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Research Paper

Molecular identification of the occurrence of magnetotactic bacteria in fresh water sediments (Czech Republic)

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Abstract

Magnetotactic bacteria (MTB) are of considerable interest because of their importance in the manufacture of various bioinspired materials. In order to find out the status of magnetotactic bacteria at three different sediment in Czech Republic, samples collected from both standing and running freshwaters were subjected to molecular diversity analysis by using 16S *rRNA* gene approach. Total community DNA from sediment sample was isolated and used for PCR, cloning and sequence analysis. Of the 24 analyzed sequences, six clones are closely related to *Magnetobacterium* sp. affiliated with the *Nitrospira* phylum which showed the dominance of *Magnetobacterium* phylotypes in the sample. This study will provide useful insight about the community structure of MTB in this particular geographical region. However more detailed and specific studies are warranted in order to properly assess the community structure of MTB's in fresh water sediments.

Key words: magnetotactic bacteria, sediment, PCR, cloning, phylogeny, 16S *rRNA* gene.

Introduction

Due to the considerable importance of magnetotactic bacteria (MTB) in the generation or manufacture of bioinspired materials, such as functionalized magnetic nanoparticles and nanotubes, magnetic tapes, printing inks, magnetic targeting of pharmaceutical drugs these bacteria are of interest to the microbiologists worldwide. Their role in cell separation and their application as contrast-enhancement agents in magnetic resonance imaging is also of interest to the biologists (Schuler and Frankel, 1999).These all properties are exhibited by these bacteria because of the presence of magnetosome crystals in them. These magnetosomes are specialized organelles consisting of magnetic iron minerals and because of these magnetosomes they are able to align and swim along with magnetic field lines (Keim *et al.*, 2007). These magnetosomes are comprised of membrane bound nano-sized crystals of magnetic iron mineral, which are arranged in one or multiple chain-like structures that enable the cell to passively align it along the external magnetic fields, a behavior known as 'magnetotaxis' (Blakemore, 1975). It is in combination with chemotaxis, aerotaxis, and perhaps phototaxis, is thought to direct the swimming of cells toward growth-favoring microoxic zones at the bottom of chemically stratified natural waters (Frankel *et al.*, 2006; Thompson *et al.*, 1994). Earlier magnetosome structure and formation were reviewed by Schüler & Frankel (1999), Schüler (2002) and by Schüller (2008).

MTB represents a diverse group of bacteria with respect to phylogeny, morphology and physiology (Spring and Schleifer, 1995). Most of the phylogenetic and morphologic diversity of the members of this group have been revealed by metagenomic analysis of 16S rRNA gene of the samples collected from natural environments. Magnetotactic prokaryotes have been affilated to two major bacterial lineages majorly, the Proteobacteria and the newly defined phylum Nitrospira. Common morphotypes include coccoid cells as well as rods, vibrios, and spirilla (Blakemore, 1975, 1982; Flies *et al.*, 2005; Lin *et al.*, 2005; Lin *et al.*, 2008; Simmons *et al.*, 2004; Spring *et al.*, 1994, 1998 and Thornhill *et al.*, 1995). Most known MTB are affiliated with the α-proteobacteria type, but magnetosome-like inclusions and magnetic orientation have also been described

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for some representatives of the δ-proteobacteria (DeLong *et al.*, 1993; Kawaguchi *et al.*, 1995; Posfai *et al.*, 2006 and Simmons *et al.*, 2004), γ-proteobacteria (Simmons *et al.*, 2004) and the Nitrospira phylum (Flies *et al.*, 2005 and Spring *et al.*, 1993). Magnetotactic bacteria are frequently found in freshwater or marine sediments. They are most abundant in the oxic-anoxic transition zone of sediments (Flies *et al.*, 2005) but have also been detected in stratified water columns or soil. Despite their high abundance and ubiquitous occurrence in many marine and freshwater habitats, most MTB's are difficult to isolate and cultivate in the laboratory, probably due to their strict lifestyle that is adapted to complex chemical gradients typically found in stratified sediments. The only available and genuinely described pure cultures of magnetotactic bacteria belongs to the genus *Magnetospirillum* and lies within the Proteobacteria phylum (Burgess *et al.*, 1993 and Kawaguchi *et al.*, 1992)while coccoid magnetotactic bacteria (Flies *et al.*, 2005) and several other morphotypes including the large magnetotactic rod, *Magnetobacterium bavaricum* containing up to nearly 103 magnetosome particles per cell (Spring *et al.*, 1992 and 1993) are not yet available in pure culture.

