, B.C. Xia, D.S. Treves, L.Y. Wu, T.L. Marsh, R.V. O'Neill, A.V. Palumbo, J.M. Tiedje, Spatial and resource factors influencing high microbial diversity in soil, Appl. Environ. Microbiol. 68 (2002) 326–334.