As only few magnetotactic bacteria strains are isolated in pure culture by now so there is worldwide increased in the efforts of finding some new culturable strains that were able to produce the magnetosome nanoparticles. So in the current study an effort has been made to check the diversity of MTB at some localities of Czech Republic with the aim to find out the possibility of some culturable MTB strains in this part of the world. In this brief study, we describe first results obtained by detailed molecular analysis of the three microcosm samples thought to be having the most abundant MTB populations.

Material and Methods

Study sites characteristics, sampling and setup microcosms

Samples were collected at a depth of 0.5-1 m from 12 different sites in Czech republic during the year 2007-2008. Selected physiochemical parametrs were measure by using portable multimeter HANNA HI9892 (dissolved oxygen, pH, temperature, redox potential) and in laboratory (Total Nitrogen, Total Phosphorus, Fe^{2+}) for both surface and interstitial water. In laboratory wet sediment was sieved and only particles < 1.5 mm were transferred to glass bottles (0.5-1 L), covered loosely and incubated in these microcosms under room temperature and low-light conditions for several months (3 replicates for each sample). Each bottle contained 2/3 of sediment and 1/3 of the overlying water originated from particular locality. This water was periodically added during the incubation. After that time, to check the presence of magnetotactic bacteria in the sediment samples, samples were taken just beneath the water sediment interface and used for enrichment by imposing a magnetic field with a bar magnet or MTB cells were enriched by attaching the south pole of a permanent magnet outside a bottle 1 cm above the sediment surface. After 3 h mixture of the water and sediment near the south pole of the magnet was collected with a pipette. MTB from three different habitats were also collected for subsequent molecular analysis because of their abundant MTB populations. Sample (microcosm) B was from a small garden pond located in small spa near Buchlovice-Leopoldov, sample D was taken from an oxbow of the Dyje River near Nové Mlýny reservoirs and sample S became from a Sitka stream, small 3rd order lowland stream near to Olomouc city.

Nucleic acid extraction, PCR amplification, cloning and sequencing

Total community DNA was extracted from 0.25 g of sediment with an PowerSoil™ DNA Isolation Kit (MoBio, Karlsbad, CA) according to the manufaturer's instructions. Bacterial 16S rRNA gene fragments (~1500 bp) were amplified using PCR with the universal primer pair 27F(AGA GTT TGA TCC TGG CTC AG) and 1492R(AAG GAG GTG ATC CAG CCG CA) (Massol-Deya *et al.*, 1995). The steps in PCR include initial denaturation at 94 °C for 5 min and 30 cycles consisting of denaturation at 94 °C for 1 min, primer annealing at 55 °C for 1 min, and extension at 72 °C for 1 min. The final elongation step was extended to 10 min. The resulting 1.5 kb PCR product was purified and cloned into pCR2.1-TOPO cloning vector and transformed into OneShot®DH5α™ TOP10 chemically competent *Escherichia coli* cells according to the manufacturer's instructions (Invitrogen, Carlsbad, CA). Randomly selected clones were sequenced using an ABI PRISM Big Dye Terminator Cycle-Sequencing Ready Reaction kit (PE-Applied Biosystems, Foster, CA) and an automatic sequence analyzer (ABI 3700).

BLAST, CLUSTAL W and phylogenetic analysis

Sequences obtained after sequencing were aligned by using Clustal W (Thompson *et al.*, 1994). The resulting sequences were compared with the Basic Local Alignment Search Tool (BLAST) in order to find out the sequence homology with the magnetotactic bacteria sequences available in the GenBank database. These sequences along with the orher homology sequences are then subjected to phylogenetic analyses using MEGA software (Kimura, 1980 and Kumar *et al.*, 2004). All partial sequences produced in this study have been deposited in GenBank under accession numbers GQ246749 (sequence B6), GQ246750 (D17), GQ246751 (D28), GQ246752 (D32), GQ246753 (S46) and GQ246754 (D47).

Results and Discussion

In the present study, occurrence and diversity of magnetotactic bacteria present in the sediment samples col-

lected from 3 different sites in Czech Republic was characterized by using comparative 16S rDNA gene sequence analysis. Out of the total analyzed sequences 38% showed similarity with the members of the phylum *Proteobacteria*, while 25% of the sequences resembles with the members of the phylum *Nitrospira*. The minor groups included the phyla *Acidobacteria* (17%), *Chloroflexi* (13%) and *Verrucomicrobia* (8%). In the phylum *Nitrospira,* all of these six phylotypes have showed a very high similarity (> 98% based on RDP database) to the known magnetotactic bacteria strains and were used further for phylogenetic analysis. On the basis of the higher abundance of magnetotactic bacteria phylotypes, the sediment sample from locality D (oxbow of the Dyje river) has showed the highest phylogenetic diversity than two remaining samples where only one-one phylotype showed a sequence similarity pattern with known MTB sequence was found. In Figure 1 it has been clearly depicted that all cloned bacterial 16S rRNA gene sequences obtained from this study were primarily closely related to *Magnetobacterium* sp. clones affiliated with the *Nitrospira* phylum. The closest relatives to the four clones from locality D (D17; D28; D32 and D47) were environmental clones retrieved from sediment of one Chinese reservoir (DQ833491), the Yellow Sea sediment (EU652666) and two *Magnetobacterium* clones from in situ reactor columns degrading benzene filled with lava granules (EF613377 and EF613379) (Kleinsteuber *et al.*, 2008). Four clones determined in this study seems to be related to each other, nevertheless, the high level of sequence divergence (up to 19%, Table 1) indicate that these cloned sequences could belong to different genera. The clone S46 from the small lowland stream was closest to the *Magnetobacterium* clone ZZ L1B6 (EF613368) which was also recovered from in situ reactor columns degrading benzene filled with lava granules (Kleinsteuber *et al.*, 2008). Finally, the clone B6 from the locality B (small spa pond) seems to be at least similar to the rest of clones retrieved from Czech sediments and is affiliated closely to clone TDNP USbc97 (Acc. No. FJ516912) retrieved from wetland in Central Spain. Our samples have showed only a limited diversity among hitherto described MTB. Long-term cultivation of sediment samples in the microcosms was recommended to enrich MTB. However, there have been some problems in subsequent purification of MTB by using the capillary racetrack (Wolfe *et al.*, 1987). This step might be led to the differences in MTB diversity between molecular and the racetrack approaches (Lin *et al.*, 2008) In this study, we used only molecular approach based on the community DNA extraction from the microcosm samples, thus no changes in community composition should occur here. *Magnetobacterium* phylotypes affiliated to the phylum *Nitrospira* comes out to be the dominant phylotype in the clone library generated from our sediments. Large magnetotactic rod-shaped bacteria tentatively named *Candidatus Magnetobacterium bavaricum*, described for the first time from freshwater lake sediments in Bavaria (Spring *et al.*, 1993), is frequently found in the sediments of some freshwater lakes. This uncultured magnetotactic bacterium is phylogenetically affiliated to the *Nitrospira* phylum and was given candidatus status due to its distinctive phenotypic traits (Spring *et al.*, 1993). *Candidatus Magnetobacterium bavaricum*has been suggested to be a chemolithoautotroph with an iron-dependent mode of energy conservation (Spring *et al.*, 1993). Besides different positioning of the environmental clones which appears to be the closest relative of our clones as mentioned above recently. In a stable isotope probing experiment with C-13 labeled acetate, *Magnetobacterium*-like organisms were found to metabolize acetate in a methanogenic sediment (Schwarz *et al.*, 2007). Uncultivated magnetotactic members of the phylum *Nitrospira* were previously detected in various oligotrophic lakes in Upper Bavaria (Spring *et al.*, 1993) and lake in northern Germany (Flies *et al.*, 2005). The general dominance of MTB clones belonging to the phylum *Nitrospira* in our sediment samples from Czech Republic and relatively high similarity to *Magnetobacterium bavaricum* (Figure 1) indicates that the occurrence of MTB belonging to this lineage is not geographically restricted to Germany only. Till now there is only one study focusing on rod-shaped bacterium producing magnetite and greigite within its magnetosomes which has been described from oxic-anoxic transition zone of a semi anaerobic estuarine basin of the Pettaquamscutt River, Rhode Island (Bazylinski *et al.*, 1995).To our knowledge this is the first study providing an evidence that MTB may also reside in the sediments of running waters. Since our long-term study revealed that coupling of anaerobic metabolism and methanogenesis appear to be an important pathway in organic carbon cycling in the Sitka stream sediments (Hlavacova *et al.*, 2005; Rulik *et al.*, 2000 and 2008), one expects that MTB might be also involved in methane dynamics as has been already suggested by Schwarz *et al.* (2007).On the basis of this study we conclude that all *Magnetobacterium* phylotype affiliated with phylum *Nitrospira* is the dominant phylotypes of MTB in freshwater sediments in Czech Republic whereas no culturable strain has been reported in this study. When our effort should face to finding of culturable MTB, it seems that further analyses of sediments from various freshwater bodies are therefore required.

On the basis of the MTB diversity in the present study, we can say that this study provides useful insight on the future research work in studying the MTB in fresh water sediments from different parts of the world. This information adds to our understanding of community structure of MTB in this particular geographical region. It also indicated that further studies are warranted in order to properly assess the effect of change in the geographical locations and climatic conditions, on the community structure of MTB's in fresh water sediments.

Table 1 - Similarity values of 16S rNA gene sequences recovered from freshwater sediments and other sequences affiliated with Nitrospira phylum. The sequences determined in this study are written in bold. Sequence similarity was calculated by converting the evolutionary distance using the equation: (1-evolutionary distance) x100.

		% sequence similarity															
Sequence	Accession no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1. B6	GQ246749	-															
2. D17	GQ246750	92.3	-														
3. D28	GQ246751	92.6	92.7	-													
4. D32	GQ246752	85.7	85.3	90.2	-												
5. D47	GQ246754	93.0	96.9	92.9	87.7	-											
6. S46	GQ246753	90.6	92.3	91.5	83.6	91.3	-										
7. clone D2CL	EU498373	87.5	86.4	85.9	78.6	86.8	85.8	-									
8. clone ZZ L1B6	EF613368	90.8	91.4	91.0	82.6	90.8	98.7	85.5	-								
9. clone ZZ L4E8	EF613377	92.1	97.7	91.6	90.3	97.7	90.5	85.2	90.1	-							
10. clone ZZ L4G12	EF613379	91.7	97.3	90.6	90.2	97.5	89.9	84.1	89.7	99.0	-						
11. M bavaricum	X71838	84.9	84.9	83.7	76.7	85.1	84.7	84.1	81.4	84.8	83.2	-					
12. clone D15 30	EU266868	95.3	92.2	91.3	83.4	91.9	90.2	87.5	87.0	91.8	91.4	86.6	-				
13. clone TDNP USbc97	FJ516912	93.6	89.2	89.9	81.7	90.0	88.9	85.7	86.0	89.2	88.3	85.2	92.9	-			
14. clone LCP 6	AF286037	85.7	86.6	84.8	76.7	85.5	89.0	84.2	88.0	85.4	84.7	82.9	88.8	86.8	-		
15. clone 35 52	DQ83349	92.1	99.1	92.6	85.1	97.2	92.6	75.5	77.7	97.1	96.8	71.9	79.3	77.0	75.4	-	
16. clone C8S 110	EU652666	91.9	91.2	93.8	85.4	90.7	91.5	87.7	87.4	89.2	88.1	86.6	91.6	91.1	88.8	79	-
17. clone FW19	AF524005	91.2	94.0	92.8	83.2	93.0	90.8	87.2	91.5	92.7	92.1	86.0	92.1	89.5	94.2	81.9	92.9

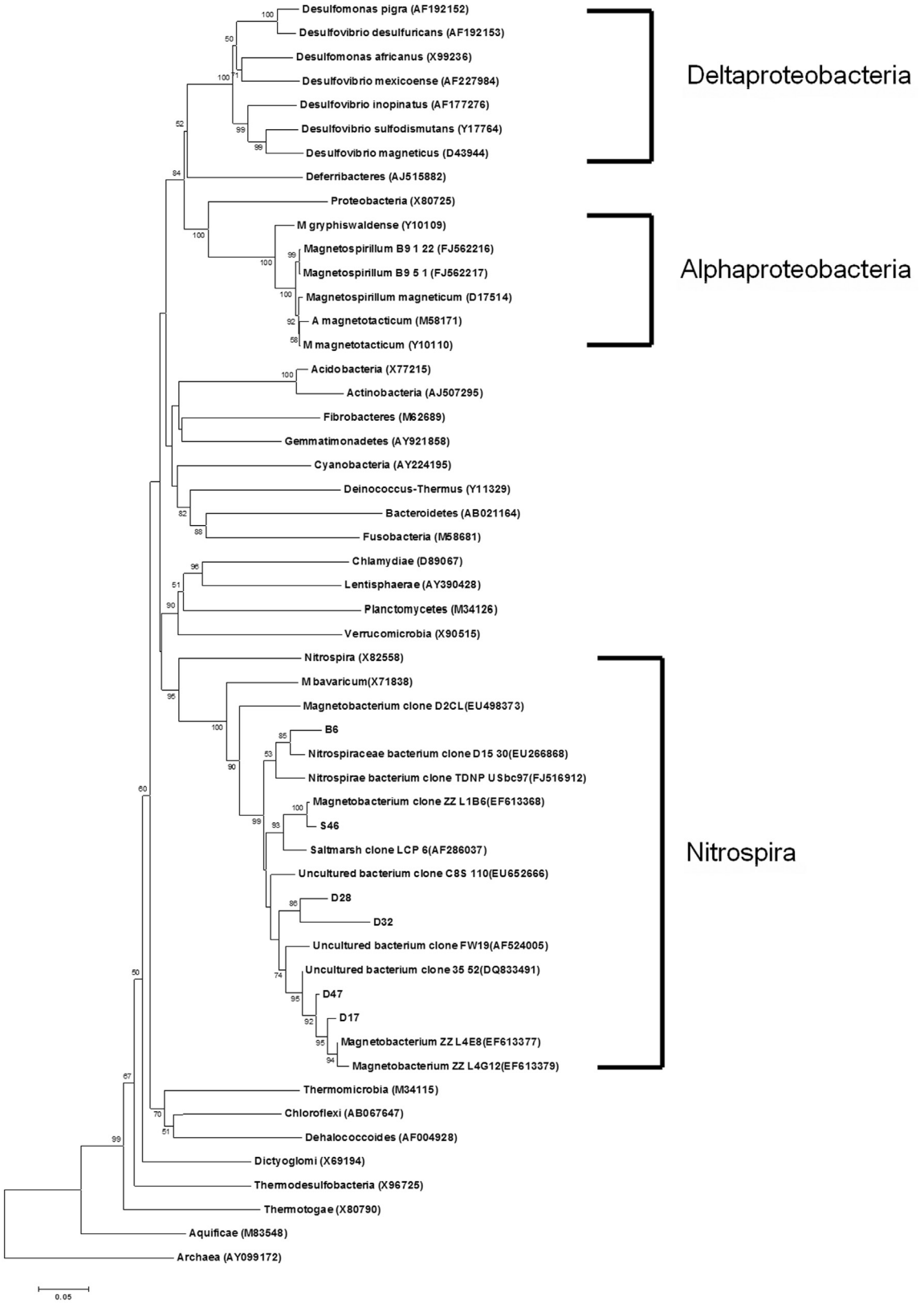

Figure 1 - Phylogenetic positions of magnetotactic bacteria-related 16S rRNA gene sequences recovered from freshwater sediments. The 16S rRNA gene sequences were compared with the most closely related sequences obtained from database (RDP-II), as well as other representatives of related bacterial groups. The phylogenetic distances of each sequence were calculated using the Kimura 2-parameter model and the tree was constructed using the neighbor-joining algorithm. The numbers at the nodes indicate the bootstrap score (as a percentage) and are shown for frequencies at or above the threshold of 50%. The scale bar represents the expected number of changes per nucleotide position.

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